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The effect of *apoE* genotype and sex on ApoE plasma concentration is determined by dietary fat in healthy subjects

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The interindividual variation in ApoE plasma concentration is considerable, mainly determined by *apoE* genotype and sex. However, a large amount of variability remains unexplained by these factors. We have evaluated whether the quantity and quality of dietary fat interacts with the *apoE* genotype and sex modifying ApoE plasma levels in young healthy subjects. Eighty-four volunteers (sixty-six apoE3/3, eight apoE4/3 and ten apoE3/2) were subjected to three dietary periods, each lasting 4 weeks. The first was a SFA-enriched diet (38 % fat and 20 % SFA), which was followed by a carbohydrate (CHO)-rich diet (30 % fat, <10 % SFA and 55 % carbohydrate) or a MUFA-rich diet (38 % fat and 22 % MUFA) following a randomised crossover design. *apoE2* carriers have the highest ApoE levels, whereas *apoE4* individuals show the lowest concentration after the SFA, CHO and MUFA diets. Women had significantly higher ApoE concentration than men only after the consumption of the SFA diet. The SFA diet increased the ApoE plasma concentration when compared with the CHO- and MUFA-rich diets in women, but not in men. In women, but not in men, the shift from the SFA- to CHO- or MUFA-rich diets significantly decreased the ApoE concentration in apoE3/2 and apoE3/3 subjects, whereas no differences were observed in women with the apoE4/3 genotype. Sex and *apoE* genotype determine ApoE plasma levels; however, this effect is dependent on dietary fat.

***apoE* genotype: Diet: Sex: ApoE concentration**

ApoE is a structural component of several lipoproteins and plays an important role in lipid metabolism through both promoting efficient uptake of TAG-rich lipoproteins from the circulation[1,2] and taking part in the cellular cholesterol efflux and reverse cholesterol transport[3]. This apoE presents three major isoforms (ApoE2, ApoE3 and ApoE4) that modulate plasma lipid levels and have been implicated in CHD. The apoE4 allele is associated with increased risk of CHD, higher total and LDL-cholesterol and lower ApoE concentrations, whereas the apoE2 allele presents the opposite effects[4,5]. Nevertheless, the apoE polymorphism is insufficient to explain the development of CHD, and several works give increasing evidence that serum concentration of ApoE, by itself, could play an independent role in this process[6–9]. Thus, circulating ApoE concentration appears to be altered in hyperlipidaemia[10–13], and case–control studies show that ApoE levels might be a cardiovascular risk factor[14–19].

The total interindividual variation in serum ApoE concentration is considerable in Caucasian populations (25–32 %)[20]. It has been reported that up to 30 % of the total variability in the ApoE concentration can be attributed to the apoE polymorphism[20,21]; however, a large amount of variability remains unexplained by this genetic factor, suggesting that other genetic or environmental components, such as sex or diet, are major determinants of its concentration. In agreement with this hypothesis, previous data have shown that cholesterol, fatty acids and oestrogens modulate liver ApoE secretion[22]. Furthermore, it has been demonstrated in both human[23,24] and animal studies[13,25] that a diet high in saturated fat and cholesterol leads to elevations in the ApoE plasma levels. These observations suggest that changes in the circulating levels of ApoE may be an important consequence of dietary habits. However, to our knowledge, no previous studies have compared the effect of both the quantity and quality of dietary fat on the ApoE plasma concentration. On the other hand, studies assessing associations between sex and ApoE concentration have shown contradictory results. Indeed, in some studies, it has been demonstrated that ApoE plasma levels were higher in women[26–28], whereas others reported higher ApoE plasma concentration in men[29] or even no difference was observed between men and women[30]. Therefore, the objective of the present study was to analyse whether the quantity and quality of dietary fat interacts with the *apoE* genotype or sex determining ApoE plasma levels in young healthy subjects.

Abbreviation: CHO, carbohydrate.

