Proceedings of the Nutrition Society (2002), **61**, 427–434 DOI:10.1079/PNS2002177

The Winter Meeting of the Nutrition Society held jointly with the Association Française de Nutrition and the Société de Nutrition et de Diététique de Langue Française was held at the Institute of Child Health, London on 13–14 February 2002

Symposium on 'Nutrition in the post-genomic era'
Plenary session 3: Genetic variation and response to food

Genetic polymorphisms and lipoprotein responses to diets

Stephanie Vincent[1], Richard Planells[1], Catherine Defoort[1], Marie-Christine Bernard[2], Mariette Gerber[3], Joanne Prudhomme[4], Philippe Vague[2], and Denis Lairon[1]*

[1]*Unité 476-Human Nutrition and Lipids at INSERM (National Institute of Health and Medical Research), 18 Avenue Mozart, 13009 Marseille, France*

[2]*CDPA, CHU Timone, Marseille, France*

[3]*CRLC Val d'Aurelle, Montpellier, France*

[4]*ORS, Marseille, France*

While human diets have markedly evolved since their origin, the human genome has only marginally changed. Nevertheless, polymorphisms of common genes are widespread. It has been substantiated that most major diseases (including cardiovascular disease, diabetes, obesity and cancers) result from the interaction between genetic susceptibility and environmental factors, including diet. In the field of lipoprotein metabolism and cardiovascular disease several gene polymorphisms for key proteins, such as apoproteins (apo) E, B, A-IV and C-III, LDL receptor, microsomal transfer protein (MTP), fatty acid-binding protein (FABP), cholesteryl ester transfer protein (CETP), lipoprotein lipase and hepatic lipase, have been identified and linked to variable responses to diets. We are carrying out an intervention study (RIVAGE) in Marseille dedicated to investigating the interactions between diets (Mediterranean or low-fat types *v*. standard Western type), risk factors for cardiovascular disease and gene polymorphisms in about 300 patients randomized into two groups over periods of 3 and 12 months. Some data obtained in about 100 patients after 3 months of dietary change are available. Among single nucleotide polymorphisms (SNP) already studied (apoE (ε2, ε3, ε4), apoB (–516C/T), apoC-III (SstI), apoA-IV (Ser347Thr), MTP (–493G/T), intestinal FABP (Ala54Thr), CETP (TaqIB) and hepatic lipase (–480C/T)), some SNP showed interactions with diets in relation to changes in particular variables after 3 months on the dietary regimens. This was the case for apoE and LDL-cholesterol and triacylglycerols, apoA-IV and LDL-cholesterol, MTP and LDL-cholesterol, intestinal FABP and triacylglycerols. These data provide evidence of the interaction between some SNP and the metabolic response to diets.

Résumé

Alors que l'alimentation de l'homme a beaucoup évoluée depuis ses origines, le génome humain est resté très stable. Pourtant, de très nombreux gènes ont des polymorphismes connus. En fait, on considère maintenant que les principales pathologies humaines (maladies cardiovasculaires, diabète, obésité et cancers) résultent d'une interaction entre des facteurs de succeptibilité génétique et des facteurs de l'environement, dont l'alimentation. Dans le domaine du métabolisme des lipoprotéines et des maladies cardiovasculaires, des polymorphimes de plusieurs gènes ont été identifiés et associés aux niveaux des paramètres lipidiques ou à des réponses variables aux régimes, comme pour les apoprotéines (apo) E, B, A-IV et C-III, le LDL récepteur, la protéine microsomiale de transport (MTP), la protéine de liason des acides gras (FABP), la protéine de transport des esters de cholésterol (CETP), la lipoprotéine lipase ou la lipase hepatique. Nous réalisons une étude d'intervention à Marseille dans le but d'étudier l'interaction

Abbreviations: apo, Apolipoproteins; % E, % energy; FABP, fatty acid-binding protein; LFLC, low-fat low-carbohydrate; LPL, lipoprotein lipase; MED, Mediterranean-type; TRL, triacylglycerol-rich lipoprotein; SNP, single nucleotide polymorphism.

***Corresponding author:** Denis Lairon, fax +33 4 91 75 15 62, email lairon@marseille.inserm.fr

entre des types d'alimentation (méditerranéenne ou pauvre en lipides et cholestérol *v.* alimentation usuelle), des facteurs de risque des maladies cardiovasculaires et des polymorphismes génétiques, chez environ 300 patients répartis au hazard dans les deux groupes et suivis pendant 3 et 12 mois. Des résultats obtenus chez envion 110 patients après 3 mois de régime sont disponibles. Parmi les SNP déjà étudiés (apoE (ε2, ε3, ε4), apoB (–516C/T), apoC-III (SstI), apoA-IV (Ser347Thr), MTP (–493G/T), FABP intestinale (Ala54Thr), CETP (TaqIB) et la lipase hépatique (–480C/T)), certains d'entre eux montrent des interactions avec les effets des régimes: c'est le cas pour apoE et le LDL-cholestérol ou les triacylglycérols, apoA-IV et le LDL-cholestérol, MTP et le LDL-cholestérol, FABP intestinale et les triacylglycerols. Ces données mettent en evidence l'interaction entre certains polymorphismes génétiques et les réponses métaboliques aux régimes.

