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Symposium on 'Implications for dietary guidelines of genetic influences on requirements'

Diet, genes and disease: implications for nutrition policy

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There is extensive evidence to show that there is considerable variation in diet and disease patterns in Europe and that many of the dietary patterns are predictive of chronic disease. Increasingly, there is evidence that this dietary effect is mediated by genetic background. The present paper examines the role of polymorphisms within three genes, those responsible for the synthesis of apoE, 5,10-methylenetetrahydrofolate reductase (MTHFR) and PPARγ. There is clear evidence to support the concept that the diet–disease link is moderated by genetic variation. The paper then considers whether this moderating effect will have implications for dietary recommendations. In the formulation of dietary reference values it has long been recognized that these values cannot cover the needs of all individuals. By setting the upper level at the mean value +2 SD, the needs of 97·5% of the population are covered. Setting a hypothetical scenario of a nutrient requirement of 200 mg/d and a polymorphism with an allelic frequency in the general population in the range of 0, 10, 20 and 30% that causes an increased nutrient requirement of 25%, there was no evidence that the traditional approach requires revision. Whilst it is recognized that genetic variability may not influence population goals, genetic variability will have to be taken into account in the clinical nutrition management of disease. To knowingly assign a patient to life-long treatment with a diet that for genetic reasons will have no success is both unethical and uneconomical. Once accepted in clinical nutrition, the diet–gene interaction will filter into the prevention of disease in public health nutrition.

Diet: Genes: Disease: Nutrition policy

It is now an established fact that diet plays an important role in the aetiology of many multifactorial diseases such as cancer and CHD. Keys (1980), in the seminal Seven Countries Study, was one of the first researchers to demonstrate an association between the incidence of CHD and serum cholesterol levels in an ecological epidemiological study. It was reported that the 10-year death rate from CHD is highly correlated with median cholesterol values of the cohorts (r 0·80), with serum cholesterol levels accounting for 64% of the variance in death rate as a result of CHD. This relationship mirrors the correlation between CHD deaths and percentage energy derived from saturated fatty acids (r 0·84). Examination of the results from the Seven Countries Study highlights that individuals who consumed what is now known as a typical 'Mediterranean diet', characteristically low in saturated fatty acids, high in MUFA and high in fresh fruit and vegetables, demonstrated the lowest incidence of CHD. Subsequently, numerous other studies have continued to investigate this relationship between fat intake, Mediterranean diets and CHD, and it is on the basis of these and similar studies that current dietary recommendations have been made.

Over the last decade outstanding strides have been made in the field of molecular biology, and a new exciting field of nutrition, 'molecular nutrition', has now evolved. Numerous publications are now available that report variations in genes coding for enzymes involved in the metabolism of various nutrients, showing differences in absorption, excretion, storage and transport of such nutrients amongst individuals of different genetic make-up (Paoloni-Giacobino *et al*. 2003). However, what does all this mean for dietary guidelines and recommendations? Will it be necessary to re-assess current thoughts and practices?

The present paper will first outline the differences seen in diet and disease across Europe and demonstrate how diet and disease are associated. Some common and

Abbreviation: MTHFR, 5,10-methylenetetrahydrofolate reductase.
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highly-studied genetic variations in apoE, 5,10-methylene-tetrahydrofolate reductase (MTHFR) and PPARγ will then be highlighted and their variation throughout Europe, how they affect metabolism and their potential impact on dietary recommendations will be examined. The implications of such variations for dietary guidelines and nutrition policy will then be assessed.

Disease patterns in Europe

According to data from the Task Force of the European Society of Cardiology on Cardiovascular Mortality and Morbidity in Europe (Sans *et al*. 1997) there are large international differences both in the levels and the trends in mortality from CVD. Data obtained from European countries between 1990 and 1992 have shown that in men aged 45–74 years the age-standardized mortality rates from IHD vary between 655 deaths/100 000 per year in Scotland (UK) to 142 deaths/100 000 per year in France, resulting in a 4·5-fold difference between these two countries. Results are even more disparate between women, with a 7·5-fold difference in incidence of IHD between France (thirty-six deaths/100 000 per year) and Scotland (UK; 273 deaths/100 000 per year). The data also show a clear north–south Europe gradient in IHD for both men and women, with the UK having exceptionally high values in Northern Ireland and Scotland (Fig. 1).

