

The genetics of serum lipid responsiveness to dietary interventions

Jose M. Ordovas

Lipid Metabolism Laboratory, JM-USDA HNRCA at Tufts University, Boston, MA 02111, USA

CHD is a multifactorial disease that is associated with non-modifiable risk factors, such as age, gender and genetic background, and with modifiable risk factors, including elevated total cholesterol and LDL-cholesterol levels. Lifestyle modification should be the primary treatment for lowering cholesterol values. The modifications recommended include dietary changes, regular aerobic exercise, and normalization of body weight. The recommended dietary changes include restriction in the amount of total fat, saturated fat and cholesterol together with an increase in the consumption of complex carbohydrate and dietary fibre, especially water-soluble fibre. However, nutrition scientists continue to question the value of these universal concepts and the public health benefits of low-fat diets, and an intense debate has been conducted in the literature on whether to focus on reduction of total fat or to aim efforts primarily towards reducing the consumption of saturated and *trans* fats. Moreover, it is well known that there is a striking variability between subjects in the response of serum cholesterol to diet. Multiple studies have examined the gene-diet interactions in the response of plasma lipid concentrations to changes in dietary fat and/or cholesterol. These studies have focused on candidate genes known to play key roles in lipoprotein metabolism. Among the gene loci examined, APOE has been the most studied, and the current evidence suggests that this locus might be responsible for some of the inter-individual variability in dietary response. Other loci, including APOA4, APOA1, APOB, APOC3, LPL and CETP have also been found to account for some of the variability in the fasting and fed states.

Dietary fats: Cholesterol: Lipoproteins: Genetics: Atherosclerosis

CHD, the leading cause of mortality in most industrialized countries, is a multifactorial disease that is associated with non-modifiable risk factors, such as age, gender and genetic background, and with modifiable risk factors, including elevated total cholesterol and LDL-cholesterol levels. There is convincing evidence showing that lowering serum lipid levels will slow the progression or even induce regression in atherosclerotic lesions (Blankenhorn *et al.* 1990).

While several types of drug therapies have been developed and have been shown to be effective in lowering serum cholesterol values, the National Cholesterol Education Program (Expert Panel on High Blood Cholesterol in Adults, 1993) has emphasized that lifestyle modification should be the primary treatment for lowering cholesterol values, with drug therapies reserved for cases where lifestyle modification is ineffective or inadequate. The modifications recommended include dietary changes, regular aerobic exercise, and normalization of body weight. The recommended dietary changes include restriction in the amount of total fat ($\leq 30\%$ energy), saturated fat ($< 10\%$ or

$< 7\%$ energy) and cholesterol (< 300 or < 200 mg/d) along with an increase in the consumption of complex carbohydrate and dietary fibre, especially water-soluble fibre. However, nutrition scientists continue to question the value of these universal concepts and the public health benefits of low-fat diets, and an intense debate has been conducted in the literature on whether to focus on reduction of total fat or to aim efforts primarily towards reducing the consumption of saturated and *trans* fats (Connor & Connor, 1997a,b; Katan *et al.* 1997a,b). While both sides agree that the intake of fruits, vegetables and high-fibre foods should be increased and that intake of saturated fat be decreased, the appropriateness of other global public health recommendations continues to be questioned.

It is well known that there is a striking variability between subjects in the response of serum cholesterol to diet. In some individuals, plasma cholesterol levels dramatically decrease following consumption of a low-fat diet, while it remains unchanged in others. Animal studies have demonstrated that the serum lipoprotein response to dietary

Abbreviations: A, adenine; apo, apolipoprotein; CAD, coronary artery disease; CETP, cholesterol ester transfer protein; G, guanine; LCAT, lecithin-cholesterol acyltransferase; LPL, lipoprotein lipase; PAI-1, plasminogen-activator inhibitor; RFLP, restriction fragment length polymorphisms; TRL, triacylglycerol-rich lipoproteins.

Corresponding author: Dr Jose M. Ordovas, fax +1 617 556 3103, email Ordovas_li@hnrc.tufts.edu

manipulation has a significant genetic component. The evidence in human subjects is less clear, primarily due to the lack of information regarding the heritability of the response to dietary intervention. Despite this uncertainty, multiple studies have already been carried out examining gene–diet interactions that could begin to explain the genetic component of the human inter-individual variability in blood lipid responses to dietary modifications. These studies have focused on candidate genes known to play key roles in lipoprotein metabolism, such as APOE, APOA4, APOB, APOA1, APOC3, LPL and CETP. The major findings of this interesting research will be presented and discussed.

Apolipoprotein A-I

Apolipoprotein (apo) A-I is the major protein of HDL and plays a crucial role in lipid metabolism. ApoA-I is the major *in vivo* activator of the enzyme lecithin–cholesterol acyltransferase (EC 2.3.1.43; LCAT; Fielding *et al.* 1972), and constitutes a key component of the reverse cholesterol transport process (Reichl & Miller, 1989). The gene for APOA1 is clustered with the APOC3 and APOA4 genes on the long arm of human chromosome 11 (Bruns *et al.* 1984; Karathanasis, 1985). This DNA region has been extensively analysed, resulting in the identification of several restriction fragment length polymorphisms (RFLP). A number of studies have shown associations between some of these RFLP and lipid abnormalities as well as increased CHD risk (Ordovas *et al.* 1991b; Paul-Hayase *et al.* 1992; Tybjærg-Hansen *et al.* 1993), but other studies have failed to do so (Marshall *et al.* 1994). Several rare genetic abnormalities at this locus have been associated with severe HDL deficiency, and some of them with premature coronary atherosclerosis (Ordovas *et al.* 1989).

A common variant due to adenine (A) to guanine (G) transition (G/A) has been described 75 base pairs upstream from the APOA1 gene transcription start site. Several studies have reported that individuals with the A allele, which occurs at a frequency of 0.15–0.20 in Caucasian populations, have higher levels of HDL-cholesterol than those subjects homozygous for the most common G allele. The magnitude of the effects, and the gender and diet interactions reported have differed among studies (for summary, see Table 1). Our own findings (Lopez-Miranda *et al.* 1994b; Mata *et al.* 1998; see Table 1) support the notion that in well-controlled dietary studies performed in normolipidaemic subjects, the A allele of this G/A polymorphism appears to be associated with hyper-response to changes in the amount and saturation of dietary fat.

It is not clear whether the putative effect of this variant on HDL-cholesterol levels is due to the G to A substitution *per se*, or to linkage disequilibrium between the A locus and a distinct and as yet unidentified effector locus. *In vitro* analysis of the effects of this polymorphism on transcription also has yielded conflicting results. Smith *et al.* (1992) reported that the A allele decreased *in vitro* transcription by 30%, consistent with their own *in vivo* turnover studies that showed decreased apoA-I synthetic rates in individuals with the A allele, although plasma HDL-cholesterol did not differ between GG and GA individuals. Tuteja *et al.* (1992) reported that substitution of A for G decreased transcription

about 2-fold and Jeenah *et al.* (1990) reported a 4-fold increase in transcription. Angotti *et al.* (1994) reported a 5 to 7-fold increase in transcription associated with the A allele, and demonstrated that this may be due to reduced binding affinity of a nuclear factor to the A allele that results in increased transcription efficiency of the apoA-I promoter.

