Apolipoprotein E polymorphisms, dietary fat and fibre, and serum lipids: the EPIC Norfolk study

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Aims To investigate whether blood lipid response to dietary fat and fibre vary according to the apolipoprotein E (APOE) gene locus.

Methods and results Regression analysis of intake of dietary fat and lipid fractions according to APOE gene loci was assessed by Pyrosequencing and validated with restriction fragment length polymorphism in 22,915 participants of the Norfolk arm of the European Prospective Investigation of Cancer. There were significant (P < 0.001) differences in serum lipids according to genotype, highest total and low-density lipoprotein (LDL) cholesterol, and lowest high-density lipoprotein and triglycerides in e4/e4 individuals. There were positive associations between total and saturated fat and serum total and LDL cholesterol, and significant inverse associations (P < 0.001) between polyunsaturated fat and dietary fibre and lipid fractions overall. Associations were in the same direction for e2, e3, and e4 expressing individuals with no significant interactions between diet and genotype group on blood lipids, except in the 3% individuals expressing e2/e4 (P < 0.05) in whom the associations were doubled.

Conclusion In this largest study to date, ApoE gene loci status does not confer exemption from population targets to reduce dietary saturated fat and increase dietary fibre in order to reduce blood lipids and risk of coronary heart disease.

Introduction

Dietary factors such as saturated fatty acids are one of the major determinants of serum cholesterol,1,2 which in turn is a risk marker of coronary heart disease (CHD). However, it has also been demonstrated that the serum lipid and lipoprotein response to dietary changes show wide variation between individuals.3,4 One reason for the variation could be genetic factors, of which apolipoprotein E (APOE) is a candidate.

APOE has three major isoforms encoded by three (e2, e3, and e4) alleles from two single nucleotide polymorphisms altering amino acids at sites 112 and 158.5–7 The APOE allele distributions in most Caucasian populations show similar patterns. The most common allele e3 (Cys112; Arg158) has a frequency between 0.70 and 0.85, e4 (Arg112; Arg158) is less frequent between 0.10 and 0.20, and the rarest e2 (Cys112; Cys158) between 0.05 and 0.10.8

APOE is primarily involved in the cellular uptake of lipoproteins through ligand-receptor interactions with the low-density lipoprotein (LDL) receptor and chylomicron remnant receptors.9 Through this interaction, APOE mediates the uptake and metabolism of lipoproteins and is thought to be a major determinant of blood lipid levels in humans.9,10 The properties of the two APOE binding domains differ according to the three isoforms, leading to functional differences.11,12 It has been well established that the presence of the APOE e4 allele is associated with increased levels of total and LDL serum cholesterol, whereas the presence of the APOE e2 allele is associated with the reverse effect.10

However, the effect of the APOE polymorphisms on the response of serum lipid levels to the intake of dietary constituents is unclear. Some intervention studies of up to 210 individuals have reported that those expressing the e4 variant are more likely to respond to increased dietary intake of fat and cholesterol, with elevation of both total and LDL serum cholesterol levels.13–17 Other studies failed to observe the same effect.18–20 Existing population based studies are also relatively small, comprising less than 1000 individuals, and the results are also inconsistent.19–24 The reported inconsistencies in results can be attributed to lack of power from small sample sizes in both these types of studies particularly in the APOE e4 group.25

We have therefore investigated relations between serum lipids and the consumption of dietary saturated, monounsaturated, and polyunsaturated fat in APOE genotypes in 22,915 free-living participants of the European Prospective...
Investigation of Cancer Norfolk study (EPIC – Norfolk). In addition we have also investigated associations between genotype and intake of dietary fibre, which also mediates serum lipid levels.

Methods

Protocol

EPIC Norfolk is a prospective cohort of men and women recruited at age 45–75 years. In 1993 a total of 35 Norfolk medical practices agreed to participate and invited 77,630 individuals to take part in the study, of whom 30,445 completed a Health and Lifestyle questionnaire prior to a health check. Twenty-five thousand six hundred and thirty nine attended this first health check from 1993 to 1997 when blood and urine samples, and data on height, weight, respiratory function, activity, anthropometry and blood pressure, were collected by trained nurses. All 30,445 participants have been followed up for their health status and as a part of follow up they were invited back for a second health check at the beginning of 1998. Fifteen thousand seven hundred and eighty three individuals attended, of whom 15,025 had attended the first health check. Permission for the study was obtained from The Norfolk and Norwich Hospital Ethics Committee.