Human intervention study: Mediterranean diet: Gene polymorphisms: Cardiovascular risk

Gene–diet interaction: from species traits to individual susceptibility

When looking at man, it is striking to observe a huge diversity in dietary habits as well as genetic traits. There is a vast record of the various diets used by populations worldwide. The hunter–gatherer way of life sustained humanity for all but the last 10 000 years of a period of about $2{\cdot}4 \times 10^6$ years. In this earlier period, given the predominance of either an animal or plant food supply, nutrient intakes varied markedly (% energy (% E); protein 19–35, carbohydrate 22–40, fat 28–58; Cordain *et al*. 2000). Other data from the paleolithic-stone age (35 000–10 000 BC) indicate that fat intake was low (21–22 % E) and intakes of carbohydrate (41–45 % E) and protein (34–37 % E) were high. For modern-age agriculture (10 000 BC to third millennium) worldwide values were 10–17 % E for protein, 40–60 % E for carbohydrate and 20–50 % E for fat. During the last century, in most industrialized countries more fat (40–50 % E), especially saturated fat, and less carbohydrate (40–45 % E), especially starches, were consumed. At the population level, there has been a very important and rapid change in these countries.

Since the time when the *Homo* genus appeared on earth (about $2{\cdot}4 \times 10^6$ years), only minor changes have occurred in the human genome. Indeed, a difference of only about 1·6 % was found between modern man and most developed primates. Despite this overall stability of the human genome, numerous minor alterations in gene structures have been found. For instance, ethnic differences for various traits have long been observed. Such differences have been reported recently for the risk for cardiovascular disease or type 2 diabetes in African or white American children (Lindquist *et al*. 2000). At the subject level, numerous single gene polymorphisms have been described and studied in the last decade. For example, in the field of lipid and lipoprotein metabolism, more than 250 single nucleotide polymorphisms (SNP) have been identified in about fifteen genes encoding for key proteins involved (Ye & Kwiterowitch, 2000; Ordovas, 2001).

From the enormous amount of data published in the literature during the last few decades, the following statements can be made regarding key metabolic effects of major nutrients:

plant or animal proteins can have distinct effects on cholesterolaemia;
amount or nature of carbohydrate elicit different responses (glycaemic and/or insulinic indexes, hypertriacylglycerolaemia;
dietary fibres, especially soluble fibres, can lower glycaemic and/or insulinic indexes or cholesterolaemia and LDL-cholesterol;
saturated fat can increase cholesterolaemia and LDL-cholesterol;
dietary cholesterol can increase cholesterolaemia and LDL-cholesterol:HDL-cholesterol;
some minerals or vitamins can have protective effects on cardiovascular risk factors;
antioxidant-rich plant-derived foods display protection against detrimental effects of oxidation.

Nevertheless, most studies have clearly highlighted the very large inter-individual variability in metabolic responses. Generally, in intervention studies $\geq$ 50 % of the subjects show changes in the main direction, a minority of the subjects do not exhibit any marked change and a few subjects show changes in the opposite direction. This observation clearly indicates that each subject can display both general traits and some particular susceptibility to a given environmental (dietary) factor. This variation is the basis of the concept of gene–diet interaction.

The new tools recently developed allow easy determination of most gene polymorphisms from a blood sample, especially SNP. This advance opens a new era where studies dedicated to interactions between diets, metabolic variables, disease risk factors and gene polymorphisms can be carried out on small or large groups of healthy subjects or patients.

In the present paper, we will first review available literature data relating to some selected key proteins involved in lipid and lipoprotein metabolism. Second we will give some preliminary data obtained during an ongoing intervention study performed in Marseille.

Single nucleotide polymorphisms and lipoprotein metabolism

The main pathways involved in lipid and lipoprotein metabolism in man are summarised in Fig. 1. Briefly, two

https://doi.org/10.1079/PNS2002177 Published online by Cambridge University Press