In summary, the mechanisms responsible for the observed effect are still unknown. This mutation may have a direct effect on liver and/or intestinal APOA1 gene expression, as suggested in previous studies, or it may be in linkage disequilibrium with a functional mutation in either of the neighbouring genes (APOC3 and APOA4). Further studies are needed to clarify these results.

Apolipoprotein A-IV

In human subjects apoA-IV is synthesized primarily in the intestine as a 46 kDa glycoprotein (Green *et al.* 1980). While the precise function of apoA-IV is still unknown, its intestinal origin and the experimental evidence from familial apoAI, apoC-III and apoA-IV deficiency (Ordovas *et al.* 1989) suggest that it plays a role in dietary fat absorption and chylomicron synthesis. *In vitro* studies have shown that the activation of lipoprotein lipase (EC 3.1.1.34; LPL) by apoC-II is mediated by apoA-IV (Goldberg *et al.* 1990), and that apoA-IV can serve as an activator of LCAT (Steinmetz & Utermann, 1985). ApoA-IV-containing lipoproteins promote cholesterol efflux from cultured fibroblasts and adipose cells *in vitro*, and there is evidence that apoA-IV may be one of the ligands for the HDL receptor (Stein *et al.* 1986; Steinmetz *et al.* 1990; Weinberg & Patton, 1990). Thus, the findings suggest that apoA-IV plays a role in fat absorption and reverse cholesterol transport.

Genetically-determined isoforms of apoA-IV have been detected in human subjects and in other mammalian species. The most common isoform detected using isoelectric focusing is the apoA-IV*1, with an allele frequency in Caucasians ranging from 0.88 to 0.95. ApoA-IV*2 (Gln³⁶⁰ → His) is the second most common isoform with an allele frequency in the range of 0.05 to 0.12 in Caucasians (Menzel *et al.* 1990; de Knijff *et al.* 1992). Additional variation within these isoforms has been detected using the polymerase chain reaction. A relatively common mutation (Thr³⁴⁷ → Ser) has been documented within subjects with the apoA-IV*1 isoform. The effect of apoA-IV genetic variation on plasma lipid levels has been studied in several populations (Eichner *et al.* 1989; Kamboh *et al.* 1991; Kaprio *et al.* 1991; de Knijff *et al.* 1992; Von Eckardstein *et al.* 1992). In some Caucasian populations, the apoA-IV*2 allele has been associated with higher levels of HDL-cholesterol and/or lower triacylglycerol levels (Menzel *et al.* 1988, 1990; Eichner *et al.* 1989), but no associations have been observed in other studies (de Knijff *et al.* 1988; Hanis *et al.* 1991; Kamboh *et al.* 1992; Bai *et al.* 1993; Crews *et al.* 1993; Zaiou *et al.* 1994).

The effect of genetic variation at this locus on dietary response has recently been examined by us and other investigators (McCombs *et al.* 1994; Mata *et al.* 1994). Our study shows that the APOA4*2 (Gln³⁶⁰ → His) allele (APOA4*2) is associated with hyporesponsiveness of LDL-cholesterol to dietary therapy consisting of reductions in

Table 1. Summary of studies examining the guanine (G)/adenine (A)-75 APOA1 polymorphism, plasma lipid levels and dietary response

Study	No. of subjects	Population	Study design	APOA1-75 effects on lipid levels
Pagani <i>et al.</i> (1990, 1992)	244	Italian men and women	Population	Higher frequency of the A allele in women with high HDL-C levels
Jeenah <i>et al.</i> (1990)	96	English men	Population	Men with the A allele had significantly higher levels of apoA-I, HDL-C and HDL2-C than those who were G/G
Smith <i>et al.</i> (1992)	54	US men and women	Metabolic	No effect on HDL-C or apoA-I levels; however, G/A subjects had lower apoA-I production rates
Pauli-Hayase <i>et al.</i> (1992a)	162	Belgian boys and young men (7–23 years old)	Population	Higher apoA-I levels associated with the A allele
Sigurdsson <i>et al.</i> (1992)	315	Icelandic men and women	Population	Higher HDL-C and apoA-I levels in men who were non-smokers
Tybjærg-Hansen <i>et al.</i> (1993)	221	Danish men	Population	The frequency of the A allele was not associated with any of the phenotypes examined
Xu <i>et al.</i> (1993)	204	Italian boys and girls (8–11 years old)	Population, diet intervention (LF–LC diet) in a subset	The A allele was associated with higher levels of TC, LDL-C, apoB and apoA-I with the effects being more marked in boys. No significant gene–diet interaction was observed
Civeira <i>et al.</i> (1993)	125	Spanish men and women	Population with high and low HDL-C levels	The allele frequencies were similar in subjects below the 25th percentile for HDL-C and apoA-I as compared with those within the 75th percentile
Saha <i>et al.</i> (1994)	287	Chinese healthy men and women	Population	The A allele was associated with higher apoA-I in men who were non-smokers. This association was absent in women
Lopez-Miranda <i>et al.</i> (1994b)	50	Spanish young men	Diet intervention (LF v. high MUFA)	After consumption of the high-MUFA diet, significant increases were noted in LDL-C in the G/A subjects but not in the G/G subjects
Talmud <i>et al.</i> (1994)	1657	European young men and women	Population (case–control)	The A allele was associated with higher HDL-C and apoA-I specially in women who were non-smokers
Needham <i>et al.</i> (1994)	148	Hypertriacylglycerolaemic and normal Caucasian and Japanese	Population (case–control)	No significant differences in allele frequency in either control–control or case–control comparisons in European and Japanese populations
Peacock <i>et al.</i> (1994)	179	Swedish MI survivors and controls	Population (case–control)	No specific effects associated with the presence of the A allele for any of the phenotypes examined
Barre <i>et al.</i> (1994)	409 subjects and twenty-two families	US Caucasian men and women	Population and family studies	No allele effect observed for HDL-C levels
Atika <i>et al.</i> (1995)	168	Japanese cases with CETP deficiency and controls	Population (case–control)	No evidence that this polymorphism has any effect on HDL-C levels regardless of CETP status
Minnich <i>et al.</i> (1995)	653 subjects and four kindred	French Canadian men and women	Population	Women carriers of the A allele had higher HDL-C and apoA-I concentrations. No allele effect was noted in men. The frequency distribution of HDL-C levels suggests linkage disequilibrium with other causative polymorphism
Meng <i>et al.</i> (1997)	86	Finnish men and women	Dietary intervention	Higher plasma HDL-C and apoA-I associated with the A allele in men. No gene–diet interaction was observed
Mata <i>et al.</i> (1998)	50	Spanish men and women	Diet intervention (high-SAT v. high-MUFA and high-PUFA diets)	Diet induced significantly greater TC and LDL-C decreases in G/A than in G/G women
Carmena-Ramon <i>et al.</i> (1998b)	69	Spanish men and women heterozygotes for FH	Diet intervention (3 % energy as fat, 10 % energy as SAT and 300 mg cholesterol/d v. NCEP-I diet)	FH subjects carrying the A allele had significantly lower TC, LDL-C and apoB baseline levels but responded to a LF diet with similar reductions in TC and LDL-C when compared with homozygotes for the G allele

