unless a different factor is given in a Codex standard or in the Codex method of analysis for that food.

3.4 PRESENTATION OF NUTRIENT CONTENT

3.4.1 The declaration of nutrient content should be numerical. However, the use of additional means of presentation should not be excluded.

3.4.2 Information on energy value should be expressed in kJ and kcal per 100 g or per 100 ml or per package if the package contains only a single portion. In addition, this information may be given per serving as quantified on the label or per portion provided that the number of portions contained in the package is stated.

3.4.3 Information on the amounts of protein, carbohydrate and fat in the food should be expressed in g per 100 g or per 100 ml or per package if the package contains only a single portion. In addition, this information may be given per serving as quantified on the label or per portion provided that the number of portions contained in the package is stated.

3.4.4 Numerical information on vitamins and minerals should be expressed in metric units and/or as a percentage of the Nutrient Reference Value per 100 g or per 100 ml or per package if the package contains only a single portion. In addition, this information may be given per serving as quantified on the label or per portion provided that the number of portions contained in the package is stated.

In addition, information on protein may also be expressed as percentages of the Nutrient Reference Value.[2]

The following Nutrient Reference Values should be used for labelling purposes in the interests of international standardization and harmonization:

Protein	(g)	50
Vitamin A	(µg)	800 [3]
Vitamin D	(µg)	5 [4]
Vitamin C	(mg)	60
Thiamin	(mg)	1.4
Riboflavin	(mg)	1.6
Niacin	(mg)	18 [4]
Vitamin B_6	(mg)	2
Folic acid	(µg)	200
Vitamin B_{12}	(µg)	1
Calcium	(mg)	800
Magnesium	(mg)	300
Iron	(mg)	14
Zinc	(mg)	15
Iodine	(µg)	150 [4]
Copper	Value to be established	
Selenium	Value to be established	

3.4.5 In countries where serving sizes are normally used, the information required by Sections 3.4.2, 3.4.3 and 3.4.4 may be given per serving only as quantified on the label or per portion provided that the number of portions contained in the package is stated.

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[2] In order to take into account future scientific developments, future FAO/WHO and other expert recommendations and other relevant information, the list of nutrients and the list of nutrient reference values should be kept under review.

[3] Proposed addition to Section 3.2.7 (Calculation of Nutrients) of the Codex Guidelines on Nutrition Labelling: "For the declaration of β-carotene (provitamin A) the following conversion factor should be used: 1 µg retinol = 6 µg β-carotene.

[4] Nutrient Reference Values for Vitamin D, Niacin and Iodine may not be applicable for countries where national nutrition policies or local conditions provide sufficient allowance to ensure that individual requirements are satisfied. See also section 3.2.4.1 of the Codex Guidelines on Nutrition Labelling.

3.4.6 The presence of available carbohydrates should be declared on the label as "carbohydrates". Where the type of carbohydrate is declared, this declaration should follow immediately the declaration of the total carbohydrate content in the following format:

"Carbohydrate ... g, of which sugars ... g".

This may be followed by the following: "x" ... g

where "x" represents the specific name of any other carbohydrate constituent.

3.4.7 Where the amount and/or type of fatty acids or the amount of cholesterol is declared, this declaration should follow immediately the declaration of the total fat in accordance with Section 3.4.3.

The following format should be used:

Total Fat		... g
of which	saturated fatty acids	... g
	trans fatty acids	... g
	monounsaturated fatty acids	... g
	polyunsaturated fatty acids	... g
Cholesterol		..mg

3.5 Tolerances and Compliance

3.5.1 Tolerance limits should be set in relation to public health concerns, shelf-life, accuracy of analysis, processing variability and inherent lability and variability of the nutrient in the product, and, according to whether the nutrient has been added or is naturally occurring in the product.

3.5.2 The values used in nutrient declaration should be weighted average values derived from data specifically obtained from analyses of products which are representative of the product being labelled.

3.5.3 In those cases where a product is subject to a Codex standard, requirements for tolerances for nutrient declaration established by the standard should take precedence over these guidelines.

4. SUPPLEMENTARY NUTRITION INFORMATION

4.1 Supplementary nutrition information is intended to increase the consumer's understanding of the nutritional value of their food and to assist in interpreting the nutrient declaration. There are a number of ways of presenting such information that may be suitable for use on food labels.

4.2 The use of supplementary nutrition information on food labels should be optional and should only be given in addition to, and not in place of, the nutrient declaration, except for target populations who have a high illiteracy rate and/or comparatively little knowledge of nutrition. For these, food group symbols or other pictorial or colour presentations may be used without the nutrient declaration.

4.3 Supplementary nutrition information on labels should be accompanied by consumer education programmes to increase consumer understanding and use of the information.

5. PERIODIC REVIEW OF NUTRITION LABELLING

5.1 Nutrient labelling should be reviewed periodically in order to maintain the list of nutrients, to be included in composition information, up-to-date and in accord with public health facts about nutrition.

5.2 A review of optional information for nutrition education including food groups will be needed as target groups increase in literacy and nutrition knowledge.

5.3 The present definition of sugars as in Section 2.6 and that of dietary fibre as in Section 2.7 and the declaration of energy as in Section 3.4.2 should be reviewed in the light of newer developments.

CODEX GENERAL GUIDELINES ON CLAIMS

CAC/GL 1-1979 (Rev. 1-1991) [1]

1. SCOPE AND GENERAL PRINCIPLES

1.1 These guidelines relate to claims made for a food irrespective of whether or not the food is covered by an individual Codex Standard.

1.2 The principle on which the guidelines are based is that no food should be described or presented in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding its character in any respect.

1.3 The person marketing the food should be able to justify the claims made.

2. DEFINITION

For the purpose of these guidelines, a claim is any representation which states, suggests or implies that a food has particular characteristics relating to its origin, nutritional properties, nature, production, processing, composition or any other quality.

3. PROHIBITED CLAIMS

The following claims should be prohibited:

3.1 Claims stating that any given food will provide an adequate source of all essential nutrients, except in the case of well defined products for which a Codex standard regulates such claims as admissible claims or where appropriate authorities have accepted the product to be an adequate source of all essential nutrients.

3.2 Claims implying that a balanced diet or ordinary foods cannot supply adequate amounts of all nutrients.

3.3 Claims which cannot be substantiated.

3.4 Claims as to the suitability of a food for use in the prevention, alleviation, treatment or cure of a disease, disorder, or particular physiological condition unless they are:

(a) in accordance with the provisions of Codex standards or guidelines for foods under jurisdiction of the Committee on Foods for Special Dietary Uses and follow the principles set forth in these guidelines;

[1] The Codex General Guidelines on Claims was adopted by the Codex Alimentarius Commission at its 13th Session, 1979. A revised version of the Codex General Guidelines on Claims was adopted by the 19th Session of the Commission in 1991. It has been sent to all Member Nations and Associate Members of FAO and WHO as an advisory text, and it is for individual governments to decide what use they wish to make of the Guidelines.

or,

(b) in the absence of an applicable Codex standard or guideline, permitted under the laws of the country in which the food is distributed.

3.5 Claims which could give rise to doubt about the safety of similar food or which could arouse or exploit fear in the consumer.

4. POTENTIALLY MISLEADING CLAIMS

The following are examples of claims which may be misleading:

4.1 Meaningless claims including incomplete comparatives and superlatives.

4.2 Claims as to good hygienic practice, such as "wholesome", "healthful", "sound".

5. CONDITIONAL CLAIMS

5.1 The following claims should be permitted subject to the particular condition attached to each:

(i) An indication that a food has obtained an increased or special nutritive value by means of the addition of nutrients, such as vitamins, minerals and amino acids may be given only if such an addition has been made on the basis of nutritional considerations according to the Codex General Principles for the Addition of Essential Nutrients to Foods. This kind of indication should be subject to legislation by the appropriate authorities.

(ii) An indication that the food has special nutritional qualities by the reduction or omission of a nutrient should be on the basis of nutritional considerations and subject to legislation by the appropriate authorities.