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Materials and methods

Human subjects

A group of eighty-four subjects (eight apoE4/3, sixty-six apoE3/3, ten apoE3/2), including both men (*n* 56; four 4/3, forty-six 3/3 and six 3/2) and women (*n* 28; four 4/3, twenty 3/3 and four 3/2), were recruited from among students at the University of Cordoba. The subjects had a mean age of 21·55 years (SD 0·40). Informed consent was obtained from all participants. All subjects underwent a comprehensive medical history, physical examination and clinical chemistry analysis before enrolment. The subjects showed no evidence of any chronic disease (hepatic, renal, thyroid or cardiac dysfunction), obesity or unusually high levels of physical activity (e.g. sports training). None of the subjects had a family history of premature coronary artery disease, or had taken medications or vitamin supplements in the 6 months before the study. Physical activity and diet, including alcohol consumption, were recorded in a personal log for 1 week and the data were used to calculate individual energy requirements. Mean BMI was 22·86 kg/m^2 (SD 0·28) at the onset of the study and remained constant throughout the experimental period. The subjects were encouraged to maintain their regular physical activity and lifestyle, and were asked to record in a diary any event that could affect the outcome of the study, such as stress, change in smoking habits and alcohol consumption or intake of foods not included in the experiment design. No changes in lifestyle were observed at the end of the study. The study protocol was approved by the Human Investigation Review Committee at the Reina Sofia University Hospital.

Diets

The study design included an initial 28-day period during which all subjects consumed a SFA-rich diet, with 15 % protein, 47 % carbohydrate and 38 % fat (20 % SFA, 12 % MUFA and 6 % PUFA). After this period, volunteers were randomly assigned to one of two diet sequences. Forty-two subjects received a MUFA-rich diet containing 15 % protein, 47 % carbohydrates and 38 % fat (< 10 % SFA, 6 % PUFA and 22 % MUFA) for 28 d. This diet was followed for 28 d by the consumption of a carbohydrate (CHO)-rich diet containing 15 % protein, 55 % carbohydrates and < 30 % fat (< 10 % SFA, 6 % PUFA and 12 % MUFA). The other forty-two subjects consumed the CHO diet before the MUFA diet. The cholesterol content remained constant (under 300 mg/d) during the three periods. Eighty per cent of the MUFA diet was provided by virgin olive oil, which was used for cooking, salad dressing and as a spread. Carbohydrate intake of the CHO diet was based on the consumption of biscuits, jam and bread. Butter and palm oil were used during the SFA dietary period.

The composition of the experimental diets was calculated using the US Department of Agriculture[31] food tables and Spanish food composition tables for local foodstuffs[32]. All meals were prepared in the hospital kitchen and were supervised by a dietitian. Lunch and dinner were eaten in the hospital dining room, whereas breakfast and an afternoon snack were eaten in the medical school cafeteria. Fourteen menus were prepared with regular solid foods and rotated during the experimental period. Duplicate samples from each menu were collected, homogenised and stored at −70°C. Protein, fat and carbohydrate contents of the diet were analysed by standard methods[33]. Dietary compliance was verified by analysing the fatty acids in LDL-cholesteryl esters at the end of each dietary period[34]. The study took place from January to March to minimise seasonal effects and academic stress.

Lipid analysis and biochemical determinations

Venous blood samples were collected into EDTA-containing (1 g/l) tubes from all subjects after a 12 h overnight fast at the beginning of the study and at the end of each dietary period. Plasma was obtained by low-speed centrifugation for 15 min at 4°C within 1 h of venepuncture. To reduce interassay variation, plasma was stored at −80°C and analysed at the end of the study. Plasma total cholesterol and TAG levels were determined by enzymatic techniques[35,36]. HDL cholesterol was determined after precipitation with phosphowolframic acid[37]. ApoA-I and ApoB were determined by immunoturbidimetry[38]. LDL-cholesterol concentration was calculated using the Friedewald formula[39]. Plasma ApoE concentration was measured using an immunonephelometric method on a BN ProSpec System with commercial kits (Dade Behring, Deerfield, IL, USA).