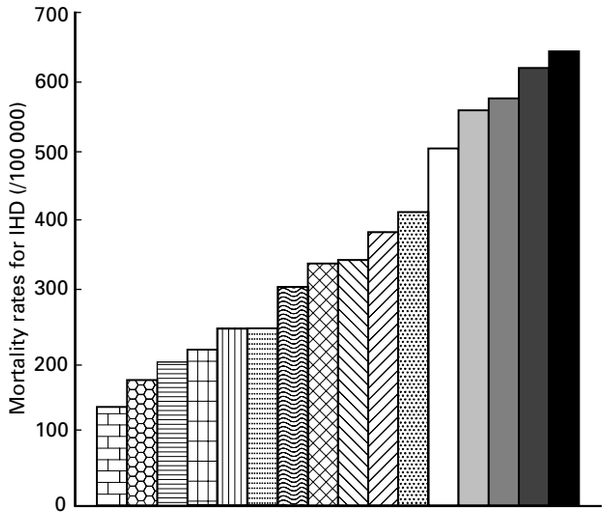

Fig. 1. Mortality from IHD in the EU 1990–2. Rates in men aged 45–74 years (/100 000 population; age-adjusted). (▦), France; (▩), Spain; (▤), Portugal; (▦), Italy; (▥), Greece; (▭), Belgium; (▨), The Netherlands; (▩), Austria; (▧), Germany; (▨), Sweden; (▭), Denmark; (□), UK, east and west; (▭), Republic of Ireland; (▩), Finland; (▩), UK, Northern Ireland; (■), UK, Scotland. (Adapted from Sans *et al.* 1997.)

While this comparison shows a cross-sectional analysis, even greater geographical variability is found when trends are examined. For the period 1970–1992 there were major differences between countries in the annual change in (IHD) mortality rates for men. IHD mortality rates for men (45–70 years) decreased in the northern and western European countries and in some of the southern European countries. There was no change in Spain and an increase of about 1 % per year in Greece. Finland had the highest rates of mortality from IHD in 1970 but demonstrated average levels in 1992. Decreasing trends were also seen in Denmark and the UK, but their decline started later than that in Finland and was not as dramatic. In contrast to the decreasing trends of the northern and central European countries, eastern European countries such as Hungary demonstrated a marked increase, rising by approximately 20 %. It should be noted that Portugal, Spain and Greece had very low rates throughout the entire 22-year period. Similar trends were observed in women, although the increasing trend in eastern Europe was less pronounced. These data show that within a constant gene pool, rates of IHD vary as the environment changes over time, indicating a clear role for nutrient intake. Equally, at any given time point with comparable environmental conditions rates of IHD differ again, indicating a clear genetic determinant of disease rates.

Variation in dietary patterns in Europe

Since the 1960s it has been known that there are differences in dietary habits between and within European populations, resulting from differing cultural norms. Keys (1980) was amongst the first to describe the 'Mediterranean diet', which is characterized by high amounts of plant foods, including fresh seasonal fruit and vegetables, cereals, beans, nuts and seeds. Olive oil is the principle source of fat. Dairy produce are consumed in low quantities, as are red meats. Poultry and fish are consumed in low to moderate amounts and wine is consumed in moderate amounts, usually with meals. Such a diet contrasts quite markedly with diets from northern European countries, which have higher levels of dairy produce and higher meat consumption, with reduced fruit and vegetable intake (Keys, 1980).

Such differences in European dietary habits are still evident. Data collated from 24 h dietary recalls collected between 1995 and 2000 from >35 000 individuals participating in the European Prospective Investigation into Cancer and Nutrition, a large cohort representing 500 000 individuals in ten European countries, demonstrate a similar pattern (Slimani *et al*. 2002) to that previously presented by Keys (1980). Data show that diets in northern Europe tend to be more heterogeneous than Mediterranean diets, with a wider variety of food groups consumed that are essentially of animal origin or highly-refined produce. Consumption in southern European countries (Italy and Greece) is characterized by high consumption of plant foods and a low intake of animal and processed foods. France and Spain tend to have a more heterogeneous diet, with their average diets characterized by a high consumption of both plant foods and animal products such as meat, eggs, fish, dairy products and alcohol. In contrast, in northern European countries (The Netherlands, Germany and UK), the diet of the general population is relatively high in potatoes, animal and processed or sweetened foods. The diets of these countries are characteristically low in consumption of fruit and vegetables, falling well below European averages. Consumption of vegetable oils is also low in Nordic countries (Slimani *et al*. 2002).

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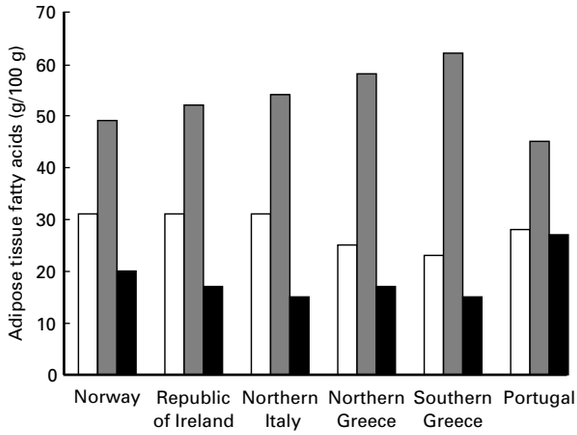

Fig. 2. Diet variation across Europe reflected in adipose tissue fatty acids (g/100 g). (□), Saturated fatty acids; (▩), MUFA; (■), PUFA. (From Corridan,1995.)

