Genetic factors affecting the consistency and magnitude of changes in plasma cholesterol in response to dietary challenge

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Received 13 November 1995 and in revised form 14 May 1996

Summary

We examined the role of common genetic variation in determining the consistency and magnitude of change in plasma total cholesterol (TC) levels in response to two separate changes from a high-saturated (SFA) to a low-saturated/high-polyunsaturated-fat (PUFA) diet, in a group of free-living healthy men and women. Consistent responders were defined as those whose mean difference in the change in TC was within one SD of the mean for all participants, and the remainder were defined as variable responders. DNA was obtained from 55 individuals and genotype determined at the apolipoprotein (apo) B locus (signal peptide, SP), apoCIII (C1100-T) and lipoprotein lipase (LPL) gene loci (HindIII). In the 38 consistent responders, the apoBSP24 allele was significantly more common than in the 17 individuals with a variable response (0.29 vs. 0.12; p<0.05). No other polymorphism showed a significant frequency difference between groups. In the group as a whole, the correlation between the change in TC level in response to the first and second dietary change was 0.28 (p=0.05), but those with one or more apoB SP24 alleles and those with the apoCIII genotype CC had a significantly higher correlation than those with other genotypes (0.46 (p = 0.05) vs. 0.12 (NS) and 0.31 (p = 0.05) vs. 0.02 (NS), respectively). In the group as a whole, mean response left TC 10% higher on the SFA than on the PUFA diet, and neither apoB nor apoCIII genotypes affected the magnitude of this response. However, individuals with the LPL HindIII genotype H+H+ had a significantly smaller change in mean TC in response to diet than those with one or more H− allele (9.3% vs. 14.4%; p=0.03). Thus variation at the apoB and apoCIII loci affects the consistency of response to change in dietary fat content, while variation at the LPL gene locus affects magnitude of response.

Introduction

The dietary intake of saturated fatty acids (SFA) is the major environmental determinant of plasma levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), both between populations and within groups of individuals.1-4 However, some individuals respond to a greater degree than others to changes in the amount of cholesterol consumed in the diet or to changes in the SFA content, and the determinants of this degree of response are unclear.5-9 The concept of hyper-responders and hypo-responders to changes in the amount of cholesterol in the diet, which was originally proposed in 198710 is now widely accepted, and since the variation in consistency of response cannot be explained by variation in compliance, this suggests strongly that genetic factors must be involved. Obvious candidate genes which might contribute to such individual differences include those coding for apolipoproteins, enzymes involved in lipid metabolism and receptors of lipoprotein particles, as well as enzymes involved in the breakdown and absorption of dietary fat in the...
the common protein polymorphisms of apoE are acid secretion.

Several studies have examined the possibility that the common protein polymorphisms of apoE are associated with such differences (reviewed in reference 11), but in most studies no consistent effect has been seen, except in those individuals with the genotype apoE4E4, who have the highest initial lipid levels and who show the greatest drop in levels in response to dietary fat reduction.12-14 The mechanism of this association is presumably due to the well-understood effects of the Cys-Arg changes in the apoE isoforms, with apoE4 having the highest fractional efficiency of cholesterol absorption from the gut,15 and highest binding affinity of lipoproteins to the LDL-receptor, leading to a larger 'up-regulation' of the LDL-receptor compared to the apoE2 or E3 isoforms.

