PASSCLAIM[1] – Bone health and osteoporosis

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■ **Summary** *Background* The EC Concerted Action PASSCLAIM aims to produce a generic tool for assessing the scientific support for health-related claims for foods and food components. *Aim* The task of the ITGB Working Group was to critically evaluate the categories of scientific evidence needed to support claims in relation to bone health and osteoporosis. *Methods* A framework was developed to describe the chain of evidence that is required to link the consumption of a food or food component to bone health outcomes. Techniques available for interrogating each link in the chain were identified and their strengths and weaknesses considered. This framework was used to determine intermediate markers of health outcome with respect to osteoporosis and to debate the level of evidence that would be required to substantiate claims of enhanced function or reduced disease risk. *Results* Use of this framework with osteoporotic fracture as the health endpoint resulted in the following judgements based on current knowledge: 1) bone mineral density (BMD) is an intermediate marker of bone health which, for people of any age and sex, can provide evidence of enhanced function; 2) for people over 50 years living in populations with a high incidence of fracture, BMD is an intermediate marker of osteoporotic fracture risk which can provide evidence of an in-creased probability of reduced disease risk; 3) because osteoporosis is defined as a state of increased fracture risk due to low bone mass and deterioration in bone microarchitecture, a claim of a definite reduction in osteoporosis or fracture risk requires similar substantiation to claims that fractures are prevented or treated, including clinical trials and animal studies; 4) data from lower in the chain of evidence, such as bone turnover and calcium bioavailability, are not, by themselves, sufficiently strongly related to bone health endpoints to provide evidence of enhanced function or reduced disease risk but can provide supporting information. *Conclusions* In the light of existing scientific knowledge, a framework has been developed as a tool for considering the scientific support for claims relating to bone health and osteoporosis. To provide a working example, the framework has been used to assess the current position with osteoporotic fracture as the health endpoint. This experience will contribute to the formulation under PASSCLAIM of a generic tool for assessing the scientific support of health claims on foods.

■ **Key words** bone health – bone mineral density – health claims – osteoporosis

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Glossary

(using definitions of bone terms selected from Department of Health, 1998 [27])

Bone mineral content (BMC)	The mass of bone mineral in a skeletal unit (g).
Bone mineral density (BMD)	The density of bone mineral in a skeletal unit (g/cm^3); when measured by absorptiometry it represents the mass of bone mineral in a scanned area (g/cm^2) and is not a true density measurement.
Bone modelling	The process of bone formation and growth.
Bone remodelling	The structured sequence of events by which old bone is replaced by new bone, and which involves resorption followed by formation and mineralisation.
Bone remodelling transient	An incremental change in measured bone mineral content/density caused by an alteration in bone remodelling rate.
Bone turnover	The replacement of old bone by new bone.
Calciotropic hormone	A hormone involved in the regulation of calcium homeostasis.
Cancellous/trabecular bone	Spongy bone with a high surface area:mass ratio found principally at the end of the long bones, and within the axial skeleton.
Collagen	The principal protein of the bone matrix.
Compact/cortical bone	Dense compact bone with a low surface area:mass ratio providing strength and structure to the skeleton.
Osteoblast	Bone forming cell.
Osteoclast	Bone resorbing cell.
Osteocyte	Cell in the connective tissue of bone.
Osteoid	Unmineralised bone tissue, bone matrix.
Osteomalacia	Skeletal disease characterised by inadequate or delayed mineralisation of bone matrix.
Osteoporosis	Skeletal disease characterised by low bone mass and microarchitectural deterioration.
Peak bone mass	The maximum bone mass achieved by mid-life.
Rickets	Disease of the immature skeleton characterised by inadequate mineralisation of bone matrix.

Introduction

The EC Concerted Action PASSCLAIM aims to produce a generic tool for assessing the scientific support for health-related claims for foods and food components. The task of the ITGB Working Group was to critically evaluate the categories of scientific evidence needed to support claims in relation to bone health and osteoporosis. The approach adopted was to consider what is meant by 'bone health' in the context of food and food components, and to develop a framework to describe the chain of evidence that is required to link the consumption of a food or food component to bone health outcomes. As a working example, the framework was used to assess the current position with osteoporotic fracture as the health endpoint, using the categories of potential health claim defined in the previous EC Concerted Action, FUFOSE [29]. Techniques available for interrogating each link in the chain were identified and their strengths and weaknesses considered. The framework was used to determine intermediate markers of health outcome with respect to osteoporotic fracture and to debate the level of evidence that would be required to substantiate claims of enhanced function or reduced disease risk. The deliberations of the Working Group and the overall conclusions are detailed in this report. This work was part of the first stage of the PASSCLAIM project and the experience gained will contribute to the formulation under PASSCLAIM of a generic tool for assessing the scientific support of health claims on foods.

Bone health

Bone is a specialised connective tissue that, together with cartilage, forms the skeletal system [1, 5]. The skeleton provides mechanical support for the body, facilitating muscle action and locomotion and protecting the vital inner organs. Bone consists of a mineral phase of crystals of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and other

ions, an organic phase (osteoid) of collagen fibres, 90 % of which is Type 1, and a ground substance formed by glycoproteins and proteoglycans. Three cell types produce and maintain bone. Osteoblasts (bone-forming cells) work at bone surfaces where they secrete osteoid, modulate the crystallisation of hydroxyapatite and influence the activity of bone-resorbing cells. Osteoclasts (bone-resorbing cells) are responsible for the resorption (destruction) of bone that is a necessary first step in the repair of bone surfaces and the remodelling of bone. Communication between osteoclasts and osteoblasts results in a controlled, coupled system of bone resorption followed by bone formation, resulting in the modelling of growing bone and the remodelling of existing tissue. This communication is achieved by local production of cytokines and other factors, and, in general, osteoclast activation is initiated by signals from the osteoblasts. Osteocytes are osteoblasts that have become embedded within the mineralised regions of bone. They continue to be metabolically active and are involved in the sensing and translation of information about the internal bone environment.

The skeleton acts as a metabolic reservoir of ions, especially calcium and phosphate, for the essential preservation of extracellular homeostasis. The concentration of ionised calcium in serum is maintained within narrow limits by the concerted action of the three calciotropic hormones, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) and calcitonin. A serum ionised calcium concentration below the set point is detected by calcium-ion sensing receptors on the parathyroid glands, invoking an increase in PTH secretion. PTH acts on bone to release calcium and on the kidney to decrease urinary calcium excretion and increase the production of $1,25(OH)_2D$. In turn, $1,25(OH)_2D$ acts on the intestine to increase calcium absorption and on bone to further release calcium. Increases in serum ionised calcium are reversed by calcitonin, secreted by the thyroid gland, and by negative feedback by $1,25(OH)_2D$ on PTH secretion.

Bone tissue is organised into two different structures, the proportions of which vary between regions of the skeleton. Cortical (compact) bone is a thick and dense layer of calcified tissue that forms the outer surfaces of most bones and the shafts of the long bones. Cancellous (trabecular) bone has a spongy appearance and consists of a lattice of thin, calcified trabeculae. A high proportion of cancellous bone is found at the ends of long bones and within flat bones and the vertebrae. Cortical and cancellous bone are constructed from the same cell types and matrix elements but they differ structurally, both in their spatial arrangement and in tissue calcification. In cortical bone, 80 %–90 % of the volume is calcified. In cancellous bone the proportion is only 15 %–25 %, the remainder being occupied by blood vessels, connective tissue and bone marrow. The main interface between bone and soft tissues occurs at the endosteal (inner) surfaces of the skeleton where the proportion of cancellous bone is greatest. As a result, the main function of cancellous bone is regarded as metabolic whereas the role of cortical bone is predominantly structural.

Bone mass increases during childhood and adolescence. Acquisition rates are highest in infancy and during the pubertal growth spurt, and are lower in the rest of childhood. The epiphyses at the end of the long bones fuse, and linear growth stops, at the end of puberty, but bone mass continues to increase for some time after, reaching a peak during young adulthood. The age at which peak bone mass is achieved varies between different regions of the body and different populations. After the age of peak bone mass, there is a slow decline in bone mass. This affects both sexes but is accelerated in women following the menopause. The rates of postmenopausal bone mineral loss average 1–2 % per annum in cortical bone and 2–3 % per annum in cancellous bone. The age-related changes in bone mass parallel those occurring in bone mineral mass and the ratio of mineral to protein in bone remains broadly similar.

Calcium, phosphorus, magnesium and zinc are the primary bone-forming minerals. At birth, an infant contains approximately 20–30 g Ca, 16 g P, 750 mg Mg and 50 mg Zn. By maturity, these mineral masses are approximately 1200 g Ca, 600 g P, 25 g Mg, 1.5 g Zn, of which approximately 99 %, 80 %, 60 % and 30 % respectively are in the bones and teeth [83]. Nutritionally, therefore, adequate supplies of these minerals, the amino acids necessary for collagen matrix formation, and other nutrients involved in bone production, skeletal metabolism and mineral homeostasis are required for skeletal growth and maintenance.

The growth and subsequent health of bone are influenced by a complex interplay of genetic, cellular, hormonal and environmental factors, including diet and physical activity. Bone health also encompasses concepts in relation to optimal size, bone composition and function although the definition of what is 'optimal' is not known. Disturbances at any level can result in bone abnormalities and disease. Many problems affecting bone health are secondary to disease processes, due for example to genetic abnormalities, hormone irregularities or cancer. Of those that affect otherwise healthy people, osteoporosis, osteomalacia/rickets and stunting are of most concern.