DNA amplification and genotyping of ApoE

Amplification of a region of 266 bp of the apoE genotype was done by PCR with 250 ng of genomic DNA, 0·2 μmol of each oligonucleotide primer (E1 5′-GAACAACTGACCCCGGTGGCGGAG-3′ and E2 5′-TCGCGGGCCCCGGCCTGGTACACTGCCA-3′) and 10 % dimethyl sulfoxide in 50 μl. DNA was denatured at 95°C for 5 min followed by thirty cycles of denaturation at 95°C for 1 min, annealing at 63°C for 1·5 min and extension at 72°C for 2 min. Twenty microlitres of the PCR product were digested with ten units of restriction enzyme *CfoI* (BRL, Gaithersburg, MD, USA) in a total volume of 35 μl. Digested DNA was separated by electrophoresis on an 8 % non-denaturing polyacrylamide gel at 150 V for 2 h. Bands were visualised by silver staining.

Statistical analysis

We used the repeated-measures ANOVA to test the effects of the apoE gene polymorphism or sex on plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, TAG, ApoA-I, ApoB and ApoE concentrations after each dietary stage. When statistical significance was found, Tukey's *post hoc* comparison test was used to identify group differences. TAG and ApoE plasma levels were log-transformed before statistical analyses. Independent sample *t* test was conducted between the two groups that consumed the MUFA, then the CHO *v.* the CHO and then the MUFA diet to test whether the MUFA–CHO differences depended on whether MUFA or CHO was first (order effects). Statistical significance was considered at $P < 0{\cdot}05$. Statistical analyses were conducted using the SPSS statistical software, version 9.0 (SPSS Inc., Chicago, IL, USA).

Results

Significant differences were observed when we compared the baseline characteristics of subjects with sex (Table 1). Women had significantly lower BMI and TAG and higher HDL-C and ApoA-I plasma levels than men. Table 2 shows age, BMI and plasma lipid and apoE levels by *apoE* genotype and sex at the beginning of the study. Mean total and LDL-cholesterol levels were significantly ($P<0·05$) higher in subjects with the apoE4/3 genotype when compared with apoE3/3 and apoE3/2 individuals in both men and women. Furthermore, we observed that the ApoE plasma concentration differed significantly ($P<0·05$) among *apoE* genotypes in both sexes: apoE2 carriers had the highest ApoE plasma concentrations and subjects with the apoE4 allele had the lowest.

The composition of the mean daily intake of the participants is shown in Table 3. Analysis of fatty acid composition of LDL-cholesterol esters obtained after each dietary period showed good adherence in the different intervention stages. After the SFA diet period, we observed a significantly greater ($P<0·005$) increase in palmitic acid in the LDL-cholesterol esters than observed after the CHO and MUFA diets: 27·3 (SD 1·4) % compared with 19·8 (SD 3·9) % and 15·2 (SD 0·4) %, respectively. A significantly greater ($P<0·05$) increase in oleic acid in the cholesterol esters was also seen after the MUFA diet (50·3 (SD 4·7) %) than after the CHO diet (38·8 (SD 9·0) %), but not after the SFA diet (47·2 (SD 4·4) %).

Changes in the diet were associated with significant lower concentration of total ($P<0·001$), LDL ($P<0·001$) and HDL cholesterol ($P<0·05$), apoA-I ($P<0·05$) and apoB ($P<0·001$) after the CHO and MUFA diets (Table 4). However, in comparison with the MUFA diet, the CHO diet was associated with significant lower plasma concentrations of HDL-C ($P<0·05$) and apoA-I ($P<0·01$). Significant differences were not observed in TAG concentrations after the different diets ($P=0·695$).

Dietary intervention had significant effects on the ApoE plasma concentration (Table 5). In comparison with the SFA diet, the CHO and MUFA diets were associated with a decrease in the ApoE plasma concentration ($-1·07$, $P<0·05$ and $-2·47$ mg/l, $P<0·001$, respectively). These differences among diets were observed in women, but not in men (Table 5). However, when the CHO diet was compared with the MUFA diet, no significant differences were observed for ApoE plasma levels in either men or women. In addition, a significant effect on the ApoE concentration was observed for the interaction between diet and sex. Thus, women had significantly higher ApoE plasma levels than men after the SFA diet ($P=0·003$), but not after the MUFA or CHO diet (Table 5).