These differences in dietary patterns across Europe are biologically manifested. For example, cross-cultural differences in the type of fat consumed are obvious when adipose tissue fatty acid composition is compared (Riemersma *et al*. 1986; Aro *et al*. 1995). Adipose tissue fatty acid composition demonstrates a north–south Europe increase in the incorporation (g/100 g) of MUFA into adipose tissue, which is accompanied by a north–south Europe decline in the incorporation of saturated fatty acids and PUFA (Corridan, 1995; Fig. 2). Such adipose tissue fatty acid patterns are indicative of the diets of these regions, with higher olive oil (monounsaturated oil) consumption in southern Europe and decreasing meat and dairy consumption (saturated fatty acids) from northern to southern Europe.

Diet and disease

Dietary fats and cholesterol play a major role in CHD development, mostly by modulating plasma lipoprotein concentrations. As previously mentioned, since Keys (1980) first published results from the Seven Countries Study it has been an established fact that CHD mortality is closely linked to plasma cholesterol fractions, which are in turn linked to the intake of saturated fats. Importantly, more recent reports from the Seven Countries Study (Kromhout *et al*. 1995) also indicate that even after 25 years follow-up the CHD mortality is still closely and directly linked to the intake of saturated fats. It is important to note that it is not total fat intake but only saturated fat intake that is positively associated with CHD, which is nicely illustrated in the comparison between Crete and Finland, with twenty-six hard coronary events in Crete (age-standardized rate per 100 000) compared with 1074 in Finland. The total intake of fat in these two countries does not differ (40% energy in Crete and 39% energy in Finland), but the intake of saturated fatty acids is 8% energy in Crete and 22% energy in Finland.

The interaction between diet and disease is strengthened by the ability to alter disease outcomes by dietary modifications. There has been an abundance of studies examining the effect of reduced-fat and modified-fat diets on risk and outcome of CHD in a variety of different populations. Schaefer (2000) has extensively reviewed these data. The general consensus at this time is that a reduction in saturated fatty acids markedly reduces CHD risk, whereas a relative increase in *n*-6 and *n*-3 MUFA consumption is beneficial in relation to CHD risk.

Although such studies have been, and still are, deemed successful and have led to current recommendations relating to fat intake, a striking variability in individual responsiveness to these diets has also been reported (Jacobs *et al*. 1983; Katan *et al*. 1986; Cobb & Teitlebaum, 1994). Some individuals demonstrate a dramatic reduction in serum cholesterol on initiation of a low-fat diet, whilst other individuals demonstrate no change at all. A study by Jacobs *et al*. (1983) classified individuals placed on such a diet as 'responders', 'non-responders' and 'hyper-responders' in relation to expected changes predicted from studies of Keys *et al*. (1957). Such disparities have led to the hypothesis that the serum lipoprotein response to dietary manipulation may have a strong genetic component. There have been many investigations into the effect of various polymorphic variations on the response to dietary manipulation. These data have been extensively reviewed (Ordovas *et al*. 1995; Ye & Kwiterovich, 2000; Vincent *et al*. 2002).

Although fat intake plays a predominantly important role in the interaction between diet and CHD, several other key risk factors have also been identified, including smoking, hypertension, obesity, alcohol and hyperhomocysteinaemia (Kromhout, 2001; Thomas *et al*. 2001; De Michele *et al*. 2002; Wilson *et al*. 2002; de Bree *et al*. 2003; Suk *et al*. 2003; Tanne *et al*. 2003); the metabolic pathways associated with all these factors may also be influenced by the underlying genetic make-up of the individual. However, how important is this genetic influence, and is it something that should be taken into account when giving and formulating dietary recommendations? To illustrate such potential influences the present paper will focus on polymorphisms within the genes expressing apoE in lipid metabolism, MTHFR in folic acid metabolism and PPARγ, part of a transcriptional complex integral to adipocyte differentiation. It will present evidence of some diet–gene interactions and discuss their potential importance in relation to public health and dietary recommendations.

ApoE

ApoE is one of the major constituents in VLDL and plays an important role in lipoprotein metabolism. ApoE has three common isoforms, E2, E3 and E4, which are coded by the alleles *apoε2, apoε3* and *apoε4* at a single locus on chromosome 19. These apoE isoforms differ in their functional properties, with the *apoε4* allele associated with increased levels of total cholesterol and LDL-cholesterol; the opposite is characteristic of the presence of *apoε2* (Eichner *et al*. 2002).