Osteoporosis, fracture and bone mineral density

Fracture burden

Osteoporosis is a skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility

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and susceptibility to fracture. The disorder is a major health problem through its relationship with these fractures, which typically occur at three skeletal sites: the hip, wrist and spine. It has been estimated from incidence rates derived in North America that a lifetime risk of a fragility fracture at one of these three sites among white women aged 50 years is around 40 %, with a comparable risk of 13 % among white men. Taking into account sites other than the hip, spine and distal forearm, the lifetime risk among women aged 50 years might be as high as 70 % [67]. Hip fractures lead to an overall reduction in survival of around 15 %, and the majority of excess deaths occur within the first 6 months following the fracture. Fractures are also associated with considerable morbidity, necessitating hospitalisation in most cases, with an average length of hospital stay approaching 30 days. Hip fractures account for over 90 % of the enormous health service budget spent on osteoporosis.

While vertebral and distal forearm fractures do not contribute as much as hip fracture to the overall economic consequences of osteoporosis, they are also associated with considerable morbidity. Epidemiological surveys using standardised measurement methods from vertebral radiographs confirm an overall prevalence of morphometrically defined vertebral deformity of around 12 % among women aged 50 to 79 years. Around 30 % of these deformities reach clinical attention as a consequence of back pain, height loss or impaired quality of life. In addition, there is an excess mortality of around 20 % at five years following vertebral fracture, which is thought to be attributed to co-morbidities that co-exist with osteoporosis.

Finally, it has become apparent that vertebral deformities are a strong predictor of the future risk of fractures of the hip, as well as those at other peripheral sites. Thus, around 20 % of men and women who have sustained a clinically diagnosed vertebral deformity will sustain a further fracture within a year following the initial vertebral fracture. If followed for ten years, around 60–70 % of such subjects will have sustained another fracture [68].

■ The relationship between bone mineral density and fracture at older ages

The ultimate determinants of fragility fracture are bone strength and trauma. Bone strength is related to the quality of bone, its architecture and its mass [104]. These characteristics cannot easily be assessed *in vivo*, but, in older adults, they correlate closely with bone mineral density (BMD)[2], measured by dual energy x-ray absorptiometry or related methods (see the Bone mineral mass

2 Bone mineral content (BMC) gives similar discrimination, but for simplicity only BMD is referred to in the text

and density section). For this reason, the World Health Organization has a working definition of osteoporosis in terms of BMD relative to the young adult mean (Table 1), and BMD assessment of older adults permits the stratification of individuals into diagnostic categories.

Several prospective studies have now demonstrated that BMD measurement of older adults predicts future fracture among women [107]. In the largest of these [22], 8134 women aged 65 years and over were followed for a two-year period. Sixty-five of them suffered a hip fracture and there was a 2.6-fold increase in the age-adjusted risk of hip fracture with each SD decrease in femoral neck density. Women with BMD in the lowest quartile therefore had an 8.5-fold greater risk of hip fracture than those in the highest quartile. These data have been incorporated into a meta-analysis [62] which confirms that the risk of fracture increases approximately two-fold for each SD decrease in BMD (Table 2). Although fracture is substantially lower among men than among women, evidence from a large cohort study in The Netherlands suggests that, at any given age, and in each gender, the relationship between BMD and fracture risk lies on the same curve. Furthermore, fracture risk also increases with age independently of BMD, to the same extent in both genders [25].

BMD measurements of older adults predict fracture as reliably as blood pressure predicts stroke, and substantially better than serum cholesterol predicts myocardial infarction [19]. As in the case of blood pressure, BMD measurements have a high specificity but low sen-

Table 1 Relative risk (95 % confidence interval) of fracture for one standard deviation decrease in bone mineral density (measured by absorptiometry) below age-adjusted mean [62]

Site of measurement	Forearm fracture	Hip fracture	Vertebral fracture	All fractures
Distal radius	1.7 (1.4–2.0)	1.8 (1.4–2.2)	1.7 (1.4–2.1)	1.4 (1.3–1.6)
Hip	1.4 (1.4–1.6)	2.6 (2.0–3.5)	1.8 (1.1–2.7)	1.6 (1.4–1.8)
Lumbar spine	1.5 (1.3–1.8)	1.6 (1.2–2.2)	2.3 (1.9–2.8)	1.5 (1.4–1.7)

Table 2 Diagnostic categories for osteoporosis based on World Health Organization criteria

Category	Definition by bone mineral density
Normal	A value for BMD that is not more than 1 SD below the young adult mean value
Osteopenia	A value for BMD that lies between 1 and 2.5 SD below the young adult mean value
Osteoporosis	A value for BMD that is more than 2.5 SD below the young adult mean value
Severe osteoporosis (established)	A value for BMD more than 2.5 SD below the young adult mean value in the presence of one or more fragility fractures

Adapted from [107]

sitivity; these characteristics of the test may be altered by changing the threshold at which the diagnosis is made. Thus, a positive test (i. e. low BMD) means a high risk of future fracture. The low sensitivity (approximately 50 %) means that half of all osteoporotic fractures will occur in women said not to have osteoporosis. For this reason, the test is more useful in the context of a case-finding strategy than for population screening. In such a strategy, the susceptible population in whom BMD measurement is indicated is identified on the basis of risk factors. These include a previous fragility fracture, long-term corticosteroid therapy, and other risk factors for osteoporosis. Although current convention utilises the World Health Organization definition of osteoporosis (Table 2), it is increasingly recognised that the assessment of fracture risk in an individual is better expressed as an absolute rather than relative risk, and should be related to a relevant time interval, for example ten years. Thus, current modelling approaches utilise age, previous fracture history, and BMD values, to generate the absolute risk of fracture over the ensuing ten years. For example, a 65 year old woman, with a previous vertebral fracture, and a BMD relative to the young adult mean (T score) of –2.5, will have a ten year risk of hip fracture around 20 %. It can be inferred from this absolute risk that use of an anti-resorptive agent such as bisphosphonate might reduce risk to 10 % over the period of use. This approach is likely to be increasingly used in the future to determine interventional, as opposed to diagnostic, thresholds.

◼ The relationship between bone mineral density at younger ages and fracture and in populations with a low fracture risk

For adults older than about 50 years, bone mass at the time of measurement reflects the combined effects of peak bone mass achieved in young adult life and subsequent bone loss. Preventative strategies for osteoporosis seek to maximise peak bone mass and reduce bone loss. Although BMD at any age is a predictor of bone strength and fracture risk at that age [44, 72], the indication that BMD predicts osteoporotic fracture risk in old age is based on studies conducted in older adults from populations with a high incidence of osteoporotic fractures, as discussed in The relationship between bone mineral density and fracture at older ages section. The evidence that this relationship can be extrapolated to young people and to other populations is weak. For instance, people in the Far East and Africa have lower BMDs than Western populations but have considerably lower rates of osteoporotic fracture [2, 88, 109].

Peak bone mass, as measured by BMD, contains elements related to the size of the skeleton, to the amount of bony tissue contained within it, to the mineral content of that tissue and to the degree to which the bony tissue is actively undergoing remodelling. Part of the variation in peak bone mass between individuals is related to inherited characteristics (60–70 %) and part to environmental factors acting during foetal life, childhood and/or adolescence (30–40 %) [82]. It is, as yet, unclear which of the various aspects of BMD is influential in determining future osteoporotic fracture risk nor is it known the extent to which a change in BMD in children and young adults, caused by a change in diet or lifestyle, can predict a change in fracture risk in later life. In addition, these uncertainties about the interpretation of changes in BMD in terms of future fracture risk are compounded by interpretative difficulties inherent in measurements by DXA and other absorptiometric techniques [80]. These problems are discussed in more detail in the Absorptiometry section.

To expand on these points further, small adult skeletal size is a recognised risk factor for osteoporosis, although in Scandinavia, taller women are at greater risk of hip fracture [45]. Anatomical variations between adults may reflect the impact of environmental effects at different stages of skeletal development and these may influence later predisposition to fractures [3]. Size in infancy predicts adult bone mass, suggesting that the environment *in utero* and in early life may be an important modulating factor for fracture risk in old age, and that bone mass in early life tracks into old age [20]. These studies provide evidence to suggest that any effect of bone mass in young people on future fracture risk may be related more to skeletal size or shape rather than to bone mineralisation *per se.* In addition, bone turnover is lower in African-American adolescents compared with Caucasians of the same age, suggesting an influence of remodelling rate on peak bone mass development and later fracture risk [93]. From these arguments it follows that, with the current state of knowledge, change in BMD or other bone mass measurements in children and adolescence cannot provide evidence of an effect on later osteoporotic fracture risk.

◼ Alterations in bone mineral density in relation to fracture risk

BMD, as described in The relationship between bone mineral density and fracture at older ages section, is a good surrogate for fracture risk in older people in countries with a high fracture incidence and, in general in this group, an intervention that results in an increase in BMD or a decrease in bone loss translates into a decrease in osteoporotic fracture risk [32]. However, despite this, changes in BMD may not accurately predict the benefits of an intervention in all situations [18, 28, 32, 106]. Improvements in bone quality and bone turnover, or non-skeletal functions such as muscle strength or balance,

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may decrease fracture risk with no change in BMD [8, 78]. Significant increases in BMD may produce poor quality bone and result in unchanged or even increased bone fragility. A well-known example of this occurs with sodium fluoride, treatment with which increases bone mineralisation but produces mechanically inferior bone and can increase fracture incidence [94]. Also, increases in BMD may cause reversible changes in bone remodelling space (see Absorptiometry: bone remodelling transients section), which may or may not have long-term benefits in terms of fracture risk, except in those experiencing bone loss.

