Foreword

Increasing knowledge of the relationship between diet and health leads to new insights into the effects of food ingredients on physiological functions and health. These insights generate interest among scientists, health care providers and consumers, and in turn stimulate the food industry to match consumers’ desire for short- and long-term health benefits through food products that promote health and well-being and reduce the risk of chronic diseases. These foods are called functional foods. The term functional food emerged about a decade ago in a number of countries, e.g. Japan and the United States, and was stimulated and supported by research on the physiological effects of food components and their consequent health benefits.

Despite the absence of a universally accepted definition for functional foods, there is growing general agreement that some foods and beverages have beneficial effects beyond normal nutrition. Functional foods have a future. However, the success of functional foods among consumers will depend on their interest and confidence in these products and will require the accurate communication of their health benefits as well as a favourable and transparent regulatory framework for approval of the new products and their associated health claims.

In 1995, the International Life Sciences Institute (ILSI) took the initiative in organising the first international symposium on functional foods, East–West Perspectives on Functional Foods, held in Singapore. The 2001 ILSI symposium discussed in this supplement, Functional Foods: Scientific and Global Perspectives, built on this by reviewing the current global status of functional foods and the scientific basis for biomarkers related to the enhancement of function and the reduction of disease risk. The symposium, which took place in Paris, also discussed the issues of human genetic variability and the safety of functional foods, as well as communication requirements from the scientific, consumer and regulatory points of view. Finally, the conference provided a future outlook on new trends in functional food science.

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Summary Report

Functional Foods: Scientific and Global Perspectives

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Key symposium messages

Session I. Global View on Functional Foods

The symposium opened with a review of the functional food concepts in place in various regions around the world. This global overview included Asia, Europe, Latin America and North America. The session touched on differences in culture and beliefs with respect to foods, functional foods and medicines, and their impact on health and disease.

In Asia, where functional foods have been regarded as an integral part of the culture for many years, there is a firm belief that foods and medicine come from the same source and serve the same purpose. However, scientists and regulators have only recently begun to agree that the functionality of functional foods should be found in whole foods rather than in their individual components. In Japan, research on functional foods started in the early 1980s. In 1991, a specific regulatory framework concerning Food for Specific Health Use (called FOSHU) was introduced, which made it possible to make limited health claims after receiving approval from the Ministry of Health. The key to success for food manufacturers is to develop products that are accepted by consumers and are consistent with the consumer’s understanding and appreciation of functional foods within the existing culture. Because the state of a person’s health may range from optimal to a state of disease, it is believed that functional foods have a major role to play in all states of health, including maintaining health and preventing disease.

In contrast to Asia, the concept of functional foods in Europe is relatively new, and at present there are no Europe-wide regulations in place. In developing health claims legislation, the EU has adopted a working definition resulting from a European consensus published in 1999: ‘a food can be regarded as functional if it is satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way which is relevant to either an improved state of health and well-being, or reduction of risk of disease’.

The target of functional foods is seen as clearly different from that of drugs, which are aimed at preventing or curing diseases.

The scientific underpinning of health claims may derive from experimental studies and/or epidemiological and intervention studies where the use of biomarkers is regarded as a key element. The EU PASSCLAIM project (Process for the Assessment of Scientific Support for Claims on Foods) is intended to provide industry, academics, consumer groups and regulators with the means to evaluate the scientific basis for health claims. Although there is increasing demand by the European consumer for healthier food products, so far few functional food products have actually reached the market place. This is due partly to the fact that developing adequate scientific support for health claims can be relatively expensive and time-consuming. Moreover, some food manufacturers struggle with how best to communicate diet- and health-related information to consumers.

Research on food and nutrition has been an important topic in the EU Framework Programmes for Research and Technology Development of the European Commission. In the 1990s a significant number of EU projects addressed issues such as fibres and pro- and prebiotics, whereas more recent EU programmes focus on areas such as antioxidants, vitamins and phyto-oestrogens, as well as the socio-economic aspects of nutrition and health.

Although there is no specific definition of functional foods in Latin America, the concept of functional foods is presently under discussion in several Latin American countries. Brazil is in the process of developing health claims, and although Argentina does not have health claims regulations in place, it does approve claims on a case-by-case basis. Provided there is adequate scientific validation, both countries have approved nutrient function and disease risk reduction claims for specific food products. Usually, locally conducted research to support product efficacy for health claims approval is requested.

The Latin American population suffers from diseases of nutrient excess as well as nutrient deficiency. Micronutrient

Abbreviations: COX-2, cyclo-oxygenase-2; CVD, cardiovascular disease; FOSHU, food for specific health use; GI, gastrointestinal; ILSI, International Life Sciences Institute; NOAEL, no observed adverse effect level; PASSCLAIM, Process for the Assessment of Scientific Support for Claims on Foods; SYNCAN, Synbiotics and Cancer Prevention in Humans.

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deficiencies exist for I, Fe and vitamin A, and sub-optimal intakes of Zn, folate, Ca and Se are reported. Nutritional excess includes energy, fats, carbohydrates, cholesterol, salt and sugar, in addition to decreased physical activity. There is thus a need in Latin America for functional foods that contain specific micronutrients through fortification, processing and genetic engineering. Although most consumers in the region are not aware of functional foods, they are concerned with their diet and health. In this respect, ‘natural foods’ are perceived as healthier than processed foods. Although the consumer associates food with healing and curing diseases, there is a clear distinction between the roles of foods and drugs.

It was stressed that basic nutrition research on the health impact of locally produced products such as grains and fruits in Latin America is inadequate. Epidemiological and clinical research is needed to correlate regional foods/diets and diseases. Also needed is a better understanding of the genetic background of the populations and what influence this may have on health and disease states.

In the United States and Canada there is also no universally accepted definition of functional foods, but 60% of the people select foods they believe are functional. The present understanding of the US population appears to be that functional foods include both nutrients and non-nutrients. Foods and drugs are clearly distinguished by their intended use and presentation, as well as by their targeted population. In the United States, functional attributes can be communicated through health claims, structure–function claims, and nutrient content claims. The Food and Drug Administration must approve health claims, and fifteen claims have been approved to date. These claims describe the relationship between a food component and a disease or health-related condition. The approval of claims has been based on an extensive review of existing scientific literature, in the form of an authoritative statement of a scientific body of the US government or the National Academy of Sciences. Nutrient content and structure–function claims are clearly defined in the regulations and do not need to be approved by the Food and Drug Administration. Canada also has undertaken initiatives to establish nutrient and health claims regulations similar to those in the United States.

Although both scientists and regulators are gaining more experience and scientific understanding of health and nutrition, the complexity of the issues continues to grow. Recognising the role of food consumption patterns in growth, development and disease risk is important to understanding how diet influences these areas. There is a myriad of possible interactions between specific dietary components and specific genetic pathways involved in health. Understanding these interactions will provide guidance in establishing dietary recommendations and communicating benefits to targeted populations.

Session II. Scientific Basis of Biomarkers and Benefits of Functional Foods: Enhancement of Function and Reduction of Disease Risk

The second session reviewed the scientific support currently available for biomarkers that indicate enhancement of physiological function and disease risk reduction. Regarding enhancement of physiological function, the session included gastrointestinal (GI) physiology, the immune system, physical performance, and behavioural and psychological functions. Regarding disease risk reduction, the session focused on biomarkers for evaluating the effects of functional foods on the risk of cardiovascular disease (CVD), obesity/diabetes, cancer and osteoporosis, all elucidated with examples. The need for well-designed placebo-controlled studies in target populations was discussed, as were the identification and validation of appropriate surrogate biomarkers. Session highlights follow.

GI tract physiology and functions are related to meal-induced responses and longer-term adaptive changes, to the mechanisms of nutrient absorption and digestion, and to the impact on metabolic effects in other organs. The digestion and absorption of food are regulated by several factors such as the hormone cholecystokinin, which is released in response to a meal, thereby preparing the GI tract to digest food and ensuring a steady rate of nutrient absorption. Cholecystokinin also signals short-term satiety and can be regarded as a mediator of satiety and feelings of hunger. A second example focused on the digestion and absorption of carbohydrates and their impact on glucose, insulin and energy metabolism. The role of non-digestible fermentable carbohydrates for GI function, including the microflora of the large intestine, and potential systemic consequences (e.g. on the immune system) highlighted the importance of this organ system. Also addressed was the role of viscosity in relation to the metabolic effects of soluble dietary fibres on nutrient absorption rate, plasma cholesterol and glycaemic response. Research was called for to elucidate the bioavailability of bioactive compounds, the role of the food matrix, and health-related outcomes originating from modulation of the gut microflora.

The functioning of the immune system and its role in protecting the host were reviewed, as were factors such as the balance between pro- and anti-inflammatory effects, inter-individual variations and available biomarkers. Although it is clear that individuals with sub-optimal immune responses are more susceptible to infectious agents, it is not clear how modulation of the immune function in healthy individuals relates to susceptibility to infections. Food components can play a key role in maintaining optimal immune function; undernutrition appears to impair this function and, consequently, susceptibility to infections. Animal and human studies have demonstrated that optimising food intake through nutrient fortification can restore resistance to infections but that excesses of particular nutrients can lead to impaired immune function. Moreover, specific dietary components can increase a particular immunological parameter while decreasing another. Among the most studied nutrients in this regard are vitamin A, vitamin E and Zn. There is emerging evidence that probiotic bacteria may improve host immune function, although, at present, human data are still controversial and the relevance of such immune modulations for human health is unclear. To correlate dietary intervention, immune function and consequent health
impact, research should also focus on whole-body parameters, such as the incidence of infections, in addition to individual immune parameters.

During the last century there were significant changes in elucidating the role of diet in exercise and physical performance. It was shown that exercise-induced reductions in muscle glycogen correlate well with the development of fatigue and that the intake of carbohydrates (versus protein or fat) improves performance. Nutrition research to identify nutrients that support energy metabolism, promote fluid balance, increase muscle mass and improve overall performance (e.g. caffeine, creatine, ribose, certain amino acids and L-carnitine) led to the development of tailored foods and drinks that are easily digestible, help sustain the exercise load and improve the recovery of athletes from intense exercise. So far it is recognised that sports nutrition research is, in scientific terms, the best-established functional food area. Accordingly, measurement techniques (for gastric emptying, rate of intestinal absorption, appearance of substrates in blood and subsequent storage) and laboratory exercise protocols that measure performance accurately have been developed and validated. As in other functional food areas, solid scientific underpinning of product claims is a prerequisite.

Interest in functional foods for the enhancement of behavioural and psychological functions is targeted to foods that can influence appetite and satiety, vitality, stress and other subjective states of mood and well-being, cognitive and mental performance, and sleep. In principle, biological markers for behavioural and psychological responses could be identified, but the complexity of these responses would make their identification far from simple. Because of progress in, for example, the field of appetite and satiety control via specific GI and metabolic responses, and in mood research via changes in plasma profiles of metabolites, these areas would present good opportunities for further research. Food intake and hunger rating profiles are often used as biomarkers for appetite and satiety. In this respect, it has been shown that proteins increase satiety more than carbohydrates and fats. There also is a clear need to distinguish between short-term postprandial responses to food ingestion and long-term effects of dietary adjustment, because they require different research methodologies. Research related to cognitive performance needs psychobiological assays. Several types of trials have measured different abilities, but no unambiguous general biomarkers are available. It is necessary to ensure that foods that provide improvements for one phenomenon do not exert deleterious effects on other bodily functions. On balance, there is no clear conceptual framework to develop and assess the efficacy of foods to improve behavioural and psychological functions, and there is a need for markers that represent real-world situations.

There appear to be many potential biomarkers for chronic diseases such as CVD, diabetes, cancer and osteoporosis, but both their ease of use and level of validation may vary significantly. It was proposed that four critical features should characterise effective biomarkers for disease risk: appropriate response in clinical or dietary trials, consistency with epidemiology, appropriate response in hereditary disease states, and plausibility of mechanism. When these criteria are applied to the plethora of biomarkers that have been proposed for CVD, only blood pressure and blood cholesterol levels stand out as valid markers. However, arterial intima-media thickness also appears to be an appropriate non-invasive marker for CVD with important potential use in dietary interventions, e.g. its positive association with blood homocysteine levels. Various indicators of endothelial function have been identified (e.g. vascular cell adhesion molecule 1, intercellular adhesion molecule 1 and p-selectin), but there is little evidence that changing these indicators will affect the disease risk. Markers are also available for CVD other than atherosclerosis, and in this context the measurement of thrombotic components, inflammatory markers and arrhythmia were highlighted.

There is an urgent need for efficacious functional foods for the control of obesity in view of the scope of the obesity epidemic, which is paralleled by a corresponding growth in type 2 diabetes. There are various non-invasive markers of obesity (e.g. BMI, dual-energy X-ray absorptiometry scanning, bioelectrical impedance, and computerised tomography and magnetic resonance imaging scanning for visceral obesity). Fat replacers, fat-binding agents and ingredients that increase energy expenditure provide clear opportunities for developing functional foods in this area. It has been suggested that specific fatty acids such as medium-chain fatty acids may have a role in weight reduction. However, there is no conclusive evidence of their efficacy, and GI implications may limit their incorporation into diets at higher levels. Results at the cellular level indicate that high Ca concentrations may increase fat oxidation, but as yet there are few supportive in vivo data. A potential promising area may be functional foods that increase satiety. Foods also can be used to reduce the risk of diabetes, and a variety of biomarkers can be applied for their evaluation (e.g. oral glucose tolerance tests, fasting blood glucose, insulin levels and insulin sensitivity). At present, however, there are few well-executed nutritional studies in this field.

In the area of bone health and osteoporosis, many of the valid biomarkers, e.g. fracture frequency and bone mineral content/density, require long-term intervention. Although bone mineral content measurements, for example, are relatively simple and non-invasive, two to four years of intervention are needed to provide meaningful data. Shorter-term markers are based on the measurement of Ca metabolism/excretion or on urinary markers of bone formation and resorption. A new technique based on $^{41}$Ca tracer technology appears to provide a highly sensitive and direct measure of calcium release/bone loss and requires relatively small numbers of subjects to achieve statistical power. Opportunities for developing functional foods in this area include in particular those that enhance Ca resorption, such as casein phosphopeptides, whey protein concentrates and non-digestible oligosaccharides.

In the field of cancer risk a number of potential biomarkers have been identified, especially for colorectal cancer, including mucosal markers (e.g. cyclo-oxygenase-2 (COX-2), DNA repair capacity, microsatellites), faecal markers (e.g. calprotectin, AP-1 activation, cytotoxicity,
Session III. Human Genetic Variability and Safety of Functional Foods

Emerging information about genetic variation and its impact on human health makes this an exciting new area related to understanding the effects of functional foods. Single nucleotide polymorphisms occur frequently, but there is little evidence that such variation in genetic expression explains a significant portion of the variability seen in the response to disease risk factors, especially those linked to dietary components. Nevertheless, the emerging information on the impact of genetic variability signals a significant potential for research. The difficulty of showing actual health effects from nutrients was acknowledged. For example, a recent trial with vitamin E and a multifunctional supplement in elderly individuals did not show any improvement in infection rates. In addition, elevated homocysteine levels are associated with increased risk for CVD, but evidence of direct causality is lacking. Variation in the gene coding for methylenetetrahydrofolate reductase, a folate-dependent enzyme responsible for remethylation of homocysteine to methionine, has been examined as a way of addressing the causality issue. The majority of epidemiological studies examining individuals with this mutation and the resulting elevation in homocysteine, however, did not find an association with CVD risk. Nevertheless, evidence is emerging that elevated homocysteine levels are positively associated with carotid artery intima-media thickness, which could be used as an intermediate marker for the progression of atherosclerosis. The benefit of folate ingestion in reducing homocysteine levels is complicated by variations in the bioavailability of different folate isomers (variations in the length of the glutamate chain). Pooled analysis of all available observational data responding to well-defined criteria may be the best way to establish true relationships.

The traditional food additive safety assessment approach of establishing a 'no observed adverse effect level' (NOAEL) may not be appropriate for assessing the safety of whole foods, including functional foods. Whole foods are complex mixtures, and it is difficult to generate acute effects, as is expected with the NOAEL approach. Moreover, this approach is based on the assumption that no effect is the best outcome, but with functional foods, an actual effect — a beneficial one — is the expected outcome. Therefore, with functional foods the standard should be 'wholesomeness' rather than safety.

The EU Novel Food Directive sets out the principle of substantial equivalence based on a history of safe use, the standard that has been applied to foods historically. New foods should be evaluated against a reference food for composition, both nutritive and non-nutritive. It is not likely that routine safety assessment protocols can be developed for whole foods and food components. Consequently, the safety assessment will need to proceed on a case-by-case basis. Better understanding of the mechanisms underlying the action of foods and food components will make it possible to move away from the use of conventional safety factors.

Consumer issues. Sessions addressing consumer issues identified communication and consumer understanding as
key factors for the success of functional foods. A first issue concerns the health claims that are justified and the level of scientific evidence needed to support such claims, and how can they be communicated effectively to the consumer. Second, the extent to which nutritionists understand the barriers to changing eating patterns has to be addressed. Pan-European surveys (e.g. the EU-funded HealthSense project) have shown that consumers have a good understanding of the link between diet and health and take the need for healthy eating seriously. Nevertheless, most consumers believe they do not need to change their diet because they feel it is healthy enough. In other words, consumers admit that a problem exists for society but not necessarily for them personally. Research reflects this by the fact that consumers rank the non-communicable chronic diseases as high concerns for society but as low concerns for themselves. Consumer perceptions of food safety issues constitute a third area of interest. Their perception of risk is a function of their own control, their possible benefit and the familiarity of the issue. Consequently, in terms of health impact, consumers tend to rank relatively unimportant or low-risk issues much higher than important or high-risk ones. For the consumer, risk is an emotional rather than a scientific issue, which suggests a mistrust of science and scientists. To improve the consumer’s understanding of risk, the scientific community needs to better understand the consumer’s perspectives in this respect.

Session IV. The Future of Functional Foods

Functional foods are a reality today and are likely to be so in the future. The key drivers behind functional food research and development are the food industry, consumers and governments. The progress of the science, particularly in the area of nutrition, is fundamental to the development of innovative food solutions for the improvement of key body functions and consequently consumer health. Past research has been strong in this field and has been able to develop sound and effective research programmes. In addition, the rapid development of the genomic sciences will allow us to improve our understanding of the effects of nutrients on gene function and health outcome and to predict individual nutritional needs. The fact that these new tools will have an impact on functional science is given; only the time frame is unpredictable. Overall, scientific research is focusing on the validation and use of biomarkers of functional improvement, the evaluation of the safety of foods and ingredients, the solid understanding of the mechanisms of action, and, of course, the discovery of bioactive ingredients.

Clearly, the success of functional foods is also dependent on the ability of the food industry to develop efficacious products that meet consumer needs. The global potential for functional foods is significant and growing because of increasing health consciousness and the self-care trends associated with ageing, knowledgeable and wealthier consumers. Today’s consumer expects foods to be convenient, safe, healthy, and above all tasty. Functional foods in particular are expected to provide a credible health benefit beyond basic attributes to ensure daily and future health. These health messages need to be communicated in a transparent, credible and understandable manner to the different stakeholders, including expert scientists with a consumer focus and the various opinion leaders, including health care providers, consumer organisations and the media.

In all of this, governments have a major role to play in the future of functional foods. They can create a favourable environment for basic and applied research programmes, promote continuous and truthful consumer education on nutrition science, facilitate integration of public health issues, competitive and innovative economic development with ethical and ecological perspectives, ensure the protection of consumers in the short and long term, and enforce regulatory systems for flexible and credible science-based claims on nutrition and health.

Finally, functional food science will create many opportunities, but its ultimate success and impact on public health will depend on the consumer’s appreciation of products based on objective criteria like taste and convenience and subjective criteria like trust and credibility.

Conclusions

The International Life Sciences Institute’s (ILSI) international symposium on Functional Foods: Scientific and Global Perspectives provided an opportunity to address progress in this field since the first such international conference, which ILSI held in Singapore in 1995. Despite significant areas of controversy on functional foods, participants in the 2001 symposium agreed with Dr John Milner’s statement that functional foods provide: ‘an unprecedented opportunity to expand the use of food to improve health, decrease the risk of disease, and increase productivity’. The meeting participants also concurred that the ability to use claims was essential to the further development and success of functional foods.

The areas of disagreement are associated with the definition of the term ‘functional foods’ and with the standards of scientific evidence required to support health claims. Differences in definition include whether nutrients and non-nutrients are included, whether dietary supplements and even drugs are included, and whether functional foods should include only unprocessed foods or should also include enhanced or fortified foods. Across the world there are two distinctly different approaches to standards of evidence. Some countries are content with a culture or belief system approach based on history of use. Other countries rely on the paradigm of scientific substantiation that includes validated biomarkers. Such markers allow physiological responses to be measured in terms of improved well-being, enhanced function or reduction in disease risk.

Several important underlying concepts were addressed. First, a large amount of information is available, especially with the coming explosion of genetic marker information, but the translation of these individual pieces of information into knowledge is moving at a much slower pace. Second, there is a critical need to demonstrate valid and relevant health effects of functional foods and food components.
in man. In this respect, a lack of validated biomarkers for specific health outcomes is a significant barrier to progress. Third, there is a need to better understand what normal physiological function is and what the range of normal variability is. Current work with single nucleotide polymorphisms is providing ever-expanding information about normal human genetic variability. However, this variability explains only a small amount of the risk associated with a specific change and, consequently, the difficulty of linking a specific health outcome to intake of a specific food or food component.

There are significant communication issues regarding functional foods that go beyond scientific understanding. Taste and convenience are still the primary reasons why consumers choose specific foods, but there is a growing interest in self-administered health care. To develop credible and effective messages for consumers, there must be in-depth understanding of consumers’ needs and wants and how they view the concept of functional foods and their consequent health benefits. This understanding is a prerequisite to generating behavioural change. The benefits of functional foods must be made accessible to both rich and poor. Most importantly, the food industry must do no harm in providing functional foods. To communicate the emerging scientific information to the consumer effectively, flexible science-based regulatory systems must be adopted by governmental institutions.
EU-funded research on functional foods

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Research on food and nutrition has been an important topic in all Framework Programmes for Research and Technological Development of the European Commission. From the Second Framework Programme (ECU 2 million for four projects on functional foods) to the Fifth Framework Programme (€51 million for thirty-three projects on functional foods), the investment in research projects on functional foods has been increasing by quite an extent. In the early 1990s, the topics were fibres, pro-, pre- and synbiotics. Nowadays, the range of subjects has been broadened to antioxidative effects, vitamins, phyto-oestrogens and the socio-economic area.

Functional foods: Framework Programmes: European Commission

Research on food and nutrition has been an important topic in all Framework Programmes for Research and Technological Development of the European Commission. Within this field of research, functional foods are of ever-increasing importance (see Fig. 1). The main emphasis here has been on the elucidation of mechanisms of action and on the proof of effectiveness in prevention and therapy.

The Second Framework Programme (1989–1994) was the first under which research on food science and nutrition was funded. In the specific programme FLAIR (Food-Linked Agro-Industrial Research), four projects on functional foods were funded with a contribution of ECU 2 million. The projects were about production and nutritional properties of fibres, the role of lectins in intestinal microbial ecology, the consumption of resistant starch, and human probiotic strains.

The subjects and number of projects within the Third Framework Programme (1991–1994) were comparable. Within the specific programme AIR (Agro-Industrial Research), five projects on functional foods were funded with a contribution of ECU 5 million. The subjects were casein complexes in reduced-fat foods, probiotics, carbohydrates/fibres and colon function (three projects).

In the Fourth Framework Programme (1994–1998), the area of functional foods was dealt with under the scope of the specific programme FAIR (Agriculture and Fisheries including agro-industry, food-technologies, forestry, aquaculture and rural development). The number of projects and the total contribution doubled, and the range of subjects broadened in comparison with the Second Framework Programme. Twelve projects with a total contribution of ECU 12 million were supported. Two projects were again in the field of gastrointestinal health: analysis of the intestinal flora, and demonstration of nutritional functionality of probiotics. Four projects were about the antioxidative effects of food components: antioxidative additives, phenols and tannins (two projects), antioxidants in tomato processing. The other projects covered the subjects of conjugated linoleic acid, phyto-oestrogens, n-3 polyunsaturated fatty acids and casein phospho-peptides. The socio-economic area was covered by projects

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Fig. 1. Research on functional foods in the Framework Programmes (FP) of the European Commission: (⃣), available budget; (●), amount thereof for functional foods. FLAIR, Food-Linked Agro-Industrial Research (FP2); AIR, Agro-Industrial Research (FP3); FAIR, Agriculture and Fisheries including agro-industry, food-technologies, forestry, aquaculture and rural development (FP4); KA1, Key Action 1 — ‘Food, Nutrition and Health’ — of Quality of Life and Management of Living Resources (FP5).
Global view on functional foods: European perspectives

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In the context of this presentation, European means the European scientific community, which, over the last decade, has been working on the concepts for functional foods, producing a number of documents including a consensus paper, guidelines and scientific publications. The Functional Food Science in Europe (FUFOSE) project has been quite a unique attempt to establish a strong scientific framework to justify the functional food concept, to discover and develop new functional foods that are primarily function-driven, and to substantiate claims scientifically. Being clearly positioned as part of nutrition, the functional food concept is, however, quite distinct from other approaches like food supplementation or food fortification, and functional foods are different from nutraceuticals, pharmafoods, vitafoods and 'aliments', all terms that are not defined conceptually. Functional foods are food products to be taken as part of the usual diet in order to have beneficial effects that go beyond what are known as traditional nutritional effects. Moreover, these beneficial effects have to be demonstrated scientifically to justify two specific types of claim: the enhanced function claim or the reduction of disease risk claim.

Functional food is a key concept for the future of nutrition as a science because it results from the implementation in nutrition of all the basic scientific knowledge that has accumulated over the past two or three decades. To the benefit of public health this progress cannot be ignored, it needs to be recognized fully and used. But, today, functional food is still mainly a scientific concept that serves to stimulate research and the development of new products.

Functional foods: Markers: Claims: FUFOSE

Nutrition: a science for the twenty-first century

At the beginning of the twenty-first century, the society of abundance, which characterizes most of the occidental/industrialized world, faces new challenges from increased costs of health care, increased life expectancy, new scientific knowledge and the development of new technologies leading to major changes in life-styles (Table 1). Nutrition, as a science of the twenty-first century, will, in addition to maintaining the emphasis on a balanced diet, target optimum (optimized) nutrition (Milner, 2000), with the objective of maximizing physiological functions in order to ensure both maximum well-being and health and simultaneously minimize the risk of disease throughout the life-span. At the same time, and because of a better understanding of the interactions between genes and nutrition (Kok, 1999), it will have to match the individual’s unique biochemical needs with a tailored selection of nutrient intakes for that individual. These interactions include polymorphism and inter-individual variations in response to diet, dietary alteration and modulation of gene expression, and dietary effects on disease risk.

Thus, at the turn of the new century, the major challenge of the science of nutrition is thus to progress from improving life expectancy to improving life quality/wellness.

On the road to optimum (optimized) nutrition, which is an ambitious and long-term objective, ‘functional food’ is, amongst others, a new, interesting and stimulating concept. It is supported by sound and consensual scientific data generated by the recently developed ‘functional food science’, which aims to improve dietary guidelines by integrating new knowledge on the interactions between food components and body functions and/or pathological processes.

Functional food: defining the concept

Functional food cannot be a single well-defined/well-characterized entity. Indeed, a wide variety of food products are, or will be in the future, characterized as functional foods, with a variety of components — both classified and not classified as nutrients — affecting a variety of body functions relevant to either a state of well-being and health and/or to the reduction of the risk of a disease. Thus no simple, universally accepted definition of functional food exists or will (ever) exist. Functional food has

Abbreviations: FUFOSE, Functional Food Science in Europe; ILSI, International Life Sciences Institute.

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Global view on functional foods: Asian perspectives

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There is an old Chinese thought meaning that medicine and food are isogenic. In Japan, a national research project started to endorse the thought scientifically, with the birth of functional foods that are now known to function to reduce the risk of life-style related diseases. The science gave rise to a national policy in which some functional foods were legally approved in terms of Foods for Specified Health Use. China has also constructed a unique system for approving some functional foods. This paper pinpoints a recent trend of functional foods characteristic to Asia, with special reference to relevant topics in Japan.

Functional foods: Japan: Health: FOSHU

Introduction

The term ‘physiologically functional food’, which first appeared in Nature news in 1993 with the headline ‘Japan explores the boundary between food and medicine’ (Swinbanks & O’Brien, 1993), gave a strong international impact. Neither the terminology nor the concept of ‘functional food’ had existed until nine years earlier. In 1984, an ad hoc research group started a systematic, large-scale national project under the sponsorship of the Ministry of Education, Science and Culture (MESC) to explore the interface between food and medical sciences (Arai, 1996). It seems that the realization of this research event first in Japan reflects an underlying thought in the minds of Japanese people throughout history, which originates in the ancient Chinese saying: ‘Medicine and food are isogenic’.

However, the advent of modern food science in Japan, which took place about 100 years ago, made its subsequent research proceed towards a purely nutritional rather than a medical science. Especially in the early twentieth century, the nutritive value of foods was of great academic concern. A number of scientists in the field of chemistry as well as food science made particular efforts to discover new nutrients.

More than 10 years after World War II, nutritional problems due to food shortage were almost solved in many countries and a new period characterized by high economic growth began. It prevailed in the time encompassing the 1960s, when the social climate focused on food preference and aversion. Such a trend of hedonism led to academic studies on the sensory properties of foods. Epoch-making advances in instrumental analysis assisted the studies greatly. Japan, among many developed countries, thus contributed to two mainstays of food science: nutrition and hedonics. In accordance with this, food industries were activated to supply a great deal of nutritionally and sensorily acceptable products to the market. We enjoyed thus our rich dietary life for a long time until almost 20 years ago, when a new, antithetic situation arose.

Functional food science and policy born in Japan

In the 1980s, as the ageing society began to manifest itself in many countries of the world, prompt increases in the so-called life-style related diseases became a matter of public concern. Growing awareness was then observed of the need for eating to beat the odds. The purpose was to prevent life-style related diseases such as diabetes, arteriosclerosis, osteoporosis, allergies, cancer, and even some kinds of infectious diseases, through improved dietary practices in daily life. This gave a strong impetus to food science in Japan. In 1984, the above-mentioned MESC project entitled Systematic Analysis and Development of Food Function commenced, where the terminology and the concept of ‘functional food’ were first proposed. It was followed by a second project (1988–1991) entitled Analysis of Body-modulating Functions of Foods. In 1992, the last in the series of MESC ‘functional foods’ projects was realized, focusing on Analysis and Molecular Design of Functional Foods. A total of 60 academic professionals participated, most from medical as well as food science fields.

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Abbreviations: FOSHU, food for specified health use; ILSI, International Life Sciences Institute; MESC, Ministry of Education, Science and Culture.
Functional foods: Latin American perspectives

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The perspectives of Latin America as a potential producer and consumer of functional foods will depend largely on the level of information and income of the population, credibility of the products, research investments and regulatory practices. The characteristics of Latin America are diverse at the regional and sub-regional levels. However, as part of the demographic and epidemiological transition currently underway, common trends can be identified such as increasing urbanization and life expectancy, the occurrence of obesity and malnutrition, increasing incidences of chronic diseases and causes of mortality rates, all of which suggests the importance of diet and functional foods in public health policies. The Latin American population in general has no knowledge of functional foods, but in the more urbanized areas there is an increasingly health-conscious consumer, aware of the importance of food for health, due to the media and local traditions. More investment in research is important to explore the existing plant biodiversity that is a rich source of new foods and bioactive compounds, some of which are already used for health improvement and well-being. Clinical validation of functional foods should consider functional food science concepts and also the diverse cultural and genetic background of the local population. In Latin American scientific and regulatory communities, the functional foods concept has been associated with foods having health benefits beyond those of basic nutrition but is not defined officially in the emerging regulatory codes. Regulation existing in some countries is focused on safety and efficacy; both functional and health claims are allowed (risk reduction) provided they have scientific validation. This allowed introduction on the market of several products with health claims, some of them submitted to post-marketing surveillance.

Functional food: Latin America: Needs and challenges

Demographic and urbanization trends in Latin America

During the last 30 years, the declining mortality and fertility rates in Latin America have had an impact on the population’s growth and structure. In 1950 the population in the Americas was 331 million and in the year 2000 it was estimated at 823 million. About one-third resides in the USA, one-third is found in Brazil (170 million) and Mexico (99 million), and the remaining third in the other countries of the region.

This demographic transition began with a decline in infant and child mortality, with most countries having halved their mortality rates among children under 1 year old in the last twenty years. Life expectancy during the last five years has stood at 70 years, with a range throughout the region from 54.1 to 79.2 years, and this change has resulted in ageing of the population, which increases chronic and degenerative diseases and disabilities. At the same time there has been an increase in the urban population, estimated now at 76%, although it varies from 85.3% for the southern cone to 48.3% for Central America (Pan American Health Organization (PAHO), 1999a). In past decades mortality rates in Latin America have declined, with an increase of 18 years in life span. Although major advances have been made in controlling infectious diseases rates are still high, similar to those of non-communicable diseases, due to differences in public health care in different social groups.

Nutrition in transition: globalization and its impact on nutrition patterns and diet-related diseases

As has happened all over the world, industrialization, urbanization and market globalization have had a strong impact on Latin Americans’ life-styles and diets, and the nutritional status of the population. The contemporary urbanization has resulted in a decline of undernutrition in...
Linkages between diet habits and the quality of life continue to surface on numerous fronts. Collectively these epidemiological, pre-clinical and clinical studies provide rather compelling evidence that numerous essential and non-essential dietary components are capable of influencing growth, development and performance and disease prevention. Scientific discoveries and widespread interest in the potential medicinal benefits of foods and food components have fostered a variety of content, health and structure–function claims. Unfortunately, defining the ideal diet is complicated by the numerous and diverse components that may influence biological processes. Inconsistencies in the literature may reflect the multi-factorial nature of these processes and the specificity that individual dietary constituents have in modifying genetic and epigenetic events. New and emerging genomic and proteonomic approaches and technologies offer exciting opportunities for identifying molecular targets for dietary components and thus determining mechanisms by which they influence the quality of life. All cells have unique ‘signatures’ that are characterized by active and inactive genes and cellular products. It is plausible that bridging knowledge about unique cellular characteristics with molecular targets for nutrients can be used to develop strategies to optimize nutrition and minimize disease risk.

**Diet: Cancer: Polymorphism: Genes: Phytochemicals**

**Introduction**

There is little doubt that nutrition and health are intimately linked. For generations, people have believed that foods could do more than merely provide energy. Beliefs in the medicinal properties of foods were highlighted in a number of the early writings of mankind. Hippocrates is frequently quoted as saying: ‘Let food be thy medicine and medicine be thy food’. Epidemiological, pre-clinical and clinical studies continue to provide fundamental insights into the dynamic relationships between nutrients — defined here as any substance in the diet that brings about a physiological effect — and health. Today, claims about the ability of foods and food components to reduce disease risks or enhance the quality of life continue to captivate our lives. In North America, passionate discussions about foods and beverages are commonplace between friends, relatives and even complete strangers.

**Unprecedented opportunities**

Inappropriate nutrition is a primary factor in unattained genetic potential, reduced physical and cognitive performance, and increased risk of some diseases. Unquestionably, strategies that optimize nutrition by the use of foods or supplements are highly commendable and considered by many to be appropriate for improving the overall quality of life. The importance of such strategies is emphasized by the recognition that about one-third of all cancers are related to dietary habits. Actually, more than half of the deaths occurring in the United States correlate with food patterns (National Center for Health Statistics, 1997). Many in North America believe that modifying their diet and the use of supplements are two of the most important options available to reduce health care costs and improve personal health (Eisenberg et al. 1998; Gilbert, 1999; Aldana, 2001).

In spite of a growing number of studies, workshops and conferences devoted to understanding the dynamic relationship existing between nutrition and disease, it remains virtually impossible to determine who will and will not benefit most, if at all, from dietary intervention. What is recognized is that the effect of food on health varies considerably among people, both in terms of its direction and magnitude (Clydesdale, 1998; Milner, 2000). Part of the difficulty in determining those who might benefit arises from the incredibly complex chemical
Gastrointestinal physiology and functions

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While the health benefit of a functional food may be a metabolic response that lowers risk for disease, the actual target for the food or food component may be on the functioning of the gastrointestinal tract (GIT). For example, slowing absorption from the intestine, as measured by examining the appearance of the nutrient or food component in the blood, the hormone response associated with absorption of the compound or excretion of the compound, may provide a health benefit. However, the food component may slow absorption by delaying gastric emptying, altering the mixing within the intestinal contents or decreasing the availability of digestive enzymes in the intestine. These measures of GIT function provide validation of the mechanisms by which the functional food or food components affect metabolism. Bioavailability of physiologically active compounds from foods will be determined by the digestibility of foods that contain these compounds, their subsequent absorption and utilization by tissues. The physical structure of foods contributes to the functional effects of foods as well as to the availability of compounds from foods. For example, recent studies have demonstrated that changing the viscosity of the gut contents alters absorption and GIT response. Additionally, food structures such as the plant cell wall change the availability of absorbable compounds along the gastrointestinal contents. The areas of probiotics and prebiotics have highlighted the potential importance of gut microflora in health. While evidence suggests biological activity relevant to disease risk reduction, the long-term implications of the microbial activity have yet to be established.

Introduction

The gastrointestinal tract (GIT) serves as an interface between the body and the external environment. The GIT is a highly specialized organ system that allows man to consume food in discrete meals as well as a very diverse array of foodstuffs to meet nutrient needs. In the GIT food is converted to compounds that can be absorbed into the body. The organs of the GIT include the mouth, oesophagus, stomach, small intestine and large intestine; in addition, the pancreas and liver secrete into the small intestine. The system is connected to the vascular, lymphatic and nervous systems to facilitate regulation of the digestive response, delivery of absorbed compounds to organs of the body and the regulation of food intake. Johnson (1997) provides an excellent overview of the gastrointestinal system. A primary function of the GIT is to extract nutrients from the complex mixture of foods as consumed. Foods contain more than essential nutrients, and the GIT has a role in metabolizing and eliminating non-nutrient and toxic compounds as well.

Functional foods include the categories of food products that are consumed for a specific health benefit or as part of a dietary plan to lower risk for chronic disease. As such, the components of these foods may exert their effects through a direct action on the GIT or become available to other target organs in the body after absorption from the GIT. In this context, three perspectives are useful to examine the importance of GIT physiology and functions in mediating the effects of functional foods: (1) meal-induced responses in the GIT caused by factors in foods, which may result in longer-term adaptive changes; (2) the ability of foods or mixtures of foods to alter the digestive and absorptive functions of the GIT in a manner that influences metabolism; and (3) the impact that the GIT, through its adaptation to diet, has on risk factors for disease. Examples are used below to illustrate each of these perspectives regarding the physiology and function of the GIT.

Meal-induced responses

Regulation of the gastrointestinal response to a meal involves a complex set of hormone and neural interactions.

Abbreviations: CCK, cholecystokinin; GIT, gastrointestinal tract; SCFA, short-chain fatty acids.
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The immune system: a target for functional foods?

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The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. The immune system is constantly active, acting to discriminate ‘non-self’ from ‘self’. The immune system has two functional divisions: the innate and the acquired. Both components involve various blood-borne factors (complement, antibodies, cytokines) and cells. A number of methodologies exist to assess aspects of immune function; many of these rely upon studying cells in culture ex vivo. There are large inter-individual variations in many immune functions even among the healthy. Genetics, age, gender, smoking habits, habitual levels of exercise, alcohol consumption, diet, stage in the female menstrual cycle, stress, history of infections and vaccinations, and early life experiences are likely to be important contributors to the observed variation. While it is clear that individuals with immune responses significantly below ‘normal’ are more susceptible to infectious agents and exhibit increased infectious morbidity and mortality, it is not clear how the variation in immune function among healthy individuals relates to variation in susceptibility to infection. Nutrient status is an important factor contributing to immune competence: undernutrition impairs the immune system, suppressing immune functions that are fundamental to host protection. Undernutrition leading to impairment of immune function can be due to insufficient intake of energy and macronutrients and/or due to deficiencies in specific micronutrients. Often these occur in combination. Nutrients that have been demonstrated (in either animal or human studies) to be required for the immune system to function efficiently include essential amino acids, the essential fatty acid linoleic acid, vitamin A, folic acid, vitamin B₁₂, vitamin C, vitamin E, Zn, Cu, Fe and Se. Practically all forms of immunity may be affected by deficiencies in one or more of these nutrients. Animal and human studies have demonstrated that adding the deficient nutrient back to the diet can restore immune function and resistance to infection. Among the nutrients studied most in this regard are vitamin E and Zn. Increasing intakes of some nutrients above habitual and recommended levels can enhance some aspects of immune function. However, excess amounts of some nutrients also impair immune function. There is increasing evidence that probiotic bacteria improve host immune function. The effect of enhancing immune function on host resistance to infection in healthy individuals is not clear.


The immune system

Introduction

The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. The immune system is constantly active, acting to discriminate ‘non-self’ from ‘self’. The immune system has two functional divisions: the innate (or natural) immune system and the acquired (also termed specific or adaptive) immune system. Both components of immunity involve various blood-borne factors (complement, antibodies, cytokines) and cells. These cells are generally termed leucocytes (or white blood cells). Leucocytes fall into two broad categories: phagocytes (which include granulocytes (neutrophils, basophils, eosinophils), monocytes and macrophages) and lymphocytes. Lymphocytes are classified as T lymphocytes, B lymphocytes and natural killer cells. T lymphocytes are further divided into helper T cells (these are distinguished by the presence of the

Abbreviations: DTH, delayed-type hypersensitivity; IFN-γ, interferon-γ; Ig, immunoglobulin; IL, interleukin; MHC, histocompatibility complex; TNF, tumour necrosis factor.

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The development of the sports food market and industrial involvement have led to numerous nutritional studies to define the type of nutrients that are most suited to support energy metabolism, fluid balance and muscle function. The key question in many of these studies was: ‘Does the product lead to a significant product/consumer benefit that can be used as a claim on the package?’ New methods and techniques have been developed, partly with sponsorship of the food industry, with the goal of measuring the effects of specific nutrients and supplements on athletic performance and metabolism. In line with this development, a wide variety of supplements and sports foods/drinks labelled with various performance or health benefit statements have been launched on the sports nutrition market. Although a variety of products have been tested clinically, there are also many products on the market with benefit claims that cannot be supported by sound nutritional and sports physiological science. The current short review highlights some of the methods and biomarkers that are used to substantiate product/consumer benefit claims for foods and drinks that are marketed as functional foods for athletes.

**Introduction**

During the last century there have been enormous changes in the understanding of the role of diet in exercise and physical performance. Almost a century ago it was considered that protein was the most important energy source for muscle. However, since the classical Scandinavian studies on the role of diet on physical performance in the 1960s, the focus has changed completely to carbohydrate. It was shown that exercise-induced reductions in muscle glycogen correlated well with the development of fatigue and that optimizing carbohydrate stores and ingesting carbohydrate during prolonged exercise improved performance. Since then, ‘carbohydrate loading’ and the consumption of carbohydrate–electrolyte drinks during exercise have become common practice among endurance athletes.

This was also the beginning of a new challenge to the food industry: the development of sports-specific food products and drinks. Sports foods/drinks should provide fluid and energy (i.e. carbohydrate) rapidly and not cause any gastrointestinal discomfort, to allow consumption prior to as well as during competition. To improve the athlete’s recovery from intense and exhausting exercise, other products were created to target the physiological functions that are involved in this recovery process. Specific blends of carbohydrate were studied to obtain evidence about effective energy fuels during exercise, with the aim of improving performance and delaying fatigue development. In line with these developments many products have been launched on the market to target the fitness and physical performance sector, mostly with attractive benefit claims.