Lipid metabolism is strongly affected by variation in a number of genes (Weggemans *et al*. 2001; Loktinov, 2003), with some studies linking the presence of the *apoε4* allele with both CHD (Lahoz *et al*. 2001; Eichner *et al*. 2002),

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and increased risk and late onset of Alzheimer's disease (for review, see Farrer *et al*. 1997).

Studies examining the frequencies of the *apoε* alleles in a variety of populations report considerable variation both globally and within Europe (Gerdes *et al*. 1992; Corbo *et al*. 1999; Panza *et al*. 1999; Schiele *et al*. 2000; Sheehan *et al*. 2000). Gerdes *et al*. (1992) have studied the frequencies of the common *apoε* alleles in forty-six study populations around the world. In this paper assessment of *apoε4* frequencies amongst different ethnic populations demonstrated considerable global variation. Population groups comprising native individuals from countries such as Denmark, Iceland, Germany and France, and Caucasian groups in Canada and the USA demonstrate an *apoε4* allelic frequency ranging from approximately 0·13 to 0·17. This range is distinctly different from that for other population groups such as the Chinese (range approximately 0·05–0·075) or Africans (range approximately 0·26–0·30). Such differences could be expected across such a wide international genetic pool. However, several other studies have reported *apoε4* variation within Europe, showing a higher frequency in northern European countries than in Mediterranean countries. A study conducted by Schiele *et al*. (2000) has demonstrated a 2-fold difference in *apoε4* allelic frequency from northern Europe (Finland) to southern Europe (Greece; Table 1).

Studies have demonstrated that a marked proportion of variability in plasma lipid levels can be explained by the genetic variation in the *apoε* genotype (Tikkanen *et al*. 1990; Savolainen *et al*. 1991; Cobb *et al*. 1992). Since such lipid markers are closely associated with CVD risk, it could be suggested that the variation in population frequency of *apoε* alleles could contribute to the variation in the incidence of the disease across Europe.

As previously mentioned, individuals carrying the *apoε4* allele have higher plasma LDL-cholesterol levels than those carrying the *apoε3* and *apoε2* alleles, with the lowest levels seen in individuals homozygous for *apoε2*. However, this association is not that straightforward, as the relationship between the *apoε4* allele and elevated serum cholesterol is greater in populations consuming diets high in saturated fats and cholesterol than in other populations with a healthier dietary lipid profile, suggesting that the higher serum LDL-cholesterol levels seen in *apoε4* carriers are manifest primarily in the presence of an atherogenic diet.

Furthermore, responsiveness to dietary fats may differ among individuals with different *apoε* genotypes (Ordovas *et al*. 1995). There have been numerous studies that have investigated the interaction between lipoprotein responsiveness to dietary change and various genotypes, particularly the *apoε* genotype. Some studies have reported greater plasma lipid response in individuals with *apoε4* and other studies have reported more muted responses (for reviews, see Ordovas *et al*. 1995; Mason *et al*. 2003). Ordovas *et al*. (1995) carried out a meta-analysis of studies investigating *apoε* genotype responsiveness in which dietary cholesterol and/or fat were modified. The studies were separated into three groups: group 1, studies with a reduction in total fat irrespective of dietary cholesterol; group 2, studies in which only dietary cholesterol was modified; group 3, studies in which dietary fat saturation or fat type were modified but not the fat amount. Their analysis demonstrates that *apoε4*-related hyper-responsiveness is apparent in group 1 studies with a simple reduction in total fat. Group 2 studies demonstrate no association and group 3 studies appear to reveal an inverse effect, suggesting that *ε4* carriers respond less well to change in fat type than either *apoε2* or *apoε3* carriers (Ordovas *et al*. 1995).

Table 1. Frequency of *apoε2*, *apoε3* and *apoε4* alleles in European populations (Schiele *et al*. 2000)

	Finland	Northern Ireland	France	Spain	Portugal	Greece
Apoε2	0·062	0·052	0·087	0·077	0·063	0·052
Apoε3	0·759	0·792	0·790	0·808	0·840	0·862
Apoε4	0·178	0·156	0·124	0·115	0·097	0·085

Thus, the simple association between saturated fat intake, lipid profile and risk of CHD first demonstrated by Keys and his colleagues (Keys *et al*. 1957; Keys, 1980) has now become more complex, with differences between individuals, as shown in the example of *apoε4*. The challenge is to decide whether this 'additional knowledge' of the intricate workings of lipid metabolism will impact on recommendations for fat intake at both population and individual levels.