The *apoE* genotype determined the ApoE plasma concentration ($P<0·001$), which was lower in subjects with the apoE4/3 genotype (25·63 (SD 2·22) mg/l), intermediate in apoE3/3 subjects (31·02 (SD 0·68) mg/l) and higher in apoE3/2 individuals (44·42 (SD 1·82) mg/l) after the SFA, CHO and MUFA diet periods. These differences among *apoE* genotypes were observed for both men and women (data not shown). On the other hand, a significant sex × diet × genotype interaction effect was found for the ApoE plasma concentration ($P<0·05$). In women, but not in men, the shift from the SFA diet to the MUFA diet significantly decreased ($P<0·05$) the ApoE plasma concentration in apoE3/3 ($-8·12$ %) and apoE3/2 ($-11·92$ %) subjects, whereas apoE4/3 individuals maintained with almost similar levels of ApoE ($-3·07$ %). Additionally, there was a significant decrease in the ApoE plasma concentration in women with the apoE3/3 and apoE3/2 genotypes after changing from a SFA to a CHO diet ($-4·67$ and $-9·71$ %, respectively, $P<0·05$), whereas no differences (1·65 %) were observed in women with the apoE4/3 genotype (Table 6). Finally, women with the apoE3/3 and apoE3/2 genotypes showed

Table 1. Baseline characteristics of plasma lipids and apo according to sex before the dietary intervention study*

(Mean values and standard deviations)

	Men (*n* 56)		Women (*n* 28)		
	Mean	SD	Mean	SD	*P*
Age (years)	21·38	1·36	22·36	1·07	0·18
BMI (kg/m^2)	23·00	2·76	20·04	2·54	0·001
Total C (mmol/l)	4·10	0·59	4·16	0·67	0·81
LDL-C (mmol/l)	2·50	0·59	2·37	0·66	0·40
HDL-C (mmol/l)	1·18	0·35	1·48	0·39	0·001
ApoA-I (g/l)	1·24	0·15	1·57	0·16	0·001
ApoB (g/l)	0·64	0·13	0·72	0·12	0·16
ApoE (mg/l)	32·83	0·96	34·7	1·41	0·24
TAG (mmol/l)	0·92	0·46	0·66	0·27	0·006

* Data were analysed by ANOVA.

Table 2. Baseline characteristics of plasma lipids and apo by *apoE* genotypes and sex*

(Mean values and standard deviations)

	ApoE4/3		ApoE3/3		ApoE3/2		
	Mean	SD	Mean	SD	Mean	SD	*P*
Age (years)							
Men	23·33	2·51	21·23	1·85	21·25	2·60	0·22
Women	23·01	1·54	22·25	1·05	21·00	1·78	0·26
BMI (kg/m^2)							
Men	22·84	2·43	23·83	2·70	24·29	2·79	0·72
Women	21·76	3·29	19·70	2·64	17·34	3·18	0·18
Total C (mmol/l)							
Men	4·89^a	0·41	4·07^b	0·64	3·74^b	0·55	0·028
Women	4·53^a	0·50	4·11^b	0·52	3·81^b	0·10	0·036
LDL-C (mmol/l)							
Men	3·20^a	0·38	2·48^b	0·59	2·10^b	0·51	0·021
Women	2·84^a	0·48	2·31^b	0·62	2·01^b	0·39	0·043
HDL-C (mmol/l)							
Men	1·28	0·27	1·19	0·29	1·10	0·22	0·59
Women	1·43	0·43	1·48	0·40	1·52	0·61	0·96
ApoA-I (g/l)							
Men	1·26	0·29	1·23	0·22	1·19	0·20	0·84
Women	1·60	0·15	1·53	0·13	1·63	0·11	0·41
ApoB (g/l)							
Men	0·77	0·08	0·64	0·15	0·57	0·12	0·09
Women	0·82	0·13	0·72	0·12	0·67	0·04	0·25
ApoE (mg/l)							
Men	23·21^a	2·56	30·81^b	1·98	43·63^c	2·31	0·023
Women	27·34^a	2·28	32·27^b	2·25	46·53^c	3·14	0·034
TAG (mmol/l)							
Men	0·91	0·12	0·88	0·49	1·14	0·39	0·30
Women	0·56	0·20	0·67	0·27	0·63	0·24	0·75

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different between genotypes ($P<0·05$; ANOVA).

