European Journal of Clinical Investigation (2002) **32**, 251–258

Influence of apolipoprotein E polymorphism on plasma vitamin A and vitamin E levels

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Abstract

Background Plasma concentrations of vitamins A and E are positively correlated with those of concurrent lipids and, on the other hand, lipid levels are influenced by apolipoprotein E polymorphism. Therefore, the effect of this polymorphism on both vitamins was analysed in an adult population.

Materials and methods Subjects were recruited from a working population. Their anthropometric, lifestyle and dietary intake variables and menopausal status were recorded. Their apolipoprotein E phenotype and their plasma vitamins A and E (by high-performance liquid chromatography) and lipid (enzymatically) concentrations were determined after an overnight fast. The associations of the phenotype with vitamins and lipids were studied in men and women separately and controlling for significant covariates.

Results The apolipoprotein E phenotype was associated with the concentrations of total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol in women, whereas no associations with lipids were found in men. Vitamin A and vitamin E levels were higher in men than in women, but only the difference in the former persisted after lipid adjustment. Apolipoprotein E2 slightly increased vitamin A levels in women, an effect which was still evident with lipid adjustment. Actually, both the apolipoprotein E phenotype and triglyceride were selected as significant predictors of this vitamin by multiple regression. This phenotype did not affect vitamin E levels in either sex.

Conclusions Lipids do not mediate the effect of gender on vitamin A levels. Apolipoprotein E polymorphism is an independent determinant of vitamin A levels in women. Pending confirmation by others, we propose that enhancement of this vitamin may contribute to the beneficial impact of the ε2 allele on human ageing and health.

Keywords Apolipoprotein E polymorphism, cholesterol, triglyceride, vitamin A, vitamin E.
Eur J Clin Invest 2002; 32 (4): 251–258

Introduction

Apolipoprotein E (apoE) is a constituent of plasma lipoproteins that is coded by three major co-dominant alleles (ε2, ε3 and ε4) whose products (apoE2, apoE3 and apoE4, respectively) give rise to six different phenotypes [1]. Common genetic variation in this locus has a profound influence on human health. Thus, apoE polymorphism is one of the strongest genetic markers associated with longevity, with apoE2 increasing and apoE4 decreasing life expectancy relative to the most frequent isoform apoE3 [2,3]. This may be explained in part by the impact of apoE polymorphism on the prevalence of atherosclerosis and neurological dysfunction, particularly Alzheimer's disease (see below), which are major causes of mortality and morbidity at advanced ages.

By influencing plasma cholesterol concentrations, apoE polymorphism is a determinant for coronary disease in the

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Received 28 May 2001; accepted 18 January 2002

general population [4–6]. Compared to apoE3, the apoE4 isoform is associated with higher levels of total and low-density lipoprotein (LDL) cholesterol and it may decrease high-density lipoprotein (HDL) cholesterol and raise triglyceride levels [4–7]. Consistent with these effects, apoE4 is linked to a greater risk of ischaemic heart disease [6,8]. Notably, this association persists even after adjustment for other traditional risk factors, including LDL and HDL cholesterol [9], suggesting that the influence of apoE polymorphism on the risk of ischaemic heart disease may also be exerted through factors other than lipid levels. ApoE2 exhibits an opposite effect on LDL cholesterol levels to that of apoE4 [4–6]. However, the cardioprotective potential of this effect may be abrogated by the tendency of apoE2 to increase the levels of triglyceride-rich lipoprotein remnants [6,7]. Thus, ε2 homozygosity can cause type III hyperlipoproteinaemia under certain circumstances [10]. As regards neurological disorders, individuals possessing the ε4 allele are more likely to develop late-onset Alzheimer's disease and cognitive decline, whereas subjects carrying the ε2 allele are at lower risk of developing these disorders [3,11–13].

Reactive oxygen-mediated processes are thought to be involved in the pathogenesis of both atherosclerosis [14,15] and Alzheimer's disease [16,17], which has led to a great interest in the potentially protective effect of antioxidant vitamins [18–21]. Cross-cultural epidemiological studies, such as the MONICA Project, have found an inverse correlation of plasma levels of vitamin E and, to a lower extent, vitamins A and C with mortality from ischaemic heart disease [22,23]. On the other hand, it has been reported that Alzheimer's patients have lower plasma levels of vitamin A [24,25] and vitamin E [24–26] than controls.