Methylenetetrahydrofolate reductase

Homocysteine is a S-containing amino acid that plays an important role in methionine metabolism. Once formed homocysteine is either remethylated to methionine or undergoes a trans-sulfuration reaction to form cysteine. The remethylation pathway involves two critical enzymes: methionine synthase catalyses the conversion of homocysteine to methionine; MTHFR is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which then acts as a substrate for the vitamin B_{12}-dependent remethylation of homocysteine to methionine (McKinley, 2000). Genetic defects in the key enzymes involved in homocysteine metabolism can cause either a severe or a more moderate accumulation of homocysteine. It is the latter increase in homocysteine that is associated with the MTHFR C677T (A222V) variant (Kang *et al*. 1993; Frosst *et al*. 1995).

The enzyme MTHFR is involved in folate metabolism and catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the C donor for the methylation of homocysteine to methionine. The MTHFR gene is located on chromosome 1 at 1 p36.3 and two common polymorphisms, C677T (A222V) and A1298C (E429A), have been described (Van der Put *et al*. 1998). The polymorphism C677T (A222V) within MTHFR, the focus of this section of the paper, has been found to have a reduced enzymic activity (Kang *et al*. 1993). For homozygous individuals, reduced MTHFR activity has been demonstrated to result in elevated plasma homocysteine concentrations under conditions of impaired folate status, and this polymorphism has, therefore, been proposed as a candidate gene for vascular disease.

The frequency of the MTHFR C677T (A222V) polymorphism has been shown to vary substantially in different

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regions of the world and amongst ethnic groups, with a reported low 677T allelic frequency of 0·07 in sub-Saharan Africans rising to frequencies of 0·24–0·48 in Caucasians, with the highest frequencies reported in Italians and Hispanics (Botto & Yang, 2000). As with the *apoε* allele described earlier, there is also considerable variation in the MTHFR 677T allelic frequency within Europe. Gudnason *et al*. (1998) noted a clear north–south gradient, with an allelic frequency of 0·23 in Baltic countries such as Finland and Estonia, rising to a frequency of 0·41 in southern European countries such as Portugal, Spain, Italy and Greece (Table 2).

Although the relationship between the MTHFR C677T (A222V) polymorphism and CHD risk has not been fully elucidated, it is hypothesized that the MTHFR polymorphism may play a key role in the pathophysiology of CHD because of its influence on plasma homocysteine levels (Eikelboom *et al*. 1999; de Bree *et al*. 2002). Epidemiological evidence has shown that common causes of hyperhomocysteinaemia include low serum or erythrocyte folate concentrations, vitamin B_{12} deficiency or TT genotype for the MTHFR C677T polymorphism in conjunction with low folate status (Danesh & Lewington, 1998; Eikelboom *et al*. 1999; Klerk *et al*. 2002). Such evidence suggests that low folate status is one of the most important determinants of elevated plasma homocysteine concentrations, and folic acid supplementation has been shown to decrease plasma homocysteine concentration in a variety of subjects (Litynski *et al*. 2002; Venn *et al*. 2003). However, much of the relationship between folate status and plasma homocysteine is dependent on the genotype of the MTHFR C677T (A222V) polymorphism, and numerous studies have been carried out to examine the effect of MTHFR genotype on risk of CVD. The results of such studies are somewhat mixed, but in general suggest that the MTHFR 677TT genotype is associated with increased plasma homocysteine levels in individuals with inadequate folate status. However, this association is no longer evident in individuals with adequate folate status who are homozygous for the variant allele (Frosst *et al*. 1995; Harmon *et al*. 1995; Fletcher & Kessling, 1998; Kluijtmans & Whitehead, 2001). Klerk *et al*. (2002) have carried out a large meta-analysis of such studies investigating the effect of the MTHFR C677T (A222V) polymorphism on the risk of CHD, using data from forty observational studies involving a total of 11 162 cases and 12 758 controls. As in previous studies, these authors have concluded that individuals with the MTHFR 677TT genotype have a higher risk of CHD, particularly in the setting of low folate status, suggesting that impaired folate metabolism (such as the MTHFR polymorphism), which results in high homocysteine levels, is causally related to increased risk of CHD.

A recent study carried out by Kluijtmans *et al*. (2003) has examined the effect of common functional variants in enzymes associated with homocysteine metabolism in a group of subjects aged 20–25 years, in order to investigate the genetic contribution to hyperhomocysteinaemia in a relatively young population. Again this study demonstrated that the MTHFR 677T allele markedly influences total homocysteine levels only in the face of low folate status. When the subjects are divided into quartiles of folate intake, there is an increase in homocysteine levels only in individuals within the lowest folate intake quartile. Further separation of the individuals within this quartile into MTHFR C677T (A222V) genotypes shows that in fact it is only those individuals with the TT genotype that have raised homocysteine, with mean serum total homocysteine levels of 10·9, 10·7 and 17·3 μmol for CC, CT and TT individuals respectively. Thus, it is now known that the MTHFR 677TT genotype is quite common in some populations. Also, this variant is associated with an increased level of homocysteine, an independent risk factor for CHD, but such an association is only evident in individuals with low folate status. Based on this information, should individuals homozygous for the MTHFR 677T allele be identified and measures taken to ensure they consume adequate folate, or should the population as a whole be targetted in an attempt to ensure that everyone has adequate folate? On the other hand, are our current recommendations already achieving this objective? These questions are among those that have been posed to public health nutritionists; questions that will be discussed later in the present paper.

