Gene–nutrient interactions: dietary behaviour associated with high coronary heart disease risk particularly affects serum LDL cholesterol in apolipoprotein E ϵ4-carrying free-living individuals

Alexandre Loktionov*, Serena Scollen, Nicola McKeown and Sheila A. Bingham

Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge, CB2 2XY, UK

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Apolipoprotein E (ApoE) genotype influence on the relationship between dietary risk factors for cardiovascular disease and blood serum lipid levels was investigated in 132 free-living individuals participating in the European Prospective Investigation of Cancer (EPIC) study. All subjects (age 40–69) were clinically healthy and provided information on their usual diet. ApoE genotype and serum lipid concentrations were determined in all subjects. Relationships of intake of dietary constituents with serum lipid levels were compared in different genotype groups. There was a significant correlation between total serum cholesterol and intake of energy derived from total fat (r 0·195; P 0·025) and saturated fat (r 0·174; P 0·046) in the cohort as a whole. However, individuals with the ApoE ϵ3/ϵ4 genotype displayed a much stronger positive correlation between LDL cholesterol level and the percentage of energy derived from intake of saturated fat (r 0·436; P 0·043). There were no significant associations in the groups with ϵ3/ϵ3 or ϵ2/ϵ2 & ϵ2/ϵ3 genotype. A significant positive correlation between alcohol consumption and HDL cholesterol level was present in individuals bearing ApoE ϵ2 allele. These findings support current public health recommendations that saturated fat consumption should be reduced in order to reduce coronary heart disease risk. Total cholesterol concentrations were positively related to saturated fat intake in the cohort as a whole, but elevated LDL cholesterol levels associated with high saturated fat intake can be expected particularly in those individuals who combine a 'risky' dietary behaviour with the presence of the ϵ4 variant of ApoE.

Genotype: Dietary fat: Serum cholesterol

The role of blood lipid concentrations, especially elevated levels of LDL cholesterol and decreased levels of HDL cholesterol, in the development of coronary heart disease is now well documented (Castelli *et al.* 1986; Castelli, 1996). Population comparisons and metabolic trials have consistently shown associations between dietary cholesterol and saturated fat intake and serum lipid concentrations (McGill, 1979; LaRosa *et al.* 1990; Clarke *et al.* 1997). However, no study of free-living individuals has confirmed these findings until now. This may be due to the effect of other dietary factors, intra-individual variation in diet and blood cholesterol from day to day, and large inter-individual variation in regulation of lipid metabolism resulting from genetic polymorphisms present in human populations. The identification of interactions between genetic background and modifiable dietary factors affecting lipid metabolism has however been little explored in large epidemiological trials.

Apolipoprotein E (ApoE) gene polymorphism involving codons 112 and 158 is a major determinant of blood lipid levels in humans (Wilson *et al.* 1994, Castelli, 1996). ApoE allele distribution shows similar patterns in most Caucasian populations. Allele ϵ3 (Cys112; Arg158) is the most common one with frequencies between 0·70 and 0·85, ϵ4 (Arg112; Arg158) is less frequent (0·10–0·20) and ϵ2 (Cys112; Cys158) is the rarest one at 0·05–0·10 (Hallman *et al.* 1991; Corbo & Scacchi, 1999; Loktionov *et al.* 1999). The ApoE protein acts as the ligand between lipoprotein particles and hepatic LDL and chylomicron receptors, and the properties of the two binding domains of the protein molecule differ in the variants encoded by the three alleles resulting in their functional differences (Weisgraber &

Abbreviation: ApoE, apolipoprotein E.

* **Corresponding author:** Dr A. Loktionov, fax +44(0)1223 252765, email alex.loktionov@mrc-dunn.cam.ac.uk

Mahley, 1996). The presence of the apolipoprotein E ε4 allele associated with increased levels of total and LDL serum cholesterol is now considered as being an important coronary heart disease risk factor (Wilson *et al.* 1994, 1996; Castelli, 1996). Some intervention studies have demonstrated that individuals bearing the ε4 variant are more likely to respond to increased dietary intake of fat and cholesterol with elevation of both total and LDL serum cholesterol levels (Tikkanen *et al.* 1990; Gylling & Miettinen, 1992; Dreon *et al.* 1995; Sarkkinen *et al.* 1998), but other groups failed to observe any difference (Savolainen *et al.* 1991; Pasagian-Macaulay *et al.* 1997). These discrepancies may be at least partially attributed to different study designs and diets applied. The ApoE genotype effect on dietary modulation of serum lipid levels has not hitherto been investigated in free-living populations unlimited in composition of their diets.