In fact, there is no *a priori* reason why it should be assumed that because fracture risk increases as BMD decreases, on an epidemiological basis, that the relationship is bi-directional and that, within individuals, an increase in bone mineral will be matched by an equivalent decrease in fracture risk [32]. Thus, although there is firm evidence that bone mineral measurements in older people are good predictors of fracture risk, the importance of increasing BMD for risk reduction is less clear. For all these reasons, there is a growing recognition that a claim that an intervention which increases BMD or decreases bone loss results in fracture risk reduction requires supporting pre-clinical studies on bone quality and strength (see the Bone quality, architecture and strength: fracture assessment section) and that clinical trials should use fracture as the primary end-point, rather than rely on evidence from bone mineral measurements [18, 32].

Osteomalacia, rickets and stunting

■ Osteomalacia

In distinction from osteoporosis, osteomalacia is characterised by defective bone mineralisation of the organic matrix and broadened osteoid seams. Bone pain, fractures, and asthenia are the main clinical manifestations. Crippling bone deformities have been described in the most severe cases. X-ray examination of the skeleton shows mineralisation defects of the cancellous matrix and cortical bone, with the occasional presence of radiolucent Looser zones adjacent to the periosteum. BMD is usually decreased. The distinction between osteomalacia and osteoporosis is often difficult and may require the histomorphometric evaluation of a bone sample [75]. Osteomalacia is generally caused by severe vitamin D deficiency, genetic abnormalities or tumours.

■ Rickets

The same causes lead to bone mineralisation defects and biological anomalies in children, which is termed rick-

ets. Rickets is a disease of the growing skeleton characterised by a number of clinical traits including deformations of the skull, ribs and limbs, possibly associated with fractures and bone pain [6, 35, 42]. It results from a defective mineralisation of the cartilage growth plate and of the bony tissue. The diagnosis of rickets requires radiological and biological explorations, besides the clinical phenotype, especially when the bone deformations are limited to the lower limbs (coxa vara, genu varum, genu valgum). X-ray examination of the skeleton shows the typical metaphyseal anomalies (widening and cupping of the growth plates, irregularity and fraying of the metaphyseal zone of provisional calcification, defective calcification of the metaphyses and epiphyses). Biological investigation shows signs suggestive of high bone cell activity, such as elevated serum alkaline phosphatase activity. In addition, it may show abnormally low serum calcium and/or phosphate concentrations, an elevated serum concentration of PTH and low serum levels of the reserve form of vitamin D, 25-hydroxyvitamin D (25(OH)D).

Vitamin D deficiency has long been regarded as the main cause of rickets, the incidence of which has been as high as 20–40 % among infants and toddlers in countries located at latitudes higher than 30° and in more tropical regions when access to solar light is limited. This incidence has dramatically decreased to below 0.1 % in countries where systematic prophylaxis has been established, using either direct vitamin D administration or vitamin D enrichment of infant formulae. However, cases of rickets are still observed in infants and toddlers, especially in breast-fed infants and in those receiving vegetal milk-substitutes, and in older children and adolescents [6, 7, 30, 35, 42, 58]. These children usually combine several of the following risk factors: heavy pigmentation of the skin, lack of sun exposure, low vitamin D intake, gastrointestinal or liver disease, treatment that decreases calcium absorption or increases vitamin D catabolism or excretion. Cases of rickets have also been observed in children with severe calcium deficiency and/or fluoride overload [99], and in premature babies with phosphate deficiency. Finally, some rare cases of rickets, with an incidence lower than 0.005 %, are due to hereditary or acquired vitamin-D resistant rickets.

■ Stunting

Stunting is a problem of inappropriately slow bone growth, generally caused by dietary deficits or malabsorption of nutrients, and which is often associated with poverty and high morbidity rates [83]. Almost one fourth of the world's children have inadequate physical development. The impairment of growth may occur prenatally, but more often it occurs in the second semester of life, during the transition from breastfeeding to fam-

ily foods. The factors that are most frequently implicated in the aetiology of stunting are nutritional deficiencies, high rates of infections and sub-optimal caregiver feeding practices. Stunting of growth may be caused by inadequate intakes of energy, protein, or micronutrients such as iron, zinc and vitamins A, C and D.

Small body size may be a problem in itself. A small adult has some functional limitations compared to a taller one, such as reduced working capacity. Where manpower is essential for subsistence this may have further consequences on health and wellbeing of the individual and his/her dependants [96]. Reproductive performance may also be affected by stature: a small woman will usually deliver a small child. Small body size is often associated with other conditions that affect the health and wellbeing of individuals: depressed immunity, impaired motor and intellectual development and deficiencies of micronutrients such as iron and vitamin A. A stunted child has also a higher risk of developing chronic diseases, such as obesity [53] and hypertension [41].

Types of claim for bone health

■ Potential claims

In the context of health claims in foods, osteoporosis is the area of bone health that is currently arousing the most interest because it is a problem of major public concern in Western countries. Issues relating to osteomalacia/rickets and stunting are likely to be covered by general nutrient claims and are not regarded as priority health issues for the majority of consumers in Europe. For this reason, this report has been focussed predominantly on the scientific evidence required to support potential claims with respect to osteoporosis.

It is important to note that specific nutrients (and overall nutrition) are only part of a spectrum of factors that modulate osteoporosis risk. Other factors include, for example, genetic disposition, hormonal status, physical activity and mobility. The effects of any food or food component under evaluation may be through a classical nutritional pathway or may be through modulation of other factors affecting bone health. Examples include the possible hormonal functions of certain phyto-oestrogens, the effects of substances that increase alertness on the individual's mobility and risk of falls, the influence of nutrients and other food components on gene expression, and overall nutritional status as a determinant of muscle development and thus of stressors which help to optimise bone strength.

■ Existing claims

Existing claims on foods, with regard to bone health, centre around only a few types of claims. These are usually based either on the traditional concepts of nutrient content and function, or occasionally on the bioavailability of relevant components. There are existing claims which make explicit reference to bone health or osteoporosis but, in general, additional information relating to enhanced function or disease risk reduction is provided only occasionally, and normally provided "off label", i. e. not formally included on the package or in the regular advertising. Existing claims on foods can be grouped as follows:

■ Nutrient function.
The main family of claims in current use relates to the nutrient function of specific micronutrients. Such claims refer mainly to the primary building blocks of bone, especially calcium and, to a lesser extent, other minerals (e. g. magnesium) and trace elements known to be essential for the formation and maintenance of bones (and teeth). They relate also to some vitamins, especially vitamin D and, to a very limited extent, vitamin K. In principle, such claims are permitted in most European markets since they are usually based on the established functionality of the food component(s) and are thus supported by a broad basis of science, and do not refer directly to health benefits. Such nutrient function claims are often linked to nutrient content claims, referring to the fraction of the RDA provided in 100 g, 100 ml or a serving of the product, or to the similarity of the content of the food product to a food recognised as rich in the nutrient (e. g. milk in the case of calcium). Typical statements in this category are worded along the lines of "This product is a source of X" or "X is essential for the development and maintenance of teeth and bones – this product contains (amount) of X."

■ Bioavailability.
Some products refer to the better utilisation of one or more of the micronutrients involved in the formation and maintenance of bone combined with some information on the function of the micronutrient(s) concerned. At present existing claims refer mainly to the promotion of calcium bioavailability. These claims are usually supported by appropriate studies, do not refer specifically to health benefits and can be considered as nutrient function claims. Typical statements include "This product contains a highly bioavailable source of X"; "X in combination with (named) other components of this product for better utilisation by the body".

■ Intermediate endpoints.
The presence/level of one or more of the micronutrients involved in the formation and maintenance of bone sometimes combined with in-

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formation on bioavailability, is indicated in some existing claims in connection with their qualitative effects on bone structure/bone strength. Typical statements include "This product contains X for stronger teeth and bones"; "X helps to keep your bones strong".

■ **Disease endpoints.** These refer either implicitly or explicitly to fighting osteoporosis or reducing fracture risk. Such claims are currently not used directly in most European countries. In a review of claims on foods current in European countries in 1997, only one, in Denmark, referred to osteoporosis "helps fight osteoporosis" [52]. The Food and Drug Administration in the USA has recently authorised a limited health claim for foods rich in assimilable calcium in terms of a reduced risk of osteoporosis in later life for specified at-risk groups and in the context of a lifestyle that includes regular exercise and a healthy diet (http://www.cfsan.fda.gov/~lrd/cf101–72.html).

Indices of bone health and their measurement

■ Introduction

The following describes the various indices of bone health that are currently available, their measurement and their relative strengths and weaknesses. They are described in an order that follows the chain of evidence required to demonstrate that a particular food or food component has a significant effect on calcium and bone metabolism and that this change brings about an improvement in bone health in terms of enhanced function, reduced disease risk or disease prevention/treatment. Fig. 1 provides, as an example, the flow of processes between the intake of a specific food or food component and the final endpoint, in this case osteoporotic fractures. This type of flow chart can be adapted to other sequences of events between a food or food component and another bone health endpoint, e. g. rickets, or describing another route to the same endpoint, for example effects on fracture via alterations in predisposition to falls. Such a flow chart can be used to determine which are the most important steps in the functional process between dietary intake and bone health, where other non-dietary factors may become relevant and have an impact, and where one might expect intermediate markers to occur that could provide valid information about the effect of the component on the final endpoint.

