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Human nutrition and food research: opportunities and challenges in the post-genomic era

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Sequencing of the human genome has opened the door to the most exciting new era for nutritional science. It is now possible to study the underlying mechanisms for diet–health relationships, and in the near future dietary advice (and possibly tailored food products) for promoting optimal health could be provided on an individual basis, in relation to genotype and lifestyle. The role of food in human evolution is briefly reviewed, from palaeolithic times to modern-day hunter–gatherer societies. The aetiology of 'diseases of modern civilization', such as diabetes, heart disease and cancer, and the effect of changes in dietary patterns are discussed. The risk of disease is often associated with common single nucleotide polymorphisms, but the effect is dependent on dietary intake and nutritional status, and is often more apparent in intervention studies employing a metabolic challenge. To understand the link between diet and health, nutritional research must cover a broad range of areas, from molecular to whole body studies, and is an excellent example of integrative biology, requiring a systems biology approach. The annual cost to the National Health Service of diet-related diseases is estimated to be in excess of £15 billion, and although diet is a key component of any preventative strategy, it is not given the prominence it deserves. For example, less than 1% of the £1.6 billion budget for coronary heart disease is spent on prevention. The polygenic and multifactorial nature of chronic diseases requires substantial resources but the potential rewards, in terms of quality of life and economics, are enormous. It is timely therefore to consider investing in a long-term coordinated national programme for nutrition research, combining nutritional genomics with established approaches, to improve the health of individuals and of the nation.

Keywords: human nutrition; food; research; diet; chronic disease; nutritional genomics

> If we knew what it was we were doing, it would not be called research… .
>
> (Albert Einstein 1879–1955)

1. INTRODUCTION

The twenty-first century marks the beginning of an exciting new era for nutrition research. Scientists are able to develop and exploit post-genomic techniques to deliver previously unimaginable data on nutrient requirements of individuals and long-awaited information on the relationship between diet and health (Sunde 2001). The ultimate goal of human nutrition research is to improve the quality of life of individuals by minimizing morbidity and maximizing longevity. This apparently simple aspiration requires a multidisciplinary approach, the use of systems biology, and an appreciation of the overriding influence of political and economic factors. It is a very complex and long-term challenge for mankind, but one that we should not ignore if the species is to thrive.

At present, the world can very broadly be divided into two main sectors.

(i) Industrialized countries where food is plentiful and the diet is varied, and the most prevalent diet-related disorders are cardiovascular disease (CVD), cancer, obesity, diabetes and osteoporosis.

(ii) Developing countries, where food is scarce and malnutrition is a major problem or where traditional foods and cultural practices are being replaced by western-style diets.

Diet-related problems are clearly very different, indeed almost diametrically opposed in these two sectors, and in developing countries there are also problems associated with the rapid adoption of western-style diets where economically possible. From a naive perspective, it could be argued that a shift in food supply from the industrialized to developing countries where food is inadequate would be an advantage to both, but such a dramatic move is beyond the remit of scientific researchers in the life sciences. Despite fundamental differences in nutrition-related disorders, the basic physiological needs of people throughout the world are similar, although phenotype differs among individuals and population subgroups according to geographical location, socio-economic and other environmental factors. Nevertheless, most aspects of nutrition and food research can be considered to have a common goal, namely to characterize and define dietary requirements of nutrients and bioactive non-nutrients for optimal health. The World Health Organization (2002) has just published a report on promoting healthy life in which eight of the top 12 risk factors, accounting for *ca.* 40% of the 56 million deaths in the world each year, are related to nutrition (table 1).

Table 1. Most important risk factors that impact on health and affect life expectancy identified by the World Health Organization (2002).
(Factors in italics are related to nutrition.)

underweight in children and mothers
iron deficiency
zinc deficiency (developing world)
vitamin A deficiency (developing world)
obesity
high cholesterol
high blood pressure
alcohol
unsafe sex
poor water, sanitation and hygiene
indoor smoke from solid fuels
tobacco

2. EVOLUTION OF NUTRITION AS A SCIENTIFIC DISCIPLINE

Nutrition is the branch of science that involves the processes by which living organisms take in and use food for the maintenance of life, growth, the functioning of organs and tissues and the production of energy. The active components of food are nutrients and energy; nutrients are essential dietary factors such as vitamins, minerals, amino acids and fatty acids (Bender & Bender 1999), and food energy is the metabolic fuel for the body. Bioactive constituents of the diet, referred to as phytochemicals or protective factors, and dietary fibre (complex carbohydrates) are not classified as nutrients, but the growing recognition of their significant impact on human health is driving a move towards their inclusion in dietary recommendations, for example non-starch polysaccharides (Department of Health 1991) and carotenoids (Institute of Medicine 2000).

Nutrition is a truly multidisciplinary science, employing a synergy of approaches to answer scientific problems. It requires a combined understanding and appreciation of several uniquely identifiable areas, such as food science, physiology, biochemistry, genetics, epidemiology, anthropology, psychology and social sciences. It is an excellent example of integrative biology (Young 2002), and there is an impressive list of Nobel Prize winners associated with nutrition (table 2). However, the very broad and diverse nature of nutrition would appear to be its Achilles' heel. Although scientists from within the nutrition community appreciate the importance of nutrition research in the post-genomic era, a wider acceptance of the key role of nutrition is required if it is to achieve its full potential in optimizing the quality of life.

Food science and nutrition are relatively young disciplines that have developed over the past 150 years, although the link between diet and health was recognized much earlier. For example, there are reports that Hippocrates (460–377 BC) gave advice about what foods his patients should eat. Nutrition has undergone several key stages of evolution, as summarized in table 3 (Carpenter *et al.* 1997). Atwater is claimed to be the father of dietary guidelines, providing leadership and vision 100 years ago in the areas of nutrient requirements, food composition and consumption, and consumer economics (Welsh 1994). Research during the first half of the twentieth century was primarily concerned with the discovery of the 'accessory food factors' (subsequently renamed vitamins), essential amino acids and minerals. Experiments were conducted on animals, and analytical techniques for food composition, metabolites and biochemical assays were developed. One of great pioneers of experimental nutrition research was Elsie Widdowson, who worked tirelessly, usually in partnership with Sir Robert McCance, from the early 1930s to nearly the end of the twentieth century, on a wide range of fundamental issues, including iron and calcium metabolism, infant physiology, normal and retarded growth, and body composition (Ashwell 1993). McCance & Widdowson (1940) were also responsible for establishing the first food composition tables; many people regard the current UK food-composition databank to be the world's gold standard (Food Standards Agency 2002) although the US–USDA equivalent is arguably more extensive.

The contribution made by nutrition research to the health of man and animals during the second half of the twentieth century was celebrated in the form of reviews presented at the Golden Jubilee of the Nutrition Society (Widdowson & Mathers 1992). As our understanding of the importance of diet in maintaining health has improved, most nutritional deficiency disorders have been eliminated in the western world. The focus of the next stage in the evolution of nutrition research was nutrient requirements for preventing diet-related chronic diseases; e.g. coronary heart disease, cancers and diabetes. The approaches included epidemiology, human metabolic studies and the development of biomarkers. Finally, as we enter the post-genomic era and begin to map variations in key diet-responsive genes, the focus is shifting towards a mechanistic understanding of the relationships between diet, phenotype and genotype. Modern-day nutrition has evolved from being an essentially descriptive discipline, relying heavily on phenomenology, into a multidisciplinary science that embodies integrative biology and will increasingly depend on computational systems biology.

3. PALAEOLITHIC MAN: NATURAL SELECTION AND DIET

Anatomically modern humans emerged some 200 000 years ago, having previously split from the common lineage with chimpanzees *ca.* 6 million years earlier. The reason for the evolutionary advantage of modern humans is unclear. The latest hypothesis revolves around a 'speech gene' called *FOXP2*, mutations of which cause a wide range of speech and language disabilities (Enard *et al.* 2002). The gene encodes a protein with 715 amino acids and resembles other members of a family of regulatory genes implicated in embryonic development. The human version of *FOXP2* has probably been in existence for less than 200 000 years, thus implicating it in the natural selection process that produced modern humans. Oral communication skills may well have facilitated the development of early humans, but the ability to use resources (e.g. to make tools), and to adapt to the environment, especially the ability to survive on a variable food supply, was arguably more important.

Genetic mutation (gain or loss of chromosomes; rearrangement, gain or loss of parts of chromosomes as a

Table 2. Nobel laureates associated with human nutrition research.

year	individual	research area
1909	Theodor Kocher	physiology of thyroid gland
1923	John James	diabetes and insulin
	Richard MacLeod	
	Frederick Grant Banting	
1929	Sir Frederick Hopkins	discovery of vitamins
1934	George H. Whipple	liver therapy for anaemia
	George R. Minot	
	William P. Murphy	
1936	Otto Loewi	protein metabolism
1937	Paul Karrer	carotenoids, flavins, vitamins A and B2
	Walter Norman Haworth	carbohydrates and vitamin C
1943	Henrik Carl Peter Dam	discovery of vitamin K
1943	George de Hevesy	isotopes as tracers for chemical processes
1945	Artturi Ilmari Virtanen	nitrogen fixation and fodder preservation
1947	Carl Ferdinand Cori	catalytic conversion of glycogen
	Gerty Theresa Cori	
1949	Lord John Boyd Orr	animal and human nutrition
1953	Fritz Albert Lipmann	co-enzyme A
	Sir Hans Adolf Krebs	citric acid cycle
1959	Arthur Kornberg	synthesis of RNA and DNA
1962	Linus Carl Pauling	chemical bonds; vitamin C
1964	Konrad Bloch	cholesterol and fatty acid metabolism
	Feodor Lynen	
1970	Norman E. Borlaug	the 'green revolution'
1985	Michael S. Brown	regulation of cholesterol metabolism
	Joseph L. Goldstein	
1988	George H. Hitchings	folate metabolism
1992	Edwin G. Krebs	protein phosphorylation and cellular regulation
1997	John E. Walker	mitochondrial ATPase

Table 3. Key stages in nutrition.