Examples of such benefits are ‘performance enhancement’, ‘more power’, ‘less gastrointestinal problems’, ‘improved recovery’, ‘less muscle cramps/pain’ and ‘reduction of body fat/increased muscle mass’. To obtain clinical evidence for a benefit, the development and use of measurement techniques that allowed measurement of real treatment effects was essential. Accordingly, techniques to measure the rate of gastric emptying, intestinal absorption, appearance of substrates in blood and their subsequent oxidation or storage, as well as laboratory exercise protocols to measure performance accurately, were developed and validated.
Functional foods: psychological and behavioural functions

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It is easier to demonstrate the consistent effects of foods on satiety than on cognitive performance. This is understandable since the satiety system incorporates physiological signalling systems that mediate the effects of foods on function. Specific manipulations of proteins, carbohydrates and fats have the potential to act as functional foods for appetite control. Because of the importance of the optimal functioning of cognitions for survival, these functions are quite strongly protected against short-term dietary and physiological perturbances. Therefore, food manipulations may be better detected through the degree of effort exerted to maintain performance rather than via changes in the actual performance itself. This procedure has not been widely used hitherto. The concept of biomarkers may have to be interpreted differently from research on physiological systems or clinical endpoints. For satiety, adjustments in the profile of hunger could serve as a biomarker or surrogate endpoint. For cognitions, correlated physiological variables may be more difficult to measure than the functional endpoint itself. Changes related to unitary functions (such as tracking) could serve as biomarkers for more complex, integrated skills (such as car driving). Since food manipulations may affect multiple functions, the challenge is to design foods with good satiety control that do not impair mental performance; or alternatively to engineer foods that optimise cognitive performance without compromising satiety. This rapidly developing field has great potential for close collaboration between academia and industry in the production of commercially successful products that show clear improvements in human functioning with the capacity to protect against disease or impairment.

Satiety: Biomarker: Cognitive performance: Functional foods

Introduction

In the First International Conference on East–West Perspectives on Functional Foods, the major emphasis was on physiological and metabolic functions. Although much of this material was relevant for well-being and quality of life, there was almost no mention of the direct effects of foods on psychological functions involving the expression of behaviour (such as food intake or appetite) or the articulation of mental state (mood) or mental abilities (cognitive performance).

In the intervening period the world-wide epidemic of obesity has been recognised, thus bringing appetite control to the forefront of functions susceptible to modulation by foods and food ingredients. Consequently, functional foods for the control of appetite (and ultimately body weight) now constitute a major health goal and research objective. In addition, the principle that foods can reliably affect cognitive performance is receiving validation and experimental support. This domain of research is still in a relative state of infancy but also rapid development.

In these areas of research it is not clear that the concept of biomarkers has the same meaning as in other domains which relate physiological functions to some health endpoint or disease protection. However, it is possible to suggest ways in which biomarkers for complex psychological and behavioural functions could be identified.

This interim report has evolved from the International Life Science Institute’s Functional Food Science in Europe (FUFOSE) Project and is designed to provide a perspective on the field and to indicate possibilities for future developments. The FUFOSE programme concentrated on the behavioural functions of appetite control, cognitive performance and mood, and this approach has been followed here.

Functional foods for satiety

The control of appetite

The ultimate objective of research in this area is to develop...
Biomarkers and functional foods for obesity and diabetes

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Obesity has reached epidemic proportions in many countries around the world. Because of the close relationship between obesity and type 2 diabetes, an epidemic of diabetes is close behind the obesity epidemic. Preventing and treating obesity is becoming an increasing priority. In the United States, over 60% of the adult population is overweight or obese and thus at increased risk of developing diabetes and cardiovascular disease. While the aetiology of obesity and diabetes is complex, diet clearly plays an important role both in the development and management of these diseases. There is interest in functional foods that could help in prevention and/or management of obesity and type 2 diabetes. This could involve food products that help management of ‘hunger’ or that increase ‘satiety’. It could also involve foods that contribute to more inefficient use of ingested energy (i.e. foods that stimulate energy expenditure more than would be expected from their energy content). As the concept of insulin sensitivity becomes generally more accepted by health care professionals and the public, foods may be targeted towards maximizing insulin sensitivity and towards ‘prevention’ of diabetes. In addition to foods that impact upon body weight, these may include foods that affect the glucose and/or insulin levels that are seen either following the ingestion of food or later in the day. The present paper reviews the complex aetiology of obesity and diabetes and considers a potential role for functional foods in prevention and treatment of obesity and diabetes.

Obesity: Type 2 diabetes: Biomarkers: Functional foods

The need for tools to manage obesity

The rate of increase in obesity in the US population has led many to label this public health threat as an epidemic. This is supported by data from the National Health and Nutrition Examination Surveys (National Center for Health Statistics, 1999) and by data from the Behavioral Risk Factor Surveillance System (Mokdad et al. 2001). All indications are that the prevalence of obesity will continue to increase over the next several years.

The obesity epidemic presents a serious threat to public health given the known relationship between obesity and other serious chronic diseases such as type 2 diabetes and cardiovascular disease (Pi-Sunyer, 1993; National Institutes of Health, 1998; Fagot-Campagna et al. 2000). In fact, there is convincing evidence that the obesity epidemic is being followed by an epidemic of type 2 diabetes (Harris et al. 1998). The metabolic syndrome (syndrome X or insulin-resistance syndrome) has been defined as a cluster of conditions including obesity (particularly visceral obesity) and insulin resistance that are frequently seen together and which impart increased risk of development of type 2 diabetes (Reaven, 1998). Thus, the vast majority of persons with type 2 diabetes are obese and weight loss may be one of the best treatments both to prevent and treat type 2 diabetes (Maggio & Pi-Sunyer, 1997).

In general, efforts to treat obesity have not met with great success, but we may be more successful than popular belief. Obesity treatment guidelines issued by the National Institutes of Health (1998) suggest an initial goal of 10% weight loss for obesity treatment. Wing & Hill (2001) have estimated that about 20% of those obese individuals who attempt weight loss are successful in achieving a 10% weight loss and maintaining that weight loss for a year. With over 60% of the US adult population being overweight or obese, there is great demand for better tools to help people achieve weight loss and maintenance of weight loss.

Treatment strategies for type 2 diabetes usually involve pharmacological treatment aimed at stimulating insulin secretion or increasing insulin sensitivity. The disease is progressive (leading to serious negative consequences such as blindness and kidney disease) and treatment aims to slow this progression (Lebovitz, 1994). Interestingly,
One of the most promising areas for the development of functional foods lies in modification of the activity of the gastrointestinal tract by use of probiotics, prebiotics and synbiotics. While a myriad of healthful effects have been attributed to the probiotic lactic acid bacteria, perhaps the most controversial remains that of anticancer activity. However, it must be emphasised that, to date, there is no direct experimental evidence for cancer suppression in man as a result of consumption of lactic cultures in fermented or unfermented dairy products, although there is a wealth of indirect evidence, based largely on laboratory studies. Presently, there are a large number of biomarkers available for assessing colon cancer risk in dietary intervention studies, which are validated to varying degrees. These include colonic mucosal markers, faecal water markers and immunological markers. Overwhelming evidence from epidemiological, in vivo, in vitro and clinical trial data indicates that a plant-based diet can reduce the risk of chronic disease, particularly cancer. It is now clear that there are components in a plant-based diet other than traditional nutrients that can reduce cancer risk. More than a dozen classes of these biologically active plant chemicals, now known as ‘phytochemicals’, have been identified. Although the vast number of naturally occurring health-enhancing substances appear to be of plant origin, there are a number of physiologically active components in animal products (such as the probiotics referred to above) that deserve attention for their potential role in cancer prevention.

Introduction

In recent years, there has been a growing interest in the concept of ‘functional foods’ by both the food industry and the consumer. Functional foods are defined as food-stuffs that improve overall health and/or reduce the risk of disease. Thus, with the consumer becoming more interested in foods which benefit health and the food industry beginning to understand the market potential of functional foods, solid research activity in the area has been initiated worldwide. In addition, when one considers the costs to society of diseases such as obesity, cardiovascular disease, diabetes, food allergies, osteoporosis and cancer, all of which may be influenced by diet, the potential of well-characterised functional foods becomes even more evident.

Although a large number of naturally occurring health-enhancing substances are of plant origin, there are a number of physiologically active components in animal products that deserve attention for their potential role in disease prevention.

One such example of a functional food, which has been the focus of increasing research activity in recent years, are probiotics: live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotics usually refer to highly selected lactic acid bacteria, e.g. Lactobacillus spp., Bifidobacterium spp. and Streptococcus spp., with defined gut survival properties and associated biological activities, that can be ingested in fermented milk products or as a supplement. The list of healthful effects attributed to probiotic bacteria is extensive and includes alleviation of lactose intolerance symptoms, serum cholesterol reduction, anticancer effects, alleviating constipation and relieving vaginitis, to name but a few. The vast majority of studies on the anticancer effects deal with colorectal cancer (Hirayama & Rafter, 2000), although there are some on breast and bladder cancer.

There is also a large amount of evidence, from epidemiological, in vivo, in vitro and clinical trial data, indicating that a plant-based diet can reduce the risk of chronic disease, particularly cancer. In 1992, a review of 200 epidemiological studies showed that cancer risk in...
Biomarkers of bone health appropriate for evaluating functional foods designed to reduce risk of osteoporosis

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Osteoporosis is a growing global problem. The health care costs and decreased productivity and quality of life are staggering. Much research is invested in life-style approaches to build peak bone mass during growth to prevent osteoporosis as well as to treat the disease in later life. Functional foods have enjoyed a niche in bone health. Foods fortified with Ca are most popular. Other bone nutrients such as vitamin D, Mg and vitamin K are sometimes added. Future products are likely to include enhancers of Ca absorption such as inulin or whey proteins. Dietary factors that reduce urinary Ca loss (plant proteins) or suppress bone resorption (possibly phyto-oestrogens) are also gaining attention. Methodologies for evaluating the effectiveness of functional foods on bone health include measures of bone quality such as bone densitometry or measures of Ca metabolism, particularly absorption. Biochemical markers for bone turnover are less satisfactory for diet-related effects. Use of a rare isotope, ⁴¹Ca, and accelerator mass spectrometry offers a new approach for assessing the ability of functional foods to suppress bone resorption.

Introduction

Biomarkers to evaluate the effectiveness of functional foods for bone health are quite advanced compared with many other diseases. Yet, simple, inexpensive and rapid methods for evaluating large numbers of people are still lacking. The appropriate choice of an outcome measure depends on the mechanism of action of the functional food and the targeted population. Functional foods designed to prevent osteoporosis may work by providing a key nutrient important to bone development and maintenance, by enhancing Ca absorption or retention, by building peak bone mass or by suppressing bone turnover and, therefore, bone loss. Because functional foods for bone health are largely targeted towards increasing Ca intake or utilization of Ca, the focus of this review will be on strategies to improve Ca nutrition and methods to evaluate their effectiveness.

Importance of adequate dietary calcium

There is a renewed interest in the importance of adequate Ca intake, because of a greater appreciation of its role in health and disease prevention. The role of Ca in bone health is best understood as the primary cation in bone (Bryant et al. 1999). Requirements of Ca are based on intakes adequate for bone accretion and maintenance. There are many other health reasons to achieve adequate Ca intake. The role of Ca and dairy product consumption in vascular tone and blood pressure control was reviewed recently (McCarron & Reusser, 1999). Observational studies suggest adequate Ca decreases risk of kidney stones, probably by decreasing urinary oxalate level (Heller, 1999). Increasing Ca and dairy food intakes appear to reduce risk of colon cancer, probably through lowering faecal bile acid and fatty acid concentrations, which lowers cytotoxicity (Holt, 1999). The reversal of dietary fat-induced hyperplasia and hyperproliferation in mammary and colonic tissues in mice when Ca and vitamin D were supplemented led to the recent suggestion that Ca is also important in preventing breast cancer (Lipkin & Newmark, 1999). A multi-centre trial showed that Ca supplementation significantly (P < 0.05) decreased premenstrual symptoms relative to those observed in a placebo-controlled group (Thys-Jacobs et al. 1998). More recently, adequate Ca intake has been associated with maintenance of body fat and body weight (Lin et al. 2000; Zemel et al. 2000).

Ca intakes for most groups over the age of 11 years fall short of the recommended intakes in North America (Food and Nutrition Board, 1997) and many other parts of the world. Ca absorption and retention are inefficient. Ca
Functional foods, trends and future

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The concept of functional foods is often considered to have emerged in Japan in the late 1980s. However, functional foods actually have a quite long history. In China, Japan and other Asian countries, many types of foods have traditionally been associated with specific health benefits. In Western societies, a well-known global brand, namely Coca-Cola, actually started as a functional food at the beginning of the twentieth century. What is probably of more recent origin is the development of nutritional science backing functional foods. During the second half of the twentieth century, exciting new nutritional insights emerged that allowed for the development of foods and beverages with a claimed health benefit, based on scientific evidence.

From a market perspective, functional foods are difficult to quantify because different definitions are used. If we view functional foods as foods that make specific health claims, then the market in the USA, Japan and Europe is estimated to be worth about €7 billion. The functional foods market can, however, be seen as part of a broader health-based/driven foods market. This includes natural and organic foods, ‘low and lite’, weight management and vitamin- and mineral-fortified products as well as functional foods. This latter market is very large with a global estimate of around €95 billion for the year 2000. The most popular foods are energy/sports drinks, probiotic dairy products, heart health spreads and ready-to-eat cereals. Major functionality claims are for gut health (especially in Japan and Europe), heart health (especially in the USA and Europe), promoting natural defences, and boosting energy levels. The outlook for the future for health-based/driven foods is bright. An overall growth rate of 10% per annum for the next five years is possible, significantly outperforming the overall foods and beverage market’s growth of about 2% per annum.

To get back to the history of functional foods, let us elaborate one of the first examples of a functional food with a specific health claim. In the late 1960s, Unilever developed spreads high in polyunsaturated fatty acids aimed at reducing blood cholesterol level. Spreads under the Becel and Flora brands were developed based on mechanistic understanding and clinical efficacy data (Keys et al. 1965) and were targeted initially at hypercholesterolaemic patients. These products developed gradually into successful mainstream consumer products. This example shows that functional foods can indeed help in addressing specific consumer needs and can actually contribute to improvement of public health. In many European countries, increasing sales of products enriched with polyunsaturated fatty acids were paralleled by more favourable ratios of polyunsaturated to saturated fat in national diets, as advocated by many nutritional authorities around the globe (National Heart Foundation of Australia, 1999; Health Council of the Netherlands, 2001). An example of the impact of health-driven foods without a specific health claim is in the ‘low and lite’ area. In The Netherlands, there has been a decrease in fat consumption in recent years. Although the decrease is moderate compared with the recommendations of health authorities, data from food consumption surveys (Anonymous, 1988; Anonymous, 1998) show that the decrease is due to changes in the composition of industrial products, rather than to changes in food choices or preferences of consumers.

More recently, the arena of cholesterol-lowering spreads was extended further by products with increased efficacy through the addition of plant sterols or stanols (Law, 2000). This again demonstrates that developments in nutritional science can be incorporated successfully into functional foods, if there is sufficient consumer awareness and need. However, sound science and perceived consumer need from a public health perspective are no guarantee for the success of functional food products, as unfortunate examples of unsuccessful market introductions show. It is hard to predict the prerequisites for success of a functional food, but factors undoubtedly include consumer need and awareness; consumer acceptance of a food solution; powerful communication of health benefits to the consumer; uncompromised taste; optimal convenience; adequate retail or out-of-home availability; proven safety and efficacy; acceptable price level; assurance and support from different sources, including scientific opinion leaders; and a clear regulatory framework for making claims, providing a level playing ground for all companies. The reader may be disappointed that nutritional science does not feature prominently in this list of prerequisites, but it is a condition sine qua non. The list does demonstrate that, from the perspective of industrial research and development but probably also from the public health perspective, research in

Abbreviations: FUFOSE, Functional Food Science in Europe.

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functional foods will only pay off if it is closely integrated with the other prerequisites for success of a functional food.

The functional foods that we find on the market today are often based on general discoveries in nutritional science, and less on a deliberate research strategy to develop functional foods. Often, research was product-driven, i.e. aimed at identifying positive aspects of existing products and functional foods were then developed by optimising these positive aspects. For the future, an approach that integrates insights into consumer needs and demands (market pull) and a structured scientific research process (science push) will give the largest chance of real innovations. The scientific research process into functional foods will be powered by technology and insights available from other disciplines, such as informatics, pharmacology, engineering, proteomics and genomics. Insights into genetic susceptibility arising from the human genome project and possibilities for individualised health monitoring using micro-engineering and sensor technology may alter dramatically the way in which we deal with food and health. For this, however, consumer acceptance is a first prerequisite. This is also true for the attainments of modern biotechnology that may also have a major impact on possibilities to optimise functional foods.

Schematically speaking, the combination of ‘market pull’ and ‘science push’ in functional foods research will result in a research funnel starting from consumer needs and narrowing down to the final functional foods products by the following stepwise approach:

1. Consumer understanding: what kind of health benefits in foods or technology solutions do consumers really want?
2. Bio-informatics: what molecules could do the job?
3. In vitro screening and in vivo testing: which molecules work best in model systems?
4. Bioavailability: is the bioactive compound digested and absorbed?
5. Functional food technology: can we source the ingredient and make an attractive food?
6. Biomarkers: can we measure relevant effects in man?
7. Human intervention studies: does it really work?
8. Communication: how do we explain the benefits?

With regard to biological benefits in functional foods, the International Life Sciences Institute’s concerted action on Functional Food Science in Europe (FUFOSE; Diplock et al. 1999) has proposed six broad groups that are considered relevant from a scientific perspective. These are (1) growth, development and differentiation; (2) substrate metabolism; (3) defence against reactive oxidative species; (4) the cardiovascular system; (5) gastrointestinal physiology and function; and (6) behaviour and psychological functions. These benefit categories indicate that functional foods should primarily be aimed at function improvement or (longer-term) disease risk reduction for ‘healthy’ people, and not at disease treatment for ‘sick’ people.

The broad categories identified by FUFOSE are still relevant, but the products that have been introduced on the market in recent years seem to indicate that although there is a place for products specifically aimed at disease reduction, there also is a trend towards products providing ‘daily health benefits’. Such functional benefits like healthy attractive skin, the ability to deal with stress and mental and physical performance may not always be exciting from a public health or medical science perspective, but they are very relevant for the consumer. In addition, providing a daily benefit can indeed contribute to longer-term health objectives. For instance, a daily benefit like satiety and all necessary nutrients from a low-calorie meal replacement has been shown to contribute to ability to lose weight and maintain a healthy dietary pattern. If products claim daily health benefits, then these will probably be in the area of ‘functional benefits’. It should be clear that the same stringent criteria for claim substantiation should be applied for functional benefit claims as for disease reduction claims. As a result, there is a challenge in further developing the science to underpin functional food development in these areas.

Returning to the market for functional foods: what sectors will grow and what trends will rule in the next five years? We predict further growth of three distinct sectors. A first sector will be products making claims backed by extensive scientific research for which endorsement is key, especially in the area of heart health and weight management. Examples have been given previously. A second sector, also with scientific backing but focusing more on daily benefit claims, will target enhancement of physical and mental performance, with sports and energy drinks becoming even more popular. A third sector will be focused around general ‘good for you’ products that will make more ‘general’ claims, based on scientific evidence around ingredients being used such as probiotics and antioxidants, but with less extensive research on specific product claims. For all of these sectors, the range of success factors mentioned previously applies. Three of these success factors seem to be paramount: taste, convenience and trust. First, consumers, except for a small minority, will not be willing to sacrifice taste for health. Secondly, products must fit consumers’ busy lifestyle; ready-to-eat or ready-to-heat and on-the-go formats will become more popular. Third, we expect a more stringent regime for claims; such that industry is only allowed to communicate what it has evidence for. Consumers want to be able to trust claims. In a world with an abundance of choices we increasingly expect brands to become beacons of trust and guidance for consumers. Around these brands marketers will aim to build better relationships with consumers by providing on-line information and advice, and perhaps service options. Although there has been speculation about pharmaceutical companies taking an interest in functional foods, we foresee that for the next five years the sector will be dominated by, in particular, diversified food conglomerates and the dairy sector. Marketed products will be based on the findings of nutrition science of the last decade with a few new options added. Only when we take an outlook of, say, ten years may we expect that some functional foods will be marketed that take into account gene polymorphisms that may be relevant for
the development of chronic diseases. Also in that time frame we may see a comeback in Europe of ingredients for functional foods produced by modern biotechnology. Apart from the developed market, we expect that in developing markets the sector of health-based/driven foods will grow. This is already occurring in the form of vitamin and mineral fortification of popular foods for the general population, but quite soon we may also see the emergence of more advanced functional foods, e.g. targeting heart health or the increasing prevalence of overweight in developing countries.

The area of functional foods is beginning to come of age. Its adolescence will be driven by exciting new scientific developments. However, the area of functional foods will only grow successfully if we are able to integrate credible science with thorough consumer understanding, uncompromised taste and convenience, and effective communication. A key challenge to ensure the bright future of functional foods is to provide solid guarantees to consumers that they can trust the safety of functional foods and their promises about better health, performance, development or growth.

References


absorption in adults averages only 30% (Heaney et al. 1988) and losses through endogenous secretions approximate 120 mg/d regardless of intake (Wastney et al. 1996). Early man is thought to have consumed liberal amounts of Ca compared with the intake of modern man (Eaton & Konner, 1985). Because osteoporosis and the other diseases described above typically occur post reproduction, there is little evolutionary pressure to adapt to the current low intake of Ca. Increasing Ca intake is a prudent solution to the Ca deficit, but improving absorption and retention could also improve Ca nutriture.

Absorption: mechanisms and methodology

Ca bioavailability is frequently equated to Ca absorption. Absorption is the first barrier to achieving Ca homeostasis. Dietary factors that affect excretion or bone resorption, i.e. net retention, will be discussed in later sections. Ca is absorbed both by active, transepithelial and by passive, paracellular processes (Fig. 1). Active absorption dominates at low Ca intake, but owing to its saturable nature and subsequent down-regulation at adequate intake, this pathway becomes less important with increasing Ca intake. Ca absorption efficiency is inversely related to Ca load over a wide range of intake, although the absolute quantity of Ca absorbed increases with increased load (Heaney et al. 1990b).

Methods to assess Ca bioavailability include Ca balance, determination of bone (or whole-body) Ca retention, and the use of intestinal loops, Caco-2 cells and isotopic tracers. These span studies in man, animal models and in vitro techniques. Ca balance studies are expensive and can lead to erroneous results unless sufficient attention is paid to ensuring the completeness of food intake and urine and faecal collections. An additional challenge is the demarcation of faeces to time intervals that can be

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**Fig. 1.** Calcium absorption from intestinal lumen through intestinal epithelium to blood. Calcium can be absorbed (1) transepithelially, by a process mediated by 1,25(OH)2 vitamin D-induced carrier, calbindin D9, or by endocytosis of acidic lysosomes; or (2) paracellularly, a passive process.
related to specific Ca intake; validity is improved markedly in this regard when faecal results are corrected for recovery of a quantitative faecal marker (Hargreaves & Rose, 1965). Appropriate uses of balance studies include comparisons of the bioavailability of different sources of Ca and to help quantify complex nutrient interactions which affect bioavailability (Mertz, 1987). Ca balance studies have contributed to the body of literature that suggests that both oxalate (Kelsay & Prather, 1983; Liebman & Doane, 1989) and phytate (Morris & Ellis, 1985; Liebman & Landis, 1989) are important inhibitors of Ca absorption. Balance studies can determine net Ca absorption, but not true absorption.

In animal models, the bone (femur) uptake method compares femur accumulation of an oral dose of an isotope tracer with a dose injected intraperitoneally. This method is based on the assumption that the intraperitoneal injection behaves as an oral dose with 100% absorption. With use of the whole-body counting technique, absorption of Ca from an oral dose of an isotopic tracer is based on extrapolating the linear portion of a retention curve to time zero (Koo et al. 1993). Koo et al. (1993) demonstrated that assessments of radiotracer activity of Ca in femur or whole body are equally accurate for comparing bioavailability of Ca among sources. Not having to collect excreta or multiple blood samples is a clear advantage of these methods.

Ca absorption can also be assessed with use of the in situ loop and everted sac methods. Studies based on these methods clearly established the existence of two Ca absorptive processes, a vitamin D-dependent, saturable, transcellular pathway that predominates in the duodenum and a non-saturable, paracellular pathway that occurs throughout the entire length of the small intestine (Pansu et al. 1983; Bronner et al. 1986). However, they are insensitive to the important factor of exposure time of the chyme to the mucosa.

Pinto et al. (1983) demonstrated that cultured Caco-2 cells, a human colon adenocarcinoma cell line, exhibit structural and functional differentiation patterns characteristic of mature enterocytes. Yee (1997) reported a strong correlation between in vitro permeability across Caco-2 cells and in vivo (small intestinal) absorption for a variety of compounds. It has also been confirmed recently that vitamin D-mediated Ca transport in these cells is a specific, transcellular process that requires transcriptional events normally mediated through the vitamin D receptor (Fleet & Wood, 1999). However, in culture, these cells form a membrane with tight junctions that interfere with measurement of transport.

Some have tried to use rise in urinary Ca to determine relative bioavailability, but this is a weak approach especially for Ca loads less than 500 mg. For example, variability in rise in urinary Ca was 77–99% compared with 38–60% for serum Ca following ingestion of three Ca salts (Heaney et al. 2001). Increments in serum Ca are also less sensitive than following the fate of isotopic tracers of Ca. Isotopic tracers have been used to label dietary sources of Ca for the measurement of Ca bioavailability in both animal models and man. Ca has two useful radioisotopes ($^{45}$Ca and $^{47}$Ca), several useful stable isotopes ($^{40}$Ca, $^{41}$Ca, $^{43}$Ca, $^{44}$Ca and $^{46}$Ca), as well as one long-lived radioisotope ($^{41}$Ca). Tracers can be followed by appearance in plasma, excretion or by whole-body retention in the case of $^{47}$Ca. Use of tracers for determining Ca absorption gives true absorption, in contrast to net absorption determined by balance. Intrinsic labelling of a broad array of hydroponically grown plants, milk made into a variety of dairy products and salts has enabled determination of Ca absorption in man from most Ca-rich foodstuffs in the Western diet (Weaver et al. 1999). Isotopic tracers can also be used to determine transfer rates, sites of absorption and pathway of absorption. When absorption is plotted v. various Ca loads, a curvilinear relationship suggests active transport and a linear relationship suggests paracellular absorption (see Fig. 2).

### Modifying calcium absorption

In a recent review, Bronner & Pansu (1999) stated: ‘It is obvious that calcium must be ionized and in solution to be absorbed’. Typically, Ca must be dissociated from its ligands in a foodstuff prior to absorption. However, there is recent evidence that Ca from a small-molecular-weight compound did not require dissociation prior to absorption (Hanes et al. 1999a,b). This was apparent because Ca and oxalic acid have very different serum appearance profiles, yet doubly labelled $^{45}$Ca-$^{14}$Cl oxalate showed parallel serum profiles of the two labels until the unabsorbed salt reached the colon, where bacterial hydrolysis presumably occurs. It is tempting to conclude that absorption of the salt occurred paracellularly, but calcium oxalate could conceivably transverse the epithelial membrane because it is apolar. The implications of this finding might be greatest for individuals with defective active Ca absorption capacity.

Solubilization is a reasonable assumption prior to Ca absorption, at least at the absorptive surfaces. Yet, in vitro solubility of Ca salts at neutral pH over a wide range had little impact on Ca absorption (Heaney et al. 1990a). For example, Ca absorption from CaCO$_3$ with a solubility of 0.14 mmol/l is as good as from tricalcium phosphate, which is nearly an order of magnitude more soluble at 0.97 mmol/l. Only salts that are at the extreme

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**Fig. 2.** Illustration of (A) saturable and (B) non-saturable components of calcium kinetics curves. At large calcium loads, active transport approaches a small value. The linear portion of the curve reflects constant and paracellular absorption.

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ends of solubility have appreciably different Ca absorption efficiencies.

Other factors that influence Ca bioavailability include the presence of inhibitors and enhancers in the diet. Functional foods for bone health may decrease the content of inhibitors and increase the content of enhancers. The most potent inhibitor of Ca absorption is oxalate (Heaney & Weaver, 1989). Fibre has been labelled as an inhibitor of Ca absorption since the early balance studies of McCance & Widdowson (1942). However, research with purified fibres has suggested that fibre does not influence Ca absorption appreciably (Heaney & Weaver, 1995). This is supported by the finding that Ca absorption efficiency from fibre-rich brassica vegetables is higher than from other foodstuffs (Weaver et al. 1999). The presence of an enhancer of Ca absorption in brassica vegetables is suspected, but not yet identified. In contrast to the lack of fibre effect, phytates associated with cereal and legume fibres can decrease Ca absorption (Heaney et al. 1991; Weaver et al. 1991). The capacity of phytate-rich extruded wheat bran cereal to bind Ca is linear over a wide range of Ca intake (Weaver et al. 1996).

Few Ca absorption enhancers have been identified. Most research has concentrated on searching for highly absorbable Ca salts. Only a few have been identified, including calcium citrate malate with a solubility of 80 mmol/l (Miller et al. 1988), calcium gluconate glycerophosphate (Schanler & Abrams, 1995) and calcium ascorbate (Tsugawa et al. 1999). Additionally, a few Ca absorption enhancers have been identified. Consumption of 15 g oligofructose/d increased stable isotopic tracer Ca absorption from 47.8% during a placebo period to 60.1% (van den Heuvel et al. 1999). Feeding of 40 g inulin/d increased apparent Ca absorption in adults participating in a balance study from 21.3 to 33.7% (Coudray et al. 1997). Certain amino acids, notably lysine, and casein phosphopeptides, digestive products from milk proteins, have Ca absorption-enhancing effects under some conditions such as in women with low absorption efficiency (Heaney et al. 1994). Yet another type of Ca absorption enhancer is the hydrolysis product of phytic acid, 1,2,3,6-inositol tetrakisphosphate, which improved 45Ca absorption efficiency from 26.2 to 30.7% in rats using calcium ascorbate as the Ca source (Shen et al. 1998).

Enhancement of Ca absorption has typically been attributed to the formation of soluble complexes with Ca which prevent precipitation by P in the gut. Other mechanisms of enhancing Ca absorption deserve to be explored. Increasing paracellular absorption is promising because it is not limited by becoming saturated, it is vitamin D-independent, and it occurs throughout the length of the intestine in contrast to active absorption which is dominant in the duodenum. If the intercellular junction spaces illustrated in Fig. 1 can be widened, more Ca could be absorbed. Or, if solvent drag could be increased, even though water would flow bidirectionally, given the large volume of blood that would serve to dilute ions extruded from the basolateral membrane, ions would have a net movement from lumen to blood. Ideal compounds would be those that could be incorporated into Ca-containing food to enhance absorption of Ca but would have only a transient effect, so that transfer of undesirable organisms and ions would be minimized. Pappenheimer & Reiss (1987) provided evidence that glucose and amino acids in the lumen of the small intestine increase solvent drag through paracellular channels by fueling the contraction of epithelial cytoskeletal elements, thereby opening tight junctions to allow mass transport of nutrients.

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**Fig. 3.** Relationship between calcium retention and calcium intake in adolescent Caucasian girls (mean and 95% confidence intervals). Calcium intakes required to achieve: (A) 70% of maximal retention at the lower edge of the confidence interval; (B) 100% of maximal retention at the upper edge of the confidence interval; and (C) 100% of maximal retention at the mean.
Modifying calcium retention

Dietary factors that alter Ca absorption or Ca excretion can modify Ca retention. Nutrients with the greatest impact on Ca loss include salt and protein, both of which increase urinary Ca output. Ironically, Ca intake influences urinary Ca output only modestly; only 6% of the variance in urinary Ca in adolescents was explained by Ca intake (Jackman et al. 1997). Data on the effect of dietary variables on Ca retention are collected largely in adults. We know little of these relationships across the life span or of the impact of race or other genetic determinants.

Perhaps a more useful way of determining the impact of dietary variables on Ca retention is to determine how Ca requirements might be affected by various dietary patterns and for different populations. The relationship between Ca retention and Ca intake has been examined for various populations (Matkovic & Heaney, 1992). For all populations, there is a linear relationship up to a certain intake after which there is a levelling off of the curve so that further increases in Ca intake produce little further gain in retention. The intake at which this plateau occurs is a reference value for setting requirements to maximize development of peak bone mass during growth and minimize bone loss during ageing. There is no consensus on whether requirements should be set at 100% of maximum retention or at some lower target. Nor is it clear how to determine intake for a given percentage retention taking into account variability. Figure 3 shows the mean and 95% confidence interval of the relationship between Ca retention and Ca intake in adolescent girls using the data of Jackman et al. (1997). Should the Ca requirement be set at extrapolated value A (70% of maximal retention taken at the lower limit of the 95% confidence interval), value B (100% maximal retention taken at the lower limit of the 95% confidence interval), value C (the mean 100% maximal retention), or some other value? The first value is arbitrary. The second value is dependent on sample size and inter-subject variability, which influences the confidence interval. The third value may be impractically high.

Regardless of how the relationship of Ca retention to Ca intake is used to set requirements, knowing the relationship is the first step. Understanding how the relationship can be influenced by dietary life-style and genetic factors is the next area of needed research. Theoretically, dietary patterns which include high bioavailable Ca, low salt and low protein can shift the curve to the left (Fig. 4), whereas diets characterized by low Ca bioavailability, high salt and high protein can shift the curve to the right (Fig. 5). Similarly, the curve might be shifted according to race, ethnic group, physical activity level, smoking and other factors.

Modifying bone turnover

Functional foods that promote health by modifying bone turnover may work by enhancing bone formation or suppressing bone resorption. Increasing Ca intake during adolescence results in increased Ca absorption, which suppresses bone resorption by the equivalent amount (Wastney et al. 2000). Phyto-oestrogens may provide some protection against bone resorption similar to oestrogen, although this is not yet well documented (Weaver et al. 2001). Suppression of bone resorption can lead to increased Ca retention.

Use of Ca isotopic tracers and kinetic analysis allows quantification of bone formation and bone resorption in units of Ca such as mg of Ca per day. Use of just one dose of the rare isotope, $^{41}\text{Ca}$, and accelerator mass spectrometry opens the possibility of determining types of diet changes that might suppress bone resorption in
individuals followed longitudinally. Approximately two months after dosing, the appearance of $^{41}$Ca in the urine directly reflects bone resorption. The sensitivity of accelerator mass spectrometry allows the tracer to be followed for years, thereby allowing assessments of changes in diet or other lifestyle factors.

Biochemical markers of bone turnover have also been used to determine qualitative changes in bone turnover (Weaver, 1998). Some common biochemical markers of bone formation include serum osteocalcin and bone alkaline phosphatase. Biochemical markers of bone resorption are typically urinary crosslinks of collagen. These assays are not in units of bone or Ca and results are highly variable. Larger sample sizes are required to find significant treatment effects. Frequently, the subtle effects of diet cannot be detected.

**Bone mineral density**

Bone mineral density is a strong biomarker for fracture. To evaluate the effectiveness of functional foods using this method, the intervention period needs to be much longer than for evaluating parameters of Ca metabolism. Ideally, interventions would have the duration of three to four sigmas. Each sigma, the period for a complete cycle of bone resorption and formation, is about four months in man. Trials of short duration fail to show the long-term impact on bone health. One study showed the benefit of one year of Ca-fortified foods on bone measures in growing children (Bonjour et al. 1997).

**Conclusions**

Increasing the Ca intake of the general population is the most effective strategy for using functional foods for bone health. Despite education efforts and the increased availability of an array of Ca-fortified foods and supplements, intake of Ca remains inadequate. The role of Ca bioavailability and the ability to maximize absorption and retention are less important under conditions of adequate intake. A variety of methods are available to evaluate the effectiveness of functional foods for bone health. Their effect on Ca absorption can be determined by *in vitro* techniques, use of animal models or in man. Use of Ca isotopic tracers offers a specific way to determine the point of Ca metabolism affected. Understanding mechanisms of Ca absorption and how to increase absorption and retention efficiency are also important.

In the next decade, we will understand better the dietary and lifestyle factors that influence Ca absorption by both transcellular and paracellular routes. We will understand better how dietary factors influence the relationship between Ca retention and Ca intake. We will understand better the genetic factors that influence Ca absorption and retention. That Ca retention has a large genetic component was demonstrated by the greater response to Ca of three generations of women who were from osteoporotic families compared with women from healthy families (O’Brien et al. 1998). A beginning in identifying a specific gene that may influence Ca absorption is the significant association of vitamin D receptor gene Fok1 polymorphism with Ca absorption and bone mineral density in children aged 7.5–12 years (Ames et al. 1999). Perhaps one day we will be able to tailor the food supply for identifiably vulnerable segments of the population.

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people consuming diets high in fruits and vegetables was only one-half that in those consuming few of these foods (Block et al. 1992). It is now clear that there are components in a plant-based diet other than traditional nutrients that can reduce cancer risk. More than a dozen classes of these biologically active plant chemicals, now known as ‘phytochemicals’, have been identified. Several examples are given below.

Functional foods from animal sources

Dairy products

Epidemiological studies regarding the effect of milk and dairy products on colorectal cancer incidence provide conflicting results. Some studies report a negative association between consumption of dairy products and disease incidence, whereas others report no or even a positive association. One reason for this discrepancy in the epidemiological observations may lie in the fact that some dairy products, such as cheese and cream, contain large amounts of animal fat, which is considered a risk factor by some for colorectal cancer. In line with this assumption, it appeared that the positive correlation between dairy products and colorectal cancer found in one study was due to cheese consumption (Iscovich et al. 1992). In addition, an international correlation study observed a negative correlation between consumption of dairy products and colorectal cancer only after adjustment for animal fat intake (McKeown-Eyssen & Bright-See, 1984). Most of the studies that reported no association did not adjust for fat intake. Hence, epidemiological studies provide weak evidence that consumption of milk and dairy products could protect against colorectal cancer.

The main hypothesis to explain this effect has been the high Ca content of dairy products. Thus, many previous studies have focused on Ca and consequently examined high Ca content of dairy products. Thus, many previous and dairy products could protect against colorectal cancer. The epidemiological studies are inconsistent (Hirayama & Rafter, 2000). Consumption of large quantities of dairy products such as yoghurt and fermented milk containing Lactobacillus or Bifidobacterium may be related to a lower incidence of colon cancer. An epidemiological study performed in Finland demonstrated that, despite the high fat intake, colon cancer incidence was lower than in other countries because of the high consumption of milk, yoghurt and other dairy products (Intestinal Microbiology Group, 1977). In two population-based case–control studies of colon cancer, an inverse association was observed for yoghurt (Peters et al. 1992) and cultured milk (Young & Wolf, 1988) consumption, adjusted for potential confounding variables. It can also be mentioned that an inverse relationship has been demonstrated between the frequency of consumption of yoghurt and other fermented milk products and breast cancer in women (van’t Veer et al. 1989). On the other hand, two companion American prospective studies, the 1980–1988 follow-up of the Nurses’ Health Study and the 1986–1990 follow-up of the Health Professionals’ Follow-up Study, did not provide evidence that intake of dairy products is associated with a decreased risk of colon cancer (Kampman et al. 1994a). In a cohort study in The Netherlands, it was shown that the intake of fermented dairy products was not significantly associated with colorectal cancer risk in an elderly population with a relatively wide variation in dairy product consumption, although a weak non-significant inverse association with colon cancer was observed (Kampman et al. 1994b).
In addition, there are some studies examining the effect of probiotics on the biomarkers for colon cancer risk (see below) in healthy volunteers and patients. Consumption of lactic acid bacteria by volunteers has been shown to reduce the mutagenicity of urine and faeces associated with the ingestion of carcinogens in cooked meat (Lidbeck et al. 1992). Mucosal cell proliferative activity in upper colonic crypts of patients with colon adenomas (believed to be a risk factor for tumour development) decreased significantly after the administration of *Lactobacillus acidophilus* and *Bifidobacterium bifidus* cultures (Biasco et al. 1991).

**Beef**

An anticarcinogenic fatty acid known as conjugated linoleic acid was first isolated from grilled beef in 1987 (Ha et al. 1987). Nine different isomers of conjugated linoleic acid have been reported as occurring naturally in food. Conjugated linoleic acid is unique in that it is found in highest concentrations in fat from ruminant animals (e.g. beef, dairy, lamb). In recent years, conjugated linoleic acid has been shown to be effective in suppressing forestomach tumours in mice, aberrant colonic crypt foci in rats and mammary tumours in rats (Ip & Scimcma, 1997).

**Functional foods from plant sources**

**Soyabeans**

Several classes of anticarcinogens have been identified in soyabeans, including protease inhibitors, phytosterols, saponins, phenolic acids, phytic acid and isoflavones. Of these, isoflavones (genistein and daidzein) are particularly noteworthy because soyabeans are the only significant dietary source of these compounds. Isoflavones are heterocyclic phenols structurally similar to the oestrogenic steroids. Because they are weak oestrogens, isoflavones may act as anti-oestrogens by competing with the naturally occurring endogenous oestrogens. This may explain why populations that consume significant amounts of soya have reduced risk of oestrogen-dependent cancer. However, more epidemiological data and clinical intervention trials are needed to investigate the role of soya in reducing cancer risk.

**Flaxseed**

There has been an increasing interest in fibre-associated compounds known as lignans. The two primary mammalian lignans, enterodiol and its oxidation product, enterolactone, are formed in the intestinal tract by bacterial action on plant lignan precursors. Flaxseed is one of the richest sources of mammalian lignan precursors. Because enterodiol and enterolactone are structurally similar to both naturally occurring and synthetic oestrogens, and have been shown to possess weakly oestrogenic and anti-oestrogenic activities, they may also play a role in the prevention of oestrogen-dependent cancers. In rodents, flaxseed has been shown to decrease tumours of the colon, mammary gland and lung. Phipps et al. (1993) demonstrated that the ingestion of 10 g of flaxseed per day elicited several hormonal changes associated with reduced breast cancer risk. However, as is the case with soya, epidemiological data are required to support the hypothesis that enterodiol and enterolactone have anticarcinogenic properties in man.

**Tomatoes**

Tomatoes have received much attention in recent years because of interest in lycopene, the primary carotenoid in this fruit, and its potential role in cancer risk reduction (Weisburger, 1998). In a prospective cohort study of more than 47000 men, those who consumed tomato products ten or more times per week had less than one-half the risk of developing advanced prostate cancer (Giovannucci et al. 1995). Interestingly, lycopene is the most abundant carotenoid in the prostate gland. Other cancers whose risk have been inversely associated with serum or tissue levels of lycopene include breast, digestive tract, cervix, bladder and skin (Clinton, 1998). Proposed mechanisms by which lycopene could influence cancer risk are related to its antioxidant function. Lycopene is the most efficient quencher of singlet oxygen in biological systems (Di Mascio et al. 1989).

**Garlic**

Garlic (*Allium sativum*) is probably the herb most widely quoted in the literature for medicinal purposes. The intact garlic bulb contains an odourless amino acid, which is converted enzymatically by allinase into allicin when the garlic cloves are crushed (Block, 1992). Allicin then decomposes spontaneously to form numerous sulfur-containing compounds, some of which have been investigated for their chemopreventive activity. Garlic components have been shown to inhibit tumourigenesis in several experimental models. However, additional reports have shown garlic to be ineffective. Inconclusive results are likely to be due to differences in the type of garlic compounds or preparations used by various investigators. Several epidemiological studies show that garlic may be effective in reducing human cancer risk (Dorant et al. 1993). However, it should be mentioned that not all such studies have shown garlic to be protective. A review of twenty epidemiological studies (Ernst, 1997) suggested that allium vegetables, including onions, may confer a protective effect on cancers of the gastrointestinal tract.