In the present study we have examined interactions between consumption of dietary constituents and serum lipid levels in relation to ApoE genotypes in a free-living cohort of participants of the European Prospective Investigation of Cancer (EPIC) study (Day *et al.* 1999).

Methods

Subjects

The subjects participated in the European Prospective Investigation of Cancer (EPIC) study (Day *et al.* 1999). This is a large free-living cohort of about 25 000 people from Norfolk, UK. As part of quality control studies on dietary methods used in the main cohort, 132 clinically healthy subjects (sixty males, seventy-two females, all Caucasians, age 40–69 years) were recruited and investigated in the present study. There were eighteen moderate smokers and 114 non-smokers among them. Dietary information was available for the subjects from Food Frequency Questionnaires (Day *et al.* 1999). Ethical permission for the EPIC study was given by the Norwich District Ethics Committee in 1992, and for the subsequent studies reported here in 1996 and 1999.

Blood collection and analysis

Subjects in the study gave three 10 ml blood samples after a 12 h fast at 3-month intervals. Serum and cell fractions (buffy coats) were separated by centrifugation. Genomic DNA was extracted from buffy coats. Serum was stored at −20°C prior to analysis for total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerols. Serum lipid measurements were performed as earlier reported (Loktionov *et al.* 1998), and the average of the three measures used in the present analysis.

Genotype determination

Genomic DNA was used for ApoE genotype determination. It was performed in all 132 subjects using a modification of the protocol based on *Hha*I restriction fragment length polymorphism detection. The detailed genotyping procedure has been described elsewhere (Loktionov *et al.* 1998).

Statistics

All results were expressed as the mean and standard error. Unpaired *t* tests were carried out to compare parameters between different genotype groups. Pearson correlation coefficients and simple linear regression between intake of different dietary constituents and serum lipid concentrations were also calculated. In some cases multiple regression analysis was employed to assess influence of confounding factors including age, gender, body mass index, and smoking status. Systat 5.2 (Systat, Evanston IL, USA) and Data Desk[4] (Data Description, Inc., Ithaca, NY, USA) statistical packages were used for statistical analyses.

Results

ApoE genotype and allele distribution

All three common ApoE alleles were found among the study subjects. The following genotype frequencies were observed: ε2/ε2 0·8 % (one female); ε2/ε3 14·4 % (nine males and ten females); ε2/ε4 3·0 % (one male and three females); ε3/ε3 64·4 % (thirty-nine males and forty-six females); ε3/ε4 17·4 % (eleven males and twelve females). No ε4/ε4 homozygotes were found. The allele distribution with 9·5 % of ε2, 80·3 % of ε3, and 10·2 % of ε4 was within the range common for Caucasian populations. It is accepted that phenotypic effects of ε2 and ε4 alleles are manifested in ε2/ε3 and ε3/ε4 heterozygotes, so we regarded these variants as ε2- and ε4-expressing respectively. There is a view that the ApoE ε2 allele is a dominant one in any combination (Bohnet *et al.* 1996). Nevertheless, we treated subjects with the ε2/ε4 genotype as a separate small group since the absence of interference of opposite effects of the ε2 and ε4 on lipid levels is still not proven.