1830s	ferrous sulphate pills recommended for the treatment of chlorosis (Blaud 1832)
1850s	glucose synthesis in the liver demonstrated (Bernard 1849)
1900s	recognition that some diseases are due to dietary deficiency (Hopkins 1906; Funk 1912)
	essentiality of minor components of foods widely accepted
1930s	lysine shown to be an essential amino acid (Osborne & Mendel 1914)
	discovery of *ca*. 40 essential nutrients
	nutrient requirements derived from data on preventing dietary deficiency diseases
1950	protective effect of foods first demonstrated (Lourau & Lartigue 1950)
1960s	bioavailability recognized to be an important issue for many micronutrients
	dietary recommendations based on promoting optimal health
1979–	genetic and biological variability in nutrient requirements recognized (Young & Scrimshaw 1979)
	goal for twenty-first century: to derive dietary recommendations for individuals in relation to genotype

result of chromosome breakage; changes in individual genes or small regions of DNA) is the ultimate source of variability on which natural selection acts. Food has undoubtedly played a major role in the evolution of humans from anthropoid primates 25 Myr ago to the appearance of modern *Homo sapiens ca*. 40 000 years ago. At that time, favourable mutations in the human genome would have been selected, including physical strength and fertility, but traits relating to health in the post-reproductive age would not have been an important part of the natural selection process. In modern times, with the exception of prenatal mortality, natural selection for effective mutation removal has been greatly reduced. Crow (1997) contends that during the past few centuries, harmful mutations have been accumulating but they have been more than compensated for by rapid environmental improvements, which have kept well ahead of any decreased efficiency of selection. Eaton *et al.* (1988) have suggested that from a genetic viewpoint, humans can be considered to be Stone Age hunter–gatherers displaced through time to a world that differs greatly from that for which our genetic constitution was selected; individuals in industrialized countries now 'forage only in supermarkets' (Diamond 2002).

Research on nutritional programming may generate mechanisms for environmental effects, including nutrition, on phenotype. Differences between genetically identical twins must be due to changes in the genome that do not affect DNA, namely epigenetic changes (Dennis 2003), but to what extent are epigenetic phenomena

responsible for increased risk of cancer and other chronic diseases, and are they involved in imprinting disturbances? Epigenetic mechanisms are central to stem cell therapy, animal cloning, complex traits and ageing (Feinberg *et al.* 2002), but are also implicated in altered risk of certain cancers. CpG island hypermethylation is a mechanism of gene silencing that can be usurped by neoplastic cells to inactivate undesirable genes. In the colon, hypermethylation often starts in normal mucosa as a function of age and is markedly increased in cancer. Age-related methylation marks the field defect that reflects an acquired predisposition to colorectal neoplasia, and the protective roles of dietary constituents such as folate and selenium are currently under investigation (Friso & Choi 2002).

In the industrial world, chronic illnesses (e.g. atherosclerosis, cancer, hypertension, type 2 diabetes and obesity) are collectively responsible for 75% of all deaths. It has been proposed that these diseases are the result of an interaction between genetically controlled metabolic processes and lifestyle factors. The latter include nutrition, physical activity and exposure to undesirable substances (e.g. tobacco smoke), all of which have changed markedly since the Industrial Revolution. The epidemiology of diet-related chronic diseases is further complicated by the fact that they are polygenic in nature and multifunctional in their aetiology. Different single nucleotide polymorphisms in our ancestors would have been conserved in social groups living in separate communities under varying environmental conditions. Phenotypic variation is undoubtedly linked to genotype, and, today, with global travel enabling the intermixing of previously isolated societies and hence a redistribution of polymorphisms, unravelling the effects of genotype on response to diet and susceptibility to disease is a major task.

The importance of diet in shaping nutrient requirements and physiology is a fiercely debated subject. Neel *et al.* (1998) proposed the 'thrifty' genotype hypothesis in which the ability to survive in the absence of an abundance of food through an innate sensitivity of insulin response was part of the natural selection process. Reaven (1998) has offered an alternative explanation, the 'not-so-thrifty' genotype, in which genes controlling the ability to conserve muscle protein by limiting gluconeogenesis from amino acids during periods of starvation would be favoured. Both hypotheses are based upon the assumption that pre-agricultural people went through periods of 'feast and famine' that had an adverse effect on reproductive ability, the recognized mechanism of natural selection. Cordain *et al.* (1999) argue that periods of starvation were not associated with hunter–gatherer groups, but are a more recent phenomenon found in agricultural communities. Pre-agricultural diets were high in protein and low in carbohydrate compared with modern diets, and this could have been the environmental pressure that was responsible for the selection of 'thrifty' genes. Brand Miller & Colagiuri (1994) suggest that an insulin-resistant genotype evolved to provide survival and reproductive advantage to populations adapted to high meat, low carbohydrate diets. They argue that primates evolved on a high carbohydrate diet, with dietary glucose being the main fuel for reproductive tissues and the brain. However, during the ice age over, approximately, the past 2 million years, the diet changed to high protein, low carbohydrate, and metabolic adaptations were needed to accommodate the low glucose supply. The phenotypic response to low glucose is insulin resistance, which maximizes gluconeogenesis, thereby ensuring adequate provision of an energy substrate for the brain, reproductive organs and mammary glands.

There is little doubt that the ability to adapt to differing levels of nutrition is crucial to survival. Throughout the course of history, supplies of food have been diverse and variable, depending on geographical location and climate. In 1865, Claude Bernard noticed that the 'constancy of the internal milieu was the essential condition to a free life' (Bell *et al.* 1968, p. 2), and in 1932 Walter Cannon coined the term *homeostasis* (resistance to change) to describe the control of physiological equilibrium. Much of today's research centres on understanding the precise limits of adaptive mechanisms, developed under constantly fluctuating intakes of energy and nutrients, whose purpose is to facilitate an adequate supply of nutrients to the appropriate cells and tissues.

Interest in evolutionary aspects of nutritional requirements was stimulated by the seminal paper on palaeolithic nutrition by Eaton & Konner (1985). Archaeological evidence indicates that as mankind evolved, the nature of the diet changed (table 4). During the Miocene era (24–25 Myr ago), fruits were the main food source. Our ancestors began to consume increasing amounts of meat (either from hunting or scavenging) long after diverging from the apes (7.5–4.5 Myr ago). Current palaeo–anthropological evidence indicates that meat eating may have begun *ca.* 2.5 Myr ago. Between 1.8 and 1.6 Myr ago, *Homo erectus* consumed large amounts of meat, as indicated by the accumulation of animal remains where they lived, the tools used, and the fact that they inhabited areas where big animals grazed. In fact, at one time in our history meat probably comprised more than 50% of the food supply. However, because plant remains are poorly preserved, it is more difficult to gauge how important they were in the diet of early man, and although fossilized fruit stones and nuts have been found, there is no evidence of tools to process plant foods. With the appearance of the Cro-Magnons in Europe 30 000 years ago, there was an increase in big game hunting. However, climate changes, population growth and over-hunting led to a period of subsistence activities, indicated by remains of shellfish, fish, small game and tools for processing plant foods. Higher strontium levels in bone confirm that there was an increased intake of vegetable foods at this time (Schoeninger 1982) and the dietary pattern of this era is closest to that of modern hunter–gatherers.

The cultivation of cereals and introduction of farming from *ca.* 10 000 years ago, in several parts of the world, was accompanied by a substantially higher intake of vegetable foods and a dramatic fall in meat consumption. This resulted in smaller stature, with skeletal evidence of sub-optimal nutrition. However, since the agricultural revolution, the animal protein content of the diet has increased in industrialized countries, and height has returned to the levels of our game-hunting ancestors just over 10 000 years ago, although the diet of today is very different from that of our most recent ancestors.

There is considerable debate about the importance of meat in the diet of early palaeolithic man. It appears that quantity and type was dependent on habitat as well as

Table 4. Diets consumed throughout the ages of mankind.

era	Myr ago	diet
Early to Mid-Miocene	24–5	primarily fruits
Late Miocene (man and apes diverge)	7.5–4.5	introduction of meat
Early Pleistocene (*Homo erectus*)	1.8–1.6	high meat intake (50%)
Middle Pleistocene (early *Homo sapiens*)	0.4–0.08	mainly plant foods
Late Pleistocene (modern *Homo sapiens*)	0.08–0.045	meat (game)
Latest Pleistocene (agricultural revolution)	0.01	plants and cereals (90%), meat (10%)
Holocene (industrial revolution)	0.0002	mixed diet

Table 5. Estimated daily nutrient intakes from the late palaeolithic diet (35% meat, 65% vegetables) and the current UK diet.

	palaeolithic diet	UK diet (adult men)[a]
total energy (%)		
protein	34	15
carbohydrate	45	44
fat	21	40
polyunsaturated : saturated fats	1.41 : 1	0.40 : 1
dietary fibre (g)	46	25
sodium (mg)	690	3376
calcium (mg)	1580	940
ascorbic acid (mg)	390	75

[a] Gregory *et al.* (1990).

stage of evolution. The need for a dietary supply of vitamin C and the presence of a colon, as a fermentation chamber, in the gut point to the fact that our early ancestors were herbivorous, and meat eating was believed to be opportunistic. The $^{13}C/^{14}C$ isotope ratio ($\delta^{13}C$) of archaeological human skeletons and foods presumed to have been commonly consumed can be used as an independent test of meat and plant food consumption. Marine foods, terrestrial plants and animal meat all have a different $\delta^{13}C$ 'signature', which is reflected in the bones of people consuming the foods (Sealy & Van der Merwe 1985; Bell *et al.* 2001). New techniques using variations in natural isotopes of elements such as calcium (Skulan & DePaolo 1999) and iron (Walczyk & von Blanckenburg 2002) to study mineral balance are currently under development, and it is possible that these may provide a useful approach to estimate the gross level of intake and source of nutrients in archaeological remains.