**Tea**

Much attention has focused on the polyphenolic constituents of tea, particularly green tea. Polyphenols comprise up to 30% of the total dry weight of fresh tea leaves. Catechins are the predominant and most significant of all tea polyphenols (Graham, 1992). The four major green tea catechins are epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate and epicatechin. Much of the work on the health effects of tea has focused on its cancer chemopreventive effects. Research results from laboratory animals tend to support a cancer chemopreventive
effect of tea components (Dreosti et al. 1997). However, the epidemiological studies are still somewhat inconclusive. It has been suggested that benefits from tea consumption are restricted to high intakes in high-risk populations (Kohlmeier et al. 1997). The consumption of five or more cups of green tea per day was shown to be associated with decreased recurrence of stage I and stage II breast cancer in Japanese woman (Nakachi et al. 1998).

**Broccoli and other cruciferous vegetables**

Epidemiological evidence has also associated the frequent consumption of cruciferous vegetables with decreased cancer risk. In a review of eighty-seven case–control studies, Verhoeven et al. (1996) demonstrated an inverse association between consumption of total brassica vegetables and cancer risk. The percentages of case–control studies showing an inverse association between consumption of cabbage, broccoli, cauliflower and Brussels sprouts and cancer risk were 70, 56, 67 and 29 %, respectively. The anticarcinogenic properties of cruciferous vegetables have been attributed to their relatively high content of glucosinolates (Verhoeven et al. 1997). Glucosinolates are a group of glycosides stored within cell vacuoles of all cruciferous vegetables. Myrosinase, an enzyme found in plant cells, converts these compounds to a variety of hydrolysis products, including isothiocyanates and indoles. While a wide variety of naturally occurring and synthetic isothiocyanates have been shown to prevent cancer in animals (Hecht, 1995), attention has been focused on a particular isothiocyanate isolated from broccoli, i.e. sulforaphane. Sulforaphane has been shown to be a good inducer of a particular phase II enzyme, quinone reductase. Indole-3-carbinol has received attention for its cancer chemopreventive properties, particularly of the mammary gland. In addition to the induction of phase I and phase II detoxification reactions, indole-3-carbinol may reduce cancer risk by modulating oestrogen metabolism.

**Citrus fruits**

Several epidemiological studies have shown that citrus fruits are protective against a variety of human cancers. Although oranges, lemons, limes and grapefruits are a principal source of such important nutrients as vitamin C, folate and fibre, Elegbede et al. (1993) have suggested that another component is responsible for the anticancer activity. Citrus fruits are particularly high in a class of phytochemicals known as the limonoids (Hasegawa & Miyake, 1996). In recent years, evidence has been accumulating in support of the cancer-preventative effect of limonene (Gould, 1997). Crowell (1997) showed this compound to be effective against a variety of both spontaneous and chemically induced rodent tumours.

However, mindful of the importance of the overall dietary pattern in cancer risk reduction, one must question the clinical implications of a single phytochemical in isolation.

**Biomarkers available for assessing diet-related changes in colon cancer risk**

Since colon cancer has been a major target for the cancer chemopreventive effects of probiotics and functional food components, I shall limit my examples of ‘biomarkers’ to this cancer form. Presently, there are a large number of biomarkers available for assessing colon cancer risk in dietary intervention studies, which are validated to varying degrees. These include colonic mucosal markers, faecal water markers and immunological markers.

**Colon mucosa biomarkers**

1. Adhesion of Gram-negative bacteria
2. Modulation of cyclo-oxygenase-2 (COX-2)
3. Proliferation
4. K-ras
5. Genetic instability
6. Apoptosis
7. DNA-repair integrity
8. Metastasis markers
9. Microsatellite instability
10. Oxidative DNA damage
11. Gene-specific damage

**Faecal water markers**

In recent years, there has been considerable interest in the role of the aqueous phase of human faeces (faecal water) in studies examining the mechanisms underlying the dietary aetiology of colon cancer. The motivation is that components of this faecal fraction are more likely to be able to exert untoward effects on the cells of the colonic epithelium than components bound to food residues and the bacterial mass.

1. Cytotoxicity
2. COX-2 induction
3. Caspase induction
4. Calprotectin levels
5. Activator protein-1 activation
6. Bile acid levels
7. Effects on metastasis
8. Genotoxicity
9. Effects on cell metabolism
10. Gene induction

**Immunological and inflammatory response markers**

In the colon:

1. Suppression of COX-2 induction by pro-inflammatory cytokines

In blood:

1. Natural killer cells
2. Lymphocyte proliferation
3. Cytokines (interleukin-2, interleukin-β, tumour necrosis factor)

Thus, in designing a dietary intervention study/clinical trial
to study anticaner effects (colon cancer) of functional foods, it is recommended to use as many of the above 'state-of-the-art' biomarkers as is feasible.

Examples of studies addressing changes in biomarker response to a functional food

Shift from dairy product-rich to dairy product-free diet

Cytotoxicity of faecal water is now an accepted risk marker for colon cancer and several studies have correlated toxicity of this faecal fraction with a higher colonic cell proliferation and increased colon cancer risk (Lapré & Van der Meer, 1992). Genotoxicity per se in human faeces is also generally accepted as a risk marker for colon cancer. Recently, Pool-Zobel et al. (1996) have demonstrated, by employing the single cell gel electrophoresis (COMET) assay, that colon carcinogenic induced genotoxicity in the colon of rats and that this genotoxicity was altered by dietary manipulations.

To determine whether the cytotoxicity and genotoxicity of the aqueous phase of human stools (faecal water) were affected by a change in dairy product intake, eighteen healthy male and female volunteers were randomly divided into two groups (Glinghammar et al. 1997). In a cross-over design, the volunteers shifted from their normal dairy product-rich to a dairy product-free diet. Nutritional analysis of the food consumed, during the study period, showed a significant decrease in energy intake from 9000 KJ/d to 7866 KJ/d ($P < 0.004$), due to decreased intake of protein and fat. Carbohydrate and fibre intakes remained unchanged during the intervention. Ca intake decreased significantly from 1488 mg/d to 372 mg/d ($P < 0.001$), with phosphate and vitamin D displaying similar significant decreases ($P < 0.001$). Cytotoxicity of faecal water, analysed by the HT-29 cytotoxicity assay, indicated a significant decrease ($P = 0.025$) in cell survival from 34% to 20% when dairy products were excluded from the participants' diet. This effect is most likely due to the decreased intake of dairy Ca and possibly phosphate.

The COMET assay, used to analyse genotoxicity of faecal waters, indicated no differences brought about by the dietary intervention. These findings indicate that a shift from a dairy product-rich to a dairy product-free diet results in a significant effect ($P = 0.025$) on an accepted risk marker for colon cancer, and may suggest that the mechanism by which dairy products are protective is at the level of tumour promotion rather than initiation.

Clinical trial to examine the effect of a symbiotic preparation on colon cancer risk biomarkers in adenoma patients

Another example of such a study is the ongoing Symbiotics and Cancer Prevention in Humans (SYNCAN) project, funded by the EU, and involving eight research centres in Europe. It involves a twelve-week, randomised, double-blind placebo-controlled trial of a food supplement containing *L. rhamnosus* GG, *Bifidobacterium* Bb-12 and Raftilose Synergyl in adenoma patients. In this study, all of the colon cancer risk biomarkers, listed above, will be measured. In parallel, a long-term tumourigenesis study in rats, using the same symbiotic combination and assaying for the same biomarkers, is being carried out. It is hoped that the results of this study will provide much needed information on the cancer-protective effects of symbiotics in man and on the underlying mechanisms.

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many of the treatments for type 2 diabetes such as insulin and sulfonylureas have been shown to produce weight gain and potentially exacerbate the disease (UK Prospective Diabetes Study Group, 1998a,b).

Prevention of type 2 diabetes has recently been shown to be a promising strategy. The Finnish Diabetes Prevention Study (Tuomilehto et al. 2001) and the larger Diabetes Prevention Program (DPP Research Group, unpublished results) both found that the risk of developing diabetes in obese, insulin-resistant subjects could be cut by more than half by lifestyle modification. The lifestyle modification consisted of modest weight loss (about 5%) and an increase in physical activity (thirty minutes of physical activity about three times per week).

**Modifying energy balance: biomarkers for obesity**

Obesity arises from an energy imbalance whereby energy intake exceeds energy expenditure—Dealing with obesity—either prevention or treatment—requires modification of one or both components of energy balance. Approaches to weight management (including a functional food approach) therefore can target multiple aspects of the energy balance systems: food intake, energy expenditure and energy storage. All of these approaches are currently being taken by pharmaceutical companies; however, developing foods for weight management may be a more attractive approach for dealing with the 61% of the population that is overweight or obese.

Regardless of the approach to weight management, a major limitation in assessing and documenting success is the lack of suitable biomarkers to assess the impact of different treatments on components of energy balance.

**Assessment of body energy stores**

Body weight can easily be assessed using a bathroom scale, but assessing the increased health risk of obesity is more difficult. The majority of data available relates BMI to morbidity and mortality (National Institutes of Health, 1998). While BMI (weight divided by the square of height) is obtained relatively easily, it does not provide information about body composition (i.e. body fat v. fat-free mass). Body composition can be assessed accurately in the laboratory, but these techniques are not readily available to the general population (Lohman, 2001). The techniques available to the general population for assessing body composition (such as bioelectrical impedance and skinfold thickness) are not considered to be highly accurate or precise (Lohman, 2001).

**Energy intake**

**Total energy intake.** It is surprisingly difficult to measure accurately the amount of energy consumed by a free-living individual. There are accurate tables for converting food eaten into energy (kJ or kg) consumed, but the difficulty is in determining the amount and composition of food eaten. The typical way of assessing food intake is to have individuals keep records of food eaten. This is frequently done using food diaries (for periods of three to seven days), 24-hour recalls of all food eaten during the previous twenty-four hours, or assessments of the frequency with which various foods are usually consumed (Westerterp, 1998). While these techniques allow estimation of energy intake, they lack both accuracy and precision (Heitman & Lissner, 1995; Westerterp, 1998). For example, when comparing self-reported energy intake with accurate measures of energy expenditure (e.g. using doubly labelled water) for individuals in energy balance, estimated energy intake is usually below measured energy expenditure (Schoeller, 1995). Proponents of the use of dietary self-reports argue that even if the absolute energy intake is not accurate, the instruments provide an accurate reflection of changes in energy intake (Willett, 1990, Kristal et al. 1994). Whether this is the case or not is unclear.

Type of food eaten. There is great controversy about the role of diet composition in the development and management of obesity. It is likely that some functional foods aimed at reducing obesity will attempt to alter diet composition. Determining diet composition accurately may be even more difficult than determining total energy intake. Perhaps changes in protein intake are more easily assessed than changes in the other macronutrients. Protein intake is correlated with urinary and blood levels of nitrogen, and these can provide a biomarker of changes in dietary protein intake.

There is no universally accepted marker for carbohydrate intake. One can obtain some sense of type of carbohydrate intake from the glycaemic index, which is the rise in blood glucose after carbohydrate intake. Amount of carbohydrate intake is also correlated with insulin level, but it is not clear that either glucose or insulin level is a useful marker of changes in carbohydrate intake. Additionally these measurements are not easily available to the general population.

There is no clear usable biomarker for fat intake. The type of fat consumed chronically can be determined from biopsies of adipose tissue or from red blood cell membrane fatty acid composition (Wardlaw et al. 1994), but these techniques are invasive and do not provide information about the amount of fat consumed. Blood levels of cholecystokinin and enterostatin change with dietary intake but it is not clear that these blood changes allow accurate quantification of changes in dietary fat.

**Assessment of energy expenditure**

Functional foods that modify energy expenditure could be useful in weight management. Assessment of energy expenditure can be accomplished accurately under laboratory conditions using indirect calorimetry or doubly labelled water (Melby et al. 1998). There are currently no biomarkers for energy expenditure that are available to the general public. A new device called the Body GEM™ has just gone on the market in the USA (Nieman et al. 2001) and appears to provide a measure of resting energy expenditure of individuals. Like more expensive hospital equipment, the Body GEM™ determines energy expenditure from measurement of the amount of oxygen consumed by the body. Such measurements can be made.
relatively inexpensively in health clubs and by personal dietitians and personal trainers. Devices like this may be useful in assessing the efficacy of products to increase energy expenditure.

**Functional foods to reduce energy intake**

One promising avenue to reduce energy intake using functional foods is through increased satiety. The goal is to provide foods that increase the sense of fullness and encourage the individual to stop eating sooner, thereby reducing total energy intake. In general, the three most promising areas to increase satiety are to: (1) modify the energy density of the diet; (2) modify the macronutrient composition of the diet; and (3) modify the glycaemic index of the diet.

**Energy density**

The energy density of the diet is the energy content per unit of weight or volume and seems to be correlated with total energy intake. The energy density of the diet is the energy content per unit of weight or volume and seems to be correlated with total energy intake. In general, the three most promising areas to increase satiety are to: (1) modify the energy density of the diet; (2) modify the macronutrient composition of the diet; and (3) modify the glycaemic index of the diet.

The energy density of the diet is the energy content per unit of weight or volume and seems to be correlated with total energy intake (Rolls & Bell, 1999, 2000; Stubbs et al. 1995a). Energy density is relatively easy to measure for most foods and can be calculated by dividing the energy content of the food in kJ by the weight or volume of the food (Rolls & Barnett, 2000). In their book *Volumetrics*, Rolls & Barnett (2000) explain the concept of energy density and provide energy density values for a range of foods.

There are substantial data to suggest that total energy intake over the short term (a few days) varies directly with the energy density of the diet (Rolls & Bell, 1999, 2000; Stubbs et al. 1995a). One physiological signal for satiety may relate to the total weight or volume of food ingested. This suggests that modifying the energy density of the diet could be a way to reduce total energy intake and reduce obesity. The main determinant of energy density is the non-caloric content of the food, primarily the water content (Grunwald et al., 2001). Foods with a high water content have a low energy density. Fibre also reduces energy density since it contributes substantially more to food weight than to caloric content. However, energy density is also affected by the macronutrient composition of the diet. Since fat is more energy-dense (38 kJ/g) than either protein or carbohydrate (17 kJ/g), reducing the proportion of fat in the diet can have a major impact on reducing the energy density of the diet. Since there are solid data showing that reducing energy density reduces energy intake (at least in the short term), functional foods aimed at modifying energy density may be useful in managing obesity. It would be helpful to have more long-term data substantiating the effect of energy density and relating energy density to changes in body weight.

**Macronutrient composition and satiety**

Researchers often refer to a hierarchy of satiety for macronutrients with protein being the most satiating and fat the least satiating, joule for joule (Hill & Prentice, 1995). High-protein diets are currently popular for weight loss and are based, in part, on the idea that high-protein diets promote satiety. Popular diet books such as *New Diet Revolution* (Atkins, 1992) and *Protein Power* (Eades & Eades, 1996) are based on this premise. The data in support of high-protein diets as facilitators of weight loss and weight maintenance are still incomplete. Some studies suggest that high-protein diets may be effective in producing short-term decreases in food intake (Rolls et al. 1988) but whether or not these diets are helpful in long-term weight maintenance or in prevention of weight gain is not clear.

With regard to satiety and carbohydrate intake, it may depend on the type of carbohydrate in the diet. While intake of simple carbohydrate or sugar has been suggested by the popular press to be associated with the development of obesity, it is not clear that high-sugar diets lead to overeating or obesity compared with low-sugar diets (Lewis et al. 1992; Hill & Prentice, 1995; Ludwig et al. 2001).

It has also been suggested that calories in liquid form may affect satiety less strongly than solid foods with the same calorie content (Mattes, 1996). While the available data are not definitive, if substantiated they could implicate caloric beverages as a facilitator of overeating and a causal factor in obesity. Most calories in beverages are in the form of carbohydrates, and there is little information about whether different types of carbohydrates in liquid form might affect energy intake differently.

Dietary fibre intake seems to best predict total energy intake, with several reports of lower total energy intake with high-fibre v. low-fibre diets (Pereira & Ludwig, 2001). There are probably several reasons why high-fibre diets are associated with lower food intake. First, high-fibre diets may trigger maximal sensory stimulation in the mouth due to the increased need for chewing. High-fibre diets also lead to slower gastric emptying and a slower rate of nutrient absorption. Finally, a high fibre content reduces the energy density of the overall diet. Regardless of the reason, increasing dietary fibre is generally thought to aid in weight management. Ludwig et al. (1999) found that high dietary fibre seemed protective against weight gain over a decade.

Modification of dietary fat type is not a commonly accepted strategy for weight loss. There is some suggestion that diets high in polyunsaturated fatty acids stimulate total fat oxidation more than diets high in saturated fatty acids (Jones & Schoeller, 1988) but this is somewhat controversial. Other fats such as short- and medium-chain triacylglycerols and n–3 fatty acids may have a greater impact on energy metabolism but it is not clear that these would play a major role in weight management.

**Glycaemic index and satiety**

The glycaemic index of a food is determined by the rise in glucose that occurs after eating that food in relation to the rise in glucose seen after eating a standardized food such as white bread (Brand-Miller et al. 1999). This obviously requires measuring glucose after consuming a food. High glucose levels following eating would stimulate insulin secretion which may increase appetite and facilitate other disease processes linked to insulin action. In their book,
The Glucose Revolution, Brand-Miller et al. (1999) provide the rationale for how glycaemic index may affect food intake and obesity, and provide tables of glycaemic index for many foods.

Whether or not the glycaemic index of the diet affects energy intake and obesity remains controversial. There is no convincing evidence that food intake is related directly to glycaemic index, although there is some evidence that high glycaemic diets are linked to weight gain (Ludwig, 2000). The glycaemic index of the total diet could be modified by eating foods with a low glycaemic index. If the glycaemic index were shown to affect food intake, a good target would be to develop more good-tasting, low glycaemic foods.

**Functional foods to increase energy expenditure**

Another way to reduce the likelihood of developing obesity or to treat obesity would be to increase total energy expenditure without increasing energy intake. While some food supplements make the claim of increasing energy expenditure, there are very few data available to support the efficacy of these products.

One product on the market that has some demonstrated efficacy is the combination of caffeine and ephedrine. This combination has been shown to increase energy expenditure modestly and is used for obesity treatment in some countries (Astrup et al. 1992). There has been recent concern about the long-term safety of ephedrine.

Recently it has been suggested that diets high in Ca may be protective against weight gain and that part of the mechanism may be an increase in energy expenditure (Zemel et al. 2000). In several datasets, high Ca intake is associated with a lower BMI (Davies et al. 2000), but there has yet been no clear demonstration that this is a causal relationship.

Oolong tea is another food that may have some impact on increasing energy expenditure (Rumpler et al. 2001), perhaps through its catechin content. Resting metabolic rate was increased by 3–4% during three days of oolong tea consumption at five cups per day. Interestingly, most of the rise in metabolic rate was from increased fat oxidation, which should have the greatest impact upon decreasing body fat stores.

**Functional foods to alter nutrient partitioning**

If some of the energy ingested is not absorbed completely, this can reduce net energy available to meet metabolic demands and can lead to weight loss. The approaches that have been taken involve blocking absorption of carbohydrates (starch blockers) or fats (fat blockers). The impact of the currently available products (e.g. Acarbose) is not clear.

**Non-absorbable fats**

Olestra is a non-absorbable fat substitute that has been shown to reduce total energy intake (Hill et al. 1998), and to be effective in producing weight loss (Bray et al. 2002). Olestra was approved by the Food and Drug Administration in 1996 for use in savoury snack products. Post market surveillance data suggest an association between Olestra intake and weight maintenance. The mechanism for a positive obesity effect may be its effects on reducing energy density, although other mechanisms cannot be ruled out.

**Functional foods to prevent or manage diabetes**

The results of the Finnish Diabetes Prevention Study (Tuomilehto et al. 2001) and the larger Diabetes Prevention Program (DPP Research Group, unpublished results) show that modest weight loss (about 5%) can reduce the development of type 2 diabetes by half in individuals at risk for this disease. There is a tremendous opportunity to develop functional foods targeted at those at risk for type 2 diabetes (i.e. insulin-resistant) to help them achieve modest weight reduction. Theoretically, any functional food that helped with weight loss could be marketed as helping prevent diabetes in an insulin-resistant population. Furthermore, it may be possible to target functional foods towards management of diabetes in those who already have type 2 diabetes. It is clear that weight control is an effective diabetes management technique and, here again, functional food aimed at weight loss could be targeted towards those with type 2 diabetes. Moreover, it may be possible to develop functional foods that impact insulin action independently of weight loss. These would probably be foods that increase the sensitivity of peripheral tissues to insulin, or insulin sensitizers.

**Insulin sensitizers**

There are currently food supplements that claim to increase insulin sensitivity, but there is very little evidence about the effectiveness of these products. Perhaps the most widely available of these is chromium picolinate, which under some circumstances appears to affect insulin action (Cefalu et al. 1999). Such products have not yet been proved to be effective in treating diabetes.

**The future: possibilities for new biomarkers**

While we see a clear opportunity to develop and market more functional foods for weight management, the lack of accurate biomarkers to assess their effectiveness is a barrier to this process. The future may hold promise for developing better biomarkers and this would greatly facilitate the development of functional foods.

It is almost certain that better and more accessible methods of measuring body composition will be available in the future. Accurate body composition, including body fat distribution, is not available on a widespread basis outside laboratory settings. This is likely to change with additional technological advances.

It is likely that we will develop better ways of assessing total energy intake and intake of specific macronutrients by adding non-metabolizable substances to the diet and monitoring their excretion. This will allow better assessment of the effectiveness of functional food for changing total energy intake or macronutrient content of the diet.
As recognition of the need to assess and treat the metabolic syndrome grows, physicians and other health care professionals will be screening patients for insulin resistance. It is likely that there will soon be consensus on how to do this, thus providing a standardized way of assessing insulin resistance that can be used to assess any change in response to functional foods.

References


foods that will protect individuals from weight gain or bring about a weight loss. The first of these objectives will be the most readily attainable. This means that foods must prevent individuals from reaching a state of positive energy balance — this means ensuring that energy intake does not exceed energy expenditure. Although it is more readily recognised that foods should have the potential to control energy intake, it should be kept in mind that foods can also enhance energy expenditure either through metabolic activity (e.g. thermogenesis) or by increasing physical activity. It is important to recognise that behaviour accounts for 100% of energy intake and between 20 and 60% of energy expenditure. Therefore the direct effect of foods on behaviour is critical.

Considering the reduction of energy uptake, adjusting the composition of the diet is a valid proposition. The use of specific macronutrient manipulations can help to suppress hunger and reduce the amount of food eaten. Alternatively, specific materials can be incorporated into food (such as yoghurts) so as to produce an intense action on satiety-signalling systems. This strategy involves the development of functional foods (nutraceuticals) for appetite control. There is a huge opportunity for foods in this sector of the food market.

**Appetite control and the satiety cascade**

The biological drive to eat is linked to the satiating power of food. Satiating power, or satiating efficiency, describes the capacity of a food to suppress and to inhibit further eating. Food causes this effect by certain mediating processes that can be roughly classified as sensory, cognitive, post-ingestive (pre-absorptive) and post-absorptive. These processes are operated by the impact of food on physiological and biochemical mechanisms, and collectively these processes have been referred to as the satiety cascade. The way in which food is sensed and processed by the biological system generates neural and hormonal signals, which are used to control appetite. It follows that any self-imposed or externally applied reduction in the food supply, creating a calorific deficit, will weaken the satiating power of food. One consequence of this will be the failure of food to suppress hunger adequately (the biological drive). The satiety cascade appears to operate as efficiently in obese people as in lean individuals — a normal appetite response to reduced calorie intake is evident in obese subjects.

Technically, satiety can be defined as inhibition of hunger and eating that arises as a consequence of food consumption. It can be distinguished from satiation, which is the process that brings a period of eating to a halt. Consequently, satiation and satiety act together to determine the pattern of eating behaviour and the accompanying profile of motivation. The conscious sensation of hunger is one index of motivation and reflects the strength of satiation and satiety. It is worth remembering that hunger is a biologically useful sensation. It is a nagging, irritating feeling that prompts thoughts of food and reminds us that the body needs energy. The identification and management of hunger are important factors underlying normal appetite function and abnormalities of appetite and body weight.

**Physiological satiety signals**

A key feature in understanding the effect of foods on satiety is the recognition of physiological satiety signals. Even before food touches the mouth, physiological signals are generated by the sight and smell of food. These events constitute the cephalic phase of appetite. Cephalic phase responses are generated in many parts of the gastrointestinal tract; their function is to anticipate the ingestion of food. During and immediately after eating, afferent information provides the major control over appetite. Afferent information from food in the mouth provides primarily positive feedback for eating while that from the stomach and small intestine provides primarily negative feedback. Initially, the brain is informed about the amount of food ingested and its nutrient content via afferent input. The gastrointestinal tract is equipped with specialised chemoreceptors and mechanoreceptors that monitor physiological activity and pass information to the brain, mainly via the vagus nerve. This afferent information constitutes one class of satiety signals and forms part of the post-ingestive control of appetite. A post-absorptive phase is also usually present in response to digested nutrients crossing the intestinal wall to enter the circulation. These products, which accurately reflect the food consumed, may be metabolised in the peripheral tissues or organs or may enter the brain directly via the circulation. In either case, these products constitute a further class of metabolic satiety signals. It has been argued that the degree of oxidative metabolism of glucose and free fatty acids in the liver constitutes a significant source of information for the control of appetite. Additionally, products of digestion and agents responsible for their metabolism may reach the brain and bind to specific chemoreceptors that influence that influence neurotransmitter synthesis or alter some aspect of neuronal metabolism. In each case the brain is informed about some aspects of metabolic state resulting from food consumption.

**Macronutrients and satiating power**

A vulnerable point within the psychobiological system is the interaction between the nature of the diet and the body’s biological responses to food. The concept of the satiety cascade implies that foods of varying nutritional consumption will engage differently with the mediating processes and will, therefore, exert differing effects on satiation and satiety. Dietary variables could overcome appetite control by a strong attractiveness mediated by sensory mechanisms, by nutrient composition or by an interaction between sensory and nutritional components. Considering protein consumption, current surveys suggest that most populations consume between 13 and 15% daily energy as protein and dietary recommendations suggest the intake of 0.8–1.0 g of protein for every kg of body weight. However, analysis of anthropological evidence suggests the possibility that our hunter-gather ancestors may have consumed diets containing 19–35% protein (Cordain et al. 2000). However, this view is not universally accepted (Milton, 2000).

Do the macronutrients protein, carbohydrate and fat contribute equally to satiating power? The answer is not
obvious. One reason is that these macronutrients are not of equal caloric density, and therefore comparisons among them should be made on the basis of their individual caloric contributions rather than upon weight. Investigations of the action of macronutrients can be made by presenting preloads in which one macronutrient is held constant and the other two are varied systematically. For example, when fat is held constant and protein and carbohydrate adjusted, it is generally found that protein provides greater satiating power than carbohydrate. Therefore, high-protein meals would be expected to give rise to intense and prolonged satiety.

**Protein and satiety**

Evidence for a specific effect of protein on satiety goes back to the ‘aminostatic’ concept of appetite control (Mellinkoff et al. 1956). Early studies examined the effects of breakfast nutritional composition and found that high-protein breakfasts were followed by a consistent and prolonged ‘sense of well-being’, and that daily energy intake computed from diaries was inversely related to the protein content of breakfast. Unfortunately though, the outcomes of both studies were compromised by methodological shortfalls, such as not controlling for the energy content or bulk of the breakfast meals. Similar tantalising but problematic findings were reported for the effect of protein on subjective hunger. Fryer et al. (1955) noted that a high-protein weight-loss diet was associated with the least reports of hunger, and Mellinkoff et al. (1956) found an inverse correlational relationship between hunger and serum amino acid levels.

Later studies enabled firmer conclusions to be drawn regarding the satiating power of protein. Booth et al. (1970) evaluated the effects of equicaloric high- and low-protein composite meals on the intake of a nutritionally intermediate cornflower pudding three hours later. Although the palatability of the food offered must be questioned, the authors nevertheless described a 26% reduction in voluntary intake after the high-protein meal. Using a completely different methodology, Butler et al. (1981) administered a small preload of an 8 g mixture of four amino acids or placebo (134 kJ) half an hour before offering a cooked midday meal. The amino acid preload led to a 10% reduction in energy intake from this meal compared with placebo.

Other studies have described the effects of high protein loads or meals on carefully measured scales of subjective motivation to eat. Spring et al. (1983) found that subjects reported feeling significantly more full after eating 227 g of turkey breast (high in protein) than after an equicaloric amount of high-carbohydrate sherbet. Likewise, Hill & Blundell (1986) found that eating a high-protein lunchtime meal led subjects to rate themselves as having less desire to eat and as feeling more full than after an equicaloric and equivolume high-carbohydrate meal. A similar action of protein was also seen in obese subjects (Hill & Blundell, 1986). Consequently, there is a good deal of evidence that high-protein foods can exert a potent modulation of human appetite. However, comparisons between different types of protein, such as egg albumin, casein, gelatin, soya protein, pea protein and wheat gluten, have failed to display differences arising from a manipulated meal (Lang et al. 1998).

**Proteins and satiety signalling**

Considering the operations of the satiety cascade, proteins in food could exert an action on satiety via the pre-absorptive or post-absorptive mechanism. In fact there is evidence for an action via both routes.

In general, proteins could intervene in the satiety cascade by triggering, or maintaining, the release of the satiety hormone cholecystokinin. There is good evidence in man that cholecystokinin intensifies normal satiety (Greenough et al. 1998) and in animals the satiety effect of protein has been shown to be mediated via cholecystokinin-A type receptors (Trigazis et al. 1997).

It is clear that intact protein can have a potent satiety-triggering effect, but in addition specific amino acids or peptides could also exert a noticeable action. Individual amino acids such as phenylanine or tryptophan (Hill & Blundell, 1988) have been shown to increase satiety and also adjust food preferences and selection. More recently, the dipeptide phenylalanine–aspartic acid, when delivered in capsule form before a meal, significantly reduced food consumption (Rogers & Blundell, 1989). Interestingly, this effect was most prominent when the dipeptide was administered an hour before the meal (Rogers et al. 1995).

There seems to be a clear potential for other functional peptides such as caseinomacropeptide to exert a satiety-enhancing effect. There is some evidence that caseinomacropeptide can release cholecystokinin and may modulate the release of other gastrointestinal hormones such as glucagon-like peptide-1, which is known to be involved in satiety (Blundell & Naslund, 1999).

Proteins and peptides may also exert effects via a post-absorptive route. Important here is the concept of a hierarchy of satiety power among the macronutrients (Stubbs, 1996). This satiating concept depends upon the limited store of protein in the body (lean body mass) and the tight coupling between protein intake and oxidation. According to this formulation the hierarchy of satiating power is protein > carbohydrate > fats.

In addition, there is evidence for a strong effect of protein on diet-induced thermogenesis. A comparison between two diets demonstrated that the diet containing 29% protein produced a greater effect on 24 h diet-induced thermogenesis than a 9% protein diet (Westeterp-Plantenga et al. 1999).

Another post-absorptive mechanism depends upon the role of amino acids as precursors of brain neurotransmitters — for example, tryptophan for serotonin and phenylalanine and tyrosine for dopamine and noradrenaline. The capacity of these neurotransmitters to modulate the expression of appetite through brain systems can be influenced by the ratio of amino acids (e.g. tryptophan to large neutral amino acids) in the plasma.

These mechanisms indicate how protein exerts effects on satiety either as an entire food, a food component or as an isolated entity. Potent effects have been demonstrated for intact protein (Blundell & Hill, 1986; Hill & Blundell, 1990), dipeptides (Rogers et al. 1990, 1991) and single
amino acids (Hill & Blundell, 1988). This evidence indicates the potential for proteins to influence the intensity of satiety and to exert control over the pattern of food consumption.

**Carbohydrates and satiety**

There are strong logical reasons why glucose should be one of the most important nutrients to be monitored. As Carlson (1991) has pointed out: ‘because the brain controls eating, it seems reasonable that hunger might be triggered by a decrease in the brain’s primary fuel’. The idea that the metabolism of glucose in the body is related to the existence of hunger and eating is represented in the glucostatic hypothesis formulated by Mayer (1953, 1955). It was postulated that the short-term articulation of energy intake with energy needs is under glucostatic control (Van Itallie, 1990). In turn, this implies that dietary carbohydrates are clearly involved in the short-term control of energy intake. Similar views are embodied in the energostatic hypothesis (Booth, 1972), metabolic control of food intake (Friedman, 1991), the glycogenostatic hypothesis (Astrup & Flatt, 1996) and the energetic concept of appetite (Blundell & Rogers, 1991a). Although the most obvious mechanism is that hunger is related directly to fluctuations in the concentration of blood glucose, the relationship between energy intake and glucose could be mediated via arteriovenous glucose differences, the rate of glucose utilisation in the liver, the activation of glucoreceptive neurons in the periphery or the brain, or change in the glycogen stores. Evidence for the role of glucose in the initiation of eating comes from the detection of transient declines in blood glucose being related to meal initiation (Campfield & Smith, 1986) and the observation that increases in energy intake follow injections of 2-deoxy-D-glucose (Thompson & Campbell, 1977). Conversely, glucose infusions (particularly into the hepatic portal system) produce an inhibition of the tendency to eat (Novin et al. 1973). After the consumption of a carbohydrate food, the glucose produced could be monitored early by glucose-detecting interoreceptors in the upper gastrointestinal tract linked to visceral afferent fibres in the vagus nerve (Mei, 1985). In addition, Oomura (1988) has described glucose-sensitive neurons (which decrease their activity in response to applied glucose) and glucoreceptor neurons (which are activated) in different parts of the brain, which presumably provide an interface between the presence of available glucose and activity in aminergic or peptidergic neural pathways (Blundell, 1991). Apart from these direct links between the detection of glucose and neural transmission, an indirect mechanism has been postulated through which carbohydrate in the diet could be related to neurochemical activity. This mechanism relates the proportion of carbohydrate to protein in the diet to the ratio of tryptophan to other large neutral amino acids in plasma, which determines the uptake of tryptophan into the brain (Wurtman, 1982). In turn, it is postulated that this leads to an activation of serotonergic neurons, which are functionally coupled to eating behaviour and food selection. The evidence for all stages in this biobehavioural loop is equivocal (Blundell & Hill, 1987), but the ingestion of pure carbohydrate certainly alters the ratios of plasma amino acids (Teff et al. 1989). The mediator of this alteration is the glucose-induced release of insulin.

It follows that many mechanisms exist to monitor the activity of glucose released by ingested carbohydrates. Therefore, it should be possible to demonstrate a relationship between consumed carbohydrates and a modulation of the expression of appetite. Although sweet carbohydrates induce some positive feedback for eating through the induction of oral afferent stimulation by sweet receptors, this should be countered by the potent inhibitory action via post-ingestive and post-absorptive mechanisms (see below for effects of carbohydrates on cognitive performance and mood).

**Carbohydrates and appetite: experimental evidence**

On the basis of studies on rats, it was argued some years ago that: ‘if the cumulative inhibitory effects of carbohydrate on feeding are indeed energostatic... then any substance that can readily be used by the animal to provide energy should produce an appropriate food intake compensation over a period of several hours after loading’ (Booth, 1972). Studies have shown that this is also the case for man. A variety of carbohydrates, including glucose, fructose, sucrose, maltodextrin and polysaccharides, exert measurable effects when given in a preload or an experimental meal. That is, they suppress later intake by an amount roughly equivalent to their energy value, although the time course of the suppressive action may vary according to the rate at which the carbohydrates are metabolised.

The preload paradigm is a sensitive procedure for disclosing the effects of glucose and maltodextrin (glucose polymer) on appetite when these carbohydrates are delivered in a natural food product. For example, in a study using the uncoupling design (Blundell et al. 1988), the actions of sweetness and energy content of a yoghurt preload were separated experimentally by using judicious combinations of glucose, maltodextrins, high-intensity sweeteners and the yoghurt base (Rogers & Blundell, 1989). After consumption, feelings of hunger were suppressed significantly by the high-energy preloads (glucose and maltodextrins) to a greater extent than after the lower-energy loads. Reduction of energy intake in the test meal was proportional to the energy difference (678 kJ) between the preloads. The accuracy of this adjustment confirms the frequent, though not universal, finding that human subjects adjust their voluntary intake in response to covert manipulations of the carbohydrate content of a food. In a more recent study, a comparison was made between preloads containing maltodextrin or sucrose compared with lower-energy loads with similar taste characteristics. At one hour after consumption, both high-energy preloads had suppressed intake compared with their low-energy counterparts.

Taken together, these and other studies demonstrate that the preload design is a sensitive procedure for assessing the satiating power of carbohydrates. It appears that accurate adjustment of subsequent energy intake (often called energy compensation) is evident for glucose and...
maltodextrin. Measurement at one hour seems to be just about perfect for detecting the effects of these carbohydrates on satiety. However, this interval may not be appropriate for other carbohydrates (or for other macronutrients).

**Carbohydrate and the satiety index**

Over twenty years ago it was suggested that: 'it could be of great value to have tables showing the energy–satiety ratio of all the common foods to indicate their potential for causing over nutrition' (Heaton, 1981). Subsequently, the term ‘satiating efficacy’ was proposed ‘as an index to compare the satiating potency of different foods’ (Kissileff, 1984).

A further measure explored by Holt et al. (1995, 1999) relates the satiety response following a food to the satiety response following a reference food (white bread). Isoenergetic preloads (1000 kJ) of thirty-eight foods were consumed, following which subjective ratings of motivation to eat were completed over two hours. Significant differences in the satiating effects of the foods were seen, indicating that different foods differ in their satiating capacities. This novel approach quantifies the effects of a food on satiety and was based on the idea of developing a satiety index analogous to the glycemic index. These studies showed that high-carbohydrate foods had a high satiety index measured by the satiety area under curve (AUC). The satiety index was positively correlated with the carbohydrate content of the foods tested.

A further measure has been termed the ‘satiety quotient’, which permits a measure of the effect of a food on satiation (within an eating episode) and satiety (the effect following eating). This allows determination of the degree of suppression of hunger that can be brought about per unit of food energy consumed (Green et al. 1997) and permits the tracking of changes over time. Using data from a variety of foods freely consumed, it was demonstrated that hunger was reduced more per kJ of carbohydrate food consumed than of high-fat food — but the strength of this varied with time. This finding confirms the proposal that, ‘joule for joule’, carbohydrate is more satiating than fat (Rolls et al. 1994).

**Evidence from nutritional interventions**

The results from short-term studies show clearly that carbohydrates exert a marked influence over the expression of appetite. Real-life intervention studies have demonstrated that this degree of appetite control has practical consequences. In one study, people identified as snackers were encouraged to eat 25 % of their daily energy intake from high-carbohydrate or high-fat snacks during separate three-week periods. The subjects incorporated the snack foods into their normal eating repertoires and maintained a normal life-style. The study was monitored very carefully to ensure compliance to the experimental requirements and to assess food intake from the snack foods and from the rest of the eating pattern. Measures of total energy intake indicated that food consumption was physiologically valid and quite in keeping with body size and the level of physical activity. First, generous consumption of these palatable high-carbohydrate foods did not generate abnormally high energy intakes; indeed daily energy intake was an average of 364 kJ less than with the high-fat snacks — a value that could lead to a weight loss of approximately 3.5 kg over the course of a year. Second, the high-carbohydrate foods significantly lowered the total daily fat intake down to a level recommended in the National Dietary Guidelines (Lawton et al. 1998). Consequently this high-carbohydrate intervention, which was easily achieved, exerted a notable control over appetite and the pattern of nutrient intake.

In a series of studies carried out in Scotland, subjects were required to increase their consumption of high-carbohydrate breakfast cereals by 60 g/d for twelve weeks (Kirk et al. 1997). This intervention resulted in a 5.4 % reduction in energy from fat and a 5.1 % increase in energy from starch. A second study with overweight men (body mass index 29.4 kg/m²), who were required to increase their intake of high-carbohydrate breakfast cereal by 90 g/d (approximately three bowls), led to an increase in carbohydrate consumption from 40 to 47 % of total energy (Crombie & Kirk, 1999). The positive changes in carbohydrate intake were accompanied by small weight losses.

Taken together, these studies show that a high intake of carbohydrate foods can lower fat intake and does not compromise energy balance.

**Fat and appetite control**

The effect of dietary fat on appetite and energy balance has been extensively researched and reviewed in the last ten years (Blundell et al. 1996). Owing to the high palatability and energy density of fatty foods, much of the evidence indicates the potential of high-fat products to cause over-consumption (Blundell & Macdiarmid, 1997). However, there exists the ‘fat paradox’ which arises from the conflict between the capacity of fat to generate potent fat-induced satiety signals and the presence of high-fat hyperphagia (Blundell et al. 1995).

Because of the potency of fatty foods to stimulate a positive energy balance (partly through the process of passive consumption), it is important to develop procedures to mitigate this action. Three possibilities will be discussed here: (1) fat substitutes or fat mimetics, (2) specific types of fatty acids or triacylglycerols, and (3) modification of fats through processes such as fractionation.

**Fat substitutes: evidence from Olestra.** Olestra is the name of one of a class of compounds called sucrose polyesters. Olestra has the organoleptic properties of natural fat but it is not hydrolysed by lipases. Therefore, following consumption, it cannot produce metabolisable energy and can be regarded as possessing no calories. However, Olestra can be used in cooking and baking in similar ways to natural fat and it can therefore be incorporated into a wide range of foods. As a substitute for fat it therefore effectively reduces the fat content of foods and the fat energy absorbed by the body. Undigested Olestra passes straight through the gastrointestinal tract. The key issue is whether or not the consumption of Olestra-containing foods — with their reduced fat and energy contents —
will lead to subsequent compensation, i.e. a later increase in the intake of fat and/or energy.

In short-term studies, one initial experiment in healthy, lean, young adult males found that the substitution of 20 or 36 g of fat by Olestra at a breakfast meal (Blundell et al. 1991; Rolls et al. 1994) led to full compensation during the course of the rest of the day. However, this study is atypical (probably because of the highly selected, well regulating subjects), and most studies have shown that the replacement of dietary fat with Olestra during one day does not cause full compensation during the next twenty-four hours. The replacement of 55 g of fat by Olestra at either lunch or dinner (Cotton et al. 1996) did not alter hunger or food intake during the test day or the following day. In a similar study, in which 55 g of Olestra was substituted in either three meals or five snacks across the course of one day, no effect was detected on hunger or food consumption. Therefore these studies indicate that Olestra can lead to a short-term reduction in the consumption of fat and energy.

Similar results have been obtained in longer-term studies. For example, when lean and obese subjects substituted an average of 26 g of fat per day for two weeks (Hill et al. 1998), total energy intake was reduced by 8 % and fat intake by 11 %. This meant that subjects compensated for only 20 % of the substituted energy.

However, the degree of compensation does appear to vary with the absolute level to which fat is reduced. When 55 g of Olestra reduced the percentage energy from fat in the diet from 32 to 20 %, a compensation of 67 % was observed (Cotton et al. 1996). However, only 21 % compensation was seen with a reduction of fat energy from 43 to 32 %. Taken together, several studies suggest that a reduction of fat energy down to generally recommended values (30–32 % of food energy per day) will not generate a strong hunger drive or tendency towards compensation. Interestingly, it does not seem to matter whether or not subjects have knowledge about the presence of a fat substitute in the foods being consumed (De Graaf et al. 1996; Miller et al. 1998). Importantly, in one long-term trial lasting for nine months, an Olestra-containing diet (reducing fat from 32 to 25 % of energy) led to a reduction of 6·3 kg in body weight. These outcomes suggest that this strategy of fat substitution could be a useful approach to functional foods for appetite control.

Enhancing satiety: fatty acids. It has been noted earlier that foods containing a high percentage of fat have the capacity to promote over-consumption in obese (Lawton et al. 1993; Green et al. 1994a) subjects. This effect, which occurs during the process of ‘satiation’, is almost certainly due to the high energy density of high-fat foods, but the somewhat weaker effect of fat on ‘satiety’ is probably due to the physiological action of fat in generating post-ingestive inhibitory signals. Therefore the demonstration of varying effects of fats on satiety signalling could be useful in the development of fat-containing foods that modulate satiety. The induction of physiological satiety signals may well depend, at least in part, on the composition of fatty acids in the particular fats used. Two prominent structural features of fatty acids are their chain length and degree of saturation. In principle it is possible to manipulate both of these aspects. At the present time only a few studies have investigated these effects.