Vitamins A (*trans*-retinol) and E (mainly α-tocopherol) are crucial for multiple physiological processes and have profound repercussions on reproduction, development and survival [27–30]. They are both lipophilic molecules, which share lipoproteins as the transport particles from the gut to the liver in the postprandial phase (as retinyl esters in the case of vitamin A), but are transported by different means after they are re-secreted into the circulation by the latter organ. Retinyl esters and tocopherol from dietary sources enter the circulation in chylomicrons via the lymphatic system [28–31]. In the plasma compartment, both retinyl esters and tocopherol distribute over all lipoproteins, vitamin E reaching a distribution proportional to the cholesterol concentration in the lipoprotein fractions [32]. Vitamin E taken up in the liver is re-secreted incorporated in very low-density lipoproteins (VLDL) [29,30]. However, the liver secretes vitamin A (free retinol) complexed with the retinol-binding protein [28,31]. Despite this, plasma vitamin A levels usually correlate with concurrent lipids, although not as strongly as vitamin E levels do [22,33–35]. Since apoE polymorphism affects plasma lipid concentrations, in the present study we analysed its relationships with plasma levels of vitamins A and E in an adult population. Given the interaction of gender with the impact of the apoE phenotype on lipid levels [36,37] the study was undertaken in men and women separately.

Methods

Study population

Subjects were recruited from the working population of the Hospital Ramón y Cajal, Madrid. Volunteers were randomly selected, the participation rate being 85%. The male-to-female ratio of the subjects that entered the study (117 men and 244 women) is representative of the working population in such a centre. The average age for males was 42·6 ± 0·9 years (mean ± SE, range 24–67 years) and for females was 40·3 ± 0·5 years (range 24–69 years). An appropriate questionnaire was provided for the subjects to record their smoking habits, pregnancy and menopausal status, oral contraceptive use and medical history. Body weight and height were measured and the body mass index (BMI) was calculated. Dieticians assessed alcohol and dietary intakes through a week-based food-frequency questionnaire [38,39]. The intakes of the following nutrient components were estimated: saturated, monounsaturated and polyunsaturated fat, cholesterol, soluble and complex carbohydrate, fibre, protein, vitamin A, vitamin E and carotene. Pregnant women and subjects taking vitamin supplements or hypolipidaemic medication were not included in the study. Among the women, none was receiving hormone replacement therapy.

Laboratory analyses

Venous blood was drawn from fasting, sitting subjects between 8·30 h and 9·30 h, and 1 mg sodium ethyldiaminetetraacetic acid mL^{-1} of blood was immediately added. After centrifugation at 4 °C, the plasma was separated; part of it was immediately stored at −80 °C until vitamin analyses and part of it was used fresh to isolate VLDL ($d < 1{\cdot}006$ kg L^{-1}) by ultracentrifugation [40]. HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with phosphotungstic acid and magnesium following the manufacturer's instructions (Boëhringer-Mannheim GmbH Diagnostic, Mannheim, Germany). LDL cholesterol was determined by subtracting VLDL and HDL cholesterol from whole plasma values. Cholesterol [interassay coefficient of variation (CV), 2·06%] and triglyceride (interassay CV, 3·42%) concentrations were measured enzymatically (Menarini, Firenze, Italy) with a Technicon Autoanalyser. ApoE phenotyping was performed by isoelectric focusing of delipidated VLDL as previously described [36,40]. The reliability of this technique was confirmed by determining both the apoE phenotype and the apoE genotype in a group of randomly selected subjects, as reported [36].

Vitamin A (*trans*-retinol) and vitamin E (α-tocopherol) concentrations were simultaneously measured by reverse-phase high-performance liquid chromatography (Waters Chromatograph) as previously described [41]. The samples were processed preserving them from light. Briefly, after precipitation of proteins with ethanol–methanol (1 : 1), samples were extracted twice with hexane and the pooled

Table 1 Plasma lipid (mmol L^{-1}) and vitamin (μmol L^{-1}) levels as a function of gender