Eaton & Konnor (1985) reviewed evidence relating to the diet of late palaeolithic man (*Homo sapiens*) and calculated representative figures for nutrient intakes. These are compared with current UK figures in table 5. Apart from a lower energy intake relating to the more sedentary lifestyle of modern man, there are some very major differences in the two diets. The dietary fibre intake of palaeolithic diets was more than twice that of UK diets, and because of the generous meat intake, iron and folate intakes were probably greater than current levels of consumption. Sodium was less than a third of the lowest estimated intake in the UK, but calcium and ascorbic acid were considerably higher. Protein was also much higher but fat considerably lower in the palaeolithic diet, presumably due to the higher intake of meat. The latter was wild game, which contained low levels of storage fat, and was eaten in conjunction with plant foods but no dairy or processed products containing added fat, so the ratio of polyunsaturated to saturated fats was much higher. The ratio of omega-6 to omega-3 essential fatty acids is one of the most striking differences between palaeolithic and modern-day diets. There has been a dramatic increase in the consumption of omega-6 fatty acids during the last 100 years owing to the development of vegetable oil technology, the introduction of sunflower and other high omega-6 oils and intensive farming of cattle fed on grains rich in omega-6 fatty acids (Simopoulos 1999).

The human genome has remained relatively unchanged since *Homo sapiens* first appeared. However, the diet consumed 40 000 years ago is probably not the one for which *Homo sapiens* was genetically programmed and, for that reason, may not be an appropriate 'gold standard' by which to judge the suitability of modern-day diets. Importantly, there is no one single palaeolithic diet; humans are omnivorous and have the capacity to subsist and thrive on a wide range of diets. Also, there is limited information on the health and longevity of early man, so it is not possible to draw conclusions about the influence of nutrition. However, studies of technologically primitive cultures, the so-called hunter–gatherer societies, are a useful surrogate for investigating the relationship between diet and health, and, in particular, to explore the effects of dietary change on risk factors for non-communicable diseases.

4. HUNTER–GATHERERS: DIET AND CHRONIC DISEASES

Research is to see what everybody else has seen, and to think what nobody else has thought.

(Albert Szent-Gyoergi; see Peter 1977)

Chronic diseases are virtually unknown in the few surviving hunter–gatherer societies whose diet and lifestyle most closely resemble that of *Homo sapiens*; therefore examining their diets may provide clues as to causal links. The dietary patterns of hunter–gatherer societies were extremely diverse because their natural habitat dictates the local food supply. They live in tundra, coniferous or temperate forest, grassland, desert, subtropical, tropical or monsoon conditions. The diet includes fish, shellfish, game and wild plants. The most common plant foods are fruit, tubers, seeds and nuts. Cordain *et al.* (2000) examined hunter–gatherer diets in terms of the contributions made by plants and animals, and estimated the likely intake of protein, carbohydrate and fat as a percentage of total energy. They propose that whenever it is ecologically possible, hunter–gatherers would consume high amounts of animal food (45–65% of total energy) and suggest that differences in the percentage of body fat of animal prey would alter protein intakes and that the maximal protein ceiling (more than 35–40% of total energy intake) influences the selection of other macronutrients. Despite the environmental diversity, the majority (73%) of hunter–gatherer societies are estimated to derive more than 50% of their subsistence from animal foods. A high intake of animal foods and the relatively low carbohydrate content of wild plant foods produces a diet that is relatively high in protein (19–35% energy), and low in carbohydrate (22–40% energy) compared with the current UK diet (14% and 44%, respectively). By difference, the fat content of the diets ranged widely from 25–58% energy, compared with the 1990 UK estimated mean intake in adult men of 40%.

The proposal by Cordain *et al.* (2000) that the diet of remote ancestors, as still consumed by hunter–gatherer societies, should be a reference standard for modern human nutrition and a model for defence against some of the chronic diseases is challenged by Milton (2000) and Walker (2001). First, it is not possible to define a generic diet, as food selection and intake is highly variable. Second, degenerative diseases occur in later life, and because humans are living longer due to the eradication of lethal infectious diseases, it is argued that the growth in chronic diseases is merely the result of increased longevity. To examine this further we can take advantage of 'natural experiments' whereby hunter–gatherer groups have replaced traditional diets with western-style diets to examine the effect of modern-day diets on chronic disease.

One very useful hunter–gatherer group is Australian Aborigines. Historians believe that Aborigines travelled to Australia from South East Asia some 40 000–50 000 years ago. They lived in small groups in a wide range of environments and hence consumed a variety of diets. Depending on local supply, they hunted and ate wild animals, reptiles, fish, shellfish, insects, fruits, vegetables, nuts and grains (Gracey 2000). We can only speculate about their health in pre-colonial days, but 'first contact' examinations found them to be physically fit and lean (body mass index (BMI) less than 20 kg m^{-2}), with a low blood pressure, low fasting glucose, low fasting cholesterol and no evidence of coronary heart disease or diabetes (Elphinstone 1971), observations that are consistent with other hunter–gatherer groups embracing a traditional lifestyle (O'Dea 1991*c*).

The hypothesis that hypertension is linked to the higher salt intake of modern-day diets relates to a mutation in the urate oxidase gene that results in hyperuricaemia, which occurred in the Miocene era. This may have had a survival advantage by helping maintain blood pressure under low dietary salt conditions (Watanabe *et al.* 2002). The relationship between salt and blood pressure is, however, controversial because of the heterogeneity in blood pressure response to salt loading and depletion. In the Cameroon, a blood pressure survey was performed in isolated Pygmy communities, still living as hunter–gatherers, and Bantu populations that rely on agriculture (Kesteloot *et al.* 1996). Dietary information was limited but the basic nutrition was not dissimilar, both groups consuming a diet high in complex carbohydrates containing plantain, manioc, bananas, mangoes, wild game, and palm and raffia wine. Spot urine sodium concentrations were higher in Bantus than Pygmies, indicating a higher salt intake. No racial differences in blood pressure were observed but there was a significant negative correlation between urinary sodium and diastolic blood pressure. A recent systematic review of the long-term effects of advice to reduce dietary salt in adults with and without hypertension (Hooper *et al.* 2002) concluded that there is a small reduction in blood pressure (1.1 mmHg systolic, 0.6 mmHg diastolic) when sodium intake is reduced but, interestingly, the degree of reduction in sodium intake and change in blood pressure were not related.

Hunter–gatherers are a valuable, albeit rapidly diminishing, resource for examining the links between diet and phenotypes predisposing to chronic disease. In particular, the effects of rapid dietary change with the introduction of western-style diets and concomitant changes in lifestyle provide important evidence for diet–health hypotheses.

5. DISEASES OF MODERN CIVILIZATION

> Quod ali cibus est alius fuat acre venenum (What is food to one may be fierce poison to others)
> (Lucretius 99–55 BC; see Bartlett 1919.)

There is substantial evidence that changes in diet are responsible, in part, for the diseases that have emerged as dominant health problems in industrialized countries over the past century. Coronary heart disease was considered rare in the USA at the beginning of the twentieth century (White 1972). Cross-country epidemiological studies have consistently demonstrated a difference in the prevalence of coronary heart disease, hypertension, diabetes and some types of cancer (Neel *et al.* 1998; Bingham 2000; Zimmet *et al.* 2001), which are related to differences in diet. For example, the recent MONICA study reports a nearly 10-fold difference in deaths from coronary heart disease in 37 populations in 21 countries worldwide (Tunstall-Pedoe *et al.* 1999). In European women, there was an eightfold difference between the highest rate in Scotland and the lowest in Spain.

In Australia, Aborigines are now the unhealthiest subgroup in the country, with very high rates of CVD and type 2 diabetes. The cause is presumed to be a combination of the switch from an active to a sedentary lifestyle, introduction of cigarette smoking and change in diet. It has been observed that the development of impaired glu-

cose tolerance, hyperlipidaemia and hyperinsulinaemia can be quite rapid in Aborigines who consume western-style diets supplied through community food stores. These diets are usually monotonous and very restricted, lacking in fresh fruit and vegetables, and containing large amounts of fat and refined carbohydrates (Gracey 2000). Quantitative dietary data on changing dietary patterns in Aborigines is limited but all evidence points to a major change in fat intake, which has stimulated the Australian Department of Health to issue guidelines directed at reducing the fat intake of Australian children (reported by Gracey 2000). The rapid adverse change in health in response to dietary change indicates a failure in homeostatic mechanisms. Clearly, the Aborigines are not able to adapt to the supply of energy and nutrients and non-nutrients that this diet provides. The observations in Aborigines stimulate two key questions. What are the dietary triggers (present and missing components and active doses) for the metabolic disturbances that lead to heart disease and diabetes, and is the phenomenon peculiar to the hunter–gatherers or is it more widespread throughout the world?

'Pre-contact' data relating to carbohydrate and lipid metabolism in Aborigines is a major gap in our knowledge. However, two avenues of investigation are open to us: (i) an examination of the few remaining groups living a traditional lifestyle; and (ii) the measurement of the effects of temporary reversion to the traditional lifestyle. In non-urbanized Aborigines, fasting glucose and cholesterol are low but fasting insulin levels are inappropriately elevated, i.e. higher than Caucasian men with a higher BMI. Fasting triglycerides are also higher than expected in view of their extreme leanness, physical activity and low fat diet (O'Dea *et al.* 1988). The plasma fatty acid profiles are also different, with higher arachidonic acid and lower linoleic acid (O'Dea & Sinclair 1985). When a group of 10 Aborigines with type 2 diabetes adopted the hunter–gatherer lifestyle for seven weeks, there was a striking improvement in all metabolic abnormailites for diabetes and major risk factors for coronary heart disease (O'Dea 1991*b*).