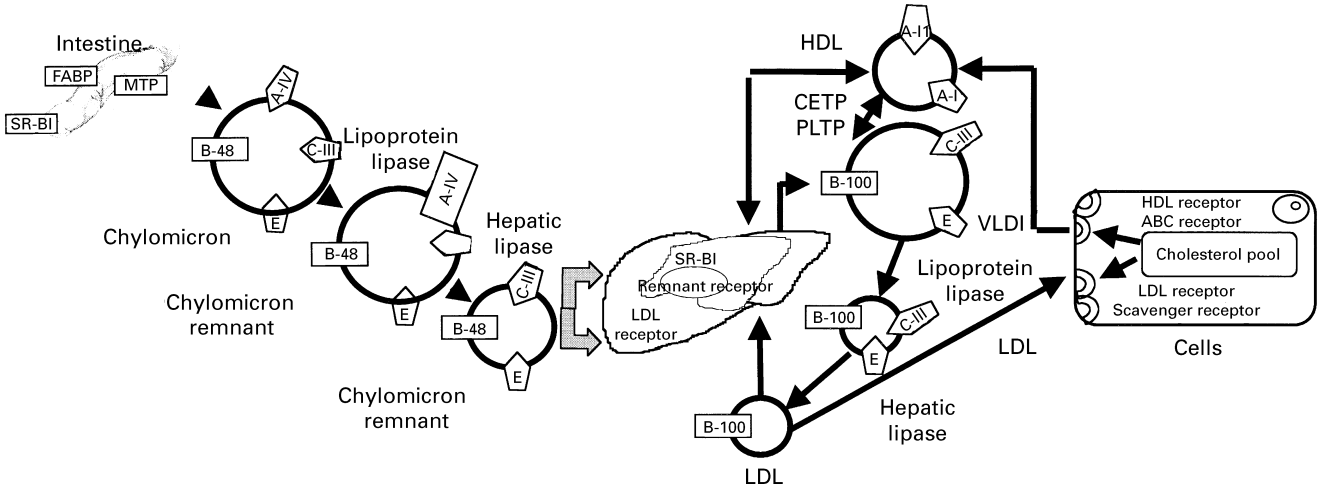

Fig. 1. Schematic representation of lipoprotein metabolism in man, with emphasis on endogenous and exogenous pathways. FABP, fatty acid-binding protein; MTP, microsomal transfer protein; SR, scavenger receptor; A-II, A-IV, B-48, B-100, C-III, E, apoproteins; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; IDL, intermediate-density lipoprotein; ABC, ATP-binding cassette protein. (Adapted from Ye & Kwiterowitch, 2000.)

main streams are involved in the intravascular transport of lipids in the form of lipid–apoprotein (apo) complexes termed lipoproteins (Ye & Kwiterowitch, 2000).

The endogenous pathway control variables measured at fasting are as follows: the liver produces and secretes VLDL bearing apoB-100, apoE, apoC-II and apoC-III; triacylglycerols then phospholipids are hydrolysed by endovascular lipoprotein lipase (LPL) and hepatic lipase to large remnants (intermediate-density lipoproteins) and then small remnants (LDL) enriched with cholesterol that accumulate in the circulation.

After digestion and absorption in the gut of dietary fat provided by meals, large triacylglycerol-rich chylomicrons that contain apoB-48, apoA-I, apoA-IV, apoE, apoC-II and apoC-III are secreted by the small intestine into the bloodstream. The result is transient accumulation of chylomicrons postprandially; hydrolysis of triacylglycerols then phospholipids by LPL and hepatic lipase generate smaller chylomicron remnants. The two kinds of endogenous and exogenous remnants are cleared from the circulation by means of receptor-driven mechanisms, i.e. liver apoB,E receptor and remnant receptor or peripheral tissue apoB,E receptor and scavenger receptor (SR).

HDL bearing apoA-I and apoA-II can exchange lipids with VLDL and chylomicrons through the transfer activity of cholesteryl ester transfer protein or phospholipid transfer protein and ensure the reverse cholesterol transport to the liver through scavenger receptor BI. The intestinal absorption of cholesterol is probably controlled by membrane transporters such as scavenger receptor BI or ATP-binding cassette protein. The production of VLDL or chylomicrons intracellularly is controlled by the action of specific proteins such as fatty acid-binding proteins (FABP) and the microsomal transfer protein (MTP).

Two major genetic defects are known to dramatically affect lipoprotein metabolism. The most well-known defect is that in the apoB,E receptor, which induces familial hypercholesterolaemia and elevated LDL levels (Brown & Goldstein, 1974). Second after this defect is that in MTP, which almost completely abolishes triacylglycerol-rich lipoprotein (TRL) production by the liver and small intestine resulting in abetalipoproteinaemia (Gregg & Wetterau, 1994). Such marked genetic defects are not within the scope of the present review, where some examples of common gene polymorphisms leading to more specific variations will be presented. The reader can refer to reviews published recently on this topic (Ye & Kwiterowitch, 2000; Ordovas, 2001).

Apoprotein E and lipoprotein metabolism

ApoE is a key apo involved in TRL (intermediate-density lipoprotein and chylomicron remnants) uptake by the liver and peripheral tissues through interaction with apoB,E and remnant receptors.

Three common polymorphisms exist through a C→T transition leading to variations in the amino acids (Cys→Arg) at 112 and 158 positions (Sing & Davignon, 1985). This transition results in three major alleles (ε2, ∈ε, ε4) with relative frequencies of 0·1, 0·8 and 0·15 respectively, with three homozygous genotypes (ε2/ε2, ε3/ε3 and ε4/ε4) and with three heterozygous genotypes (ε2/ε3, ε2/ε4 and ε3/ε4). The apoE-ε2 gene product binds poorly to the remnant receptor, leading to accumulation of TRL in the circulation. Conversely, the apoE–4 gene product has high affinity and increases TRL uptake by the liver, thus inducing a down-regulation of the apoB,E receptor level, reduced LDL uptake and increased LDL accumulation (Sing & Davignon, 1985). It is noteworthy that such apoE polymorphisms account for about 8–10 % of the total plasma cholesterol variation (Schaefer *et al.* 1994) and that only a few subjects with apoE-ε2/ε2 display frank type III hyperlipidaemia.