The effect of polymorphisms at the apoE locus in determining differences in variability in response to dietary changes is not as well documented, and no consistent picture has yet emerged. In the North Karelia Dietary Intervention study,16 individuals with different apoB signal peptide genotype,17 and Xbal genotype showed different response to changes in the fat content of the diet.18,19 The Xbal polymorphism was associated with a difference in response in the plasma levels of apoAl and HDL-C, but this association has not been examined in other studies. The signal peptide polymorphism is caused by the deletion of 9 bp, coding for the three amino acids leucine-alanine-leucine in the hydrophobic core sequence, giving a 24-amino-acid peptide compared to the 'wild-type' 27-amino-acid sequence. On changing from a high-fat to a low-fat diet, the mean triglyceride levels of the whole sample fell slightly, but individuals who were homozygous for the common SP27 amino-acid allele had a significantly greater reduction than individuals with one or more SP24 alleles.17 A similar effect associated with signal peptide genotype on triglyceride levels has been noted in a dietary study from the US,20 although the differences were small and did not reach statistical significance. The effects associated with other polymorphisms at the apoB locus have also been small and/or non-reproducible.21-23 Only two studies have examined the effect of polymorphisms at the apoAI-CIII-AIV locus, and no significant effects have been reported.19,22 The major problems with interpreting these studies are the different ages, and genetic and cultural (dietary) backgrounds of the participants, the different dietary protocols used and the difficulty of ensuring compliance. Taken together, these data suggest that although the effects associated with apoB genotype (but not apoE genotype) are likely to be making a small contribution, other genes involved in determining the strong genetic effects that determine response to dietary change have yet to be identified.

The major goal of this study was to identify genotypes which might determine either the magnitude of the response, or the consistency of the response, to dietary change. Three genes were investigated: apoB, apoCIII, and LPL, and one polymorphism for each gene was chosen on the basis of its having a high rare-allele frequency and because of previous reports of being associated with fasting lipid or lipoprotein levels or extent of atherosclerosis. For the apoB gene, this was the signal peptide (SP) length polymorphism,24-27 for the apoCIII gene, the C1100 to T polymorphism,28,29 and for the LPL gene, the HindIII polymorphism.30-34 The effect of these polymorphisms was examined in a dietary intervention study,35 where levels of dietary saturated fatty acids were changed twice in a group of free-living healthy men and women, and in whom the response to change of total cholesterol had been determined, thus allowing an estimation of the degree of consistency in response.

Methods

Subjects and dietary protocol

The sample of individuals used in this study was as described previously.35 Briefly, it consisted of 55 healthy White Caucasian individuals (32 female, 23 male) mean age (±SE) 52.2 (± 1.1) mean BMI 25.67 (± 0.51) living in New Zealand who had taken part in a dietary response study. The individuals were all free of manifest signs of diabetes, familial hypercholesterolaemia or other major concomitant disease such as uraemia and collagenosis. None of the individuals were receiving hypolipidaemic treatment. The dietary protocol has been described previously,35 but briefly, subjects were randomized to receive two dietary sequences of a high-saturated-fat period (S) or a low-saturated high-polyunsaturated fat diet (P) in the order SPSP or PSPS. Each phase (S or P) of the double crossover was continued for 6 weeks. Diet S was high in SFA, and was designed to provide 38% total energy as fat with 26% from SFA, 10% from mono-unsaturated fat and 2% from PUFA. Diet P was low in SFA and high in PUFA, providing 23% total energy from PUFA, 6% from MUFA and 9% from SFA. Detailed dietary instructions, menu suggestions and recipes were provided and reinforced during personal interviews and telephone calls throughout the study. Compliance was excellent as determined by weighed food inventory, with the percentage of SFA as energy consumed during the P and S periods estimated as being 10% ± 2 and
21% ± 2, respectively, and percentage PUFA being 12.5% ± 4 and 4% ± 1, respectively.

**Serum lipoprotein determinations**

Prior to randomization and at week 4 and week 6 of each diet period, a fasting venous blood sample was taken for analysis of lipoproteins and analysis of fatty-acid composition. The major serum lipoproteins [total cholesterol, (TC), triglycerides (Tg), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)] were determined by a combination of preparative ultracentrifugation and precipitation of apolipoprotein-B-containing lipoproteins, followed by lipid analyses in the lipoprotein fractions as described.

**DNA preparation and analysis**

DNA was extracted from fresh, whole blood collected after an overnight fast, by the salting-out method. Genotype was determined by the polymerase chain reaction (PCR) using previously published methods and amplifying oligonucleotides. Taq polymerase was from Bethesda Research Laboratories, and reactions were performed on a Hybaid Intelligent Heating Block. ApoCIII-C1100-T genotype was carried out as described by amplification and the use of allele specific oligonucleotides. LPL HindIII genotyping was as described, using restriction-enzyme digestion and separation of the fragments on an agarose gel. ApoB signal-peptide genotype was determined as described, by direct electrophoresis of the amplified fragments on a 10% acrylamide gel.