LF, low fat; LC, low cholesterol; CETP, cholesteryl ester transfer protein; FH, familial hypercholesterolaemia; MI, myocardial infarction; HDL-C, LDL-C, HDL- and LDL-cholesterol respectively; MUFA, monounsaturated fatty acids; SAT, saturated fat; PUFA, polyunsaturated fatty acids; TC, total cholesterol; NCEP, National Cholesterol Education Program; A, adenine; G, guanine; G/A, guanine to adenine transition; apo, apolipoprotein.

total fat and cholesterol. McCombs *et al.* (1994) have demonstrated that this effect may be due exclusively to the reduction in dietary cholesterol. Moreover, in the study by Mata *et al.* (1994) we observed that subjects with the APOA4*2 allele tended to have greater decreases in HDL-cholesterol concentrations following a low-fat low-cholesterol diet. To follow up on this observation, Jansen *et al.* (1997a) have examined the effect of this polymorphism on HDL-cholesterol response in forty-one healthy male subjects (thirty-three APOA4*1/1 and eight APOA4*1/2). These subjects were given three consecutive diets (high-saturated fat, low-fat and high-monounsaturated fatty acid diets) for 4 weeks each. After consuming the saturated-fat diet, carriers of the APOA4*2 allele had a greater decrease in HDL-cholesterol and apoA-I. In these subjects, replacement of a high-carbohydrate diet with a diet containing monounsaturated fatty acid resulted in a greater increase in HDL-cholesterol and apoA-I as compared with homozygotes for the APOA4*1 allele. These findings suggest that in APOA4*2 subjects, a high-carbohydrate diet may induce an apparently increased atherogenic lipid profile (LDL-cholesterol does not decrease, but HDL-cholesterol does decrease). Thus, these subjects may benefit particularly from a diet relatively high in monounsaturated fatty acids.

The mechanism by which this mutation may exert the observed effect is still unknown. The apoA-IV*2 isoform binds to lipoproteins with higher affinity than apoA-IV*1, which may result in delayed hepatic clearance of chylomicron remnants, as shown in metabolic studies (Rader *et al.* 1993). Given the important role that this apoA-IV may have in lipid absorption, it is possible that mechanisms involving intestinal fat absorption and/or the metabolism of triacylglycerol-rich lipoproteins (TRL) may be differentially affected by each isoform.

The APOA4*2 allele has also been studied in relation to the changes in cardiovascular risk factors associated with urbanization in developing countries. We demonstrated in a population-based study in Costa Rica that lifestyles associated with an urban environment, such as increased smoking and saturated fat intake, elicit a more adverse plasma lipoprotein profile among subjects who are carriers of the APOA4*2 allele than in APOA4*1 homozygotes, which could make them more susceptible to CHD (Campos *et al.* 1997). These results may be difficult to reconcile with those from the dietary metabolic studies. However, it should be noted that the changes associated with 'modernization' (i.e. increased fat and cholesterol and smoking, and decreased physical activity) are more complex (gene-environment interaction) than those taking place during a well-controlled dietary protocol carried out in a metabolic unit (gene-diet interaction).

An APOA4 variant observed within the APOA4*1 allele, the APOA4 (Thr³⁴⁷ → Ser), is present in the population with frequencies ranging from 0.16 to 0.22. To study the influence of this mutation on the LDL-cholesterol response to diets, Jansen *et al.* (1997b) re-examined the data from the study presented previously (Jansen *et al.* 1997a). Their results indicated that carriers of the ³⁴⁷Ser allele presented a greater decrease in total cholesterol, LDL-cholesterol and apoB concentrations when they were switched from the low-fat low-cholesterol diets than homozygous carriers of

the ³⁴⁷Thr allele. Similarly, the change from a low-fat low-cholesterol diet to a monounsaturated fatty acid-rich diet resulted in a greater increase in those same variables.

We have recently examined the influence of these APOA4 polymorphisms on dietary response in subjects with familial hypercholesterolaemia (sixty-seven heterozygous men and women; Carmena-Ramon *et al.* 1998a). The APOA4*2 allele was associated with lower LDL-cholesterol and apoB levels, independent of diet effects. No differences in total cholesterol, LDL-cholesterol, HDL-cholesterol and apoB levels were observed between subjects homozygous for the APOA4³⁴⁷Thr allele and those carriers of the APOA4³⁴⁷Ser allele. After dietary intervention, Ser/Ser subjects showed significant reductions in plasma triacylglycerols and VLDL-cholesterol levels, but no changes were found in carriers of the Ser allele.

The combined information for the Thr³⁴⁷ → Ser and the Gln³⁶⁰ → His suggests that the responsiveness of LDL-cholesterol to changes on dietary fat is as follows: ³⁴⁷Ser/³⁶⁰Gln > ³⁴⁷Thr/³⁶⁰Gln > ³⁴⁷Thr/³⁶⁰His. The mechanisms by which these mutations may exert the observed effects are still unknown. The apoA-IV*2 isoform binds to lipoproteins with higher affinity than apoA-IV*1, which may result in delayed hepatic clearance of chylomicron remnants, as shown in metabolic studies. The substitution of Ser for Thr at position 347 induces changes in the secondary structure, and a slight increase in hydrophilic profile at this position, which could result in a decrease in its affinity for lipids on the TRL particles. This could facilitate the exchange with apoC-II, thereby increasing LPL activity over those particles, which would in turn accelerate clearance of remnants. The increased influx of dietary cholesterol would downregulate the LDL receptors, with the consequent increases in LDL-cholesterol concentrations. Thus, consumption of fat-rich diets would produce a greater increase in LDL-cholesterol in ³⁴⁷Ser carriers.

Apolipoprotein E

ApoE in serum is associated with chylomicrons, VLDL and HDL, and serves as a ligand for the LDL receptor and the LDL-receptor-related protein (Mahley, 1988; Beisiegel *et al.* 1989). When apoE deficiency is present, there is marked accumulation of cholesterol-enriched lipoproteins of density < 1.006 g/ml containing apoB-48 and apoA-IV, as well as apoB-100 (Schaefer *et al.* 1986). Moreover, in this disorder there is delayed clearance of both apoB-100 and apoB-48 within TRL. These findings support the concept that apoE is important for the clearance of these lipoprotein particles. Genetic variation at the APOE locus results from three common alleles in the population, E*4, E*3 and E*2, with frequencies in Caucasian populations of approximately 0.15, 0.77, 0.08 respectively (Davignon *et al.* 1988). Population studies have shown that plasma cholesterol, LDL-cholesterol and apoB levels are highest in subjects carrying the apoE4 isoform, intermediate in those with the apoE3 isoform, and lowest in those with the apoE2 isoform (Ordovas *et al.* 1987; Schaefer *et al.* 1994). It has been suggested that APOE allelic variation may account for up to 7% of the variation in total cholesterol and LDL-cholesterol levels in the general population (Davignon *et al.*

1988). This relationship between LDL-cholesterol levels and APOE genetic variation is not independent of environmental and ethnic factors. The association of the apoE4 isoform with elevated serum cholesterol levels is greater in populations consuming diets rich in saturated fat and cholesterol than in other populations. These findings indicate that the higher LDL-cholesterol levels observed in subjects carrying the apoE4 isoform are manifested primarily in the presence of an atherogenic diet characteristic of certain societies, and that the response to dietary saturated fat and cholesterol may differ among individuals with different apoE phenotypes.