In two studies in which chain length was manipulated, medium-chain triacylglycerols have been shown to have greater satiating power than long-chain triacylglycerols (Rolls et al. 1988; Stubbs & Harbron, 1995). A further study kept chain length constant and varied the degree of saturation (Lawton et al. 2000). This short-term study indicated that a meal containing largely monounsaturated fat induced a weaker satiety than polyunsaturated fat or a blend of saturated fat and monounsaturated fat. A second study produced a similar but less decisive outcome. Taken together, these and other studies suggest that monounsaturated fatty acids may exert a relatively weak effect on satiety. On the other hand, polyunsaturated fatty acids such as linoleic acid are worthy of further investigation for their relatively strong effect.

Enhancing satiety: novel treatments. It is known that fat, introduced directly into the gastrointestinal tract, can lead to an inhibition of eating that is mediated, at least in part, by the slowing of gastric emptying (Welch et al. 1985). The importance of these gastrointestinal responses means that if ingested fat (or fat-containing products) can resist digestion and reach the intestine, they may be able to induce a stimulation of satiety. One possible mechanism involved here is the so-called ‘ileal brake’ phenomenon. This concept has been invoked to explain the action of a specific treatment of fatty acids incorporated into yoghurt as an emulsion.

This emulsion, known, as Olibra, is a food ingredient containing fractionated palm oil and fractionated oat oil in the proportion 95:5. This emulsion contains more palmitic and linoleic acids, and less oleic acid, than milk fat. A yoghurt containing 5 g of the Olibra fat was given to subjects as a breakfast. In separate studies in lean (Burns et al. 2000) and obese (Burns et al. 2001) subjects, this single administration significantly reduced food intake throughout the whole day. This effect, which was accompanied by an increased sense of fullness and decreased hunger, was therefore maintained for up to eight hours. This is an extremely powerful effect. If an action of this intensity can be maintained with repeated administration, then the product could well become a functional food for appetite control. However, long-term weight-loss trials are required before any specific obesity-controlling effect can be claimed. Still, this novel manipulation suggests a potential route for developing fats with an enhanced action upon satiety.

Interim summary for satiety

Analysis of experimental studies indicates that all foods share certain common features (weight, volume, texture) which contribute to their effects on satiation and satiety. However, the nutritional composition also plays a key role and markedly modulates the intensity and duration of satiety and the strength of satiation. This means that the macronutrient composition of foods can favour consumption (and over-consumption) and also limit the amount of food willingly consumed and the motivational feelings associated with eating. The macronutrients
protein, carbohydrate and fats differ in their mechanisms of inducing satiety and also vary in their impact on the size and frequency of eating episodes. Therefore many opportunities exist for targeted manipulations of proteins, fats and carbohydrates in the development of functional foods for appetite control.

Biomarkers for appetite

A biomarker can be defined as a surrogate endpoint that can be used instead of some clinical endpoint. How can this be applied to a behavioural or psychological function such as appetite control? If the ultimate objective of a functional food for appetite control is the reduction or maintenance of food intake, then a surrogate or ‘proxy’ for this could be the profile of subjective hunger that oscillates with the pattern of food intake and is usually well correlated with the amount of food consumed (when hunger fails to correlate with food eaten there are usually good reasons). Consequently, it can be proposed that subjectively perceived hunger — translated into an objective end-point by means of the visual analogue rating scale — can be regarded as an objective biomarker for appetite control.

Functional foods for cognitive performance

Measurement of cognitive processes

In principle, a large number of mental or cognitive tasks have the potential to disclose the effects of foods. However, in practice, a limited number of tests have been used. Performance tasks reflect a number of cognitive functions; for example, perception, memory, attention and arousal, information processing, accuracy and speed of movement (Bellisle et al. 1998). These tasks represent single components of performance, which form part of more complex skills and abilities (see Table 1); for example, car-driving ability or operating machinery. Cognitive performance encompasses not only measures of speed (reaction time) but also of processing accuracy (measures of accurate and inaccurate detection). Interventions may elicit

<table>
<thead>
<tr>
<th>Function</th>
<th>Example of tests</th>
<th>Common components of task</th>
</tr>
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<tbody>
<tr>
<td>Reaction time (decision time and movement time)</td>
<td>Simple (SRT) or choice (CRT)</td>
<td>Stimulus appears (visual or auditory) and S must make a single response, usually by depressing a key. In CRT one of a number of stimuli may appear and S must make one of two responses (e.g. left hand, right hand) according to the type of stimulus</td>
</tr>
<tr>
<td>Vigilance (also known as attention), rapid information processing or continuous performance</td>
<td>Search tests, e.g. – categoric search – Digit symbol substitution – Stroop – Bakan</td>
<td>Detection of stimulus items from particular categories S must replace digits with symbols S must attend to certain features of stimuli and ignore others A series of numbers are presented visually in rapid succession on a VDU screen. S has to respond by depressing a key when a sequence of three odd or three even digits is detected</td>
</tr>
<tr>
<td>Visual information processing</td>
<td>Critical flicker fusion threshold Immediate recall</td>
<td>S must detect flicker and fusion of light S shown a list of stimuli at a given rate (e.g. one per second). At end of presentation S recalls the stimuli</td>
</tr>
<tr>
<td>Frontal executive</td>
<td>Verbal memory Spatial memory Associative memory Word recognition Pattern comparison</td>
<td>S must remember (recall) series of items in forward or reverse order S must process and indicate if stimulus (phrase, equation or expression) is true or false</td>
</tr>
<tr>
<td>Working memory (short-term memory)</td>
<td>Driving simulation Pursuit rotor</td>
<td>Tracking and deviation on a course are monitored continuously S must trace a shape (maze) with a stylus under time pressure. Error score computed</td>
</tr>
<tr>
<td>Immediate memory</td>
<td>Digit span</td>
<td>S must detect flicker and fusion of light S shown a list of stimuli at a given rate (e.g. one per second). At end of presentation S recalls the stimuli</td>
</tr>
<tr>
<td>Reasoning</td>
<td>Arithmetic, logical, grammatical or semantic</td>
<td>S must remember (recall) series of items in forward or reverse order S must process and indicate if stimulus (phrase, equation or expression) is true or false</td>
</tr>
<tr>
<td>Psychomotor performance (tracking ability)</td>
<td>Visuo-spatial motor task Tapping task</td>
<td>S must trace a shape (maze) with a stylus under time pressure. Error score computed</td>
</tr>
</tbody>
</table>

CRT, choice reaction time; S, subject; SRT, simple reaction time; VDU, visual display unit.
changes in function in some or all of these performance components.

In choosing a task much depends on whether an immediate (short-term) effect of food is expected or whether a chronic long-term adaptation to a diet is being examined. The former and arguably more simple approach is the focus of most current research. Measures that are frequently utilised in assessing the effects of nutrients include reaction time, attention and memory. Other tests have also been used but selection is frequently based on accessibility rather than likely effect of a nutrient on a particular cognitive process. The proposed mechanism of action of a nutrient in terms of its effects on blood glucose or other neurotransmitter systems and the likely effects of changes in these neurobiological parameters on cognitive processes have not been considered. Thus a failure to detect an effect of a particular macronutrient on a particular aspect of cognitive function may be due to a true lack of effect, because the neuropsychological processes needed to perform the task are unaffected by the nutrient ingested. The effect of compensatory effort in experimental situations — ‘the Hawthorne effect’ (Roethlisberger, 1997) — is well known and can account for null results. Alternatively, the intervention may affect only a small component of the task (e.g. reaction time or decision time) such that an effect on overall performance is not detected. Recent advances in computer technology have meant that these tasks can be administered in a structured, carefully controlled fashion, with extremely accurate measurement of processing and reaction time, correct responses and error rates. Examination of the pattern of effects on sub-processes within a task or on simple unitary measures of cognition may be helpful in determining the mechanism of action or neurotransmitter systems involved or affected by the nutrient ingested.

**Effects of foods on cognitive performance**

Various studies have examined the effects of nutritional interventions across a range of cognitive domains. The effects of these manipulations on each of the major areas of cognitive performance are summarised below.

**Reaction time.** Reaction time may be measured as a response to a variety of stimuli. Visual or auditory stimuli are presented and response time is measured. Simple or choice reaction time can be employed, requiring the participant to respond selectively to stimuli which have particular features, e.g. frequency, colour, spatial position, tone, etc. Thus the reaction time task can comprise more than one cognitive process. In addition, the reaction time recorded can be broken down into movement and detection components.

Studies that examine the effects of macronutrients on reaction time are described in Table 2. The main conclusions of this body of research can be summarised as follows.

1. Effects are more clearly demonstrable in the morning (particularly breakfast studies).
2. Nutritional interventions, which facilitate a rise in blood glucose, enhance performance of reaction time tasks.
3. The findings of studies of macronutrients given in combination suggest that fat may slow reaction time; carbohydrate can impair peripheral processing and reaction time, depending on the time of day and type of carbohydrate ingested as well as ratio of carbohydrate to protein.
4. Foods that facilitate speed of reaction should enhance glucose availability, e.g. high glycaemic index. There have been no studies that specifically compare the effects of foods with different glycaemic indices. One study (Kaplan et al. 2001) found similar improvements in memory after high- and low-glycaemic-index carbohydrates in the elderly.

**Attention and vigilance.** Vigilance or attention tasks have been included in a large number of studies that aim to assess the effects of nutrients on performance. The nature of the task employed often differs and it is sometimes difficult to determine whether the cognitive processes that may be involved in each task are similar or comparable. Digit symbol substitution and the Bakan test have frequently been employed (see Table 3). However, some studies do not adequately describe the specific test used.

The effects of nutrients on attention and vigilance, as described in Table 3, can be summarised as follows.

1. Attention can be difficult to disrupt in the first half of the day but seems susceptible to fat ingestion at lunch and later. It can be disrupted by high carbohydrate and effects of this are more clear in terms of response time measures.
2. Effects could be due to an impairment of either detection or movement, but work on reaction time measures would suggest that movement is the most vulnerable cognitive process.
3. Although participants appear to be slower, there is some suggestion that they are also more accurate, which could imply criterion shifts.
4. Protein appears to influence susceptibility to distraction.
5. The suggestion that attention can be enhanced by energy requires verification.

**Memory.** Examination of functional food effects on measures of memory has focused on short-term or working memory (see Table 4). Tests employed usually require immediate recall of a word list or similar stimuli presented at a fixed rate, after which the subject is required to repeat or write down as many of the stimuli as possible in a fixed period of time. The number of items in the word list is usually twenty to thirty in normal volunteer samples and seven plus or minus two words should be the average number recalled. Some samples, notably students, recall almost double this rate, partly due to the effects of training and intelligence. Two effects are common in memory tasks. Primacy and recency effects relate to the increased likelihood of recalling the earliest and latest stimuli from a list, although few studies examine the order of words recalled or request that the subject recalls the stimuli in the order presented. The nature of the stimulus words are
also important since efficiency of recall can be affected by differences in the frequency, concreteness and imagery of the words used as stimuli. It is therefore important that memory tasks select and match stimuli on these features. Other types of memory tasks sometimes employed include delayed recall, where the subject is required to recall a list presented some time earlier (usually ten to twenty minutes) and has had to perform other tasks in the interim period. Recognition recall requires the subject to identify stimuli that were presented earlier from a list that includes unseen, distractor stimuli. Recognition recall is generally easier than free recall. Pattern comparison is another form of memory task that examines spatial memory performance. Paired associate learning involves remembering two paired but semantically unrelated stimuli and recalling one of the pair when the other is presented. Although there are many variants of memory tasks, those used in the assessment of nutrients have been limited.

A summary of the effects of nutrients on memory, outlined in Table 4, is given in the following.

1. Glucose appears reliably to improve memory, particularly in vulnerable subjects such as the young and elderly.
2. Pure carbohydrate in other forms improves memory but can impair peripheral processing, attention and reaction time, depending on the time of day, type of carbohydrate ingested and ratio of carbohydrate to protein. It is also associated with feelings of fatigue.
3. The carbohydrate:protein of a meal can produce depletion or enhancement of tryptophan (Klassen et al. 1999). Where tryptophan availability is enhanced, faster memory scanning has been demonstrated in stress-prone subjects who may have receptor sensitisation of the serotonergic system or 5-hydroxytryptamine (5-HT) deficiency (Markus, 1999).
4. Tryptophan depletion selectively impairs memory consolidation in normal volunteers (Riedel et al. 1999).
5. Thus memory tasks appear to be important measures of cognitive function, which are susceptible to nutritional manipulations.

Other aspects of performance. A large number of cognitive tests can be used to examine the effects of nutrients on performance and which measure functions other than attention and memory.

Psychomotor performance involving hand–eye coordination can be used to reflect the kind of cognitive processes involved in operating machinery or driving. These tasks may feature reaction time measures but are generally more complex performance indicators than the tests reviewed in the section on reaction time above. The effect of macronutrients on these performance measures is shown in Table 5. The simplest form of psychomotor task, which has been used reasonably widely in this area, is the tapping task. Dieters performed better on a finger-tapping test in the morning (Deijen et al. 1989). Lloyd et al. (1994, 1996) also used a tapping task to assess breakfast and lunch manipulations.

More complex psychomotor tasks involve tracking and control. Driving can be considered to be an ecologically valid psychomotor task and real or simulated driving tasks are used to establish the effects of drugs or other interventions on actual driving and driver safety. There have been a small number of studies examining nutrient effects on performance, which have used tracking or driving simulator tasks. Performance of a tracking task was better in a group of cadets after repeated consumption of a tyrosine-rich drink than in the group supplied with a carbohydrate-rich drink (Deijen et al. 1999). Smith & Rich (1998) examined the effect of no snack, a chocolate bar or an equicaloric portion of cheese and biscuits on simulated driving. Following consumption of the chocolate, subjects drove more carefully and hit the side of the track less often. It is difficult to determine from this manipulation whether any specific ingredient in the chocolate was predominantly affecting the performance although the caffeine content is not likely to be high enough to have an alerting effect. An alternative explanation is that mood may have been modified differentially across the three conditions (foods were not matched for taste, appearance and palatability) and this could have mediated the performance effects observed.

Other tests assess perceptual processes such as vision. Such tests have been used widely in assessing the importance of the essential fatty acid, docosahexaenoic acid, in neural maturation, visual acuity and brain function in ageing (Horrocks & Yeo, 1999). The effect of docosahexaenoic acid supplementation on cognitive performance in healthy young adults has not been examined specifically. Another widely used test in psychopharmacology is Critical Flicker Fusion Threshold, which involves the discrimination of flicker from fusion and is an index of cortical arousal and visual information processing capacity. Visual information processing (measured by Critical Flicker Fusion Threshold) was increased significantly by a decaffeinated Coca-Cola drink with added tryptophan, compared with a decaffeinated Coca-Cola drink alone (Cunliffe et al. 1998). This effect of amino acid manipulation suggests impairment of performance follows protein consumption. Fat ingestion has also been shown to decrease flicker fusion frequency, indicating increased central fatigue or decreased visual information processing capacity (Cunliffe et al. 1997).

Other cognitive tasks such as reasoning tasks (involving logical, semantic and grammatical reasoning), problem-solving tasks and arithmetic tasks have also been employed in studies of nutrient effects. Addition and sentence verification tasks are not affected by missing breakfast (Dickie & Bender, 1982; Smith & Kendrick, 1992; Smith et al. 1994a). Smith et al. (1994b) showed that a high-fat lunch did not affect logical reasoning. In comparison with performance after a diet soft-drink (containing aspartame), subjects solved more arithmetic problems in a shorter time after consuming calorie-containing yoghurts (Kanarek & Swinney, 1990).

Providing a supplementation of branched-chain amino acids has also been shown to prevent impairment of performance of shape rotation and identification tasks and to improve performance of the Stroop task after exercise
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample</th>
<th>Study design</th>
<th>Cognitive testing</th>
<th>Effect on performance of task</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Spring et al. (1983) | 184 healthy adults      | Two meals for BK or lunch:  
- HCHO (non-dairy sherbet)  
- HP (turkey breast)  | Auditory RT  
Assessment 2 h after consuming the meal | No effect  | No pre-meal measure of performance  
Between-subjects design  
Foods were not BK or lunch standard items |
| Lieberman et al. (1983) | Young men               | Two lunches:  
- HCHO (pita bread)  
- HP (turkey breast)  | Auditory RT  
Assessment after lunch | Slower RT to an auditory stimulus after the HCHO meal than after the HP meal  | No baseline measures of cognitive performance |
| Smith & Miles (1986 b,c) | Forty-eight subjects | Two conditions:  
- lunch  
- no lunch  | Serial self-paced CRT (detection RT and movement time)  
Assessment before and after lunch | Lunch consumption impaired detection time (RT) on CRT | Movement time slower in early afternoon than in morning, independent of meal consumption |
| Smith et al. (1988) | Five M and six F        | Three iso-energetic lunches  
(3.77 MJ in M, 2.72 MJ in F):  
- HCHO, high in starch  
(40% starch, 15% sugar, 15% protein, 30% fat)  
- HCHO, high in sugar  
(15% starch, 40% sugar, 15% protein, 30% fat)  
- HP (14% starch, 1% sugar, 55% protein, 30% fat)  | RT-focused attention test  
Assessment before and 75 min following lunch | Slower RT to peripheral targets after the HCHO meals than after the HP meal |  
Smaller RT to an auditory stimulus after the HCHO meal than after the HP meal  
The MF/CHO meal was most similar in size and macronutrient content to the subject’s habitual lunch. Results may reflect responses to changes in normal intake rather than to the specific macronutrient composition of the meals |
| Lloyd et al. (1994) | Eighteen subjects       | Three iso-caloric lunches  
(about 2.92 MJ):  
- LF/HCHO (2-90 MJ, 29/54 % en)  
- MF/MCHO (2-95 MJ, 45/42 % en)  
- MHF/LCHO (3-01 MJ, 62/24 % en)  | SRT  
Assessment 30 min before lunch, 30 min, 90 min and 150 min after finishing lunch | Longer RT after LF and HF lunches, compared with the MF lunch, at 90 min post ingestion |  
The MF/CHO meal was most similar in size and macronutrient content to the subject’s habitual lunch. Results may reflect responses to changes in normal intake rather than to the specific macronutrient composition of the meals |
| Smith et al. (1994c) | Experiment 1:  
forty-eight university students (twenty-four M, twenty-four F)  | Six conditions:  
- no BK + decaffeinated coffee  
- cereal toast BK (equivalent to LF) + decaffeinated coffee  
- cooked BK (equivalent to HF) + decaffeinated coffee  
- no BK + decaffeinated coffee  
- cereal toast BK (equivalent to LF) + caffeinated coffee  
- cooked BK (equivalent to HF) + caffeinated coffee  | SRT  
Five-choice serial response time (CRT)  
Assessment 30 min before BK, 60 and 120 min after the start of BK | No effect of BK on performance on SRT or CRT  | Between-subject design  
BK differs from lunch  
Caffeine enhanced performance of SRT but had no effect on CRT |
| Smith et al. (1994c) | Experiment 2:  
forty-eight university students (twenty-four M, twenty-four F)  | Four conditions:  
- three-course meal (5.02–6.28 MJ) + decaffeinated drink  
- three-course meal (5.02–6.28 MJ) + caffeinated drink  
- no meal + decaffeinated drink  
- no meal + caffeinated drink  | SRT  
Five-choice serial response task (CRT)  
Assessment before the meal and 90 min and 180 min after the start of the meal | No effects on tasks  | Effects are different from those observed after lunch or BK |
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Meals</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells &amp; Read (1996)</td>
<td>Eighteen males (groups A and B: nine M in each)</td>
<td>Two meals: – HF/LCHO (3.18 MJ, 55/40 % en) – LF/HCHO (3.60 MJ, 88/7 % en) Group A: ate meals at 10.30 (brunch) Group B: BK (08.15) and test meal at 12.30 (lunch)</td>
<td>SRT: Serial choice reaction Overall little change in performance</td>
</tr>
<tr>
<td>Cunliffe et al. (1997)</td>
<td>Sixteen healthy volunteers (nine M, seven F), aged 20–47 years (mean, 29.7 years)</td>
<td>1672 kJ at BK of approx. 200 ml: – pure CHO (maltodextrin) – pure fat (long-chain triacylglycerol emulsion) Control: mixed CHO, fat and protein (55 % CHO, 15 % protein, 30 % fat, same sources of CHO and fat)</td>
<td>Simple visual reaction time Hourly for 4 h post ingestion Slower after CHO No effect of fat on RT</td>
</tr>
<tr>
<td>Lloyd et al. (1996)</td>
<td>Sixteen healthy habitual BK eaters (fourteen F, two M), age 26 years</td>
<td>Four conditions: – no BK – three iso-caloric BK (2.51 MJ): – LF/HCHO (27/62 % en) – MF/MCHO (44/47 % en) – HF/LCHO (56/34 % en) Habitual BK (1.05 MJ, 25 % fat, 65 % CHO)</td>
<td>SRT: Assessment beginning 30 min before, and 30 min, 90 min and 150 min after BK No clear difference in performance between the four conditions BK provided in the study was higher in en than usual BK</td>
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<tr>
<td>Fischer et al. (2001)</td>
<td>Fifteen young males</td>
<td>1670 kJ at breakfast, spoonable creams of: – pure fat – pure CHO – pure protein</td>
<td>Simple and choice RT Hourly for 3 h post ingestion Fat improved RT performance compared with baseline at 1, 2 and 3 h, and reduced error rate No effect of CHO on SRT CHO impaired CRT at 2 h Protein improved CRT at 2 h</td>
</tr>
<tr>
<td>Lluch et al. (2000)</td>
<td>Thirty-two volunteers (sixteen M and sixteen F; seventeen LF and fifteen HF consumers)</td>
<td>Two iso-caloric BK (1.72 MJ) and lunches (3.10 MJ for F, 4.14 MJ for M) – LF/HCHO (BK: 22/67 % en, lunch: 20/61 % en) – HF/LCHO (BK: 48/42 % en, lunch: 54/32 % en)</td>
<td>Simple and choice RT Assessment just before and 30 min after finishing lunch Slower RT after HF lunch than after LF lunch No effect of habitual diet on performance</td>
</tr>
</tbody>
</table>

BK, breakfast; CHO, carbohydrate; CRT, choice reaction time; en, energy; F, female; HCHO, high carbohydrate; HF, high fat; HP, high protein; LCHO, low carbohydrate; LF, low fat; M, male; MCHO, medium carbohydrate; MF, medium fat; RT, reaction time; SRT, simple reaction time.
<table>
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<tr>
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<th>Study design</th>
<th>Cognitive testing</th>
<th>Results</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Spring <em>et al.</em> (1983)</td>
<td>184 healthy adults</td>
<td>Two meals for BK or lunch:</td>
<td>Dichotic shadowing (sustained and</td>
<td>Impairment on the sustained selective attention task after</td>
<td>No pre-meal measure of performance</td>
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<td></td>
<td>(129M, fifty-five F), 18–65 years</td>
<td>- HCHO (non-dairy sherbet)</td>
<td>selective aspects of attention)</td>
<td>HCHO lunch compared with HP in subjects over 40 years of age</td>
<td>Between-subjects design</td>
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<td></td>
<td></td>
<td>- HP (turkey breast)</td>
<td>Assessment 2 h after consuming the</td>
<td></td>
<td>Foods were not BK or lunch standard items</td>
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<tr>
<td>Lieberman <em>et al.</em></td>
<td>Young men</td>
<td>Two lunches:</td>
<td>DSST</td>
<td>Poorer performance on a DSST after the HCHO meal than after</td>
<td>No baseline measures of cognitive performance</td>
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<td></td>
<td>(1983)</td>
<td>- HCHO (pita bread)</td>
<td>Assessment after lunch</td>
<td>the HP meal</td>
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<td>Smith <em>et al.</em> (1988)</td>
<td>Five M and six F</td>
<td>Three iso-energetic lunches</td>
<td>Two tasks:</td>
<td>Slower RT to peripheral targets after the HCHO meals than</td>
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<td>(3.77 MJ in M, 2.72 MJ in F):</td>
<td>- focused attention test</td>
<td>after the HP meal</td>
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<td>- HCHO, high in starch (40 % starch, 15 % sugar, 15 % protein, 30 % fat)</td>
<td>- search test</td>
<td>Susceptibility to distracting stimuli greater after the HP</td>
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<td>- HCHO, high in sugar (15 % starch, 40 % sugar, 15 % protein, 30 % fat)</td>
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<td>lunch than after the HCHO meals</td>
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<td>- HP (14 % starch, 1 % sugar, 55 % protein, 30 % fat)</td>
<td>Assessment before and 75 min</td>
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<td>Deijen <em>et al.</em> (1989)</td>
<td>Two groups:</td>
<td>During three weeks, diet group</td>
<td>Sustained visual attention task</td>
<td>No effect on attention type tasks</td>
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<td></td>
<td>- control (n=9)</td>
<td>had a daily intake of 110 g protein, 320 g CHO and 80 g fat</td>
<td>Pattern comparison</td>
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<td>- diet (n=10)</td>
<td>- BK: 70 g protein (63 %), 25 g CHO (8 %)</td>
<td>DSST</td>
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<td></td>
<td>21–27 years</td>
<td>- lunch: 10 g protein (9 %), 100 g CHO (31 %)</td>
<td>Assessment: baseline scores</td>
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<td>(last day of 3 weeks, after BK and dinner) and post-treatment scores (2 months later)</td>
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<td>Benton <em>et al.</em> (1994)</td>
<td>Seventy young female</td>
<td>Glucose drink (50 g) after baseline and 25 min later (25 g) or CHO-free placebo drinks (sweetened with aspartame and acesulfame K)</td>
<td>Reaction to Bakan test</td>
<td>Improved decision time</td>
<td>Between-subjects design, no dietary restrictions on test day</td>
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<td></td>
<td>students</td>
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<tr>
<td>Kelly <em>et al.</em> (1994)</td>
<td>Experiment 1: two groups of six subjects</td>
<td>Four lunches varying in energy and fat and carbohydrate content</td>
<td>Choice of tasks:</td>
<td>Performance on the task was poorer after compared with before</td>
<td>Limited number of subjects and dimensions measured</td>
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<td>- LF/low en (1-80 MJ)</td>
<td>the meal</td>
<td>Subjects were allowed to choose among the tasks</td>
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<td>No systematic variations in performance as a consequence of</td>
<td>Unrestricted access to caffeine and nicotine</td>
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<td>- DSST</td>
<td>either the energy or macronutrient content of the meals</td>
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<tr>
<td>Lloyd <em>et al.</em> (1994)</td>
<td>Eighteen subjects</td>
<td>Three iso-caloric lunches</td>
<td>Bakan task (sustained attention)</td>
<td>No effect on sustained attention</td>
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<td>(fifteen F, three M),</td>
<td>(about 2.93 MJ):</td>
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<td></td>
<td>mean age 27 years</td>
<td>- LF/HCHO (2.90 MJ, 29/54 % en)</td>
<td>Assessment 30 min before</td>
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<td>- MF/MCHO (2.95 MJ, 45/42 % en)</td>
<td>lunch, 30 min, 90 min and 150 min after finishing lunch</td>
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<td>Study</td>
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<td>Meals</td>
<td>Tasks</td>
<td>Results</td>
<td>Design</td>
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</tbody>
</table>
| Smith et al. (1994a) | Forty-six university students (twenty M, twenty-six F) | Four lunches:  
- small HF: 530 g, 5.40 MJ, 55% fat  
- large HF: 840 g, 5.44 MJ, 58% fat  
- small LF: 600 g, 3.51 MJ, 19% fat  
- large LF: 840 g, 3.68 MJ, 23% fat | Repeated digit vigilance task  
Focused attention task  
Categoric search task  
Assessment before lunch and 90 min after the start of the meal | Slower but more accurate response after HF lunch than after LF lunch on selective attention tasks at 90 min  
No effect of fat content or weight of the meals on vigilance tasks | Between-subjects design |
| Smith et al. (1994c) | Experiment 1: forty-eight university students (twenty-four M, twenty-four F) | Six conditions:  
- no BK + decaffeinated coffee  
- cereal toast BK (equivalent to LF) + decaffeinated coffee  
- cooked BK (equivalent to HF) + decaffeinated coffee  
- no BK + caffeinated coffee  
- cereal toast BK (equivalent to LF) + caffeinated coffee  
- cooked BK (equivalent to HF) + caffeinated coffee | Repeated-digits vigilance task  
Assessment 30 min before BK, 60 and 120 min after the start of BK | No effect of BK on performance on sustained attention tasks | Between-subject design |
| Wells et al. (1995) | Experiment 1: five males | Two testing days with two consecutive 3 h duodenal infusions of:  
- 100 g Intralipid/l followed by isotonic saline (9 g NaCl/l)  
- isotonic saline (9 g NaCl/l) followed by 100 g Intralipid/l (cross-over balanced design) | Sustained attention task  
Assessment at seven time points: one before the first infusion and six during the two infusions | Lipid affected the speed and accuracy of performance in the sustained attention task | Between-subjects design |
| Experiment 2: eight males | Fixed BK (3.18 MJ) and two iso-energetic lunches (3.66 MJ):  
- LF/HCHO (7.76% en)  
- HF/HCHO (8.18% en) | Sustained attention task  
Responses to stimuli in the sustained attention task slower after the HF than after the LF meal |  |
| Wells & Read (1996) | Eighteen males (groups A and B: nine M in each) | Two meals:  
- HF/LCHO (3.18 MJ, 55/40% en)  
- LF/HCHO (3.60 MJ, 88/7% en)  
- HF/LCHO (3.60 MJ, 64/18% en) | Bakan task  
Assessment beginning 30 min before, and 30 min, 90 min and 150 min after BK | Increase in the frequency of false alarms in the Bakan test after the LF/HCHO brunch |  |
| Lloyd et al. (1996) | Sixteen healthy habitual BK eaters (fourteen F, two M), age 26 years  
Habitual BK (1.05 MJ, 25% fat, 65% CHO) | Four conditions:  
- no BK  
- three iso-caloric BK (2.51 MJ):  
  - LF/HCHO (27/62% en)  
  - MF/MCHO (44/47% en)  
  - HF/LCHO (56/34% en)  
- Habitual BK (1.05 MJ, 25% fat, 65% CHO) | Bakan task  
Assessment beginning 30 min before, and 30 min, 90 min and 150 min after BK | No clear difference in performance between the four conditions |  |
| Lluch et al. (2000) | Thirty-two volunteers (sixteen M and sixteen F; seventeen LF and fifteen HF consumers) | Two iso-caloric BK (1.72 MJ) and lunches (3.10 MJ for F, 4.14 MJ for M):  
- LF/HCHO (BK: 22/67% en, lunch: 20/61% en)  
- HF/LCHO (BK: 48/42% en, lunch: 54/32% en) | Attention  
Assessment just before and 30 min after finishing lunch | No effect on attention |  |
Kanarek & Swinney (1990)  
**Experiment 1:** sixteen males (19–22 years) 
Four conditions: BK (1·26 MJ) followed by:  
– lunch + calorific snack (confectionary snack)  
– no lunch + calorific snack  
– lunch + non-caloric snack (drink)  
– no lunch + non-caloric snack (drink) 
**Attention Assessment 15 min following eating of the snack** 
Subjects blind to calorific content of snacks  
Subjects responded faster in the attention task after the calorific snack than after the non-caloric snack  
Practice effects observed; therefore, a practice day introduced in the second experiment  
Pre-lunch performance not assessed (in both experiments) 

**Experiment 2:** eight males 
Same conditions, but calorific snack replaced by fruit-flavoured yoghurt  
No effect on attention

Smith & Miles (1986a)  
Forty-eight university students (eighteen M, thirty F) 
Two conditions:  
– lunch (three-course meal from university refectory, chosen by subjects)  
– no lunch  
Detection of repeated numbers Assessment before and after lunch 
Deficit in performance following lunch consumption on number task  
Caffeine also consumed following lunch

Smith & Miles (1986b)  
Forty-eight subjects 
Two conditions:  
– lunch  
– no lunch  
Stroop colour and word task (selective attention) Assessment before and after lunch 
Stroop not influenced by lunch

Smith & Miles (1986c)  
Twenty-four university students 
Four conditions:  
– lunch (soup, two sandwiches, fruit pie and fruit)  
– no lunch  
– with or without noise (75 dB v. 40 dB) 
Detection of odd or even digits 
Hit rate positively correlated with extraversion, negatively correlated with neuroticism  
Post-lunch dip in hits detected and RT in the 40 dB (quiet) but not 75 dB (noisy) condition

Smith et al. (1994c)  
Forty-eight university students (twenty-four M, twenty-four F) 
Four conditions:  
– three-course meal (5·02–6·28 MJ) + decaffeinated drink  
– three-course meal (5·02–6·28 MJ) + caffeinated drink  
– no meal + decaffeinated drink  
– no meal + caffeinated drink  
Repeated-digits vigilance task Assessment before the meal and 90 min and 180 min after the start of the meal 
No effects on task  
Effects are different from those observed after lunch or BK

Fischer et al. (2001)  
Fifteen young males  
1670 kJ at breakfast, spoonable creams of:  
– pure fat  
– pure CHO  
– pure protein  
Combi test (peripheral attention) Hourly for 3 h post ingestion 
Best performance after fat  
Protein better than CHO  
CHO — more accurate  
Protein — more efficient

**Table 3. Continued**

<table>
<thead>
<tr>
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<th>Study design</th>
<th>Cognitive testing</th>
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<td>Experiment 1: sixteen males (19–22 years)</td>
<td>Four conditions: BK (1·26 MJ) followed by: – lunch + calorific snack (confectionary snack) – no lunch + calorific snack – lunch + non-caloric snack (drink) – no lunch + non-caloric snack (drink)</td>
<td>Attention Assessment 15 min following eating of the snack Subjects blind to calorific content of snacks</td>
<td>Subjects responded faster in the attention task after the calorific snack than after the non-caloric snack Practice effects observed; therefore, a practice day introduced in the second experiment Pre-lunch performance not assessed (in both experiments)</td>
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<td>Two conditions: – lunch (three-course meal from university refectory, chosen by subjects) – no lunch</td>
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<td>Deficit in performance following lunch consumption on number task</td>
<td>Caffeine also consumed following lunch</td>
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<tr>
<td>Smith &amp; Miles (1986b)</td>
<td>Forty-eight subjects</td>
<td>Two conditions: – lunch – no lunch</td>
<td>Stroop colour and word task (selective attention) Assessment before and after lunch</td>
<td>Stroop not influenced by lunch</td>
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<tr>
<td>Smith &amp; Miles (1986c)</td>
<td>Twenty-four university students</td>
<td>Four conditions: – lunch (soup, two sandwiches, fruit pie and fruit) – no lunch – with or without noise (75 dB v. 40 dB)</td>
<td>Detection of odd or even digits</td>
<td>Hit rate positively correlated with extraversion, negatively correlated with neuroticism Post-lunch dip in hits detected and RT in the 40 dB (quiet) but not 75 dB (noisy) condition</td>
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<tr>
<td>Smith et al. (1994c)</td>
<td>Forty-eight university students (twenty-four M, twenty-four F)</td>
<td>Four conditions: – three-course meal (5·02–6·28 MJ) + decaffeinated drink – three-course meal (5·02–6·28 MJ) + caffeinated drink – no meal + decaffeinated drink – no meal + caffeinated drink</td>
<td>Repeated-digits vigilance task Assessment before the meal and 90 min and 180 min after the start of the meal</td>
<td>No effects on task Effects are different from those observed after lunch or BK</td>
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<td>Fischer et al. (2001)</td>
<td>Fifteen young males</td>
<td>1670 kJ at breakfast, spoonable creams of: – pure fat – pure CHO – pure protein</td>
<td>Combi test (peripheral attention) Hourly for 3 h post ingestion</td>
<td>Best performance after fat Protein better than CHO CHO — more accurate Protein — more efficient</td>
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<tr>
<td>Deijen</td>
<td>Two groups:</td>
<td>During three weeks, diet group had a daily intake of 110 g protein, 320 g CHO,</td>
<td>Pattern comparison Memory scanning task Assessment: baseline scores (last day of 3 weeks, after BK and dinner) and post-treatment scores (2 months later)</td>
<td>Diet group performed more slowly in the morning on memory scanning task than the control group</td>
<td></td>
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<tr>
<td>et al.</td>
<td>– control (n = 9)</td>
<td>80 g fat</td>
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<tr>
<td>(1989)</td>
<td>– diet (n = 10)</td>
<td>– BK: 70 g protein (63 %), 25 g CHO (8 %)</td>
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<td>Two iso-energetic diets:</td>
<td>Memory scanning task after controllable or uncontrollable stress</td>
<td>Performance in HS subjects was better with the CHO-rich diet than with the protein-rich diet, but only after controllable stress</td>
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<td>Markus</td>
<td>Forty-three subjects:</td>
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<td>et al.</td>
<td>twenty-two HS,</td>
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<td>(1998)</td>
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<td>Deijen</td>
<td>Twenty-one cadets</td>
<td>Five daily doses of an iso-caloric drink (1·07 MJ): – protein-rich drink (2 g</td>
<td>Memory task Group with the tyrosine-rich drink performed better on a memory task than the group consuming the CHO-rich drink</td>
<td>Between-subjects design</td>
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<tr>
<td>et al.</td>
<td>(two groups, of ten and eleven, receiving</td>
<td>CHO-rich drink</td>
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<td>(1999)</td>
<td>two different drinks)</td>
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<td>Benton</td>
<td>Seventy young female students</td>
<td>Glucose drink (50 g) after baseline and 25 min later (25 g) or CHO-free placebo drinks (sweetened with aspartame and acesulfame K)</td>
<td>Memory task Improved recall after glucose drink No differential effect on primacy or recency</td>
<td>Between subjects design, no dietary restrictions on test day</td>
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<td>et al.</td>
<td>Eighty female students</td>
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<td>(1999)</td>
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<td>Kelly</td>
<td>Experiment 1: two groups of six subjects</td>
<td>Four groups: – BK + 50 g glucose drink (n = 28) – BK + placebo drink (n = 25)</td>
<td>Brown–Petersen memory task Poorer performance after fasting Glucose improved memory in fasted subjects</td>
<td>Between subjects design Nature of BK consumed not known/controlled</td>
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<td>et al.</td>
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<td>– fasted + 50 g glucose drink (n = 12) – fasted + placebo drink (n = 15)</td>
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<td>(1994)</td>
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<td>Lloyd</td>
<td>Experiment 1: two groups of six subjects</td>
<td>Four lunches varying in energy and fat and carbohydrate content: – LF/low en</td>
<td>Number recognition task Repeated acquisition task Assessment before and after consuming the lunch (Experiment 1); after BK and lunch (Experiment 2)</td>
<td>In both experiments: – performance on the tasks was poorer after than before the meal – no systematic variations in performance as a consequence of either the energy or macronutrient content of the meal</td>
<td>Limited number of subjects and dimension measured Subjects were allowed to choose among the tasks Unrestricted access to caffeine and nicotine</td>
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<tr>
<td>et al.</td>
<td>Eighteen subjects</td>
<td>(about 2·93 MJ): – LF/HCHO (2·90 MJ, 29/54 % en) – MF/HCHO (2·95 MJ, 45/42 % en) – HF/HCHO (3·01 MJ, 62/24 % en)</td>
<td>Free recall task (memory) Assessment 30 min before lunch, 30 min, 90 min and 150 min after finishing lunch</td>
<td>No effect on memory</td>
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<td>(1994)</td>
<td>(fifteen F, three M), mean age 27 years</td>
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Smith et al. (1994)
Experiment 2: forty-eight university students (twenty-four M, twenty-four F)
Four conditions:
- no BK + decaffeinated coffee
- cooked BK (equivalent to HF) + decaffeinated coffee
- no BK + caffeinated coffee
- cooked BK (equivalent to HF) + caffeinated coffee
Four memory tasks:
- free recall
- delayed recognition memory
- logical reasoning
- semantic reasoning
Assessment 60 min before BK, 45 and 105 min after the start of BK
BK improved performance on free recall and delayed recognition memory tasks
No effect on semantic memory task
Impairment of the accuracy of performing the logical reasoning task
Between-subjects design

Lloyd et al. (1996)
Sixteen healthy habitual BK eaters (fourteen F, two M), age 26 years
Habitual BK (1·05 MJ, 25 % fat, 65 % CHO)
Four conditions:
- no BK
- three iso-caloric BK (2·51 MJ)
- LF/HCHO (27/62 % en)
- MF/MCHO (44/47 % en)
Free recall task
Assessment beginning 30 min before, and 30 min, 90 min and 150 min after BK
No clear difference in performance between the four conditions
BK provided in the study was higher in energy than usual BK
Between-subjects design

Lluch et al. (2000)
Thirty-two volunteers (sixteen M and sixteen F; seventeen LF and fifteen HF consumers)
Two iso-caloric BK (1·72 MJ) and lunches (3·10 MJ for F, 4·14 MJ for M):
- LF/HCHO (BK: 22/67 % en, lunch: 20/61 % en)
- HF/LCHO (BK: 48/42 % en, lunch: 54/32 % en)
Associative memory (code substitution)
Assessment just before and 30 min after finishing lunch
No effect on memory task
No effect of habitual diet on performance
Practice effects observed
Pre-lunch performance not assessed (in both experiments)
Between-subjects design

Kanarek & Swinney (1990)
Experiment 1: sixteen males (19–22 years)
Four conditions: BK (1·26 MJ) followed by:
- lunch + caloric snack (confectionary snack)
- no lunch + caloric snack
- lunch + non-caloric -snack (drink)
- no lunch + non-caloric snack (drink)
Digit span recall (forwards and backwards)
Assessment 15 min following eating of the snack
Subjects recalled more digits in the backwards digit span test after the caloric snack than after the non-caloric snack
Subjects recalled more digits in the backwards digit span test after the caloric snack than after the non-caloric snack
Practice effects observed
Pre-lunch performance not assessed (in both experiments)
Between-subjects design

Parker & Benton (1995)
Thirty-three university students (seventeen M, sixteen F), mean age 21 years
Two conditions:
- no BK
- milk-based nutritional beverage
Spatial memory
Immediate recall
Assessment 2 h after BK
Subjects who ate a BK took less time to finish memory tasks than subjects who did not have BK
No effect of BK on number of errors
Between-subjects design

Smith et al. (1994c)
Forty-eight students (twenty-four M, twenty-four F)
Four conditions:
- three-course meal (5·02–6·28 MJ) + decaffeinated drink
- three-course meal (5·02–6·28 MJ) + caffeinated drink
- no meal + decaffeinated drink
- no meal + caffeinated drink
Free recall task
Delayed recognition memory task
Assessment before the meal and 90 min and 180 min after the start of the meal
No effects on memory tasks
Effects are different from those observed after lunch or BK
Between-subjects design

Smith et al. (1999)
144 volunteers (seventy-two M, seventy-two F), mean age 21 years
Four conditions:
- no BK + decaffeinated coffee
- no BK + caffeinated coffee
- cereal BK + decaffeinated coffee
- cereal BK + caffeinated coffee
Spatial memory task
Working memory
Assessment before BK, 80 min and 120 min after the beginning of BK
Subjects who consumed the cereal BK performed better on the spatial memory task than those in the no BK condition
Between-subjects design

BK, breakfast; CHO, carbohydrate; en, energy; F, female; HCHO, high carbohydrate; HF, high fat; HP, high protein; HS, high-stress-prone; LCHO, low carbohydrate; LF, low fat; LS, low-stress-prone; M, male; MCHO, medium carbohydrate; MF, medium fat.
(Hassmen et al. 1994). Since exercise increases the plasma concentration ratio of free tryptophan to other large neutral amino acids leading to an elevation of 5-HT in the brain, branched-chain amino acids may therefore minimise central and mental fatigue during and after sustained exercise.