(iii) Terms such as "natural", "pure", "fresh", "home made", "organically grown" and "biologically grown" when they are used, should be in accordance with the national practices in the country where the food is sold. The use of these terms should be consistent with the prohibitions set out in Section 3.

(iv) Religious or Ritual Preparation (e.g. Halal, Kosher) of a food may be claimed provided that the food conforms to the requirements of the appropriate religious or ritual authorities.

(v) Claims that a food has special characteristics when all such foods have the same characteristics, if this fact is apparent in the claim.

(vi) Claims which highlight the absence or non-addition of particular substances to food may be used provided that they are not misleading and provided that the substance:

(a) is not subject to specific requirements in any Codex Standard or Guideline;

(b) is one which consumers would normally expect to find in the food;

(c) has not been substituted by another giving the food equivalent characteristics unless the nature of the substitution is clearly stated with equal prominence; and

(d) is one whose presence or addition is permitted in the food.

(vii) Claims which highlight the absence or non-addition of one or more nutrients should be regarded as nutrition claims and therefore should invoke mandatory nutrient declaration in accordance with the Codex Guidelines on Nutrition Labelling.

Eur J Nutr (2005) [Suppl 1] 44 : I/5–I/30
DOI 10.1007/s00394-005-1104-3

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Contents

* Process for the Assessment of Scientific Support for Claims on Foods.

EJN 1104

Executive summary

The Process for the Assessment of Scientific Support for Claims on Foods (PASSCLAIM) had the following principal objectives:

- to evaluate existing schemes which assess scientific substantiation;
- to produce a generic tool for assessing the scientific support for health claims for foods;
- to establish criteria for markers which can be used to explore the links between diet and health.

It has involved more than 160 experts from academia, industry, public interest groups and the regulatory environment. It has been supported by the Fifth European Community Framework Programme for Research and Technological Development and was co-ordinated by ILSI Europe.

Through an iterative process of discussion in expert groups and workshops, a set of criteria which define requirements for assessing the quality of scientific data reporting the impact of foods and food components on health and well-being have been proposed and progressively refined. As a basis for the development of the criteria, seven comprehensive reviews were produced covering examples of areas of diet, health and performance in which health claims are likely to be made. An eighth paper reviewed existing processes and regulations.

The criteria:

- emphasise the need for direct evidence of benefit to humans in circumstances consistent with the likely use of the food in order for a case to be made;
- recognise the usefulness of markers of intermediate effects when ideal endpoints are not accessible to measurement;
- stress the importance of using only those markers which are of proven validity; and
- highlight the necessity of ensuring that the magnitude and character of effects on which claims are based are statistically and biologically meaningful.

The criteria are presented in summary form, with an outline of the context within which the detailed assessment of the scientific evidence is to be undertaken. The criteria and the context within which they are to be assessed are further discussed and explained in depth in the present document. Whereas requirements relating to safety and other aspects of legislation are part of the context in which foods carrying claims are presented, and must be complied with, they are not part of the PASSCLAIM process and are excluded from the scope of the criteria.

The context within which a claim and the case made in its support should be assessed, involves considering existing legislation and dietary guidelines; the need for review in the light of evolving science; and the comprehensibility of the claim to consumers. These aspects are not thought to be part of the scientific criteria reviewed by PASSCLAIM. They nevertheless provide the background against which the scientific validity of claims should be justified.

Criteria for the scientific substantiation of claims

1. The food or food component to which the claimed effect is attributed should be characterised.
2. Substantiation of a claim should be based on human data, primarily from intervention studies the design of which should include the following considerations:
   - 2 (a) Study groups that are representative of the target group.
   - 2 (b) Appropriate controls.
   - 2 (c) An adequate duration of exposure and follow up to demonstrate the intended effect.
   - 2 (d) Characterisation of the study groups' background diet and other relevant aspects of lifestyle.
   - 2 (e) An amount of the food or food component consistent with its intended pattern of consumption.
   - 2 (f) The influence of the food matrix and dietary context on the functional effect of the component.
   - 2 (g) Monitoring of subjects' compliance concerning intake of food or food component under test.
   - 2 (h) The statistical power to test the hypothesis.
3. When the true endpoint of a claimed benefit cannot be measured directly, studies should use markers.
4. Markers should be:
   - biologically valid in that they have a known relationship to the final outcome and their variability within the target population is known;
   - methodologically valid with respect to their analytical characteristics.
5. Within a study the target variable should change in a statistically significant way and the change should be biologically meaningful for the target group consistent with the claim to be supported.
6. A claim should be scientifically substantiated by taking into account the totality of the available data and by weighing of the evidence.

This document presents a consensus view of criteria which, if met, provide a reasonable assurance that scientific data underpinning health claims made for foods are adequate for the purpose and that the claims can be considered valid. It also discusses the relative strengths and limitations of types of scientific approaches and data

that are relevant to different health and disease states. The discussion provides guidance on the interpretation of the criteria.

The criteria describe the standards by which the quality and relevance of the scientific evidence including new data should be judged, and thus the extent to which claims based on them can be said to be scientifically valid. As the view of a broad-based partnership of scientific and other experts, the criteria provide a basis for harmonising the requirements for, and the assessment of, scientific data supporting health claims made on foods which has a potential for positive impact across a spectrum of stakeholder activities, including those of interest groups within (consumers, health professionals and industry) and across (national and international regulatory agencies) geographic regions.

By raising the level of awareness of the essential attributes of the scientific data supporting health claims, the criteria have the potential to increase public confidence in the role of diet in maintaining and improving health and well-being. By defining the quality and type of scientific data required to substantiate health claims, the criteria will assist industry, including small and medium sized enterprises, to identify the scope for new products offering health benefits to consumers. Where there is a lack of specific expertise or resource to undertake development projects, the need for sound evidence bases, as illustrated by these criteria, could be seen as a stimulus for industry and government to encourage and support co-operative initiatives. Thus a harmonised regulatory approach to health claims for foods, operating within a EU single market in an ethos of increased consumer awareness of nutrition, along with confidence in the validity of claims, will provide a driver for innovative production of healthier foods appropriate for modern and changing lifestyles and needs. Collectively these factors should benefit public health and increase the competitiveness of the European agri-food industry in the global market.

Abbreviations

BMD	Bone mineral density
EC	European Commission
FAO	Food and Agricultural Organization
FOSIE	Food Safety in Europe
FUFOSE	Functional Food Science in Europe
HDL	High-density lipoproteins
IUPAC	International Union of Pure and Applied Chemistry
LDL	Low-density lipoproteins
PASSCLAIM	Process for the Assessment of Scientific Support for Claims on Foods
QC	Quality control
RCT	Randomised controlled trial
SME	Small or Medium sized Enterprise
WHO	World Health Organization

Introduction

Background and objectives

Much attention is now being paid to health claims on foods, including enhanced function claims, reduction of disease risk claims and also nutrient function claims. There are already on the market many food products with claims about health effects beyond the simple provision of nutrients. One important basis for claims is the increasing number of reports of the effects of dietary components on body functions. However there is no scientific consensus as to how claims based on these reports should be evaluated at European level [1]. In the absence of such a consensus, different national and international bodies are applying various approaches in their attempt to regulate an evolving market. The resultant fragmentation of the regulatory framework for claims leads to diverse and, perhaps, contradictory messages to consumers about diet and health, and uncertainty for the industry. With this background, ILSI Europe initiated the Concerted Action 'Process for the Assessment of Scientific Support for Claims on Foods' (PASSCLAIM). Its objective is to define criteria for assessing the scientific support for claims made in relation to foods. There are three main reasons for assessing the scientific substantiation of claims: 1) to provide truthful information and to support consumer confidence in foods with claims, 2) to satisfy regulatory requirements, and 3) to allow fair market competition. The availability of agreed criteria for this process should facilitate the achievement of these goals in a harmonised fashion.

The project builds on a previous major EU project, 'Functional Food Science in Europe' (FUFOSE). The main thrust of the FUFOSE Consensus Document on Scientific Concepts of Functional Foods in Europe, produced as the final deliverable from the FUFOSE project, was a scheme to link claims for functional foods to solid scientific evidence [2]. FUFOSE suggested that any claim for 'enhanced function' and 'reduced risk of disease' should be scientifically justified. The key importance of valid markers of exposure, enhanced function or reduction of disease risk was highlighted (Fig. 1).