Forty two millilitre of non-fasting blood was collected at both health checks in EDTA, citrated and plain monovettes, and separated into plasma, serum, buffy coats and red blood cells as described previously. Serum lipids were analysed for total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride on an RA-1000 (Bayer Diagnostics, Basingstoke). LDL cholesterol was calculated using the Friedewald formula; when serum TG exceeded 4 mmol/L, LDL cholesterol was not calculated.

Genotype determination

DNA for genotyping was extracted from an extra 9 mL blood in EDTA collected at the second health check. For those individuals who did not give blood at the second health check, DNA for genotyping was extracted from remnant red blood cell samples and buffy coats collected at the first health check.

APOE genotype was assessed using Pyrosequencing. PCR was performed to obtain an amplicon of 185 bp covering the area of interest, amino acids 112 and 158, with primers 5′-GGG CGC CGA CAT GGA GG-3′ and a 5′-biotin labelled primer 5′-Biotin-CCC CGG CCT GGT ACA CTG-3′ designed by Pyrosequencing Assay Design Software (Pyrosequencing AB, Sweden). A 50 μL PCR mixture was prepared with 1 x PCR Buffer, 2 mM MgCl2, 5% DMSO, 0.125 mM of each dNTP with 75% of dGTP replaced by dITP, 10 pmol of each primer, two units of Taq Gold and 12 ng of DNA. The annealing temperature was 62 °C and PCR was performed for 45 cycles in a DNA Thermal Cycler (PTC-225; MJ Research, Inc., Watertown, MA, USA). The detailed Pyrosequencing sample preparation procedure has been described elsewhere. The amplicon was denatured to isolate single stranded DNA on the beads then immersed in 15 pmol of the two sequencing primers in annealing buffer (Pyrosequencing, AB, Sweden). The two sequencing primers were 5′-GAC ATG GAG GAC GTG-3′ for site 112 and 5′-CCG ATG ACC TGC AGA-3′ for site 158 designed using PSQ Primer design software (Biotage). The Pyrosequencing (Pyrosequencing, AB, Sweden) machine was prepared as recommended by the manufacturer and the samples were loaded into the machine. The dispensation order for the machine was: G T A C G A C T C G C G T.

Validation and reproducibility

One hundred and thirty five samples were genotyped using RFLP (restriction fragment length polymorphism) and the Pyrosequencer to check for reproducibility, and were fully concordant.

Dietary data

A food frequency questionnaire (FFQ) consisting of 131 items was sent to all participants prior to the first health check. An in-house computer program (Compositional Analyses from Frequency Estimates), with an associated database, was developed for data entry, analysis, and conversion to nutrient intakes using databases published by the Royal Society of Chemistry. Participants also completed 7 day food diaries, but due to the time and resources required for coding, only data from 6,416 were available at the time of this analysis. The direction of change between diet and blood lipids was the same for both sets of data in these 6,416 individuals but FFQ data was used to gain sufficient power for the analysis by separate genotype.

Statistics

The genotype data was combined with data from the first health check in the main EPIC database. The combined database of 24,513 individuals with genotype was then issued for statistical analysis. After excluding those subjects with missing or incomplete data on diet or lipids from the first health check, complete data were available for analysis of 22,915 participants.

To increase statistical power, the six APOE genotype groups (e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e2/e4) were classified into four groups for analysis. The e3/e3 genotype has long been regarded as the wild type due to the high population frequency of this group. It has also been well documented that the e2 allele lowers and the e4 allele increases serum total and LDL cholesterol.

To estimate the genotype effects on serum lipid response to diet, the different deviations from the wild type were classified together, e2 expressing (e2/e2, e2/e3, e4 expressing (e3/e4, e4/e4) and e3/e3. Although one study showed that the e2 allele on serum lipids to be greater than the e4 allele, other studies have not investigated this. Therefore the e2/e4 genotype was considered as a separate group.

The differences between the means and standard deviation (S.D.) were assessed using ANOVA. Categorical significant differences were accessed with the χ² test. Regressions between diet and blood lipids were adjusted for sex, age, body mass index (BMI), smoking status (current, past, and never), exercise index, use of lipid lowering drugs, total energy intake, and for non-fat components of energy intake, alcohol, and carbohydrate. Regression coefficients (β) and the standard errors (S.E.) were normalized to show the change of serum lipid for every S.D. change of dietary constituent intake. The results were expressed as two-tailed test of significance (P-value) and the 95% confidence intervals (95% C.I.).