* A group of eighty-four subjects (eight apoE4/3, sixty-six apoE3/3, ten apoE3/2), including men (*n* 56; four apoE4/3, forty-six apoE3/3 and six apoE3/2) and women (*n* 28; four apoE4/3, twenty apoE3/3 and four apoE3/2).

Table 3. Daily intake during each experimental diet period

	SFA diet	CHO diet	MUFA diet
Protein (percentage of energy intake)			
Calculated	15	15	15
Analysed	18·0	17·5	17·7
Fat (percentage of energy intake)			
Saturated			
Calculated	20	10	10
Analysed	22·2	9·1	9·1
Monounsaturated			
Calculated	12	12	22
Analysed	11·1	13·2	24·1
Polyunsaturated			
Calculated	6	6	6
Analysed	5·1	5·2	4·9
Carbohydrates (percentage of energy intake)			
Calculated	47	57	47
Analysed	44·2	54·5	44·1
Complex	27·1	33·3	27·5
Simple	17·1	21·2	16·6
Cholesterol (mg/d)			
Calculated	285	285	285
Analysed	272	275	277
Fibre (g/d)			
Calculated	30	30	30
Analysed	25·8	26·0	24·6
Energy (MJ)	10·2	10·2	10·2

CHO, low-fat, high-carbohydrate diet.

higher ApoE plasma levels than men after the SFA-rich diet, but not after the MUFA or CHO diet (Table 6). No significant differences between men and women were observed in apoE4/3 subjects after the three dietary periods.

Plasma lipids were analysed in both men and women according to the apoE genotype at the end of each diary stage (Tables 7 and 8). Significant differences in the HDL-cholesterol ($P=0{\cdot}016$) and TAG ($P=0{\cdot}030$) levels were observed between women with the apoE3/2 genotype and those with the apoE3/3 and apoE4/3 genotypes when changing their diet from SFA to MUFA or CHO (Table 8). In women with the apoE3/2 genotype, the decrease in the HDL-cholesterol plasma concentration was significantly greater than that in women with the apoE3/3 or apoE4/3 genotype when these women switched from a SFA diet to a CHO or MUFA diet (apoE3/2 −16 and −13 % *v.* apoE3/3 −8 and −7 % and apoE4/3 −4 and −1 %, respectively). In addition, women with the apoE3/2 genotype had an increase in TAG concentrations after changing their diet from SFA to CHO or MUFA, whereas women with the apoE3/3 or apoE4/3 genotype maintained with almost similar levels (apoE3/2 14 and 16 % *v.* apoE3/3 3 and 7 % and apoE4/3 −4 and −1 %, respectively). These differences in the HDL-cholesterol or TAG levels were not observed for men.

Table 4. Plasma lipid levels of all subjects (n 84) after dietary intervention (Mean values and standard deviations)

	SFA		CHO		MUFA	
	Mean	SD	Mean	SD	Mean	SD
Total cholesterol (mmol/l)	4·24[a]	0·62	3·68[b]	0·60	3·75[b]	0·64
LDL-C (mmol/l)	2·61[a]	0·60	2·17[b]	0·56	2·20[b]	0·60
HDL-C (mmol/l)	1·23[a]	0·29	1·12[b]	0·25	1·18[c]	0·29
ApoA-I (g/l)	1·35[a]	0·24	1·25[b]	0·22	1·29[c]	0·24
ApoB (g/l)	0·68[a]	0·15	0·59[b]	0·15	0·60[b]	0·15
TAG (mmol/l)	0·82	0·37	0·82	0·35	0·79	0·31

C, cholesterol.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different between genotypes ($P<0{\cdot}001$; repeated-measures ANOVA).

Discussion

The present results show that sex and apoE genotype determine ApoE plasma levels; however, this effect is dependent on dietary fat. Thus, women had significantly higher ApoE concentration than men only after the consumption of the SFA diet. Furthermore, we have observed that women with the apoE3/2 and apoE3/3 genotypes have a greater increase in the ApoE plasma levels when a SFA-rich diet is consumed instead of a CHO- or MUFA-rich diet.