	Men (n = 117)	Women (n = 244)	P-value*
Total cholesterol	5·61 ± 0·10	5·31 ± 0·06	< 0·05
Total triglyceride	1·46 ± 0·06	0·99 ± 0·04	< 0·0001
VLDL cholesterol	0·37 ± 0·02	0·18 ± 0·01	< 0·0001
VLDL triglyceride	0·97 ± 0·05	0·51 ± 0·03	< 0·0001
LDL cholesterol	4·03 ± 0·09	3·69 ± 0·06	< 0·01
HDL cholesterol	1·23 ± 0·03	1·42 ± 0·02	< 0·0001
Vitamin A	2·07 ± 0·04	1·64 ± 0·03	< 0·0001
adjusted for lipids	2·01 ± 0·04	1·67 ± 0·02	< 0·0001
Vitamin E	30·01 ± 0·58	27·10 ± 0·40	< 0·001
adjusted for lipids	27·70 ± 0·44	28·18 ± 0·29	NS

Values (mean ± SE) are after adjustment for their respective set of covariates selected from anthropometric, lifestyle and dietary variables (see Methods) or from these variables and lipid (total cholesterol and triglyceride) levels when indicated.

NS, not significant.

*P-value by Newman–Keuls test.

extracts were evaporated under nitrogen. The dry residue was redissolved with 200 μL of methanol. Chromatography was carried out using methanol–water (95 : 5) as eluent at a flow-rate of 2·0 mL min^{-1}. Detection was at 340 nm for the first 3 min and at 280 nm thereafter. Retinyl acetate was used as internal standard for both vitamins [41], since the recoveries of retinyl acetate and tocopheryl acetate at the end of the procedure were not significantly different (89 ± 3% and 92 ± 4%; mean ± SE, n = 12, respectively).

Plasma levels of uric acid and urea nitrogen were measured by automated methods.

Statistical analyses

Allele frequencies were determined by the gene-counting method. Frequency distributions of the phenotypes were analysed by the χ^2 goodness of fit test.

To analyse the differences between apoE phenotypes, subjects were divided into three groups: the E2 group (E2/2 and E3/2 subjects), the E3 group (E3/3 subjects) and the E4 group (E4/4 and E4/3 subjects). Subjects with an apoE4/2 phenotype were not included in this analysis. Significant covariates (P < 0·05) for each dependent variable were identified using stepwise multiple regression to select backwards the most parsimonious set of covariates. The possible covariates considered were age, BMI, smoking, alcohol consumption and the dietary intakes indicated above. For women, menopausal status and oral contraceptive use were also considered. Each dependent variable was then adjusted for its respective set of covariates. Analysis of variance (ANOVA) was used to examine the effects of the apoE phenotype. The differences between two groups were assessed by the Newman–Keuls test. Before statistical comparisons, total and VLDL triglyceride and VLDL cholesterol concentrations were log transformed given their skewed distribution.

To ascertain the independent predictors of plasma vitamin A and vitamin E concentrations, stepwise multiple regression analysis was performed. For this, the independent variables were selected backwards among the apoE phenotype, plasma lipid levels, anthropometric and lifestyle variables, menopausal status, dietary components and the concentrations of the other plasma constituents mentioned above. All these variables were previously analysed for co-linearity. The apoE phenotype was represented as two dummy variables, each one coded, respectively, with the number (0,1,2) of alleles ε2 and ε4 in each phenotype. The goodness of the fit of the models was tested by an F ratio. A partial F was used to test the statistical significance for a variable to improve the model. The proportion of the variance of vitamin levels attributable to each independent variable (R^2) in the final model was calculated as the ratio of the sum of squares due to this variable to the covariate-adjusted total sum of squares (total sum of squares minus the covariate sum of squares).

Statistical analyses were performed using the STATGRAPHICS software, version 5 (Statistical Graphics Corporation).