Since 1983, the interaction between lipoprotein responsiveness to dietary manipulation and apoE phenotype or genotype has been the subject of several studies. Some investigators reported greater plasma lipid responses in subjects carrying the APOE4 allele (see Table 2), while others failed to find significant associations between APOE genotype and plasma lipid response (see Table 2 and Glatz *et al.* 1991; Ginsberg *et al.* 1994; Friedlander *et al.* 1995). There are important differences among these studies that could account for some of the discrepancies observed. These studies differed in gender, age and baseline lipid levels, and all these variables are known to play an important role in the variability of dietary response. Dreon *et al.* (1995) have shown that the apoE-dependent mechanism may be specific for large buoyant LDL particles. Consequently, baseline LDL particle distribution will also play a significant role in the outcome of different studies, and this variable should be controlled in future studies. In addition, Lehtimäki *et al.* (1995) have demonstrated that the association between serum lipids and apoE phenotype is influenced by diet in a population-based sample of free-living children and young adults.

Overall, a significant diet \times APOE gene interaction was reported in studies with men alone. In studies involving men and women, significant effects were noted only in men, suggesting a significant gene \times gender interaction. Another difference between the negative studies and those reporting significant APOE gene \times diet interactions related to the baseline lipid levels of the subjects. Positive findings were frequently observed in those studies which included subjects who were moderately hypercholesterolaemic and/or had significant differences in base total cholesterol and LDL-cholesterol among the APOE genotype groups, suggesting that the significant gene \times diet interaction is apparent only in subjects who are susceptible to hypercholesterolaemia. With regard to differences in dietary interventions, significant interactions were more commonly observed among studies in which total dietary fat and cholesterol was modified. It is possible that dietary cholesterol may play a significant effect in this gene–diet interaction. It should also be noted that some reports have shown that cholesterol absorption is related to APOE genotype.

In a preliminary report Loktionov *et al.* (1998) examined the effects of the APOE alleles and tea drinking on blood lipids and blood coagulation factors in sixty-five clinically-healthy men and women. This 10-week randomized study revealed that subjects bearing at least one APOE4 allele had significantly elevated total cholesterol, LDL-cholesterol and triacylglycerol levels. Moreover, mean plasminogen-

activator inhibitor (PAI-1) activity was higher in APOE4 subjects than in APOE3/3 or APOE3/2 subjects. These findings suggest that elevated PAI-1 activity may be an additional factor involved in the increased cardiovascular risk associated with the APOE4 allele. In terms of interactions, tea drinking was associated with significant decreases in HDL-cholesterol levels of APOE3/3 subjects as well as decreases in triacylglycerol levels and PAI-1 activities of APOE2/3 subjects. These results indicate that tea drinking has a beneficial effect on plasma lipid levels and coagulation factors, especially in subjects carrying the APOE2 allele. It should be noted that fruits and vegetables also contain polyphenols similar to those in tea, and high consumption of fruits and vegetables has been reported to decrease PAI-1 activity. The molecular mechanisms involved in the interaction between APOE gene variability and PAI-1 are still unknown, but one possible link lies on the LDL-receptor-related protein receptor, which is known to bind several ligands, including apoE, tissue-type plasminogen activator and PAI-1. As for the hyper-response observed for the APOE2 allele in relation to triacylglycerol levels, it should be noted that previous studies using dietary manipulation (including type of carbohydrates and fibre rather than type and amount of dietary fat and cholesterol) resulted in APOE gene–diet interactions by which APOE2 subjects were more responsive to these dietary modifications than APOE3/3 and APOE4 subjects (see Table 2). Moreover, APOE2 carriers are significantly more responsive to hydroxymethylglutaryl-CoA reductase (*EC* 1.1.1.34) inhibitors than APOE3/3 and APOE4 subjects (Carmena *et al.* 1993; Ordovas *et al.* 1995). However, as pointed out by the authors, these results were obtained in a small group and should be interpreted with care, and should be confirmed in larger studies in which genotyping is part of the initial subject selection criteria.

In recent decades, evidence has accumulated to demonstrate that postprandial lipaemia is a major determinant of blood lipoprotein concentrations and cardiovascular risk (Dallongeville & Fruchart, 1998). The postprandial response is highly heterogeneous, and multiple factors such as age, exercise, body weight, fasting lipid levels, diet and genetics have been noted to be responsible for this variability. The APOE gene has been implicated as one of the genetic factors responsible for these effects. The apoE2 isoform is considered to decrease remnant clearance because of decreased affinity for the receptors. Conversely, the apoE4 isoform should induce a faster clearance. However, studies that have compared postprandial triacylglycerol responses across different APOE genotypes have produced conflicting results, especially regarding the effects associated with the APOE4 allele (Kesaniemi *et al.* 1987; Weintraub *et al.* 1987; Brown & Roberts, 1991; Superko & Haskell, 1991; Nikkilä *et al.* 1994). Postprandial response was examined at 4 and 8 h by Boerwinkle *et al.* (1994) in a large sample (n 474) of individuals taking part in the Atherosclerosis Risk in Communities Study, following a single high-fat meal containing vitamin A. Postprandial plasma retinyl palmitate response was significantly different among APOE genotypes, with delayed clearance in subjects carrying the APOE2 allele, compared with APOE3/3 and APOE3/4 subjects; however, measurements of other lipid

Table 2. Summary of studies examining APOE genotype-diet interactions with changes in dietary fat and/or cholesterol