■ Bioavailability of the functional component

These studies are required to demonstrate that a product contains a functional component that is highly available for utilisation by the body or contains a functional
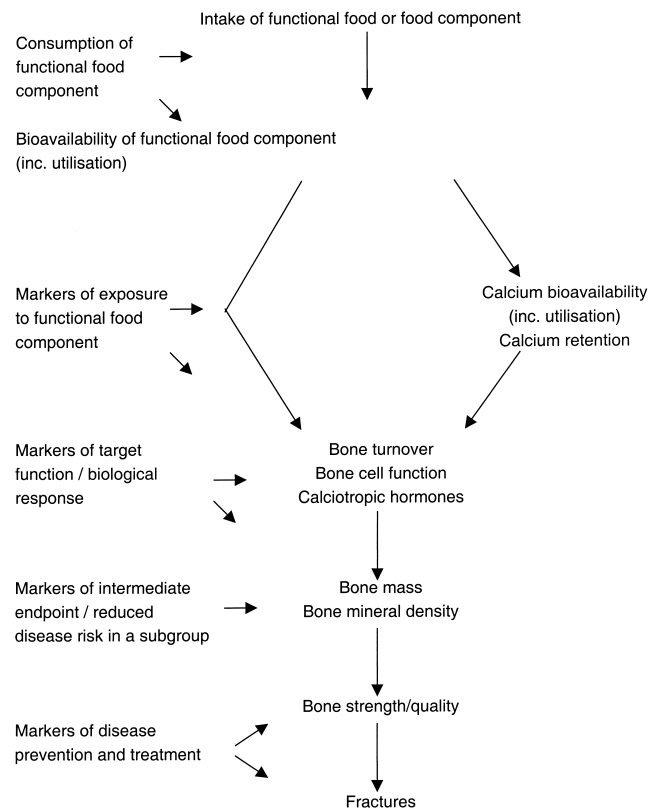

Fig. 1 Schematic outline of relationships of intake of a functional food and food component to bone health. With reference to FUFOSE categories [29]

component with an enhanced bioavailability/biopotency. These studies need to provide evidence that consumption of the food or food component produces a significant increase of the functional component or its metabolites in the human body (serum, urine, tissues) or that there is an increased effect on a functional parameter at a given intake. The bioavailability of a functional component can be considered to be the fraction of an ingested functional component contained in food that can be utilised by the human body to elicit a particular function/effect. The assessment of bioavailability in nutrition research is complicated by various factors such as endogenous levels of the functional component, the food matrix the functional component is consumed in, and limited knowledge of its kinetics and metabolites.

It is beyond the scope of this report to review the specific methods that are used to assess bioavailability. However, some general approaches should be mentioned, such as response of a physiological variable, body retention, blood (urine) response and uptake by tissues, and measurements of absorption rates. In the future, *in vitro* methods to assess bioavailability may become more important. Basic information on the bioavailability of a single functional component should be addressed under conditions that are standardised rel-

ative to age, gender, health status and fasting/postprandial state. Practically speaking, the assessment of the bioavailability of a given functional component should be looked at with respect to the component on its own and in the complex matrix of the food stuff, to take account of factors such as fat/water solubility, pH, chelating agents and the processing of the food stuff. In addition, several physiological factors are likely to affect bioavailability, such as digestion, gut transit time, and presence of gastrointestinal disorders. As there are so many variables, it is necessary to assess the bioavailability of a particular functional component in the food matrix under standardised conditions and in a form that is representative of how the food will be used by the consumer, e. g. raw or cooked, solid or liquid. If it can be demonstrated that the impact of the particular functional component at a given amount in the tested foodstuff is superior to others, this will provide evidence that the product contains a functional component with an enhanced bioavailability/biopotency.

In conclusion, a standardised and clearly defined protocol is required to assess the bioavailability of a functional component contained in foodstuff in order to obtain information that can be used to qualify a component of a particular food product for a certain claim with respect to its bioavailability.

■ Calcium retention and bioavailability

Background

Calcium is one of the major bone-forming minerals, representing 40 % by weight of the mineral in the skeleton. Because 98–99 % of the calcium in the body is in bone, changes in whole-body calcium retention, brought about by alterations in the balance between calcium absorption and excretion, imply changes in bone mineral mass. For this reason, calcium bioavailability studies not only give evidence about the supply of calcium for utilisation by the body, for a nutrient function claim, but also on bone mineral mass, which could provide supporting information with respect to a potential health claim in this area (see the Claims relating to osteoporosis and fracture, strengths and weaknesses of methods for specific levels of health claim section).

Calcium bioavailability may be defined as that proportion of calcium in a food or diet which is absorbed and utilised for normal metabolic functions, e. g. bone mineralisation. Fractional absorption is a useful indicator of calcium bioavailability. The fraction of calcium absorbed is considered to be available for utilisation for metabolic functions. Whether or not it is retained depends on need. Efficiency of calcium absorption and retention is determined by usual dietary calcium intake relative to physiological need. It is higher when there is increased demand for calcium for bone mineralisation, e. g. in infancy, childhood and adolescence and pregnancy, or for milk production during lactation. Absorption efficiency declines in older adults.

Calcium bioavailability is difficult to predict from knowledge of the chemistry of the source, or even from the results of *in vitro* testing or animal studies. While such studies may provide useful information on factors that may influence calcium absorption they do not provide quantitative estimates of calcium bioavailability in humans. Hence direct measurement of bioavailability in humans is required.

Methods

■ **General.** Calcium bioavailability may be measured in single meal studies in human subjects by tracer methods that use either stable or radio-isotopes. This requires uniform labelling of the food calcium with tracer isotope by intrinsic or extrinsic labelling. Intrinsic labelling involves the incorporation of the tracer isotope in the food in the normal physiological manner, e. g. hydroponic production of plants using labelled nutrient medium, feeding or injecting label to animals, or, for added calcium, by uniformly labelling the calcium source before addition to food. Extrinsic labelling involves addition of label to a food and allowing sufficient time for equilibration of tracer isotope with indigenous calcium. With intrinsic labelling care should be exercised since the food calcium is not always uniformly labelled using this approach [51].

Since calcium absorption is subject to considerable inter-individual variation, it is useful to compare absorption from the test food/meal with a referent food (e. g. cow's milk is commonly used) within the same subjects. Because the fractional calcium absorption varies inversely with calcium load (dose) it is important to feed similar calcium loads in the test and referent meals. It should be noted that the effects of inhibitors and enhancers of absorption and retention of calcium may be overestimated by single meal studies since changes in calcium absorption/retention will be partly offset by adaptive changes of calcium absorption which occur over a period of about one to two weeks [24].

■ **Double label stable isotope method.** Mean true fractional absorption of calcium can be measured accurately with the double label stable isotope method [100]. One label is added to the food (as either an intrinsic or extrinsic label) and the other is administered intravenously about one hour after the food is consumed. Fractional absorption is estimated from the urinary excretion of the two isotopes between 24 and 72 hours after dosing. It is important not to include urinary isotope excretion during the first 24 hours post dose in the estimation to ensure the required equilibrium is attained

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[21, 110]. Better precision can be obtained by carrying out the absorption study using a randomised crossover within subject comparison of calcium absorption from the test food and referent food/meal. A minimum of 4 weeks is allowed between the two absorption tests in the same subject.

■ **Single label isotope methods.** Calcium absorption and retention can be measured accurately by a single label radioactive isotope method using ^{47}Ca [47], a gamma-emitting isotope with a half life of about 4.7 days. The tracer is included in a food (as either an intrinsic or extrinsic label) and administered in a test meal. Body content of ^{47}Ca tracer is monitored from whole body radioactivity over two to three weeks. Calcium absorption and retention from the test meal can be estimated by extrapolation of the time course of the body loss of tracer during the second and third weeks post dose. Better precision can be obtained by carrying out the absorption study using a randomised crossover within subject comparison of calcium absorption/retention from the test food and referent food/meal. A minimum of 3–4 weeks is allowed between the two absorption tests in the same subject.

Calcium absorption can be measured by a single label radioactive isotope method using ^{45}Ca [50, 51], a beta-emitting isotope with a half-life of about 163 days. The method has been calibrated for women using a double isotope method and a predictor equation was developed that allows estimation of true absorption from the concentration of ^{45}Ca in serum exactly 5 h after oral dosing, height, and weight.

■ **Calcium homeostasis**

Anomalies of calcium homeostasis are often associated with bone diseases. They may reflect causal factors of the bone disease, like vitamin D deficiency, primary or secondary hyperparathyroidism, decreased intestinal absorption or increased loss of calcium, phosphate or magnesium. Alternatively, they may be the consequence of the bone disease, like the elevated urinary calcium excretion resulting from an excessive resorption of the skeleton. It is possible that an effect of a functional food or food component on bone health may manifest itself through modulation of calcium homeostasis. It is also important to demonstrate that any functional food or food component does not alter calcium homeostasis unacceptably. Investigations of elements of the calcium homeostatic system are, therefore, valuable as supporting evidence in any claim with respect to bone health.

The principal parameters of calcium homeostasis include serum concentrations of calcium, phosphate, and magnesium. Bias and inaccuracy of commonly used methods are considered satisfactory as regards the normal range and the variations observed in pathological conditions. For example, mean biases and 95 % confidence intervals for serum calcium range between -0.4 ± 0.44 % and 1.3 ± 0.62 %, with an inaccuracy below 4.7 % [102], while serum calcium concentrations usually range between 2.10 and 2.56 mmol/l in healthy adult populations. However, measurement of total calcium in plasma often does not allow a precise evaluation of the interactions within the extracellular calcium-PTH system. Indeed, only "free" calcium, which is about half of total calcium, influences the parathyroid gland function via its binding to the calcium ion sensing receptor [14, 76]. The precision of this evaluation may be enhanced by adjustment of total calcium for albumin concentration, because about a third of total calcium is bound to albumin, and because much of the population and individual variations in total calcium concentration result from its dependence on albumin [76]. In addition, anions like bicarbonate, citrate, or phosphate, complex 12 % of total calcium and thus interfere with free calcium concentrations. It is, therefore, preferable to use direct assays of free calcium concentration (ionised calcium), where possible.