Several studies have investigated the relationship between apoE polymorphisms and responses to diets. Healthy men ingesting a low-fat diet *v.* their usual high-fat high-cholesterol diet showed extents of reduction in LDL-

https://doi.org/10.1079/PNS2002177 Published online by Cambridge University Press

cholesterol in the following order: ε4/ε4 = ε4/ε3 > ε3/ε3 > ε3/ε2 (Dreon *et al*. 1995). In a cohort of men and women with CHD it was demonstrated only in patients with the apoE-ε2 allele that a high sucrose intake is associated with high plasma triacylglycerol concentration and that saturated fat or fibre intake predicts serum cholesterol levels (Erkkilä *et al*. 2001). The response to a fat test meal was investigated in normolipidaemic patients with type 2 diabetes by Reznik *et al*. (1996); accumulation of TRL remnants (retinyl palmitate) postprandially was exacerbated in patients with the ε2/ε3 or ε3/ε4 genotype. Finally, the response to drinking alcohol was evaluated in a cohort of healthy men and women (Corella *et al*. 2001); men and women with the apoE-ε2 allele had lower LDL-cholesterol levels, while men with the apoE-ε4 allele had highest LDL-cholesterol levels. We investigated the influence of the combination of apoE-ε2 and a rare apoE-ε3 « Christchurch » mutant 136Arg→Ser in a few related patients (Vialettes *et al*. 2000); this genotype was associated with type V hyperlipoproteinaemia and exacerbated postprandial lipaemia and TRL-remnant accumulation.

Taken together, information available on apoE polymorphisms clearly indicates that apoE-ε2 and -ε4 variants are associated with disturbed lipid metabolism.

Apoprotein B and lipoprotein metabolism

ApoB is the main apo associated with VLDL and LDL (apoB-100) and chylomicrons (apoB-48). It is essential for lipoprotein assembly, and apoB-100 serves as the principal ligand ensuring interaction between LDL and apoB,E receptor.

Several common mutations have been found in the human apoB gene, e.g. insertion or deletion at the signal peptide region (three genotypes), exon 26 (3611Arg→Gln or without sequence change), exon 29 (4154Glu→Lys) and exon 4 (Thr→Ileu), with allele frequencies ranging from 0·11 to 0·46. The functional consequences of such mutations are either an apoB secretion defect or truncated forms with a binding defect to the receptor, inducing opposite clinical traits such as hypobetalipoproteinaemia (secretion defect) or hypercholesterolaemia and LDL-cholesterol (binding and clearance defect).

Several studies have investigated the influence of some apoB polymorphisms on responses to changes from high-fat to low-fat diets (or vice versa), and these studies have been the subject of a meta-analysis published recently (Rantala *et al*. 2000). The two polymorphisms mostly associated with diet-induced changes in LDL-cholesterol are X-/X- at exon 26 (XbaI site) and R-/R- at exon 29 (EcoRI site). Other polymorphisms showed weaker interaction. Lopez-Miranda *et al*. (1997) reported that subjects with the X-/X- genotype at the apoB locus have a greater postprandial response (retinyl palmitate and apoB-48 levels) to a fat meal than the subjects with the other allele.

Apoprotein A-IV and lipoprotein metabolism

ApoA-IV is an apo secreted by the small intestine and specifically associated with chylomicrons and chylomicron remnants. Among possible roles are interactions between chylomicrons and HDL particles.

A common SNP has been identified on the apoA-IV gene as a G→T substitution resulting in a 360His→Glu substitution. The absence or presence of the restriction site (Fnu4HI) gives apoA-IV-1 or apoA-IV-2 variants with three genotypes (1/1, 1/2 and 2/2). The frequency of apoA-IV-2 form in the USA and France is 7–9 % (Weinberg, 1999). The apoA-IV-2 form has increased affinity for lipid particles and displays more competition towards apoE and apoC-II binding (Hockey *et al*. 2001). Higher fasting triacylglycerols and lower HDL-cholesterol have been reported in homozygous women.

Several recent studies have reported an interaction between apoA-IV genotypes and metabolic responses. The postprandial response to a fat meal has been shown to be higher in apoA-IV-1/2 carriers compared with apoA-IV-1/1 carriers for plasma, chylomicron and VLDL-triacylglycerols (Hockey *et al*. 2001). This finding is in line with other data showing that apoA-IV-1/2 carriers have lower intestinal cholesterol absorption rates than apoA-IV-1/1 carriers (Weinberg *et al*. 2000).

The effects of this polymorphism on LDL-cholesterol change in response to high-fat *v*. low-fat diets have been recently investigated (Weggemans *et al*. 2000). It appears that carriers of the apoA-IV-2 allele have a lower increase or lower decrease in LDL-cholesterol level in response to a high-fat high-cholesterol diet or a low-fat low-cholesterol diet respectively. However, some studies have been unable to show different responses.

Intestinal fatty acid-binding protein and lipoprotein metabolism

Intestinal FABP (FABP-2) is a cytosolic intracellular protein capable of binding non-esterified fatty acids and other lipid moieties and delivering them to the membrane. A common SNP in the intestinal FABP gene has been found at exon 2, codon 54, with a G→A substitution resulting in a 54Ala→Thr substitution (Baïer *et al*. 1995). The frequency of the 54Thr variant has been evaluated as 0·29 in Pima Indians and 0·27 in European students. This mutation gives three genotypes. The functional changes resulting from the mutation are higher fatty acid binding and transport and triacylglycerol secretion from intestinal cells with the 54Thr variant. The clinical traits associated with the 54Thr mutation are higher insulinaemia and insulin resistance, higher fasting LDL-cholesterol and apoB levels, and higher BMI and fasting triacylglycerols (Baïer *et al*. 1995; Agren *et al*. 1998; Hegele, 1998). The LDL-cholesterol and apoB-lowering effects of high-soluble-fibre diets were more pronounced in subjects with the 54Thr intestinal FABP variant (Hegele *et al*. 1997).