Study	Subjects	Experimental design*	Diet period	Observed effects
Fisher <i>et al.</i> (1983)	Nine normolipidaemic males (three E2, five E3, one E4)	Maize oil v. coconut oil plus LC v. HC diets, combination	9 d	No effect on TC, LDL-C, HDL-C or TG levels
Miettinen <i>et al.</i> (1988)	Sixteen men (six E2 and ten E4)	LF-LC diet v. LF-HC diet	6 weeks	E4 allele associated with greater TC, LDL-C and HDL-C response. E2 associated with increased HDL-C response
Tikkanen <i>et al.</i> (1990a)	110 Men and women (twelve E2, forty-eight E3 and fifty E4)	Baseline (HF-HC diet) v. LF high-P:S diet, controlled	6 or 12 weeks	Significant APOE allele effect on plasma lipid levels. Greater reductions in TC occurred in subjects homozygous for the APOE4 allele
Savolainen <i>et al.</i> (1991)	Forty-four healthy middle-aged men and women (twenty-three E3 and twenty-one E4)	LF-LC diet v. HF-HC diet, controlled	4 weeks	The absolute and percentage lipid changes on the two diets were equal in E3 and E4 subjects
Boerwinkle <i>et al.</i> (1991)	Seventy-one men (thirteen E2, forty-eight E3 and ten E4)	LC diet v. HC diet, counselled	3 weeks	The average responses in lipid levels were not significantly different among APOE genotypes
Gaddi <i>et al.</i> (1991)	Twenty men and women, FH (seven E2, nine E3 and four E4)	LF-LC diet v. soyabean-protein diet, controlled	4 weeks	The plasma cholesterol reduction was higher in patients with E3/E3 or E3/E4 v. an almost negligible effect with E3/E2
Mantari <i>et al.</i> (1991)	117 Dyslipidaemic middle-aged men (placebo group Helsinki Heart Study)	Diet therapy, counselling	15 months	Baseline lipid levels were not affected by the E allele. E4 subjects exhibited a greater reduction in TC and LDL-C
Lehtimäki <i>et al.</i> (1992)	Thirty-six healthy students (eight E2, eleven E3 and sixteen E4)	Usual (no eggs) v. usual plus eggs, counselling	3 weeks	The increases were similar in groups E3/2, E3/3, and E4/3. Stronger responses were observed in the small group of E4/4 subjects
Juustupa <i>et al.</i> (1992)	Nineteen (twelve E3 and seven E4)	High-fibre diets (oat bran v. wheat bran)	8 weeks	Only E3 subjects had hypocholesterolaemic response to oat bran. No change was found in E4 subjects
Miettinen <i>et al.</i> (1992)	Twenty-nine middle-aged men (eight E2, nine E3 and twelve E4)	Normal diet v. a diet low in fat and cholesterol, counselled	5 weeks	The apoE subtype (E2/2, 1, E2/3, 2 etc.) was positively associated with cholesterol absorption and the LDL-apoB and -cholesterol levels and negatively with cholesterol synthesis and FCR for LDL-apoB
Jenkins <i>et al.</i> (1993)	Sixty-seven men and women (thirteen E2, thirty-eight E3, sixteen E4)	High-fibre diet (oat bran v. wheat bran)	2 weeks	Carriers of the E2 allele appear to be more responsive than non-carriers to a dietary change involving increased fibre intake
Cobb <i>et al.</i> (1992)	Sixty-seven normolipidaemic men and women (thirteen E2, forty-four E3 and eight E4)	A 'Western' diet with a low P:S value or a 'therapeutic' diet, with a high P:S value, retrospective pooled analysis of six controlled studies		Women of the E3/2 phenotype stand to benefit the least from a high P:S diet because of reduction in the more 'protective' HDL-C, whereas men of the E4/3 phenotype showed the greatest improvement in LDL:HDL
Cobb & Risch (1993)	Sixty-seven normolipidaemic men and women (thirteen E2, forty-four E3 and eight E4)	Low P:S diet or high P:S diet, retrospective pooled analysis of six controlled studies		ApoE phenotype was not a significant predictor of responsiveness
Hunninghake <i>et al.</i> (1993)	Ninety-seven male and female patients with moderate hypercholesterolaemia	Diet high in fat and cholesterol v. LF diet, counselled	9 weeks	ApoE phenotype was not a significant predictor of responsiveness
Martin <i>et al.</i> (1993)	Thirty young normal male subjects (five E3/2, eleven E3/3, fourteen E4/3)	LC diet v. HC diet, controlled	35 d	The LDL-C response to dietary cholesterol did not differ among the APOE genotypes. APOE genotype has significant and opposite effects on plasma CETP and HDL-C responses to dietary cholesterol in men
Lopez-Miranda <i>et al.</i> (1994c)	128 Men and women (seventeen E2, ninety-four E3 and seventeen E4)	HF-HC v. LF-LC diets, retrospective controlled and counselling protocols	4-24 weeks	The plasma LDL-C reduction was higher in male subjects with the E4 allele
Sarkinen <i>et al.</i> (1994)	Forty hypercholesterolaemic men and women (three E2, twenty-seven E3 and seventeen E4)	High-SAT diet v. high-MUFA diet, counselled	6 months	ApoE phenotype was not a significant predictor of responsiveness
Dreon <i>et al.</i> (1995)	102 Normal men (ten E2, sixty-four E3 and twenty-eight E4)	HF v. LF diets, counselled	6 weeks	The plasma LDL-C reduction was higher in subjects with the E4 allele
Clifton <i>et al.</i> (1995)	120 Normolipidaemic men and women	LF v. LF plus two liquid supplements (one that contained HF-HC and one that was fat-free), counselled and supplement	2 and 3 weeks	The plasma LDL-C reduction was higher in male subjects with the E4 allele

Table 2. Summary of studies examining APOE genotype-diet interactions with changes in dietary fat and/or cholesterol. *Continued*

Study	Subjects	Experimental design*	Diet period	Observed effects
Schaefer <i>et al.</i> (1995)	Thirty-two men and women	HF-HC diet v. NCEP step II, controlled	6 and 24 weeks	The plasma LDL-C reduction was higher in male subjects with the E4 allele
Zambon <i>et al.</i> (1995)	122 Hypercholesterolaemic men and women (twenty-seven E2, forty-eight E3 and forty-seven E4)	High-MUFA diet v. LF diet, counselled	12 weeks	ApoE phenotype was not a significant predictor of responsiveness
Park <i>et al.</i> (1996)	Seventeen subjects (three E2 and fourteen E3)	HF diets containing different proportions of specific saturated fatty acids	4 weeks	ApoE phenotype was not a significant predictor of responsiveness
Lefevre <i>et al.</i> (1997)	103 Men and women (eleven E2, fifty-seven E3 and thirty-five E4)	HF-HC v. AHA Step I v. low-SAT (no changes in dietary cholesterol), controlled	8 weeks	ApoE phenotype was not a significant predictor of responsiveness
Pasagian-Macaulay <i>et al.</i> (1997)	488 Healthy women (seventy-one E2, 288 E3, 120 E4 and nine E2/4)	LF dietary intervention, counselled	6 months	The magnitude of the changes in TC and LDL-C was not dependent on APOE genotype
Dixon <i>et al.</i> (1997)	125 Children aged 4–10 years (twenty-five E4, ninety-four E3 and six E2)	LF dietary intervention, counselled	3 months	The magnitude of the changes in TC and LDL-C was not dependent on APOE genotype
Lehtimäki <i>et al.</i> (1997)	Fifty-eight healthy men and women (five E2, thirty-five E3 and eighteen E4)	Fasting, controlled	1 week	In men, the changes in plasma LDL-C during fasting differed significantly between APOE genotypes
Loktionov <i>et al.</i> (1998)	Sixty-five healthy men and women aged 20–73 years (seven E2, forty-five E3 and thirteen E4)	Tea (six mugs of black tea/d) and then a crossover of placebo (consisting of water, caffeine, milk and sugar)	4 weeks	Tea drinking was associated with significant decreases in HDL-C levels of E3/3 subjects as well as decreases in TG levels and PAI-1 activities of APOE2/3 subjects
Tso <i>et al.</i> (1998)	Thirty-six healthy premenopausal women (seven E2, twenty-two E3, six E4 and one E2/4)	HF diets containing different proportions of specific saturated fatty acids, controlled	4 weeks	Responsiveness regulated in part by APOE polymorphism

LF, low fat; LC, low cholesterol; HF, high fat; HC, high cholesterol; P-S, polyunsaturated; saturated; CETP, cholesteryl ester transfer protein; FH, familial hypercholesterolaemia; HDL-C, LDL-C, HDL- and LDL-cholesterol respectively; E2, includes E2/2 and E2/3; E3, includes E3/3; E4, includes E3/4 and E4/4; MUFA, monounsaturated fatty acids; PAI-1, plasminogen activator inhibitor-1; SAT, saturated fat; TC, total cholesterol; TG, triglycerols; FCR, fractional catabolic rate; apo, apolipoprotein; AHA, American Heart Association; NCEP, National Cholesterol Education Program.