**Statistical analysis**

Statistical analysis used the SPSS/PC+ computer program. The allele frequency for each polymorphism was estimated using the 'gene counting' method, and determined separately for the consistent-responders and the variable-responders. 2x2 contingency tables were used to compare allele frequencies between the two groups, with the SP24 allele being more frequent (0.29 vs. 0.12; p<0.05) in those with a consistent compared to a variable response.

The baseline characteristics of the sample and the major effects of the dietary change on plasma total cholesterol (TC), in the whole group and in groups
Figure 1. Schematic representations of the three gene loci, apoB, apoAI-CIII-AIV and lipoprotein lipase, showing the location of polymorphisms used in this study. Rectangles represent transcribed region of gene. Black and shaded boxes within rectangles represent translated and untranslated regions, respectively. For the LPL gene exons are numbered in ascending order along direction of transcription (from 5' to 3').

Table 1 Genotype and allele frequencies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Rare allele frequency</th>
<th>Rare allele frequency in consistent/variable response (38/17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoB SP 27/24</td>
<td>35 14 6</td>
<td>24 = 0.236</td>
<td>0.289/0.118*</td>
</tr>
<tr>
<td>ApoCIII C/T</td>
<td>38 15 2</td>
<td>T = 0.173</td>
<td>0.171/0.176</td>
</tr>
<tr>
<td>LPL HindIII H+/H−</td>
<td>45 9 1</td>
<td>H− = 0.100</td>
<td>0.105/0.088</td>
</tr>
</tbody>
</table>

*p2 = 3.84; p = 0.05.

Table 2 Association between genotypes and response to dietary change and with baseline lipid traits (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Total sample</th>
<th>apoB SP</th>
<th>apoCIII</th>
<th>LPL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>27/27</td>
<td>27/24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>35</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>ΔTC1</td>
<td>0.65 ± 0.08</td>
<td>0.66</td>
<td>0.64</td>
<td>0.58</td>
</tr>
<tr>
<td>ΔTC2</td>
<td>0.61 ± 0.05</td>
<td>0.66</td>
<td>0.52</td>
<td>0.61</td>
</tr>
<tr>
<td>ΔTC mean</td>
<td>0.63 ± 0.05</td>
<td>0.66</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Correlation (r)</td>
<td>0.28*</td>
<td>0.12</td>
<td>0.46*</td>
<td>0.31*</td>
</tr>
<tr>
<td>ΔTC1 and ΔTC2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.20 ± 0.10</td>
<td>6.28</td>
<td>6.08</td>
<td>6.30</td>
</tr>
<tr>
<td>Tg</td>
<td>1.45 ± 0.15</td>
<td>1.58</td>
<td>1.20</td>
<td>1.46</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>4.04 ± 0.10</td>
<td>4.00</td>
<td>4.10</td>
<td>4.26</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.39 ± 0.04</td>
<td>1.36</td>
<td>1.44</td>
<td>1.41</td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>0.59 ± 0.07</td>
<td>0.69</td>
<td>0.41</td>
<td>0.48</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.03; ***p < 0.01.
TC, total cholesterol. All data are in mmol/l.
The relationship between the consistency and the magnitude of the change in total cholesterol levels over the two separate periods was determined in groups of individuals with different genotypes, and for each locus, this was done by combining individuals homozygous for the rare allele with those heterozygous for that allele, and results are presented in Table 2. As previously reported, the correlation between the change in total cholesterol on the first and second occasions was low but statistically significant ($r = 0.28$, $p < 0.05$), but it can be seen that this correlation was significantly greater in the individuals with more than one apoBSP24 allele than those with only the SP27 allele ($r = 0.46$, $p < 0.05$ and $r = 0.12$, NS). Similarly for the apoCIII gene, this correlation was significantly greater in the individuals with the genotype CC compared to those with one or more T allele ($r = 0.31$, $p < 0.05$, and $r = 0.01$, NS, respectively). These results are presented graphically in Figures 2a and 2b, respectively. There was no effect on this correlation associated with the LPL HindIII polymorphism.