Table 2. Frequency of the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677T allele in regions of Europe (Gudnason *et al.* 1998)

Region of Europe	MTHFR 677T allelic frequency
Baltic	0·233
UK	0·353
Central	0·312
Southern	0·410

PPARγ

Dietary fat is an important macronutrient in all mammalian diets, as it provides a source of energy and hydrophobic components that are utilized for the synthesis of complex lipids involved in structural membrane composition and signalling processes within the body. However, more recently it has been noted that dietary fat has profound effects on gene expression that can lead to changes in cell metabolism, growth and differentiation. The effect of dietary fat on gene expression reflects an adaptive response to changes in the quantity and type of fat digested (Jump & Clarke, 1999).

Work examining the molecular basis of fatty acid regulation on gene expression has focused on several key players, including PPAR. PPAR are single polypeptide nuclear receptors containing two Zn finger motifs in the DNA-binding domain. On activation PPAR form a heterodimeric complex with another nuclear receptor, the retinoic X receptor, and bind to definitive DNA *PPAR* response elements in the promoters of specific target genes, thus regulating their expression (Jump & Clarke, 1999). Three PPAR family members have been identified, PPARγ, -α and -δ, each with slightly different ligands and targets. PPARγ is believed to be a key player in metabolic syndromes such as obesity and diabetes, influencing lipid metabolism by targetting genes encoding hydroxymethylglutaryl-CoA synthase, apoA-I and lipoprotein lipase. Putative

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Table 3. Consideration of a hypothetical common polymorphism that, when present, results in a 25% increase in the requirement of a hypothetical nutrient. The calculated increase in requirement for this hypothetical nutrient is presented for allelic frequencies of 0, 10 and 30%, with the final population reference value weighted to take account of these frequencies

Average requirement (mg/d)	Frequency of allele	Percentage increase in nutrient requirement as a result of allele	SD (%) of requirements	+2 SD		
				Original	Allele	Adjusted on a population basis
200	0	–	15	260	–	260
200	10	25	15	260	325	267
200	20	25	15	260	325	273
200	30	25	15	260	325	280

naturally-occurring ligands for PPARγ have been proposed and include oxidized linoleic acid and the PG metabolite 15-deoxy-$\Delta^{12,14}$–PG J_2. Some workers have suggested that a single specific ligand may not exist, suggesting that PPARγ may be a fatty acid sensor, with the affinity of fatty acids for the receptor varying according to their chain length and extent of desaturation (Jump & Clarke, 1999).

An investigation into mutations in the coding region of the *PPARγ* gene (both γ1 and γ2 isoforms) has been carried out by Yen *et al.* (1997). Among other non-significant mutations a missense mutation has been identified at nucleotide C34G (P12A). The allele frequency of the PPARγ2 C34G (P12A) variant is 0·12 in Caucasian Americans, 0·10 in Mexican Americans, 0·08 in Samoans, 0·03 in African Americans and 0·01 in Chinese (Yen *et al.* 1997).

The physiological response to consumption of fat will depend on the quantity and type of fat consumed, cell-specific fatty acid metabolism, specific nuclear and membrane receptors and involvement of specific transcription factors in gene expression (Jump & Clarke, 1999). PPARγ is one such transcription factor that has the ability to modify gene expression depending on the fatty acids it derives from the diet. It is, therefore, not surprising that researchers have now begun to investigate the effect of polymorphic variations, such as the PPARγ2 C34G (P12A) mutation, on levels of gene expression and metabolic profiling associated with varying dietary fatty acid intakes. The interaction between dietary polyunsaturated fat:saturated fat and the PPARγ2 C34G (P12A) polymorphism in a large UK population has been examined by Luan *et al.* (2001). As the GG genotype is uncommon (approximately 2% of the UK population), analyses compared the CC (Pro–Pro) genotype individuals with CG (Pro–Ala) and GG (Ala–Ala) genotypes combined. Determination of fasting insulin levels in CG and GG genotypes or CC genotypes stratified from the lowest to the highest quartiles of the dietary polyunsaturated fat : saturated fat has demonstrated that there is a clear tendency for fasting insulin levels to decrease in both CG and GG genotype individuals but not in CC genotypes ($P = 0{\cdot}0097$). When BMI is included as a covariate the observed interaction between genotype and dietary polyunsaturated fat:saturated fat for fasting insulin is attenuated, suggesting that the effect is mediated through obesity. Again, the capacity of genetic variability to be linked to a disease can be seen, the strength of which is clearly related to the diet.