* Controlled indicates food provided, counselling indicates dietary advice provided and combination indicates some food and dietary advice provided.

variables, such as triacylglycerols, and triacylglycerol in TRL were not sensitive enough to detect these effects. Another study by Nikkilä *et al.* (1994) carried out in CHD cases and controls showed that in CHD patients with the apoE2/3 phenotype triacylglycerol levels were highest and still increasing after 7 h, reflecting delayed chylomicron remnant clearance. The same effect was observed also in normotriacylglycerolaemic non-insulin-dependent diabetic patients (Reznik *et al.* 1996) and in non-diabetic normolipidaemic subjects (Orth *et al.* 1996), although in the latter report the delayed chylomicron remnant was observed only on APOE2/2 individuals. The findings associated with the APOE4 allele have been more discordant. In an earlier report heterozygosity for this allele was associated with a lower lipaemic response relative to other phenotype groups (Weintraub *et al.* 1987); however, in a more recent study the APOE4 allele was associated with prolonged postprandial responses of lipids and apolipoproteins in TRL (Bergeron & Havel, 1996). This topic has been revisited recently by Wolever *et al.* (1997), who examined the long-term effect of soluble-fibre foods on postprandial fat metabolism in dyslipidaemic subjects (sixteen with APOE3/3 and seventeen with APOE3/4 genotypes). These subjects consumed low-fat (20 % energy), high-fibre (>5.7 g/MJ) diets for two 4-month periods separated by a 2-month wash-out period, according to a randomized crossover design. One diet contained foods rich in insoluble fibre and the other diet was rich in soluble fibre. They carried out a 1 d postprandial study during the last 2 weeks of each diet. Subjects ingested a standard fibre-free fatty liquid meal containing retinyl palmitate as a marker of intestinally-derived lipoproteins. Blood samples were obtained at hourly intervals for 10 h. Their results suggest that a long-term increase in dietary soluble fibre does not affect postprandial fat metabolism in subjects with the APOE4 allele; however, soluble fibre enhanced fat absorption in APOE3/3 subjects, which could be due to an increased bile acid pool and increased micelle formation.

Several mechanisms have been proposed to explain these apoE-related differences in individual response to dietary therapy. Some studies have shown that intestinal cholesterol absorption is related to apoE phenotype, with apoE4 carriers absorbing more cholesterol than non-apoE4 carriers. Other mechanisms, such as different distribution of apoE on the lipoprotein fractions, LDL-apoB production, bile acid and cholesterol synthesis, and postprandial lipoprotein clearance, may also be involved.

Apolipoprotein C-III

Plasma apoC-III is a component of chylomicrons, VLDL and HDL. This protein is synthesized primarily in the liver, and to a lesser extent in the intestine (Zannis *et al.* 1985). *In vitro*, apoC-III inhibits LPL (Wang *et al.* 1985), and also inhibits the binding of apoE-containing lipoproteins to the LDL receptor, but not to the LDL-receptor-related protein (Kowal *et al.* 1990; Weisgraber *et al.* 1990). In agreement with the observations *in vitro*, the overexpression of the human APOC3 gene in transgenic mice resulted in severe hypertriacylglycerolaemia (Ito *et al.* 1990). The APOC3 gene is closely linked to the APOA1 and APOA4 genes on

the long arm of chromosome 11 (region 11q13; Bruns *et al.* 1984). Several RFLP have been described at this locus. The S2 allele of the SstI RFLP 3' to the APOC3 gene has been associated in some studies with hypertriacylglycerolaemia and increased coronary artery disease (CAD) risk (Ferns *et al.* 1985; Ordovas *et al.* 1991a,b). A PvuII RFLP located in the first intron of the APOC3 gene has also been associated with variation in HDL-cholesterol levels. Recent studies have demonstrated the presence of five DNA polymorphisms (C⁻⁶⁴¹ → A, G⁻⁶³⁰ → A, T⁻⁶²⁵ → deletion, C⁻⁴⁸² → T, and T⁻⁴⁵⁵ → C) in the promoter region (Dammerman *et al.* 1993). These mutations were in strong linkage disequilibrium with the Sst I site in the 3' untranslated region (Dammerman *et al.* 1993; Li & Leff, 1994). An insulin response element has been mapped to a forty-two nucleotide fragment located between -490 and -449 relative to the transcription start site, and *in vitro* studies demonstrated that transcriptional activity of the APOC3 gene was downregulated by insulin only in the construct bearing the wild-type promoter, but not in those constructs containing the C⁻⁴⁸² → T, and T⁻⁴⁵⁵ → C variants (Li & Leff, 1994; Li *et al.* 1994). These results may provide the molecular basis to understand the increased levels of apoC-III found in subjects carrying the S2 allele and its association with hypertriacylglycerolaemia. Our own studies show that following an increase in dietary monounsaturated fatty acids, S1S1 subjects responded with an increase in LDL-cholesterol levels, whereas S1S2 subjects experienced a significant decrease (Lopez-Miranda *et al.* 1997a). These findings suggest that the APOC3 locus is involved in LDL-cholesterol responsiveness to dietary fat. This interaction could begin to explain the different effects associated to this polymorphism that have been reported in the literature.

Apolipoprotein B

ApoB is the main protein component of LDL and is the ligand which mediates the recognition of LDL by the LDL receptor. In human subjects, apoB-100 is synthesized by the liver, although some synthesis may occur in the intestine (Hoeg *et al.* 1990; Levy *et al.* 1990; Lopez-Miranda *et al.* 1994a). ApoB-48 is the primary form synthesized by the intestine by a mRNA editing mechanism (Driscoll & Casanova, 1990; Lau *et al.* 1991). The APOB gene has been mapped to the region 2p24-p23 on chromosome 2 (Law *et al.* 1985). Since apoB is the major protein of LDL and an important component of VLDL, it will be expected that genetic variation at this locus could influence plasma cholesterol and/or triacylglycerol levels. Some of the polymorphic sites such as the XbaI site, the EcoRI, the MspI, the insertion/deletion (I/D) and the 3'VTR polymorphisms have been utilized as markers in population or case-control studies in an attempt to correlate individual alleles or haplotypes with lipid levels or CHD risk. In general, the outcome of these studies has not been unanimous (Berg, 1986; Law *et al.* 1986; Talmud *et al.* 1987; Genest *et al.* 1990; Xu *et al.* 1990b; Hixson *et al.* 1992; Saha *et al.* 1992).

The XbaI RFLP is a silent mutation involving the third base of the threonine codon 2488 (ACC → ACT) in exon 26 (Carlsson *et al.* 1986). This RFLP has been associated with the variability in plasma lipid levels (Berg, 1986; Law *et al.*

1986; Talmud *et al.* 1987; Aalto-Setälä *et al.* 1988, 1991). The allele lacking the XbaI recognition site (X⁻) has been associated with lower total cholesterol, LDL-cholesterol and triacylglycerols. Paradoxically, this same X-allele has been found to be more common among CAD cases than in controls (Hegele *et al.* 1986; Monsalve *et al.* 1988; Myant *et al.* 1989). It has been suggested that the hypothetical causal mutation associated to the XbaI polymorphism, may result in a structural change in apoB that affects the egression of LDL from the arterial wall (Bohn & Berg, 1994).