Results

Table 1 shows the plasma levels of lipids and vitamins A and E in men and women adjusted for anthropometric, lifestyle and dietary factors, plus menopausal status in women. Total cholesterol and triglyceride, VLDL cholesterol and triglyceride and LDL cholesterol levels were significantly higher in men than in women, whereas HDL cholesterol levels were higher in women than in men. Men also had higher plasma concentrations of vitamins A and E (Table 1). Consistently with previous reports [22, 33–35], both vitamin A and vitamin E were positively correlated with plasma lipids in the whole population. Vitamin A was more strongly correlated with triglyceride (r = 0·45, P < 0·0001) than with cholesterol (r = 0·21, P < 0·0001) levels, whereas vitamin E was correlated with cholesterol (r = 0·69, P < 0·0001) to a

Table 2 Plasma lipid (mmol L^{-1}) and vitamin (μmol L^{-1}) levels as a function of the apoE phenotype in men

	ApoE phenotype		
	E2 (n = 16)	E3 (n = 90)	E4 (n = 10)
Total cholesterol	5·45 ± 0·27	5·79 ± 0·11	5·56 ± 0·34
Total triglyceride	1·70 ± 0·21	1·55 ± 0·09	1·53 ± 0·27
VLDL cholesterol	0·52 ± 0·07	0·38 ± 0·03	0·37 ± 0·10
VLDL triglyceride	1·14 ± 0·18	1·05 ± 0·08	1·05 ± 0·23
LDL cholesterol	3·77 ± 0·25	4·20 ± 0·11	4·14 ± 0·32
HDL cholesterol	1·15 ± 0·06	1·22 ± 0·03	1·03 ± 0·08
Vitamin A	2·02 ± 0·10	2·10 ± 0·04	2·27 ± 0·12
adjusted for lipids	2·01 ± 0·10	2·10 ± 0·04	2·25 ± 0·12
Vitamin E	28·58 ± 1·80	30·36 ± 0·76	31·86 ± 2·29
adjusted for lipids	29·43 ± 1·19	30·10 ± 0·50	32·84 ± 1·49

Values (mean ± SE) are after adjustment for their respective set of covariates selected from anthropometric, lifestyle and dietary variables (see Methods) or from these variables and lipid (total cholesterol and triglyceride) levels when indicated.

All comparisons were not significant by ANOVA.

greater extent than with triglyceride (r = 0·50, P < 0·0001) levels. Given these associations, vitamin levels in men and women were compared after the adjustment, also taking into account their lipid levels. Lipid-adjusted vitamin E was not significantly different between sexes, whereas lipid-adjusted vitamin A remained lower in women than in men (Table 1), indicating that the difference in the latter vitamin was independent of the lipid levels.

We then studied the association of the apoE phenotype with the variations in vitamin and lipid levels in these subjects. The number of subjects possessing each phenotype were the following: E2/2, 0; E3/2, 46; E3/3, 270; E4/3, 41; E4/4, 3 and E4/2, 1, with no significant differences in the relative frequencies between men and women (χ^2 = 1·68, P = 0·43, 2 d.f.). The resulting allele frequencies were: ε2, 0·065; ε3, 0·868 and ε4, 0·067. This low prevalence of the ε4 allele as compared to that in other Caucasian populations [4,5] is in agreement with previous findings [36,42]. The distribution of the observed frequencies of the different apoE phenotypes was in Hardy–Weimberg equilibrium in the total population (χ^2 = 0·38, P = 0·83, 2 d.f.), in men (χ^2 = 0·26, P = 0·88, 2 d.f.) and in women (χ^2 = 0·40, P = 0·82, 2 d.f.).

Adjusted lipid and vitamin levels as a function of the apoE phenotype were analysed in men and women separately. In men, no significant associations were found between lipid levels and the apoE phenotype (Table 2). Similarly, the concentrations of vitamins A and E, either nonlipid or lipid-adjusted, were not associated with this polymorphism (Table 2). In women, however, the concentrations of total, LDL and HDL cholesterol varied significantly with the apoE phenotype (Table 3). ApoE2 decreased total and LDL cholesterol, whereas apoE4 exerted an opposite effect on these variables. Besides, apoE4 significantly lowered HDL cholesterol as compared to the E2 group. On the other hand, vitamin E was not significantly different between the apoE phenotypes, independent of the adjustment used (Table 3). However, the variation in vitamin A levels in women was significantly associated with the apoE phenotype, the apoE2 carriers having a slightly higher concentration than the E3 and E4 subjects. The difference in the concentrations of vitamin A among the apoE phenotypes remained after the adjustment for lipid levels (Table 3).