**Interim summary for cognitive performance**

In summary, acute interventions with high carbohydrate and low protein are sedating and anxiolytic. Protein-rich, carbohydrate-poor interventions tend to produce arousal and improve reaction time and vigilance. Nutritional interventions that facilitate a rise in blood glucose enhance performance of memory and reaction time tasks. The decline in glucose in hypoglycaemia impairs performance. Fat appears to decrease alertness but this effect is often delayed and the habitual diet is important in producing this response. Acute effects vary with time of day. The effect size for glucose is larger than for other macronutrient manipulations and confounding variables are more easily controlled. Chronic or longer-term intervention studies are rarer but suggest that a protein-rich diet can increase negative and decrease positive affect. Longer-term (habitual diet) effects on performance are important because of their relationship to certain disease states that carry with them the risk of cognitive impairment. Poor glucose regulation is associated with poor cognitive performance although this has been examined only in healthy elderly (Kaplan et al. 2000) and diabetic (Strachan et al. 1997) populations.

**Biomarkers for cognitive performance**

Biomarkers for cognitive function are probably more difficult to measure than the actual function itself. We are currently lacking a strong theoretical base to relate neurochemical or physiological activity to cognitive performance. Biomarkers need to be identified in order to elucidate the mechanisms by which food components may affect performance. However, these mechanisms are unlikely to be simple and therefore biomarkers are not an easy route to identifying the effects of food on specific functions.

It is also clear that cognitive performance is normally well protected by a regulatory process that maintains a stable output. This means that effectiveness of overt task behaviour is difficult to disrupt or enhance but the cost of maintaining efficient processing and performance capacity may vary. Decrements are therefore difficult to measure except in circumstances where large manipulations or interventions have an effect on control mechanisms, e.g. drugs with strong effects on the central nervous system. Any genuine effects will tend to be subtle ones, and can only be detected reliably by measuring both overt performance and the underlying ‘maintenance costs’ of performance protection. These should be detectable as ‘latent decrements’ — various markers of increased regulatory strain — seen, for example, in sympathetic dominance in the autonomic nervous system, subjective strain (increased effort, strain, fatigue as after-effect) and in impairment in secondary aspects of performance in complex tasks where only the primary component is usually protected. In this case a measure of increased selectivity (narrowed attention) can be derived based on the relative performance in the primary and secondary tasks. Decrements should also be revealed by the use of tasks in which upper control is a key factor, e.g. as used to assess frontal lobe problems or fatigue after-effects (involving effort, planning, sequential management). Thus any assessment of cognitive performance should include measures of costs as well as of overt performance, and assess both upper and lower function (Hockey, 1997). One solution might be to build a complex task from simpler components, allowing one to test effects of cognitive components alone (i.e. minimal control) and also combined (with additional control requirement to manage the two and plan shifts of focus between them). These behavioural actions can be regarded as biological activities although they clearly differ from a physiological index such as blood glucose.

There are also some physiological indices that may be good candidates as biomarkers of cognitive function. These include measures of autonomic nervous system activity, heart rate and electroencephalography, although these are complex, variable and, currently, difficult to work with in experimental settings where cognitive performance is examined simultaneously. Blood glucose level or rate of change has been used as a biomarker in a number of studies. However, the action of glucose on the cholinergic system may increase synthesis of acetylcholine (Owens & Benton, 1994). Hence the performance-enhancing effects of glucose on reaction time may not be solely related to the increased availability of neural fuel. Alternatively the effect may be produced by increased glucose uptake in the frontal cortex in response to cognitive load. The increase in heart rate associated with high cognitive load supports this assertion. Other studies suggest that stable performance is related to a balanced glucose metabolism and state of metabolic activation. Future markers might include metabolites of neurotransmitters, e.g. 5-HT, but the development of these will require an understanding of their involvement in simple and complex cognitive activities. Moreover, the use of physiological biomarkers relies on the assumption that peripheral measures reflect central activity.

**Functional foods for mood**

**Effect of food on mood and mood on performance**

There are some well-documented effects of food on mood and of mood on performance (for a review, see Rogers et al. 1995). Many studies have related the effects of food on mood to the modulation of serotonin by carbohydrate (e.g. Wurtman et al. 1981) but effects of consuming other foods on mood have also been documented, e.g. chocolate’s positive effects on mood have been attributed to its orosensory properties (Macdiarmid & Hetherington, 1995). Carbohydrate produces a reduction in depression in vulnerable samples such as premenstrually depressed women and those with seasonal affective disorder (Wurtman
<table>
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<tr>
<th>Reference</th>
<th>Sample</th>
<th>Study design</th>
<th>Cognitive testing</th>
<th>Results</th>
<th>Comments</th>
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<tr>
<td>Deijen <em>et al.</em> (1989)</td>
<td>Two groups: – control (n = 9) – diet (n = 10) 21–27 years</td>
<td>During three weeks, diet group had a daily intake of 110 g protein, 320 g CHO, 80 g fat – BK: 70 g protein (63 %), 25 g CHO (8 %) – lunch: 10 g protein (9 %), 100 g CHO (31 %)</td>
<td>Finger tapping Assessment: baseline scores (last day of 3 weeks, after BK and dinner) and post-treatment scores (2 months later)</td>
<td>Diet group performed better in the morning on finger tapping than the control group</td>
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<td>Deijen <em>et al.</em> (1999)</td>
<td>Twenty-one cadets (two groups, of ten and eleven, receiving two different drinks)</td>
<td>Five daily doses of an iso-caloric drink (1·07 MJ): – protein-rich drink (2 g tyrosine) – CHO-rich drink</td>
<td>Tracking task Assessment before the combat course and on the sixth day of the course</td>
<td>Group with the tyrosine-rich drink performed better on tracking task than the group consuming the CHO-rich drink</td>
<td>Between-subjects design</td>
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<tr>
<td>Kelly <em>et al.</em> (1994)</td>
<td>Experiment 1: two groups of six subjects</td>
<td>Four lunches varying in energy and fat and CHO content: – LF/low en (1·80 MJ) – MF (5·16 MJ) – HF (7·05 MJ) – LCHO/low en (1·81 MJ) – HCHO/high en (3·51 MJ)</td>
<td>Choice of four psychomotor tasks available: – differential reinforcement of low-rate schedule of point presentation Assessment before and after consuming the lunch (Experiment 1); after BK and lunch (Experiment 2)</td>
<td>In both experiments: – performance on the tasks was poorer after than before the meal – no systematic variations in performance as a consequence of either the energy or macronutrient content of the meals</td>
<td>Limited number of subjects and dimension measured Subjects were allowed to choose among the tasks Unrestricted access to caffeine and nicotine</td>
</tr>
<tr>
<td>Lloyd <em>et al.</em> (1994)</td>
<td>Eighteen subjects (fifteen F, three M), mean age 27 years</td>
<td>Three iso-caloric lunches (about 2·93 MJ): – LF/HCHO (2·90 MJ, 29/54 % en) – MF/MCHO (2·95 MJ, 45/42 % en) – HF/LCHO (3·10 MJ, 62/24 % en)</td>
<td>Two-finger tapping task Assessment 30 min before and 30 min, 90 min and 150 min after finishing lunch CFFT Hourly for 4 h post ingestion</td>
<td>Reduced after CHO and fat</td>
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<td>Cunliffe <em>et al.</em> (1997)</td>
<td>Sixteen healthy volunteers (nine M, seven F), age 20–47 years (mean, 29.7 years)</td>
<td>1672 kJ at BK approx. 200 ml: – pure CHO (maltodextrin) – pure fat (long chain triacylglycerol emulsion) Control: mixed CHO, fat and protein (55 % CHO, 15 % protein, 30 % fat same sources of CHO and fat)</td>
<td>Two-finger tapping task Assessment 30 min before l unch, 30 min, 90 min and 150 min after finishing lunch CFFT Hourly for 4 h post ingestion</td>
<td>No clear difference in performance between the four conditions</td>
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<tr>
<td>Lloyd <em>et al.</em> (1996)</td>
<td>Sixteen healthy habitual BK eaters (fourteen F, two M), age 26 years Habitual BK (1·05 MJ, 25 % fat, 65 % CHO)</td>
<td>Four conditions: – no BK – three iso-caloric BK (2·51 MJ) – LF/HCHO (27/62 % en) – MF/MCHO (44/47 % en) – HF/LCHO (36/34 % en)</td>
<td>Two-finger tapping task Assessment beginning 30 min before, and 30 min, 90 min and 150 min after BK</td>
<td>No clear difference in performance between the four conditions</td>
<td>BK provided in the study was higher in energy than usual BK</td>
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Eight tasks:
- grammatical reasoning spatial information (e.g., Manikin)
- perceptual input (e.g., simultaneous pattern comparison)
- motor speed (e.g., tapping task)
Assessment just before and 30 min after finishing lunch

Slower RT after HF lunch than after LF lunch
No effect of habitual diet on performance

Thirty-two volunteers (sixteen M and sixteen F; seventeen LF and fifteen HF consumers)

Two iso-caloric BK (1.72 MJ) and lunches (3.10 MJ for F, 3.77 MJ for M):
- LF/HCHO (BK: 22/67 % en, lunch: 20/61 % energy)
- HF/LCHO (BK: 48/42 % en, lunch: 54/32 % en)

Kanarek & Swinney (1990) Experiment 2:
Eight males
Four conditions: BK (1.26 MJ) followed by
- lunch + fruit flavoured yoghurt
- no lunch + fruit flavoured yoghurt
- lunch + non-caloric snack (drink)
- no lunch + non-caloric snack (drink)

Arithmetic reasoning
Subjects solved more arithmetic problems and solved these problems in less time after consuming the yoghurt than after the drink
Reading performance not assessed

Smith & Miles (1986a) Forty-eight students (eighteen M, thirty F)

Two conditions:
- lunch (three-course meal from university refectory, chosen by subject)
- no lunch

Proportion estimation task
Deficit in estimation task in the afternoon, irrespective of lunch consumption
Caffeine also consumed following lunch

Subjective feelings which decrease and increase, respectively, after particular macronutrients, e.g. feelings of coldness or fullness which may be related to the satiating and thermogenic effects of macronutrient ingestion (Cunliffe & Donohoe, 1999). Some subjective feelings and mood changes reported post ingestion in studies of performance and cognitive function are likely to interact with food consumed. Thus it is important to examine the effect of mood on performance from the perspective of mood change in subjective state after nutritional intervention. Indeed, the pre-sense of both nutritional-related mood change (e.g., post-meal caloric intake) and with a degree of statistical complexity, relate these changes to effects on cognitive function, to determine whether mood change is a correlate or a consequence of the food ingested. This mood change can also have positive effects after nutritional intervention. These changes have been observed in samples of highly restrained women or those currently dieting (Rogers & Green, 1993). Negative mood is also present in such samples and it is difficult to untangle the effects of dietary change from the effects of mood. Indeed, the presence of both nutritional-related mood change (e.g., post-meal caloric intake) and with a degree of statistical complexity, relate these changes to effects on cognitive function, to determine whether mood change is a correlate or a consequence of the food ingested.
Palatability

The precise relationship between palatability (often regarded as the hedonic dimension of food) and appetite has been debated for some time now (Ramírez, 1990). The original work of Hill and colleagues (Hill et al., 1984) was instrumental in raising the issue by demonstrating that subjective hunger was higher after a preferred, fixed preload compared with a non-preferred, fixed preload. Therefore, it has been proposed that palatability has an important influence on the initiation and termination of eating events (Blundell & Rogers, 1991b). The palatability of a food is as a result of the integration of orosensory and post-ingestive stimuli. Like hunger, palatability is a hypothetical construct; that is, an explanatory concept that cannot be observed directly itself, but is inferred from operationally defined and measurable events (Blundell & Rogers, 1991a,b). Therefore, the term 'palatability', like hunger, can be conceived of in two ways: either as an intervening variable not measurable directly or, when construed as the perceived pleasantness of food, as a subjective experience that can be monitored objectively by means of a rating. Consequently, in reference to human appetite, palatability appears to be considered to be equivalent to perceived pleasantness of food. Nonetheless, the relationship of pleasantness (or palatability) to actual consumption remains to be determined. With reference to the mediating processes involved in the satiety cascade, it is important to know how these mechanisms are related to fluctuations in perceived palatability and hunger. For example, is high palatability a sufficient or necessary condition for the initiation of eating? It is suggested that the answer to both situations must be no, since there are occasions when even highly palatable food will not be consumed and unpalatable food will be consumed. Another important issue here is what is the relationship between palatability and the termination of eating (satiety)? That is, what is the relationship between palatability and hunger? It is known that mood has a strong influence on cognitive performance; therefore, the relationship amongst mood, satiety, palatability and performance is important.

Effect of palatability on satiety

Work on the relationship between palatability and satiety has demonstrated that hunger was increased at the end of a preferred meal compared with a non-preferred meal (Hill et al., 1984). This phenomenon has recently been confirmed (Yeomans, 1996; Yeomans et al., 1999). Moreover, with respect to the profile of hunger during eating, there was a tendency for hunger to increase during the early stages of eating when the most palatable food was consumed whereas fullness did not follow the same pattern. Therefore, these data suggest that the manipulation of palatability leads to stimulation of appetite, which in turn could increase food intake through the process of satiation. What is less clear is how palatability affects subsequent hunger and food intake (i.e. satiety). From the data that exist there seems to be some ambiguity about the effects of palatability on satiation and satiety (Hill et al., 1984; Johnson & Vickers, 1992; Rogers & Schutz, 1992; De Graaf et al., 1999).

Effect of palatability on performance

Whilst the relationship between palatability and food intake has been examined in many studies, the relationship between palatability and cognitive performance has not. In fact, our search has not identified any studies that have measured directly the effects of palatability per se on cognitive performance. It is, however, logical that palatability could influence cognitive performance via the mediating effects of mood (e.g. palatable food-induced increase in endorphins). That is, if an increase in palatability causes an increase in positive mood, then performance could also be enhanced. Despite the lack of evidence on this topic, some experiments intended to examine the effects of foods (e.g. macronutrient content, energy value, energy density) on cognitive performance have inadvertently allowed taste or palatability characteristics to vary.

Habitual diet

The composition of the habitual diet could have important consequences for the physiological and behavioural patterns of the participant. For example, phenotypes of habitual diet composition (low-fat vs. high-fat) have recently been identified using data from a national database (Macdiarmid et al., 1996). It has been suggested that individuals habitually consuming different diets vary in certain physiological and behavioural characteristics (Blundell & Cooling, 2000). Therefore, habitual diet may have an important influence on the effect of food on satiety, cognitive performance and mood as well as sleep (Lé Noury et al., 2000).

Habitual diet and satiety

In line with the habitual diet composition, the familiarity with the preload could also have an important influence on the outcome. The effects of the interventions in the macronutrient (and energy) content of the food/diet on appetite control and performance may be dependent on the habitual diet of the participant. For example, a high-fat preload may have a different effect on a habitual, high-fat consumer compared with a habitual low-fat consumer (e.g. perceptions of palatability). Tournier & Louis-Sylvestre (1991) have hypothesised that a learned adjustment to the calories in liquid food would occur with repeated exposure. One study demonstrated that informed and uninformed subjects ate similar weights of low- and high-energy sweet preloads (Rolls et al., 1989). This suggests that, when ingesting familiar foods, individuals may rely on previous experiences with these foods to determine how much they should eat (Booth et al., 1982). Clearly participants would not be able to do this with unfamiliar foods.

Habitual diet and performance

Habitual diet does not seem to influence performance in short-term studies where nutritional interventions are made (Lluch et al., 2000). However, performance seems to be optimal when the food consumed most resembles the usual diet, e.g. medium-fat, medium-carbohydrate (Lloyd et al., 1994, 1996). Naturalistic studies do, however,
suggest that the habitual diet, which influences parameters such as cholesterol level, may influence levels of performance. Low plasma cholesterol was associated with slower movement and decision time on a choice reaction time task (Benton, 1995; Muldoon et al. 1997). However, this linear relationship was found only in females; males exhibited a non-linear relationship, suggesting that more than one mechanism may be involved. The interaction of habitual diet and cognitive performance in man requires further investigation. There may be an interaction between cholesterol, dieting and cognitive function because dieting lowers cholesterol.

Evidence from animal studies suggests that there may be differences between the chronic and acute impact of diets varying in fat content. Studies in animals suggest that the structure of the brain may be modified over time by dietary fatty acids (Wainwright et al. 1994). A high-fat diet generally disrupts cognitive function. In addition, an enriched environment reduced the learning impairment associated with frontal lobe function whereas glucose enhanced memory performance by exerting beneficial effects on hippocampus-related memory function (Greenwood & Winocur, 1997).

Some physical illnesses are associated with impairment of cognitive function and dietary intake is a risk factor for the development of these conditions, e.g. atherosclerosis, type 2 diabetes and hypertension. Obesity is a significant risk factor in the development of all these conditions. Rogers (2001) has reviewed the long-term impact of diet on mood and cognitive function in relation to these conditions. Antioxidants, vitamins and other nutritional supplements have been reported to prevent or delay age-related cognitive impairment (Kalmijn et al. 1997; Lethem & Orrell, 1997). Epidemiological studies support these findings (e.g. Gale et al. 1996) although some controlled studies have shown little or no effect of supplementation (Smith et al. 1999, 2000; Cockle et al. 2000).

Summary
This domain of research possesses great potential for collaborations between academia and industry for the development of such products with functional properties. The current world-wide epidemic of obesity has generated an urgent need for the development of functional foods with the capacity to control appetite and influence the regulation of body weight. Some foods already exist and others are under development. There is a clear market need for such products. At the same time, the requirement of foods to produce vitality and influence dynamic life-style creates a drive to produce foods with the capacity to empower people to cope well with an ever more challenging technological environment.

The physiological system underlying the function of satiety is better understood than that responsible for competitive performance. Key sites in the brain — and the periphery — have been identified, and both genetic and pharmaceutical tools have contributed to understanding the network. Consequently, some clear targets (and processes) exist to guide the development of functional foods for satiety. In the field of cognitions, rather less is known about the detail of the neural network although developments in pharmaceuticals have identified certain neurochemical systems and receptors. However, it is likely to be more difficult to develop foods that can exert specific and selective actions at such critically localised targets. Moreover, because of the importance of maintaining an adequate level of cognitive performance, people can protect themselves from degradation in performance by increasing effort. Consequently, measurement of both applied effort and the performance endpoint are required to assess the impact of nutritional manipulations.

A corollary of this is that, because of the requirement to maintain a continuous high level of cognitive performance, it is rather difficult for foods to enhance performance even further (the ceiling effect). Therefore, an appropriate strategy for foods may be to develop products that can protect people from an enforced performance decrement due to fatigue or stress.

Another strategic issue concerns the unavoidable simultaneous effect of foods on different domains of psychological behavioural functioning. In the field of pharmaceuticals it is well known that drugs developed for the treatment of one psychological condition — for example, anxiety — can have collateral effects on appetite and satiety. The capacity of some anxiolytic and neuroleptic drugs to increase eating and body weight may contribute to a form of iatrogenic-induced obesity. In the field of functional foods it is therefore important to develop foods that enhance satiety without exerting detrimental effects on mood or cognitive performance; and to create more products for sustaining cognitive performance that do not compromise satiety.

A further challenge in this area is also to go beyond unitary cognitive functions to complete integrated performance relevant to modern life-styles such as vehicle driving, technological monitoring and operating industrial equipment. Only a very few studies (e.g. Moser et al. 1983) have examined the effects of food materials on a complicated skill such as car driving. This type of cognitive task clearly requires the interpretation of a number of cognitive functions such as reaction time, perceptual ability, information processing, tracking ability and memory.

The identification of biomarkers in the field of psychological and behavioural functions requires more thought and careful consideration. A biomarker can be conceptualised as an indicator of normal biological processes, a parameter associated with the psychological/behavioural response or a surrogate endpoint. In other areas of physiological functioning relevant to functional foods, a convenient biomarker could be a measured variable in blood or some other tissue that correlated with a functional endpoint or disease state. A good example could be a measure of intestinal flora for bowel disease. However, in this particular field it may be more difficult to measure some underlying physiological or neural variable than it is to measure the functional endpoint itself. For both satiety and cognitive performance we have suggested how the issue of biomakers could be approached.
Acknowledgements
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Using such techniques it was observed that the rates of gastric emptying, digestion and absorption are of crucial importance with respect to digestive tolerance during exercise. These factors were also found to determine the rate of nutrient/substrate supply to the active muscles and the subsequent conversion/oxidation for the generation of ATP. This has led to a large number of studies on the effect of various food applications on gastrointestinal function, tolerance and performance. Specific foods were shown to be functional in terms of benefits mentioned above. More recently the field of interest has shifted from macronutrients and fluids to isolated nutritional or non-nutritional food components (FC). Some examples are caffeine, creatine, ribose, l-carnitine, certain amino acids, antioxidants, lactate, pyruvate, glycerol, sodium bicarbonate and hydroxycitric acid (Maughan, 1999; Spriet, 1999; Wagenmakers, 1999a; Volek, 2000; Brouns, 2002). The present short review discusses some of the ‘most favourable’ claims as well as aspects of their validation.

**Performance-limiting factors: a target for supplementation**

There are a number of sectors in sports nutrition that are of great interest to the food industry. Basically these sectors are related to performance-limiting factors in which nutrition is thought to play a role. Research on the effect of selected FC has focused on how to improve or minimize the impact of these limitations. A number of examples are listed below. The nutrients listed have been used in studies and are given as examples only. The outcome of these studies may have been positive (P), negative (N), led to mixed results (M) or is still hypothetical (H).

1. **Muscle mass ⇒** protein synthesis-stimulating FC (e.g. branched-chain amino acids in combination with a carbohydrate (P), arginine (N), creatine (H) or β-hydroxy-β-methylbutyrate (M)).

2. **Fat mass ⇒** FC that induce fat loss, improving fat-free mass (e.g. hydroxycitric acid (N), caffeine (M), l-carnitine (N), chromium picolinate (N), chitosan (H), pyruvate (N) and l-tyrosine (N)).

3. **Bone and joint conditions ⇒** FC to improve bone mass, cartilage thickness, synovial fluid condition (e.g. specific amino acids such as proline (H), lysine (H), mineral combinations (H), phyto-oestrogens (H), glucosamine (H), vitamin K (H) and cartilage preparations (H)).

4. **Dehydration ⇒** drinks composed for rapid rehydration and fluid retention (e.g. hypertonic or isotonic drink formulation containing 30–70 g carbohydrate/litre plus 20–30 mmol Na/litre (P)).

5. **Glycogen depletion ⇒** carbohydrate types to maximize muscle and liver glycogen resynthesis (e.g. glucose and glucose polymers for muscle (P), fructose for liver (P), carbohydrate in combination with amino acids (P)).

6. **Carbohydrate availability and oxidation ⇒** FC such as selected carbohydrate types that are expected to be digested rapidly and absorbed completely in favour of complete oxidation (excellent energy source) during exercise (P).

7. **Low fat oxidation rate ⇒** FC that influence the rate of lipolysis, and improve fat uptake in muscle and mitochondria and ultimately fatty acid oxidation rate (e.g. caffeine (M), hydroxycitric acid (N), l-carnitine (N) and oil containing medium-chain triacylglycerols (N)).

8. **Adenine nucleotides depletion ⇒** FC that are thought to enhance resynthesis as well as storage of ATP and creatine phosphate (e.g. ribose (N) and creatine monohydrate (P)).

9. **Immunosuppression ⇒** supply of FC that increase resistance to disease and reduce exercise-induced inflammations (e.g. specific vitamins such as vitamins C and E (H), minerals such as Zn (H), trace elements such as Se (H), colostrum extracts (H), glutamine (H), achinacea (H), polysaturated fatty acids (H) and probiotics (H)).

10. **Neurostimulation ⇒** supply of neuromodulating FC that are thought to improve reaction times, shorten neuromuscular impulse transmission, improve the availability of precursors for hormones and brain peptides, and improve cognitive function in stress conditions (e.g. caffeine (P), specific amino acids such as tyrosine (P), γ-aminobutyric acid (H) and tryptophan (H), branched-chain amino acids (H), choline (H) and phosphatidylcholine (lecithin; H)).

11. **Suppressed hormone secretion ⇒** FC thought to enhance release of hormones or improve sensitivity to hormones that are involved in protein synthesis, substrate metabolism and recovery from exercise (e.g. tryptophan (H, M), branched-chain amino acids (H, M), tyrosine (H, M), arginine (H, M), ornithine (H, M) and γ-aminobutyric acid (H, M)).

12. **Blood flow ⇒** FC that are thought to enhance blood flow by vasoactive effects (e.g. arginine (M) and l-carnitine (H)).

13. **Gastrointestinal distress ⇒** supply of foods and drinks that are optimally tolerated by the digestive system during exercise (e.g. hypotonic food formulas composed of rapidly digestible and completely absorbable nutrients (P)).

14. **Poor nutritional status ⇒** FC to ensure optimal nutrient status of vitamins, trace elements and minerals (e.g. specific micronutrient supplements (P)).

15. **Poor performance ⇒** FC that may increase endurance or maximal oxygen uptake and thereby improve performance (e.g. carbohydrate (P), coenzyme Q10 (N) and branched-chain amino acids (N)).

16. **Muscle damage ⇒** FC that may reduce the occurrence of muscle damage during exercise and improve recovery from it (e.g. antioxidants like vitamin E (M) and β-carotene (M)).

17. **Muscle cramps ⇒** FC to support neuromuscular events and reduce indices of cramp (e.g. Mg (H), Zn (H) and ribose (H)).

18. **Injuries ⇒** FC to prevent the development of injuries and speed up the recovery process from injury (e.g. glucosamine (H) and chondroitin (H)).
Examples of claims that are made for products whose target is to improve performance-limiting factors are ‘more power’, ‘improved performance’, ‘more muscle mass’, ‘less body fat’, ‘faster recovery’, ‘quick rehydration’, ‘reduces muscle cramps’ and ‘improved hormone release’. Clearly, the food and supplement industry tries to make such claims on the product packaging as well as in advertising by promoting the product ‘as a solution with a proven benefit’. The ultimate goal is to give athletes the feeling that consumption of the product will improve performance, or result in a benefit that is of importance to health and well-being.

The basic question in this respect is: ‘What is the evidence?’ There are valid examples of nutritional interventions in exercise and sport that indeed resulted in significant effects on the study endpoint of interest. However, there are also a very large number of products available on the market promoted with benefit statements that have never been validated by sound scientific studies.

Below, a short discussion is given on some of the above-mentioned examples. Test methods and biomarkers thought to be suitable for the measurement of interventions in which any of such nutritional factors are tested against a suitable placebo are discussed briefly. References are given for the reader who is interested in detailed information.

Muscle/fat mass

The importance of a relatively high muscle mass and low fat mass for most power and strength events is based on the observation of a significant relationship between muscle cross-section and maximal muscle strength measured either as maximal strength or dynamic strength. The relative strength or power of an athlete declines with increasing fat mass. As such, all athletes who move/replace their own body weight will benefit from having a high lean body mass/low fat mass. Therefore, athletes involved in strength and power events will benefit from training regimens and nutritional factors that boost muscle protein synthesis and reduce fat mass (Lemon, 1993). Athletes involved in sports in which low body fat is a key factor, such as bodybuilding, will do almost anything to reduce fat mass and increase visible muscle mass (Kleiner et al. 1990; Walberg-Rankin et al. 1993).

Methods used to measure muscle mass/fat mass and muscle protein synthesis concern the following biomarkers.

1. Selected muscle mass by muscle circumference measurements (surrogate marker, not reliable due to a number of confounding factors, e.g. level of subcutaneous fat, muscle glycogen and water content).
2. Site-specific fat mass by circumference measurements (as above, not reliable).
3. Selected muscle mass or site-specific fat mass by scanning techniques such as computed tomography (CT) and magnetic resonance imaging (MRI; reliable but complex and expensive, CT is invasive, MRI non-invasive; Gadian, 1995).
4. Total muscle mass by body composition techniques. The reliability depends strongly on the method of choice. So-called mechanistic methods, such as underwater weighing, isotope dilution or both methods combined, are the most reliable (non-invasive, although underwater weighing may be problematic in some groups, is time-consuming, requires special laboratory facilities and is relatively expensive). The descriptive methods, such as dual X-ray absorptiometry (DXA), bio-impedance analyses (BIA) and skin folds (SF), which are derived from the mechanistic ones, are less reliable with respect to the absolute values (non-invasive although DXA uses a small doses of X-rays, fast, DXA relatively expensive, BIA and SF are cheap). Body composition changes resulting from the intervention are also detected most accurately with mechanistic methods. Finally, imaging techniques, such as CT and MRI, are used increasingly (accurate but interpretation of images may be complicated, CT invasive, MRI non-invasive, special laboratory facilities needed, often time-consuming, expensive). For more comprehensive overviews of methods and systematics, see Wang & Heymsfield (1995) and van Marken Lichtenbelt & Fogelholm (1999).

5. Protein synthesis, muscle protein degradation and turnover rates by measuring the incorporation rate and quantity of selected markers such as stable isotope-labelled amino acids: leucine, glycine, phenylalanine (reliable but complex and expensive; Wagenmakers, 1999b; Rennie & Tipton, 2000).

Dehydration/hydration

The importance of appropriate hydration and rehydration stems from a number of observations. With continuous exercise the water content of all body compartments will decrease as a result of fluid loss by sweating and insensible water loss from the lungs. Depending on the exercise intensity, training status, climatic circumstances and body size, sweat losses may range from a few hundred millilitres to more than two litres per hour. Such large sweat losses will reduce circulating body fluids. Because a normal plasma volume is of prime importance in maintaining an appropriate blood flow through ‘exercising tissues’, it may be deduced that a significant decrease in plasma volume will impair blood flow. This will in turn lead to a reduced transport of substrates and oxygen, which are needed for energy production, to the muscles. Also, the transport of metabolic waste products and heat, from the muscle to ‘eliminating organs’ such as the liver and skin, will be impaired. The decreased heat transfer from the muscles to the skin results in an increased core temperature in all cases where heat production exceeds heat dissipation from the body. This may lead to a decreased energy-production capacity, fatigue, impaired performance and health threats (Brouns, 1997).

Clearly, not only the total amount of body water (TBW) is important, but also the water distribution. Therefore determination of intracellular water (ICW) and extracellular water (ECW), or the ratio between these two, is often practised.

Methods used to measure the hydration status of the whole body concern the following biomarkers.

1. TBW content by dilution of stable isotopes, deuterium or the more expensive oxygen-18 (accurate, non-invasive, time-consuming, special laboratory facilities
needed, moderately expensive; Westerterp et al. 1995; Westerterp, 1999).

2. ECW content by bromide dilution (accurate but
bromide dilution space is not identical to ECW and
a correction factor is needed, non-invasive, time-
consuming, moderately expensive; van Marken
Lichtenbelt et al. 1996).

3. TBW and ECW by bio-impedance spectroscopy
(accuracy is still a matter of controversy but non-
invasive, fast and cheap; van Marken Lichtenbelt,
2001).

Methods used to measure shifts in body fluids concern
the following biomarkers.

1. Changes in plasma volume by determination of
haemocrit and haemoglobin values (reliable and easy
to measure; Dill & Costill, 1974).

2. ECW:ICW with the dilution techniques mentioned
above (unsuitable for short-term interventions).

3. Bioelectrical impedance (see above; Boileau &
Horswill, 2000; van Marken Lichtenbelt, 2001). The
method may pose problems during the study of
short-term (sports) intervention, because of its sensi-
tivity to changes in body temperature and electrolyte
concentrations in body fluids.

4. Muscle biopsy analysis (not reliable for rehydration
effects).

Methods used to determine fluid uptake rate by the body
concern techniques to measure biomarkers of gastric
emptying and intestinal absorption. It should be noted
that gastric emptying alone does not give a valid answer
with respect to rehydration efficacy since it is only the
first factor in a chain of determinants of bioavailability.
For example, water is emptied rapidly from the stomach
but is absorbed slowly in the gut. The best data are
obtained from a battery of tests in which gastric emptying,
gut segmental absorption and fluid retention measurements
are combined.

Gastric emptying may be measured by:

1. Intubation techniques applying the double sampling
technique of George (invasive, needs routine, reliable
to quantify during exercise, cheap; Beckers et al.
1988).

2. Use of stable isotopes (non-invasive, complex, can be
used during exercise but quantification not possible,
only qualitative differences can be shown, expensive;
von Nieuwenhoven et al. 1999).

3. Gamma scintillation (non-invasive, complex, isotopes
required, reliable but not suitable during exercise,
expensive; Leiper & Maughan, 1988; Beckers et al.

Intestinal absorption may be measured by:

1. Intubation/perfusion techniques using non-absorbable
markers (invasive, routine required, difficult during
exercise, reliable to quantify for the test segment but
not for the whole intestine, relatively cheap; Leiper
& Maughan, 1988).

2. Determining the appearance of labels in blood using
stable isotopes (non-invasive, but complex and diffi-
cult to quantify due to label shifts; Leiper & Maughan,
1988).

Glycogen depletion/muscle glycogen content

Since the classical Scandinavian studies using muscle
biopsy techniques, a lot of work has focused on the import-
ance of muscle glycogen for energy homeostasis and per-
formance. Several lines of evidence show that intense
and lasting muscle work cannot be performed without
appropriate availability of carbohydrate. As soon as
specific muscles or muscle fibres become glycogen-
depleted, they will be impaired in their ability to perform
repeated high-intensity contractions. Glycogen depletion,
caused either by exercise or a combination of exercise
and low carbohydrate intake, leads to a reduction in
work capacity to a level of about 50 % of the normal maxi-
mal working capacity. Alternatively, when the carbo-
hydrate stores in muscle and liver are increased by diet
manipulation, athletes are able to perform longer at high
exercise intensity. Thus, the availability of carbohydrate
and the size of the glycogen stores are important and limit-
ing factors for endurance performance.

Methods used to measure muscle and liver glycogen
concern the following biomarkers.

1. Muscle biopsy analysis for muscle glycogen (reliable
and easy but invasive, cheap; Hultman, 1967).

2. NMR for muscle glycogen (reliable for qualitative
changes, difficult for quantification, expensive, com-
plex but non-invasive; Price et al. 1999).

3. NMR for liver glycogen (as above; Price et al. 1999).

Hormone-releasing agents

There are observations that intensive training may induce
lower circulating levels of stress hormones, insulin and
glucagon. This is most probably caused by enhanced sensi-
tivity of tissues/cells for the hormones. It has also been
observed that intense training/over-training may lower
secretion of androgens and growth hormone, most probably
because of endocrine disruption effects. However, the
physiology of many hormones as well as the effects of
exercise on hormonal adaptations are still incompletely
understood (McMurray & Hackney, 2000) and make it
difficult to link secretion modifications of many of them,
if any, to health performance benefits. Nevertheless, a sub-
stantial number of products are promoted to enhance
hormone release for athletic benefits (Brouns, 2002). For
example, it has been hypothesized that the ingestion of
arginine and ornithine may stimulate the release of
growth hormone, which is thought to stimulate muscle
growth.

Reliable measurement of blood hormone levels after
supplementation is complex. Because of diurnal effects,
24 h profiles will be required to make any statement on
the physiological relevance of observations.

Gastrointestinal tolerance

A substantial number of endurance athletes (40–60 %)
may be prone to developing gastrointestinal disturbances during exercise, especially when exercising in the heat and developing dehydration (Brouns, 1991). To avoid such problems recommendations are given to reduce the intake of poorly fermentable dietary fibres and fat-rich foods prior to competition and to ingest easily digestible and absorbable energy sources during exercise. Because the drink/food composition can have a significant influence on the occurrence of gastrointestinal symptoms (Brouns, 1991a,b, 1997; Brouns & Beckers, 1993; Brouns & Kovacs, 1997a,b; van Nieuwenhoven et al. 2000), the industry tries to make claims related to this aspect.

The tolerance to such products during exercise can be assessed as follows.

1. By questionnaire. Registration of the occurrence of gastrointestinal symptoms during exercise after consumption of the test product in comparison to a control product (for fluids, usually artificially sweetened and coloured water; for solids, a normal food item such as an equicaloric amount of bread). Symptoms scored usually are related to degree of fullness, stomach pains, regurgitation, intestinal cramps, borborygmy, flatulence and diarrhoea/loose stools. It has to be noticed that controlled laboratory studies do not mimic the situation in the field. Laboratory studies have the advantage of controlling variables such as work intensity, environmental temperature and humidity, and quantitative timed intake of the test product. Field conditions make these controls very difficult but are more reliable with respect to competition stress and fluctuating exercise intensities during a competition. In addition, the food intake two or three days prior to the test may be of influence on the occurrence of lower intestinal symptoms. This means that food intake should be standardized along with exercise programmes and the consumption of caffeine- and alcohol-containing beverages during the days prior to the test (easy but very labour-intensive testing, reliability of laboratory data for field circumstances is speculative).

2. By motility measurements. These can be done reliably in a laboratory but the outcome of the data in terms of relevance for competition circumstances is speculative. Moreover, data are specific for the tested biomarkers/endpoints only and do not allow one to make statements on gastrointestinal tolerance in general.

3. Oesophageal sphincter pressure/reflux episodes (invasive, needs routine, reliable, can be measured ambulatory; Schoeman et al. 1995).

4. Contractile activity of the stomach and duodenum by antroduodenal manometry (invasive, needs routine, reliable, can be measured ambulatory but not suitable during exercise; Penning et al. 2001).

5. Intestinal transit rate by breath hydrogen from small intestine (non-invasive, easy, allows ambulatory measurement, cheap; Levitt, 1969) or whole-gut transit measurements using scintigraphic techniques (non-invasive, reliable, but expensive and exposure to radioactivity and measurement takes a long time; Charles et al. 1995).

**Fat utilization and oxidation**

An enhanced oxidation of fatty acids during exercise will induce a sparing of endogenous carbohydrate stores. The latter may reduce the development of glycogen depletion and hypoglycaemia and improve endurance capacity. Many attempts have been made to modify fat metabolism (Brouns & van der Vusse, 1998; Hawley et al. 1998; Jeukendrup et al. 1998; Jeukendrup, 1999).

Biomarkers to measure fat metabolism are mostly related to (1) the rate of lipolysis, (2) the rate of fat uptake in muscle and mitochondria and (3) ultimately the fatty acid oxidation rate.

**Fat lipolytic rate**

Methods used to determine lipolytic rate concern the following biomarkers.

1. Determination of release of fatty acids and glycerol from adipose tissue by microdialysis (invasive, needs routine, reliable; Arner, 1999; Frayn, 1999; Henriksson, 1999).

2. Determination of the rate of appearance of glycerol (and fatty acids) in the circulation (surrogate marker, reliable, requires stable isotopes, expensive).

3. Determination of changes in plasma fatty acid and glycerol concentrations (surrogate marker, easy, reliable only in combination with stable isotopes; Coggan, 1999; Landau, 1999; van Hall, 1999).

**Fatty acid uptake rate by muscle**

Methods used to determine fatty acid uptake concern the following biomarkers.

1. Determination of rate of free fatty acid disappearance from blood by stable isotopes (surrogate marker, reliable, requires stable isotopes, expensive; Coggan, 1999; Landau, 1999; Rennie, 1999; van Hall, 1999).

2. Determination of net uptake by muscle using arteriovenous techniques (invasive, reliable; McDonald, 1999) or microdialysis (Arner, 1999; Henriksson, 1999).

**Fatty acid oxidation rate**

1. Determination of respiratory quotient (surrogate marker, easy, reliable for group means, less reliable for quantification of small changes within a subject).

2. Quantification by appearance of label in expired gas, using stable isotopes (reliable, non-invasive, expensive; Coggan, 1999; Rennie, 1999; van Hall, 1999; van Hall et al. 1999).

3. Quantification of intramuscular fat use by biopsy analysis (invasive, difficult to quantify, not reliable for intervention effects; Hoppeler et al. 1999) or by NMR (non-invasive, complex technique, not reliable for quantification of treatment effects; Boesch et al. 1999).
Carbohydrate oxidation

An enhanced oxidation of ingested carbohydrate during exercise will spare the endogenous carbohydrate stores and this is generally thought to enhance endurance capacity. It has been shown that when carbohydrate oxidation rates drop below critical levels during exercise, this will coincide with fatigue (Coyle et al. 1986). In an attempt to optimize the carbohydrate oxidation from ingested carbohydrate sources, numerous studies have been performed in which the timing, amount and type of carbohydrate were varied. These studies were reviewed recently by Hawley et al. (1992) and Jeukendrup & Jentjens (2000). Carbohydrate consumed during exercise is oxidized in small amounts during the first hour of exercise (~20 g) and thereafter reaches a peak rate of ~1 g/min (Jeukendrup & Jentjens, 2000). Even ingestion of very large amounts of carbohydrate will not result in higher oxidation rates. Generally, the timing of ingestion has little or no influence as long as the amount of carbohydrate ingested is sufficient (60 g/h). Glucose, sucrose, maltose, maltodextrins and amylpectin starch are oxidized at high rates (up to a maximum of 1 g/min) whereas fructose, maltotose, maltodextrins and amylpectin starch are oxidized at 25–50% lower rates. Combinations of carbohydrates, however, may give higher exogenous carbohydrate oxidation rates than single carbohydrates (Adopo et al. 1994).

Methods to determine carbohydrate oxidation concern the following biomarkers.

1. Oxidation of ingested carbohydrate is usually measured by using a $^{13}$C (or $^{14}$C) isotope of the carbohydrate of interest.
2. A $^{13}$C or $^{14}$C carbohydrate tracer (i.e. glucose, fructose) is ingested and excretion of $^{13}$C (or $^{14}$C) in breath is measured. In combination with a measurement of total CO$_2$ production, an accurate measure of exogenous carbohydrate oxidation can be obtained. The drawback of this method is usually the cost of the tracer, although in some cases the natural $^{13}$C enrichment of the carbohydrate (e.g. from cornstarch or cane sugar) can be of use for testing (reliable, complex method, expensive).

Nutritional status

There are a number of high-risk sports for marginal nutritional intakes that may impact on performance capacity and health (Brouns, 2002). The endpoints used for nutritional status in healthy athletes mostly concern circulating levels of vitamins, minerals and trace elements. Development of analytical techniques has made it possible to study ‘biochemical vitamin deficits’ that occur at an early stage of poor intakes. These measurements include the determination of plasma vitamin levels by HPLC and enzymatic stimulation tests (easy but invasive and also expensive). Although minerals are fixed components of tissues such as bone or muscle, this does not necessarily mean that they are freely available for metabolic purposes. The major fraction of the ‘metabolic’ mineral pool is present in blood plasma and interstitial fluid. The amount of minerals circulating in body fluids is a resultant of different ongoing processes. Absorption from food, on the one hand, and uptake or release by tissues as well as losses/excretions (by sweat, urine, faeces), on the other, determine the actual mineral content in the blood. This mineral level remains within a narrow range. Any mineral shortage will, in the first instance, be compensated by reduced excretion of tissues and increased release from tissues. With a continuing shortage, plasma mineral levels will start to fall. The latter will influence the uptake or release of minerals by cells and thus the cell mineral status. During prolonged periods of mineral deficits, cell growth and cell function will become impaired, as will performance capacity. However, a short period with a relative shortage of one or more minerals, e.g. as may occur during an ultra-endurance event or during a multi-day cycling event, will not necessarily mean that health and performance will become affected measurably.

With respect to trace minerals, it is possible to obtain ‘a certain figure’ or ‘a certain level’ from samples such as serum, tissue, hair and nails. The question one should ask is about how representative these samples are for whole-body status and for muscle in particular. Moreover, hair and nail analyses give a figure related to intake in the past and do not necessarily correlate with current status, while in the case of hair analysis environmental factors such as water trace minerals content (swimmers, frequent showering) and use of hair products (shampoos, gels, colour agents) may be confounding factors.

Growing knowledge over the years has indicated different sample sites as being most representative for specific trace elements. This makes it practically impossible to perform a simple body fluid analysis with the goal of making a statement on trace element status, as is the case for vitamins and minerals as well.