The importance of the *apoE* polymorphism on lipid metabolism and CHD is well known. Several matched case–control studies have shown a modest but significantly increased prevalence of the *apoE4* allele in CHD patients from various populations[40,41]. Nevertheless, even though the *apoE4* allele appears to be a significant genetic risk factor for CHD, individuals with the *apoE4* allele do not inevitably develop this disorder. It has been recently proposed that together with the qualitative modification of the ApoE structure due to the *apoE* alleles, quantitative variations in the ApoE plasma levels could play a major role in determining risk[6–9,42]. In agreement with this hypothesis, it has been observed that ApoE concentration determines serum lipid levels by modulating lipoprotein production, lipolytic conversion and receptor-mediated clearance, independently of the ApoE polymorphism[43]. Furthermore, circulating ApoE concentration appears to be altered in hyperlipidaemic human and animal subjects[11–13,27], and several case–control studies show that ApoE plasma levels, *per se,* may be a risk factor for CHD[14–17].

A wide total interindividual variation in the ApoE plasma concentration has been observed in previous reports, which has been principally attributed to the *apoE* common polymorphism[20,21]. In accordance with the present results, previous studies have shown that *apoE2* carriers have the highest levels, whereas *apoE4* individuals show the lowest concentration[20,21]. However, a large amount of variability (65–75 %) remains unexplained by this genetic factor, suggesting that other variables could be involved in determining the ApoE concentration. In several tissues, *apoE* expression is up-regulated by oestrogens[44], so sex was proposed to be one of these variables. However, the data available to date are contradictory: whereas some studies found that ApoE levels are higher in women when compared with men[26–28], others reported the opposite relationship[29] or no differences[30]. These inconsistencies may be explained by the variety of methods used for ApoE measurements, different ages of the subjects or environmental factors, such as diet[45,46]. We suggest this latter one as a source of variability due to an increasing male to female gradient in the serum ApoE concentration when the subjects consumed a SFA-rich diet. It has been demonstrated that a SFA-rich diet leads to elevations in the ApoE plasma levels[23,24], but different

Table 5. ApoE plasma concentration (mg/l) by sex at the end of each dietary period
(Mean values and standard deviations)

	SFA		CHO		MUFA		
	Mean	SD	Mean	SD	Mean	SD	*P*
Total (*n* 84)	34·61[a]	4·87	33·54[b]	3·97	32·14[b]	4·96	0·008
Men (*n* 56)	33·01[c]	7·82	32·86	8·98	31·43	8·20	0·15
Women (*n* 28)	36·21[a,d]	6·54	34·22[b]	6·83	32·86[b]	8·35	0·009

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
[c,d] Mean values within a row with unlike superscript letters were significantly different between men and women ($P<0·05$).

responses between men and women were not previously analysed. The present results suggest the existence of a SFA–oestrogen interaction that could increase the ApoE levels in women. Thus, oestrogens may enhance the SFA-raising effect, resulting in a higher difference on the ApoE plasma levels between sexes, as we have observed. These results require confirmation in future studies since experimental data to corroborate this hypothesis do not exist.

To our knowledge, this is the first intervention study comparing the effect of both the quantity and quality of dietary fat on the ApoE concentration. In spite of a small sample size, we found that a SFA-rich diet increases the ApoE plasma levels in comparison with a CHO- and MUFA-rich diet, a counter-intuitive finding, in view of the healthful properties of ApoE. Metabolically, ApoE interacts with different receptors in the liver, inducing the uptake of TAG-rich particles from the circulation[3]. Thus, we would expect that ApoE plasma levels would be a protective influence against CHD. Surprisingly, ApoE levels are increased in patients with CHD[17,19,47–49] and non-insulin-dependent diabetes[50,51]. These observations illustrate the fact that an elevation in the ApoE plasma concentration increases cardiovascular risk and could explain why the SFA-rich diets, which have been associated with dyslipidaemia and a higher cardiovascular risk, are associated with a higher ApoE plasma concentration. The mechanisms behind this association are unclear. It has been observed that ApoE may be beneficial up to a point, but a further increase may then lead to alterations in lipid metabolism[7]. Accordingly, the increase in the ApoE expression of human apoE in transgenic mice stimulates VLDL production by the liver and inhibits VLDL lipolysis by lipoprotein lipase[52]. In addition, increased levels of ApoE might render the excess ApoE unavailable for receptor interaction, resulting in the inhibition of remnant clearance[53]. Therefore, it is necessary to highlight that an optimal ApoE plasma concentration is necessary to maintain lipid homeostasis. It is not well understood how SFA increases the ApoE plasma levels; however, it was proposed that this effect could be related to a post-transcriptional mechanism, possibly decreasing both receptor-mediated uptake and hydrolysis of ApoE-containing particles[54]. In agreement with this hypothesis, a greater enrichment of TAG-rich lipoproteins with apoE and C-III has been observed after the consumption of meals rich in SFA[55].