Urinary calcium excretion is another marker of calcium homeostasis and bone metabolism. Changes in urinary calcium excretion result from alterations in the tubular reabsorption of calcium, especially its sodium-dependent reabsorption in the proximal tubule and loop of Henle, and even more its reabsorption in the distal tubule and collecting duct, the latter being influenced by parathyroid hormone (PTH), metabolic acidosis and phosphates. Changes in urinary calcium also reflect changes in dietary calcium intake, in the intestinal capacity to absorb calcium, or in bone mineralisation status. Urinary calcium excretion is best appraised by calcium measurement in 24-hour urine collections. If these are not possible, measurement of the calcium/creatinine ratio in urine samples may be used, keeping in mind the large intra-individual variations which limit the use of this index for monitoring changes in bone metabolism [103].

Finally, investigation of calcium homeostasis should include the measurement of its two main hormonal regulators, PTH and $1,25(OH)_2D$. Two specific and sensitive methods are now available to assay intact 1–84 PTH rather than its active or inactive circulating fragments. Both the immunoradiometric (IRMA) and immunochemoluminometric assays (ICMA) use antibodies directed at two distinct epitopes of the intact PTH peptide, on its N-terminal and C-terminal ends. These methods are now in routine use and have greatly improved the evaluation of PTH secretion and of its adaptation to the level of free calcium in extracellular fluids. More recent studies have suggested that these assays recognise not only the complete 1–84 PTH, but also a PTH fragment, likely to be 7–84 PTH, which accumulates

in the serum of uramic patients and may inhibit part of the PTH effects. New assays have therefore been developed which detect only the complete 1–84 PTH [71].

The relationship between vitamin D deficiency and osteomalacia/rickets is classical knowledge. There is, however, increasing evidence of an association between vitamin D deficiency and low bone mineral density, body sway and bone fractures [63, 69, 79]. Monitoring of vitamin D status is therefore important for any study of the effect of a food or food component on bone health, either by providing evidence of an effect on the vitamin D system, or to properly describe and take account of the characteristics of the study subjects. The measurement of the active form of vitamin D, $1,25(OH)_2D$, is used to evaluate the individual's adaptive ability to changes in dietary calcium, phosphates and vitamin D intakes, and to changes in calcium homeostasis and bone metabolism. However, $1,25(OH)_2D$ is not a marker of steady-state vitamin D status, because of its short half-life and the tight regulation of its production. As a consequence, the circulating concentration of the reserve form of vitamin D, 25(OH)D, is regarded as the best marker of vitamin D status.

Several assay methods for 25(OH)D are available with sufficient sensitivity and accuracy, provided that adequate quality controls are performed regularly. At present, the main difficulty is to find a consensual definition of vitamin D deficiency when no overt clinical signs are present in apparently healthy populations. For example, there are seasonal variations in the serum concentration of 25(OH)D in normal populations, the lowest values being found at the end of the winter-spring season. These low winter values have recently been suggested to reflect some degree of vitamin D deficiency, although they are found in 'normal' populations and are not associated with parallel variations in bone mass, in healthy adults or older populations [64, 77]. It has been suggested that an alternative approach to this problem would be to define vitamin D deficiency in terms of an 'optimal' 25(OH)D concentration below which the circulating PTH concentration rises above a 'reference range'. However, these 'optimal concentrations' and desirable 'reference ranges' are still a matter of debate [73, 95].

■ Bone turnover and bone cell function

General

Bone turnover markers are usually able to provide an early indication of an effect on bone and they may be quite sensitive. On the other hand, an early response does not rule out the need for long-term studies, and the sustainability of the response should also be investigated, in view of the time required for the skeleton to achieve a new steady state as a result of an intervention (see the Absorptiometry section). Most indices of bone turnover can be measured with good reproducibility and at acceptable cost by many laboratories. Their main limitations are poor specificity of response and the lack of validated connections with the functional outcome.

Accelerated turnover is a positive occurrence during periods of rapid bone remodelling and growth, but is negative during older adulthood. Osteoporotic patients often have accelerated turnover and increased osteoclast activation frequency. However, increased bone turnover is only a marker of fracture risk in older people. Individuals who have a greater rate of bone loss with the same BMD have increased risk, possibly due to alterations of trabecular geometry. Furthermore, a decrease of bone turnover in such people, although beneficial, may not automatically lead to a decrease in fracture risk.

Bone turnover markers are available to investigate both bone formation and bone resorption. In theory, the parallel assessment of formation and resorption permits investigation of whether a positive or negative bone balance is achieved and whether the two functions are coupled. The investigation of bone turnover is a field that is rapidly progressing, and it is likely that in the next few years new indices will be identified and improved assay methods developed. The following sections provide a brief overview of the current position.

Methods

■ Bone formation markers.
Markers of bone formation assess the synthetic activity of osteoblasts or the metabolism of procollagen, the main synthetic product of osteoblasts.

Osteocalcin (OC) has been used for many years as an indicator of osteoblastic activity [27], but there are many uncertainties in the interpretation of the data. Osteocalcin is relatively unstable in serum and is cleaved within a C-terminal site. It is therefore important to use assays which recognise these cleaved products (termed N-terminal mid fragments) [39]. The osteocalcin molecule also exhibits considerable immunological heterogeneity, and these complications, combined with the fact that no internationally recognised assay standard exists, make osteocalcin measurements difficult to interpret meaningfully. Osteocalcin, in its fully functioning form, has three gamma-carboxylated side chains which require vitamin K for their synthesis. Measurement of the degree of carboxylation of this protein can provide a useful index of vitamin K status and other aspects that may influence bone health [90, 98].

Bone alkaline phosphatase (bone ALP) has traditionally been a difficult assay to perform, technically speaking, but the advent of relatively specific monoclonal antibodies has led to new immunoassays [36]. The most

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recent development is a capture immunoassay that measures enzyme activity, thus allowing direct comparisons with total alkaline phosphatase measurements [43]. This bone ALP assay, which is robust and easy to perform, appears to be sensitive to changes in bone metabolism.

Collagen represents over 90 % of bone matrix proteins. The procollagen type I C-propeptide (PICP [65]) has proved to be rather insensitive to changes in bone remodelling, whereas more recent data using PINP have been more encouraging. These differences are probably associated with the way that the two analytes are cleared from the circulation. There remains some controversy, however, about which molecular forms of PINP should be measured, and some smaller components that were initially thought to be degradation products [66] are now believed to represent single chain forms of PINP which should be included in the analyses [13].

■ **Bone resorption markers.** Bone resorption markers describe osteoclast activity and collagen metabolism.

Tartrate resistant acid phosphatase (TRACP) is an enzyme synthesised by osteoclasts required in the first step of bone matrix resorption. The measurement of its serum level involves the use of isotype specific antibodies following the dissociation of the enzyme from its circulating form as a high-M, calcium-containing complex [46].

Because of its high content in the bone matrix, collagen type I is the primary source of analytes for monitoring bone resorption. These include the pyridinium collagen crosslinks (deoxypyridinoline DPD and pyridinoline PYD [86]) and the peptides associated with crosslinking at either the N-terminal (NTX [48]) or C-terminal ends (CTX [9]). The recent discovery of age-related changes in the crosslinking regions involving isoaspartyl transformations [11, 33] has provided an opportunity to gain additional information on bone collagen metabolism with specific assays that reflect the age of the molecules being degraded. Most pyridinium crosslink and telopeptide assays have been performed in urine and the results expressed relative to creatinine. Serum assays for NTX [87] are now available and methods for the measurement of both free and total pyridinium crosslinks in serum have been described [54]. The latter are, however, technically demanding and unsuitable for large-scale clinical trials. It was hoped that, because of their lower intrinsic variability compared with urinary assays, serum measurements could provide more reliable assessments of bone metabolism. Initial data for serum NTX, however, have indicated that the lower variability of the serum assay is matched by smaller differences between groups and in response to a change [31]. Serum assays of bone crosslinking components may not, therefore, provide any real advantages over urinary measurements. Newer bone resorption markers are also being developed. These include serum bone sialoprotein which, although interpretation of the data is still not fully understood, appears to be useful in monitoring pathological bone resorption [57, 92].

Human peripheral blood mononuclear cells, particularly CD14(+) monocytes, differentiate into osteoclasts when cultured *in vitro* in the presence of specific factors and are capable of extensive bone resorption. *Ex vivo* models based on human osteoblast cultures may facilitate a greater understanding of the mechanisms underlying *in vivo* effects and therefore offer promising approaches to the study of functional foods.

Interpretation

■ **Bone turnover markers during growth.** In pre-pubertal children, bone formation and resorption markers are able to predict growth velocity in the short term, particularly when periods of rapid growth occur [12]. Bone turnover markers can also be used to evaluate bone deposition. In a study of healthy young girls serum levels of bone formation markers (OC, bone ALP) were shown to be correlated with bone formation measured by calcium kinetics, while urinary levels of NTX and serum levels of TRACP were correlated with bone resorption [105]. In 6–14 year-old children reduced bone turnover assessed by decreased serum levels of TRACP was associated with increased BMD [93]. It is also known that increased turnover may be associated with increased forearm fractures in adolescents.