ApoE genotype, serum lipids and diet

Serum lipid levels for different genotype groups are shown in Table 1. As expected, comparison between the three most common ApoE variants demonstrated slightly lower concentrations of total and LDL cholesterol in the ε2-expressing subjects, whereas ε3- and ε4-expressing groups displayed generally higher values. It was difficult to interpret high serum lipid levels in the ε2/ε4 group since there were only four individuals in it; however obviously higher consumption of fat found in this small group (see Table 2) could be a contributing factor. Higher triacylglycerol concentrations were observed in the ε4 group. No significant differences ($P < 0{\cdot}05$) between the sexes in total and LDL cholesterol levels were found, whereas significant differences ($P < 0{\cdot}05$) existed between the sexes in HDL cholesterol and triacylglycerol concentrations among ε3 and ε4 group subjects, females displaying significantly higher HDL cholesterol and lower triacylglycerol levels ($P < 0{\cdot}05$). There was no significant difference ($P < 0{\cdot}05$) in age distributions and body mass index values between genotype groups (Table 1). Comparisons of average intake of dietary constituents did not reveal any significant differences ($P < 0{\cdot}05$) between three main ApoE genotype groups (Table 2).

Table 1. Comparison of age, body mass index, and serum lipid concentrations in free-living subjects with different ApoE genotypes
(Data are means and standard errors)

ApoE Genotype	Number of subjects	Age (Years)		BMI (kg/m^2)		Total cholesterol (mmol/l)		LDL cholesterol (mmol/l)		HDL cholesterol (mmol/l)		Triacylglycerols (mmol/l)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
All types	132	53·6	1·5	26·2	0·6	6·09	0·20	4·05	0·19	1·35	0·07	1·80	0·17
Males	60	53·7	2·1	27·1	0·9	6·21	0·26	4·19	0·23	1·18[d]	0·08	2·12[g]	0·29
Females	72	53·5	2·1	25·5	1·0	5·99	0·30	3·93	0·29	1·50[d]	0·10	1·53[g]	0·19
ε2-expressing (ε2/ε2&ε2/ε3)	20	55·8	4·9	27·4	1·7	5·63[a]	0·48	3·62	0·54	1·35	0·19	1·90	0·44
Males	9	55·1	6·5	26·7	2·3	5·41[b]	0·50	3·50[c]	0·45	1·25	0·19	1·90	0·66
Females	11	56·4	9·1	28·1	3·2	5·82	0·82	3·72	1·00	1·42	0·36	1·90[h]	0·71
ε3-expressing (ε3/ε3)	85	53·2	1·9	26·0	0·8	6·11	0·24	4·13	0·24	1·36	0·09	1·64[i]	0·19
Males	39	53·4	2·8	26·8	1·2	6·33[b]	0·28	4·35[c]	0·29	1·20[e]	0·11	1·97[j,k]	0·32
Females	46	53·0	2·6	25·3	1·2	5·93	0·38	3·94	0·35	1·49[e]	0·13	1·35[h,k]	0·20
ε4-expressing (ε3/ε4)	23	53·3	2·8	25·9	1·4	6·24	0·49	4·11	0·49	1·33	0·16	2·24[i]	0·60
Males	11	53·3	3·7	28·1	1·6	6·21	0·71	4·18	0·64	1·07[f]	0·10	2·84[j,l]	1·06
Females	12	53·3	4·6	23·9	2·2	6·26	0·79	4·06	0·80	1·56[f]	0·21	1·70[l]	0·55
ε2/ε4 combination*	4	53·5	9·2	27·5	9·3	7·14[a]	2·15	4·22	0·41	1·51	0·79	2·16	1·68

* Limited number of subjects in this group prevented us from analysing males and females separately.
[a]P 0·0174; [b]P 0·0039; [c]P 0·0084; [d]$P < 0·0001$; [e]P 0·0017; [f]P 0·0002; [g]P 0·0007; [h]P 0·0315; [i]P 0·0060; [j]P 0·0017; [k]P 0·0002; [l]P 0·0007. Letters indicate pair-wise comparisons between genotype groups or between males and females within genotype groups.