O'Dea (1991*a*) has reviewed the impact of westernization on Australian Aborigines, drawing upon 55 peer-reviewed articles. Her conclusion is that reduced physical activity and consumption of an energy-dense diet, characteristic of the western lifestyle, result in obesity (energy intake is greater than energy expenditure), and that the high fat diet, rich in refined carbohydrates, maximizes insulin resistance. When Aborigines make the transition from hunter–gatherer to a western lifestyle, many exhibit an android pattern of fat deposition (waistline fat deposits) and develop type 2 diabetes. They have impaired glucose tolerance, hypertriglyceridaemia, hypertension and hyperinsulinaemia. In effect, it appears that the insulin-resistant genotype selected for survival with a hunter–gatherer lifestyle becomes a distinct disadvantage. The metabolic pathways tuned to operate under conditions of high protein, low carbohydrate and low fat exacerbate insulin resistance when high-energy diets, rich in refined carbohydrates and fat, are consumed. Importantly, there are marked improvements in carbohydrate and lipid metabolism in both diabetic and non-diabetic individuals after temporary reversion to the traditional hunter–gatherer diet and lifestyle for periods as short as two weeks.

This is compelling evidence to support the hypothesis that diets high in fat and refined carbohydrates, particularly via their association with obesity, are responsible for the appearance of type 2 diabetes and other chronic diseases in Australian Aborigines, but how widely applicable are these findings to other populations in the world? In the United States, Van Dam *et al.* (2002) undertook a 12 year prospective cohort study in 42 504 male health professionals (between 40 and 75 years old, without diagnosed diabetes, cardiovascular health or cancer at baseline). They identified two major dietary patterns, 'prudent' diets (higher in vegetables, fruit, fish, poultry and whole grains) and 'western' diets (higher in red meat, processed meat, fried potatoes, high-fat dairy products, refined grains, sweets and puddings). After adjusting for potential confounders (including BMI, physical activity and smoking) they found that the western dietary pattern, combined with low physical activity or obesity, was associated with a particularly high risk for type 2 diabetes. The protective effect of wholegrain cereals appears to be greater in non-obese (BMI less than 30) than obese men (Fung *et al.* 2002).

Hegele (1999) has evaluated the genetic determinants of complex diseases and their intermediate phenotypes in Canadian Aboriginal communities. The frequency of type 2 diabetes or impaired glucose intolerance in Oji-Cree who live in northwestern Ontario is 50%, whereas it is only 1% in the Inuit from the Northwest Territories. There are some major differences in these two groups, in respect to lifestyle and diet; the Oji-Cree live on reservations, their lifestyle has changed from very physically active to very sedentary, and their diet has changed from predominantly wildlife, roots and berries to high animal fat, processed food provided by company stores. The Inuit, however, still adhere to a more traditional lifestyle and consume foods high in omega-3 fatty acids (e.g. at least three meals of arctic fish each week), but *ca.* 80% of adults smoke cigarettes. Candidate genes that are associated with increased risk for atherosclerosis were examined in the two Aboriginal communities and in white Canadians. Deleterious alleles whose frequencies differed between the ethnic subgroups were observed, and there were a higher number of deleterious alleles in Oji-Cree and Inuit than in white Canadians; this difference was greater than that observed between the two Aboriginal groups, which suggests that factors other than the alleles measured were the primary determinants of disease susceptibility. The major difference in the type of fat consumed suggests that omega-3 fatty acids may be important in protecting against disease.

The polygenetic and multifactorial nature of chronic diseases and the fact that certain genetic variants may be relatively more important determinants of susceptibility to CVD makes the interpretation of allele frequency data difficult. It is likely that other unmeasured, genomic variants may contribute to resistance to disease, and the total genetic component of variation is clearly due to the aggregate of several effects. The full impact of environmental effects on genotype–phenotype–disease progression is not yet appreciated (figure 1). Current medical practice places too great an emphasis on the use of drugs for disease prevention or control because it is an easy alternative to implementing changes in diet and lifestyle, but the latter can, in fact, be more effective, and has obvious physiologi-

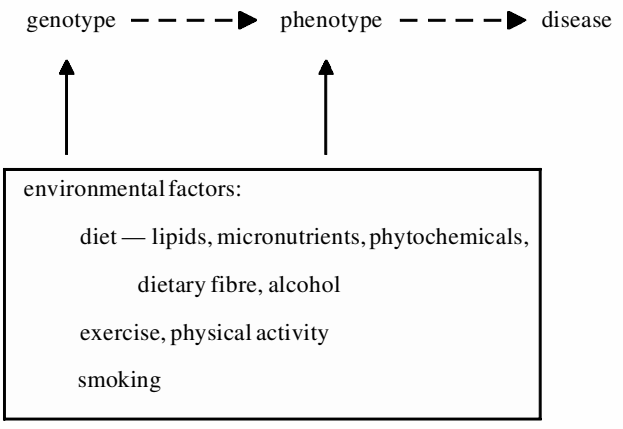

Figure 1. Genotype–phenotype–disease relationship.

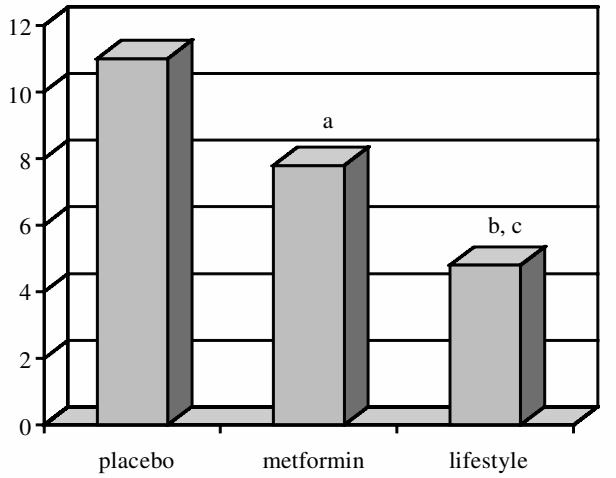

Figure 2. Effect of lifestyle intervention and metformin on incidence of type 2 diabetes (cases per 100 persons per year) in 'high risk' individuals. a, reduction in incidence compared with placebo (95% confidence interval (CI)) 31% (17 to 51); b, reduction in incidence compared with placebo (95% CI) 58% (48 to 66); c, reduction in incidence compared with metformin (95% CI) 39% (24 to 51).

cal and economic benefits. Knowler *et al.* (2002) compared lifestyle intervention with the drug metformin in 3234 adults selected as being at 'high risk' for type 2 diabetes. The goals for the lifestyle intervention were a 7% mass loss over 24 weeks by means of a low energy, low fat diet, and 150 min of physical activity per week. After a 2-year follow-up, the success rate was 38% for the mass loss and 58% for physical activity; the metformin compliance was 72%. The incidence of diabetes fell by 58% with the lifestyle intervention, and 31% with metformin, as compared with the placebo group (figure 2). This illustrates the power of positive changes in diet and lifestyle to prevent disease.

Since the 1950s, when the serum cholesterol lowering effect of omega-6 polyunsaturated fatty acids (PUFAs) was first recognized (Ahrens *et al.* 1954; Keys *et al.* 1957), PUFAs have become the cornerstone of dietary strategies to prevent CVD. However, there is mounting evidence that omega-3 fatty acids have wide-ranging effects on human health, and the omega-6 : omega-3 ratio in the diet may be particularly important. Many food products were developed in the mid-to-late twentieth century that were enriched with omega-6 fatty acids, partly aimed at improving the health of consumers and partly due to the widespread availability of oils rich in PUFAs. Agricultural practices also contributed to a rise in omega-6 and fall in omega-3 fatty acids. For example, in the USA 10 years ago, the ratio of omega-6 to omega-3 in free-range eggs was 1.3 : 1, whereas eggs from hens housed indoors had a ratio of 19.9 : 1, but by enriching the feed with fishmeal or flax, the ratio fell substantially (Simopoulos & Salem 1992).

Omega-3 fatty acids, particularly docosanhexaenoic acid (DHA), are essential for the development of retina and brain tissue in the premature infant. Clinical trials have demonstrated hypo-triglyceridaemic, anti-inflammatory and anti-thrombotic effects. Epidemiology has shown that they are associated with reduced risk of CVD and cancer. Simopoulos (1999) summarizes the characteristics of the Cretan diet and concludes that it is very similar to the palaeolithic diet, being low in saturated fat, very low in *trans* fatty acids, high in vitamins C and E, and high in omega-3 fatty acids. The Lyon prospective randomized single blind secondary prevention trial employed a diet rich in fish, fruits and vegetables, enriched with omega-3 fatty acids, and observed a more than 70% reduction in death rate (Renaud *et al.* 1995). However, this study has been strongly criticized; it is not possible to draw a definitive conclusion concerning the effect of fatty acids because of other changes introduced with the high fruit and vegetable intake.