Several studies have dealt with postprandial responses. The 54Thr homozygotes showed increased postprandial insulin (Baïer *et al*. 1995) or triacylglycerol responses (Agren *et al*. 1998), but no marked difference was observed for glucose, non-esterified fatty acids or triacylglycerols in another study (Pratley *et al*. 2000).

Lipoprotein lipase and lipoprotein metabolism

LPL is the key enzyme responsible for TRL-triacylglycerol lipolysis within blood vessels; from VLDL or chylomicrons it generates TRL remnants that can be taken up by the liver or peripheral tissues and thus cleared from the circulation.

Among more than fifty known polymorphisms, several common ones have been found in the LPL gene, e.g. in exon 6 (291Asn→Ser) with a 4–6 % frequency in Western populations, or in intron 8 (HindIII). LPL activity can be reduced (291Asn→Ser), and these polymorphisms are associated with higher fasting triacylglycerols and lower HDL-cholesterol levels (Gerdes *et al*. 1997; Senti *et al*. 2000). In heterozygous carriers of the 291Asn→Ser mutation, postprandial levels of large VLDL apoB–48, triacylglycerols and retinyl palmitate were higher than those in non-carriers (Mero *et al*. 1999). Regarding the effects of chronic diets, Humphries *et al*. (1996) reported that the carriers of the HindIII polymorphism, in response to a poly-unsaturated fatty acid diet, showed an increased extent of plasma cholesterol and triacylglycerol reduction.

Miscellaneous

The reader should refer to recent literature reviews on the effects of other gene polymorphisms studied such as those encoding for apoA-I, apoC-III, cholesteryl ester transfer protein, hepatic lipase or scavenger receptor BI (Ye & Kwiterowitch, 2000; Ordovas, 2001).

To summarize, most studied polymorphisms of genes encoding for key proteins involved in lipid and lipoprotein metabolism have been associated with some documented effects on fasting and/or postprandial metabolic markers in man. This finding consistently supports the concept that interaction between genetic succeptibility and inappropriate diet is a key determinant of cardiovascular disease risk.

Marseille RIVAGE study

This ongoing study is being conducted in Marseille through the combined efforts of the Center for Diagnosis and Prevention of Cardiovascular Disease at Timone University Hospital, the Inserm Research Unit for Human Nutrition and Lipids, several hospital analytical departments and epidemiologists at Inserm (Montpellier) and Regional Health Observatory.

Aim

The aim of the RIVAGE project is to instigate a primary prevention action concerning cardiovascular risk factors, in the form of a dietary intervention follow-up:

to evaluate the effects of a Mediterranean-type (MED) diet;
to compare this diet with the usually-prescribed low-fat low-cholesterol (LFLC) diet as recommended by the American Heart Association or the European Arteriosclerosis Society;
to determine influences of gene polymorphisms encoding for key proteins involved in lipoprotein metabolism or thrombosis.

Patients

About 250–300 patients attending the Center for Diagnosis and Prevention of Cardiovascular Disease, who volunteer for the project and have one or more cardiovascular disease risk factors are to be included. Inclusion criteria are fasting cholesterolaemia (2·5–3·0 g/l), triacylglycerolaemia (1·8–4·0 g/l) or glycaemia (1·1–1·25 g/l), BMI $\geq$ 27 kg/m^2, hypertension (systolic blood pressure 140–180 mmHg; diastolic blood pressure 90–105 mmHg), sedentary lifestyle, smoking, family history of a cardiovascular event, not currently taking medication (diabetes, dyslipoproteinaemia, hypertension).

Diets

The diets to be prescribed for 12 months to the two study groups are either the LFLC diet or an MED diet. The composition of the LFLC is (% E): protein 10–15, carbohydrate 55–60, fat 30 (saturated–monounsaturated–polyunsaturated fat (1:1:1, by wt)), cholesterol $\leq$ 300 mg/d, total fibre 20 g/d, vitamins and minerals (French recommanded dietary allowances). The composition of the prescribed MED diet is (% E): protein 10–15, carbohydrate 50–55, fat $\leq$ 37 (saturated–monounsaturated–poly-unsaturated fat (1:2:1, by wt), with emphasis on *n*-3 fatty acids (fish)), cholesterol $\leq$ 300 mg/d, total fibre (with emphasis on soluble fibres (whole grains, legumes)) 25–30 g/d, vitamin C (fruit and vegetables) 100 mg/d, vitamin E 5–10 mg/d, other vitamins and minerals (French recommended dietary allowances), carotenoids (fruit and vegetables) 7 mg/d and as much polyphenols as possible (Martin, 2001).

The compliance to the recommended diets is being achieved through booklets and the menus provided, interviews during visits and phone calls. Compliance is being checked by 3 d dietary records, assays of blood fatty acids and carotenoids.

Biochemical variables

The fasting variables measured at entry or after dietary intervention for 3 and 12 months are: glycaemia, insulinaemia; triacylglycerols, TRL-triacylglycerols; cholesterol, LDL-, HDL- and TRL-cholesterol; apoB, apoA-I, apoC-III and TRL-apoC-III; homocysteine, polyphenols, fat-soluble vitamins and carotenoids; thrombosis factors (fibrinogen, tissue plasminogen activator, plasminogen activator inhibitor 1, factor VII).

Some variables are also measured 2·5 and 5 h post-prandially after intake of a standardized test-meal at 08.00 hours (breakfast with 40 g fat): triacylglycerols, TRL-triacylglycerols; apoB-48; retinyl palmitate.