Interaction between dietary constituents and serum lipids in subjects with different ApoE genotypes

In the cohort as a whole there were weak but significant positive correlations between total serum cholesterol and daily intake of dietary constituents including total energy (r 0·173, P 0·047), total fat (r 0·219, P 0·012), saturated fat (r 0·216, P 0·013), monounsaturated fat (r 0·216, P 0·013), polyunsaturated fat (r 0·197, P 0·023), cholesterol (r 0·195, P 0·025) and total protein (r 0·180, P 0·039). This effect, however, disappeared for protein and polyunsaturated fat when expressing the contribution of nutrients as a percentage of total energy intakes. It was still present for total fat as energy % (r 0·195, P 0·025), saturated fat as energy % (r 0·174, P 0·046), and monounsaturated fat as energy % (r 0·181, P 0·038). Multiple regression analysis incorporating influences of age, gender, body mass index and smoking revealed that only influence of total fat intake was still statistically significant (P 0·028). No marked relationships between diet and concentrations of LDL cholesterol, HDL cholesterol, and triacylglycerols were found.

ApoE genotype variants were then analysed separately. There were no significant correlations between dietary factors and serum lipids in the ε2 and ε3 groups apart from a positive association of alcohol intake with HDL cholesterol in the ε2 group (r 0·563, P 0·010). Negative correlations between alcohol intake and total cholesterol, LDL cholesterol and triacylglycerols were also present in this group, but they were not statistically significant.

In the ε4-expressing subjects, positive correlations between diet and blood lipids were found for total fat

Table 2. Daily intake of main dietary constituents calculated from food questionnaires in free-living subjects with different ApoE genotypes
(Data are means and standard errors)

Dietary constituents	All subjects (ε2/ε2&ε2/ε3)		ApoE genotype groups: ε2-expressing (ε3/ε3)		ε3-expressing (ε3/ε4)		ε4-expressing combination		ε2/ε4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy (kJ)	8760	258	9193	813	8631	321	8691	471	9749	1818
Total fat (g)	81·1	3·3	82·1	9·8	80·4	4·2	78·6	6·2	106·5	20·4
Total fat (energy %)	33·2	0·6	31·7[a]	7·1	33·2	0·8	33·0	1·5	40·0[a]	3·0
Monounsaturated fat (g)	28·7	1·2	29·2	3·5	28·3	1·6	27·3	2·2	39·6	8·1
Monounsaturated fat (energy %)	11·7	0·3	11·3[b]	0·6	11·6[c]	0·3	11·4[d]	0·5	14·9[b,c,d]	1·8
Polyunsaturated fat (g)	14·1	1·2	13·0	1·4	14·4	0·8	13·3	1·0	18·7	6·1
Polyunsaturated fat (energy %)	5·9	0·2	5·2	0·3	6·0	0·2	5·7	0·4	6·5	1·2
Saturated fat (g)	31·8	1·4	33·0	4·5	31·1	1·8	31·7	2·9	41·9	5·7
Saturated fat (energy %)	12·9	0·4	12·5	0·9	12·8	0·4	13·2	0·8	16·5	1·4
Cholesterol (mg)	297·9	12·9	341·2	45·8	280·3	14·8	310·4	25·4	385·4	88·9
Protein (g)	86·4	2·2	92·3	5·4	84·4	3·0	88·4	4·4	87·8	14·2
Protein (energy %)	17·3	0·3	18·2	0·8	17·1	0·4	17·5	0·5	15·6	0·8
Carbohydrates (g)	253·9	7·5	268·0	23·0	251·1	9·4	250·9	14·2	259·9	48·9
Carbohydrates (energy %)	46·9	0·6	47·4	1·7	47·1	0·8	46·5	1·4	43·2	2·3
Alcohol (g)	7·9	0·9	10·3	3·6	7·1	0·9	9·2	2·5	5·5	4·7
Alcohol (energy %)	2·6	0·3	2·7	0·8	2·5	0·4	3·0	0·7	1·2	1·0

[a]P 0·0493; [b]P 0·0296; [c]P 0·0381; [d]P 0·0408. All these values fail to reach statistical significance if Bonferroni correction is applied.