When we take into account the influence of the apoE genotype on this complex sex–diet interaction, we observed that the ApoE-raising effect of the SFA diets was only observed in women with the apoE3/2 and apoE3/3 genotypes, but not in men. Carriers of the E2 allele had higher mean apoE–HDL concentrations and lower apoE–VLDL concentrations than individuals carrying the E3 allele, whereas subjects with the E4 allele showed the inverse profile[56]. This fact is in accordance with the known preference of apoE4 for apoB-containing lipoproteins and the preferential association of the apoE2 isoform with HDL particles. Furthermore, it has been reported that women, compared with men, have more ApoE in lipoproteins than in VLDL, mainly HDL particles[26]. Therefore, it is logical that the SFA-raising effect associated with women could be higher in those carriers of the apoE2 allele, as we have observed, since ApoE is mainly associated with HDL particles in women with this genotype. In agreement with this hypothesis, a higher response for the HDL-cholesterol plasma concentration was observed in women with the apoE3/2 genotype after changing their diet from SFA to CHO or MUFA. However, the mechanism of how

Table 6. ApoE plasma concentration (mg/l) in subjects with different apoE genotypes and sexes at the end of each dietary intervention period *
(Mean values and standard deviations)

	Men (*n* 56)		Women (*n* 28)	
Genotype–diet	Mean	SD	Mean	SD
apoE4/3 (*n* 8)				
SFA	24·71	1·55	26·63	1·86
CHO	25·13	2·96	27·07	3·64
MUFA	23·12	1·17	25·81	2·47
apoE3/3 (*n* 66)				
SFA	30·43[c]	5·76	34·22[a,d]	5·34
CHO	29·62	7·14	32·62[b]	4·77
MUFA	29·33	6·84	31·44[b]	7·74
apoE3/2 (*n* 10)				
SFA	43·61[c]	5·87	48·40[a,d]	5·79
CHO	43·13	7·24	43·70[b]	6·76
MUFA	41·68	5·69	42·65[b]	3·65
P				
Diet	0·128		0·041	
Genotype	0·001		0·001	
Interaction	0·215		0·012	

[a,b,c] Mean values within a row with unlike superscript letters were significantly different between diet groups for a given genotype ($P<0·05$).
[c,d] Mean values within a row with unlike superscript letters were significantly different between men and women for a given diet period ($P<0·05$).
* Men (four apoE4/3, forty-six apoE3/3 and six apoE3/2) and women (four apoE4/3, twenty apoE3/3 and four apoE3/2).

Table 7. Plasma lipids (mmol/l) and apo (g/l) in men at the end of each dietary period according to the *apoE* genotype
(Mean values and standard deviations)