Gene polymorphisms

Selection of gene polymorphisms was done according to literature data and on the basis that mutant allele frequency is at least 10 %, with few exceptions.

The selected gene polymorphisms, determined using common restriction fragment length polymorphism methods

https://doi.org/10.1079/PNS2002177 Published online by Cambridge University Press

(as published), are: apoE, 112/158Cys→Arg (Hixson & Vernier, 1990); apoB, –516C→T (Van't Hooft *et al*. 1999); apoC-III, 3175C→G (Salas *et al*. 1998); apoA-IV, Ser347,A→T (Ostos *et al*. 1998); intestinal FABP, Ala54→Thr (Baïer *et al*. 1995); MTP, –493G→T (Karpe *et al*. 1998); cholesteryl ester transfer protein, TaqI B1:B2 (Fumeron *et al*. 1995); hepatic lipase, –480C→T (Jansen *et al*. 1999); LPL, 291Asn→Ser (Gerdes *et al*. 1997).

Other SNP determinations are under development (apoA-V, sterol response element-binding protein 1a, 7α-hydroxylase, apoB,E receptor, β3-receptor, plasminogen activator inhibitor 1, factor VII, etc.)

Marseille RIVAGE study: available data

Currently, 117 patients have been fully investigated after 3-month dietary interventions, with sixty-four patients on the MED diet and fifty-three patients on the LFLC diet. Mean ages and sexes were not different between groups. Compliance was very good, with only a few patients showing bad compliance or dropping out (excluded).

Lipid and lipoprotein variables. Fasting cholesterolaemia decreased after the 3-month dietary intervention in both groups, but with a more marked amplitude in the MED group (–0·25 g/l *v*. –0·09 g/l for the LFLC group). Fasting LDL-cholesterol showed the same pattern with a more marked reduction in the MED group (–0·12 g/l) than in the LFLC group (–0·04 g/l). Fasting HDL-cholesterol showed a slight increase in the MED group (+ 0·01 g/l) and a slight decrease in the LFLC group (–0·08 g/l).

Fasting triacylglycerols were more reduced after the 3-month dietary intervention in the MED group (–0·14 g/l) than in the LFLC group (–0·09 g/l). Postprandial triacylglycerol 0–5 h area under the curve was reduced after the dietary intervention, but more markedly (4·3-fold) in the MED group than in the LFLC group. Postprandial apoB-48 0–5 h area under the curve (a specific marker for chylomicron and remnant accumulation) was reduced in both cases, but to a greater extent (2-fold) in the MED group than in the LFLC group.

Changes in lipoprotein variables and gene polymorphisms. We analysed the relative changes in lipoprotein variables after the 3-month dietary intervention (all subjects pooled or grouped according to diet) with regard to alleles of the gene locus of interest.

With all patients pooled, we did not observe any marked effect of apoE-ε2 or -ε4 alleles compared with major apoE-ε3 allele on the relative decrease in cholesterolaemia. Conversely, ε4 allele carriers showed a much more marked LDL-cholesterol reduction (about 3-fold) than ε2 or ε3 carriers, while ε2 allele carriers exhibited a much more marked reduction in fasting triacylglycerols (about 4-fold). A tentative conclusion could be that patients with an apoE-ε2 or -ε4 allele, i.e. 39 % of included patients, exhibit better lowering of triacylglycerols or LDL-cholesterol under the dietary intervention. This finding is in line with results of some published studies (Dreon *et al*. 1995; Reznic *et al*. 1996; Corella *et al*. 2001).

With all patients pooled we observed that the relative reduction in LDL-cholesterol was about 4-fold higher in carriers of apoA-IV T allele than in the others. Interestingly, a more marked extent of LDL-cholesterol reduction was observed in patients with the T allele in the MED group than in the same carriers in the LFLC group. From these data it can be suggested that patients with the apoA-IV T allele, i.e. 37 % of the patients included, showed a better reduction in LDL-cholesterol under dietary intervention, especially with the MED diet.

With all patients pooled, we found that the lowering of LDL-cholesterol with the intervention diets was about 3-fold greater in patients homozygous for the MTP T allele. Moreover, the reduction in triacylglycerolaemia was more marked in these patients. Thus, patients with the MTP T/T alleles, i.e. 11 % of the patients included, exhibited better lowering of LDL-cholesterol and triacylglycerolaemia with dietary intervention.

With all patients pooled, we observed that the relative reduction in triacylglycerolaemia was about 5-fold greater in heterozygous and homozygous carriers of the intestinal FABP A substitution than in intestinal FABP G carriers. Less-marked differences were observed for postprandial triacylglycerol responses. This finding suggests that in patients with the intestinal FABP 54Thr allele, i.e. 52 % of the patients included, a much better lowering of triacylglycerolaemia is achieved with dietary intervention.

RIVAGE study: preliminary conclusion. The data available currently indicate that the two dietary interventions do improve some lipid and lipoprotein variables, with overall more marked effects with the MED diet than with the usual LFLC diet. Moreover, we found that several common polymorphisms of genes encoding for key proteins involved in lipid and lipoprotein metabolism are associated to different extents of change in variables such as fasting cholesterolaemia and LDL-cholesterol, fasting triacylglycerolaemia and postprandial lipaemia.