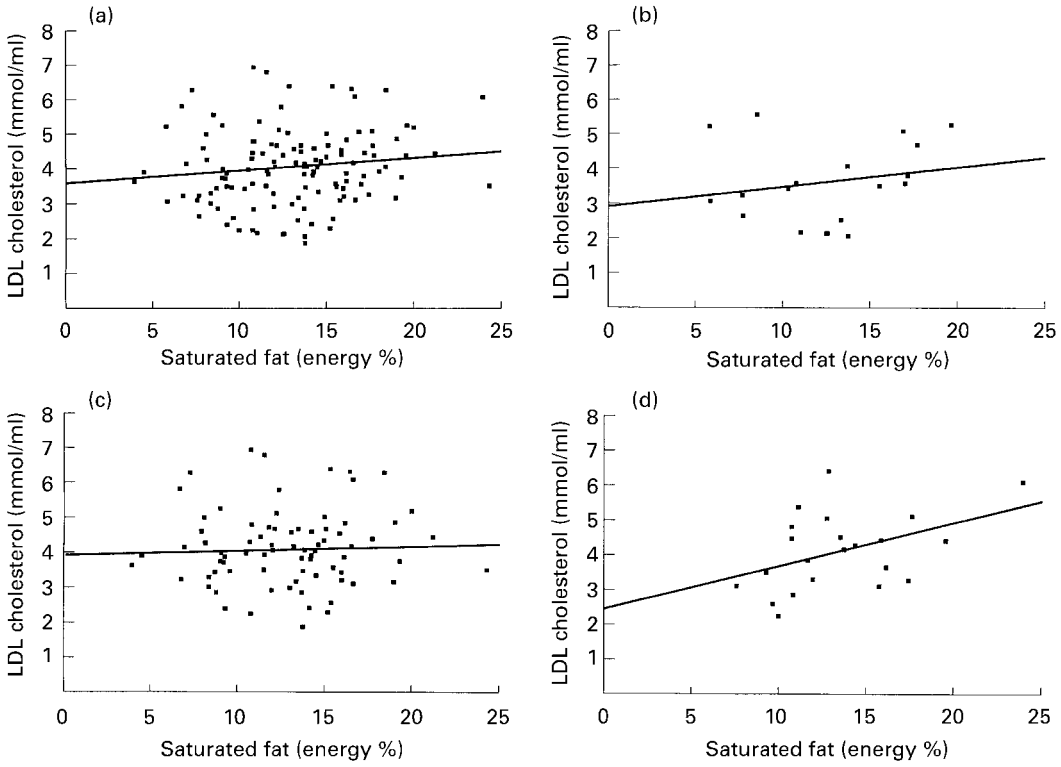

Fig. 1. Scatterplots of LDL cholesterol serum levels and energy percent provided by daily intake of saturated fatty acids (SF) with the corresponding regression lines showing: (a) All 132 study subjects. Regression line corresponds to: LDL = 0·038 × SF + 3·552 (P = 0·119). (b) Twenty individuals bearing ApoE ϵ2 allele (ϵ2/ϵ2 & ϵ2/ϵ3 genotypes). Regression line corresponds to: LDL = 0·057 × SF + 2·906 (P = 0·401). (c) Eighty-five individuals with ApoE ϵ3/ϵ3 genotype. Regression line corresponds to: LDL = 0·011 × SF + 3·984; (P = 0·718). (d) Twenty-three individuals with ApoE ϵ3/ϵ4 genotype. Regression line corresponds to: LDL = 0·123 × SF + 2·447 (P = 0·043).

intake and total and LDL cholesterol (r 0·422, P 0·045 and r 0·494, P 0·016 respectively) and for saturated fat intake and total and LDL cholesterol (r 0·479, P 0·021 and r 0·565, P 0·006 respectively). Monounsaturated fat consumption was also positively correlated with total and LDL cholesterol concentrations (r 0·419, P 0·047 and r 0·453, P 0·034 respectively), whereas it was inversely related with HDL cholesterol (r −0·413, P 0·050). There were also inverse associations between HDL cholesterol and polyunsaturated fat (r −0·504, P 0·014) and protein (r −0·421, P 0·045). Alcohol intake was positively correlated with triacylglycerol concentration (r 0·460, P 0·027). There were also positive associations between energy intake and serum lipids for total cholesterol (r 0·447, P 0·033) and LDL cholesterol (r 0·475, P 0·026), and a negative association for HDL cholesterol (r −0·482, P 0·046). However, when energy was controlled for by expressing intakes as a percentage of total energy, only the relation between saturated fat and LDL cholesterol remained significant (r 0·436, P 0·043). This relationship was even stronger in multiple regression controlling for age, gender, body mass index and smoking status (P 0·006). Fig. 1 shows scatterplots with regression lines demonstrating the relationship between saturated fat intake as a percentage of total energy and LDL cholesterol concentration in the individuals with different genotypes. There is a convincing relationship only in the ϵ4 individuals. The LDL cholesterol serum concentration in this group changed by 0·123 mmol/l for every 1 % of change in energy provided by saturated fat. Comparison of the genotype groups by multiple regression analysis revealed a highly significant difference (P 0·005) between ϵ3- and ϵ4-expressing individuals when age, gender, BMI and smoking status were taken into account.