In particular with respect to disease risk reduction claims, it was noted that the true disease endpoint often cannot be measured directly for ethical or practical reasons. Therefore, the identification and validation of suitable markers were considered as key issues. Markers were classified as related to 1) exposure, 2) a target function or biological response, and 3) an appropriate intermediate endpoint of an improved state of health and well-being, or reduction of the risk of disease, or both.

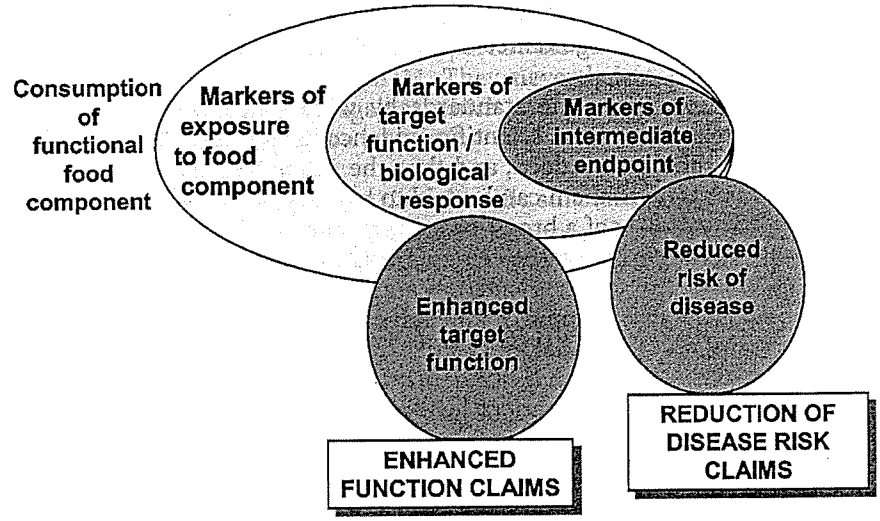

Fig. 1 FUFOSE concept of scientific evidence and corresponding health claims

The main objective of PASSCLAIM has been to produce a guidance tool to assess the scientific support for claims for foods and food components. The main outcome of the project is a set of common criteria that can be used as a basis for assessment of the scientific substantiation of claims. The way to develop valid scientific study designs and to identify, validate and use markers to explore the effects of diet on health was dealt with by seven expert groups, each focussed on a specific theme, and produced comprehensive reviews covering examples of areas of diet, health and performance. In addition, an eighth expert group comprehensively and critically evaluated existing legislation and voluntary codes of practice used to assess the scientific substantiation of claims around the world; this has been presented in a comprehensive review.

The PASSCLAIM project focussed on beneficial effects of foods and food components on health. Safety is a prerequisite for all foods. Considerations of nutritional safety are particularly relevant for foods for which claims are made relating to nutrition and health. However, safety is not a consideration in the data supporting the scientific validity of the claims themselves and safety issues were not within the scope of the PASSCLAIM project. Safety was the subject of another major European Commission (EC) concerted action, Food Safety in Europe (FOSIE) – Risk Assessment of Chemicals in Food and Diet [3]. The discussions in both projects underlined the need to look at risk assessments and benefit assessments in combination. As a consequence, a programme has been initiated to develop a common basis for the comparison of risks and benefits associated with a given food product or product modification.

Enhanced function claims were defined in FUFOSE [2] as claims that concern specific beneficial effects of nutrients and other substances on physiological and psychological functions or biological activities beyond their established role in growth, development and other normal functions of the body. In Codex Alimentarius working groups, nutrient function claims, referring to the normal physiological effects of nutrients in growth, development and normal functions of the body, have been included under health claims. In Codex terms, "Other function claims" are more or less equivalent to enhanced function claims. In the proposed EU regulation [4], "health claims describing a generally accepted role of a nutrient or other substance" would include both nutrient function claims and other function claims (Table 1).

A major legislative issue so far has been the fact that claims about prevention, alleviation and cure of diseases are confined to medicinal products. Accordingly, the mention of food effects in relation to disease on food labels or in other promotional material has been regarded as a medicinal claim. However, disease risk reduction by means of healthy diets is a well-established concept in nutrition and is a basis for official dietary recommendations. Accordingly, authorities in the USA have allowed generic disease risk reduction claims for certain foods since 1993. A major breakthrough in developments in Europe is that recently the EU Commission has also appreciated that foods may contribute to the reduction of the risk of disease and that such effects should be regulated in the context of food legislation [4]. This has provided the basis for the current development of an EU regulation on nutrition and health claims for foods, including the possibility to use disease risk reduction claims. The distinction between "the prevention of a disease" and "the reduction of the risk of a disease" is still being discussed.

The FUFOSE conclusions and principles are now taken to the next logical stage, which is that of applying the principles. The project 'Process for the Assessment of Scientific Support for Claims on Foods (PASSCLAIM)' starts with, and builds upon, the principles defined

Table 1 Health claims classification according to FUFOSE, Council of Europe, Codex Alimentarius and the proposed EU regulation

FUFOSE (1998)	Council of Europe (2001)	Codex Alimentarius (2003)	Proposed EU regulation (2003)
Nutrient function claims not considered	Nutrient function claims not considered	Nutrient function claims	Health claims related to the generally accepted role of nutrients and other substances
A. Enhanced function claims	A. Enhanced function claims	Other function claims	
B. Disease risk reduction claims	B. Disease risk reduction claims	Disease risk reduction claims	Health claims related to disease risk reduction

Nutrient function claims (sometimes referred to as structure function claims), enhanced function claims, and other function claims are closely related, but have been introduced at different stages of the claim development discussion. The dotted lines indicate that there is no absolute delineation between "nutrient function claims" on the one hand and "enhanced function/other function claims" on the other hand. A "new" function of a nutrient may be regarded as an enhanced/other function until, through further documentation, practice and familiarity, it becomes generally recognised as a "nutrient function claim". A function of a non-nutrient would be regarded as "other function" according to Codex, but as science advances, it may later fall under "generally recognised effects of nutrients and other substances" according to the proposed EU regulation [1]

within the FUFOSE project. The Concerted Action PASSCLAIM (QLK1–2000–00086) was supported by the EC, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 (KA1) on Food, Nutrition and Health, and is coordinated by ILSI Europe.

In the context of this report, the term "health claim" is understood in the sense defined by Codex Alimentarius – i. e. "any representation that states, suggests, or implies that a relationship exists between a food or a constituent of that food and health". In this paper, the word "claim" means "health claim" and includes all claims related to health, well-being and performance (including both physical and mental performance). The term "food component" includes components such as ingredients and food additives intentionally added to foods, as well as components that are part of the natural composition of foods.

Structure

Experts from academia, industry, public interest groups and regulatory bodies in 24 countries have contributed to the PASSCLAIM Project. In order to meet the project objectives, eight expert groups ("Individual Theme Groups" or ITGs) were set up involving experts from academia, regulatory bodies and the food industry. Representatives of public interest groups were also approached. Seven of the expert groups reviewed the scientific basis for claims in various areas of health and disease with a focus on markers. One group critically evaluated existing international approaches to the scientific substantiation of claims.

The development of criteria for the scientific substantiation of claims, based on the results of the expert groups, was the focus of a first and a second plenary meeting. A first set of draft interim criteria was discussed and modified at the first plenary meeting [5]. The interim criteria were then tested through practical application by the second phase expert groups and further developed at the second plenary meeting [6].

The structure of the project is illustrated in Fig. 2. The steps taken by the different expert groups were to:

- collate examples of potential types of claims in different areas from the perspective of physiological functions and, if it was relevant, disease states;
- describe the scientific requirements for the quality of data needed to support these claims and to evaluate the relevance of the scientific support;
- assess the usability of markers for the scientific substantiation of the claims;
- develop a list of criteria for use in assessing the portfolio of evidence submitted to substantiate identified and potential claims.