The Bonferroni correction for multiple tests was used to demarcate significant regressions to be used for further analysis by genotype. Each serum lipid was tested with six dietary constituents therefore the demarcating value for significance was P < 0.008. Using this demarcation, associations between polyunsaturated and saturated fat and total and LDL cholesterol and fibre and triglycerides and HDL cholesterol were investigated further. As there was little difference between results using total fat or fat as percentage of energy, the convention of analysis as percentage energy for the fat components is reported here.

Results

**APOE genotype and allele distribution**

Genotype frequencies for the 22,915 samples were: e2/e2 0.6%; e2/e3 12.4%; e2/e4 2.6%; e3/e3 58.6%; e3/e4 23.5%; e4/e4 2.3%. The allele distribution of 8.1% e2, 76.55% e3 and 15.35% e4 was similar to that reported for Caucasian populations in the United Kingdom, of 8% e2, 77% e3 and 15% e4.
**APOE genotype and coronary heart disease risk factors**

Mean serum lipids and CHD risk factors in different genotype groups were very similar in the male and female populations. There was no significant genotype distribution difference between the two sexes ($\chi^2 P = 0.73$), so basic statistics with the sexes combined for different genotype groups are shown Table 1. Sex, age, BMI, systolic blood pressure, smoking status, use of hypertension lowering drugs, and physical activity index were not significantly different between the different genotype groups. The percentages of individuals prescribed lipid lowering drugs at baseline was low, 1.6% overall, with a significant difference between the genotype groups, lowest in the individuals carrying the e2 allele, and highest for individuals carrying the e4 allele ($P < 0.001$).

Apart from the e2/e4 group, mean serum total and LDL cholesterol concentrations were lowest in the e2 expressing groups ($P < 0.001$) and highest in the e4 expressing groups ($P < 0.001$). The mean serum HDL cholesterol was highest in the e2 expressing groups and lowest in the e4 expressing group ($P < 0.001$). The mean serum triglyceride was lowest in the e3 group and highest in the e2 group ($P < 0.001$). In the e2/e4 group the mean total, LDL and HDL cholesterol was intermediate between groups e2/e3 and e3/e3 (Table 1 and Figure 1).

**Associations between dietary constituents and serum lipids**

Table 2 shows that there were highly significant associations between serum lipids with nearly all the tested dietary constituents. Mean intakes in the 22 915 participants were as follows: total fat 32.45 (5.83)% of energy intake, monounsaturated fat 11.40 (2.34), polyunsaturated fat 6.14 (1.98), saturated fat 12.47 (3.31)% of energy intake, cholesterol 0.28 (0.12) g/day and fibre 18.50 (6.56) g/day. Results were in a similar direction but somewhat stronger in females compared with males (data not shown). Fat and monounsaturated fat and cholesterol intake were positively associated with total cholesterol and LDL cholesterol, whereas polyunsaturated fat and fibre were inversely associated. Fibre was also inversely associated with triglycerides ($P < 0.001$) and monounsaturated fat positively associated with triglycerides ($P < 0.001$).

Associations with $P < 0.008$ were analysed further by genotype and assessed for differences in the four expressing genotype groups. Table 3 shows that most associations in Table 2 remained significant in the e3/e3 group. Associations were in the same direction for e2 and e4 groups with no evidence for differences between coefficients ($Z = >p0.05$). β coefficients were of greater magnitude in the e2/e4 group, with significant differences between the e3/e3, e4 expressing and the e2/e4 group for total serum cholesterol and total fat (% energy) ($Z=2.37, 2.29$ respectively, $P < 0.05$) and also between the e3/e3 and the e2/e4 group for total serum cholesterol and monounsaturated fat (% energy) ($Z=2.99, P < 0.05$). There were no other significant diet lipid differences between other genotype groups. There were significant ($P < 0.014$) but small differences in mean intakes as percentage energy of total fat and saturated fat between the different genotype groups that was mainly attributable to the 137 participants who were e2/e2, and a small difference ($P = 0.012$) in cholesterol but no differences in dietary fibre, or monounsaturated fat as a percentage of energy (data not shown).

**Discussion**

There were significant differences in blood lipids in mean serum total and LDL cholesterol between the e2, e3/e3, and e4 genotype groups, as has been shown previously.\(^{10,39}\) However the present study with approximately 23 000 participants was much larger than previous ones and thus more individuals in the rarer groups of e4/e4, e2/e2 and of e2/e4 were investigated. The mean total, LDL and HDL cholesterol of the e2/e4 group was consistently intermediate between the mean levels of the e2/e3 and e3/e3 groups, suggesting that the up regulatory effect of the e2 genotype is more acute than the down regulatory effect of the e4 genotype, supporting the suggestion that e2 allele has phenotypic dominance.\(^{35}\)