Adenine nucleotides depletion

A number of important metabolic functions are related to the availability of appropriate levels of phosphocreatine and total adenine nucleotides (TAN; Balsom et al. 1994; Williams et al. 1999; Brouns, 2002). The maintenance of an appropriate level of ATP, through a rapid repophorylation of ADP from phosphocreatine, supports the creatine phosphate shuttle to enhance the exchange of high-energy phosphate from the site of the mitochondria to the site of the cytosol. It also helps reduce acidosis in muscle cells by buffering hydrogen ions, and indirectly helps regulate the activation of carbohydrate breakdown (glycolytic) processes in muscle by activation through the products resulting from the hydrolysis of phosphocreatine, which are inorganic phosphate and free creatine.

In the case of depletion of the phosphocreatine store during all-out exercise, a number of metabolic changes may occur in muscle that will lead to a change in TAN: (1) depletion of the creatine phosphate store; (2) breakdown of ATP → ADP → AMP → end products; (3) increase in muscle and blood lactate; (4) increase in muscle and blood ammonia; and (5) increase in blood xanthine, hypoxanthine, adenine and uric acid. After exercise the breakdown products mentioned might be lost from muscle.
This results in a decreased TAN content. The resynthesis of adenine nucleotides is a slow process, causing recovery to a normal level to take up to three or four days. It has been hypothesized that oral supply of ATP or ribose can lead to a more rapid recovery of the TAN pool after intensive training sessions or competitions.

Markers of TAN are:

1. Changes in plasma levels of ammonia, uric acid, xanthine and hypoxanthine (surrogate markers for qualitative changes in TAN, easy to perform, not reliable to make any statements about muscle TAN contents; Op t’Einde et al. 2001).
2. Muscle TAN levels by biopsy analysis (invasive, relatively easy, reliable) or NMR (non-invasive, complex methodology, only for qualitative changes, not reliable for quantification; Heerschap et al. 1999).

### Muscle/tissue damage

There are a number of excellent reviews that highlight the effect of exercise-induced free radical formation on various tissues, as well as their measurement in biological fluids (Halliwell & Gutteridge, 1985; Jenkins, 1988; Kanter, 1994; Duthie, 1999; Jackson, 1999; Poulsen et al. 1999; Li, 2000). Muscle soreness after an intensive bout of exercise is caused by an inflammation process. The micro trauma (disruption at the Z band level of the sarcomeres) that results from acute overload cannot be avoided by antioxidant systems, because it is mechanical in nature. However, the repair process of the mechanically damaged muscle fibres involves an inflammatory process, which causes muscle pain, stiffness and loss of muscle strength, especially two to five days after the sport event (Smith & Miles 2000). It is suggested that free radicals play an important role during this inflammatory process and that supply with adequate amounts of antioxidants may lessen both the severity and the duration of this delayed muscle soreness.

Endurance exercise in polluted air, such as running a major city marathon on a hot summer’s day in the smog, has been suggested to lead to damage to the lung tissue induced by ozone (Folinsbee, 2000). Free radical formation and reactions are also suspected here to be the mediating mechanism. Accordingly, nutritional substances such as vitamin E supplementation are suggested to reduce such damage (Evans, 1991) and lung function impairment (Folinsbee, 2000).

Biomarkers used in tissue damage assessments are listed below.

1. Free radical formation (complex assays, not reliable for tissue damage quantification; Duthie, 1999; Poulsen et al. 1999; Powers & Lennon, 1999).
2. Histological damage by biopsy analysis (reliable, invasive, but difficult to quantify for whole muscle).
3. Damage markers in the circulation: myoglobin, muscle enzymes (creatlinphosphokinase; surrogate markers, reliable for qualitative changes, difficult as quantitative measure, long-lasting post-damage adaptation effects (up to six months) make cross-over studies very difficult, easy to measure; Clarckson, 1992; Volfinger et al. 1994).
4. Strength performance and subjective pain (surrogate marker, easy to measure, no quantification of damage possible; Clarckson, 1992).

### Muscle cramps

Low resting and exercise plasma Mg levels have repeatedly been reported in athletes who are involved in regular endurance exercise. This has been thought to lead to impaired energy metabolism, greater fatigue and to the occurrence of muscle cramps. Cramps are also thought to be caused by acute energy deficits as in the case of phosphocreatine depletion and reductions of ATP during very intensive, all-out metabolism. Accordingly, attempts have been made to study the effects Mg, creatine and ribose supplementation. The clinical endpoint here, ‘muscle cramp’, seems to occur infrequently and is very difficult to quantify. Many factors may be involved in its aetiology.

1. Mineral levels in body fluids and muscle and TAN levels do not seem to correlate with cramp occurrence (invasive, easy to measure but unreliable; Maughan, 1986).

### Performance

For many products, the claim will be ‘enhanced performance’. Of course, there are various forms of performance and no single laboratory or field test could be used to generalize across all sports performances. Often the distinction is made between endurance performance and high-intensity exercise or sprint performance. In addition, in the literature, the terms ‘endurance performance’ and ‘endurance capacity’ are often used as synonyms. However, endurance capacity refers to the exercise time to volitional fatigue whereas endurance performance relates to completing a certain task (running a certain distance, cycling a certain distance) as fast as possible. The latter is obviously a more realistic approach since there are very few events where athletes are asked to exercise for as long as they can. Studies have investigated the reliability of both of these test protocols and found coefficients of variation of between 1 and 3% for performance trials and up to 26% for time to exhaustion measurements (Jeukendrup et al. 1995). Performance trials have been developed and validated for the treadmill (self-paced runs for a fixed distance), intermittent running (Loughborough Intermittent Shuttle Run Test; Nicholas et al. 2000), the cycle (self-paced time trial; Jeukendrup et al. 1997), the rowing ergometer (Schabert et al. 1999) and various other intermittent sports such as soccer, squash (Romert et al. 2001) and tennis (Vergauwen et al. 1998). Studies that try to simulate a real event, especially in the field rather than in the laboratory, are harder to conduct. The most complicated types of performance belong to unpredictable team games or sports involving complex decision-making and motor skills. It is hard to find a way to measure adequately all components of performance, and it is complicated to design a protocol in which the same event is conducted twice, before and after an intervention, or with a treatment.
and a placebo. Despite the difficulties in conducting studies, strategies that enhance carbohydrate availability have been shown to enhance cycling and running endurance, cycling and running performance, and the performance in complex games such as tennis, soccer and ice hockey.

When studying the effects of a nutrition supplement on performance, an ergometer, treadmill or similar tool should introduce negligible random error (variation) in its measurements. Random error in the performance measurement should also be minimized by choice of an appropriate type of test. Tests based on physiological measures (e.g. maximum oxygen uptake, anaerobic threshold) and tests requiring self-selection of pace (e.g. constant-duration and constant-distance tests) usually produce a random error of at least 2 to 3% in the measure of power output (Paton & Hopkins, 2001). Random error for measures of power in ‘all-out’ sprints, incremental tests and very short (1–5 min) constant-power tests to exhaustion may be as low as 1%. Measures with such low error might be suitable for tracking the small changes in competitive performance that matter to elite cyclists.

In all performance tests it is extremely important that all confounding factors are removed or standardized where possible (music, encouragement, feedback). In addition it must be kept in mind that some of these performance tests may not always pick up the small improvements in performance that are relevant to an elite athlete.

1. Sprint performance can be assessed by a Wingate test (easy to perform, reliable).
2. Endurance capacity can be assessed by a time to exhaustion test (poor reproducibility, limited practical value).
3. Endurance performance can be assessed by a constant-duration or constant-distance test (good reproducibility, needs extremely strict standardization).
4. Sport-specific performance and field tests could include measurements of performance in ‘real life’ such as during a soccer match or simulations of a particular sport or discipline in field conditions (very few established tests, needs extremely strict standardization; a review describing details of sport-specific tests is given by Kearney et al. 2000).

Claims and legal aspects

Generally, no food regulation exists (yet) to control performance or health benefit statements with respect to the supportive scientific evidence. Also, depending on the dosage, many of the FC used are in the grey zone between foods and drugs. The question whether an effective dosage of a certain FC that never will be consumed in that particular quantity with the normal diet is nutritional or pharmacological is relevant in this respect. Many of these products are sold as dietary supplements, mostly by direct mail order systems and the Internet, which are difficult to control.

Development of the sports foods and drinks market is highly attractive to the food industry. However, one should be aware of the fact that current developments in the field of consumer protection, regulatory environment and product liability aspects do call for a more careful and well defined product development and marketing programme than is usually the case. Obtaining scientific support for a product claim is an essential issue in this respect.

The establishment of a code of practice among sports food/supplement companies is another must, as long as an appropriate legislation is not in place.

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molecule CD4 on their surface) and cytotoxic T cells (these are distinguished by the presence of CD8 on their surface). All cells of the immune system originate in bone marrow. They are found circulating in the bloodstream, organised into lymphoid organs such as the thymus, spleen, lymph nodes and gut-associated lymphoid tissue, or dispersed in other locations around the body.

**Innate and acquired immunity**

Innate immunity is the first line of defence against infectious agents. It is present prior to exposure to pathogens and its activity is not enhanced by such exposures. Innate immunity is concerned with preventing entry of infectious agents into the body and, if they do enter, with their rapid elimination. Elimination can occur by:

1. direct destruction of pathogens by complement, by toxic chemicals (e.g. superoxide radicals and hydrogen peroxide) released by phagocytes or by toxic proteins released by natural killer cells;
2. engulfing pathogens by the process of phagocytosis, which is made more efficient by coating the invading pathogen with host proteins like complement or antibodies, and their subsequent destruction.

Acquired immunity involves the specific recognition of molecules (antigens) on an invading pathogen, which distinguish it as being foreign to the host. The recognition of antigens is by antibodies (immunoglobulins (Ig) produced by B lymphocytes) and by T lymphocytes. T lymphocytes are only able to recognise antigens displayed on cell surfaces. Therefore, infection of a cell by an intracellular pathogen is signalled to T lymphocytes by cell surface expression of peptide fragments derived from the pathogen. These fragments are transported to the surface of the infected cell and expressed there in conjunction with proteins termed major histocompatibility complex (MHC); in man MHC is termed human leucocyte antigen. It is the combination of the pathogen-derived peptide fragment bound to MHC that is recognised by T lymphocytes. There are two classes of MHC, MHC I and MHC II, and the source of the peptide bound to each differs. MHC I binds peptides that originate from pathogen proteins synthesised within the host cell cytosol; typically these are from viruses or certain bacteria. The peptides bound to MHC II are derived from pathogens that have been phagocytosed by macrophages or endocytosed by antigen-presenting cells (macrophages, dendritic cells, B lymphocytes). The MHC–peptide complex is recognised by the T cell receptor on T lymphocytes. T lymphocytes expressing CD8 recognise MHC I, while T lymphocytes expressing CD4 recognise MHC II. Thus, intracellular pathogens stimulate cytotoxic T lymphocytes to destroy the infected cell, while extracellular pathogens stimulate a helper T cell-mediated response.

The acquired immune system includes a component of memory, such that if the antigen is encountered again (i.e. there is re-infection) the response is faster and stronger than the initial response. Although the immune system as a whole can recognise tens of thousands of antigens, each lymphocyte can recognise only one antigen and so the number of lymphocytes specific for a particular antigen must be very low. However, when an antigen is encountered it binds to the small number of lymphocytes that recognise it and causes them to divide so as to increase the number of cells capable of mounting a response to the antigen; this is the process termed lymphocyte expansion or proliferation. B lymphocytes proliferate and mature into antibody-producing cells (plasma cells) and T lymphocytes proliferate and are able directly to destroy virally infected cells (cytotoxic T lymphocytes) or control the activity of other cells involved in the response (helper T cells). The B lymphocyte response to antigen is termed humoral immunity and the T cell response is termed cell-mediated immunity.

**Integration of the immune response**

Communication within the acquired immune system and between the innate and acquired systems is brought about by direct cell-to-cell contact involving adhesion molecules and by the production of chemical messengers. Chief among these chemical messengers are proteins called cytokines, which can act to regulate the activity of the cell that produced the cytokine and/or of other cells. Each cytokine can have multiple activities on different cell types. Cytokines act by binding to specific receptors on the cell surface and thereby induce changes in growth, development or activity of the target cell.

When an immunological stimulus is encountered, the innate response, including its inflammatory component, responds initially, acting directly to eliminate it by the activities of complement, phagocytosis, etc. Cytokines (e.g. tumour necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-6) produced by the cells involved in the innate response, especially monocytes and macrophages, will regulate this response and also act systemically on the liver to promote acute phase protein synthesis, on skeletal muscle and adipose tissue to promote proteolysis and lipolysis, respectively (this is believed to be the body’s way of providing fuels to the immune system) and on the brain to reduce appetite and induce fever. These cytokines will also interact with T lymphocytes. Antigen-presenting cells, which include activated monocytes and macrophages, will present antigen to T lymphocytes and so the acquired immune response will be triggered. Now there will be a cell-mediated response to the antigen. T lymphocytes will produce cytokines which will regulate the activity of the cells involved in the innate response (monocytes, macrophages, natural killer cells), promote the proliferation of B and T lymphocytes and promote antibody production by B lymphocytes. By virtue of the integrated innate and acquired responses the source of the antigen should be eliminated and a component of immunological memory will remain (Fig. 1).

**Biomarkers of immune function**

There is a wide range of methodologies by which to assess the immune response and the impact of nutrient supply on immune function (for a discussion see Cunningham-Rundles, 1998). Assessments can be made of cell functions...
**ex vivo** (i.e. of the isolated cells outside the body and studied in short- or long-term culture), of indicators of immune function **in vivo** (e.g. by measuring the concentrations of proteins relevant to immune function in the bloodstream), of responses to an immunological challenge (e.g. inoculation with an antigen, a vaccine or live bacteria) or, in human studies, of the incidence and severity of infectious diseases. Clearly, animals offer greater access to the immune system, but it is important that observations made in animal studies be confirmed in man. Although **ex vivo** measures of immune function are made frequently, changes in these may not necessarily result in an altered immune response **in vivo**. Thus, in order to ascertain the effect of an intervention on the immune response, measures reflecting **in vivo** activity of the immune system are superior to **ex vivo** measures. However, the two approaches should be used in combination to understand better the mechanism of impact of an intervention.

**Ex vivo measures**

Animal studies often investigate the functions of immune cells isolated from the blood, thymus, spleen, lymph nodes, peritoneal cavity and, in some cases, from the bone marrow, lungs, liver and gastrointestinal tract. Human studies are more limited and routinely only the blood pool is sampled. In some cases other sources of human immune cells have been studied: for example, studies of asthma and respiratory illness often use cells collected by bronchoalveolar lavage. Measures of immune function that can be made on cells cultured **ex vivo** include:

1. Phagocytosis of bacteria, sheep red blood cells or yeast particles by neutrophils, monocytes and macrophages; this can be coupled with measures of bacterial killing.
2. Respiratory burst (superoxide generation) by neutrophils, monocytes and macrophages in response to...
bacteria or bacterial peptides; this can be coupled with measures of bacterial killing.

3. Natural killer cell activity, measured as killing of tumour cells known to be specific targets for natural killer cells.

4. Cytotoxic T lymphocyte activity, measured as killing of virally infected cells known to be specific targets for cytotoxic T cells.

5. Lymphocyte proliferation. This is the increase in number of lymphocytes in response to a stimulus. Most often this is measured as the incorporation of radioactively labelled thymidine into the DNA of the dividing lymphocytes, although a number of other measures are available. Agents used to stimulate lymphocyte proliferation include concanavalin A and phytohaemagglutinin, which stimulate T lymphocytes. These agents are all known as mitogens and the process as mitogen-stimulated lymphocyte proliferation. If the individual has been sensitised to an antigen (or allergen), then the antigen can be used to stimulate lymphocyte proliferation.

6. Production of cytokines by lymphocytes, monocytes and macrophages. This usually requires the cells to be stimulated. For lymphocytes, mitogens (or antigens, if the individual has been sensitised) are used, while for monocytes and macrophages bacterial lipopolysaccharide is most often used.

7. Production of total or specific Ig by lymphocytes.

8. Cell surface expression of molecules involved in antigen presentation (e.g. MHC) and in cellular activation (e.g. cytokine receptors).

**In vivo measures**

Measures of immune function that reflect in vivo activity include:

1. Size of lymphoid organs.

2. Cellularity of lymphoid organs.

3. Number and types of immune cells circulating in the bloodstream.

4. Cell surface expression of molecules involved in antigen presentation (e.g. MHC) and in cellular activation (e.g. cytokine receptors).

5. Circulating concentration of thymulin and its activity.

6. Circulating concentrations of total Ig and of the Ig subclasses.

7. Circulating concentrations of Ig specific for antigens after an antigen challenge.

8. Concentration of secretory IgA in saliva, tears and intestinal washings.

9. Circulating concentrations of cytokines.

10. Delayed-type hypersensitivity (DTH) response to intradermal application of an antigen to which the individual has already been exposed; this measures the cell-mediated immune response. The response is measured as the size of the swelling (termed induration) around the area of application at a period (usually forty-eight hours) after the application.

11. Resistance to challenge with live pathogens; the outcome is usually survival, although this can be coupled with some of the above measures and with measures of the numbers of pathogens found in various organs (e.g. spleen, lymph nodes, liver). This has been used in animal experiments.

12. Incidence and severity of infectious diseases. This has been used in some human studies to suggest interactions between nutrient status and immune function.

**In vitro studies**

In addition to the above approaches, which can be used to assess the impact of a nutrient supplied in the diet on immune function, in vitro studies adding the nutrient in pure form directly to immune cells in culture can be used. Each of the cell functions listed above under ‘ex vivo measures’ can be studied in this way. In vitro studies use conditions that are highly controlled, although they are often rather unphysiological in nature. For example, the cells are cultured in isolation from the other types of cell that they would come into contact with in the body (this is also a problem with many ex vivo measures) and the concentrations of the pure nutrient added to the cultures are often greatly in excess of those that can be attained in vivo. Also, the exact form of the nutrient added directly to cell cultures might be different from the form that is available to the cells in vivo. Nevertheless, in vitro studies are useful to identify the potential effects of dietary components and to study their mechanisms of action. However, it is necessary that effects identified in in vitro studies be confirmed in controlled dietary studies.

**There is significant inter-individual variation in immune biomarkers**

Variation in cellular immune responses among individuals is not a great problem in animal studies since these most often use inbred strains. However, it is important to note that some responses do differ among animal species and even among different strains within a species. It is possible that immune cells from different species and strains will exhibit different sensitivities to the amount of a nutrient in the diet. Thus, extrapolations from animal studies to man should be made cautiously. There is wide variation in immune cell responses among healthy human subjects (Table 1; see also Yaqoob et al. 1999), and immune responses can be affected by the presence of disease. Individuals with deficient immune responses are more susceptible to infectious agents and suffer greater morbidity and mortality as a result of infections. Such individuals have one or more key immune responses that fall below the threshold that represents ‘normality’. However, it is not at all clear whether variation in immune responses within the ‘normal’ range results in variable susceptibility to infection. Understanding the relationship between the variations in immune responses and in susceptibility to infection is complicated by a number of other factors. For example, vaccination is widely used to prime the immune system to efficiently eliminate certain pathogens that may or may not exist in the environment at some later stage, antibiotics are used widely to help the host
Table 1. Variation in immune responses among healthy human subjects. Blood was collected from healthy human volunteers (aged 40 to 60 years) in the fasting state. Phagocytosis and oxidative burst in response to Escherichia coli were determined in whole blood by flow cytometry (see Thies et al. 2001a); data are expressed as the % of active cells. Mononuclear cells (a mixture of lymphocytes and monocytes) were prepared using standard procedures and were cultured under standard conditions in the presence of either bacterial lipopolysaccharide (LPS; 15 μg/ml) or concanavalin A (Con A; 25 μg/ml; see Yaqoob et al. 2000; Thies et al. 2001b). After 24 h of culture, the concentrations of cytokines in the cell culture supernatants were measured by specific ELISA (see Yaqoob et al. 2000; Thies et al. 2001b). LPS was used to stimulate production of tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6, while Con A was used to stimulate the production of IL-2, interferon-γ (IFN-γ) and IL-4. Lymphocyte proliferation was determined as the incorporation of [3H]thymidine over the last 18 h of a 66 h culture period (see Yaqoob et al. 2000; Thies et al. 2001b); data are expressed as cpm of thymidine incorporated/2 × 10⁵ cells in the initial culture.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Function</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>Phagocytosis of <em>E. coli</em> (% of cells)</td>
<td>41–93</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Oxidative burst to <em>E. coli</em> (% of cells)</td>
<td>68–98</td>
<td>90</td>
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<tr>
<td>Monocyte</td>
<td>Phagocytosis of <em>E. coli</em> (% of cells)</td>
<td>3–47</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Oxidative burst to <em>E. coli</em> (% of cells)</td>
<td>76–98</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>TNF-α production (ng/ml)</td>
<td>1.2–48.8</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>IL-1β production (ng/ml)</td>
<td>0.5–14.5</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>IL-6 production (ng/ml)</td>
<td>0.9–83.8</td>
<td>36.5</td>
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<tr>
<td>Lymphocyte</td>
<td>Proliferation (cpm/2 × 10⁵ cells)</td>
<td>475.3–70 583</td>
<td>32241</td>
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<tr>
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<td>IL-2 production (IU/ml)</td>
<td>2.2–43.7</td>
<td>8.5</td>
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<tr>
<td></td>
<td>IFN-γ production (IU/ml)</td>
<td>4.2–493.1</td>
<td>127.3</td>
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<tr>
<td></td>
<td>IL-4 production (pg/ml)</td>
<td>3.5–485.7</td>
<td>69.6</td>
</tr>
</tbody>
</table>

SD, standard deviation.
of the nutrient to be studied in a highly controlled setting, and because the immune system of experimental animals is more accessible than that of man. In man, studies of the immunological impact of nutrient deficiencies have been imposed by habitual consumption of diets deficient in one or more nutrients, or, more rarely, through individuals with diseases that result in inability to absorb nutrients. These studies have made it clear that there are a number of nutrients whose availability at an appropriate level is essential if the immune response is to operate efficiently. Nutrients that have been demonstrated (in either animal or human studies) to be required for the immune system to function efficiently include essential amino acids, the essential fatty acid linoleic acid, vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, Zn, Cu, Fe and Se (for reviews see Gross & Newberne, 1980; Chandra, 1991; Kuvibidila et al., 1993; Scrimshaw & SanGiovanni, 1997; Calder & Jackson, 2000). Practically all forms of immunity may be affected by deficiencies in one or more of these nutrients and the deficient animal or individual becomes more susceptible to infections. Animal and human studies have demonstrated that adding the deficient nutrient back to the diet can restore immune function and resistance to infection (for reviews see Chandra, 1991; Scrimshaw & SanGiovanni, 1997; Calder & Jackson, 2000).

Vitamin E and zinc: well researched examples of the impact of the dietary supply of micronutrients on immune function

Vitamin E. Vitamin E is the major lipid-soluble antioxidant in the body and is required for protection of membrane lipids from peroxidation. Since free radicals and lipid peroxidation are immunosuppressive, it is considered that vitamin E should act to optimise and even 'enhance' the immune response (for reviews see Bendich, 1993; Meydani & Beharka, 1998). The effects of vitamin E depletion are more marked if animals are fed a diet containing a high level of polyunsaturated fatty acids (Corwin & Schloss, 1980). Vitamin E deficiency increases susceptibility of animals to infectious pathogens (for references see Meydani & Beharka, 1998). Vitamin E supplementation of the diet of laboratory animals enhances antibody production, lymphocyte proliferation, natural killer cell activity, and macrophage phagocytosis (for references see Meydani & Beharka, 1998). Adding vitamin E to the diet of aged mice increased lymphocyte proliferation, IL-2 production and the DTH response (Meydani et al. 1986). A high level of vitamin E in the diet (500 mg/kg food) also increased natural killer cell activity of spleen cells from old, but not young, mice (Meydani et al., 1988). Dietary vitamin E promotes resistance to pathogens in chickens, turkeys, mice, pigs, sheep and cattle (for references see Meydani & Beharka, 1998; Han & Meydani, 1999); some of these studies report improved immune cell functions in the animals receiving additional vitamin E (Han & Meydani, 1999). Vitamin E prevented the retrovirus-induced decrease in production of IL-2 and interferon-γ (IFN-γ) by spleen lymphocytes and in natural killer cell activity in mice (Wang et al. 1994). In another study, young and old mice were fed diets containing adequate (30 mg/kg diet) or high (500 mg/kg diet) levels of vitamin E for 6 weeks and infected with influenza A virus. Young or old mice fed the high level of vitamin E had lower lung titres of virus than old mice fed the adequate vitamin E diet (Hayek et al. 1997). The high level of vitamin E caused increased production of IL-2 and IFN-γ by spleen lymphocytes from influenza-infected old mice (Han et al. 1998). These observations suggest that increasing vitamin E intake above habitual levels might enhance immune function and improve resistance and that vitamin E supplementation might be particularly beneficial in the elderly.

Studies in man also suggest a relationship between vitamin E supply and immune function. Canadian 3-year-olds with the lowest serum vitamin E levels had the lowest lymphocyte proliferative responses and serum IgM concentrations (Vobecky et al. 1984). Chavance et al. (1989)
found a positive association between plasma vitamin E levels and DTH responses, and a negative association between plasma vitamin E levels and incidence of infections, in healthy adults aged over sixty. Administration of vitamin E to premature infants enhanced neutrophil phagocytosis (Baehner et al. 1977; Chirico et al. 1983) but decreased the ability of neutrophils to kill bacteria (Baehner et al. 1977); this latter effect is most likely due to a vitamin E-induced decrease in the production of free radicals and related reactive species. Supplementation of the diet of elderly subjects with 800 mg vitamin E/d for 4 weeks increased lymphocyte proliferation, IL-2 production and the DTH response, but did not affect IL-1 production, the number of CD4\(^+\) cells or circulating Ig concentrations (Meydani et al. 1990). In a more recent study, 60, 200 and 800 mg vitamin E/d increased the DTH response in elderly subjects, with 200 mg/d having the maximal effect (Fig. 3; Meydani et al. 1997). The 200 mg/d dose increased the antibody responses to hepatitis B, tetanus toxoid and pneumococci vaccinations (Fig. 3; Meydani et al. 1997). In some cases the 800 mg vitamin E/d supplement decreased the antibody response to below that of the placebo group (Fig. 3). The authors concluded that 200 mg of vitamin E daily represents the optimal level for the immune response. Some studies report that high levels of vitamin E in the human diet (>300 mg/d) decrease the ability of neutrophils to undergo phagocytosis (Boxer, 1986) and to kill bacteria (Baehner et al. 1977; Prasad, 1980) and decrease monocyte respiratory burst and IL-1\(\beta\) production (Devaraj et al. 1996).

**Zinc.** Zn deficiency in animals is associated with a wide range of immune impairments (for reviews see Fraker et al. 1993; Wellingshausen et al. 1997; Shankar & Prasad, 1998). Zn deficiency has a marked impact on bone marrow, decreasing the number of nucleated cells and the number and proportion of cells which are lymphoid precursors (for reviews see Fraker et al. 1993; Fraker & King, 1998). In patients with Zn deficiency related to sickle cell disease, natural killer cell activity is decreased, but can be returned to normal by Zn supplementation (Tapazoglou et al. 1985). In acrodermatitis enteropathica, which is characterised by reduced intestinal Zn absorption, thymic atrophy, impaired lymphocyte development, decreased numbers of CD4\(^+\) cells and reduced lymphocyte responsiveness and DTH are observed (Chandra & Dayton, 1982; Fraker et al. 1986). Moderate or mild Zn deficiency or experimental Zn deficiency (induced by Zn consumption of <3.5 mg/d; habitual intakes among adults in the UK are 9 to 12 mg/d) in man results in decreased thymulin activity, decreased natural killer cell activity, a lowered CD4\(^+\):CD8\(^+\), and decreased lymphocyte proliferation, IL-2 production and DTH response; all can be corrected by Zn repletion (Shankar & Prasad, 1998). Experimental Zn deficiency in man decreased IL-2, IFN-\(\gamma\) and TNF-\(\alpha\) production by mitogen-stimulated lymphocytes but did not affect IL-4, IL-6 or IL-10 production by these cells or IL-1\(\beta\) production by lipopolysaccharide-stimulated cells (Beck et al. 1997).

Low plasma Zn levels predicted the subsequent development of lower respiratory tract infections and diarrhoea among Indian infants (Bahl et al. 1988). Indeed, diarrhoea is considered a symptom of Zn deficiency. Malnourished, Zn-deficient children given Zn (2 mg/kg body weight daily for 10 days) had increased thymus size as judged by radiography (Golden et al. 1977). Topical application of Zn to malnourished children improved the DTH response in the area of skin on which the application was made (Golden et al. 1978). Zn administration (2 mg/kg body weight daily) to malnourished children decreased the incidence of diarrhoea by more than 50\%, decreased the incidence of respiratory and skin infections, and resulted in threefold increased growth compared with children given low-dose Zn (3.5 mg daily; Castillo-Duran et al. 1987). There are now a number of studies showing that Zn supplementation decreases the incidence of childhood diarrhoea and respiratory illness (for references see Scrimshaw & SanGiovanni, 1997; Shankar & Prasad, 1998; Calder &
Jackson, 2000), although some studies fail to show benefit of Zn supplementation in respiratory disease (for references see Calder & Jackson, 2000). As well as decreasing the risk of young infants developing diarrhoea (Rosado et al. 1997), Zn supplementation (20 mg/d) to malnourished children reduced diarrhoea-induced growth faltering (Roy et al. 1999). Zn administration to preterm low-birth-weight infants (1 mg/kg daily for 30 days) increased the number of circulating T lymphocytes and lymphocyte proliferation (Chandra, 1991). Providing 5 mg Zn/d to low-birth-weight, small-for-gestational-age infants for 6 months increased measures of cell-mediated immune function and decreased the incidence of gastrointestinal and upper respiratory tract infections (Lira et al. 1999); a Zn dose of 1 mg/d was without effect.

As observed for vitamin E, excessive Zn intakes impair immune responses. For example, giving 300 mg Zn/d for 6 weeks to young adult human subjects decreased lymphocyte and phagocyte function (Chandra, 1984). High Zn intakes can result in Cu depletion, and Cu deficiency impairs immune function (for reviews see Prohaska & Failla, 1993; Failla & Hopkins, 1998).

**Probiotics: functional food components that impact on immune function**

Indigenous bacteria are believed to contribute to the immunological protection of the host by creating a barrier against colonisation by pathogenic bacteria. This barrier can be disrupted by disease and by use of antibiotics, so allowing easier access of the host gut by pathogens. It is now believed that this barrier can be maintained by providing supplements containing live ‘desirable’ bacteria: such supplements are called probiotics (for extensive reviews see Goldin, 1998; Naidu et al. 1999). Probiotic organisms are found in fermented foods, including traditionally cultured dairy products and newer kinds of fermented milks. The organisms included in commercial probiotics include lactic acid bacteria (Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium) and Bifidobacterium spp. These organisms colonise the gut only temporarily and so their regular consumption is necessary. In addition to creating a barrier effect, some of the metabolic products of probiotic bacteria (e.g. lactic acid and a class of antibiotic proteins termed bacteriocins, produced by some bacteria) may inhibit growth of pathogenic organisms (Fig. 4). Also, the desirable bacteria may compete for nutrients with the pathogens (Fig. 4). Finally, there is some evidence that probiotic bacteria may enhance the gut immune response against pathogenic bacteria (Fig. 4).

Studies in rats and mice reveal that lactic acid bacteria administered orally increase the numbers of T lymphocytes, CD4⁺ cells and antibody-secreting cells, including those in the intestinal mucosa, and enhance lymphocyte proliferation, natural killer cell activity, IL-1, TNF and IFN-γ production, antibody production (including secretory IgA), phagocytic activity and the respiratory burst of macrophages and the DTH response (for a review see Naidu et al. 1999). However, not all strains of lactic acid bacteria are equally effective (Naidu et al. 1999). Animal studies also show that orally administered lactic acid bacteria protect against challenges with pathogenic bacteria such as Salmonella typhimurium, reverse some of the immunosuppressive effects of malnutrition and cause the symptoms of enterocolitis to be less severe (Naidu et al. 1999). Co-colonisation of rats with

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**Fig. 4.** Potential roles of probiotic bacteria in the human intestinal tract. Probiotic bacteria may act in a variety of ways to prevent the growth and colonisation of pathogenic bacteria.
Lactobacillus plantarum and Escherichia coli resulted in higher circulating concentrations of total IgA and of E. coli-specific IgA and IgM than if the rats were colonised with E. coli alone; there was also increased expression of the IL-2 receptor in the lamina propria (Herias et al. 1999). Adults fed fermented milk containing lactobacilli and bifidobacteria and exposed to S. typhimurium showed increased total and S. typhimurium-specific IgA concentrations in their serum (Link-Amster et al. 1994). Administration of probiotic bacteria reduced the incidence and severity of diarrhoea in children attending daycare centres and the duration of diarrhoea in infants hospitalised with gastroenteritis; in some studies this was associated with an increase in levels of IgG, IgA and IgM and in anti-rotavirus IgA secreting cells in the bloodstream (for references see Naidu et al. 1999). Probiotic bacteria have also been shown to reduce the incidence of antibiotic-induced diarrhoea in children (Arvola et al. 1999; Vanderhoof et al. 1999). Although some studies have shown that consumption of probiotics can protect against traveller’s diarrhoea, other studies do not demonstrate such protection (for references see Naidu et al. 1999).

Healthy Japanese children consuming a probiotic formula containing bifidobacteria had increased faecal levels of total and anti-poliovirus IgA than prior to taking the formula (Fukushima et al. 1998). Healthy adults consuming probiotic bacteria showed enhanced phagocytosis by neutrophils and monocytes (Schiffrin et al. 1997; Yoon et al. 1999). Adults fed fermented milk containing lactobacilli and bifidobacteria and exposed to S. typhimurium showed increased total and S. typhimurium-specific IgA concentrations in their serum (Link-Amster et al. 1994). Administration of probiotic bacteria reduced the incidence and severity of diarrhoea in children attending daycare centres and the duration of diarrhoea in infants hospitalised with gastroenteritis; in some studies this was associated with an increase in levels of IgG, IgA and IgM and in anti-rotavirus IgA secreting cells in the bloodstream (for references see Naidu et al. 1999). Probiotic bacteria have also been shown to reduce the incidence of antibiotic-induced diarrhoea in children (Arvola et al. 1999; Vanderhoof et al. 1999). Although some studies have shown that consumption of probiotics can protect against traveller’s diarrhoea, other studies do not demonstrate such protection (for references see Naidu et al. 1999).

The immune system as a target for functional foods?

As described earlier, deficiency of total energy or of one or more essential nutrients impairs immune function and increases susceptibility to infectious pathogens. This is most likely because these nutrients are involved in the molecular and cellular responses to challenge of the immune system. Providing these nutrients to deficient individuals restores immune function and improves resistance to infection. For some nutrients (e.g. vitamin E) the dietary intakes that result in greatest enhancement of immune function are greater than recommended intakes. However, excess intake of some nutrients (e.g. vitamin E, Zn) also impairs immune responses. Thus, four potential general relationships appear to exist between the intake of a nutrient and immune function (Fig. 5). These different types of relationship might in part reflect interactions between nutrients such that an excess of one nutrient negatively affects the status of a second nutrient (e.g.

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**Fig. 5.** Potential patterns of relationship between nutrient supply and immune function. All patterns assume that a deficiency of the nutrient impairs the immune response. In (a) the recommended intake of the nutrient is an intake at which the maximal immune response occurs and intakes somewhat above the recommended intake do not impair immune function. In (b) the recommended intake is below the intake at which the maximal immune response occurs and intakes somewhat above the recommended intake do not impair immune function. In (c) the recommended intake of the nutrient is an intake at which the maximal immune response occurs and intakes somewhat above the recommended intake impair immune function. In (d) the recommended intake is below the intake at which the maximal immune response occurs and intakes somewhat above the recommended intake impair immune function.

**Fig. 6.** Different components of the immune system may respond to intakes of a particular nutrient in different ways.
There are likely to be interactions between similar classes of nutrients (e.g. \( n \rightarrow 6 \) and \( n \rightarrow 3 \) polyunsaturated fatty acids) that have yet to be unravelled fully, and there are most likely interactions between nutrients which contribute to oxidative stress (e.g. polyunsaturated fatty acids) and those which protect against it (e.g. vitamin E).

It is often assumed when defining the relationship between nutrient intake and immune function that all components of the immune system will respond in the same dose-dependent fashion to a given nutrient. This is not correct, at least as far as some nutrients are concerned, and it appears likely that different components of the immune system show an individual dose–response relationship to the availability of a given nutrient (Fig. 6). Indeed, some immune functions might be relatively insensitive to nutrient supply.

Some consider that the immune system does not respond optimally to challenge in apparently healthy, free-living human individuals even if they are not deficient in any single nutrient. Individuals may be marginally deficient in one or more nutrients or they may be consuming some nutrients (e.g. fat) in excess. There is now much interest in optimising the immune response in such individuals not simply by correcting marginal deficiencies but by increasing the intake of certain nutrients and probiotics. At this stage the immuno-enhancing effect of such supplementation is generally unproven. Indeed, as far as micronutrients are concerned, such supplementation might even be dangerous. Before the immune system can be considered a genuine target for disease prevention, much more needs to be known about the role of variation in the immune response among apparently healthy individuals in determining their susceptibility to infection; about the influence of altered supply of specific nutrients and of nutrient combinations on aspects of immune function in different populations; about the impact of genotype, gender, age and early life experiences on immune function and on determining the sensitivity of the immune system to nutrients; and, most importantly, about whether enhanced immune responses really translate into increased resistance to infection.

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The sensory, macronutrient composition and physical properties of the diet modulate the response to a meal during the cephalic, gastric and intestinal phases of digestion. In the cephalic phase the sight, smell and taste of foods stimulate the secretion of digestive juices into the mouth, stomach and intestine, essentially preparing these organs to digest the foods to be consumed. Experiments, in which animals are sham-fed so that food consumed does not actually enter the stomach or intestine, demonstrate that the cephalic phase accounts for about 20% or more of the secretion into the gut. The gastric and intestinal phases occur when food and its components are in direct contact with the stomach or intestine, respectively. During these phases the distension of the organs with food, as well as the specific composition of the food, can stimulate a GIT response. The GIT is the richest endocrine organ in the body, containing a large array of peptides; however, the exact physiological function of each of these compounds has not yet been established. Five peptides, gastrin, cholecystokinin (CCK), secretin, gastric inhibitory peptide and motilin, are established as regulatory hormones in the GIT and several factors affect their release and subsequent actions. Among the established gastrointestinal peptides, secretin stimulates secretion of fluid and bicarbonate from the pancreas, gastrin stimulates secretion in the stomach, gastric inhibitory peptide inhibits gastric acid secretion and stimulates insulin release, and motilin stimulates the motility of the upper GIT (Bloom, 1983). In addition to the various factors causing release of these hormones and the response to them, physiologists are also interested in the interactions among hormones, as well as with the nervous system, since the response to a meal involves release of many factors. Of these established hormones, CCK provides a useful example to examine some implications of meal-induced responses.

CCK is released from cells of the upper small intestine into the blood. Dietary protein and fat stimulate its release from the intestine, while digestible carbohydrates have no effect on release of the hormone. Gastric acid inhibits CCK secretion. Once released CCK can inhibit gastric emptying, stimulate secretion of acid in the stomach and pancreatic juice into the small intestine over several hours. Several factors can delay the release of CCK, the presence of non-digestible polysaccharides appears to modulate the pattern of CCK release. This meal-induced response of CCK has implications for understanding the potential effects of fibre on insulin release and/or satiety (Holt et al. 1992; Rushakoff et al. 1993; Burton-Freeman, 2000). The subjective measures of satiety recorded by subjects after consuming test meals were correlated with the CCK response. Thus meals that elicit the highest CCK response are perceived by human subjects as the most filling. This example illustrates that the meal-induced response causes secretion of digestive juices essential for the digestion of food components; however, the response also influences other metabolic responses to the meal. The GIT is one of the richest endocrine organs in the body, and CCK is just one example of a variety of hormones released during the response to a meal (Bloom, 1983; Johnson, 1997). These hormones have metabolic effects beyond the GIT. The system is simultaneously preparing the GIT to digest and absorb the meal that has been consumed in an efficient manner and also is signalling short-term satiety so that feeding is terminated at an appropriate point.

**Digestive and absorptive functions of the gastrointestinal tract**

The GIT breaks down food into absorbable compounds through mechanical and biochemical processes. Chewing breaks food into smaller particles that can mix more readily with the GIT secretions. In the mouth saliva lubricates the food bolus so that it passes readily through the oesophagus to the stomach. The sensory aspects of food stimulate the flow of saliva, which not only lubricates the bolus of food but also is protective and contains digestive enzymes. Swallowing is regulated by sphincter actions to move the bolus of food into the stomach. The motility of the stomach continues the process of mixing food with the digestive secretions, now including gastric juice, which contains acid and some digestive enzymes. The action of the stomach continues to break down food into smaller particles prior to passage to the intestine. The stomach, which after a meal may contain over a litre of material, regulates the rate of digestion by metering chyme into the small intestine over several hours. Several factors can slow the rate of gastric emptying; for example, solids take longer to empty than liquids, mixtures relatively
high in lipid take longer to empty, and viscous or thick mixtures take longer to empty than watery, liquid contents.

Once digesta is in the small intestine, peristaltic motor activity propels it along the length of the intestine and segmentation allows mixing with digestive juices in the intestine, which include pancreatic enzymes, bile acids and sloughed intestinal cells. Digestion of macronutrients, which began in the mouth, continues in the small intestine primarily through the action of enzymes. Each of the macronutrients has a unique set of enzymes that break the macromolecules into sub-units that can be taken up by the absorptive systems in the intestinal cells. The phytochemicals that have been investigated for lowering risk of chronic disease are part of the cell matrix in plant foods and depend on the mechanical and biochemical disruption of the food matrix to become available for absorption from the GIT. Thus factors that alter the rate and site of digestion and absorption will alter their bioavailability as well. To illustrate how the GIT functions of digestion and absorption are important for bioavailability and subsequent metabolism, a useful example is to consider some of the factors that affect carbohydrate digestion and utilization.

Carbohydrates are categorized as digestible or nondigestible. Digestible carbohydrates are the various sugar-containing molecules that can be digested by amylase or the saccharidases of the small intestine to sugars that can be absorbed from the intestine. The predominant digestible carbohydrates in foods are starch, sucrose, lactose (milk sugar) and maltose. Foods may also contain simple sugars such as glucose or fructose that do not need to be digested before absorption from the gut. α-Amylase, which hydrolyses the (1→4) linkages in starch, is secreted in the mouth from salivary glands and from the pancreas into the small intestine. The action of amylase produces smaller carbohydrate segments that can be hydrolysed further to sugars by enzymes at the brush border of the intestinal cells. This hydrolysis step is closely linked with absorption of sugars into the intestinal cells.

The glucose absorbed from digestible carbohydrate stimulates the release of insulin. In healthy, normal-weight individuals, the postprandial increase in blood insulin concentration is correlated with the intake of carbohydrate (Lee & Wolever, 1998; Lu et al. 2000). Insulin is important for regulation of energy metabolism in the body and its release after a meal promotes energy storage. Thus, in healthy individuals, the postprandial release of insulin results in glucose and amino acid uptake by cells and stimulates glycolysis and synthesis of glycogen, protein and fatty acids. The rate and pattern of carbohydrate digestion and absorption will influence the appearance of glucose in blood and stimulation of insulin secretion. Consequently there is considerable interest in how factors that influence carbohydrate digestion and absorption might affect energy metabolism during the postprandial period. Slowing gastric emptying, which slows entry of digestible carbohydrate into the small intestine, blunts the glycaemic and insulinaemic response to a meal. The hormone CCK, discussed above, also modulates the release of insulin. In healthy individuals dietary factors that enhance CCK reduce insulin and glucose response (Liddle et al. 1988; Rushakoff et al. 1993; Liddle, 2000). CCK may function by stimulating an early release of insulin before plasma glucose begins to increase. This priming of the system results in an overall lower glycaemic and insulinaemic response to a meal (Bloom, 1983). In certain types of obesity, cells become insulin-resistant, which results in elevated plasma levels of insulin, leading to non-insulin dependent diabetes mellitus. To help manage this condition, dietary factors such as viscous polysaccharides or α-amylase inhibitors that can blunt the postprandial rise in glucose have been investigated.