More recently, reliable cross-national data of the prevalence of severe mental depression (Weissman *et al.* 1996) were used by Hibbein (2002) to examine the possible association between high fish consumption and lower prevalence of depression. Low plasma concentration of DHA predicts low cerebrospinal fluid (CSF) 5-hyroxyindolacetic acid (5-HIAA), the major metabolite of serotonin; a low concentration of CSF 5-HIAA is strongly associated with depression and suicide (Mann 1998). Hibbein is careful to point out that cultural, economic, social and other factors could confound this simple correlational relationship. In direct opposition to Hibbein's argument, Bosch (2002) argues that low serum cholesterol is associated with increased likelihood of suicidal behaviour, and that people living in countries with a high fish consumption tend to have a lower serum cholesterol and hence lower CSF 5-HIAA (Bosch 2002). There is obviously a need to test both these hypotheses by integrating data on cholesterol and DHA intake. However, the various observations on effects of omega-3 fatty acids illustrate their importance in the diet, in particular the need to increase the ratio of omega-3 to omega-6 fatty acids, both with regard to mental health (Adams *et al.* 1996) and preventing chronic diseases. Further research is required to understand underlying mechanisms, including the complex interactions with other dietary constituents, and differences in individual response. An intriguing finding was recently reported from a cross-sectional study examining married couples' risk of the same disease (Hippisley-Cox *et al.* 2002). Partners of people with specific diseases are at an increased risk of the disease themselves; the strongest link is with depression, with an odds ratio (adjusted for age, smoking and BMI) of 2.08, closely followed by peptic ulcer disease (odds ratio 2.01). This highlights the role of

Table 6. Incidence of most common cancers in England (1999) and possible dietary links.

		putative dietary and lifestyle modulators	
cancer	incidence	antagonistic	protective
breast	34 176	obesity in post-menopausal women; alcohol; processed and/or well-cooked meat	obesity in pre-menopausal women; omega-3 fatty acids (when vitamin E intake is low)
lung	31 067	tobacco smoking	fruits and vegetables
colorectal	28 451	obesity; alcohol; high intake of red and processed meat	physical activity; dietary fibre; fruits and vegetables; folate; calcium; vitamin D
prostate	20 842	animal products	selenium; lycopene; vitamin E

shared environmental conditions, notably diet. Although no information was collected on habitual diet, it is likely that the types of fat consumed by partners (and hence the omega-3 : omega-6 fatty acid ratio) might be similar.

Dietary factors are believed to account for *ca*. 30% of cancers in developed countries (World Cancer Research Fund 1997; Key *et al.* 2002). The cancer–diet hypothesis was derived from the following macroscopic population-level observations.

(i) Diets in industrialized countries are relatively rich in animal products, fats and sugar, and there are high rates of colorectal, breast, prostate, endometrial and lung cancer compared with developing countries where the diets are based on starchy staples with a low intake of animal products, fat and sugar.
(ii) Migrants from one country to another generally acquire cancer rates of the new host country.
(iii) In many industrialized countries, diets changed substantially during the second half of the twentieth century. Intakes of meat, dairy products, vegetable oils, fruit juice and alcohol increased, and starchy staples (bread, potatoes, rice and maize) fell. Over the same period of time, certain types of cancer have become more common, e.g. colorectal cancer has increased by *ca*. 35% in the UK since 1960.

The hypothesis has been supported by many higher-grade individual level case-control and cohort studies. Key *et al.* (2002) recently reviewed the effect of diet on risk of different cancers. The incidence of common cancers and possible dietary factors that may increase or decrease risk are shown in table 6. The general conclusions are as follows:

(i) obesity is associated with a higher risk of cancer of the oesophagus, colorectum, breast, endometrium and kidney;
(ii) alcohol increases the risk of cancers of the oral cavity, pharynx, larynx, oesophagus, liver and breast;
(iii) a high intake of fruits and vegetables decreases the risk of gastrointestinal (GI) tract cancers; and
(iv) the influence of meat, dietary fibre and vitamins is inconsistent.

In addition, certain non-nutrient components of plant foods (phytochemicals) have been shown to have a potentially protective effect against cancer (Johnson *et al.* 1994; Johnson 2002). They may act as antioxidants (e.g. carotenoids and flavonoids), increase the activity of phase 2 detoxification enzymes (e.g. isothiocyanates), inhibit the synthesis of nitrites by bacteria in the stomach (e.g. sulphur-containing compounds), affect signal transduction pathways and apoptosis (e.g. phytosterols), or have anti-oestrogenic effects (e.g. isoflavones). Although there is a growing body of research underway investigating the effects of phytochemicals, the supporting epidemiological data are not yet available.

There are a number of caveats to bear in mind when examining the relationship between diet and cancer. First, detailed investigations of the diets of individuals do not always support the findings of population studies: (i) because of confounding lifestyle and genetic effects; and (ii) because of the difficulty of collecting good quality, reliable dietary information. Second, temporal effects are critical, in that a diet consumed today may be very different from that consumed at the time the cancer first appeared. Prospective studies, such as the European Prospective Investigation into Cancer (EPIC), a large multicentre cohort study in 10 European countries (Riboli & Kaaks 1997; Gerber *et al.* 2002), should generate more informative data on diet and cancer. Dietary components that have been studied in connection with the cancer preventative properties of fruits and vegetables include vitamins A, C, E, folates, carotenoids, selenium, magnesium, polyphenols (e.g. quercetin, phytoestrogen and other flavonoids), glucosinolates (e.g. sulforaphane and other isothiocyanates), monoterpenes and sulphur-compounds found in allium vegetables (Gerber *et al.* 2002).

In the 10-year period 1989–1998, the age standardized incidence rates for cancer increased by 1.6% in men and 6.3% in women (CancerStats 2002). The fastest growing cancers in men are malignant melanoma (42%) and prostate cancer (38%), while in women it is kidney cancer (22%), non-Hodgkin's lymphoma (18%) and breast cancer (18%). Breast and prostate cancer occur with higher frequency in western societies and commonalities between them suggest similar aetiological risk factors. Grover & Martin (2002) propose that diet plays a significant causative role, citing changes in cooking practices that introduce higher levels of genotoxins, e.g. polycyclic aromatic hydrocarbons and heterocyclic aromatic amines formed when foods are cooked at very high temperatures, combined with a fall in the intake of protective factors. Most recently, there is considerable concern about the carcinogenic potential of acrylamide (Tareke *et al.* 2000), which is formed by the Maillard reaction from glucose and asparagine, a major amino acid in potatoes and cereals, at temperatures above 100 °C (Mottram *et al.* 2002; Stadler *et al.* 2002).

Consumer interest in food and concern over food safety issues has never been greater. There is growing sensitivity surrounding environmental contaminants, including acrylamide, dioxins and heavy metals, and natural bioactive components of the diet, such as phytoestrogens. There is also growing appreciation that risk evaluation must take into account differences in age, gender, lifestyle, diet, metabolism and genetic makeup. The dietary element is critical, bearing in mind the growing body of evidence demonstrating a protective effect from certain foods and dietary constituents. Environmental issues are high on the political agenda, and issues such as sustainability, organic farming and genetic modification are emerging as strong drivers for agricultural practices, which may in turn have a significant impact on raw food supplies. As lifestyle changes in the twenty-first century make consumers increasingly reliant on processed foods and fast food outlets, we should not forget the 100-year old vision of Atwater who emphasized the importance of the whole diet, and stated that 'for the great majority of people in good health, the ordinary food materials … make a fitting diet, and the main question is how to use them in the kinds and proportions fitted to the actual needs of the body' (Welsh 1994).

A recent evaluation of the impact of dietary guidelines based on target consumption of food groups (US Department of Agriculture 2000) suggests that adherence to the cluster of recommended nutrition-related behaviours may be associated with a lower risk of cancer, primarily for cancers of the colon, lung, breast and uterus in postmenopausal women (Harnack *et al.* 2002). Because obesity and physical activity are known to be important in carcinogenesis, these are included in the nutrition-related behaviour score, together with smoking, but this rather dilutes the efficacy evaluation of the dietary guidelines. The impact of diet will be different depending on the cancer site and individual genotype. As new and improved data on dietary composition, dietary patterns and genetic factors accumulate, it should be possible to refine dietary advice for cancer prevention, ideally on an individual basis.

Chronic diseases are unavoidable given the extended lifespan of humans in the twenty-first century that has resulted from the eradication of contagious diseases and major advances in medical treatment. They are not only due to inappropriate diet, although the consumption of energy-dense diets, especially those with low levels of omega-3 fatty acids and complex carbohydrates and not meeting the five-a-day fruits and vegetables target, are undoubtedly associated with increased risk. When coupled with an inappropriate lifestyle, the chances of becoming overweight or obese are magnified and hence the risk of type 2 diabetes, certain cancers and CVD rises considerably. Preventing or delaying chronic diseases requires a concerted effort by health professionals and consumers, focused on both diet and lifestyle.

6. THE TECHNOLOGICAL REVOLUTION—DEVELOPMENT OF -OMICS

> If I have seen further than others, it is by standing upon the shoulders of giants.
>
> (Isaac Newton, letter to Robert Hooke, 5 February 1676; see Peter 1977)

Population comparisons provide useful evidence for detecting putative environmental effects, such as dietary composition, on health, but depend on the quality and consistency of the data for disease prevalence and the ability to assess habitual diets. Nutrient requirements, optimal health and susceptibility to chronic disease are inextricably linked to genotype, and require the application of nutritional genomics technologies (Guengerich 2001; Elliott & Ong 2002). Post-genomics embraces an ever-growing number of terms, including microarrays, genomics, proteomics, transcriptomics, metabolomics, metabonomics and methylomics. The techniques are increasingly being used to investigate the health-promoting (or damaging) effects of food components by generating fundamental mechanistic information to explain phenomenological data. Post-genomic approaches are in their infancy because the techniques are new, and several complex technical issues need to be resolved before they will become routine. However, because of the great importance of the data they generate, the '-omics revolution' that has very recently emerged probably represents the most significant era of nutrition research. Sadly, this is not widely appreciated in scientific circles, as evidenced by the fierce competition for equipment and funding needed to take the nutritional sciences forward and the continuing fragmentation of food research among the various stakeholders. A national champion for nutrition research, with sufficient vision and political acumen to harness and exploit the exciting opportunities, has yet to emerge.