In terms of plasma lipid response to changes in dietary fat, it has been reported that subjects carrying the X⁺X⁺; or X⁺X⁻ genotypes responded to a low-fat low-cholesterol diet with greater reductions in plasma total cholesterol, LDL-cholesterol, apoB (Tikkanen *et al.* 1990b; Talmud *et al.* 1992), and surprisingly HDL-cholesterol levels than subjects with the X⁻X⁻ genotype (Xu *et al.* 1990b). We have recently evaluated whether the APOB XbaI polymorphism was associated with the inter-individual variability observed during postprandial lipaemia (Lopez-Miranda *et al.* 1997b). For this purpose, fifty-one healthy young male volunteers (twenty X⁻/X⁻ (X⁻) and thirty-one X⁺/X⁻ or X⁺/X⁺ (X⁺)), homozygous for the APOE3 allele, were subjected to a vitamin A-fat load test. Subjects with the X⁻ genotype had significantly greater retinyl palmitate and apoB-48 postprandial responses on both the large and the small TRL fractions compared with X⁺ subjects. These findings suggest that subjects with the X⁻/X⁻ genotype at the APOB locus have a greater postprandial response than X⁺ subjects. These differences observed in postprandial lipoprotein metabolism could explain some reported associations of this polymorphism with CAD risk. As indicated previously, this specific mutation does not result in an amino acid change at the affected codon, and it cannot have a direct functional effect. However, the mutation is in strong linkage disequilibrium with the APOB(Val⁵⁹¹→Ala) polymorphism (Ag a1/d), which may be the functional sequence change. An alternative hypothesis relates to the position of this mutation near the apoB-48 editing site at position 2153. The mechanism of this editing appears to involve a form of cytidine deaminase (EC 3.5.4.5). The 'mooring' sequence model for the editosome suggests that the recognition and binding sequences of nuclear factors that identify the specific site for editing and 'moor' the editing activity are distal to and different from the editing site. It is possible that the XbaI mutation, close to the editing site, could affect these 'mooring' sequences, modifying the editing activity on the apoB mRNA. This might result in changes on apoB-48 synthesis and intestinal TRL secretion, as shown in our study (Lopez-Miranda *et al.* 1997b).

The three-codon (leu-ala-leu) I/D polymorphism within the apoB signal peptide (Boerwinkle & Chan, 1989) has been found to be associated with variation in total cholesterol and LDL-cholesterol and/or triacylglycerol levels (Xu *et al.* 1990b; Visvikis *et al.* 1993) as well as CHD risk (Hixson *et al.* 1992); however, these effects have not been confirmed by other investigators (Peacock *et al.* 1992a,b; Bohn *et al.* 1994). Xu *et al.* (1990a) reported that subjects with the I/I genotype had the highest triacylglycerol

levels and D/D subjects had the lowest, while consuming a high-fat high-cholesterol diet. This effect disappeared when the subjects were consuming a low-fat low-cholesterol diet. These results were not confirmed by Boerwinkle *et al.* (1991) in a study in which subjects received two levels of dietary cholesterol without modification of dietary fat. In a more recent study, the D allele was found to be associated with reduced postprandial lipid response as compared with individuals homozygous for the I allele, suggesting that this mutation in the signal peptide may affect apoB secretion during the postprandial state (Talmud *et al.* 1996). More recently, the association between free fatty acid concentrations and TRL in the postprandial state has been reported to be influenced by a common deletion polymorphism of the apoB signal peptide (Byrne *et al.* 1996). The same polymorphism was involved in postprandial responses of lipoparticles (Régis-Bailly *et al.* 1995).

A plausible mechanism to explain the observed interaction between this I/D polymorphism, postprandial free fatty acids and TRL has been proposed (Byrne *et al.* 1996). *In vitro* studies have shown that apoB is synthesized and then either assembled into lipoproteins and secreted, or is degraded intracellularly. The free fatty acid oleate may increase secretion by protecting intracellular apoB from degradation. Using a yeast expression system it has been shown that the twenty-four amino acid signal peptide mediates apoB translocation into the endoplasmic reticulum less efficiently than the twenty-seven amino acid signal peptide, which may result in reduced apoB secretion. If this mechanism held *in vivo*, then in subjects homozygous for the twenty-seven amino acid signal peptide, increased free fatty acids might result in increased protection of apoB from degradation and increased VLDL production. However, in subjects carrying the twenty-four amino acid signal peptide, increased free fatty acids fail to regulate VLDL production because of accelerated intracellular degradation of apoB.

The MspI (CGGG→CAGC) polymorphism in exon 26 results in an amino acid change (Arg³⁶¹¹→Gln; Huang *et al.* 1988). We have previously found a significant association between the less common allele (M2) and the presence of premature CAD (Genest *et al.* 1990), with this allele being nearly twice as frequent in subjects with CAD (0.105) as in the control population (0.057). However, no associations of this allele with alterations in plasma apoB or LDL-cholesterol levels in subjects with CAD were noted (Genest *et al.* 1990). No associations between this RFLP and variability in dietary response have been reported. An EcoRI RFLP described in exon 29 consists of a single base pair mutation (GAA→AAA; Blackhart *et al.* 1986) that results in an amino acid change from Gln⁴⁵¹⁴→Lys. This RFLP is in linkage disequilibrium with the MspI described previously and it has similar phenotype associations.

A 3'-VNTR region approximately 300 base pairs distal to the 3' end of the APOB gene results in approximately seventeen different alleles. Some initial reports suggested that larger numbers of repeats were associated with increased CAD risk (Hegele *et al.* 1986; Friedl *et al.* 1990; Genest *et al.* 1990). However, other studies did not observe such an association (Heliö *et al.* 1991).

Lipoprotein lipase

LPL is a heparin-releasable enzyme, bound to glycosaminoglycan components of the capillary endothelium. It plays a key role in lipoprotein metabolism by catalysing the hydrolysis of 1,3-ester bonds of triacylglycerols chylomicrons and VLDL. The active form of LPL is constituted by two identical subunits each of approximately 60 000 Da and it requires apoC-II as a cofactor, whereas apoC-III acts as an inhibitor. LPL is synthesized in the adipose and muscle tissue, as well as in macrophages (Nilsson-Ehle *et al.* 1980). The gene for LPL has been located to the short arm of chromosome 8 (region 8p22; Oka *et al.* 1990; Mattei *et al.* 1993). Several common RFLP have been reported at this locus, including a PvuII RFLP located in the intron between exons 6 and 7 (Fisher *et al.* 1987), a HindIII located in the intron between exons 8 and 9 (Heinzmann *et al.* 1987) and a cytosine to G transversion at nucleotide 1595 of the cDNA sequence. Unlike the PvuII and HindIII RFLP, the latter mutation alters the structure of the protein, and it results in the production of a truncated protein (Ser⁴⁴⁷-Stop; Stocks *et al.* 1992). In population studies, these variants have been found to be associated with variability in plasma lipid levels and also with severity of coronary atherosclerosis (Thorn *et al.* 1990; Chamberlain *et al.* 1991; Peacock *et al.* 1992b). The HindIII RFLP has also been reported to be associated with the variability in lipid response to changing from a high-saturated-fat diet to a low-saturated-fat diet (Humphries *et al.* 1996).