Phase One expert groups

Initially, in 2001, four 'Phase One' expert groups were set up [5]. The following summary reflects the discussions and conclusions of these expert groups.

Synthesis and review of existing processes [1]. This group critically evaluated most of the existing international codes of practices and regulations in relation to the scientific substantiation of claims with a view to identifying common ideas, definitions, best practice and methodology to underpin current and future developments. The group summarised the regulatory approaches to claims as set out by seven countries and two international organisations. A common feature in all these approaches is the requirement for solid scientific substantiation. The group focussed on processes existing up to 2002 for assessing the scientific substantiation of claims, which includes identification of all relevant studies, evaluation and interpretation of the totality of the evidence and the concept of "significant scientific agreement". The group proposed a procedure for reviewing the evidence in support of claims, a protocol for extracting data from individual research papers in a systematic and consistent manner and a template for the documentation of evidence.

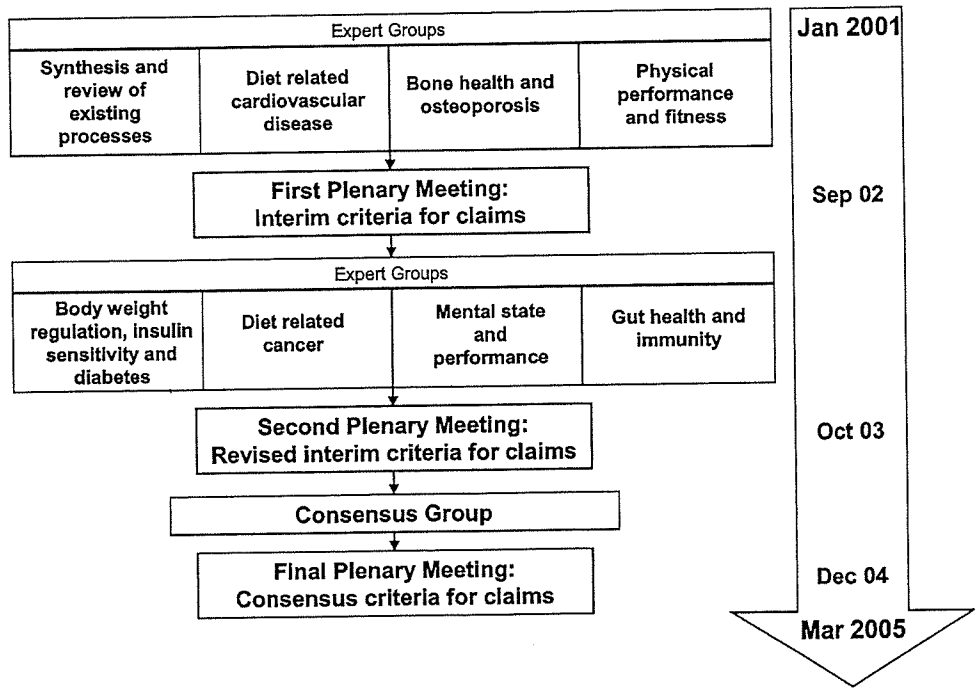

Fig. 2 Structure of the PASSCLAIM project

Diet-related cardiovascular disease [7]. From the wealth of publications in one of the most researched areas of food and health, the group concluded that LDL cholesterol and blood pressure are well-established markers generally accepted as related to changes in risk of cardiovascular diseases. Claims for enhanced function could be made for diet-related changes in LDL cholesterol and blood pressure and, since the relationship with disease risk is well-established, changes in these markers would also support disease risk reduction claims. HDL cholesterol, fasting triacylglycerol and plasma homocysteine are established as examples of markers sensitive to dietary factors and are validated methodologically, but it is as yet not clear to what extent changes in these markers reflect enhanced function and reduction of disease risk. For haemostatic function and oxidative damage, there is a need to develop and validate markers of enhanced function and disease risk reduction that are sensitive to dietary changes.

Bone health and osteoporosis [8]. Although bone health problems encompass many skeletal disorders, the group focussed on osteoporosis because this is a major public health issue in the EU. Bone mineral density (BMD), a measure of the calcium content in bones, was identified as an example of a marker of enhanced function in relation to bone strength for people of any age and sex. For people over 50 years of age living in countries with a high risk of fracture, BMD was considered to be a good marker of fracture risk, meaning that changes in BMD caused by a food component could provide evidence of a reduction in disease risk, that is reduced risk of fractures.

Physical performance and fitness [9]. The group reviewed claims relating to muscular strength and power, endurance, energy supply and recovery, hydration status, flexibility, tissue growth, and general immune functions. Many methods for measuring these fitness parameters as evidence for claims were examined, including tests of muscle strength, energy metabolism, food intake, body composition, gastrointestinal function and immune function. A database of methods including advantages and disadvantages of use of these methods was generated. The group concluded that for all physical performance and fitness domains, there are markers and endpoints available that fulfil the criteria for the substantiation of claims. For most areas, reliability and validity were considered to be good. On the other hand, with respect to immune functions in relation to physical performance and fitness, interpretation of the available markers and endpoints was considered problematic.

First Plenary Meeting

The information resulting from the Phase One expert groups provided the building blocks for a first draft set of interim criteria for the scientific substantiation of claims on foods and food components. This was the starting point for discussions at the first Plenary Meeting, held in Berlin, Germany in September 2002, and interim criteria were the main output from the meeting.

The reports of "Phase One" expert groups and an interim set of criteria were published [5].

Phase Two expert groups

In the Second Phase of the project, the "interim criteria" [5] were used by four further expert groups ("Phase Two" expert groups) during 2002–2003 to explore the following additional areas [6], and the principal conclusions from these groups are summarised below.

Body weight regulation, insulin sensitivity and diabetes [10]. The biological functions underlying these three conditions were characterised and related to the corresponding diseases overweight, metabolic syndrome and diabetes. The group was able to identify good markers and reliable measurement methods for the modulation of each key target function with its range of associated functions. Regarding body weight regulation, the target function is body fat deposition, which can be measured with both laboratory and field methods. A number of associated functions involved in the regulation of body fat can be measured as well. Insulin sensitivity is the target function in the metabolic syndrome and validated methods are available for its measurement. Measurable functions associated with insulin sensitivity include lipotoxicity, body fat composition, oxidative stress, inflammation and vascular function. In diabetes mellitus, the target function is regulation of blood glucose level, associated with functions such as glucose delivery to the bloodstream, glucose utilisation, and insulin secretion and sensitivity.

Diet-related cancer [11]. It has been suggested that approximately one third of all cancers are caused by inappropriate intakes and imbalances of food components. It is therefore of key importance to develop clear criteria to substantiate cancer risk reduction claims for foods or food components. The group focused on tumours of the colon, lung, breast and prostate. Eighteen markers were identified that represent events at various points in the chain from the initial exposure to carcinogens to the overt malignant tumour. The true endpoint in this area – the malignant human tumour – usually cannot be measured as a basis for claims. Pre-cancerous lesions, such as polyps in the colon, were regarded as a good example of a strong marker, and the recurrence of polyps in humans was regarded as the only good marker currently available on which to base reduction of disease risk claims. The development of markers of events in the pathogenic process, which can be used as surrogate endpoints, is therefore essential.

Mental state and performance [12]. Foods and drinks can influence brain functions and affect mental state and performance. Claims relating to several aspects of mental function can be substantiated using validated scientific instruments (tests, questionnaires etc.). The group examined mood, arousal (including activation, vigilance, attention and sleep), motivation and effort, perception, memory and intelligence. For each of these functions, a critical review of validated instruments was presented. In the area of mental effects, the final endpoint (improved function) can often be assessed directly using appropriate tests as opposed to physiological or other intermediate markers. In other cases, markers can be used as in other fields. The group concluded that validated methodologies exist to generate sound scientific evidence supporting the beneficial influence of many foods and nutrients on a broad variety of mental functions.