The major limitations currently faced include access to appropriate hardware and availability of suitable human tissue. The short-term solution to the latter problem has been to use tissue culture and animal models. Data handling and interpretation are further problems, requiring a dedicated team of bioinformaticians with an appreciation of biological issues. However, the key requisite for successful nutrition research is the ability to design and undertake appropriate experiments to demonstrate causality through testable hypotheses. Nutritionists must be trained to differentiate between inductive and deductive reasoning and not lose sight of the fact that the '-omics technologies' are a tool in this process, not a substitute.

Nutrient requirements depend upon a number of factors, as summarized in table 7. Deriving dietary reference values for population subgroups requires several assumptions: (i) the variation in physiological requirement follows a normal or symmetrical distribution or if skewed (e.g. iron) it can be dealt with using a transformation approach; (ii) the mean value for each nutrient is an estimate derived from the coefficient of variation; and (iii) the population is healthy. In the absence of specific indices of status, global changes in the expression of genes of known function in response to a nutrient deficiency (or excess) can be measured. The dominant technology for analysis of expression of individual mRNAs involves the isolation of mRNA, conversion to cDNA using reverse transcription and fluorescent nucleotide tags, and hybridization to probes on chips. Different dyes (e.g. red and green) are incorporated into samples collected from tissue exposed to different 'dietary treatments' and a direct comparison made using differential analysis. Blanchard *et al.* (2001) applied this technique to a rodent model of early zinc deficiency and reproducibly identified modest changes in the mRNA

Table 7. Factors affecting nutrient requirements.

host-related/physiological variables
gender
age: biological stage of growth
pregnancy
lactation
immune function
genotype: single nucleotide polymorphisms
dietary factors
physico-chemical form of nutrient
composition of the diet
interactions with other dietary constituents; availability for absorption
pathological states
metabolic disorders
neoplasia and other chronic diseases
infectious diseases
drugs
environmental factors
physical activity; exercise
climate
tobacco consumption
exposure to micro-organisms and chemical contaminants

abundance of intestinal genes that influence signalling pathways, growth, transcription, redox and energy use. The influence of dietary zinc supply on the expression of some of the genes, including recognized zinc-regulated genes (metallothionein 1, zinc transporter 2 and uroguanylin), was confirmed by real-time, quantitative PCR. This is a powerful new approach for identifying long-awaited markers of nutritional status for many micronutrients, needed to derive dietary requirements of nutrients for individuals and populations. The challenge that we currently face is the replacement of the model systems used so far (animals and cell cultures) with tissues from human *in vivo* intervention studies; where these are not easily accessible, surrogate markers need to be identified.

Another example of the application of microarrays to study the effect of diet on health is research on the role of dietary fibre in human health. Short chain fatty acids (SCFA) are produced by microbial fermentation of dietary fibre in the colon and are an energy source for colonic epithelial cells. They also induce pathways of cell maturation, including cell cycle arrest, differentiation and apoptosis, but the underlying mechanisms are unclear. Microarray technology of 8063 sequences was used to investigate genetic reprogramming of colon cells in response to SCFAs (Mariadason *et al.* 2000). High throughput analysis of gene expression profiling was used to characterize and distinguish the mechanisms of response of SW620 colonic epithelial cells to pharmacological and physiological inducers of cell maturation, including butyrate. Cell phenotype was examined and common patterns in altered profile of cell cycle gene expression observed, including G_o–G_1 cell cycle arrest, triggering of an apoptotic cascade, and upregulation of β-catenin-Tcf, but there was no overall consistency in the genes involved. The authors concluded that molecular profiling of the response to different agents could be used to design chemopreventive strategies for colon cancer, and that the databases, analyses, gene clusters identified, sequences, models of lineage-specific differentiation of colonic epithelial cells and response to other chemopreventive agents would be made available in order that the results of the experiments could be fully exploited. This illustrates another aspect of post-genomic nutrition, namely the importance of electronic sharing of data.

A third example of the use of microarrays, also pertaining to the GI tract, is the effect of commensal (non-pathogenic) flora on pathogen resistance and intestinal function. In recent years, it has become apparent that the molecular cross talk between commensal bacteria and intestinal epithelial cells plays a pivotal role in gut physiology. To begin to unravel the mechanisms, Hooper *et al.* (2001) used DNA microarrays to measure global intestinal transcriptional responses to the colonization of germ-free mice with *Bacteriodes thetaiotaomicron*, a dominant anaerobic bacterium found in the gut of adult mice and humans. This is one of the first studies to use molecular array technology and laser capture microdissection to investigate the impact of commensal bacteria on intestinal gene expression. Kelly & Conway (2001) comment on the most pronounced transcriptional change, namely enhanced expression of the small proline rich repeat protein 2a (sprr-2a) mRNA that was localized to the villus epithelium. Upregulation was reported to be 102- and 10.6-fold on the two sprr-2a probe sets (25 mer oligonucleotides), whereas quantitative PCR showed a 205-fold upregulation. This exemplifies the importance of quantitative methods, such as real-time PCR, to verify expression-profiling studies. This approach can be used to identify candidate genes and further experiments designed to disentangle cause and effect relationships.

Changes in gene expression may not, however, reflect changes in protein expression or function. To determine the consequences of changes in gene expression, we have to make use of proteomics. The technology is more complex than mRNA measurement, but is crucial to our understanding of molecular events because the levels of expression of individual proteins do not always correlate with levels of mRNA, and many important regulatory signals involve post-translational changes in proteins, e.g. phosphorylation and oxidation–reduction changes. Current technology involves the separation and quantification of proteins by two-dimensional (2D) gel electrophoresis and identification by mass spectrometry, usually with matrix assisted laser desorption ionization methods. Developments are underway to replace the 2D gels with protein separation using chips and microfluidics, or direct hybridization of proteins to specific antibodies. Phage display systems, where libraries of proteins are created and packaged in bacteriophage for expression, could be developed to produce large collections of specific antibodies for this purpose (Guengerich 2001). Perhaps the most important difficulty faced by nutritionists wanting to use proteomics is the inefficiency of current extraction procedures, especially for membrane-bound proteins. As the technology improves, proteomics will be able to generate data to explain the fundamental mechanisms underpinning the metabolism of nutrients and food components in individuals.

During the twentieth century, useful generalizations were made about the hereditability of most traits for 'average' dietary intakes, but the explanation for individual dif-

ferences in phenotype is linked to genotype, as first discussed systematically by Vernon Young over 20 years ago (Young & Scrimshaw 1979). There are in the order of two million common polymorphisms (Sachidanandam *et al.* 2001), of which perhaps 200 000 lie in the exonic (coding) region of genes. Developmental biologists believe that gene variants are essential for the adaptation and survival of life forms when challenged by changing environments. Undesirable mutations are thought to be 'silenced' by chaperone proteins, e.g. heat-shock protein 90 (hsp90), one of the most abundant proteins in animals, plants and fungi (Queitsch *et al.* 2002). Chaperones bind unstable proteins and buffer normal development in the face of destabilizing environmental factors, and mutations are therefore dormant. However, as humans age, proteins accumulate more and more damage and their chaperones have to deal with an increasing number of problems. Csermely (2001) suggests that improvements in housing, public health and medical care in the past century will reduce the frequency of environmental stresses and therefore mutations should remain tightly cloaked, but the opportunities to remove undesirable mutations through natural selection have been reduced and thus most of today's generation may carry more hidden mutations than our ancestors. Csermely suggests that when these silent mutations emerge they trigger degenerative diseases, such as cancer, heart disease and diabetes (Soti & Csermely 2002).

Twin studies are a useful means of separating environmental from genetic effects, and to identify the polymorphisms that affect nutrient requirements and susceptibility to diet-related diseases. They can be used to distinguish between the effects of 'normal' genes that directly affect a trait, such as blood pressure, from environmentally sensitive genes that modify how sensitive the trait is to external influence. For example, Birley *et al.* (1997) examined the plasma lipid (low-density lipoprotein level) response of twins given a low fat diet and observed a greater effect in homozygotes with a polymorphism at or near the GLYA locus on chromosome 4 that influences the sensitivity of plasma lipid levels to dietary fluctuations in fat intake. An overfeeding study conducted in identical twins demonstrated that genetic factors play an important role in the responsiveness to changing energy balance conditions; leptin receptor, beta$_2$ adrenergic receptor and glucocorticoid receptor gene polymorphisms were associated with an augmented clustering of metabolic abnormalities in response to overfeeding (Ukkola & Bouchard 2001). Extreme phenotypes, or 'experiments in nature', are also a powerful tool in human biology. O'Rahilly (2002) studied rare extreme human phenotypes of obesity and insulin resistance (figure 3) to identify the PPPIR3 mutation, involved in glycogen synthesis in striated muscle.

Nutritional genomics will provide data on how diet influences cellular processes, including gene and protein expression, post-translational modification, and actions of proteins at target site, and will reveal important single nucleotide polymorphisms and epigenetic effects initiated by the diet that increase an individual's risk for specific chronic diseases. The large body of data that will be generated must be handled using a systems biology approach; thus the latter is a resource that needs to be developed to support post-genomics technologies and to take nutritional science a quantum leap forward.