During the different stages of growth, size and mineralisation progress somewhat independently. Bone markers can provide information about each of the two processes. In a study on pubertal children, for example, bone resorption markers (Pyd, Dpd) were inversely related to the size and volume of the bone in the axial and appendicular skeleton measured with computed tomography, while bone formation markers (bone ALP and OC) were related to the material density of bone, defined as the amount of bone per pixel at the midshaft of the femur [70].

■ **Bone turnover markers and bone mineral density in postmenopausal women.** Bone turnover is an important determinant of BMD after menopause. In a cross-sectional study on 653 healthy European women, those in the low quartile of BMD had higher bone turnover. A combination of three markers (OC, NTX, CTX) explained more than 50 % of the variability in BMD [38]. In another study on 60–90 year-old women, 10 % of BMD variability was explained by serum NTX [91]. Bone turnover markers can also be used to identify rapid bone losers, i. e. women who lose more than the average 1 % per year, usually in the order of 3–5 % [15].

■ **Bone turnover markers and risk of fracture in postmenopausal women.** Elderly women with osteoporotic

fractures have higher bone turnover than those without fracture. The EPIDOS study showed that subjects with higher bone turnover had higher risk of fracture. Bone turnover was 85 % greater in women with reduced skeletal mass [37]. Turnover was a risk factor independent of BMD. In the OFELY study, baseline levels of bone markers in the highest quartile were associated with increased risk of fracture in a five-year follow-up of postmenopausal women [40]. In the EPIDOS study, only resorption markers had this predictive value. However, a recent study has shown that the ratio of undercarboxylated to carboxylated OC is predictive of fracture risk [61].

■ **Use of bone turnover markers in the establishment of health claims.** There are several methodological problems in the use of bone turnover markers to evaluate health claims related to bone. Firstly, the timing of response. Bone resorption markers respond rapidly to treatment (3 months) while bone formation markers respond more slowly (6 months) [61]. Secondly, the size of response. If bone turnover markers are to be used to decide about the effect of an intervention, it is necessary to estimate the size of changes that can be expected. Bone turnover markers do not respond uniformly to different treatments and the reduction is different for different indices and different individuals. For example, hormone replacement therapy results in a greater than 50 % reduction in all bone resorption markers. However, treatment with bisphosphonates produces a reduction in NTX and CTX but no change in free crosslinks. Individuals with a higher bone turnover respond to therapy better than those with a lower bone turnover. There are confounding factors that affect bone turnover, such as seasonality (indices are increased during winter, with 12 % variability). In addition, reproducibility factors have to be considered. Usually there is greater variability in urinary than serum indices, and there is considerable variability within an individual during the day and from day to day.

In conclusion, the value of the biochemical markers of bone turnover is that they can be obtained inexpensively and quickly. Such indices are useful in providing supporting evidence in evaluations of the effects of foods and food components on bone, but at the current state of knowledge cannot be considered by themselves as primary indicators of bone health or surrogates of change in fracture risk.

■ Bone mineral mass and density

Absorptiometry

■ **General.** Absorptiometry permits the precise *in vivo* measurement of bone mineral in healthy individuals.

Single energy absorptiometry is used for measuring the bones of the arms and legs; dual energy instruments are required for axial (spine, hip) and whole-body measurements in order to correct for overlying soft tissue of variable composition [80, 101]. Absorptiometry is based on the attenuation of energy from a beam of penetrating photons during a scan across the skeletal region of interest [80]. Radiation exposure is low. The effective dose per measurement (ED, a measure which takes into account surface exposure and penetrating power of the energy beam together with the depth and vulnerability of exposed tissues at the scan site) is generally within natural background radiation levels [55, 80]. As a consequence, absorptiometry can be used to study healthy children and pre-menopausal women, as well as other population groups less vulnerable to radiation, and is currently regarded as the method of choice.

The precision of absorptiometry is high. The coefficients of variation of repeated bone scans of phantoms are about 1–2 %, or less with the latest DXA instruments. The precision of repeated measurements is poorer when people are scanned, particularly if they are repositioned each time or if they are osteoporotic, but generally short-term reproducibility is 2–5 %. In addition, long-term reproducibility is good [80]. Such good precision facilitates the longitudinal monitoring of individuals, although, even in osteoporosis, changes in bone mass can be sufficiently slow that measurements have to be spaced by several months or years for significant increments to be detectable [23]. It is generally accepted that 6–12 months is a minimum for any study seeking to evaluate the short-term impact of an intervention on bone mineral status, and that 2–3 years, with serial measurements during that time, are necessary for the evaluation of longer term effects.

The accuracy of absorptiometry is more problematical. Accuracy is affected by choice of calibrating materials, assumptions built in to the computer algorithms about bone-edge detection and intra-osseous fat, the depths of tissues in the scan path, the non-uniformity of soft tissues overlying bone, and differences in clothing and bedding [80]. As a consequence, both absolute and relative values can differ between manufacturers and between instruments. Use of different versions of computer software can affect results [59] and updates of pre-existing programs necessitate re-analysis of data. Reference data also vary between manufacturers, which can result in differences in interpretation and in evaluation of osteoporotic fracture risk [60]. Possibly the greatest problems with accuracy are found at the extremes of tissue depth (generally equivalent to less than 10 cm or more than 25 cm water), such as are encountered in children, anorexics, lean male athletes and the obese, and in situations where tissue depth changes over time, such as in growing children or in those who are losing weight [80]. It is important that both the instrument and the

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software version used for analysis are specified in published accounts of absorptiometric studies.

One of the major limitations of absorptiometry is that the result represents an integration of absorption over all elements within the bone envelope, such as the medullary cavity, and is not confined to osseous tissue per se. Similarly, absorptiometry cannot differentiate between cancellous and cortical bone, nor exclude abnormalities, such as osteophytes, crush fractures or calcifications, which interfere with the measurement of bone mineral content. Other methods, such as computed tomography and X-radiography are necessary if structural information about bone is required. Some modern DXA instruments have the facility to examine morphological features of the spine in order to identify and exclude complicating factors.

■ **Size effects.** As stated above, absorptiometry is based on the attenuation of energy from a beam of penetrating photons during a scan across the skeletal region of interest. Calibration materials are used to convert the cumulative attenuation between opposing bone edges to mass of mineral within the bone envelope (bone mineral content, BMC). Absorptiometric data are generally expressed as bone mineral density (BMD) in order to minimise measurement errors connected with positioning, movement and bone edge detection, and to make some adjustment for size differences between individuals. This index is obtained by dividing BMC by the area of the scanned bone envelope. Absorptiometric BMD is a mathematical construct and is not a true density measurement; the units are g/cm^2.

As discussed in the section The relationship between bone mineral density and fracture at older ages, BMD is useful for fracture risk assessment in older people and long-term patient monitoring, since it is a highly reproducible measure and is simple to use. However, BMD represents only a partial correction for bone size, and scanned bone area can be an influential determinant of BMD as well as of BMC [4, 80, 84]. In epidemiological studies, failure to correct fully for bone area can lead to spurious or inflated relationships with other variables that are themselves related to size, such as dietary intake, obesity and energy expenditure [17, 84]. Correction for weight, height or body mass index does not necessarily remove the problem. Size-related artifacts potentially affect the majority of published epidemiological studies that have investigated the relationship between BMD and dietary intakes of calcium or other nutrients [84]. It is possible, as discussed in the section The relationship between bone mineral density at younger ages and fracture and in populations with a low fracture risk, that bone size is important in determining fracture risk, and that the fact that size and dietary intake are correlated may indicate associations between bone health and dietary components, but such studies are difficult to interpret because epidemiological investigations are unable to distinguish between cause and effect.

■ **Bone remodelling transients.** Differences in bone mineral content and density imply differences in the retention of calcium in the skeletal pool. This pool corresponds, in adults, to the calcium released and laid down during bone remodelling. Remodelling is the process whereby the skeleton undergoes continual renewal by a phased sequence of bone resorption and formation [34, 56, 74, 81]. In the adult, 95 % of bone turnover occurs by remodelling and approximately 10–15 % of skeletal surfaces are in the process of being remodelled at any one time. During this time, there is a temporary net deficit of mineral in the volume of bone undergoing remodelling and hence in whole-body bone mineral [56, 74]. It is estimated that, in normal adults, the reversible calcium deficit represents about 1.3 % of total body bone calcium, equivalent to around 14 g of calcium. Since bone turnover is greater in cancellous bone, the reversible calcium deficit is greatest in cancellous regions (about 4 %). When resorption conditions vary, there is a transition period where the number or size of new resorption cavities is modified but restoration of existing resorption pits is maintained at the previous rate. This produces a quantitative change in the remodelling space, a corresponding alteration in the reversible calcium deficit, and results in a rise or fall in the total amount of mineralised tissue per unit volume of bone until a new steady state is established [56].

Absorptiometry is a static measurement which provides a 'snapshot' of bone at any one moment. If bone turnover decreases with no overall change in bone volume, BMD values rise by a few per cent because of the smaller number of resorption cavities. The rise continues for some time until a new steady state is achieved because of the time lag between resorption and formation [56, 74]. Similarly, a decrease in reversible calcium space caused by an increase in bone turnover leads to a decrease in BMD. Such phenomena are known as bone remodelling transients.