Gut health and immunity [13]. Many parameters of digestion can be measured, such as absorption and secretion, bowel habit and transit time, the gut flora, gastric emptying and motility, but interpretation is complicated by the large individual variability within what is considered to be a normal range. The group defined normal function as far as possible and methods for measuring it. The well recognised but ill-defined concept of gastrointestinal well-being was also discussed and identified as an important area for future method development. The immune system was seen as being difficult to make quantitative judgements about. No single test can define immune function but measurement of several parameters in combination can be used to assess functional capacity.

Second Plenary Meeting

The interim criteria resulting from the First Plenary Meeting were developed further at the Second Plenary Meeting held in Bordeaux, France in October 2003, taking into consideration the outcome of the Phase Two expert groups. Their reports formed the starting point for discussions at the meeting. The meeting resulted in proposals for a number of changes to the interim criteria and a summary of the discussion and comments from the meeting participants have been published [6].

Consensus Group

In the third phase of the PASSCLAIM project during 2004, a Consensus Group* was formed whose role was to refine and clarify the criteria for the assessment of the scientific support for claims on foods and food components taking into account the input from the expert groups, from the working groups and general discussions at the First and Second Plenary Meetings, and from individual comments.

* The members of the Consensus Group are the authors of this document.

Final Plenary Meeting

The draft set of criteria proposed by the Consensus Group was reviewed by the Final Plenary Meeting held in Lisbon, Portugal in December 2004, resulting in the final set of criteria presented in this document.

A number of general points have been discussed which relate to the context in which claims must be assessed. Fulfilling these is a prerequisite to the assessment of the portfolio of submitted evidence. After these contextual conditions are discussed, the proposed final criteria are presented, accompanied by summaries of the motives, explanations, comments and discussions behind their development.

The criteria relate specifically to the assessment of scientific evidence and information submitted to support claims on foods. They have not been developed as guidelines for study protocols and the acquisition of that evidence, but they nevertheless indicate the quality and nature of the evidence such protocols should produce.

Context for the scientific substantiation of claims

Evidence for the substantiation of claims needs to address some core principles which should be followed in providing an evidence-based justification for a claim. Some general aspects are outside the scope of the PASSCLAIM project because they do not deal with the science base. Others have generic implications relating to the scientific evidence and these are reviewed here. The specific characteristics of the evidence to be presented in the scientific substantiation of claims are considered in later sections of this document.

Foods and food components for which a claim is made should comply with existing legislation and fit into a healthy diet

Foods with claims should comply with all relevant regulations, including those relating to safety. The need for compliance with all relevant legislation and dietary guidelines in their respective markets relates primarily to ensuring the safety, including nutritional safety, of foods. A major purpose of food legislation is to ensure that under normal conditions of use a food is safe for the consumer. Accordingly, foods and food components for which claims are made need to be assessed for possible undesired side effects, including undesirable nutritional effects, according to the same standards that are applied to other foods and food components. They should also fit into a healthy diet.

Regulations should in principle reflect the evolving science base taking into account new scientific developments as appropriate

Regulatory and advisory agencies should be alert and responsive to the continuous research and improving scientific knowledge concerning the functionality of foods. The developing science base would be expected to lead not only to new claims but also to the need periodically to re-assess existing claims.

FUFOSE [2] and the expert groups within PASSCLAIM have demonstrated the principles for the scientific substantiation of a wide range of health benefits, and have illustrated these with specific examples, relating to physiological, psychological and mental functions [12, 14], of which several have been accepted as bases for claims by national authorities [1]. The decision on the justification of a claim should give due weight to current knowledge and the evidence-base for outcomes, and the primary basis for allowing a claim should be the soundness of the science or evidence-base.

A claim should reflect its scientific basis and, at the same time, should be understandable, and not be misleading for the intended consumer

A claim needs to be scientifically correct and understood by the intended consumers. Conversely, the need to use consumer friendly language should not conceal any lack of scientific substantiation of the proposed claim. If only part or parts of the population can be expected to experience the claimed benefit, this should be clearly stated and those who would be expected to experience the benefit should be identified. Otherwise consumers may be misled.

Claims can help to translate scientific learning into useful communication to the consumer. Ultimately, in this context, the investment is only justified if research demonstrates a clear health benefit that is communicated to the consumer. As such, claims may be a valuable means of promoting public understanding of science.

Criteria for the scientific substantiation of claims

Criterion 1. The food or food component to which the claimed effect is attributed should be characterised.

The food or food component for which a claim is made must be sufficiently characterised and described in the submission to allow an assessment of the validity of the scientific case made in support of the claim. The proper design of a programme of scientific studies requires that the food or food component be sufficiently characterised at the outset to enable comparability between studies and to ensure that the levels of exposure can be linked quantitatively to the claimed effect. Knowledge of effects, either beneficial or adverse, may need also to be related to the particular composition of the food matrix (see criterion 2 (f)).

Some aspects of this criterion would be covered by

existing legislation (Context for the scientific substantiation of claims) but it is important to emphasise that information should be provided on the origin and nature (including processing methods) of the component or product for which the claim is being made. Furthermore, evidence should be provided that the food or component of the food for which the claim is made is sufficiently standardised to ensure that the composition of the marketed product reflects fully and consistently the composition and nature of the material for which the data (see criterion 2) are provided. It is also important that the characterised food or food component relates to the food or component as it is consumed.

Criterion 2. Substantiation of a claim should be based on human data, primarily from intervention studies the design of which should include the following considerations:

- **2 (a) Study groups that are representative of the target group.**
- **2 (b) Appropriate controls.**
- **2 (c) An adequate duration of exposure and follow up to demonstrate the intended effect.**
- **2 (d) Characterisation of the study groups' background diet and other relevant aspects of lifestyle.**
- **2 (e) An amount of the food or food component consistent with its intended pattern of consumption.**
- **2 (f) The influence of the food matrix and dietary context on the functional effect of the component.**
- **2 (g) Monitoring of subjects' compliance concerning intake of food or food component under test.**
- **2 (h) The statistical power to test the hypothesis.**

2. Substantiation of a claim should be based on human data, primarily from intervention studies.

A claim can only be considered substantiated if there is a body of evidence that demonstrates an effect in the target population (see Table 2).

There are many forms of human studies, which can broadly be classified into intervention and observational. Supporting evidence may be based on animal, in vitro or modelling experiments.

Intervention studies include the randomised controlled trial (RCT) in healthy subjects, or in patients in which case they are called clinical trials, and studies looking at physiological or psychological effects. Of all studies, RCTs are thought to provide the best standard of evidence. An RCT is a study in which people are allocated at random to receive one of two or more interventions, one of which would usually be an inactive or control intervention. They are often the final piece of evidence for a claim, after data have been gathered from observational and other types of study. Endpoints can include markers of risk as well as physiological changes and other health outcomes. Since reproducibility of an effect is fundamental to progress in biological science, more than one RCT are desirable.

Table 2 Categories of evidence that may be used in the substantiation process

Intervention
Randomised controlled trials
Clinical trials
Physiological and psychological trials
Observational
Prospective (cohort)
Cross-sectional (analytical)
Case-control
Supporting
Animal
In vitro cell and molecular
Studies of genotype
Modelling (of mechanism)

Physiological and psychological studies have a long and distinguished history in the testing of hypotheses linking food and food components to health. These studies are also hypothesis driven and have to meet rigorous standards of research governance and laboratory practice, statistical design and ethical probity and still form one of the major inputs into understanding the role of diet and health. In the historical context, such studies were the predecessors of the modern RCT study design. In the current context, they provide means for a detailed characterisation of effects and their possible mechanistic bases. They require healthy subjects in a highly controlled environment and allow integration of cellular and molecular studies into whole body metabolism. They also provide a good basis for dose-response studies. In addition, they may be carried out in a randomised fashion, with or without cross-over between conditions.

Clinical studies (i. e. studies in patients) might be used for substantiation of claims for the general population although they are essentially studies of people who are ill and who may be receiving treatment such as drugs and whose physiological functions may be disturbed in many ways. There are potential problems with these studies because ill health can affect dietary intake, nutritional state and metabolism and there can be difficulties selecting an appropriate control group. However, there is often a continuous spectrum in a physiological variable, such as blood pressure, cholesterol, or bowel habit between healthy and disease states, and subjects

"at risk" are legitimate targets for claims. In these circumstances clinical studies can usefully contribute to the process.