Non-digestible carbohydrates cannot be digested by the enzymes in the small intestine and are the primary component of dietary fibre. Most of these non-starch polysaccharides are part of the plant cell wall. The most abundant polysaccharide in plant tissue is cellulose, which is a glucose polymer with β(1→4) links between the sugars. Amylase, the starch-digesting enzyme of the small intestine, can hydrolyse only α linkages. The non-digestible carbohydrates also include hemicelluloses, pectins, gums, oligofructose and inulin. While nondigestible, they do affect the digestive process because they provide bulk in the intestinal contents, hold water, can become viscous or thick in the intestinal contents, and delay gastric emptying. Viscosity is correlated with the ability of polysaccharides to lower plasma cholesterol levels (Gallacher & Hassel, 1995; Carr et al. 1996; Gallacher et al. 2000). Slowing gastric emptying is associated with blunting of the glucose and insulin response to a meal as well as enhancing feelings of satiety. In addition, non-starch polysaccharides are the primary substrate for growth of the micro-organisms in the large intestine and contribute to stool formation and laxation. Products of microbial action include ammonia, gas and short-chain fatty acids (SCFA). SCFA are used by cells in the large intestine for energy and some appear in the circulation and can be used by other cells in the body for energy as well. Thus, while dietary fibre is classified as non-digestible carbohydrate, the eventual digestion of these polysaccharides by microbes does provide energy to the body. Current research is focused on the potential effect of SCFA on the health of the intestine and their possible role in the prevention of gastrointestinal diseases.

In summary, the rate at which macromolecules in food are digested and absorbed influences metabolism in the body. Additionally, physical properties of foods such as viscosity will influence the bioavailability and utilization of nutrients and other compounds in foods by the body.

The potential impact of the gastrointestinal tract on health

The examples above illustrate that the GIT modulates metabolic responses through meal-induced responses to diet properties and composition, and through the rate of digestion and absorption. These examples indicate that the physiology and function of the GIT can influence risk factors for non-communicable diseases and thus the GIT has an important role in maintaining health. Research on dietary fibre has illustrated that actions within the GIT modify risk factors for non-communicable diseases since
fibre is non-digestible and exerts its effects on metabolism during its transit through the GIT. For example, the ability of certain fibres to reduce plasma cholesterol is associated with an increase in viscosity of digesta (Carr et al. 1996) and with increased excretion of bile acids due to binding or adsorption by fibre fractions (Buhman et al. 2000). Non-starch polysaccharides are the primary substrates for microflora in the large intestine.

The fact that fibre is utilized by the GIT microflora has stimulated research on the potential impact of the metabolism of this microflora on reducing the risk of disease and promoting health. Fermentable carbohydrates increase the microbial mass in the large intestine, which contributes to stool bulk and aids laxation (Cummings et al. 1992; Chen et al. 1998). Fermentation also produces SCFA, which provide energy from highly fermented polysaccharides. SCFA are utilized by the intestinal epithelial cells, liver and muscle and have a trophic effect on the metabolic enzymes, including cell signal transduction and the enzymes associated with microbial metabolism of this microflora on reducing the risk of disease. SCFA markedly the molar ratio of SCFA produced. The fermentation of carbohydrate also be associated with metabolic enzymes, including cell signal transduction pathways in colonic mucosal cells (Pajari et al. 2000) and the enzymes associated with microbial metabolism of mutagenic compounds (Cummings et al. 2001).

Approximately 10^{12} micro-organisms reside in the GIT, most of which are in the large intestine. Some of these microbes are considered harmful and have been associated with intestinal diseases, while others are considered beneficial and have been associated with decreasing risk for chronic disease as well as the synthesis of vitamins, facilitating mineral absorption and immune stimulation. The microflora of the intestine is complex and diverse and attempts to modify the composition by dietary means have not been very effective. However, feeding cultured products that contain certain strains of Bifidobacterium or Lactobacillus have been reported to have both beneficial and impact health. Typically, the response is seen only while the product containing a live culture is fed, suggesting that the bacteria do not colonize the GIT permanently but remain metabolically active during their transit thorough the GIT. At the simplest level these cultures are reported to improve lactose digestion in lactose-intolerant individuals and, at a more complex level, stimulate several facets of immune response (Salminen et al. 1998; Gill et al. 2000). The functional significance and sustainability of the immuno-stimulation have not been reported. However, certain live cultures have been reported to help ameliorate symptoms of diarrhoea. The emerging research in this area suggests that we still have a poor understanding of the significance of the microbial population in the GIT and its role in promoting health and reducing risk for disease.

Conclusions and need for future research

While the health benefit of a functional food may be a metabolic response that lowers risk for disease, the actual target for the food or food component may be on the functioning of the GIT. This situation raises interesting challenges for assessing the response to functional foods and the validation of health claims. For example, there may be a health benefit from slowing absorption from the intestine; slower absorption might be measured by examining the time course for appearance of the nutrient or food component in the blood, measuring the hormone response associated with absorption of the compound, or measuring parameters related to excretion of the compound. However, the food component may slow absorption by delaying gastric emptying, altering the mixing within the intestinal contents or decreasing the availability of digestive enzymes in the intestine. These measures of GIT function provide validation of the mechanisms by which the functional food or food component affects metabolism. Such validation is essential to demonstrate that the food or food component has a specific effect on health or risk for disease. For example, health claims for sources of dietary fibre and reduced risk of cardiovascular disease are strengthened by demonstrating the mechanisms by which fibre lowers plasma cholesterol levels. Research is needed to validate the links between various aspects of gastrointestinal function and subsequent health effects of functional foods.

Understanding the GIT response to foods and meals is critical to understanding the metabolic effects of functional foods. Three important research needs are evident from a review of the function and physiology of the GIT in relation to foods. A primary need is to determine the bioavailability of physiologically active compounds from foods. Bioavailability will be determined by the digestibility of foods that contain these compounds, their subsequent absorption and their utilization by tissues. For certain compounds their mode of action will be in the GIT and the focus will be on interaction with organs of the GIT; however, in other cases, absorptive mechanisms will be important in determining the metabolic effects of functional foods. The physical structure of foods has often been overlooked in understanding the metabolic response to diet. However, this structure contributes to the functional effects of foods as well as to the availability of compounds from foods. For example, recent studies have demonstrated that changing the viscosity of the gut contents alters absorption and GIT response. The implications of these studies need to be investigated further. Additionally, food structures such as the plant cell wall change the availability of absorbable compounds along the gastrointestinal contents and raise interesting research questions about the importance of plant cell walls beyond their content of dietary fibre. A third area of research with emerging importance relative to the GIT concerns the function of microflora along the intestines. Studies on probiotics and prebiotics suggest that this microflora is responsive to dietary influences. Understanding the influence of the GIT microflora on health promotion and
disease prevention is a complex but important research agenda.

References


composition of the food supply (Leclercq et al. 2001). Likewise, increasing evidence points to genetic variability as a significant determinant of the response to foods and food components. While delineating the most effective diet for an individual is a daunting task, recent scientific discoveries as reviewed briefly below reinforce the belief that a personalized dietary intervention approach to health promotion and disease prevention is feasible.

Functional foods: a health perspective

The intriguing term ‘functional foods’ has arisen from a general belief in the health benefits of foods. While this term has no legal meaning, it nevertheless signifies a proactive appreciation that some foods may confer health benefits (Milner, 2000). While functional foods have been defined in various ways, the International Life Sciences Institute (ILSI) North America defines them as: ‘Foods, that by virtue of physiologically active food components, provide health benefits beyond basic nutrition’. Classifying which foods fall within the health benefits category and which do not has been an interesting challenge for some, especially since many factors can influence the overall response to a food including the quantity consumed, accompanying foods ingested, duration of exposure, physiological state, etc. At this point, it is best to indicate that all foods are probably functional in ‘some’ capacity and that, under specific circumstances, some may provide immediate and/or long-term benefits.

Widespread interest in functional foods continues to abound in North America among scientists, legislators and consumers. About 60 % of adults residing in the United States are believed to select foods for health purposes, regardless of their age or gender (http://www.IFIC.org). While younger individuals may be selecting foods for mental and physical performance, older individuals appear to be selecting foods for their potential merits in reducing disease risk or improving the quality of life. Regardless of why foods are chosen, it is refreshing to have a positive proactive belief, since during the past few decades campaigns to alert people about diet and health issues have largely been negative.

Market value of functional foods in the United States

Increasing consumer demand for healthful foods and beverages, coupled with scientific discoveries about the physiological consequences of selected foods and ingredients, has fostered the development of an active functional foods and beverage market. Some of these products certainly extend beyond conventional fortified items because they contain specific ingredients designed to have health or structure–function benefits for the consumer.

Statistics about the size of the US natural foods market vary considerably depending on the source of information and which products are included or excluded, as evident by a recent report entitled ‘The Natural/Organic Food Market in the United States’ (http://atnriae.agr.ca/info/us/e3164.htm#MARKET OVERVIEW). Many estimate that natural and organic foods account for annual sales of between $16·3 billion and $29·7 billion, which undeniably represents a large variance. The market value of functional foods is also largely ill defined. Frost & Sullivan (http://www.food.frost.com) suggest the total market for functional ingredients, functional foods, functional beverages, dietary supplements and foods for special dietary use is approximately $50 billion. Trade publications for the food industry in 2000 indicated that sales of natural products including foods, beverages and supplements grew by about 7 % over the previous year.

Health and structure–function claims in North America

The United States’ Federal Food, Drug and Cosmetic Act does not provide a statutory definition of functional foods. Thus, the Food and Drug Administration (FDA) has no authority to establish a formal regulatory category for such foods. The primary determinant of the regulatory status of these foods is thus their intended use. The distinction between a food and a drug has been blurred because of recent scientific findings suggesting that the former may have some medicinal properties. Nevertheless, according to the FDA, a drug is a substance that is used to prevent, treat, cure or mitigate disease. Foods, on the other hand, and according to Webster’s Dictionary, are defined as protein, carbohydrates, fats, minerals, vitamins and other supplementary substances that nourish and sustain life. According to the FDA, a food is defined as: ‘Articles used for food or drink for man or other animals, chewing gum, and articles used for components of any such article’.

In the United States, food claims can take a number of approaches, i.e. those related to content, health or to structure–function. In marketing these foods, manufacturers may come under one of several existing regulatory options. The first decision manufacturers will make that will help determine their product’s regulatory status is whether the product is a food or a drug. Thus, manufacturers and retailers have a range of legal and regulatory categories into which their products may be classified. Fifteen health claims have been approved for manufacturers to describe the relationship between a food substance and a disease or health-related condition. Some of the broad areas

Table 1. Areas associated with health claims in the United States

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<th>Area</th>
<th>Description</th>
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<tr>
<td>Calcium and osteoporosis</td>
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<td>Dietary lipids and cancer</td>
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<td>Sodium and hypertension</td>
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<td>Dietary saturated fat and cholesterol and the risk of CHD</td>
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<tr>
<td>Fibre-containing grain products, fruits and vegetables and cancer</td>
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<tr>
<td>Fruit, vegetables and grain products that contain fibre, particularly soluble fibre, and the risk of CHD</td>
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<tr>
<td>Fruits and vegetables and cancer</td>
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<tr>
<td>Folate and neural tube defects (1996)</td>
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<tr>
<td>Dietary sugar alcohols and dental caries (1996)</td>
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<tr>
<td>Soluble fibre from certain foods and the risk of CHD (1997)</td>
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<td>Soya protein and the risk of CHD (1999)</td>
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<td>Stanols and heart disease</td>
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CHD, coronary heart disease.
addressed by these health claims are presented in Table 1. Health claims may arise in three ways. First, the 1990 Nutrition Labeling and Education Act allows the FDA to authorize a health claim for a food or dietary supplement based on the FDA’s careful review of the scientific literature. Second, the 1997 Food and Drug Administration Modernization Act allows a health claim to be made for a food based on an authoritative statement of a scientific body of the US government or the National Academy of Sciences. And third, the 1999 court decision in the case of Pearson vs. Shalala allows certain qualified health claims to be used. The relatively small number of health claims for foods that have been approved serves to emphasize the incomplete or inconsistent information that precludes firm conclusions.

Structure–function claims have been allowed for foods under the US Federal Food, Drug and Cosmetic Act, but because they are allowed differently for dietary supplements and conventional foods their use is stirring considerable controversy in the USA. Some current uses and controversies about structure–function claims focus on how best to identify an action of the food or bioactive food component without simultaneously imply a health or drug outcome. Thus, structure–function statements are often vague and imply the food or components helps maintain normalcy, whatever that might signify.

Historically, the Food Regulations under the Canadian Food and Drugs Act were developed with the objectives to ensure that food that was sold was safe and nutritious and for the prevention of fraud. The recognition of the health effects of various food constituents has sparked legislative interest in functional foods. In Canada foods containing the beneficial ingredients, whether naturally occurring or as a result of addition of an isolated component, are termed ‘functional foods’. The proposed Health Canada definition of a functional food (http://www.hc-sc.gc.ca/main/drugs/zfiles/english/ffn/fds/cdc_e.html) is one that is: ‘similar in appearance to, or may be, a conventional food, is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions’. Currently a proposal is pending that would allow health claims in Canada.

Regardless of the claims that are allowed, consumers are eager to learn more truthful information about food, food components and health. To assist in clarifying the issue, ILSI North America’s Technical Committee on Food Components for Health Promotion developed in 1997 a Road Map as a strategy to ‘improve the health of the public through consumer acceptance of safe food products that provide significant health benefits’. A number of approaches were identified to accomplish this goal including: the creation of a comprehensive science base, the promotion of public trust, the development of consumer preferred functional foods, the optimization of a regulatory framework and the creation of marketplace incentives to develop functional foods. Because of the scientific and legal mandates involving organizations such as ILSI, it was apparent that other groups were needed if progress was truly going to be made. Delightfully, progress has occurred during the past four years in not only showcasing this area but also with building a number of important and effective linkages. Undeniably the database regarding foods and their components as factors in health has continued to blossom during this period. Likewise, new and exciting linkages have surfaced with various groups with a commitment to research and outreach about foods and health. The turn of the twenty-first century was a point to not only reflect on what had been accomplished by the Road Map, but to rekindle interest in foods and bioactive components as a continuing focus. While the exact plan remains to be resolved, key components will probably be to stimulate fundamental science to evaluate the health effects of functional foods, develop a framework to utilize science for decision making and optimize the scientific impact of these undertakings through key collaborations.

Numerous bioactive food components may be involved

Numerous foods are already associated with health promotion and disease prevention. The diversity of these foods suggests a variety of components may be involved. It has been estimated that about 25 000 different chemical compounds occur in fruits, vegetables and other plants eaten by man. To date, more than 500 compounds have been identified as potential modifiers of the cancer process. Both essential and non-essential allochemicals occurring in plants, as well as zoochemicals found in animal products, may be physiologically important modifiers of a host of biological processes. Compounds encompassing such diverse categories as carotenoids, dithiolthiones, flavonoids, glucosinolates, isothiocyanates, allyl sulphydryls and fermentable fibres have been found to influence a variety of cellular processes that would be expected to influence health (Hasler et al. 2000; Milner, 2000; Prosky, 2000; Go et al. 2001). Many have been reported to modify the redox status of cells and therefore may have far-reaching implications as determinants of health and well-being (Diplock et al. 1998; Clarkson & Thompson, 2000; Roberfroid, 2000). Numerous reviews extolling the merits and/or possible risks of numerous bioactive food components have been presented in recent years (Potter, 1997; Abdulla & Gruber, 2000; Gill & Cross, 2000; Milner, 2000).

Moving beyond observational studies

While observations about dietary bioactive food components and health are exceedingly tantalizing, the future of nutrition research probably resides in the ability to move beyond these studies to a more probing and molecular approach that will allow for tailored recommendations to individuals (Greenwald & Milner, 2001). DellaPenna (1999) coined the term ‘nutritional genomics’ to describe work at the interface of plant biochemistry, genomics and human nutrition aimed at understanding and manipulating nutrient reactions and interactions at the molecular or genomic level. For the purposes of this review, nutritional genomics refers to the study of any genetic or epigenetic interaction with a nutrient that leads to phenotypic changes. Unquestionably, the study of nutritional genomics offers
the potential to identify definitively which components in foods influence growth, development and health, and to clarify their specific mechanisms of action. About 26 000 to 38 000 genes were proposed in the first draft of the human genome. While this is only about double the number found in the fruit fly and worm, it points to the importance of the expression of these genes and to their regulation (Paabo, 2001). A web site (http://www.cgap.nci.nih.gov/), developed jointly by the National Cancer Institute and the National Library of Medicine, was part of the recently launched Cancer Genome Anatomy Project (CGAP). The database offers scientists a powerful new tool to study how various factors including dietary components might alter a host of cellular events. Queries to the CGAP database are examined quickly through a gene index system that a few years ago might have taken years or even lifetimes to compile. Genomic data for man and mouse, including expressed sequence tags, gene expression patterns, single nuclear polymorphisms, cluster assemblies and cytogenetic information, are included. In addition to genetic information, this web site contains informatics tools to query and analyse the data and information on methods and resources for reagents developed by the project. All CGAP resources — including cDNA libraries, clones and sequence data — are publicly accessible to scientists.

Rather compelling evidence already exists revealing that a variety of nutrients can influence genetic and epigenetic processes that determine cellular metabolism, differentiation and apoptosis (Bradlow et al. 1999; Knowles & Milner, 2000; Pan et al. 2000; Blanchard et al. 2001; Merrill et al. 2001). It is known that cell homeostasis is regulated by a finely controlled balance among proliferation, growth arrest, differentiation and apoptosis (programmed cell death). Disruption in this balance can lead to profound phenotypic changes ranging from growth suppression to the transformation of normal into neoplastic cells. Dysregulation of apoptosis is frequently accompanied with the pathogenesis arising from a wide array of conditions including neurodegeneration, autoimmunity, heart disease, cancer and others. Several nutrients are already identified as factors influencing cellular homeostasis. For example, while vitamin A has repeatedly been shown to be involved with differentiation, other nutrients such as vitamin D and lignan may also play a role (Ward et al. 2000; Gray et al. 2001; Lee & Pelletier, 2001). Since various nutrients can influence the same process, it becomes of increasing importance to understand the ideal balance of these nutrients that brings about a desired effect and whether a shift in this balance leads to changes in physiological processes and/or phenotypic characteristics. These dynamic interactions are not limited to differentiation but are also evidenced by the ability of diverse nutrients such as plant sterols, Se and butyrate arising from fermentable fibres to promote apoptosis in intestinal cells (Awad & Fink, 2000; Chapkin et al. 2000; Schrauzer, 2000). One of the major issues to be clarified in the future is the minimum quantity of these dietary components required to bring about phenotypic effects and if genetic differences within tissues determines their physiological consequences. Unfortunately, far too often many pre-clinical studies utilize concentrations of bioactive food components that would be virtually impossible to achieve with typical daily eating behaviours. Historically, evidence has surfaced that cells are effective in acclimatizing to insults resulting from exposures to excessive quantities of nutrients (Fafournoux et al. 2000; Jackson, 2000). It remains to be determined if intermittent intake of nutrients offers advantages or if sustained intake is a method to promote health and well-being.

Excesses are not the only way that nutrients may change cellular events. Caloric restriction is associated with a reduction in a number of age-specific chronic diseases (Turturro et al. 1994). Evidence already exists that the transcriptional silencing of selected genes by DNA methylation plays a crucial role in ageing and a number of diseased states (Issa, 2000). Evidence with yeast indicates that caloric restriction can lead to the silencing of a variety of genes (Guarente & Kenyon, 2000).

While the study of nutritional genomics is still in its infancy, it is starting to reveal that nutrient excesses and deficiencies can bring about a host of genomic and proteomic changes. Regardless of whether the molecular target is at the transcription, translation or post-translational level, the net result is an up- or down-regulation of specific gene products. Unravelling the multitude of interactions among nutrients with these key events makes the challenge of nutritional genomics extremely daunting. Inter-individual differences probably reflecting genetic polymorphisms may mask the response to an individual nutrient and thereby complicate this undertaking to an even greater degree (Hegele et al. 1997; Hegele, 1998; Rapuri et al. 2001). In some cases nutrients that are generally thought to be protective may actually increase risk (Hilakivi-Clarke et al. 1999; Ross, 2000). Deciphering the dynamic relationship between dietary components and genes is fundamental to optimizing health. With the information gained, it should be possible to determine why inconsistencies occur in the nutrition and health literature and to develop meaningful and tailored strategies to assist individuals.

Complementary and overlapping mechanisms appear to account for the response to bioactive food components. These biological responses encompass such diverse functions as serving as an antioxidant, promoting the activity of detoxification enzymes, blocking carcinogen formation and metabolism, shifting hormonal homeostasis, retarding cell division, and inducting apoptosis. Since more than one of these processes may be influenced simultaneously, it is difficult to determine which is most important in explaining any phenotypic changes.

Some of the most compelling evidence that diet can influence the cancer process comes from the intervention study by Clark et al. (1996) with Se yeast as a supplement. Based on these findings and a host of pre-clinical studies, the National Cancer Institute and a network of researchers known as the Southwest Oncology Group initiated the largest-ever prostate cancer prevention study, The Selenium and Vitamin E Cancer Prevention Trial, to determine if these two dietary supplements can offer protection (http://newscenter.cancer.gov/pressreleases/SELECTQandA.html). Although much of the attention
on Se during the past decade has focused on its anti-
oxidant activity, this trace element is known to bring
about a diverse set of biological effects including
suppressing cell proliferation, enhancing immuno-
competence, blocking carcinogen metabolism, and
induction of apoptosis. By identifying which one of
the events is most important in altering the phenotypic
characteristics under specific circumstances, it should
be possible to identify who might and might not benefit
from exaggerated intakes of this trace element and
during what periods of life that efficacy might be
greatest. The characterization of the specific molecular
target(s) for this and other nutrients represents a major
hurdle for the science of nutrition. Nevertheless, it is
fundamental to truly understanding the involvement of
nutrients in cancer prevention as well as in other
health anomalies.

Another nutrient with apparent significance in the cancer
process is folate (folic acid). Its essential role in the
de novo biosynthesis of purines and pyrimidines, and thus in
DNA replication and cell division, and for the synthesis
of S-adenosylmethionine, a methyl donor for more than
100 biochemical reactions including methylation of
DNA, places it in a unique position relative to DNA
stability (Kim et al. 1997; Molloy & Scott, 2001). These
biosynthetic pathways, each of which is important to
DNA metabolism, appear to compete when the dietary
methyl supply is inadequate, as in folate deficiency, poss-
ibly resulting in altered DNA methylation (an epigenetic
event), disruption of DNA integrity, disruption of DNA
repair and, consequently, increased risk for several dis-
orders (Molloy & Scott, 2001). Hypomethylation and
DNA strand breaks arising from folate inadequacy may
actually promote the incorporation of viruses such as
human papilloma virus into human DNA (Choi &
Mason, 2000). Additional research is need to determine
if the response to folate inadequacy is also observed
when methyl donor supply is suppressed by depression in
choline, vitamin B12, pyridoxine and a variety of other
nutrients.

A number of non-essential phytoneutrients have also been
found to impact health (Clydesdale, 1998; Milner, 2000).
Recent studies have revealed that the use of transgenic
and knockout animals offers exciting opportunities to
define the mechanisms by which nutrients including phyto-
chemicals function. Diethiolethione represents one class of
nutrients that has been reported to influence a variety of
molecular targets associated with cancer (Kensler et al.
2000). One of the major mechanisms of protection against
carcinogenesis, mutagenesis and other forms of toxicity
mediated by carcinogens is the induction of enzymes
involved in their metabolism, particularly phase 2 enzymes
such as glutathione S-transferases, UDP-glucuronosyl
transferases and quinone reductases. The use of a knockout
animal model has revealed that 1,2-dithiole-3-thione trig-
gers nuclear accumulation of the transcription factor Nrf2
and its enhanced binding to the antioxidant response
element, leading to transcriptional activation of a score
of genes involved in carcinogen detoxification and attenu-
ation of oxidative stress (Ramos-Gomez et al. 2001). More
recently, Gupta et al. (2001) found that polyphenols in
green tea were effective in reducing the incidence and
metastasis in the transgenic adenocarcinoma mouse pros-
tate model and increased the overall longevity of these
animals. Additional studies are needed that employ a
range of gene expressions to determine what impact
genetic backgrounds have on the response to individual
nutrients.

Foods and food components may also influence the
microenvironment within the gastrointestinal tract. Inulin
and oligofructose are intriguing dietary fermentable fibres
that may have an impact on a number of processes directly
and indirectly, and thereby influence health (Roberfroid,
1993). Inulin and oligofructose are fructans with a degree of
polymerization of 2 to 60 and 2 to 20, respectively. Owing to
t heir structural conformation, they are resistant
to hydrolysis by human alimentary enzymes and therefore
are fermented almost exclusively by colonic bifidobacteria
and bacteroides. This fermentation increases faecal bac-
terial biomass, decreases caeco-colonic pH and produces
a large amount of fermentation products, among which are
short-chain fatty acids. While the long-term impli-
cations of changing the intestinal microflora remain
unknown, the changes are consistent with an induction of
genes in these micro-organisms and with a reduction in
gastrointestinal distress (Roberfroid, 1993; Kleessen et al.
2001).

The ability of several nutrients to influence the same
biological processes, as mentioned above, raises issues
about possible synergy, as well as antagonistic interactions,
among dietary components. Future studies must character-
ize nutrients in terms of their relative effectiveness, dose
dependency, temporality, consistency and specificity. The
defining of diet-specific molecular targets in terms of gen-
etic and epigenetic events that lead to phenotypic changes
should assist in the development of new and creative diet-
ary intervention strategies for not only reducing diseases
but also improving the overall quality of life.

Dynamics between biomarkers and long-term intervention

Unquestionably, scientifically sound and probing inter-
vention studies must be viewed as the cornerstone for
developing nutrition guidance for individuals. Regrettably,
the sheer number of long-term intervention studies that will
be needed to define nutrient interactions will surely be
impractical in terms of speed of discovery and overall
cost to society. Alternative procedures will thus be required
to predict benefit and risk of selected interventions. These
approaches will necessitate the use of sensitive and reliable
biomarkers. Factors similar to those evaluated by environ-
mental toxicologists (Suk & Collman, 1988; Sakai, 2000)
will be needed to evaluate the benefits/risks of functional
foods and/or their components. Fundamental to this process
will be biomarkers that evaluate: (1) the bioactive food
component capable of modifying a molecular target
(intake/exposure biomarker), (2) biological responses that
evaluate directly or indirectly disease risk or health main-
tenance (effect biomarker), and (3) factors modifying the
response such as genetic and the environment (suscepti-
bility biomarker; Milner, 1999). To assess adequately
whether a food or its component has a physiological
effect it is imperative that stringent experimental design characteristics are followed. Several factors, including appropriateness of controls, randomization of subjects, blinding, statistical power of study, presence of bias, attrition rates, recognition and control of confounding factors (e.g. weight change or nutrition status) and appropriateness of statistical tests and comparisons, are addressed in a guidance document published in the Federal Register (1999). Each of these factors must become the mainstay for all investigations. Pre-clinical investigations will require that many if not most of these same factors be considered in their experimental design. Whatever biomarkers are established, it is clear that they must be readily accessible, easily and reliably assayed, differentially expressed in normal and diseased conditions, directly associated with disease progression, modifiable and, most important, ‘predictive’ (Fig. 1). Similar to the US Department of Agriculture’s pyramid that is used for dietary guidance, it is likely that the early predicative biomarkers will not be at the top because of the latency of the observation but be focused at the base where they will be more specific and timelier. Thus, the future of nutritional biomarkers is likely to reside in the enhanced use of molecular technologies to help decode who will and will not benefit from intervention strategies and, just as important, who might be placed at harm.

Genetic polymorphisms and dietary variability

Increasing genetic polymorphisms are thought to have a role in the ability of individuals to withstand exposure to exogenous carcinogens or to inhibit initiation, promotion or proliferation in carcinogenesis. It is certainly plausible that polymorphic differences have been a contributing factor in the inconsistencies surrounding dietary components and health (Cotton et al. 2000). For example, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC study), there was a low prevalence of polymorphisms in genes coding for activation (phase I) enzymes CYP1A1 (0.07) and CYP2E1 (0.02) and a high prevalence in genes coding for detoxification (phase II) enzymes GSTM1 (0.40) and NQO1 (0.20) (Woodson et al. 1999). Seven of ten members of this sample carried the VDR–Taq1 polymorphism (t) associated with lower risk for prostate cancer, which may account in part for lower cancer rates in Finland compared with the United States (Woodson et al. 1999). Further, in a nested case–control study within the ATBC Study, glutathione peroxidase 1 (hGPX1), an Se-dependent enzyme involved in detoxification of hydrogen peroxide, was found to have a polymorphism exhibiting a proline to leucine replacement at codon 198. This polymorphism conferred a relative risk for lung cancer risk of 1.8 for heterozygotes and 2.3 for homozygote variants, compared with homozygote wild types (Ratnasinghe et al. 2000).

The future

Research in nutrition and health in this new millennium must give top priority to studies that will aid in understanding the basic molecular events by which nutrients influence biological process. A well-coordinated, multidisciplinary effort among scientists — including nutritional scientists, molecular biologists, geneticists, statisticians and clinical cancer researchers — may be needed to advance this molecular approach to nutrition-related cancer research. Many research questions and issues will need to be addressed for this approach to become a reality, including the minimum intake of bioactive components to bring about phenotypic change and how genetically controlled processes, including acclimatization, may change the overall response. While the challenges to researchers will be enormous, the potential rewards in terms of morbidity and mortality could also be enormous.

References


codon 198 polymorphism variant increases lung cancer risk. 
metropolitan areas, but at the same time has caused increased adoption of inadequate dietary patterns and decreased physical activity. Diseases of both deficiency and excess are simultaneously important in the public health scenario. This has been called the nutrition transition.

Increased consumption of diets high in fat (particularly saturated fat), low in complex carbohydrates and less dense in micronutrients, combined with a more sedentary life-style, is responsible for spread of diet-related disorders including obesity, diabetes, cardiovascular diseases, hypertension, osteoporosis and cancer. This situation increases disabilities and premature death, placing an additional burden on public health budgets. Over the next ten years it is estimated that 11 million people in Latin America and the Caribbean will die from cardiovascular diseases, 5·7 million from cancer and 3·4 million from external causes. Nearly 23 % of cardiovascular deaths and 39 % of cancer deaths will occur among people under 60 years of age, representing premature mortality that can be prevented or postponed (PAHO, 1999).

Data show that many known risk factors for non-communicable diseases are becoming highly prevalent in Latin American and Caribbean countries. Infant malnutrition may coexist with a high prevalence rate of overweight in some adult populations, as high as 30–55 % in some groups. Obesity among women is 3 % in Haiti, 8–10 % in eight countries and 29 % in Mexican-Americans. The prevalence of overweight among 1–5-year-old children ranges from 6 % in Haiti to 24 % in Peru, among thirteen countries. Prevalences of overweight in children and obesity in women are greater in urban areas and in households of higher economic status (Martorell et al. 1998). Studies in Brazil have shown overweight rates as high as 30 % in school children and in urban areas. The prevalence of hypertension has been estimated to range between 8 and 30 % and even higher (40 %) in existing studies. High prevalence of hypercholesterolaemia (30–40 %) has been documented in some regions. The few existing studies on physical activity reported rates of sedentary life as high as 50–90 %. Even diabetes, previously uncommon in many developing countries, seems to be growing in importance with prevalence estimates of between 5 and 10 %. Much higher figures, up to 18 %, have been found in some countries of the Caribbean. In the year 2000 the number of people who suffered from diabetes in the Americas was estimated as 35 million, of which 19 million (54 %) live in Latin America and the Caribbean.

Micronutrient deficiencies

The nutrition transition changes in dietary habits are also causing nutritional deficit, resulting in marginal micronutrient deficiencies, well-known classical clinical deficiencies or both. But diseases of both deficiency and excess are simultaneously important.

Estimates of 1995 indicated over 168 million people at risk of iodine deficiency disorders (63 million affected by goitre), 15 million children under 5 years old suffering from various levels of vitamin A deficiency (20 % prevalence) and 94 million suffering from deficiency anaemia (40 % prevalence in women; PAHO, 2000). Classical deficiency affects millions of people and several countries have fortification programmes, some of which are even mandatory, like the addition of Fe to flour in Brazil. In urban areas, research has indicated that priorities are deficiencies of Fe, Zn and vitamin A in infants and pre-school children and of Fe, folate, Ca and vitamin A in adolescent, pregnant and lactating women (Trugo et al. 1997).

Costa Rica has better nutritional status than most other Latin American countries, with low levels of anaemia; yet half of Costa Rican adolescent girls showed anaemia and low folate intake. This shows the importance of studying specific population groups not only concerning anaemia, but also for neural tube defects and cardiovascular diseases (Monge et al. 2001). In Brazil, too, evidence based on consumption and clinical and biochemical surveys has shown the prevalence of Fe deficiency, followed by low consumption (or low availability) of folate, Zn and Ca, a trend of reduced fibre intake and excessive Na consumption (Cozzolino, 1997). Regarding vulnerable groups and critical nutrients, the requirement may be above the potential available in foods.

As for the relationship between micronutrient status and chronic degenerative diseases, one should focus on folates, antioxidant nutrients and cardiovascular diseases, and on Ca and osteoporosis.

Opportunities and challenges

Foods for deficiency and for excess

Nutrition is recognized as one of the major health determinants. We have already seen that the epidemiological and nutritional situation in Latin America represents a considerable challenge for everyone involved in the different aspects of food and nutrition: university, industry and government staff, responsible for research, production and regulation, respectively.

There are needs and opportunities to produce foods for the classical clinical deficiencies, and also for the already known marginal or emerging micronutrient deficiencies of particular population sub-groups. Enriched, restored and fortified foods, as well as foods with a high bioavailability of nutrients, must be available either for government nutrition programmes or on the market. Considering that in Latin American countries the diet is frequently based on a staple like cassava, corn, potato or beans with low nutrient density, there is borderline ingestion of several nutrients, such as folic acid, Zn, Se and Ca. Thus optimum consumption of these micronutrients in some groups may only be achieved by some kind of diet supplementation.

In this respect genetic engineering is an important tool to increase nutrient contents and bioavailability, and in some cases may be the best approach. Some examples are the introduction of vitamin A and also the increase in Fe content of rice. The production of foods that may improve immunological protection of infants, to prevent intestinal infections, is another way to introduce functionality in
foods and address public health concerns in less developed countries. A group from the Biomedical Science Institute of the author’s university has engineered a Lactobacillus to express Escherichia coli intimin that stimulates serum immunoglobulin A production and thus may protect infants from diarrhoea.

At the same time there is a need for foods to address specific health conditions that have become critical in Latin American countries nowadays, like the ones associated with cardiovascular problems, diseases like cancer, diabetics, obesity, osteoporosis and others. Latin America also needs foods that may help specific sub-groups of the population gain control of the risk of disease and of their well-being.

The concepts of food functionality and functional food science, as defined in Europe, seem to be the right approach to generate the knowledge necessary to help industry offer new foods in order to answer these challenges and help governments to define nutrition and adequate regulatory policies.

Bioactive phytochemicals in foods and plants

Another aspect to be considered is associated with phytochemical compounds of foods and other plants. A growing number of studies have linked diets rich in fruit and vegetables with good health, and correlations have also been found between the ingestion of some compounds, like flavonoids, carotenoids and glucosinolates, and lower risks of cancer, cardiovascular and some other chronic diseases. In Latin America there are very few data concerning the nutrient composition of foods and virtually none on non-nutrient components. Considering the low fruit consumption and the identified marginal deficiencies of micronutrients, the consumption of protective compounds may be low, calling for more research.

One can say that the discoveries of the fifteenth century introduced Latin America into the global economy and that globalization has proceeded from then on. Since then food products such as corn, beans, amaranth, quinoa, potatoes, tomatoes, cocoa, guava, papaya, avocado and many others have become an important part of the diet in many regions of the world, including Europe. The great potential of unknown or under-exploited Latin American plants is still waiting adequate research. It is estimated that in the Amazon region there are 50,000 plants representing 25% of total biodiversity, but less than 2% have been studied. Many of them may be important sources of phytochemicals, flavours and colours.

Latin American plants are usually rich in flavonoids, anthocyanins and carotenoids, and some have interesting amounts and combinations of specific carotenoids like zeaxanthin, lutein and others. Some fruits like guava and palm fruits like açai (Euterpe oleracea) have three times more lycopene than tomatoes and are also rich sources of anthocyanins (Rodriguez-Amaya, 1999). Camu-camu (Myrciaria dubia) fruit may contain 2% ascorbic acid. Brazil nuts are the richest source of Se. Roots from some regions produce functional oligosaccharides, polysaccharides and important oils, sterols, and saponins. Maca (Lepidium meyenii), guaraná (Paulinia cupana) and mate tea (Leia paraguayensis) are known for their energy-giving properties. Many of them have important physiological and pharmacological actions. Several of these fruits are no longer just harvested but are cultivated industrially, and soon they will be economically significant.

Claims, efficacy validation and human variability

A great variety of products are available in open markets in Latin America, even in highly urbanized areas like São Paulo. They are whole plants, plant organs like leaves, tubers, seeds, fruits presented in several forms from powder to extracts, and juices, alone or in combination. They are very popular because they are part of the cultural tradition, they are perceived as ‘natural’ and therefore healthy, and because they are cheap. These products are sold with claims for important diseases, to help nutrition or simply for bad humour. Not much is known about the composition, safety and activity of most of them. Only recently have research projects been undertaken in a more systematic manner and these products submitted to review by health authorities.

Latin America has a very diverse cultural and genetic background. The indigenous population, for instance, can represent 40% or more in some regions and less than 1% in others. Populations with African ancestors in the Americas may vary from less than 3% in Mexico to 44% in Brazil and 84% in the Dominican Republic. The first genetic profile of the Brazilian population showed that the black people are whiter and the white people are blacker than first thought (white are partly genetically black and vice versa). Interesting data are emerging in Brazil to suggest that genes vary from region to region, where the regions also have different cultural and dietary habits (Pena, 2000). Here, differences in Apo E4 genes and LDL A+ A+ genes are linked to coronary diseases (Cavalli et al. 2000; Salazar et al. 2000) and Apo E4 genes to high cholesterol and diabetes mostly in black people (Otta et al. 1996). This is important, for instance, in studies on cholesterol reduction by diet. All this clearly shows the importance of genetic background and human ecology considerations when evaluating the efficacy of functional foods. Even if urbanization has homogenized some habits, there is still, by and large, a significant variation in dietary habits and organism physiology in our populations, pointing to the importance of and need for local research.

Functional foods should be viewed in Latin America beyond short-term commercial prospects and consider long-term research and development, regional needs, and the cultural habits and economical level of consumers.

Legislation on functional foods in Latin America: regulatory status

Concerning functional foods, adequate legislation is essential to protect public health and stimulate technological development. In Latin America and the Caribbean, regulation concerning functional foods, functional supplements and functional and health claims differs greatly
from country to country. There is no official or legal definition of functional foods or a specific regulation for functional foods/ingredients. In general, basic nutrient content/function claims are allowed and subject to some norms, but only a few countries allow or have norms for health claims. Even so, health authorities have allowed product claims by on a case-by-case basis in several countries. Brazil is the only country to have a well-defined regulation for functional and health claims for either nutrient or non-nutrient components and also for the demonstration of safety and efficacy (Agência Nacional de Vigilância Sanitária, 1999). In almost all countries functional or health claims associated with non-nutrient compounds or non-essential nutrients are neither prohibited nor regulated. As a consequence, there is no legislation on efficacy and safety and also a lack of criteria for scientific substantiation of claims, except for Brazil. Even so, all over Latin America, in popular markets and even in supermarkets, hundreds of foods and drugs are sold together with curative allegation.

Considering the regional economic level, cultural identity and public health situation, the development of common functional foods concepts and norms seems to be very important. Brazilian legislation can be used as a starting point in this direction since it has been based on internationally acceptable principles and is considered to be up-to-date. In Brazil functional foods have not been defined as such but the norms were based on the idea of a food that is food and not a drug, that is part of a normal diet and that can produce benefits beyond basic nutrition. Legislation essentially rules on demonstration of the safety and efficacy of novel foods (foods not used before in Brazil) and foods/ingredients that have a claim in the label. All of these products should be registered and approved by health authorities. Safety demonstration is a priority and should be based on risk analysis, including risk assessment, management and information. Efficacy concerning the claims should be based on scientific evidence obtained from the literature or by new research and must represent scientific consensus, as has been

| Table 1. Examples of food claims approved and not approved in different Latin American countries |
| Spreads with phytoesterols | Brazil | ‘Helps to maintain healthy level of cholesterol’<br>‘When associated with a healthy diet and life-style’ (research in Brazil suggested and concluded) |
| Milk containing phytosterol (different manufacturers) | Argentina | ‘Helps in reducing cholesterol’<br>‘As part of a healthy diet’ |
| Milk containing n − 3 fatty acids (EPA and DHA) | Brazil | ‘Helps to control triglyceride levels, blood fluidity, inflammatory and immune response’<br>When associated with a healthy diet and life-style:<br>‘Enhances immune defence’<br>‘Reduces inflammatory processes, control the level of triglycerides and cholesterol.<br>Increases blood fluidity’<br>In doing so they:<br>‘Contribute to prevention or retardation of atherosclerosis and associated diseases of the circulatory and nervous systems’ |
| | Argentina | ‘Has less saturated fatty acids that increase cholesterol and more n − 3 and n − 6 that help reducing blood cholesterol’<br>‘Good for healthy persons who have balanced nutrition and life-style’<br>‘Low in saturated fat and enriched with n − 3 and n − 6 fatty acids’<br>‘Needed to keep cholesterol low and healthy heart’ |
| Milk with n − 3 and n − 6 fatty acids from vegetable oil | Argentina | ‘Is a functional ingredient that naturally helps your son to have a better digestive system performance’<br>‘Allows better absorption of vitamins’<br>‘Helps for a better absorption of nutrients to strengthen your defences’<br>‘Contributes to a healthy intestinal flora’<br>‘Helps maintaining the intestinal flora balanced’ |
| Probiotics (lactobacilli and bifidobacteria) | Brazil | ‘Helps in maintaining the intestinal equilibrium flora balanced’<br>‘Helps in reducing harmful bacteria’<br>‘Helps in increasing the beneficial flora’<br>‘Allegations not approved:’<br>‘Increases antibodies’<br>‘Strengthens natural defences against daily aggression and stress’ |
| Breakfast biscuits | Argentina | ‘Slow releasing energy. Energy is released slowly during all the morning’<br>‘Shows a glycaemic curve on the label’ |
discussed by the US Food and Drug Administration and by the International Life Science Institutes in several forums and in the Codex Alimentarius.

In Brazil, both functional (enhanced function) and health claims are defined and allowed. As for health claims, only health maintenance and risk reduction claims are allowed, with both prevention and cure allegations being prohibited. In this case, however, the product can be considered as a drug and assessed as such. The label on the product should be clearly understandable by consumers, not misleading, it should inform the limitation of efficacy, and should be in accordance with public health policies. In some specific cases products should clearly inform restriction of their use to specific groups or to individuals with specific physiological conditions, as well as any adverse side-effects that may occur. These rules also apply for marketing and advertising materials.

Regarding products in pharmaceutical form that contain bioactive compounds normally present in foods, the label should inform that the product ‘does not prevent or cure any disease’. Mixtures of foods and food components with herbs that have pharmacological action are considered drugs or phytotherapeutic agents, and submitted to pharmaceutical regulations.