	Total C		LDL-C		HDL-C		ApoA-I		ApoB		TAG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
apoE4/3 (*n* 4)												
SFA	4·67[a]	0·44	3·03[a]	0·28	1·25	0·20	1·29	0·23	0·79[a]	0·09	0·83	0·28
CHO	4·22[a]	0·35	2·71[a]	0·27	1·10	0·11	1·12	0·14	0·71[a]	0·17	0·82	0·10
MUFA	4·15[a]	0·59	2·42[a]	0·58	1·17	0·13	1·11	0·18	0·73[a]	0·17	0·77	0·12
apoE3/3 (*n* 46)												
SFA	4·21[b]	0·63	2·61[b]	0·60	1·17	0·29	1·24	0·20	0·65[b]	0·15	0·91	0·41
CHO	3·61[b]	0·59	2·20[b]	0·53	1·09	0·24	1·16	0·19	0·56[b]	0·14	0·86	0·38
MUFA	3·76[b]	0·58	2·22[b]	0·54	1·14	0·28	1·20	0·20	0·57[b]	0·14	0·83	0·29
apoE3/2 (*n* 6)												
SFA	3·91[c]	0·39	2·35[c]	0·44	1·10	0·16	1·24	0·14	0·56[c]	0·12	0·95	0·31
CHO	3·29[c]	0·29	1·77[c]	0·25	1·00	0·14	1·23	0·27	0·47[c]	0·12	1·08	0·38
MUFA	3·40[c]	0·58	1·90[c]	0·47	1·07	0·18	1·19	0·20	0·50[c]	0·16	0·90	0·38
P												
Diet	0·001		0·001		0·025		0·001		0·001		0·492	
Genotype	0·039		0·034		0·651		0·760		0·048		0·595	
Interaction	0·062		0·041		0·784		0·052		0·147		0·254	

C, cholesterol.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different between genotype groups for a given diet group ($P<0·05$; repeated-measures ANOVA).

apoE expression is regulated by saturated fatty acid in women with the apoE3/3 and apoE3/2 genotypes is not yet known. New studies are needed, therefore, to confirm the present findings in a larger sample size and to characterise the molecular mechanism responsible for this complex interaction. It is important to note that one of the limitations to genetic association studies is the difficulty in corroborating findings observed in populations with different characteristics. We must be cautious therefore when extrapolating the results to a more general population. We included only healthy young normolipaemic individuals in order to avoid the effect of other factors (age, BMI, etc.) on lipid response to the content and quality of dietary fat. Studies conducted with conditions representing impaired metabolism, as in dyslipoproteinaemic patients, will generally be more successful in finding differential effects across *apoE* genotypes, and such studies may be helpful in clarifying apoE–nutrition relationships.

In conclusion, the present paper deals with the study of the principal factors determining the ApoE plasma levels. The present results show that circulating ApoE concentration is determined by both sex and apoE genotype; however, this effect can be modulated by dietary intervention. Since ApoE plasma levels appear to be altered in CVD, the present results can thus give interesting therapeutic data to give individual specific dietary recommendation.

Table 8. Plasma lipids (mmol/l) and apo (g/l) in women at the end of each dietary period according to the *apoE* genotype
(Mean values and standard deviations)

	Total C		LDL-C		HDL-C		ApoA-I		ApoB		TAG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
apoE4/3 (*n* 4)												
SFA	4·34[a]	0·51	2·81[a]	0·38	1·30	0·20	1·60	0·15	0·82	0·12	0·58	0·20
CHO	4·20[a]	0·31	2·64[a]	0·23	1·24	0·33	1·49	0·10	0·74	0·11	0·53	0·28
MUFA	3·86[a]	0·27	2·32[a]	0·38	1·28	0·27	1·52	0·09	0·73	0·08	0·54	0·17
apoE3/3 (*n* 20)												
SFA	4·22[b]	0·68	2·54[b]	0·65	1·38	0·27	1·53	0·13	0·71	0·12	0·61	0·17
CHO	3·53[b]	0·62	2·01[b]	0·61	1·26	0·27	1·40	0·13	0·61	0·12	0·63	0·18
MUFA	3·61[b]	0·70	2·03[b]	0·61	1·28	0·33	1·46	0·17	0·62	0·11	0·65	0·26
apoE3/2 (*n* 4)												
SFA	3·99[c]	0·20	2·31[c]	0·10	1·31	0·42	1·71	0·10	0·67	0·11	0·76	0·26
CHO	3·30[c]	0·55	1·78[c]	0·11	1·10	0·56	1·44	0·13	0·64	0·09	0·87	0·19
MUFA	3·52[c]	0·05	1·88[c]	0·43	1·12	0·55	1·31	0·12	0·61	0·09	0·88	0·19
P												
Diet	0·001		0·001		0·002		0·001		0·001		0·011	
Genotype	0·019		0·043		0·959		0·283		0·107		0·335	
Interaction	0·014		0·010		0·016		0·054		0·105		0·030	

C, cholesterol.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different between genotype groups for a given diet group ($P<0·05$; repeated-measures ANOVA).

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