Diet, genes and disease: implications for dietary recommendations

It is evident from the preceding section that genetic make-up most definitely modulates the links between diet and risk factors for disease. However, it is important to note that the geographical distribution of polymorphisms in Europe, such as those previously described, is such that several 'disadvantageous' polymorphisms are not primarily located in one region, which would defy the basic concepts of evolution. Thus southern France has a high rate of MTHFR 677T allele, which offers one level of disadvantage, while northern Europe has a high rate of the *apoε4* allele, which offers a different level and form of disadvantage.

All the genetic variations (such as *apoε4* and MTHFR C677T) that are only now being fully elucidated must raise the question as to whether present dietary recommendations might need to be altered. Table 3 examines this issue for a hypothetical scenario in which there is a 0, 10, 20 or 30% prevalence of an allele in the general population, which when present raises the requirement of a hypothetical nutrient (with an average requirement of 200 mg/d) by 25%. With a CV of the standard deviation of 15%, the traditional RDA would be 260 mg/d. With a 25% increase in requirement as a result of the presence of the hypothetical allele, the original value rises to 250 mg/d, and retaining a 15% CV for the standard deviation then 2 SD increases the RDA to 325 mg/d. Finally, making the population adjustment based on the frequency of the allele the new RDA, if the allele is taken into account, would be 267, 273 and 280 mg/d for frequencies of 10, 20 and 30% respectively.

The original RDA of 260 mg/d was set to account for only 97·5% of the population, and as such recognizes that values will be above this amount. In fact, if the original RDA is recalculated and the 99th percentile is taken into account (approximately the mean +3 SD) then the requirement of the 99th percentile of the population would be met by 275 mg/d, enough to accommodate the likely highest true prevalence of any allele of 20%. Such calculations easily demonstrate that many alleles will be found that provide little reason to revise existing RDA, but there will always be exceptions. Of course, eventually polymorphisms will be encountered that will lead to lower requirements, which balances out the status quo when looked at on a population basis.

Notwithstanding the fact that those who developed the concept of RDA built in a 'genetic variability safeguard', it

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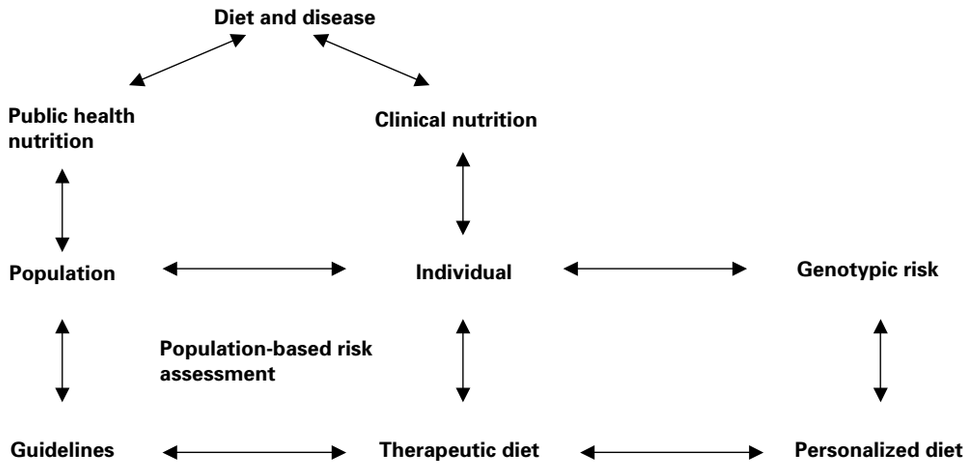

Fig. 3. Comparison of pathways for population dietary guidelines (public health nutrition) and individualized therapeutic advice (clinical nutrition) and the links between the latter and the use of genotypic risk for personalized diets.

remains possible that a case will be found where a marked proportion of the population are found to have a very large increase in requirement, a hypothetical situation that grows more likely as more diet–gene and gene–diet interactions are investigated. However, if such a situation occurs are the public health nutrition experts ready to correctly modify and implement current guidelines? To illustrate how public health nutrition experts vary in their response to such a problem, consider the case of Fe requirements for menstruating women, where it is known that there is a very skewed Fe requirement as a result of a very skewed pattern of menstrual Fe losses (the main determinant of such requirements). Some expert committees have retained the concept of protecting the highest element of the population resulting in requirements for Fe in the USA to be 18 mg/d (Food and Nutrition Board/Institute of Medicine, 2000). In contrast, other expert committees, recognizing the difficulty that this approach places on the food supply, have altered the traditional strategy to cover only 75% of the population, allowing an Fe requirement of 14 mg/d in the UK (Department of Health, 1991). In effect, there is no agreement at present on how to deal with distributions of nutrient requirements that are heavily skewed to the upper end.