With regard to the magnitude of the effect of change in total cholesterol levels on the two separate periods, as can be seen in Table 2, only for the LPL HindIII genotype was there evidence of an effect, with the S-P change being consistently larger in the 10 individuals with one or more $H^+$ allele, and for the change on the second occasion this difference reached a statistical significance ($p = 0.03$). When data was expressed as percentage change between the two periods, the 38 individuals homozygous for the $H^+$ allele experienced on average a 10% increase (11.1% and 9.6% on first and second change, respectively), whereas the 10 individuals with one or more $H^+$ alleles showed a 15% increase (15.5% and 14.4%, respectively). On combining the data from the two dietary periods (ΔTC mean), and adjusting the change in total cholesterol observed for individual differences in age, baseline BMI and gender, the LPL HindIII genotype explained 6.3% of the sample variance ($p = 0.03$). For none of the other polymorphisms was there any consistent or significant association with change in TC between the two dietary periods.

The effect of each genotype on baseline lipid traits was examined, and as shown in Table 2, significant differences in baseline plasma lipid level were only seen for the apoCIII C-T polymorphism. In this sample, those with one or more T allele had significantly higher plasma triglycerides and VLDL cholesterol, and significantly lower LDL cholesterol. These differences were consistently observed for all dietary periods (not shown), but there were no significant effects associated with this apoCIII genotype and change in any lipid trait between the two dietary periods. For the apoB SP genotype, as shown in

![Figure 2. Correlation of the change in total cholesterol (TC) on the first and second dietary change in individuals with different genotypes. a ApoB signal peptide genotype; b ApoCIII C1100-T genotype.](https://academic.oup.com/qjmed/article-abstract/89/9/671/1522625/1522625)
0.51 mmol/l on the two saturated-fat periods (p < 0.05). For plasma Tg there were similar effects; compared to those with the genotype H+H+, individuals with one or more H− alleles had non-significantly higher (0.31 mmol/l) levels on the P diet but significantly higher levels (0.56 mmol/l, p = 0.05) on the saturated diet. The result of this was that the 10 H− individuals had a significantly greater (p = 0.05) mean fall in both TC (0.88 mmol/l, 12.8%) and Tg (0.35 mmol/l, 12.7%) on changing from the saturated to the polyunsaturated diet.

**Discussion**

The main feature of this study was that dietary changes were repeated twice, which allowed an estimation of the degree of consistency in response. Studies in humans have demonstrated the existence of hypo- and hyperresponders to dietary change,2-10 and that these traits are stable over several years10 but the genes responsible for these differences are unknown. The variability gene concept as proposed by Berg37 made a distinction between genetic variation determining the level of a plasma lipid trait in an individual and genetic variation determining that individual’s ability to vary in response to environmental changes. The design of this study enabled us to look for the effect of ‘variability genes’ by examining the correlation between the change in TC on the first and second occasions was examined. Of the three genes examined, this correlation was significantly greater in those with one or more apoB SP24 allele and those with the apoCIII genotype CC compared to those with other genotypes. In support of this, the frequency of the SP24 allele was more than twice as high in the group of individuals showing a consistent response to dietary change compared to the variable responders. The data would predict that individuals who had both the apoCIII CC genotype and also one or more of the apoB SP24 alleles would be likely to have a significantly greater correlation in response than those with other genotypes. In this small sample there are 13 such individuals, and in this group the correlation between the change in TC on the two challenges was 0.56 (p = 0.05) compared to a correlation of 0.12 (NS) in the 42 individuals with the other genotypes. Although this post hoc comparison needs to be confirmed in a separate study, and the precise mechanism is yet to be determined, it does suggest that the combined information about these two genotypes might identify individuals who would have a particularly good response to dietary change.