Observational studies are often loosely referred to as epidemiological studies. They include prospective (cohort) studies, case control and cross-sectional (analytical) studies. In a cross-sectional study, observations on suspected causes and outcomes are made at one point in time. Variables, such as salt intake in individuals and blood pressure, can be measured in a group of subjects and an assessment made of whether they are associated. Gross national measures of dietary intake, for example red wine consumption, can be related across different populations to national death rates of cardiovascular diseases. An important drawback from the cross-sectional approach is that it is not known whether exposure to the putative cause being measured actually preceded the outcome of interest. Case-control studies aim to address this limitation by comparing subjects with and without disease and assessing past exposure in both groups in relation to suspected causes. In this type of study, however, recall bias arising from the retrospective estimation of exposure is a drawback. This shortcoming can be addressed by a prospective study design in which a group of subjects without disease (a "cohort") are followed in time and their exposure to putative causative factors and the subsequent development of disease are monitored with a view to establishing whether the temporal incidence of disease can be related to exposure to the factors of interest. The main remaining drawback with this type of study is the difficulty of accounting for unknown confounding factors, which influence the incidence of disease.

The probability of a causal relationship in human studies can, according to Bradford-Hill, be analysed with reference to five key features [15, 16] if these are applied to the interpretation of data relating to evidence supporting claims for food then they are:

- Temporality: exposure to the possible cause must precede the outcome;
- The strength of the relationship: the stronger the association, the more probable that it is causal;
- A dose-response effect;
- Consistency across all lines of evidence and studies;
- Existence of an analogy.

The strength of evidence derived from observational studies differs depending on methodology. If they are all well designed, well performed and well analysed findings in prospective cohort studies should receive more weight than data from case-control and cross-sectional studies.

For studies of food, the anatomy and physiology of humans and animals are generally not sufficiently comparable to allow evidence from animal experiments to provide the basis for claims for humans. There are major differences in the amount and composition of food intake and in longevity, lipid metabolism, gastrointestinal function and microbiology amongst species. Animal studies can, however, provide insights that may be used in the design of human studies and may be necessary in circumstances where the use of human subjects is unethical. They may provide supporting evidence in cases where the comparability of specific parameters between animal and human has been established.

In vitro cellular and molecular studies often provide supportive evidence of the effect of food and food components on cell function. They do not on their own indicate a health benefit or change in physiology that might be the basis for a claim. However, such studies can provide insights into mechanisms and can lead to the identification of markers for use in other studies. They are considered especially useful for looking at the genetic control of metabolism. Such studies should:

- use cell lines appropriate to human tissue;
- have functionally relevant genes and proteins expressed;
- use defined exposures to the food or food component;
- be repeatable in more than one experimental system.

Many laboratory or computer based models are now used in nutrition to circumvent the long and costly procedure of human studies, to dissect out mechanisms and predict behaviour in biological systems. Such models can provide additional evidence for the substantiation process.

For all studies and methodologies, quality and power may take precedence over the type of study in weighing evidence for the substantiation process (criterion 6).

International and national expert review panels, such as the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO), from time to time publish a collective view on diet and a particular aspect of health. These reviews, which may be based on many of the same strands of evidence as would be used in the substantiation process for a claim, can be used to inform this process and provide valuable background information, especially to support generic claims.

Mechanism. A mechanism expressed in terms of a physiological, psychological or cellular function that explains the association between observed dietary intake and resulting health effects, adds credibility to a claim, and provides strategies for the development of markers (see criterion 3). Historically, however, elucidation of mechanisms has often followed the demonstration of health benefits of food components or foods and the implementation of public policy. A classic example of this would be the recommendation more than 50 years ago, resultant upon the Seven Countries Studies, of a reduction in dietary saturated fat to decrease the risk of coro-

nary heart disease. The mechanisms involved in fat, lipid metabolism and atheroma are still not yet fully understood. For substantiation of a claim, it is, therefore, currently more important to demonstrate a consistent effect of a food or food component on health across a range of studies than to have a scientifically substantiated mechanism.

A mechanism, therefore, is not essential, but could be important in studies where markers were being used as surrogates because the relevant health endpoints such as, for example, prevention of fracture or reduction of risk of cancer, cannot be assessed directly. A problem with mechanisms that should also be taken into consideration is that the understanding of them tends to evolve as experimental data are forthcoming. At a point in time, therefore, one mechanism may be accepted, but later another may be better demonstrated. This then alters the perception of the nature of health itself and the understanding of the role of diet, which can change from generation to generation.

Nevertheless, an understanding of mechanisms is valuable because it allows the development of products more specifically to alter physiological systems with benefit to health. Therefore, human intervention studies designed for the development of mechanistic hypotheses, including collection of data on absorption, distribution, metabolism and excretion of the food or food component under test, should be encouraged.

Although a clear mechanism is not essential to progress claims in relation to dietary components, most if not all of the PASSCLAIM expert groups detailed the physiological, metabolic and molecular events that link markers with physiological and health effects. In other words, for many claims substantial bodies of knowledge already exist which allow mechanisms to be proposed and hypotheses for the effect to be described.

The design of studies should include the following considerations:

2(a) Study groups that are representative of the target group

Study groups should match as nearly as possible the target group, considering, as is appropriate for the food or food component and outcome under study, physiological and other variability arising from, for example, age, gender, diet, activity and smoking habits and other lifestyle factors. Where relevant, genotype should be taken into account.

Results gathered from a study group will be extrapolated to the group targeted by the claim. This could be either the whole population, or a specific sub-group (elderly, obese, smokers, runners, students, pregnant women). The effects induced by a food or food component in the study group are expected to occur in the targeted group, therefore the physiology or psychology of the study group should be representative of the target group. When the functions and the mechanisms involved in the claimed effect are distributed in the same way in the whole population there is no need to have specific data on sub-groups.

When a claim is specifically addressing a target group, obese people for example, studies on cohorts from this target group are essential. The appropriateness of the study group must always be considered on a case by case basis.

Identification of genotype pertinent to the physiological or psychological process under study is becoming increasingly feasible, and important for interpretation of results. For example there are now well recognised polymorphisms in the genes controlling the metabolism of folic acid, isoflavones and lipoproteins, which may affect the outcome of studies.

The issue generally is to avoid a study group that is not representative of the target population. For example, reduction of osteoporosis in post-menopausal women cannot be extrapolated from studies on young women nor from studies on men.

In all human studies the following factors should be considered and addressed when relevant:

- Age
- Gender
- Ethnic origin
- Genotype relevant to the function under study
- Lifestyle factors, for example – smoking, physical activity, alcohol consumption
- Body weight and height
- Menstrual cycle
- Usual diet
- Environmental conditions such as climate

2(b) Appropriate controls

Defining an appropriate control is often not easy in dietetic and nutritional studies. The amount of food consumed every day is roughly constant and when a new food is added to a diet, another may be left out or eaten in a smaller quantity. Therefore the addition of a food or food component may induce an effect by itself by the removal or displacement of another food. This is known as a passive effect and was the original explanation for the effect on cholesterol that is seen when dietary saturated fat is substituted with polyunsaturated fat.

The second difficulty is that many foods cannot be studied in a 'blinded' way. For example it would be difficult to find a suitable control in a study supporting the beneficial effect of consuming fruits and vegetables. An appropriate design and randomisation is required, including, in cross-over studies, adequate wash-out periods, and the control will be a usual food providing similar nutrients. On the other hand, when a component can be hidden in a product then the use of a control product without the component is recommended. Whenever possible a control product should be used.

The postulated active principals of the tested food must be either absent, or present at a known concentration, in the food given to the control group. This concentration must be significantly different from (usually significantly lower than) that in the test food.

Not only the food or food component, but the process of the study itself can have objective or subjective effects or both, on the study outcome. These may not actually be related to specific effects emanating from the test substance. Such placebo or nocebo phenomena may happen for both control and test products and need to be considered in the study design.