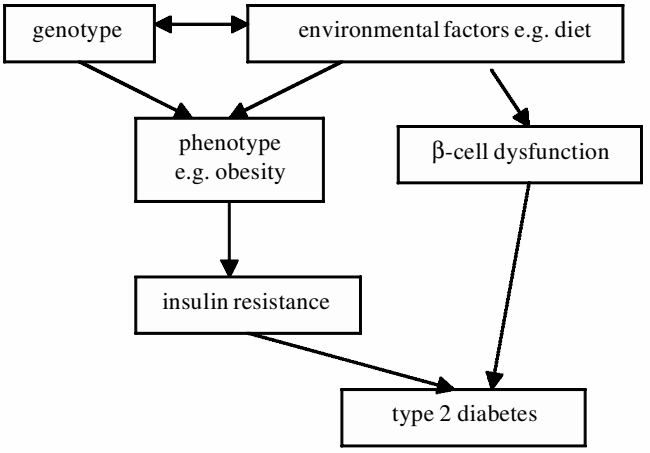

Figure 3. Example of interaction between genotype, environmental factors and phenotype.

7. NUTRIENT–GENE INTERACTIONS

The expression of phenotype is dependent on the combination of genotype and environmental exposure (figure 1). For example, a number of micronutrients and phytochemicals play a critical role in determining genomic stability on exposure to dietary carcinogens, activation/detoxification of carcinogens, DNA repair, DNA synthesis and apoptosis. Key nutrients include folate, vitamins C, D, E, zinc and selenium (Friso & Choi 2002); phytochemicals include carotenoids, flavonoids and glucosinolates. Dietary recommendations have traditionally been based on preventing deficiency disorders, and mainly restricted to nutrients, although some recent recommendations are encompassing protection against chronic disease, e.g. the vitamin C requirements in the USA are based on consideration of its antioxidant properties as well as its role in preventing scurvy (Institute of Medicine 2000). Future recommendations must take into account prevention of degenerative disease and slowing of the ageing process, both partly caused by damage to nuclear and mitochondrial DNA. Research on the biology of ageing has gathered momentum in the past decade with the introduction of genetic approaches in model systems. It has long been known that energy restriction increases the lifespan and attenuates chronic diseases of ageing in rodents; it decreases plasma glucose concentration and insulin-like growth factor and this information is being used to target a new category of drugs that could prevent or postpone diseases of ageing (Longo & Finch 2003). There is a growing body of evidence implicating a number of key regulators in the ageing process: gene silencing, cumulative damage inflicted by reactive oxygen species, effectiveness of DNA polymerases and germline genomic stability (Martin *et al.* 2003), and the challenge for nutritionists is to determine the impact of diet on these mechanisms.

Examples of common polymorphisms that may affect dietary requirements are shown in table 8. An interaction has been demonstrated between folate status and a mutation of a key enzyme in one-carbon metabolism, methylenetetrahydrofolate reductase enzyme (MTHFR C677T). The reduced MTHFR activity leads to an increased level of cytosolic 5,10-methylenetetrahydrofolate available for thymidylate synthesis, which may protect cells from DNA damage induced by uridylate misincor-

Table 8. Examples of common polymorphisms that may influence dietary requirements.

candidate gene	estimated allele frequency in the UK	phenotype/proposed site of metabolic effect
methylenetetrahydrofolate reductase (*MTHFR*)	677T 37%	hyperhomocysteinaemia; DNA methylation
glutathione-*S*-transferase	M1 *ca*. 50%	reduced ability to detoxify environmental or endogenous toxins; higher phytochemical intake beneficial
angiotensin-converting enzyme (*ACE*)	DD 40%	raised blood pressure
apolipoprotein E (*APOE*)	E4 20%	raised cholesterol
apolipoprotein A (*APOA*)	GG 3%, GA 26%	type of dietary fat affects serum cholesterol, depending on genotype and sex
ATP-binding cassette transporter 1 (*ABCA1*)	R219K 46%	carriers have *lower* triglyceride levels and coronary artery disease
peroxisome proliferator-activated receptor-γ-2	PPARγ2Pro12Ala 32%	obese carriers have lower HDL-cholesterol
β_2-adrenergic receptor (*BAR-2*)	Arg16 35%	women have higher BMI and more pronounced fall in NEFAs after exercise
insulin-like growth factor (*IGF-1*)	192 bp 88%	increased risk of type 2 diabetes *in non-carriers*
vitamin D receptor (*VDR*)	BB 17–23%	reduced calcium absorption
haemochromatosis (*HFE*)	C282Y 5–19%	increased iron absorption

poration. Thus folate-replete men who are homozygous for the TT mutation are reported to have a twofold reduction in risk of colorectal cancer compared with wild-type or heterozygous individuals (Ma *et al.* 1997; Chen *et al.* 1999; Slattery *et al.* 1999). However, homozygotes with inadequate folate intake have elevated plasma homocysteine, an independent risk factor for atherosclerosis (Cortese & Motti 2001), which is associated with increased risk of neural tube defects (Ueland *et al.* 2001) and colon cancer (Levine *et al.* 2000). Folate has also been implicated in the development of breast cancer. In a case-control study Campbell *et al.* (2002) found that the *C677T* mutation was associated with an increased risk of early onset breast cancer, which contradicts the observation by Sharp *et al.* (2002) that low MTHFR actually protects against breast cancer. The latter was a small case-control study in a Scottish population, and it raises a question about diet–gene interactions; neither study attempted to measure folate intake or status, and it is possible that this may explain the conflicting findings, as is the case with colorectal cancer. The importance of characterizing both the diet and the nutritional status in addition to genotype cannot be overstated.

The health-promoting effect of fruits and vegetables is related to their micronutrient contribution to the diet. Glutathione-*S*-transferase plays a key role in the removal of carcinogens through adduct formation; thus higher intakes of dietary components that enhance its activity, such as isothiocyanates, may be protective. There is convincing evidence of an interaction between glucosinolate consumption and *GST* genotype (Le Marchand 2002). Smokers null for *GSTT1* and *GSTM1* with evidence of isothiocyanate excretion had a much-reduced risk of lung cancer compared with individuals who had a low consumption of brassicas (London *et al.* 2000).

Several polymorphisms are implicated in lipid metabolism, but the effect of diet on risk of CVD is complex. Recent data on *APOA* polymorphisms and PUFAs illustrate the interaction between type of dietary fat and genotype that affects blood cholesterol (Ordovas *et al.* 2002*a*). Results from the Framingham study show that dietary fat modifies the effect of the *–514T* polymorphism of the hepatic lipase (*HL*) gene; in individuals consuming less than 30% energy from fat, the T allele is associated with higher high-density lipoprotein (HDL) cholesterol, and TT subjects appear to have an impaired adaptation to higher animal fat diets that could result in higher cardiovascular risk (Ordovas *et al.* 2002*b*). Obese, but not lean, carriers of the *PPARγ2Pro12Ala* polymorphism have lower HDL-cholesterol and a tendency towards hyperlipidaemia (Swarbrick *et al.* 2001); thus they are at higher risk of CVD. The proposed mechanism is that the Ala isoform of PPARγ2 is less effective at activating target genes, including lipoprotein lipase, the activity of which is strongly correlated with plasma HDL levels. The effect of the Ala allele on blood lipid profile may only be revealed in the obese state due to the larger adipose tissue mass and therefore relative abundance of the fat-specific PPARγ2 isoform. Natural ligands for the PPARγ2 receptor include fatty acids; therefore the effect of this common variant may be altered by the character of the diet, particularly the polyunsaturated : saturated (P : S) fat ratio. Luan *et al.* (2001) demonstrated that when the dietary P : S ratio was low, the BMI in the Ala allele carriers was greater than in the Pro homozygotes, but when the P : S ratio was high the BMI was significantly lower. Because most people consume a diet with an intermediate P : S ratio, the effect of this polymorphism will not be observed unless the results are stratified according to P : S ratio. Exercise is another important modifier of diet–gene interactions. Meirhaeghe *et al.* (2001) studied common polymorphisms in the *BAR-2* gene in relation to obesity. After adjustment for age, sex and smoking, a significant interaction was observed between *BAR-2* genotype and BMI in determining fasting nonesterified fatty acid (NEFA) concentrations, but the level of physical activity significantly modified the effect. These gene–nutrient interactions emphasize the difficulties encountered when examining the effect of common polymorphisms on health outcomes. In the absence of good quality data on dietary and lifestyle

factors, it is quite possible to draw incorrect conclusions or to miss important genotype–phenotype–disease associations.

The list of common polymorphisms that impact on health is steadily growing. Some mutations are desirable: for example, the wild-type for *IGF-1* is the 192 bp mutation (allele frequency 88%), but it is non-carriers who have an increased risk of type 2 diabetes (Vaessen *et al.* 2001). There are many, as yet unidentified, genetic factors that predispose individuals to osteoporosis, and the importance of the effect of a vitamin D receptor (VDR) polymorphism that reduces calcium absorption (Ames *et al.* 1999) is not clear. There is much debate surrounding iron nutrition and optimal levels of intake; the influence of mutations of the haemochromatosis (*HFE*) gene on iron absorption and levels of iron in body tissues is attracting attention, but penetrance appears to be low (Beutler *et al.* 2002). Nutrition is in its infancy with regard to the study of diet–gene interactions and a great deal of research must be undertaken before we can fully understand the interrelationships between genotype, diet and risk of chronic disease.

Although, in specific instances, polymorphisms in single genes explain some of the variation in phenotype, the majority of chronic diet-related diseases are polygenic in origin; thus their aetiology is very complex. To undertake association studies, information is needed on candidate genes and common polymorphisms in these genes, bearing in mind the fact that common variants have more clinical impact than highly penetrant but rare alleles. The genes are selected on the basis of biological hypotheses; usually this means being involved in a relevant metabolic pathway. However, diet–disease interactions are difficult to study because of the uncertain yet lengthy gestational period of chronic diseases and the, as yet unresolved, ethical questions surrounding the genotyping of individuals (Beskow *et al.* 2001). Another major problem with association studies is the lack of information on diet and physical activity, both of which have a major impact on phenotype and disease progression, as already discussed. Nutritional genomics offers a powerful approach for elucidating the polygenic basis for variations in nutrient requirements and risk of degenerative disease. However, we should be aware of the challenges before us, including the need to find suitable biomarkers for gene expression studies because of the inaccessibility of many tissues and cells in human volunteers and the inability to measure the expression of membrane-bound proteins using the current proteomics techniques. Prospective epidemiological trials will continue to provide information about the effect of diet on chronic disease and phenotype–genotype relationships, but interactions are often most evident in studies employing a metabolic challenge. Appropriate experimental protocols are a vital component of hypothesis testing and the skills required to undertake human dietary intervention studies should not be underestimated.