Other relatively common mutations at this locus have been recently reported to be associated with mild disturbances in lipid profiles. A missense mutation (Asn²⁹¹Ser) in exon 6 has been found with relative high frequency (2–4%) in Western populations, and appears to be enriched in familial combined hyperlipidaemia (Reymer *et al.* 1995; Hoffer *et al.* 1996) and low HDL-cholesterol (Pimstone *et al.* 1995). *In vivo* and *in vitro* measurements of LPL activity indicate that this mutation is associated with approximately 50–70% of normal LPL catalytic activity. Pimstone *et al.* (1996) have shown that normolipidaemic Asn²⁹¹Ser carriers exhibited a more pronounced postprandial response compared with non-carriers, as shown by higher chylomicron triacylglycerol and retinyl palmitate peaks. It is possible that carriers of this mutation may be unable to respond to a high-fat diet by an increase in their LPL activity as normal subjects do. Thus, a high-fat diet challenge may unmask a hidden defect in lipolysis in these subjects that may not be evident in the fasting state. Another common variant (Asp⁹Asn) has been associated with elevated triacylglycerol levels and with increased progression of coronary atherosclerosis (De Bruin *et al.* 1996; Jukema *et al.* 1996; Maily *et al.* 1996); however, to date no reports have appeared regarding the response of these subjects to a fat challenge.

Cholesteryl ester transfer protein

Cholesteryl ester transfer protein (CETP) mediates the exchange of neutral-lipid core constituents (cholesteryl ester in triacylglycerol) between plasma lipoproteins. The facilitation of the transfer of cholesteryl ester from HDL to TRL results in a reduction in HDL-cholesterol levels, but

CETP may also promote the reverse cholesterol transport. Thus, the overall effect of CETP expression on atherogenesis is uncertain. The gene has been located on chromosome 16 adjacent to the LCAT gene (16q21). A common TaqI polymorphism has been identified in intron 1 (TaqIB; Kondo *et al.* 1989; Freeman *et al.* 1990; Kessling *et al.* 1991; Kuivenhoven *et al.* 1997). The presence of the cutting site has been referred to as B1 and its absence as B2. The B2 allele has been shown to be associated with lower lipid-transfer activity (Hannuksela *et al.* 1994) and higher HDL-cholesterol concentrations (Freeman *et al.* 1994). Fumeron *et al.* (1995) found that alcohol intake modulates the effect of the TaqIB polymorphism on plasma HDL and the risk of myocardial infarction. They found that HDL-cholesterol was increased in subjects with the B2B2 genotype only when they ingested at least 25 g alcohol/d. In the latter study, the cardio-protective effect of the B2B2 CETP genotype was restricted to subjects who consumed the highest amounts of alcohol.

Several reports have demonstrated a significant gene × smoking interaction associated with this RFLP, but only one study has examined the relationship between this polymorphism and dietary response. Dullaart *et al.* (1997), in a study of patients with insulin-dependent diabetes, demonstrated that the VLDL-cholesterol + LDL-cholesterol : HDL-cholesterol value fell in response to a linoleic acid-enriched low-cholesterol diet in B1B1 homozygotes but not in B1B2 heterozygotes.

An interesting gene–drug interaction has been reported (Kuivenhoven *et al.* 1998) in 807 men with angiographically-documented coronary atherosclerosis who were participants in a cholesterol-lowering trial designed to induce the regression of coronary atherosclerosis and were randomly assigned to treatment with either pravastatin or placebo for 2 years. The presence of the TaqIB polymorphism was associated with both higher plasma CETP concentrations and lower HDL-cholesterol concentrations. In addition, they observed a significant association between this marker and the progression of coronary atherosclerosis in the placebo group. This association was abolished by pravastatin. Pravastatin therapy slowed the progression of coronary atherosclerosis in subjects homozygous for this polymorphism but not in those who were homozygous for the most common allele. This common DNA variant appeared to predict whether men with CAD will benefit from treatment with pravastatin to delay the progression of coronary atherosclerosis.

The evidence of the association of the CETP TaqIB polymorphism with HDL-cholesterol levels and its interaction with smoking is unequivocal. Similar evidence for a gene–diet interaction is beginning to emerge. However, the mechanism is unknown. The TaqIB polymorphism is within the non-coding region of the CETP, thus suggesting that the TaqIB may be in linkage disequilibrium with another polymorphism that may affect CETP activity, and consequently, plasma HDL-cholesterol levels.

Conclusion

Evidence accumulated during the last 10 years supports the concept of gene–diet interactions in human subjects. Several

candidate genes, including APOE, APOA4, APOA1, APOC3, APOB, LPL and CETP have been examined under different experimental conditions. However, because of conflicting results, further studies are still required to reconcile the available information.

We should be cautious concerning the interpretation of studies of association between allelic variants, and common phenotypes (Altshuler *et al.* 1998). Great attention should be placed on the population admixture, which can cause an artificial association if a study includes genetically-distinct subpopulations, one of which coincidentally displays a higher frequency of disease and allelic variants. Consideration of the ethnic backgrounds of subjects and the use of multiple independent populations can help avoid this problem. The most persuasive tests, however, involve family-based controls such as the transmission disequilibrium test. In this test, if a given allele contributes to disease, then the probability that an affected person has inherited the allele from a heterozygous parent should vary from the expected Mendelian ratio of 50:50; the association of a neutral polymorphism due to admixture displays no such deviation. A second source of concern is multiple-hypothesis testing, aggravated by publication bias. Authors who test a single genetic variant for an association with a single phenotype base statistical thresholds for significance on a single hypothesis. But many laboratories search for associations using different variants. Each test represents an independent hypothesis, but only positive results are reported, leading to an overestimate of the significance of any positive associations. Statistical correction for multiple testing is possible, but the application of such thresholds results in loss of statistical power. Another concern, that is specific for gene–diet associations, is that most studies were not initially designed to examine gene–diet interactions, and the conclusions were derived from re-analysis of previously obtained data using new information from genetic analysis carried out *a posteriori*. Future studies need to be carefully designed in terms of sample size, taking into consideration the frequencies of the alleles examined. Moreover, we do not really know yet the specific dietary factors responsible for most of the effects already reported. Thus, baseline and intervention diets should be carefully controlled in terms of dietary cholesterol, individual fatty acids, levels of fat, as well as fibre and other minor components of the diet such as phytosterols. It is also important to emphasize that some allele effects may be apparent primarily during the postprandial state; consequently, studies should be designed to test gene–diet interactions, both in the fasting and fed states. Beyond gene–diet interactions, attention should be paid to gene–gene interactions. However, the large number of study subjects required and subsequent costs involved may not make such studies feasible. Two alternatives to examine these complex interactions in human subjects are possible: one possibility would be to select study participants based on their genetic variants; the second possibility would be to make use of the large cohort studies for which dietary information has been collected. The latter approach would take the concept of gene–diet interactions beyond the metabolic unit into the real world.

Although animal experiments have not been reviewed here, the use of animal models will play a crucial role in

mapping new genes involved in dietary responsiveness and atherogenesis. Furthermore, the genetic heritability of dietary responsiveness has not been carefully studied in human subjects (Tall *et al.* 1997). Thus, future studies will need to include siblings and families, with the dual purpose of getting a more accurate measure of heritability and the performance of wide genome scans to search for new responsiveness loci.

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