Diet, genes and disease: implications for public health nutrition

Nutrition intervention operates at the level of the population in public health nutrition and at the level of the individual in clinical nutrition. As outlined in Fig. 3, the population advice of public health nutrition will be as dietary guidelines, and almost certainly these types of guidelines will always be necessary. In the clinical nutrition model the advice is at the level of the individual but the risk assessment is based on population studies. A patient with a plasma total cholesterol level of 6·45 mmol/l will be deemed to have a given risk based on epidemiological or population data. As has been discussed earlier, there are increasing data to suggest that the risk associated with a given level of cholesterol is related to genotypic risk, and if a therapeutic diet is warranted, that the appropriate diet should be personalized, based on a knowledge of the genotypic responsiveness to particular diets. In the course of time, as genotypic data filter into the practice of clinical nutrition, the probability of such data entering the realm of public health nutrition rises. Consider, for example, a hypothetical situation in which a particular polymorphism or haplotype is found to dramatically influence the probability of colo-rectal cancer in individuals with chronic low fibre intake? Would it be ethical not to include this profound knowledge in the public health nutrition management of the disease? To some extent, the answer to this question is indicated in the approach used in the genetic disorder of phenylketonuria.

Phenylketonuria is an inborn error of metabolism caused by an autosomal recessive deficiency of the hepatic enzyme phenylalanine hydrolase. Failure to convert phenylalanine to tyrosine leads to an increase of phenylalanine in the body fluids, resulting in severe mental retardation unless dietary phenylalanine is restricted (Waters, 2003). Phenylketonuria is a major but rare defect, with a frequency of approximately one in 4500 new births in the Republic of Ireland and one in 24 000 new births in Greece, and >400 different disease-causing mutations have been identified to date and are listed in the phenylalanine hydrolase mutation detection consortium database (www.pahdb.mcgill.ca; Scriver *et al.* 2003). Such mutations can cause reductions in enzyme activity as a result of direct alteration of the catalytic activity of the enzyme, or more commonly alterations in post-translational folding causing reduced stability or accelerated degradation (Waters, 2003). With the knowledge of the genetic cause of this disease and the fact that it can be treated simply through dietary modifications, society has chosen to screen every newborn baby at birth, by the Guthrie test. There is no choice in whether or not this test is carried out and it is carried out on each baby regardless of the likelihood of testing positive. The nutritional management of the individual is at an individual level.

The important message is that newborn screening for phenylketonuria leads to the identification of the very small percentage of the population at genetic risk and only their diet needs to be modified accordingly. Society as a whole is not expected to adopt an unpalatable phenylalanine-free diet, simply because there is a one in 24 000 chance of disease. The phenylketonuria screening process is relatively unique, but it should be possible to learn from this approach when considering a more subtle genetic alteration in a quite marked proportion of the population. Take, for example, lactose intolerance, a disorder that produces a painful digestive condition commonly associated with nausea, cramps and diarrhoea. This disorder results from the physiological decline in activity of the lactase-phlorizin hydrolase in intestinal cells after weaning. This enzyme hydrolyses lactose to glucose and galactose. For many years, epidemiogical data have indicated that the frequency of lactose intolerance varies widely between different races, with individuals of Asian origin presenting with a frequency of approximately 95% lactose intolerant compared with 79% in African American individuals and only 5% in Caucasians of northern European and Scandinavian descent (Sahi, 1994). Such disparity between different ethnic groups would suggest an obvious genetic component. However, it is only recently that genetic variants associated with hypolactasia have been identified. Enattah *et al*. (2002) identified two variants on *LCT*, the gene encoding for lactase-phlorizin hydrolase, one of which (C13910T approximately 14 kb upstream from the *LCT* locus) completely associates with, and biochemically verifies, lactose intolerance in a variety of different populations, including Finns and African Americans. Although the genetic cause of lactose intolerance is only now becoming fully understood, it must be pointed out that public health policy has in fact been implementing population-specific guidelines on lactose intake for many years, without needing to understand the intricate genetic explanation of the disorder. So perhaps, like the case of lactose intolerance, the result of many genetic variants may have already been taken into account when formulating dietary recommendations. However, there will inevitably be more insight into common and widespread disorders, such as lactose intolerance, in the course of time, and it would be wise for those involved in public health nutrition to begin considering how more targetted information arising from nutrition–genomic research can be successfully implemented.

Conclusion

Geographic variation in disease patterns in Europe is attributable to variation in both diet and genetics, with the former being presently better understood. The genetic basis of nutrient requirements that modulate disease risk factors are unlikely to require major revisions of existing dietary advice to populations. However, it is likely that an increased understanding of the genetic variation, disease risk factors and responsiveness to therapeutic diets is likely to first have an influence on clinical nutrition and subsequently on public health nutrition.

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