Bone remodelling transients complicate the interpretation of absorptiometric studies [49, 81]. For example, an observed rise in BMD in an older person after intervention with a particular food might be due to reduced bone turnover rather than to increased bone mass. In this instance, the decrease in turnover might of itself indicate reduced fracture risk, because bone loss might be diminished. However, such an interpretation cannot be applied to children and young people. Calcium supplementation studies in children have demonstrated higher BMD values [27, 81]. However, circulating osteocalcin concentrations have been shown to be reduced in some studies, indicating lower rates of bone formation, and the effects appear to be reversed after supplement withdrawal [27, 81]. This is strongly suggestive of an alter-

ation in bone remodelling rate and subsequent alterations in reversible remodelling space and cannot be equated with a permanent increase in bone mass with a subsequent reduction in fracture risk. More research is needed to understand the relevance and importance of reversible calcium space in the interpretation of absorptiometric data, and indicates the importance of including measures of bone turnover in research protocols involving absorptiometry.

Other methods for measuring bone mineral mass and density

Quantitative computed tomography (QCT) and quantitative bone ultrasound (QUS) are modern techniques that are being used increasingly for bone mineral assessment, although there are no prospective data linking these measures to fracture incidence and their value in longitudinal studies has only recently been evaluated.

QCT measures the amount of mineral in a unit volume of the region of interest (e.g. cancellous areas of the spine) and more closely equates to a true density (g/cm^3). Bone ultrasound measures the attenuation of ultrasound across the heel or patella, and has the advantage of not involving ionising radiation. There is insufficient published information to determine whether these techniques, or older methods such as radiogrammetry, are likely to be affected by size-related artifacts and this possibility should be borne in mind when considering studies in which these methods are used to relate bone mineral to dietary factors.

QCT is regarded as less precise than DXA but can be used to follow changes specifically in cancellous bone, where rates of change are faster [85]. QUS is considered less precise than DXA, though more recent instruments appear to have precision similar to DXA. In addition, there is evidence that QUS provides different information to absorptiometry and may not necessarily reflect longitudinal changes in bone mineral status as measured by DXA, especially in response to drug treatment, dietary intervention or changes in physiological state such as lactation [59]. Where changes are seen, the time to observe a significant effect is considerably longer with QUS than with DXA, by 2–3 fold [89, 97], and larger sample sizes are required.

■ Bone quality, architecture and strength: fracture assessment

Animal models

The evaluation of an intervention on bone quality, architecture and strength with the aim of assessing change in fracture risk cannot be reliably made with currently available techniques applied to human studies. The most, if not the only, relevant variable that matters in terms of efficacy for any preventive or curative intervention in osteoporosis is change in bone strength. Therefore, like for drugs [108], the scientific evaluation of the effect of any food or food component on bone strength can only be made in pre-clinical studies. Animal models have been developed that allow the reliable assessment of the relationship between change in bone mass and strength. These models have been shown to be highly predictive of outcome in adequately designed clinical studies where both bone mineral density and fracture incidence have been recorded [10].

The aims of pre-clinical studies, in the context of claims relating to osteoporosis, would be to establish the relationship between the consumption of a food or food component and the formation of new bone tissue with normal architecture, and, of particular importance, a commensurate increase in bone strength, and to understand the mechanism in order to provide the rationale for its use in humans. These pre-clinical studies should be made in animal models appropriate for the intended use of the food or food component.

If postmenopausal osteoporosis is the outcome of interest, the animal model should have at least the following characteristics: increased bone turnover after oophorectomy; bone loss leading to an osteoporotic state that is not spontaneously reversible; bone loss affecting both cortical and cancellous tissue at relevant skeletal sites such as the vertebral body, femoral neck, metaphysis and diaphyses of the long bones; and increased skeletal fragility. Other animal models are available for other aspects of bone health in relation to osteoporosis. For example, there are animal models that mimic the condition of malnutrition and osteoporosis as observed in elderly, and growing animals can be used to study the effect of a food and food component on bone mass acquisition.

Various experimental manoeuvres may be used to induce changes in bone mass in experimental animals. These include oophorectomy, orchidectomy, corticosteroid use, manipulation of the dietary intake of calcium in lactating and non-lactating animals, immobilisation by using plaster-casts, hemicordectomy, sciatic denervation and other manipulations to unload the skeleton. Some of these experimental models have high predictive value. An example is the case of the oophorectomised adult rat in which the results of several therapeutic interventions mimic observations made in postmenopausal osteoporotic women. Though less well characterised, more recent models of osteoporosis are worthy of consideration. Some rodent strains have low peak bone mass or display spontaneous signs of osteoporosis associated with accelerated ageing. Apart from models of human osteoporosis, several other experimental systems *in vivo* are appropriate for selecting food component(s) of interest and dose finding.

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A series of relevant end-points can be evaluated using either invasive or non-invasive techniques. These include histomorphometry, culture of bone forming or bone resorbing cells *ex vivo*, the chemistry and biochemistry of bone tissue, the assessment of biochemical indices of skeletal turnover in blood and urine, metabolic balance of calcium combined with radioactive calcium kinetics, radiogrammetry of bone radiographs, neutron activation for whole body calcium, single and dual photon absorptiometry, quantitative computed tomography and dual energy X-ray absorptiometry and the assessment of bone strength [10].

Measurement of bone strength in animal models

Several methods of biomechanical testing can be used for the evaluation of the structural or material properties of bones harvested from large and small animals. Care should be taken to preserve and test the specimens in standardised conditions. Changes in the mechanical properties of the diaphyses of the long bones can be tested using three- or four-point bending or torsion tests. The advantage of point bending tests is that the precise location on the long bone can be chosen where the mechanical properties will be tested. Moreover, data produced using this type of test generally show minimal dispersion, and, therefore, yield means with relatively low standard deviations and standard errors. As a consequence, fewer numbers of animals need be used in order to achieve sufficient statistical power. The advantage of using a torsion test, on the other hand, is that it permits the bone to break at its weakest point. Thus, the site on the bone where the mechanics are measured are not predetermined, but the bone behaves more in accordance with a pathophysiological state. Compression or a combination of bending compression tests (producing a load-deflection curve) can be used to assess the mechanical resistance of the vertebral bodies and other sites. The structural and material properties of bone can be determined directly by analysing the load-deformation relationship (e. g. stress-strain curve) provided that the loaded surface can be accurately measured. This analysis provides information on:

- Stiffness. This property measures the ability of bone to accept and transmit mechanical loads. It is dependent on the cross-sectional diameter of the bone such that the value is proportional to the fourth power of the radius.
- Failure load (ultimate or breaking strength). This measurement corresponds to the maximal load that bone can sustain. For the bending test, the maximal load is usually expressed as the bending moment.
- Energy absorption. This is the measure of bone toughness and corresponds to the area under the load-deflection curve up to the maximal load.

The relative contribution of cancellous and cortical tissue to overall bone strength varies markedly according to the skeletal site. The two types of bone tissue can be influenced in different ways by the type of manoeuvre used to induce osteoporosis and by the agent being tested. Because of this, biomechanical tests should be performed at several skeletal sites. Important sites include the vertebral bodies, and the mid-shaft regions of the long-bones. Testing at other sites may be appropriate, particularly when it corresponds to testing sites where densitometric and/or histomorphometric evaluations have been made. At spinal sites it may also be appropriate to undertake tests on both whole vertebrae and on cores of cancellous bone with or without removal of the end-plates.

The results of biomechanical testing should take into account changes in bone size and shape. A combined evaluation of the geometric and mechanical alterations permits the distinction between changes in intrinsic property of bone during the treatment period from modifications due to changes in the size and shape of the skeleton. This assessment is particularly important for agents that accumulate in bone that may alter the quality of the organic or crystal phase. The distinction can be made in bending and torsional tests on long bone diaphyses by the measurement of cross-sectional moment of inertia (a measure of the distribution of bone tissue around the central or neutral axis) whereas at the proximal femur, both the axis length and the cross-sectional moment of inertia at the femoral neck should be measured by an image analysis system. Calculation of the moment of inertia from cylindrical or overall approximation of the cross-sectional geometry of the long bones should be avoided because of the intrinsic errors of accuracy.

For intact vertebral bodies, information on ultimate strength and intrinsic stiffness should be obtained by compressive tests undertaken in a cephalo-caudal axis. Data should be analysed in relation to the effects of the intervention on bone mass, microarchitecture and the material property of skeletal tissue. Some fractures in humans result from an accumulation of microdamage in cortical or cancellous bone due to cyclical loading at forces which are below the fracture threshold. Such microdamage involves the accumulation of cracks (in cortical bone) or microfractures (in individual trabeculae) over time. When a sufficient number of cracks or microfractures accumulate a complete fracture may occur, sometimes with little or no trauma. The ability to model 'stress fractures' or 'fatigue fractures' is difficult and may require designing an apparatus which fixes an animal's limb such that it can undergo repetitive impact loading, or alternatively, placing an animal on a continuous treadmill such that the lower extremities experience continual cyclical loads.

Finally, pre-clinical studies permit the assessment of the effects of an intervention on not only the macroar-

chitecture but also the microarchitecture of skeletal tissue using microcomputerised tomography.

■ Fracture assessment and morbidity

Most fractures are clinically obvious and readily documented. Distinction should be made between high and low energy-induced fracture. In the case of vertebral fractures, definition is less obvious. Small vertebral deformities occur more frequently than complete crush fractures. Studies of vertebral fracture depend critically on the method of assessment and on the way of expressing the results. Different information is obtained when the number of new vertebrae deformed or the number of patients with new vertebral deformities is documented. Quantitative morphometry can be applied to standardised lateral radiographs or from absorptiometric images. Images should be read blindly from treatment allocation, whenever possible at a centre specialised in vertebral morphometry. Indeed, not all vertebral deformities are due to osteoporosis. A centre with experience permits the triage of radiographic vertebral abnormalities. Studies of vertebral fracture frequency using imaging technique should report on [108]:

- The number of patients with one new deformity;
- The number of patients in whom established deformities ascribed to osteoporosis deteriorate;
- The number of new vertebral deformities;
- The number of progressive vertebral deformities.