The claim must be assessed on the product as it is intended to be consumed. This means that normally the test and control material should be the same as, or closely represent the food or food component as it is intended to be marketed and purchased.

Subjects should be selected on the basis that the appropriate control group is one with a typical diet, and not a special diet that might interfere with the intended benefit. For example, it might not be appropriate to use vegetarians to test the effect of an added fibre.

2(c) An adequate duration of exposure and follow up to demonstrate the intended effect

There are two aspects to this criterion. These are ensuring (i) that there has been a suitable period of exposure to the food or food component (period of intake), and (ii) that the duration of observation is long enough for the expected effect to occur, and, if necessary, to show that the benefit is sustained.

The effects of a food may appear after consumption on a single or few occasions; for example the effect of glucose on memory performance or the effect of low glycaemic index foods on post-meal satiety. Alternatively a food may need to be consumed over a number of weeks before an effect occurs; examples of this include changes induced by prebiotics on intestinal function; or by stanols or sterols on cholesterol metabolism. Sometimes months or years might be needed to observe key effects; for example changes in bone density in response to calcium: any evidence of a reduced risk of certain cancers: and the impact of low glycaemic index foods on the risk of diabetes and obesity. A human intervention study must ensure that the product is ingested long enough to allow the claimed effect to appear. In many instances this will not be practicable and alternative approaches to assessing the claimed benefits are needed (criterion 3).

Equally important is that effects can appear in people after variable delays following intake of the food. In the simplest situation, an effect appears after a predictable time delay, increases to reach a plateau and then decreases and disappears. Other effects are bi-phasic: a change in a biological parameter can be followed by an opposite change. Some but not all effects are cumulative over time. Some substances may progressively induce tolerance, so that the observed effect becomes attenuated. There may be certain periods during which effects would occur and need to be observed. Intervention studies should consider if and how all these possibilities should be addressed.

The sustainability and nature of the effect with continuing and discontinuing intakes need to be characterised. For example, a pro- or prebiotic may produce a change in gut bacteria within a few days but the sustainability of this effect with continued ingestion of the prebiotic, or the persistence of the effect if consumption of the prebiotic stops, need to be known.

A further example is the functional effect of low glycaemic index foods, which can be assessed at various intervals after single or repeated intakes. Post-ingestive glycaemic and insulinaemic effects should be studied in the hours following intake and satiety should also be studied over the hours that follow ingestion. Changes in body fat, especially visceral fat, can be observed following repeated daily intake over weeks or months. A decreased risk of developing the metabolic syndrome, also called the insulin resistance syndrome, can be assessed over months or years of regular intake. The risk of developing diabetes mellitus and cardiovascular diseases should also be assessed over several years. Similar arguments can be made in relation to diet and the prevention of cancer, which is a multistage process occurring over many years.

2(d) Characterisation of the study groups' background diet and other relevant aspects of lifestyle

The substantiation of claims should include characterisation of the study groups' background diet and adjust not only for diet but also for lifestyle factors that might affect the outcome of the study (see 2(a) above). If a control has been used, and the study groups are randomised, then adjusting for background factors becomes less important. The baseline diet of the target population and the study group must be taken into account when planning or evaluating an intervention. If the baseline diet has not been described, it is important that the decision to disregard this should be scientifically justified. As has been mentioned in criterion 2(b) above there can be a "study effect" simply occurring because a subject has entered a trial, and these and other factors may influence outcome.

Humans are exposed to many active substances in their diet. Intervention studies dealing with one functional agent should determine whether or not the active substance is already present in the diets of the population or sub-groups of interest. Consideration should also be given as to whether any substance provided by the diet could potentially interact with the tested substance to amplify or decrease its effect. For example, when testing the functional effect of antioxidant vitamins on the reduction of cancer risk, it is necessary to

know the habitual dietary intake of these vitamins so as to assess the extent of the dietary change induced by the experimental manipulation and to control for different levels of intake in different sub-groups of the study population.

The difficulties in determining dietary intake are frequently underestimated and dietary assessment needs a rigorous approach based on a high degree of competence. Methodological challenges exist both for the collection of information on foods consumed, and for the assessment of the composition of these foods. Various methods of dietary assessment have been developed and their strengths and limitations have been reviewed by many authors [17–20], in particular in a Joint FAO/WHO Report [21]. Independent markers of intake or exposure are helpful for assuring the fidelity of dietary intake data. For example plasma levels or urinary excretion can also be used as main sources of data or in association with intake assessment [22–25]. As an example, a recent study used plasma vitamin levels and urinary potassium excretion as markers of fruit and vegetable intake [26]. Other independent markers include doubly labelled water for energy expenditure, and urinary nitrogen, sodium and sulphate. Body weight and weight changes may also be important measurements to make.

In situations where valid markers of exposure do not exist, intakes of individual food constituents can be estimated on the basis of the amount and composition of the foods consumed. This requires not only that people report their food intake reliably but also that reliable information on the composition of foods for that population is available. The conventional food composition tables often differ from country to country, in some cases due to true differences in the foods consumed in different regions, in other cases due to differences in methodology and/or the frequency of accounting for changes in food composition over time. A similar issue exists for the classification of foods where food categories are often very broadly defined, for example the inclusion of potato crisps, pretzels and nuts under the generic heading of "snacks", and where definitions, for example, of meat cuts, differ. An EU "Network of Excellence" (EuroFIR – European Food Information Resource) started in 2004 attempts to harmonise both food composition tables and food classification approaches in order to improve comparability of results.

The demands associated with a valid description of intake were considered in the context of the FUFOSE project [2, 14]. Although several methods for assessing intake exist, their validity has been questioned in recent years, particularly after the development of the doubly-labelled water methodology to measure body energy expenditures and therefore body energy needs. This method disclosed important discrepancies between what people report in dietary surveys, and the measured level of their energy needs. About 20 % of the general population underreport, and some people, particularly those with weight control problems (who constitute a growing proportion of modern populations), underreport by up to 50 %. Underreporting is not consistent for all foods. For example it affects fats and sugars more than proteins.

These well-documented levels of misreporting of food intake, especially in obese subjects, underline the need for the accurate determination of dietary intake [17, 27–30]. The task of characterising the habitual intake of a population or study group is not easy and precautions should be taken to maximise the validity of the data. Both retrospective and prospective methodologies are available but are subject to systematic error due, in particular, to underreporting of true intake. Subjects might also report according to expected instead of real intake. Furthermore, the act of recording is thought to influence the respondent's food choices and intake. Riccardi et al. [10] suggest that the Dietary Record method, which consists of a prospective/concurrent self-monitoring of food and drink intake over a specified period, could be used to determine baseline status and to track intake patterns during and after treatment. However, the respondent burden with this method is heavy and food selection and intake may be altered.

2(e) An amount of the food or food component consistent with its intended pattern of consumption

The amount of food or food component that will be tested should match its intended use and the way and frequency with which it will be eaten. Where dose response studies are performed, the range of doses must include the amount of food or food component expected to be consumed.

There is a tendency in some experimental studies to use diets or individual food components at levels that are too high to be achieved in daily practice with the intended food. Such studies are unrealistic and their results need to be confirmed at more achievable intakes. For example, extreme diets may be used in weight reduction programmes and in studies intended to demonstrate the benefits of foods or food components to high levels of physical performance. The role of these in promoting health and in serving as the bases for claims need to be considered carefully in the light of population exposure to the food components in question, particularly in groups that may be at risk of excessive intakes. An intake response relationship can identify an optimum effective intake, but this is not crucial to substantiate a claim.

2(f) The influence of the food matrix and dietary context on the functional effect of the component

The functional effect of a food or food component depends on the active component gaining access to the functional target site. For systemic effects this means

that the component needs to be taken up by the gut mucosa, transferred into the body and then distributed to the respective sites where its effects are active: the overall efficiency of this process, which is usually expressed as a percentage, is regarded by nutritionists as the bioavailability of the component. Bioavailability is influenced by a variety of factors arising from characteristics of the host, the diet as a whole, and the food itself. Host factors, and the need to characterise and control for them would be an aspect of data evaluation under criterion 2(b).