The study is proceding in order to achieve inclusion of at least 250 patients, to examine relationships between about fifteen different SNP and responses to diets, to evaluate possible specific relationships between SNP and a particular diet, and to determine the effects of SNP combinations.

Conclusion

In conclusion, gene–diet interaction is a key emerging field for research which is expected to provide better knowledge of the relative importance of genetic traits and dietary habits in relation to disease risks for most people.

Given the broad scope and complexity of this subject, efforts are needed to identify the most relevant gene polymorphisms influencing metabolic variables and risk factors. The expected consequences of such new knowledge are the ability to improve dietary recommendations by taking into account known variability within a given population, by targeting specific recommendations to particular groups at risk, and even to provide dietary advice (or drug therapy) to individual subjects on the basis of identified genetic susceptibility.

Acknowledgements

The Marseille RIVAGE study is supported by Marseille Public Hospital, Inserm, Ministry of Research (agro-food sector), Provence Regional Council, Bouches-du-Rhone Council and agro-food companies.

References

Agren JJ, Valve R, Vidgren H, Laakso M & Uusitupa M (1998) Postprandial lipemic response is modified by the polymorphism at codon 54 of the fatty acid-binding protein 2 gene. *Arteriosclerosis, Thrombosis and Vascular Biology* **18**, 1606–1610.

Baïer LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, Mochizuki H, Bennett PH, Bogardus C & Prochazka M (1995) An amino acid substitution in the human intestinal fatty acid-binding protein is associated with increased fatty acid binding, increased fat oxidation and insulin resistance. *Journal of Clinical Investigation* **95**, 1281–1287.

Brown MS & Goldstein JL (1974) Familial hypercholesterolemia: defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy–3-methylglutaryl coenzyme-A reductase-activity. *Proceedings of the National Academy of Sciences USA* **71**, 788–792.

Cordain L, Miller JB, Eaton SB, Mann N, Holt SHA & Speth JD (2000) Plant–animal subsistence ratios and macronutrient energy estimations in worldwide hunter–gatherer diets. *American Journal of Clinical Nutrition* **71**, 682–692.

Corella D, Tucker K, Lahoz C, Coltell O, Cupples LA, Wilson PWF, Schaefer EJ & Ordovas JM (2001) Alcohol drinking determines the effect of the APOE locus on LDL-cholesterol concentrations in men: the Framingham Offspring study 1–3. *American Journal of Clinical Nutrition* **73**, 736–745.

Dreon DM, Femstrom HA, Miller B & Krauss RM (1995) Apolipoprotein E isoform phenotype and LDL subclass response to a reduced fat diet. *Arteriosclerosis, Thrombosis and Vascular Biology* **15**, 105–111.

Erkkilä AT, Sarkkinen ES, Lindi V, Lehto S, Laakso M & Uusitupa MIJ (2001) APOE polymorphism and the hypertriglyceridemic effect of dietary sucrose. *American Journal of Clinical Nutrition* **73**, 746–752.

Fumeron F, Betoulle D, Luc G, Behague I, Ricard B, Poirier O, Jemaa R, Evans A, Arveiler, D, Marques-Vidal P, Bard JM, Fruchart JC, Ducimetière P, Apfelbaum M & Cambien F (1995) Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. *Journal of Clinical Investigation* **96**, 1664–1671.

Gerdes C, Fisher RM, Nicaud V, Boer J, Humphries SE, Talmud PJ & Faergeman O (1997) Lipoprotein lipase variants D9N and 291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations. Study in the fasting and postprandial states; the European atherosclerosis research studies. *Circulation* **96**, 733–740.

Gregg RE & Wetterau JR (1994) The molecular basis of abetalipoproteinemia. *Current Opinion in Lipidology* **5**, 81–86.

Hegele RA (1998) A review of intestinal fatty acid binding protein gene variation and the plasma lipoprotein response to dietary components. *Clinical Biochemistry* **31**, 609–612.

Hegele RA, Xolever TM, Story JA, Connelly PW & Jenkins DJ (1997) Intestinal fatty acid-binding protein variation is associated with variation in the response of plasma lipoproteins to dietary fibre. *European Journal of Clinical Investigation* **27**, 857–862.

Hixson JE & Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *Journal of Lipid Research* **31**, 545–548.

Hockey KJ, Anderson RA, Cook VR, Hantgan RR & Weinberg RB (2001) Effect of the apolipoprotein A-IV Q360H polymorphism on postprandial plasma triglyceride clearance. *Journal of Lipid Research* **42**, 211–217.

Humphries SE, Talmud PJ, Cox C, Sutherland W & Mann J (1996) Genetic factors affecting the consistency and magnitude of changes in plasma cholesterol in response to dietary challenge. *Quarterly Journal of Medicine* **89**, 671–680.

Jansen H, Chu G, Ehnholm C, Dallongeville J, Nicaud V & Talmud PJ (1999) The T allele of the hepatic lipase promoter variant C-480T is associated with increased fasting lipids and HDL and increased preprandial and postprandial LpCIII:B: European Atherosclerosis Research Study (EARS) II. *Arteriosclerosis, Thrombosis and Vascular Biology* **19**, 303–308.

Karpe F, Lundahl B, Ehrenborg E, Eriksson P & Hamsten A (1998) A common functional polymorphism in the promoter region of the microsomal triglyceride transfer protein gene influences plasma LDL levels. *Arteriosclerosis, Thrombosis and Vascular Biology* **18**, 756–761.