It has been consistently reported that alcohol intake [33,35,43,44] and oral contraceptive use [45–48] increase vitamin A levels. Actually, in the present study both variables were selected as covariates for vitamin A concentrations in women and, hence, these concentrations were adjusted for such variables prior to the analysis. However, to confirm that they did not have a confounding effect on the difference in vitamin A between the apoE phenotypes, further analyses were performed. Both alcohol consumption and the distribution of oral contraceptive users did not significantly differ between the three apoE phenotypes (not shown). When women consuming alcohol (n = 48) were excluded from the analysis, the group possessing apoE2 still had significantly higher vitamin A levels than the groups not possessing it (1·77 ± 0·07, 1·58 ± 0·03, 1·57 ± 0·07, for the E2, E3 and E4 groups, respectively; P by ANOVA < 0·05, P by Newman–Keuls test < 0·05), a difference that persisted after lipid adjustment (1·75 ± 0·07, 1·59 ± 0·03, 1·53 ± 0·07; P by ANOVA < 0·05, P by Newman–Keuls test < 0·05). Similarly, the exclusion of oral contraceptive users (n = 23) did not eliminate such an influence of apoE2 on vitamin A concentrations, either without (1·77 ± 0·07, 1·58 ± 0·03, 1·60 ± 0·06; P by ANOVA < 0·05, P by Newman–Keuls test < 0·05) or with lipid adjustment (1·75 ± 0·06, 1·59 ± 0·02, 1·58 ± 0·05; P by ANOVA < 0·05, P by Newman–Keuls test < 0·05).

The present results indicate that vitamin A levels in women varied as a function of the apoE phenotype and that this association was independent of the effect of such a polymorphism on lipid levels. Moreover, such an association was not due to confounding variables, as studied. Nevertheless, other variables, not considered to adjust the data, could possibly mediate the effect of apoE2 on vitamin A levels. It

Table 3 Plasma lipid (mmol L^{-1}) and vitamin (μmol L^{-1}) levels as a function of the apoE phenotype in women

	ApoE phenotype			
	E2 (n = 30)	E3 (n = 180)	E4 (n = 34)	ANOVA
Total cholesterol	4·93 ± 0·18[a]	5·25 ± 0·07[ab]	5·56 ± 0·16[b]	$P < 0·05$
Total triglyceride	1·00 ± 0·07	0·91 ± 0·03	0·94 ± 0·07	NS
VLDL cholesterol	0·19 ± 0·03	0·16 ± 0·01	0·19 ± 0·02	NS
VLDL triglyceride	0·52 ± 0·06	0·45 ± 0·02	0·47 ± 0·05	NS
LDL cholesterol	3·24 ± 0·16[a]	3·65 ± 0·06[b]	4·02 ± 0·15[c]	$P < 0·01$
HDL cholesterol	1·52 ± 0·05[a]	1·45 ± 0·02[ab]	1·34 ± 0·05[b]	$P < 0·05$
Vitamin A	1·79 ± 0·07[a]	1·60 ± 0·03[b]	1·61 ± 0·06[b]	$P < 0·05$
adjusted for lipids	1·77 ± 0·06[a]	1·60 ± 0·02[b]	1·60 ± 0·06[b]	$P < 0·05$
Vitamin E	27·35 ± 1·03	26·63 ± 0·42	28·23 ± 0·95	NS
adjusted for lipids	27·94 ± 0·77	26·71 ± 0·31	27·12 ± 0·72	NS

Values (mean ± SE) are after adjustment for their respective set of covariates selected from anthropometric, menopausal status, lifestyle and dietary variables (see Methods) or from these variables and lipid (total cholesterol and triglyceride) levels when indicated. When statistically significant differences were found by ANOVA, comparisons between each pair of groups were performed by the Newman–Keuls test. Values not sharing any superscript are significantly different ($P < 0·05$) by this test.

NS, not significant.

has been reported that plasma concentrations of vitamin A are increased in uraemia [49]. The possibility existed that urea nitrogen and/or uric acid levels were associated with vitamin A levels in the general population also. Thus, the associations of plasma urea nitrogen and uric acid with vitamin A were explored by univariate analysis. It was found that the concentrations of urea nitrogen ($r = 0·25$; $P < 0·001$) and uric acid ($r = 0·22$; $P < 0·001$) were also correlated with that of vitamin A. However, neither of them varied with the apoE phenotype (results not shown).