The consistent response associated with the apoB
SP genotype is in support of the findings in the North Karelia dietary intervention study, where those with one or more SP24 allele showed a smaller change in plasma lipid traits in response to dietary change.\textsuperscript{18,19} It is possible to propose a cellular mechanism for the signal-peptide effect. It is known that under most conditions the majority of the apoB protein that is synthesized in the liver is degraded intracellularly, and that such degradation is reduced by factors such as increased influx of free fatty acids, leading to the increased secretion of lipoprotein particles.\textsuperscript{40,41} We have shown in a yeast model system that compared to SP27, a marker protein linked to SP24 is poorly secreted from the endoplasmic reticulum.\textsuperscript{42} Therefore, extrapolating from this we have proposed that in the liver (and also possibly the intestine) of an individual with the SP27 allele the rate of secretion of Tg-rich lipoproteins can fluctuate over a wide range in response to changes in dietary fat intake. By contrast, in an individual with the SP24 allele a greater proportion of the apoB protein is destined to be degraded, and the proportion which is ‘rescuable’ by influx of dietary lipids is fixed and is low. This leads to reduced variability in secretion and thus reduced variability in fasting levels of plasma lipoproteins in response to dietary change. In support of this model, we have recently reported that individuals with the apoB SP24 allele have lower levels of post-prandial triglyceride-rich lipoproteins of both intestinal and hepatic origin after a fat meal,\textsuperscript{43} confirming \textit{in vivo} that the apoB SP24 protein is not responsive to up-regulation by a fat meal, whereas the level of lipoprotein secretion controlled by the SP27 protein is modifiable.

Many studies have shown associations between variation at the apoAI-CIII-AIV gene cluster and hyperlipidaemia (e.g. references 44–48). Of the known polymorphisms in this gene cluster, we chose the recently discovered C1100-T polymorphism in the apoCIII gene (that does not alter the amino acid sequence), because of the relatively high rare-allele frequency and because in two studies of healthy individuals and patients the T allele was associated with significantly higher Tg levels.\textsuperscript{28,29} This association with plasma Tg levels was confirmed in this sample of healthy men and women, and in addition, those with the genotype CC and thus the lowest Tg levels showed a more consistent response to dietary change as estimated from the higher correlation in change in plasma TC compared to those with one or more T allele. Although individuals with high plasma lipids are statistically likely to show greater fluctuations in response to dietary change, the C1100-T genotype was not associated with effects on baseline TC or on the magnitude of the change in TC, and thus the molecular mechanism of the effect on consistency is unclear.

It is however plausible that variation in the apoCIII gene will affect the metabolism of apoB-containing particles, since apoCIII is a component of both VLDL and HDL, and is an exchangeable apolipoprotein between lipoprotein particles.\textsuperscript{49} \textit{In vitro} studies have shown that apoCIII inhibits the hydrolysis of triglyceride in VLDL by LPL\textsuperscript{50–52} and the uptake of VLDL particles by hepatocytes.\textsuperscript{53} Recent studies with human apoCIII transgenic mice have reported hypertriglyceridaemia and elevated levels of cholesterol in these animals,\textsuperscript{54,55} confirming an important role for apoCIII in lipid metabolism \textit{in vivo}, probably acting through displacement of apo E on the lipoprotein particles by high levels of apoCIII, leading to reduced hepatic uptake. Since the C1100-T polymorphism is unlikely to be of functional relevance, the most likely mechanism is that it is a marker of another sequence variation at the apoCIII-AIV intragenic region that contains DNA sequence elements responsible for control of expression of the apoproteins in the liver or intestine.\textsuperscript{56} One possible molecular mechanism of the effect seen would be if the transcription of apoCIII mRNA from the T allele, and thus levels of apoCIII protein secreted from the liver, were more sensitive to stimulation by dietary saturated fat; in this case, individuals with only the C allele would be predicted to have a more consistent response to dietary change. Since levels of apoCIII were not measured in this study, no direct evidence in support of this hypothesis can be obtained.