Discussion

Atherosclerosis and coronary heart disease pathogenesis incorporates numerous interacting mechanisms, undergoing regulation by both intrinsic (genetic) and modifiable (environmental and life-style) factors (Ross, 1993). Our findings of a significant relation between total and saturated fat consumption and total and LDL cholesterol presented in this paper support current public health recommendations that saturated fat consumption should be reduced to 10 % total energy in the population as a whole in order to reduce the currently high serum LDL and total cholesterol levels in the UK (COMA 1991, 1994). However we observed differential responses according to the ApoE genotype, and individuals expressing both the ϵ2 and the ϵ3 variants of the gene did not display any substantial diet-dependent changes in their serum lipid levels, suggesting that homeostatic mechanisms controlling lipid distribution and metabolism act efficiently in these people.

The presence of the ApoE ϵ4 allele seems to damage this

mechanism so that the serum lipid concentrations become more dependent on dietary factors in the affected subjects. This observation provides further evidence corroborating the ϵ4 allele presence as an important coronary heart disease risk factor. When controlling for energy expenditure and body size by expressing results as a percentage of total energy intake, there was a much stronger and direct relationship between dietary saturated fat and LDL cholesterol levels in these people. This implies that individuals with this genotype and serum lipid patterns that are unfavourable for coronary heart disease may be able to respond to dietary interventions to a greater extent compared to similar persons with other ApoE variants. Indeed, simple calculations show that in these subjects a 50 % change in saturated fat consumption would result in almost 20 % change in LDL cholesterol serum level. In contrast, if the whole cohort were taken, regardless of genotypes, a 50 % change in saturated fat intake would result only in approximately 5 % changes in both total and LDL cholesterol levels.

Light to moderate alcohol consumption is now widely regarded as a protective factor against coronary heart disease risk (Thun *et al.* 1997; Muntwyler *et al.* 1998; Berger *et al.* 1999). Several groups earlier reported alcohol-induced decrease in total cholesterol, LDL cholesterol and triacylglycerol levels and simultaneous increase in HDL cholesterol concentration (Linn *et al.* 1993; Mayer *et al.* 1993; Choudhury *et al.* 1994). However, alcohol raises blood pressure at intakes above about 4 units per day increasing the risk of stroke (COMA, 1991, 1994). Our data also indicate that people with different ApoE allele combinations are likely to respond differently to alcohol intake. The 'protective' pattern of serum lipid level changes related to moderate alcohol consumption was manifested in the ϵ2-bearing subjects and less pronounced in the ϵ3/ϵ3 majority. In contrast, in the ϵ4 group alcohol was associated with increased serum triacylglycerols, although raised serum triacylglycerol levels are a less clearly defined risk factor for coronary heart disease (Patsch, 1993; Harjai, 1999). The observation is again consistent with the fact of greater coronary heart disease risk associated with the presence of the ϵ4 allele. These results, however, need confirmation in a larger cohort since about 25 % of the participants of the present study either completely abstained from alcohol or reported occasional intake of very low amounts.

This study has demonstrated the importance of assessing gene–nutrient interactions in strengthening the evidence relating diet to cardiovascular disease risk. Unlike previous studies, these associations have been found in a free-living population. However, members of the general public are unlikely to have the opportunity at present to be genotyped for ApoE. In the absence of population-based genotyping facilities, current advice for the population, as a whole to restrict saturated fat consumption in order to reduce the risk of coronary heart disease should be followed.

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