To further establish an independent contribution of the apoE phenotype to the concentration of vitamin A in women, stepwise multiple regression analysis was performed. For this, the above mentioned plasma constituents were also considered among the independent variables. By this analysis, the apoE phenotype was selected as an independent predictor of vitamin A levels ($P < 0·01$). The other significant variables were triglyceride ($P < 0·0001$) and urea nitrogen ($P < 0·0001$) levels, oral contraceptive use ($P < 0·001$) and alcohol intake ($P < 0·01$; R^2 of the model = 0·32, $P < 0·0001$). ApoE polymorphism explained 3·3% of the variance of this vitamin. Triglyceride was the main predictor of this vitamin, accounting for 18·3% of its variance, whereas the contribution of the other significant terms to the variance was of the same order as that of apoE polymorphism.

When multiple regression analysis was performed for women's vitamin E levels as the dependent variable, the variables selected were cholesterol ($P < 0·0001$) and triglyceride ($P < 0·0001$) concentrations, polyunsaturated fatty acid intake ($P < 0·01$) and age ($P < 0·05$; R^2 of the model = 0·52, $P < 0·0001$). Thus, the apoE phenotype was not selected as an independent determinant of vitamin E levels. Cholesterol and triglyceride levels were the main predictors of the concentration of vitamin E, explaining 32·6% and 11·5%, respectively, of its variance, whereas the rest of the variables explained less than 3% each.

Discussion

Given that the levels of vitamins A (free retinol) and E are positively correlated with those of concurrent lipids, in the present work the relationships between these vitamins and apoE polymorphism were examined in men and women from a working population. Men had higher concentrations of vitamins A and E than women, but the bases for these differences were distinct. The higher vitamin E levels in men were attributable to their higher plasma concentrations of both cholesterol and triglyceride as compared to women, in agreement with the essentially lipoprotein-mediated transport of this vitamin in plasma [29]. In contrast, the difference in vitamin A levels between men and women was not mediated through their lipid levels. This is consistent with the weaker correlation of vitamin A with lipids than that of vitamin E. Therefore, the present results indicate a different relationship for each of these vitamins with circulating lipids.

To get a deeper insight into these relationships, the effect of the apoE phenotype as an important determinant of lipid levels was analysed. This phenotype was not found to be significantly associated with variations in vitamin E levels in either men or women. The lack of effect in men was in correspondence with the lack of effect of the apoE phenotype on their lipoprotein levels. However, in women this phenotype did affect cholesterol levels, which were correlated with vitamin E ($r = 0·66$, $P < 0·0001$). The absence of an effect of apoE polymorphism on vitamin E in women can be attributed to the fact that as much as two-thirds of the variability of this vitamin was not explained by cholesterol levels, as determined by multiple regression.

The main finding of the present work was an independent influence of apoE polymorphism on vitamin A levels in women, with apoE2 having a slight increasing effect. Particularly, such an influence was not dependent on the impact of this isoform on lipids. Firstly, the raising effect of apoE2 on vitamin A cannot be explained by the relationship

of this vitamin with triglyceride levels observed in women, since, in agreement with our previous observation [36], the apoE phenotype was not significantly associated with variations in total and VLDL triglyceride. Secondly, cholesterol levels, although different as a function of the apoE phenotype, were not independently associated with this vitamin. The possible confounding influences of anthropometric parameters, lifestyle factors, dietary intakes, menopausal status and the circulating levels of some other plasma constituents in the effect of apoE2 on plasma vitamin A were also discarded. The mechanism responsible for such an effect of the apoE phenotype on vitamin A levels remains to be clarified.

The reason for the lack of influence of apoE polymorphism on vitamin A levels in men is unclear. It is possible that some gender-specific factor(s) modulate the impact of this polymorphism on vitamin A levels. Such an interaction with gender was reported previously regarding the effect of apoE polymorphism on lipoprotein levels [36, 37]. For example, we have found that in our population the apoE phenotype influenced LDL and HDL levels in women, whereas in men it influenced VLDL, but did not affect cholesterol-rich lipoprotein levels [36]. In the present study, the low number of subjects with the E2 or E4 isoforms, which have a low prevalence, probably masked such an effect on men's VLDL. This same reasoning can be followed not to rule out the possibility of an impact of apoE polymorphism on vitamin A levels in men.

