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## Symposium on ‘Diet and bone health’

# Altered bone metabolism in inflammatory disease: role for nutrition

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Osteoporosis is a major public health problem, and as life expectancy and the world's population continue to increase will become even more important. Thus, there is an urgent need to develop and implement nutritional approaches and policies for the prevention and treatment of osteoporosis. Patients with some chronic inflammatory diseases appear to be more likely to develop osteopenia, and in some cases earlier in life, which is of particular concern as the incidence of inflammatory diseases in the Western world is increasing. While the cause of bone loss in patients with inflammatory disease is multifactorial, nutrition may have a role. Many of these patients may have one or more nutritional deficiencies, which can lead to altered rates of bone metabolism. On the other hand, some nutritional factors may attenuate the inflammatory process itself, and thus may indirectly benefit bone metabolism and bone health in patients with inflammatory disease. The present review will consider these issues, particularly in the context of inflammatory bowel disease, coeliac disease and atherosclerosis.

### **Bone health: Bone metabolism: Inflammatory disease: Nutrition**

Osteoporosis is a global health problem that will become increasingly important as individuals live longer and the world's population continues to increase in number<sup>(1,2)</sup>. Fragility fractures, the hallmark of osteoporosis, are a major cause of morbidity and mortality. Such fractures can have a serious impact on the quality of life of patients with osteoporosis. There is an increase in incidence rates of osteoporotic fractures with age, and the demographic changes and increasing life expectancy within many countries will have a huge impact on the number of fractures that can be expected to occur<sup>(1,3)</sup>. Osteoporosis risk is also increased in a number of diseases with an inflammatory component, such as inflammatory bowel disease (IBD)<sup>(4,5)</sup>, coeliac disease<sup>(4,6)</sup> and rheumatoid arthritis<sup>(7)</sup>. The bone loss associated with some of these diseases is a major risk factor for osteoporotic fractures of the spine, wrist and hip, which can have a deleterious effect on the quality of life of patients, especially for young patients who have a normal life expectancy. It is possible that even patients who do not experience a fracture may have a reduction in quality of life. For example, for

post-menopausal women with osteoporosis, while as expected those with fractures show a substantially reduced health-related quality of life than those without fractures, the mere presence of low bone mineral density (BMD) induces a reduction in quality of life<sup>(8)</sup>. Nutrition undoubtedly has a role in the prevention of osteoporosis in the general population<sup>(9–11)</sup>, and may also have a specific role in both the aetiology and treatment of inflammation-induced bone loss in patients with certain chronic diseases. The present review will first briefly define the principal disease of bone mass (i.e. osteoporosis), as well as considering its epidemiology and risk factors, including some diseases with an inflammatory component. It will then focus on the evidence of increased risk of osteoporosis in inflammatory diseases and its aetiology. In particular, the evidence that deficiencies of bone-active nutrients contribute to the osteopenia in some of these conditions will be reviewed. Finally, it will consider how inflammation itself influences bone metabolism and its regulation as well as how certain dietary factors may have a role in preventing or minimising inflammation-induced bone loss.

**Abbreviations:** BMD, bone mineral density; CD, Crohn's disease; hs-CRP, high-sensitivity C-reactive protein; IBD, inflammatory bowel disease; RANK, receptor for activated NF- $\kappa$ B; RANKL, RANK ligand; OPG, osteoprotegerin; UC, ulcerative colitis.

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## Osteoporosis: a health concern for populations and patients

Osteoporosis is defined as a systemic skeletal disease characterised by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture<sup>(12)</sup>. For the purposes of clinical diagnosis a working party of the WHO has redefined osteoporosis according to bone mass, at least for women. Their diagnostic criteria for osteoporosis, based on bone mineral content or BMD include: normal, within 1 SD of the young-adult reference mean for the population; osteopenia, between -1 SD and -2.5 SD of the young-adult mean; osteoporosis, >-2.5 SD below the young-adult mean; established osteoporosis, the same mass definition but associated with a fragility fracture<sup>(13)</sup>. It is still not clear whether these densitometric criteria are as indicative of fracture risk in certain patient groups<sup>(5,6)</sup>. Fragility fractures are particularly common in the spine, hip and distal forearm, although they can occur throughout the skeleton.

Osteoporotic fractures constitute a major public health problem. Currently, in the USA alone ten million individuals aged >50 years already have osteoporosis, and a further 34 million more have low bone mass, placing them at increased risk from this disorder<sup>(3)</sup>. A study of fracture occurrence in the UK indicates that the population risk is similar<sup>(14)</sup>. Moreover, one in eight EU citizens aged >50 years will fracture their spine this year<sup>(1)</sup>. The estimated remaining lifetime risk of fractures in Caucasian women at age 50 years, based on incidence rates in North America, is 17.5, 15.6 and 16% for hip, spine and forearm respectively; the remaining lifetime risk for any fragility fracture approaches 40% in women and 13% in men<sup>(15)</sup>.