The present study is cross-sectional with consequent limitations concerning the effect of change on blood lipids and therefore risk of CHD. However, it is notable that highly significant associations between diet and serum lipid fractions in this large cross-sectional prospective study were seen in the directions predicted from carefully controlled intervention studies on blood lipids.\(^{1,2}\) The positive association between monounsaturated fat and serum total and LDL cholesterol was unexpected, as some studies suggest monounsaturated fat has no effect or a negative effect on serum LDL cholesterol.\(^{1,40,41}\) However, positive associations between oleic acid and LDL and total cholesterol (and with total and saturated fat) have been shown in a previous but smaller cross-sectional analysis in women in the Framingham study.\(^{42}\) As predicted from the Keys and Hegsted equations, the magnitude of the inverse association with polyunsaturated fat on serum LDL cholesterol was approximately half that of the positive effect of saturated fat.\(^{1,2}\) However, every 3% of energy as saturated fat increased LDL cholesterol by only about 1.52% ($b = 0.06, Table 2$, of the average 3.96 mmol/L, Table 1). From the Keys equation, a change of 5% would be expected.\(^2\) This smaller effect than predicted is likely due to known attenuation in dietary assessment introduced by the use of a FFQ rather than more detailed methods.\(^{43}\)

Insufficient dietary diaries were available for analysis of lipid nutrient associations by genotype, but the directions of change were similar using both methods. Despite a number of intervention and association studies, there is controversy as to whether the association of ApoE phenotypes with serum lipids is related to dietary factors.\(^{13-25}\) Inconsistencies in results have been attributed to differences in study design and small sample sizes leading to lack of statistical power, particularly in the e4 groups.\(^{25}\) In this study of nearly 23 000 individuals, all genotype groups increased serum LDL cholesterol in response to dietary saturated fat, with highly significant β coefficients for every genotype group, but there were no significant difference in the magnitude of the associations according to genotype. The magnitude of responses for fat were up to six-fold greater in the e2/e4 groups and there was a significant difference ($P < 0.05$) between the regression coefficients for total and monounsaturated fat when compared
Table 1  The number (n) and percentage (%) in each genotype group and mean and standard deviation (S.D.) of some coronary heart disease risk factors and serum lipids