Of the three genes examined, only the HindIII polymorphism of the LPL gene was consistently associated with differences in the magnitude of the TC response to the change in proportion of saturated fatty acids in the diet. Individuals with one or more H\textsuperscript{−} alleles had a larger response than those with just the H\textsuperscript{+} allele (14.2% mean change vs. 9.3%). It would thus be expected that the frequency of this H\textsuperscript{−} allele would be higher in the individuals identified as consistent hyperresponders, and this was observed (H\textsuperscript{−} frequency 0.11 in hyperresponders vs. 0.088 in the others), but this difference did not reach statistical significance in this small sample. Since LPL has its major effect on the metabolism of the Tg-rich lipoproteins, it would be expected that the TC effect should be a result of a change in plasma Tg in response to diet. This effect was observed, with those with the genotype H\textsuperscript{+}H\textsuperscript{+} showing only a small and non-significant average fall in Tg of 4.6% on changing from the saturated to the polyunsaturated diet, while those with one or more H\textsuperscript{−} alleles showed a fall of 12.7% (p = 0.05). This suggests that dietary differences may be a possible reason for why the association between LPL...
genotypes and lipid traits is not always consistent or of similar magnitude between studies in different communities and countries.\textsuperscript{32-34} One possible explanation for the effect seen with LPL genotype would be if the HindIII polymorphism was associated with a significant effect on baseline plasma cholesterol levels, and as can be seen from Table 2, those with one or more H\textsuperscript{+} alleles had slightly but not significantly higher plasma total cholesterol levels at baseline. However, the association between LPL genotype and response to dietary change was still significant when baseline total cholesterol was included as a covariate in an analysis of variance (p = 0.03). The mechanism of this LPL-genotype-mediated effect is unclear, but as for the apoCIII gene, it is likely that the HindIII genotype is a marker for a functional change elsewhere in the LPL gene that leads to differences in the activity or mass of LPL present in the different individuals, with the H\textsuperscript{+} allele producing either less LPL or LPL that is less active than from the H\textsuperscript{-} allele. If this were the case, the extra load of saturated fatty acid present in the dietary challenge would result in greater secretion of the VLDL from the liver, which would overwhelm the 'partial' LPL deficiency in these individuals. To date, there is no definitive proof of this mechanism, and so far studies have reported only a small and non-significant difference in LPL activity or mass associated with the HindIII polymorphism (e.g. reference 33). It has recently been proposed that LPL bound to remnant particles may act as a ligand for their rapid clearance by the liver via a receptor-mediated mechanism.\textsuperscript{57,58} It is also therefore possible that variation of the LPL gene locus might be affecting the clearance of lipoproteins through such a mechanism. We are currently searching for variation in the LPL gene that would explain these effects.

In this small sample, only seven and nine individuals were carriers of the apoE2 and apoE4 isoforms, respectively and no significant effects of apoE genotypes were seen on dietary response.\textsuperscript{35} Other studies have reported that such effects are restricted to those with the apoE genotype E4E4\textsuperscript{18} and such individuals are so rare in the general population (estimated to be 2\% in most Caucasian populations) that this genotype has little impact on determining response in general. The data suggest that variation at the apoB and apoCIII gene loci are having a larger effect than at the apoE locus, at least in this sample of healthy middle-aged men and women. Overall, these data lend support to the variability and level gene concept, and implicate apoCIII and apoB (but not apoE) as being genes where variation determines the variability or consistency of plasma lipid trait levels, and the LPL gene as one determining the magnitude of the response of TC seen when the individual experiences changes in diet. A detailed understanding of the molecular mechanisms behind these effects may be useful in identifying and counselling certain individuals, in order that they may avoid particular diets which may specifically increase their chance of developing hyperlipidaemia and thus risk of coronary artery disease.

Acknowledgements
We thank Ms Gina Shoesmith for typing the manuscript, and Mr Lee Huang and Miss Grace Chu for technical assistance. Studies conducted at UCL Medical School were funded by the British Heart Foundation (RG16). Studies conducted at the University of Otago were funded by grants from the National Heart Foundation of New Zealand and the Anderson and Telford Charitable Trust.

References