In Brazil, like in many other countries in Latin America, there is a great use of phytotherapeutics. These include plants, plant organs, extracts, pills, etc. that by tradition or by research are known to cure or alleviate some diseases, and are classified and regulated as drugs by health authorities. Two years ago, before the existence of norms in Brazil, there were a large number of these ‘natural’ products being sold as foods or food supplements with unproved and undemonstrated curative claims for all known diseases. Some of them were complex mixtures of vegetable extracts containing highly active pharmacological compounds such as amphetamines. The legislation issued allowed organization of the market through the analysis for safety and efficacy of the existing 2000 products, among which only 430 are still being produced by 115 manufacturers.

**Foods with functional or health claims in Latin America**

During the last two years, a number of new foods that may be considered as functional foods, since they have functional or health properties acknowledged by health authorities, have appeared on the market in some countries. The most important are spreads and milks with added phytosterols, milk containing long-chain \( n = 3 \) fatty acids and milks with added \( n = 3 \) and \( n = 6 \) fatty acids from vegetable oils. Milk and products with oligofructose, margarine and yoghurts containing fibre, cookies engineered to have low glycaemic index, and milks fermented with selected *Lactobacillus* and *Bifidobacterium* strains have also appeared. Other products are those containing soyabean proteins and isoflavones, low-cholesterol eggs, and energy and isotonic drinks containing caffeine and other herbal extracts.

A number of products in pharmaceutical form should also be mentioned. These are mostly fibre-containing products, antioxidants and oils containing freeze-dried vegetable/fruit extracts.

Some of the claims approved and not approved in different countries can be used as examples to illustrate the on-going legal situation in Latin America, and are shown in Table 1. As shown by these examples, almost all of the claims can be classified more as functional claims than as claims related to a disease, reflecting the present position of the health authorities. They are mostly based on internationally accepted scientific knowledge but some of them may be too sophisticated even for a well-informed public.

Latin American consumers, in general, do not know what functional foods are, although in the more urbanized areas there is an increasing number of health-conscious consumers aware of the importance of diet for health and well-being. This awareness comes either from the media or cultural tradition. Concepts associated with vitamins, obesity, cholesterol, fibre, fermented milk and physical activity are disseminated, even if the general nutrition knowledge is poor. However, long and complex claims are difficult to understand and may confuse consumers. It is necessary to disseminate the best available information and advice about diet, as part of a sound alimentary education.

In several cases, marketing information carried by the media to promote a product goes far beyond what was approved, misleading the consumer. To solve this problem some countries like Brazil are also regulating propaganda on foods. This may help give credibility to the assessment process and to the functional foods area.

While middle- and upper-class people can get their health foods at the supermarket, lower-class people can get theirs in other places where they find their functional ‘garrafas’, known as natural and therefore healthy products for nutrition and healing. They both seem clearly to understand how to make use of the concept of improving their health and well-being through food.

**Conclusions**

The occurrence of diet-related diseases of deficiency and excess points to the importance of the development of functional food (science). In urban areas, due to the media and cultural tradition, there is increasing consumer awareness of the importance of foods for health. The perspectives of Latin America as a potential producer and consumer of functional foods will depend on the level of information and income of the population, on the credibility and pricing of products, on the nutrition and regulatory policies and on research investments. Functional food science must be viewed in Latin America beyond the short-term commercial prospects and should consider long-term research and development.

**References**


Cavalli SA, Hirata MH, Salazar LA, Diament J, Forti N, Giannini


In this project, several interesting results were obtained. In particular, research into novel phytochemicals elucidated the occurrence of new anticarcinogens, e.g. fucosterols in seaweed and auraptene in citrus fruits. The discovery and characterization of potent antioxidants of plant origin, e.g. curcuminooids in ginger, were also reported. Another unique example was offered by oryzacystatin, a cysteine proteinase inhibitor found in the rice, Oryza sativa L., which was molecularly cloned (Abe et al. 1987) and demonstrated to inhibit the proliferation of human herpes virus in infected animal cells (Aoki et al. 1995). These studies were aimed at finding factors with body-defending rather than body-modulating functions.

For body modulation, food allergies and countermeasures were studied extensively. The background was that rice-associated allergy in the form of atopic dermatitis was increasing continually in Japan, a major rice-consuming country, and countermeasures were needed urgently. In response to such a social need, an experiment was undertaken to design and produce hypoallergenic rice grains by enzymatic hydrolysis of main allergenic proteins occurring in the globulin fraction. The development of well-controlled conditions for the hydrolysis gave an immunologically and clinically satisfactory product (Watanabe et al. 1990) and the production process was industrialized to manufacture it. ‘Fine Rice’ was approved as the first Food for Specified Health Use (FOSHU) product (described below) after careful intervention tests in well-controlled conditions for the hydrolysis gave an immunologically and clinically satisfactory product (Watanabe et al. 1990) and the production process was industrialized to manufacture it. ‘Fine Rice’ was approved as the first Food for Specified Health Use (FOSHU) product after careful intervention tests in many medical centres. These basic and applied studies were followed by the discovery and characterization of soybean and wheat allergens to develop hypoallergenic soybean and flour products (Tanabe et al. 1996).

The orthodox way of producing functional foods is to maximize beneficially functional factors that are responsible for modulation of the immune, endocrine, nerve, circulatory or digestive system in the body. Sometimes, however, a unique approach can be adopted. Uniqueness is found in minimizing non-beneficial functional factors by enzyme technology or even genetic engineering. The importance of using this concept for the design and production of functional foods has been emphasized since 1984 when the first project began.

The Japan Ministry of Health and Welfare in 1991 initiated the world’s first policy of legally permitting the commercialization of selected functional foods in terms of FOSHU, as mentioned above. The new policy is defined by new legislation and also characterized by approving the presentation of a health claim for each FOSHU product. Such legal framework is also expected to hinder the presentation of ill-defined and misleading advertisements in commercial products. Thus, since 1993, some selected food products have been approved to claim a certain degree of medical representation never before permitted for any food. The first was hypoallergenic rice grains (‘Fine Rice’ by commercial name) developed after extensive immunological studies in the MESC project, produced by enzyme technology and industrialized after clinical intervention tests (Watanabe et al. 1990). A great many FOSHU products have been approved up to now (Table 1).

### Glance at Asian functional foods

Asian food processing is characterized by fermentation. This is reflected in the production of functional foods as well. Actually, FOSHU items (Table 1) include fermented milk, which originates in Caucasus and is produced industrially for particular purposes. A good example is offered by a FOSHU product in which reasonable amounts of the lacto-tripeptides Val–Pro–Pro and Ile–Pro–Pro, angiotensin-converting enzyme inhibitors, have been derived from β-casein by lactic acid bacteria. Another interesting example is provided by fermented soyabean, natto by Japanese name, which has long been consumed in some Asian districts as well as in Japan. Very recently, a mutant of Bacillus natto was developed that can produce a high quantity of menaquinone-7 (vitamin K₇) as a cofactor of γ-glutamyl acid carboxylase for biosynthesis of the Ca-carrying protein, osteocalcin. The natto produced using this mutant is thus expected to reduce the risk of osteoporosis and was approved as a FOSHU product. Also, rice whose wild species per se can be found in a district near Tibet is often used as a material for fermentation. In Japan, it was found that a rice bran extract treated with a mixed Lactobacillus–Saccharomyces culture produces a large amount of γ-aminobutyric acid as a functional food factor for reducing the risk of neural disorders as well as hypertension, although the product remains to be approved.

In China as well, the functional food policy has made a good progress. Chinese functional foods are legally approved, with the logo, in sky blue colour, issued by the Ministry of Public Health. The functions to be considered include twenty-four items as follows (Yi et al. 1999):

1. immune regulation,
2. postponement of senility,
3. memory improvement,
4. promotion of growth and development,
5. anti-fatigue,
6. body weight reduction,
7. oxygen deficit tolerance,
8. radiation protection,
9. anti-mutation,
10. anti-tumour,
11. blood lipid regulation,
12. improvement of sexual potency,
13. blood glucose regulation,
14. gastrointestinal function improvement,
15. sleep improvement,
16. improvement of nutritional anaemia,
17. protection of liver from chemical damage,
18. lactation improvement,
19. improvement for beauty,
20. vision improvement,
21. promotion of lead removal,
22. removal of ‘intense heat’ from the throat and moistening of the throat,
23. blood pressure regulation and
24. enhancement of bone calcification.

For some categories among these, a variety of foods are considered to be approved (Table 2). These foods are also...
used as medicines. Thus, distinguishing between foods and traditional medicines is not an easy task. The difficulty apparently reflects the Chinese tradition that medicine and food share a common origin.

Some food factors may be studies for purely medical rather than food use. In an extensive screening study covering common vegetables and fruits in south-east Asian countries, anti-tumour promoting potential, detected by the inhibitory rate of tumour promoter-induced Epstein-Barr virus activation, was generally higher in the plants from tropical zones than in those from Japan. Moreover, plants in the families Zingiberaceae, Rutaceae, Labiatae and Cruciferae, which are used as traditional medicines, were shown to contain potent anti-tumour promoters at high rates (Arai et al. 2001). For functional food scientists, Asia may thus be like a treasure house.

### Table 1. Data on 252 foods for specified health uses

<table>
<thead>
<tr>
<th>Health claim*</th>
<th>Functional factor</th>
<th>Number of products approved†</th>
<th>Forms of products on the market</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Prebiotics</td>
<td>Lactosucrose</td>
<td>61</td>
<td>Soft drink, yoghurt, biscuit, cookie, table sugar</td>
</tr>
<tr>
<td></td>
<td>Fructo-oligosaccharides</td>
<td>15</td>
<td>Table sugar, tablet candy, pudding, soyabean curd</td>
</tr>
<tr>
<td></td>
<td>Soyabean oligosaccharides</td>
<td>9</td>
<td>Soft drink, table sugar</td>
</tr>
<tr>
<td></td>
<td>Xyle-oligosaccharides</td>
<td>5</td>
<td>Soft drink, vinegar, chocolate, tablet candy</td>
</tr>
<tr>
<td></td>
<td>Galacto-oligosaccharides</td>
<td>4</td>
<td>Table sugar, vinegar</td>
</tr>
<tr>
<td></td>
<td>Isomalto-oligosaccharides</td>
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<td>Table sugar</td>
</tr>
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<td>Raffinose</td>
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<tr>
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<td>Lactulose</td>
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<td>Soft drink</td>
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<tr>
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<td>Arabinose</td>
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<td>Table sugar</td>
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<td>Lactic acid bacterium drink</td>
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<td>26</td>
<td>Fermented milk</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus delbrueckii and Steptococcus salivar ius</td>
<td>6</td>
<td>Lactic acid bacterium drink</td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium lactis</td>
<td>4</td>
<td>Yoghurt</td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium breve Yakult</td>
<td>4</td>
<td>Fermented milk</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus rhamnosus GG</td>
<td>2</td>
<td>Fermented milk</td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium longum</td>
<td>4</td>
<td>Fermented milk</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus acidophilus and B. longum</td>
<td>58</td>
<td>Fermented milk</td>
<td></td>
</tr>
<tr>
<td>Dietary fibres</td>
<td>Indigestible (resistant) dextrin</td>
<td>28</td>
<td>Sausage, cookie, drink, miso soup, curd</td>
</tr>
<tr>
<td></td>
<td>Psyllium husks</td>
<td>21</td>
<td>Powdered drink, noodle, cereal</td>
</tr>
<tr>
<td></td>
<td>Wheat bran</td>
<td>2</td>
<td>Cereal</td>
</tr>
<tr>
<td></td>
<td>Galactomannan</td>
<td>1</td>
<td>Jelly</td>
</tr>
<tr>
<td></td>
<td>Partially hydrolysed guar gum</td>
<td>3</td>
<td>Powdered drink, boiled rice</td>
</tr>
<tr>
<td></td>
<td>Agar</td>
<td>2</td>
<td>Noodle, jelly</td>
</tr>
<tr>
<td></td>
<td>Beer yeast</td>
<td>1</td>
<td>Fermented milk</td>
</tr>
<tr>
<td>II Soya protein and peptide</td>
<td>Low-molecular-weight sodium alginate</td>
<td>7</td>
<td>Soft drink, soup</td>
</tr>
<tr>
<td></td>
<td>Protein yeast</td>
<td>3</td>
<td>Biscuit</td>
</tr>
<tr>
<td></td>
<td>Sitosterol ester</td>
<td>1</td>
<td>Margarine</td>
</tr>
<tr>
<td>III Peptides</td>
<td>Sardine peptides (containing VY)</td>
<td>9</td>
<td>Soft drink, soup</td>
</tr>
<tr>
<td></td>
<td>Lacto-tripeptides (VPP and IPP)</td>
<td>5</td>
<td>Lactic acid bacterium drink</td>
</tr>
<tr>
<td></td>
<td>Dried bonito oligopeptides (containing IKP)</td>
<td>2</td>
<td>Fermented soyabean curd</td>
</tr>
<tr>
<td></td>
<td>Casein dodecapeptides (FFVAPEPEVFGK)</td>
<td>4</td>
<td>Soft drink</td>
</tr>
<tr>
<td></td>
<td>Eucommia leaf glycoside (geniposidic acid)</td>
<td>2</td>
<td>Soft drink</td>
</tr>
<tr>
<td>IV Diacylglycerol</td>
<td>Diacylglycerol and sitosterol</td>
<td>4</td>
<td>Cooking oil</td>
</tr>
<tr>
<td></td>
<td>Calcium citrate malate</td>
<td>3</td>
<td>Soft drink</td>
</tr>
<tr>
<td></td>
<td>Heme</td>
<td>4</td>
<td>Soft drink, jelly</td>
</tr>
<tr>
<td>V Casein phosphopeptide</td>
<td>Menasquinone-7 producing Bacillus subtilis</td>
<td>2</td>
<td>Fermented soyabean (natto)</td>
</tr>
<tr>
<td></td>
<td>Soy isoflavone</td>
<td>1</td>
<td>Tea drink</td>
</tr>
<tr>
<td>VI Manitol, palatinose and tea polyphenols</td>
<td>3</td>
<td>Chocolate, chewing gum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xylitol</td>
<td>1</td>
<td>Chewing gum</td>
</tr>
<tr>
<td>VII Wheat albumin</td>
<td>Globin digest</td>
<td>1</td>
<td>Soft drink</td>
</tr>
<tr>
<td></td>
<td>Guava polyphenols</td>
<td>1</td>
<td>Soft drink</td>
</tr>
</tbody>
</table>

* Claim I, foods that improve gastrointestinal conditions; Claim II, foods for those with high serum cholesterol; Claim III, foods for those with high blood pressure; Claim IV, foods for those with high serum triacylglycerol; Claim V, foods related to mineral absorption and transport; Claim VI, non-cariogenic foods; Claim VII, foods for those who begin to feel concerned about their blood sugar level.

† As of 29 May 2001.
new concept is of extreme importance in view of the demands of the elderly population for improved quality of their late life, increases in life expectancy and costs for health care, and technical advances in the food industry.

As recognized in the Japan MESC research project, up-to-date knowledge in the biosciences, including biochemistry and molecular biology together with sophisticated biotechnologies based on these, supports the hypothesis that foods can modulate various functions in the body and thus participate in the maintenance of a state of health that reduces the risk of life-style related diseases. Such a hypothesis exists at the origin of the concept of "functional food" for the development of a new discipline, "functional food science", in Europe as well as in Japan.

What may be most important at present is to evaluate the function. Since any functional food must be based on science, its evaluation should essentially depend on data from biochemistry, physiology, molecular and cell biology, and other modern biosciences. A key approach to the development of a functional food is the identification and validation of relevant markers, including biomarkers that can predict potential benefits relating to a target function in the body. If the markers represent an event directly involved in the process, these should be considered as functional factors. On the other hand, if the markers represent correlated events, they should be considered as indicators. In detail, possible markers have been tentatively listed in relation to target functions (Diplock et al. 1999).

There are many options for selecting appropriate markers. A promising option may be the use of accumulated knowledge about genomics. As the human genome project has been completed (Venter et al. 2001), the so-called post-genomics, involving the use of genomics data, will follow, which may not be unrelated to functional food science. A number of sophisticated biotechnologies will become available, one of the most useful being DNA tip technology (Marton et al. 1998). It is helpful because of its high throughput potency to assess the possible effects and safety of functional food factors by total gene expression profiles.

Current genome programmes are proceeding on food organisms as well. These include rice, wheat, corn, soya-bean, and other major plant seeds for food use. A variety of post-genomics sciences, to be born shortly before and after the completion of the genomics, will lead to the development of new biosciences such as nutrigenomics. The development will support advances in proteomics, providing key information about the molecular design of functional food proteins. It will thus be possible to easily define their specific functional regions involved in the prevention of such life-style related diseases as diabetes, hypertension, hypercholesterolaemia, allergies, cancer and even infectious diseases. Among a number of trials, proteomics for the design of antiviral proteins may serve as an example. One such study involves a three-dimensional NMR analysis of solution structures of oryzacystatin, an anti-herpes protein of rice origin described above (Abe et al. 1987; Aoki et al. 1995). The analysis

### Table 2. Representative Chinese functional foods for body modulation in specified categories (Yi et al. 1999)

<table>
<thead>
<tr>
<th>Immune regulation</th>
<th>Blood lipid regulation</th>
<th>Blood lipid regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>American ginseng (Panax quinquefolius)</td>
<td>Hawthorn fruit (Crataegus pinnatifida)</td>
<td>Hawthorn fruit (C. pinnatifida)</td>
</tr>
<tr>
<td>Ginseng (Panax ginseng)</td>
<td>Soyabeans (Glycine max)</td>
<td>Chinese yam ( Dioscorea opposita)</td>
</tr>
<tr>
<td>Acanthopanax (Acanthopanax senticosus)</td>
<td>Peach seed (Prunus persica)</td>
<td>Buckweat poria (Fagopyrum esculentum)</td>
</tr>
<tr>
<td>Barbary wolfberry fruit (Lycium barbarum)</td>
<td>Chrysanthemum flower (Chrysanthemum morifolium)</td>
<td>Cocoas poria (Poria cocos)</td>
</tr>
<tr>
<td>Astragalus (Astragalus membranaceus, A. membranaceus var. mongholicus)</td>
<td>Super vinegar</td>
<td>Extract of pumpkin (Cucurbita moschata)</td>
</tr>
<tr>
<td>Chinese caterpillar fungus (Cordyceps sinensis)</td>
<td>Spine date seed (Z. jujuba var. spinosa)</td>
<td>Extract of pig pancreas</td>
</tr>
<tr>
<td>Gingko leaf (Ginkgo biloba)</td>
<td>Fish oil</td>
<td></td>
</tr>
<tr>
<td>Walnut (Juglans regia)</td>
<td>Corn oil (Zea mays)</td>
<td></td>
</tr>
<tr>
<td>Chinese date (Ziziphus jujuba)</td>
<td>Extract of flax seed (Linum usitatissimum)</td>
<td></td>
</tr>
<tr>
<td>Royal jelly</td>
<td>Safflower seed (Carthamus tinctorius)</td>
<td></td>
</tr>
<tr>
<td>Chinese angelica (Angelica sinensis)</td>
<td>Blood and urine glucose reduction</td>
<td></td>
</tr>
<tr>
<td>Glossy ganoderma (Ganoderma applanatum)</td>
<td>Fivelleaf gynostemma (Gynostemma pentaphyllum)</td>
<td></td>
</tr>
<tr>
<td>Postponement of senility</td>
<td>Hawthorn fruit (A. membranaceus, A. membranaceus var. mongholicus)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chinese yam ( Dioscorea opposita)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Buckweat poria (Fagopyrum esculentum)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cocoas poria (Poria cocos)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract of pumpkin (Cucurbita moschata)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract of pig pancreas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Powder of spirulina (Spirulina princeps)</td>
<td></td>
</tr>
</tbody>
</table>

### International view on functional food science and policy

Meanwhile, the implementation of the Ministry of Health and Welfare’s FOSHU policy as well as the initiation of functional food science in Japan had a strong impact on many countries of the world, particularly in Europe. Early in 1995, the UK Ministry of Agriculture, Fisheries and Food defined, though temporarily, functional foods as those that had components incorporated to give specific medical or physiological benefits, other than nutritional effects (Richardson, 1996).

Deeper interest was paid by the European body of the International Life Sciences Institute (ILSI Europe). ILSI Europe addressed the present status by claiming that we stand today at the threshold of a new frontier in nutritional science, and also stating that the concept of food is changing from a past emphasis on eating to get rid of hunger into a current emphasis on promising uses of foods to reduce the risk of chronic illness (Bellisle et al. 1998). This
provides information about the minimal effective region (Nagata et al. 2000), the use of which will contribute to molecular breeding of new rice cultivated with an antiviral function.

It is also noted that an ad hoc team headed by the present author (S.A.) has begun to make a database of functional foods under the sponsorship of the Japan MESC.

Finally, it is stressed that Asian people have always recognized the importance of sensory properties of foods. They still think that every food must be sensorily acceptable. Even functional foods cannot be ruled out. The science should include studies on the senses of taste and smell. The significance of this is emphasized since such sensations have been found to be related more or less to functions of the endocrine and exocrine systems, as well as to those of the digestive system. An interesting example can be found in red pepper, whose unique irritable component, capsaicin, has a ‘hot’ flavour in seasoning. This substance, shortly after entering the body per os, interacts with vanilloid receptors such as Ca channels in sensory neurons inducing a specific dynamic action, possibly with an anti-obesity result. Capsaicin can thus be regarded as a functional factor with such a bilateral effect. Sensory abnormalities and disorders are other targets of functional food science, because insights into syndromes of these kinds will supply patients with organoleptically acceptable tailor-made foods having body-modulating functions at the same time.

The design and development of functional foods should not be carried out independently of purely nutritional and sensory or cognitive properties of foods. Future advances in relevant sciences for giving rise to foods with integral attributes would distinguish functional foods from medicines. Research along this line will add a newer dimension of research along this line will add a newer dimension to molecular breeding of new rice cultivated with an antiviral function.

The reader is advised to consult recently published papers to learn more about the state-of-the-art concerning functional foods (Arai et al. 2001; Kwak & Jukes, 2001). The former paper is the proceedings of a symposium, A Mainstay of Functional Food Science in Japan — History, Present Status, and Future Outlook, which was organized by the Japan Branch of the International Union of Food Science and Technology in collaboration with ILSI Japan.

References


thus to be understood as a concept (Roberfroid, 2000). Moreover, if it is function-driven rather than product-driven, the concept is likely to be more universal and not too much influenced by local characteristics or cultural traditions (Diplock et al. 1999).

In scientific as well as in more marketing-oriented literature, functional food has as many definitions as the number of authors referring to it. These definitions go from simple statements, such as:

1. foods that may provide health benefits beyond basic nutrition (IFIC Foundation, 1995) and
2. foods or food products marketed with the message of the benefit to health (Riemersma, 1996),

to very elaborate definitions such as:

1. food and drink products derived from naturally occurring substances consumed as part of the daily diet and possessing particular physiological benefits when ingested (Hillian, 1995);
2. food derived from naturally occurring substances, which can and should be consumed as part of the daily diet and which serves to regulate or otherwise affect a particular body process when ingested (Smith et al. 1996);
3. food similar in appearance to conventional food, which is consumed as part of the usual diet and has demonstrated physiological benefit and/or reduces the risk of chronic disease beyond basic nutritional functions (Health Canada, 1997); and
4. food that encompasses potentially helpful products, including any modified food or food ingredient that may provide a health benefit beyond that of the traditional nutrient it contains (Food and Nutrition Board, 1994).

But whatever definition is chosen, 'functional food' appears as a quite unique concept that deserves a category of its own, a category different from nutraceutical, f(php)armafood, medifood, designer food or vitafood, and a category that does not include dietary supplements. It is also a concept that belongs to nutrition and not to pharmacology. Functional foods are and must be foods, not drugs. Moreover, their role regarding disease will, in most cases, be in 'reducing the risk' rather than 'preventing' it (Roberfroid, 2000).

**Functional food: a European consensus**

The unique features of a ‘functional food’ are (Bellisle et al. 1998; Knorr, 1998):

1. a conventional or everyday food,
2. consumed as part of the normal/usual diet,
3. composed of naturally occurring (as opposed to synthetic) components, perhaps in unnatural concentrations or present in foods that would not normally supply them, and
4. having a positive effect on target function(s) beyond nutritive value/basic nutrition,
5. that may enhance well-being and health and/or reduce the risk of disease or provide health benefit so as to improve the quality of life including physical, psychological and behavioural performances, and
6. have authorized and scientifically based claims.

It is in this general context that the European Commission’s Concerted Action on Functional Food Science in Europe (FUFOSE), which actively involved a large number of the most prominent European experts in nutrition and related sciences, has been coordinated by the International Life Sciences Institute (ILSI) Europe. It developed in early 1996 to reach a European Consensus on ‘Scientific Concepts of Functional Foods’, which was published in 1999 (Diplock et al. 1999).

As already indicated above, and because functional food is a concept rather than a well-defined group of food products, that consensus document proposes ‘a working definition’:

‘A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease. A functional food must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet: it is not a pill or a capsule, but part of the normal food pattern.’

Diplock et al. (1999)

The main aspects of that working definition are:

1. the food nature of functional food — not a pill, a capsule or any form of dietary supplement;
2. the demonstration of effects to the satisfaction of the scientific community;
3. the beneficial effects on body functions, beyond adequate nutritional effects, that are relevant to an improved state of health and well-being and/or reduction of risk (not prevention) of disease, and
4. the consumption as part of a normal food pattern.

This definition encompasses all main features of functional foods identified above, and it is aimed at stimulating research and development in the field of nutrition so as to contribute adequately to the scientific knowledge that will be required to define optimum (optimized) nutrition by elaborating new dietary guidelines. But it should be emphasized that a functional food will not necessarily be functional for all members of the population, and that matching individual biochemical needs with selected food component intakes may become a key task as we progress in our understanding of the interactions between genes and diet (Kok, 1999).
From a practical point of view, a functional food can be:
1. a natural food,
2. a food to which a component has been added,
3. a food from which a component has been removed,
4. a food where the nature of one or more components has been modified,
5. a food in which the bioavailability of one or more components has been modified or
6. any combination of these possibilities.

**Strategy for development of functional food: functional food science**

Being foods, functional foods need to be safe according to all criteria defined in current food regulations. But in many cases, new concepts and new procedures will need to be developed and validated to assess functional food risks. In Europe, some, but certainly not all, functional foods will be classified as ‘novel food’ and, consequently, will require the decision tree assessment regarding safety that is described in the EU Novel Food Regulation (European Commission, 1997).

But that regulation does not concern nutritional properties or physiological effects of these novel foods. It is strictly a safety regulation. The requirement for safety is a prerequisite to any functional food development. Indeed the risk versus benefit concept, that is familiar to pharmacologists developing new drugs, does not apply to functional foods except, maybe, in very specific conditions for disease risk reduction when the scientific evidence is particularly strong.

As described in the European consensus document:

‘The design and development of functional foods is a key issue, as well as a scientific challenge, which should rely on basic scientific knowledge relevant to target functions and their possible modulation by food components. Functional foods themselves are not universal and a food-based approach would have to be influenced by local considerations. In contrast, a science-based approach to functional food is universal… The function-driven approach has the science base as its foundation — in order to gain a broader understanding of the interactions between diet and health. Emphasis is then put on the importance of the effects of food components on well-identified and well-characterized target functions in the body that are relevant to well-being and health issue, rather than, solely, on reduction of disease risk.’

Diplock et al. (1999)

By reference to the new concepts in nutrition outlined above, it is the role of ‘functional food science’ to stimulate research and development of functional foods (Fig. 1).

Referring to basic knowledge in nutrition and related biological sciences, such a development requires the identification and, at least partly, the understanding of the mechanism(s) by which a potential functional food or functional food component can modulate the target function(s) that is/are recognized or proven to be relevant to the state of well-being and health, and/or the reduction of a disease risk. Epidemiological data demonstrating a statistically validated and biologically relevant relationship between the intake of specific food components and a particular health benefit will, if available, be very useful. The conclusion of the first step will be the demonstration of a functional effect that should serve to formulate hypotheses to be tested in a new generation of human nutrition studies aimed to show that relevant (in terms of dose, frequency, duration, etc.) intake of the specified food will be associated with improvement in one or more target functions, either directly or indirectly in terms of a valid marker of an improved state of well-being and health and/or reduced disease risk. If well supported by strong scientific evidence, the conclusion could be a recommendation for improved or new dietary guidelines.

The new-generation human nutrition studies should be hypothesis-driven. But, in many cases, and even though they will have to follow the same basic rules in terms of quality of protocol design, quality of data management and value of statistical analysis, they will differ quite substantially from what is classically referred to as clinical studies. The main differences are that nutrition studies aim at testing the effect of a food as part of the ordinary diet, that they may concern the general population or generally large, at-risk target groups, that they are not diagnostic- or symptoms-based, and that they are not planned to evaluate a risk versus benefit approach. Most of these studies will rely on change(s) in validated/relevant markers to demonstrate a positive modulation of target functions after (long-term) consumption of the potential functional foods. A (double) blind-type of design based on parallel groups, rather than crossing-over, will generally be appropriate. Finally, the long-term consequences of the interaction(s) between functional foods and body function(s) will have to be monitored carefully.

**Markers: a key to the development of functional foods**

The development of functional foods will, in most cases, rely on measurements of ‘markers’. These markers need to be identified and validated for their predictive value of potential benefits to a target function or the risk of a particular disease. Markers of correlated events are ‘indicators’, whereas markers representing an event directly involved in the process are ‘factors’ (Diplock et al. 1999). When related to the risk of a disease, indicators...
and even factors might, in some instances, be equivalent to ‘surrogate markers’, defined as a biological observation, result or index that predicts the development of a chronic disease (Keystone, 1997). The more is known about the mechanisms leading to health outcomes, the more refined will be the identification of the markers and their appreciation. The markers should be feasible, valid, reproducible, sensitive and specific. They can be biochemical, physiological, behavioural or psychological in nature. But dynamic responses might be as useful as, or more useful than, static or single point measurements. In many cases, a battery of markers might be needed in order to create a decision tree from multiple tests.

These markers, most of which still need to be identified and validated (Fig. 2), should relate to:

1. exposure to the food component under study by measuring the serum, faecal, urine or tissue level of the food component itself or its metabolite(s), or the concentration of an endogenous molecule that is influenced directly by consumption of the food component;
2. target function(s) or biological response, such as the change in serum or other body fluids of the concentration of a metabolite, specific protein, enzyme or hormone, etc. (these first two markers are either indicators or factors);  
3. an appropriate endpoint of an improved state of well-being and health and/or reduction of a disease risk (such a marker is likely to be a factor rather than an indicator); and  
4. individual susceptibility or genetic polymorphism controlling the metabolism and/or the effect of the food component under study (Kok, 1999).

To develop these markers further, a state-of-the-art literature review will be necessary to identify, define and characterize potential markers. Furthermore, the basic scientific knowledge underpinning these markers should be evaluated. The next step will include the assessment of their relevance to physiological functions, well-being and health, and eventually disease risk. A validation will then be necessary both for the methodology and the biological relevance. Finally, the classification as indicator or factor will be made and potential dietary modulations will be demonstrated. New techniques such as those used by molecular and cellular biologists will be useful in identifying target groups who could benefit from the consumption of specific functional foods.

**Functional foods and claims: a communication and scientific challenge**

As stated in the European consensus on scientific concepts of functional foods:

‘As the relationship between nutrition and health gains public acceptance and as the market for functional foods grows, the question of how to communicate the specific advantages of such foods becomes increasingly important. Communication of health benefits to the public, through intermediates such as health professionals, educators, the media and the food industry, is an essential element in improving public health and in the development of functional foods. Its importance also lies in avoiding problems associated with consumer confusion about health messages. Of all the different forms of communication, those concerning claims — made either directly as a statement on the label or package of food products, or indirectly through secondary supporting information — remain an area of extensive discussion.’

Diplock et al. (1999)

It is also the opinion of Hudson (1994) that: ‘the links between nutrition science and food product development will flow through to consumers only if the required communication vehicles are put in place’. But the communication of health benefits and other physiological effects of functional foods remains a major challenge because:

1. science should remain the only driving force,
2. messages — claims — must be based on sound, objective and appropriate evidence, and
3. evidence must be consistent, able to meet established scientific standards and plausible.

Moreover, communication in nutrition generally comes from multiple sources that are sometimes contradictory, creating an impression of chaos. And chaotic information often generates ignorance and easily becomes misinformation. Regarding functional foods, claims associated with specific food products are the preferable means of communicating to consumers. In application of the fundamental

![Fig. 2. Schematic representation of the markers that can be used to discover and develop new functional foods.](image-url)
principles, any claim must be true and not misleading, it must be scientifically valid, unambiguous and clear to the consumer. These basic principles should be safeguarded without, however, becoming a disincentive to the production of functional foods or to the acceptance of these foods by consumers. Even though a general definition of ‘claim’ as widely accepted in the field of nutrition is: ‘any representation, which states, suggests or implies that a food has certain characteristics relating to its origin, nutritional properties ... or any other quality’ (Codex Alimentarius, 1991), one of the difficulties in communicating the benefits of functional foods is that distinct types of claim exist, and that especially the term ‘health claim’, traditionally used to communicate the benefits of foods, is defined differently in different part of the world. Seeking for clarity, Codex Alimentarius (1991) has classified and defined four different categories of claim, but excluding the term ‘health claim’, as:

1. relating to dietary guidelines,
2. relating to nutrient content,
3. being comparative (reduced, less, more) and
4. describing nutrient function (contains ..., that contributes to the development of ...).

These claims refer to known nutrients and their role in growth, development and normal function as well as to the concept of adequate nutrition. They are based on established, widely accepted knowledge but they do not refer to a particular effect over and above that expected from consuming a balanced diet. These claims are thus not really helpful to communicate the specific benefits of functional foods. Indeed the claims for functional foods should be based on the scientific classification of markers (indicators and/or factors) for target functions and on the effects on these markers. If such an effect, which goes beyond what could be expected from the established role of diet in growth, development and normal function in the body, concerns a target function or a biological activity without direct reference to a particular disease or pathological process, claims will be made for ‘enhanced function’. But, if the benefit is clearly a reduction of the risk of a disease or pathological process, claims will be made for a ‘disease risk reduction’. These two types of claim, which are specific for functional foods, are the ‘type A’ and ‘type B’ claims, respectively, as they are described in the European consensus on scientific concepts of functional foods (Diplock et al. 1999). The type A claim is similar to the ‘structure–function claim’, whereas the type B claim can be regarded as equivalent to the ‘health claim’ in the USA. Type B claim also corresponds to ‘health claim’ in Sweden (Swedish Nutrition Foundation, 1996). In its last proposed draft recommendations for the use of health claims, Codex Alimentarius (1999) has included type A and type B claims and defined them as follows:

1. Type A or claims that concern specific beneficial effects of the consumption of foods and their constituents on physiological or psychological functions or biological activities but do not include nutrient function claims. Such claims relate to a positive contribution to health or to a condition linked to health, to the improvement of a function or to modifying or preserving health.
2. Type B or ‘risk of disease reduction’ claims that concern the reduction of a disease risk related to the consumption of a food or a food constituent in the context of the daily diet that might help reduce the risk of a specific disease or condition.

One of the major issues, still to be resolved especially with these two types of claim, concerns the biological level at which evidence can be accepted as ‘satisfactorily demonstrating’ an enhanced function or a reduction of disease risk. This evidence should rely on all data available that can be grouped in three categories:

1. biological observations,
2. epidemiological data and
3. intervention studies, mostly based on markers.

But for any given specific food product, supporting evidence for enhanced function or reduction of disease risk might not be available or even not necessary from all three areas (Diplock et al. 1999). All supporting evidence should, however, be:

1. consistent in itself;
2. meet accepted scientific standards of statistical as well as biological significance, especially dose–effect relationship, if relevant;
3. plausible in terms of the relationship between intervention and results, especially in terms of mechanism(s) of action; and
4. provided by a number of sources (including obligatorily human studies) that give consistent findings able to generate scientific consensus.

Food technology and its impact on functional food development

From the point of view of food processing (Knorr, 1998; Diplock et al. 1999), the development of functional foods will often require an increased level of complexity and monitoring of food processing because

1. new raw materials including those produced by biotechnologies,
2. emerging thermal and non-thermal technologies,
3. new safety issues and
4. integration throughout the entire food chain, especially to ensure preservation and/or enhancement of functionality,

will have to be considered carefully.

The following main areas for technological challenge have been identified.

1. The creation of new food components in traditional and novel raw materials that add or increase functionality. Examples of such challenges are genetic modification, the use of under-utilized or unconventional natural sources (e.g. algae, seaweed) and the development of bioreactors based on immobilized enzymes or live micro-organisms.
2. The optimization of functional components in raw material and in foods, to ensure maximal preservation of the component(s), modify their function, increase their bioavailability, etc. Examples of such challenges are the development of membrane-processing techniques, the use of controlled and modified atmospheres, the use of high hydrostatic pressure, high-intensity electric field pulse technology and ultrasound treatments.

3. The effective monitoring, throughout the entire food chain, of the amount and functionality of the component(s) in raw materials and foods. Examples of such challenges are the monitoring of microbial viability and productivity for probiotic functions, the development of sensitive markers to record changes in speciation and the interactions with food components during processing, especially fermentation.

**Future trends and conclusions**

By reference to the conclusions of the FUFOSE concerted action (Diplock* et al.* 1999), future trends are as follows.

1. Components in foods have the potential to modulate target functions in the body so as to enhance these functions and/or contribute to reduce the risk of diseases, and ‘functional food science’ will contribute to human health in the future provided evidence is supported by sound scientific (mostly human) data.

2. Nutritionists and food scientists have the possibility, through the development of functional foods, to offer beneficial opportunities related to well-being and health and reduction of the risk of diseases. But the success of this new approach to nutrition will require the identification, characterization and development of methodologies to measure, and the validation of, relevant markers being indicators or factors to be used in human nutrition studies. The design of such studies still needs to be analysed carefully and developed specifically by reference to, but differently from, classical clinical studies that have been elaborated to help in the development of drugs, not food products.

3. Major target functions in the body that are or can be modulated by specific food products will have to be identified or characterized. The basic science to understand these functions, and how they relate to well-being and health or a particular pathological process, needs to be developed so as to give the necessary scientific base to develop new functional food products.

4. Progress in food regulation, which is the means to guarantee the validity of the claims as well as the safety of the food, will have to be made.

On the road to optimized nutrition that will be one of the major challenges of nutrition in the twenty-first century, functional foods have their own role to play. But the development of claims for already existing food products, as well as the development of new products and their own claims, should remain first a scientific challenge and not only a marketing challenge. This is the condition for success to the benefit of both human health and the food industry.

**References**


on the consumer acceptance of healthy foods and on functional food science.

Food and nutrition science is dealt with in Key Action 1, ‘Food, Nutrition and Health’, of the specific programme Quality of Life and Management of Living Resources of the Fifth Framework Programme (1998–2002). The calls under FP5 have resulted in thirty-three projects with a contribution of €51 million. The names and contract numbers of the projects can be read from Table 1.

### Table 1. Projects on functional foods

<table>
<thead>
<tr>
<th>Contract Number</th>
<th>Project Description</th>
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<tbody>
<tr>
<td>QLK1-1999-42</td>
<td>Nutritional enhancement of probiotics and prebiotics: technology aspects on microbial viability, stability, functionality and prebiotic function</td>
</tr>
<tr>
<td>QLK1-2001-67</td>
<td>Functional food, gut microflora and healthy ageing</td>
</tr>
<tr>
<td>QLK1-1999-76</td>
<td>Conjugated linoleic acid (CLA) in functional food: a potential benefit for overweight middle-aged Europeans</td>
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<tr>
<td>QLK1-2000-86</td>
<td>A process for the assessment of scientific support for claims on foods</td>
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<tr>
<td>QLK1-2000-108</td>
<td>Development and application of high-throughput molecular methods for studying the human gut microbiota in relation to diet and health</td>
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<tr>
<td>QLK1-1999-124</td>
<td>Functional properties, bioactivities and bioavailability of phytochemicals, especially anthocyanins, from processed foods</td>
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<tr>
<td>QLK1-2001-135</td>
<td>Functional assessment of interactions between the human gut microbiota and the host</td>
</tr>
<tr>
<td>QLK1-2000-146</td>
<td>Probiotic strains with designed health properties</td>
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<tr>
<td>QLK1-2001-173</td>
<td>Local Mediterranean food plants: potential new nutraceuticals and current role in the Mediterranean diet</td>
</tr>
<tr>
<td>QLK1-1999-179</td>
<td>European research on functional effects of dietary antioxidants</td>
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<tr>
<td>QLK1-2001-221</td>
<td>Isoflavones for reducing risk of coronary heart disease among postmenopausal women</td>
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<tr>
<td>QLK1-2000-266</td>
<td>The role of dietary phyto-oestrogens in the prevention of breast and prostate cancer</td>
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<tr>
<td>QLK1-2000-324</td>
<td>Barley β-glucan and wheat arabinoxylan soluble fibre technologies for health-promoting bread products</td>
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<tr>
<td>QLK1-1999-346</td>
<td>Symbiotics and cancer prevention in humans</td>
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<tr>
<td>QLK1-2000-431</td>
<td>The prevention of osteoporosis by nutritional phyto-oestrogens</td>
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<tr>
<td>QLK1-1999-505</td>
<td>Health implications of natural non-nutrient antioxidants (polyphenols): bioavailability and colon carcinogenesis</td>
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<tr>
<td>QLK1-2000-535</td>
<td>Design of foods with improved functionality and superior health effects using cereal β-glucans</td>
</tr>
<tr>
<td>QLK1-2000-563</td>
<td>Probiotics and gastrointestinal disorders: controlled trials of European Union patients</td>
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<tr>
<td>QLK1-1999-576</td>
<td>Folate: from food to functionality and optimal health</td>
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<tr>
<td>QLK1-2000-623</td>
<td>Towards a strategy for optimal vitamin D fortification</td>
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<tr>
<td>QLK1-1999-706</td>
<td>Functional foods against colon cancer: development of a genomics- and proteomics-based screening assay</td>
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<tr>
<td>QLK1-1999-888</td>
<td>Nutraceuticals for a healthier life: n-3 polyunsaturated fatty acids and 5-methyltetrahydrofolate</td>
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<tr>
<td>QLK1-2001-1179</td>
<td>Molecular analysis and mechanistic elucidation of the functionality of probiotics and prebiotics in the inhibition of pathogenic micro-organisms to combat gastrointestinal disorders and to improve human health</td>
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<td>QLK1-2001-1273</td>
<td>Biosafety evaluation of probiotic lactic acid bacteria used for human consumption</td>
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<td>QLK1-1999-1376</td>
<td>Increase in nutritional value of food raw materials by addition, activity or in situ production of microbial nutraceuticals</td>
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<td>QLK1-2000-1423</td>
<td>Enhancing the content of beneficial fatty acids in beef and improving meat quality for the consumer</td>
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<td>QLK1-2002-2362</td>
<td>Production of CLA-enriched dairy products by natural means</td>
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<td>QLK1-2002-2433</td>
<td>Seaweed antioxidants as novel ingredients for better health and food quality</td>
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<td>QLK1-2002-2453</td>
<td>Improving health through dietary phyto-oestrogens: a pan-European network on consumers’ issues and opportunities for producers</td>
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<td>QLK1-2001-70508</td>
<td>Development of new innovative functional foods containing microcrystalline chitosan</td>
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<tr>
<td>QLK1-2002-71361</td>
<td>Development of new food additives extracted from the solid residue of the tomato processing industry for application in functional foods</td>
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<tr>
<td>QLK1-2002-71714</td>
<td>Developing lignan-enriched functional food from linseed</td>
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<tr>
<td>QLK1-2002-72376</td>
<td>Microencapsulation of probiotic products for human and animal consumption</td>
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CLA, conjugated linoleic acid.