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>e2/e2 (n = 137, 0.6%)</th>
<th>e3/e2 (n = 2842, 12.4%)</th>
<th>e3/e3 (n = 13 428, 56.6%)</th>
<th>e4/e3 (n = 5385, 23.5%)</th>
<th>e4/e4 (n = 527, 2.3%)</th>
<th>e2/e4 (n = 596, 2.6%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M (%)</td>
<td>45.85</td>
<td>46.00</td>
<td>45.88</td>
<td>45.23</td>
<td>48.40</td>
<td>45.69</td>
<td>0.733</td>
</tr>
<tr>
<td></td>
<td>F (%)</td>
<td>54.15</td>
<td>46.40</td>
<td>54.12</td>
<td>54.77</td>
<td>51.60</td>
<td>54.31</td>
<td>0.733</td>
</tr>
<tr>
<td>Age – Mean (SD)</td>
<td>58.71(9.29)</td>
<td>58.73 (9.33)</td>
<td>58.72 (9.30)</td>
<td>58.14 (9.07)</td>
<td>58.26 (9.16)</td>
<td>0.571</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m^2) – Mean (SD)</td>
<td>26.31 (3.85)</td>
<td>25.96 (3.43)</td>
<td>26.45 (3.96)</td>
<td>26.27 (3.85)</td>
<td>26.04 (4.03)</td>
<td>26.25 (3.74)</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg) – Mean (SD)</td>
<td>135.42 (18.32)</td>
<td>135.25 (17.98)</td>
<td>135.58 (18.35)</td>
<td>135.18 (18.40)</td>
<td>134.93 (17.51)</td>
<td>134.61 (18.91)</td>
<td>0.235</td>
<td></td>
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<tr>
<td>Chol (mmol/L) – Mean (SD)</td>
<td>6.18 (1.17)</td>
<td>5.74 (1.10)</td>
<td>6.19 (1.14)</td>
<td>6.36 (1.17)</td>
<td>6.59 (1.32)</td>
<td>6.05 (1.23)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/L) – Mean (SD)</td>
<td>3.96 (1.03)</td>
<td>3.44 (0.94)</td>
<td>3.99 (1.00)</td>
<td>4.17 (1.05)</td>
<td>4.38 (1.14)</td>
<td>3.74 (1.00)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/L) – Mean (SD)</td>
<td>1.42 (0.43)</td>
<td>1.46 (0.43)</td>
<td>1.42 (0.43)</td>
<td>1.39 (0.41)</td>
<td>1.34 (0.39)</td>
<td>1.43 (0.44)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Tg (mmol/L) – Mean (SD)</td>
<td>1.82 (1.11)</td>
<td>2.30 (1.33)</td>
<td>1.86 (1.12)</td>
<td>1.86 (1.14)</td>
<td>2.01 (1.57)</td>
<td>1.98 (1.33)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>Current</td>
<td>11.54</td>
<td>11.55</td>
<td>11.81</td>
<td>11.06</td>
<td>9.34</td>
<td>11.92</td>
<td>0.778</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>42.52</td>
<td>42.36</td>
<td>42.36</td>
<td>42.85</td>
<td>42.55</td>
<td>43.16</td>
<td>0.778</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>45.93</td>
<td>46.09</td>
<td>45.83</td>
<td>46.09</td>
<td>48.11</td>
<td>44.93</td>
<td>0.778</td>
</tr>
<tr>
<td>Physical activity index 1 (%)</td>
<td>30.27</td>
<td>30.22</td>
<td>30.36</td>
<td>30.41</td>
<td>29.94</td>
<td>29.08</td>
<td>30.83</td>
<td>0.774</td>
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<td>2 (%)</td>
<td>28.62</td>
<td>28.34</td>
<td>28.48</td>
<td>29.14</td>
<td>27.84</td>
<td>29.39</td>
<td>0.774</td>
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<tr>
<td></td>
<td>3 (%)</td>
<td>22.79</td>
<td>22.46</td>
<td>22.60</td>
<td>23.29</td>
<td>22.16</td>
<td>24.28</td>
<td>0.774</td>
</tr>
<tr>
<td></td>
<td>4 (%)</td>
<td>18.32</td>
<td>18.88</td>
<td>18.51</td>
<td>17.63</td>
<td>20.92</td>
<td>15.50</td>
<td>0.774</td>
</tr>
<tr>
<td>Hypotensive drugs</td>
<td>Yes (%)</td>
<td>18.4</td>
<td>18.2</td>
<td>17.9</td>
<td>19.1</td>
<td>18.7</td>
<td>21.8</td>
<td>0.203</td>
</tr>
<tr>
<td>Hypolipidaemic drugs</td>
<td>Yes (%)</td>
<td>1.6</td>
<td>1.1</td>
<td>0.5</td>
<td>1.4</td>
<td>2.3</td>
<td>2.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

BMI, Body mass index; SBP, Systolic blood pressure; Chol, Cholesterol; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; Tg, Triglycerides.
with those of the e3/e3 individuals. Due to the comparatively small size of the e2/e4 group, it is possible that this difference occurred by chance.

Fibre was inversely associated with all blood lipid fractions, except for a positive association with serum HDL. The inverse associations between fibre and serum total and LDL cholesterol are supported by numerous studies that show fibre intake to consistently reduce serum total and LDL cholesterol concentrations. In intervention studies, fibre has not previously been shown to reduce serum triglyceride levels. However, in the Framingham study of women, there were cross-sectional inverse associations between fibre and serum triglycerides. The effect of fibre on cholesterol is attributed to fermentation of fibre polysaccharides in the large bowel and the consequent absorption of the short chain fatty acid propionate. As
Table 3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total fat en (%)</th>
<th>MUFA en (%)</th>
<th>PUFA en (%)</th>
<th>Sat fat en (%)</th>
<th>Fibre (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>e2/e2</td>
<td>0.047 (0.028)</td>
<td>0.056</td>
<td>0.103</td>
<td>0.126</td>
<td>0.036</td>
</tr>
<tr>
<td>e2/e3</td>
<td>0.023 (0.017)</td>
<td>0.039</td>
<td>0.076</td>
<td>0.111</td>
<td>0.023</td>
</tr>
<tr>
<td>e3/e4</td>
<td>0.016 (0.015)</td>
<td>0.024</td>
<td>0.045</td>
<td>0.098</td>
<td>0.014</td>
</tr>
</tbody>
</table>

The effect of diet and serum lipids on APOE polymorphisms is as follows:

- Despite the large size of this study, the largest to date, there was little evidence that, apart from the small (3%) of individuals who are of e2/e4 genotype, different APOE genotypes respond differently to increased dietary saturated or total fat intake. ApoE gene loci status does not confer exemption from targets to reduce dietary saturated fat and increase dietary fibre in order to reduce blood lipids and risk of CHD in populations as a whole.

Conflict of interest: none declared.

References