It is vital that vision and commitment, over a sustained period, are provided to develop and fully exploit post-genomic opportunities for nutrition research. In parallel, it is imperative that food composition and dietary intake are characterized by studying consumer behaviour and *in vivo* metabolism. Although perceived by some to be less intellectually challenging than molecular genetics and cell biology, good quality data on dietary intake, eating behaviour, and whole body metabolism of nutrients and phytochemicals are absolutely essential for the understanding and interpretation of nutritional genomics data and to provide dietary advice for improving health.

When dealing with complex biological systems, the ultimate example being the human organism, it is imperative that researchers use a systems-oriented approach to interrogate and interpret their data. Understanding biological systems requires the integration of experimental and computational research. Data must be collated on the structures of systems (gene/metabolic/signal transduction networks and physical structures), dynamics, methods of control (including homeostasis), and the development of suitable models and methods to generate systems with desired properties. Computational systems biology is reviewed by Kitano (2002). Understanding the food/nutrition/health continuum not only requires a multidisciplinary approach (figure 4), but it could also be the catalyst that promotes the study and understanding of integrative systems biology needed to fully harvest the fruits of genome research.

Various estimates suggest that the costs of chronic diseases in the UK that have a dietary link are in excess of £15 billion (table 9); this excludes less common conditions that can be modulated by diet, such as arthritis. Although diet is only part of any preventative strategy, a significant proportion of illnesses could be delayed or avoided through improvements in diet. Even taking into account 'five-a-day' initiatives in schools and the community (to increase fruit consumption), for example the latest Department of Health £10 million programme, only a very small fraction of expenditure is directed towards prevention. There are some encouraging signs, for example the updated plans for the Welfare Food Scheme (£142 million per year), renamed 'healthy start', have broadened the help given to low-income parents to provide fruit to children and pregnant and lactating mothers (Department of Health 2002). A recent report of a working party of the Royal College of Physicians (2002) underlines how under- and over-nutrition are closely linked to illness and disease processes, and describes the multidisciplinary approach required by clinicians in both preventative and therapeutic nutritional care in the community and the hospital; the report is described as a 'wake-up call to the medical profession to take clinical nutrition seriously'. The escalating problem of obesity and diabetes and rising incidence of some cancers, including prostate and GI tract cancers, indicates that food selection and dietary patterns in a growing number of people are not optimal. There is considerable debate about the precise dietary advice that is required, and the methods to be employed to motivate individuals to introduce dietary change. Perhaps the most controversial topic is the role of the food industry, including manufacturers, suppliers, retailers and caterers: to what extent can they be held responsible? They are, after all, in the business of selling food to consumers. What should their role be in improving the health of the nation? Does the government need to introduce legislation, such as a tax on fast food to improve our diet? Comparisons are often made with the tobacco industry, where public health advice and legislation have been singularly ineffective at reducing cigarette smoking. This

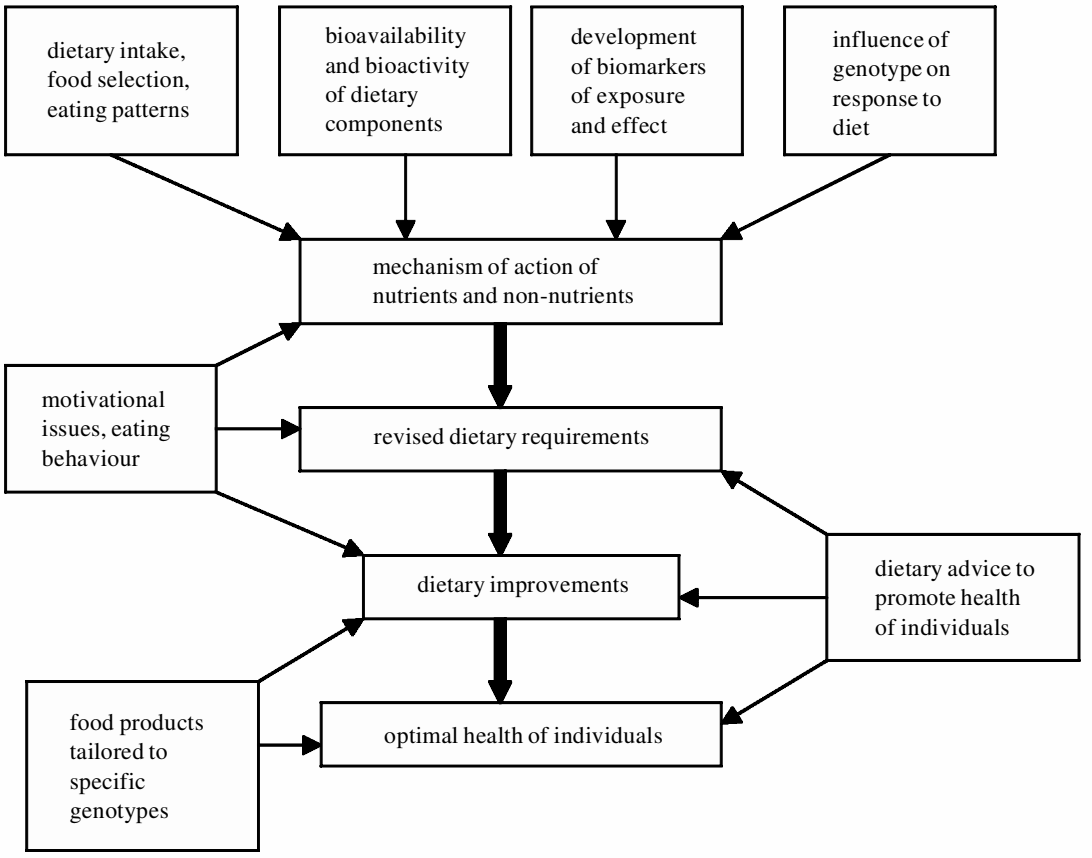

Figure 4. Nutrition research: a roadmap for the twenty-first century.

Table 9. Estimated NHS expenditure (total budget *ca*. £40 billion) on diet-related diseases and disorders in the UK.

disorder	relevant information	annual cost
type 2 diabetes	currently affects 1.4 million people in the UK; predicted to double by 2010	£5.2 billion
cancer	difficult to quantify costs; *ca*. 6–9% of total NHS budget is spent on cancer treatment, 70% of which is during the terminal stages	£2.4–3.6 billion
obesity	responsible for 30 000 premature deaths per year	£2.5 billion
coronary heart disease	less than 1% (£11.6 million) spent on prevention	£1.6 billion
stroke	7.7 million lost working days	£1.4 billion
osteoporosis	affects three million people in the UK; it is estimated that 61 million Americans will suffer from osteoporosis by 2020	£900 million
iron deficiency anaemia	affects 30% of the world's population	£25 million

is not, however, an appropriate comparison because unlike tobacco, food is essential for life, and the use of tobacco relates to an addiction problem, whereas food is not addictive: or is it?

The new era of nutrition research has much to offer the UK population, with respect to both quality of life and financial gain. However, taking nutrition research a quantum leap forward involves significant investment. Therefore, we need to consider establishing a national nutrition programme involving all the research councils, major funding agencies, and governmental and non-governmental departments. Regional nutrition strategies for Wales (Food and Well Being) and Scotland (Draft Diet and Nutrition Strategy) are being developed by FSA Wales and FSA Scotland, respectively, in partnership with regional health organizations and local government. These, quite rightly, address health issues specific to the region, but where is the equivalent activity for England and Northern Ireland? Most people in the UK live in England, where inequalities in health are likely to be more pronounced than in Scotland or Wales, presenting an even greater challenge. A national nutrition strategy in conjunction with regional nutrition initiatives would harness and direct existing efforts, generate a coordinated research programme and ensure national funding is optimally deployed. Clearly, this initiative is justified by cost alone, although quality of life should be a high priority for the twenty-first century. Whatever emerges as the key driver, enforced improvements in diet (aimed at preventing diet-related diseases) will eventually be unavoidable as the UK will not be able to afford the necessary health care resulting from inappropriate lifestyles and diet. Vision and

commitment are required at the most senior level to introduce a longer-term strategic approach, rather than waiting until the emergence of a financial crisis.

The UK situation is no different to that faced by many other countries, in that there is a growing recognition that modern-day nutrition research has much to offer, but the potential rewards will only be realized with concerted effort from within the nutrition community and support from outside. Therefore it may be timely for more academics to step outside laboratories and libraries and enter the political arena to raise the profile of nutritional science. The justification for introducing preventative approaches to chronic disease through diet and lifestyle has been clearly articulated by nutritionists, but ultimately the drive for dietary change needs to come from consumers. This would stimulate the food industry to introduce healthier products, but the process could be accelerated if the industry were more proactive in its product development, marketing and pricing strategies to encourage consumers to select healthier diets. The knowledge gained from appropriately directed and coordinated human nutrition and food research can help individuals achieve their maximum potential and live a long and healthy life. It must, however, be accompanied by widespread changes in attitudes and practice in the home and school environment, local communities, institutions, government, healthcare, marketing and media activity, and, last but not least, the food and catering industries.

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