This criterion (2(f)) relates to the influence that physico-chemical properties of the food, the diet and the intestinal luminal milieu would have on the stability of the active component and on the efficiency with which it is released from the food either to be absorbed for systemic effects, or to have effects within the intestinal lumen (e. g. on the microflora) or at the intestinal mucosa. The food matrix, both in its raw state and after storage (e. g. freezing), or culinary preparation can have a significant influence on the "activity" or release of the key component. This can be measured in food free aqueous systems *in vitro*, and such systems enable comparison of the release of components from different dietary matrices. This "intrinsic availability" can vary considerably and is particularly relevant to assessing non-systemic, i. e. gut related effects of foods. It is relevant also as a component of the evaluation of evidence relevant to nutritional bioavailability. Weighting the relevance of these components of bioavailability needs to be considered on a case by case basis. There are few generalisable points applicable to all foods and food components. Thus a claim obtained with one particular diet or food matrix cannot necessarily be extrapolated to a second product containing the same component within a different matrix: extension of a claim to a product with another composition requires evidence that the component remains functionally effective to the extent claimed.

It might be necessary to substantiate the claimed effect for each individual product separately. Where differences in the matrix are small, and where evidence indicates that differences are unlikely to affect the availability of the key component for which the claim is made, it may not be necessary to substantiate the claim *in vivo* separately for each product. On the other hand, transfer of the key component to a totally different matrix, say from a fruit juice to a biscuit or cereal product, might well need further studies to demonstrate efficacy, and possibly to redefine dose or intake-response relationships. As an extreme example, a lipid soluble component would be expected to need dietary fat to ensure absorption, and provision in an aqueous environment would not seem to be a sensible development. On the other hand a minor change, such as a change in flavour variety, would not necessarily be considered a significant change in the matrix.

These considerations further emphasise why the characteristics of the food supporting the claim must be provided and must be consistent all along the studies supporting the claim (see criterion 1).

It may be possible to develop validated *in vitro* models to support the equivalence of different food matrices and to reduce the need for in vivo studies to show efficacy in every case.

On a similar basis, it might be important to consider the overall context of the diet in which the food is going to be eaten or even the type of meal, that is to say – breakfast, snack food or major meal, at which the food will be eaten.

2(g) Monitoring of subjects' compliance concerning intake of food or food component under test

In any study of diet and health it is essential to know the actual dietary intake of the subjects and to confirm that they have taken the food or food component in question in the right amount at the right time and over the specified period. If the subjects have done this, they can be said to have complied with the protocol and the study will therefore be an adequate test of the benefit of the food. Monitoring to confirm compliance is essential for assurance that the study is valid. Poor compliance can result in failure to demonstrate an effect, and an assumption, on a false basis, of "non-responsiveness", i. e. that the functional effect does not occur. Such a "false negative" result clearly does not show the absence of an effect, but unless one knew that the compliance of study participants was poor this would not be realised. Similarly, this insight also helps one appreciate that further systematic study is needed to establish whether there is a positive effect or not.

Examples of compliance measures include blood or tissue levels of the known component or its metabolites, such as red cell membrane phospholipid composition, breath hydrogen excretion in the case of fermented components, and urinary excretion of metabolites. Another approach is to add to the food in question a marker that can be detected in blood, urine or breath, which will allow compliance to be determined. Examples of such markers are para-aminobenzoic acid or lithium, which are excreted in urine or a bacterium that can be readily detected in faeces in the case of a probiotic food.

A more difficult question relates to levels of compliance and what standards need to be set that should be achieved to designate adequate compliance. Clearly, 100 % compliance with a protocol is usually not achieved in human intervention studies. In the analysis of the data of a randomised study, one may choose to exclude data of subjects whose adherence to the intervention or treatment protocol was below a certain, arbitrarily chosen minimum level. This may, however, cause selection bias and spurious results. A highly valued approach is to evaluate the 'intention-to-treat' effect [31]. This includes

data on all subjects, including those whose adherence was low or even nil. In this type of analysis the risk of bias is minimal. As compared to the first approach, the conclusions based on the latter depict more closely the expected effect of the intervention in 'real life', where the food might not be eaten daily or in the optimum amounts.

Where studies of dietary compliance have been attempted, the results have often suggested that compliance was much less than was expected and exclusion of non-compliant subjects can make a major difference to interpretation of results. Some changes in the diet such as those in relation to fat intake can be monitored rather more easily than global changes in the diet, for example, reduction in meat intake that might be used in studies of cancer prevention. Consequently, the development of markers of dietary intake is greatly needed to progress in this area.

2(h) The statistical power to test the hypothesis

Studies providing evidence for a claimed effect of a food should indicate the statistical criteria that were used in the design of the intervention trials.

When assessing a study design, one needs to estimate the study size, or power, needed to achieve a level of statistical significance. This minimal effect size will usually be the one that is biologically or practically relevant. To estimate the study size some prior knowledge of the statistical characteristics (for example the expected variance) of the outcome measure is needed.

Once the study has been carried out, estimates of the size of the effect and its statistical significance are calculated to allow valid conclusions to be drawn. Note that statistical power may turn out to differ from *a priori* estimates if, for example, the variance in the outcome variable turned out to be different to that expected [32]. In cases where the magnitude of effect is substantial but falls short of statistical significance the data will not normally, on their own, be sufficient to substantiate a claim. However, they may be valuable for the purpose of guiding further research and should not be discarded entirely. In comparing studies that differ in their outcomes, greater weight should be given to those trials that have the best design and adequate numbers of subjects.

Randomised controlled trials should comply with Consolidated Standards of Reporting Trials (CONSORT) guidelines [31] and consider Directive 2001/20/EC of the EU on good clinical practice [33].

Criterion 3. When the true endpoint of a claimed benefit cannot be measured directly, studies should use markers.

Whenever possible the claimed benefit, that is the true endpoint, should be measured directly. However, even though the ideal or target endpoint for human intervention studies of health, performance and well-being may be identified, it may not be measurable in practice. There are several possible reasons for this. There could be a long-time period between the introduction of the intervention and the desired outcome (for example a reduced incidence of a disease as evidence of a reduced risk); it might not be feasible or ethical to access the appropriate target tissues or biochemical processes (for example in the vascular wall or bronchial mucosa). Alternatively, although it is possible to measure the desired outcome, such as the components of measuring energy metabolism, protein turnover, lipoprotein and lipid metabolism, and glucose kinetics, the processes of actually doing so in a large-scale study would be excessively demanding of expertise and resource, which might be unpractical.

FUFOSE has recommended that when the definitive endpoint cannot be determined, more easily measured markers may be used as proxies or surrogates for the real or desired outcome. The robustness of such markers and their relevance to the key measure or target endpoint (meeting the quality indicators described in criteria 4 and 5) need to be assured. The FUFOSE consensus indicated how this could be achieved [2].

FUFOSE classified markers of relevant functional outcomes according to whether they:

- *Relate to the exposure to the food component being studied*, such as a serum, faecal, breath, urinary or tissue marker. For instance, the increased level of red blood cell folate is a marker of exposure to folate in food and the increased level of blood tryptophan is a marker of exposure to tryptophan in food. Markers relating to exposure to the food component can give some indication, but not absolute proof, of the bioavailability of the food component, or its presence, or that of a functional derivative or metabolite, at the functional target site.
- *Relate to the target function or biological response* such as changes in body fluids, levels of a metabolite, protein or enzyme (for example, the reduction in levels of plasma homocysteine as a possible response to dietary folate) or changes in a given function (for example, blood pressure in response to dietary caffeine).
- *Relate to an appropriate intermediate endpoint of an improved state of health and well-being or reduction of risk of disease, or both*, such as the measurement of biological processes that relate directly to the endpoint (for example, the extent of narrowing of the carotid artery as evidence of cardiovascular disease, or bone mineral density as a marker for risk of bone fracture). The target endpoint itself, if it were accessible, should be measured in some way. If this is possible, such measurement can be used as a basis for the validation of markers of intermediate endpoints to be used in subsequent studies.