The incidence of vertebral and hip fractures increases exponentially with advancing age<sup>(16)</sup>. This relationship is of particular concern as it is projected that the number of elderly ( $\geq 80$  years, in whom the incidence of osteoporotic fracture is greatest) in the EU population will grow from 8.9 million women and 4.5 million men in 1995 to 26.4 million women and 17.4 million men in the year 2050<sup>(1)</sup>. As mentioned earlier, because of the increase in incidence rates of osteoporotic fractures with age, these demographic changes and increasing life expectancy will have a huge impact on the number of fractures that can be expected to occur. For example, the number of hip fractures occurring each year in the EU alone is estimated to rise from 414 000 currently to 972 000 by the year 2050, representing an increase of 135%<sup>(1)</sup>. Patients with hip fracture have an overall mortality of 15–30%<sup>(17)</sup>, with the majority of excess deaths occurring within the first 6 months after the fracture. The incidence of osteoporosis and fracture in patients with inflammatory disease will be dealt with later (pp. 197–198).

From an economic perspective the expenses of hospital care and rehabilitation associated with osteoporotic fractures are a considerable fiscal drain for the healthcare system, exceeding those of other highly-prevalent pathologies of the elderly, such as myocardial infarction<sup>(18)</sup>. Osteoporosis costs national treasuries >€3500 × 10<sup>6</sup> annually in hospital healthcare alone<sup>(1)</sup>. Thus, while there

is little doubt that osteoporosis is a serious health concern at a population level, it may also impact heavily on the health and quality of life of patients with inflammatory disease, especially since these patients may experience accelerated bone loss earlier in life and will have to cope with osteopenia and osteoporosis in addition to the medical complications arising from the inflammatory disease itself.

### Risk factors for osteoporosis

Low bone mineral mass is the main factor underlying osteoporotic fracture. Bone mass in later life depends on the peak bone mass achieved during growth and the rate of subsequent age-related bone loss. Development of maximal bone mass during growth and reduction of loss of bone later in life are the two main strategies for preventing osteoporosis<sup>(19)</sup>. Consequently, any factor that influences the development of peak bone mass or the loss of bone in adulthood will affect later fracture risk. Several factors are thought to influence bone mass. These factors can be broadly grouped into those that cannot be modified, such as gender, age, body (frame) size, genetics and ethnicity, and those that can be modified, such as hormonal status (especially that of sex and calciotropic hormones), lifestyle factors including physical activity levels, smoking and alcohol consumption patterns, and diet. The interaction of these genetic, hormonal, environmental and nutritional factors influences both the development of bone to peak bone mass at maturity and its subsequent loss.

A number of disorders and diseases (e.g. hyperparathyroidism, hyperthyroidism, gastrointestinal disease, chronic liver disease, chronic renal disease, connective tissue disorders) and drugs (e.g. glucocorticoids, heparin) are also clearly related to accelerated bone loss. These effects are superimposed on those described earlier. While there are many diseases that increase risk of osteoporosis, for the purposes of the present review only those that have an inflammatory component will be dealt with.

### Evidence of altered bone metabolism and bone mass and increased risk of fracture in inflammatory disease

Defining 'inflammatory disease' is in itself difficult as there are many diseases that have an inflammatory component. For the purposes of the present review, two diseases of gastrointestinal origin, IBD and coeliac disease, will be considered initially as examples of diseases with an inflammatory component and in which there is an associated osteopenia. In a later section two further examples of diseases with an inflammatory component, rheumatoid arthritis and atherosclerosis, will be briefly considered.

Coeliac disease is an abnormal immuno-mediated response to the ingestion of gluten and other peptides from different cereals (wheat, barley and rye) in subjects who are genetically-susceptible<sup>(20)</sup>. The disease involves the small intestine and is characterised by histological mucosal alterations. Its treatment is the lifelong withdrawal of gluten from the diet. There is very good evidence for reduced BMD in patients with coeliac disease and this evidence base has been summarised elsewhere<sup>(4,6,20)</sup>. One review of

the evidence suggests that >75% of untreated adult patients with coeliac disease suffer from a loss of bone mass, and that coeliac disease should be considered one of the most frequent predisposing conditions to metabolic osteopathy<sup>(6)</sup>. While there are a number of relatively small studies that report variable estimates of excess fracture risk in patients with coeliac disease, data from two population-based reports show a slight increase in fracture prevalence (for reviews, see Scott *et al.*<sup>(4)</sup>, Corazza *et al.*<sup>(6)</sup> and Bianchi & Bardella<sup>(20)</sup>).