In interventions in patients with established osteoporosis, change in standing height can be taken as a useful index of vertebral fracture. The method of choice is to use the Harpenden stadiometer with an appropriate quality control procedure. In addition, with the development of validated instruments of morbidity, assessment of indices of quality of life should be considered.

Claims relating to osteoporosis and fractures: types of evidence and general study designs

■ Types of evidence

The clinical consequence of osteoporosis is fracture and any complication that arises [108]. The demonstration that a food or food component reduces the risk of fracture, the actual health endpoint, requires long-term studies involving large numbers. It is, therefore, desirable to use surrogates of change in fracture risk as much as possible. The use of validated surrogates of change in fracture risk could help to establish a correlation between a food or food component and the health endpoint (or the absence of such a correlation) sufficient for a claim of enhanced function or increased probability of reduced disease risk to be supported within a shorter

period of time and with more realistic use of resources in terms of personnel and funds for product development. However, any claim that a food or food component actually reduces osteoporosis or fracture risk will have to use the incidence of fractures as the endpoint to substantiate that claim, supported by the full complement of pre-clinical and clinical evidence, in the same way as any pharmaceutical agent.

As discussed in the section The relationship between bone mineral density and fracture at older ages, there is a good relationship between BMD and fracture risk. This is currently the best surrogate available to assess the effect of an intervention on fracture risk. Nevertheless, some pharmaceutical interventions have shown a discrepancy between change in BMD and reduction in fracture risk. Change in BMD is a less reliable surrogate of reduction in fracture risk in interventions aimed at restoring normal bone in patients with advanced osteoporosis than in interventions aimed at preserving normal bone. In the first case, pre-clinical studies made in validated animal models of osteoporosis, previously shown to be predictive of human response to antiosteoporotic therapy, can assess whether change in BMD is associated or not with commensurate change in bone strength [108]. Several techniques can be used to assess change in bone mass in response to intervention. The most widely used is dual X-ray absorptiometry. Available osteodensitometers allow change in BMD to be monitored at several sites of the skeleton, including lumbar spine, proximal femur and forearm (see the section Absorptiometry).

Indices of calcium and bone metabolism can be useful to assess the effect of an intervention (see sections on Calcium homeostasis and Bone turnover and bone cell function). These include biochemical markers of bone formation (e.g. bone ALP and OC), bone resorption (e.g. urinary pyridinoline crosslinks and related peptides) and calcium homeostasis. Changes in biochemical markers of bone turnover can occur earlier than changes in BMD. Nevertheless, changes in biochemical markers of calcium homeostasis or bone turnover in response to an intervention cannot be extrapolated to a long-term effect on BMD.

■ General principles of study designs

Population sampling

The target population should be fully defined, including such considerations as gender, age, ethnicity, pre- or postmenopausal status, nutritional status, and medication. In children and adolescents, pubertal stages should be clearly determined. Characterisation of the study population may also need to include background diet, physical activity, smoking, family history of osteoporotic (particularly hip) fracture. Confirmatory trials

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in other ethnic populations may often be needed since environmental conditions could influence the outcome of interventions with foods or food components. Inclusion and exclusion criteria need to be precisely defined in order to increase homogeneity and thereby the statistical power of the study. Stratification can also be considered as an approach to avoid confounding environmental determinants or common risk factors. It is obvious that too restrictive inclusion and exclusion criteria may limit the ultimate use to the specific population studied and, therefore, limit the claim of the food product tested. The use of bone active pharmaceutical agents may or may not be an exclusion criterion, and even may be an essential part of the enrolment strategy where the food product is evaluated as an "adjuvant" to antiosteoporotic medication.

Protocol

The aim of the study should be clearly defined with a description of the endpoints and criteria of effect. Where multiple techniques are utilised, the criteria of effect should be distinguished from changes in variables providing information on the mechanism of the effect. Pivotal, rather than supporting or circumstantial evidence for effect should be based on prospective intervention studies, randomised, double-blind and controlled. The control wing may be randomised to placebo or to a food product already proven to be active in a previous placebo controlled study. Cross-over study designs can be considered, taking into account the assumed duration of the effect on bone metabolism.

Study duration, sample size and data analysis

The study duration should take account of the likely rate skeletal change and the reproducibility of the technique

applied. The following elements should be considered in the determination of the sample size for the study:
- Nature of the end point;
- Precision of the technique for the population under study;
- Difference between treatment wings considered to be physiologically or clinically relevant;
- Duration of the study;
- Anticipated number of drop-outs.

The null hypothesis to be tested will affect the sample size. The study should have sufficient power (i. e. to be of sufficient size) to reject the null hypothesis in favour of the alternative hypothesis. The power should be 80 % or greater and the probability of erroneously rejecting the null hypothesis, where in fact it is true, should be set at = 0.05 or less.

Data collation should be done before breaking the blinded code that gives the treatment allocation. Primary analysis should normally be based on intention to treat, including, if possible, the change in endpoints of all drop-outs. The reasons for withdrawal should be reported as should any differences in the characteristics of drop-outs compared with those subjects who completed the trial.

Claims relating to osteoporosis and fracture: strengths and weaknesses of methods for specific levels of health claim

■ Overview

Table 3 provides an overview of the consensus of the Working Group about the types of evidence that, in the light of current scientific knowledge, could be used to substantiate a health claim with respect to bone health and osteoporosis. This is based, by way of illustration, on

Table 3 Summary of evidence to support claims in relation to osteoporosis

	Component Bioavailability	Calcium Bioavailability	Bone Metabolism/Calciotropic Hormones	Bone Mineral Density (BMD)	Bone strength/quality	Fracture
Nutrient claim (cmpt)	XX					
Nutrient claim (Ca)	X	XX				
Enhanced function	X	X	X	XX		
Reduced risk in subgroup (people aged > 50y only)	X	X	X	XX		
Prevention/treatment	**X**	**X**	**X**	**X**	**X**	**XX**

Double cross indicates evidence that is essential to support claim to that level
Single cross indicates evidence that would provide contributory information but which on its own cannot support a claim to that level

Nutrient claim (cmpt) claim with respect to enhancing the bioavailability of the component
Nutrient claim (Ca) claim with respect to the component enhancing calcium bioavailability
Enhanced function similar to FUFOSE Type A
Reduced risk similar to FUFOSE Type B, but in a defined subgroup
Bold prints indicate claims that would be regarded as medicinal rather than health

the FUFOSE categories of enhanced function and reduced disease risk. It was the view of the Working Group that, because osteoporosis is defined as a state of increased fracture risk due to low bone mass and deterioration in bone microarchitecture, a claim that osteoporosis or fracture risk *is* reduced would need similar substantiation to claims that fractures are prevented or treated. However, a claim that osteoporosis or fracture risk *may* be reduced would need a different level of evidence.

■ Nutrient and nutrient function

The Working Group agreed that claims relating to the content or bioavailability of specific nutrients essential for the formation and maintenance of bone, or to the effects of a specific food or food component on the bioavailability of these nutrients would require relevant substantiating evidence (Table 3 and Fig. 1). This would include, as appropriate: analysis of the food as eaten; food consumption data and contribution of the component to intake as part of the whole diet; absorption, distribution, metabolism and excretion studies of the component in the food matrix as eaten; studies on the bioavailability of calcium over the short and long-term (see sections on Types of claim for bone health and Indices of bone health and their measurement).

■ Enhanced function

The Working Group agreed that BMD, measured by DXA or other validated methods, is a sufficiently strong intermediate functional marker of bone health that, for people of any age and sex, it can provide evidence of enhanced function and could be used to substantiate a claim such as "increases bone density". Data from lower in the chain of evidence (Table 3 and Fig. 1) can provide supporting evidence and mechanistic plausibility. This includes studies of bone turnover and calcium bioavailability/retention which, by themselves, are not sufficiently strongly related to bone health endpoints to provide evidence of enhanced function.

■ Reduced risk of disease

The Working Group agreed that BMD can be considered as an intermediate marker of osteoporotic fracture risk but only for people older than about 50 years living in populations with a high incidence of fracture. As low bone mass is only one of many risk factors, it was considered that an alteration in BMD in this population subgroup can only demonstrate an increased probability that fracture risk is reduced, and not that fracture risk is definitely decreased. BMD measures can, therefore, provide evidence of an increased probability of a reduction in osteoporotic fracture risk, and could be used to substantiate a claim such as 'may reduce the risk of osteoporosis in a defined subgroup of the population' or 'may reduce the risk of fracture in a defined subgroup of the population'. Data from lower in the chain of evidence (Table 3 and Fig. 1) can provide valuable supporting evidence and mechanistic plausibility but cannot provide evidence of reduced reduction of fracture risk.

■ Prevention/treatment

The Working Group considered that any claim that a food and food component in a specific product 'reduces the risk of osteoporosis' or 'reduces the risk of fracture' implies prevention or treatment of a disease and, therefore, is likely to be regarded as a medicinal rather than a health claim. Because osteoporotic (or fragility) fracture is the clinically significant disease endpoint, trials with fracture incidence as an outcome will be required for claims of prevention (primary or secondary) or treatment e. g. "reduces the risk of osteoporotic fracture" or "prevents osteoporotic fracture". Data from lower in the chain of evidence (Table 3 and Fig. 1), including preclinical studies, will be needed to provide supporting evidence and mechanistic plausibility.

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