Lindquist CH, Gower BA & Goran MI (2000) Role of dietary factors in ethnic differences in early risk of cardiovascular disease and type 2 diabetes. *American Journal of Clinical Nutrition* **71**, 725–732.

Lopez-Miranda J, Ordovas JM, Ostos MA, Marin C, Jansen S, Salas J, Blanco-Molina A, Jimenez-Pereperez JA, Lopez-Segura F & Perez-Jimenez F (1997) Dietary fat clearance in normal subjects is modulated by genetic variation at the apolipoprotein B gene locus. *Arteriosclerosis, Thrombosis and Vascular Biology* **17**, 1765–1773.

Martin A (2001) Apports Nutritionnels Recommandés à la Population Française. Paris: Lavoisier Tec et Doc.

Mero N, Suurinkeroinen L, Syvänne M, Knudsen P, Yki-Järvinnen H & Taskinen MR (1999) Delayed clearance of postprandial large TG-rich particles in normolipidemic carriers of LPL Asn 291Ser gene variant. *Journal of Lipid Research* **40**, 1663–1670.

Ordovas JM (2001) Genetics, postprandial lipemia and obesity. *Nutrition Metabolism and Cardiovascular Disease* **11**, 118–133.

Ostos MA, Lopez-Miranda J, Ordovas JM, Marin C, Blanco A, Castro P, Lopez-Segura F, Jimenez-Pereperez J & Perez-Jimenez F (1998) Dietary fat clearance is modulated by genetic variation in apolipoprotein A-IV gene locus. *Journal of Lipid Research* **39**, 2493–2500.

Pratley RE, Baier L, Pan DA, Salbe AD, Storlien L, Ravussin E & Bogardus C (2000) Effects of an Ala54Thr polymorphism in the intestinal fatty acid-binding protein on responses to dietary fat in humans. *Journal of Lipid Research* **41**, 2002–2008.

Rantala M, Rantala TT, Savolainen MJ, Friedlander Y & Kesäniemi YA (2000) Apolipoprotein B gene polymorphisms and serum lipids: meta-analysis of the role of genetic variation in responsiveness to diet. *American Journal of Clinical Nutrition* **71**, 713–724.

Reznik Y, Pousse P, Herrou M, Morello R, Mahoudean J, Drosolowsky MA & Fradiu S (1996) Postprandial lipoprotein metabolism in normotriglyceridemic non-insulin-dependent diabetic patients: influence if apolipoprotein E polymorphism. *Metabolism: Clinical and Experimental* **45**, 63–71.

Salas J, Jansen S, Lopez-Miranda J, Ordovas JM, Castro P, Marin C, Ostos MA, Bravo MD, Jimenez-Pereperez J, Blanco A, Lopez-Segura F & Perez-Jimenez F (1998) The SstI polymorphism of the apolipoprotein C-III gene determines the insulin response to an oral-glucose-tolerance test after consumption of a diet rich in saturated fats. *American Journal of Clinical Nutrition* **68**, 396–401.

Schaefer EJ, Lamon-Fava S, Johnson S, Ordovas JM, Schaefer MM, Castelli WP & Wilson PWF (1994) Effects of gender and menopausal status on the association of apolipoprotein E

phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. *Arteriosclerosis and Thrombosis* **14**, 1105–1113.

Senti M, Bosch M, Aubo C, Elosua R & Marrugat J (2000) Relationship of abdominal adiposity and dyslipemic status in women with a common mutation in the lipoprotein lipase gene. The REGICOR investigators. *Atherosclerosis* **150**, 135–141.

Sing CF & Davignon J (1985) Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *American Journal of Human Genetics* **37**, 268–285.

Van't Hooft FM, Jormsjo S, Lundahl B, Tornvall P, Eriksson P & Hamsten A (1999) A functional polymorphism in the apolipoprotein B promoter that influences the level of plasma low density lipoprotein. *Journal of Lipid Research* **40**, 1686–1694.

Vialettes B, Reynier P, Atlan-Gepner C, Mekki N, Lesluyes-Mazzochi L, Luc G, Lairon D & Malthiery Y (2000) Dietary fat clearance in type V hyperlipoproteinaemia secondary to a rare variant of human apolipoprotein E: the apolipoprotein E3 (Arg136Ser). *British Journal of Nutrition* **83**, 615–622.

Weggemans RM, Zock PL, Meyboom S, Funke H & Katan MB (2000) Apolipoprotein A4–1/2 polymorphism and response of serum lipids to dietary cholesterol in humans. *Journal of Lipid Research* **41**, 1623–1628.

Weinberg RB (1999) Apolipoprotein A-IV-2 allele: association of its worldwide distribution with adult persistence of lactase and speculation on its function and origin. *Genetic Epidemiology* **17**, 285–297.

Weinberg RB, Geissinger BW, Kasala K, Hockey KJ, Terry JG, Easter L & Crouse JR (2000) Effect of apolipoprotein A-IV genotype and dietary fat on cholesterol absorption in humans. *Journal of Lipid Research* **41**, 2035–2041.

Ye QS & Kwiterowitch PO (2000) Influence of genetic polymorphisms on responsiveness to dietary fat and cholesterol. *American Journal of Clinical Nutrition* **72**, Suppl., 1275S–1284S.