It could be estimated that the apoE phenotype explained approximately 3% of the variability of women's vitamin A levels, which was of the same order as that estimated for oral contraceptive use and alcohol intake, two variables whose enhancing effects on vitamin A concentration are well recognized [33,35,43–48]. However, the main predictor of vitamin A levels among the variables studied herein was triglyceride, which explained 18% of its variance, suggesting a link between this vitamin and triglyceride metabolism. In keeping with this, it has been reported that oral administration of retinol or other retinoids causes an increase in circulating triglyceride [50–52]. Retinoids enhance the transcription of the human apoC-III gene – whose product antagonizes the catabolism of triglyceride-rich lipoproteins [53] – via the retinoid X receptor (RXR) [54], which recognizes the vitamin A derivative 9-*cis*-retinoic acid as a natural ligand [28]. Thus, the relationship between vitamin A and triglyceride levels may reside on a delay of triglyceride-rich lipoprotein catabolism caused by the vitamin.

Despite the fact that cholesterol levels showed a weak, not independent, relationship with vitamin A, this can also potentially modulate cholesterol metabolism. Once 9-*cis*-retinoic acid has activated RXR, this forms heterodimers with other nuclear receptors, including the liver X receptors (LXR), the farnesoid X receptor (FXR) and the peroxisome proliferator-activated receptors (PPAR), that control the transcription of several genes involved in triglyceride and cholesterol homeostasis [55–58]. Repa *et al.* have demonstrated recently that the activation of RXR inhibits cholesterol absorption by a dual mechanism: the RXR/LXR-mediated up-regulation of the transporter ATP-binding cassette A1 (ABCA1) to increase cholesterol efflux by the intestine, and the RXR/FXR-mediated repression of the enzyme CYP7A1 to reduce the synthesis and, hence, the intestinal pool of bile acids [59]. It has been reported that subjects carrying the ε2 allele have lower cholesterol absorption as compared with subjects homozygous for the ε3 allele or carrying the ε4 allele [60,61]. Thus, it is tempting to speculate that, through 9-*cis*-retinoic acid, an increased concentration of vitamin A would mediate the inhibition of cholesterol absorption in apoE2 subjects. Although the results of the present study do not suggest a substantial repercussion of vitamin A on cholesterol levels, the determination of the possible involvement of RXR-mediated processes in the impact of apoE2 on cholesterol homeostasis deserves further investigation.

Vitamin A plays a crucial role in multiple and relevant physiological processes such as embryonic development, growth, vision, immunity and survival of vertebrates [27,28]. On the other hand, the ε2 allele has been related with increased longevity, whereas the opposite applies for the ε4 allele [2,3]. The effects of these alleles on the risks for atherosclerosis and Alzheimer's disease may in part explain such associations. The lowering effect on LDL cholesterol confers antiatherogenic potential to the ε2 allele [4–6], although in certain circumstances this influence is offset by the accumulation of triglyceride-rich lipoprotein remnants [10]. With regard to Alzheimer's disease, it has been reported that allele ε2 is protective against the late-onset form of this pathology [3,11–13]. Thus, the effect of apoE2 on vitamin A levels raises the question of its possible contribution to the beneficial impact of the ε2 allele on such disorders. Consistently with this hypothesis, cross-cultural epidemiological studies, such as the MONICA Project, have found that vitamin A is independent and negatively related with ischaemic heart disease mortality [22,23]. Moreover, some studies have reported reduced plasma concentrations of vitamin A in Alzheimer's disease [24,25] and vascular dementia [25] patients.

In summary, in the population studied herein, the variation in lipid levels accounted for the difference in vitamin E between men and women, but not for that observed in vitamin A, which is consistent with the different nature of the relationships of each of these vitamins with lipids. On the other hand, apoE polymorphism influenced vitamin A levels in women, with the E2 isoform having an increasing effect. This influence was independent of different variables related with vitamin A concentration and, in particular, it was not related with the association existing between triglyceride and vitamin A levels. Pending confirmation of these findings by others, it is hypothesized that the enhancement in vitamin A contributes to the beneficial effects of the ε2 allele on human ageing and health.

Acknowledgements

The study was supported by grants from the Fundación Ramón Areces and the Fondo de Investigación Sanitaria

(94/0540 and 99/0286), Spain. The authors wish to thank Antonia Arbiell and Ángela Murúa for excellent technical assistance, and Dr Victor Abraira for statistical advice.

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