Biochemical markers that reflect the processes of bone resorption and bone formation, and thus bone turnover, can be measured in blood and urine<sup>(21)</sup>. Utilisation of such markers in studies of patients can provide some insight into how altered rates of bone metabolism may underpin the osteopenia and osteoporosis associated with diseases that have an inflammatory component, such as coeliac disease and IBD. There is also good evidence from studies that have used such biochemical markers that bone turnover is perturbed in patients with coeliac disease<sup>(6)</sup>. For example, it has been shown that as many as one-third to two-thirds of adult patients with coeliac disease have levels of biochemical markers of Ca and bone metabolism that are considered abnormal<sup>(22)</sup>.

Crohn's disease (CD) and ulcerative colitis (UC), collectively referred to as IBD, are chronic aggressive disorders that have a combined incidence of about 15–25/100 000 in western Europe<sup>(23)</sup>. CD and UC are important diseases that are increasing in frequency, disabling for many patients and generating a major burden in the healthcare system<sup>(24)</sup>. The disease normally starts in childhood or youth with a peak between 20 and 30 years of age. Although there are many similarities in relation to pathomechanisms and clinical course, the disorders have very distinct features. UC is characterised by inflammation with superficial ulcerations limited to the mucosa of the colon. It normally starts in the rectum and continuously spreads throughout the large intestine. CD, however, is characterised by a discontinuous pattern, potentially affecting the whole gastrointestinal tract. In contrast to UC the inflammation is transmural with large ulcerations and occasional granuloma<sup>(24)</sup>. Osteopenia and osteoporosis are recognised complications of IBD, and while there is some uncertainty about their exact prevalence, it is generally accepted that the prevalence of reduced BMD is increased in patients with IBD<sup>(5,25)</sup>. Given the variation in site, extent and severity of disease between patients with IBD, the variation of all these factors with time and associated drug therapy (especially steroids) it is not altogether surprising that results of studies of osteoporosis in IBD are less consistent than those in coeliac disease<sup>(4)</sup>. In a comprehensive systematic review of osteoporosis in IBD it is highlighted that the prevalence of severe demineralisation in patients with IBD depends on which evidence is considered; it has been reported to be as low as 2–16% (data from larger controlled studies) and as high as 18–42% (from uncontrolled studies)<sup>(26)</sup>. The findings from four large population-based studies describing fracture risk in IBD have produced relatively similar results<sup>(27–30)</sup>. While each of the studies has its limitations, the findings from these four studies collectively seem to suggest that patients with IBD

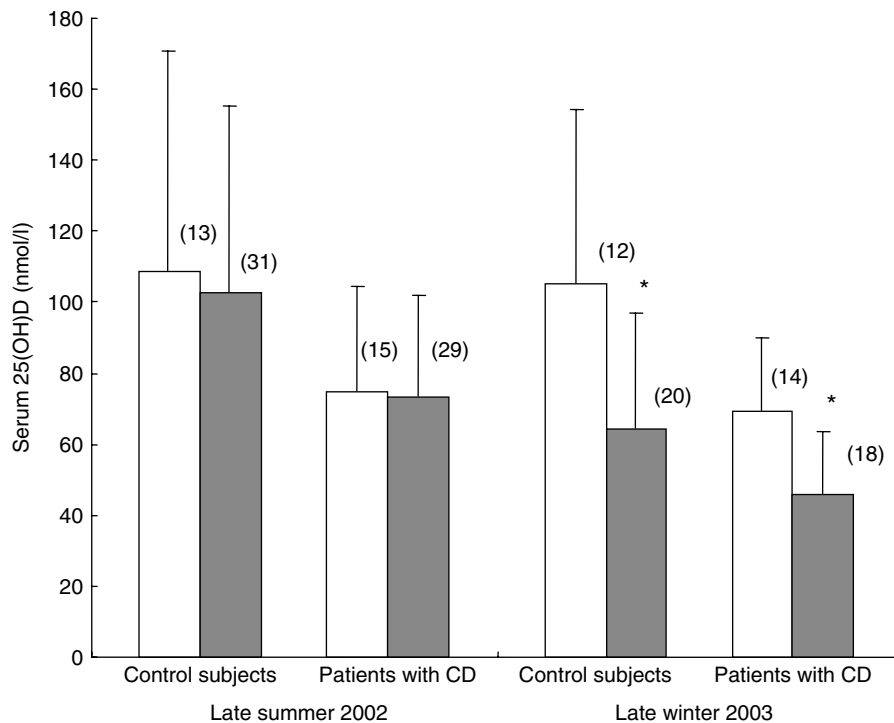
may have increased fracture risk, but the magnitude of the excess risk is small and most evident in the elderly<sup>(5)</sup>. In addition, fracture rates are generally similar in CD and UC, and osteoporosis appears to occur at similar rates in male and female patients with IBD<sup>(5)</sup>.

Studies of bone marker levels in patients with IBD have produced conflicting results. While some studies have reported increased levels of bone resorptive markers without a compensatory increase in formation markers<sup>(31–35)</sup>, other studies have reported reduced levels of markers of bone formation and no difference in resorptive markers<sup>(36)</sup>, elevated levels of both types of markers<sup>(37–40)</sup> or indeed no difference in markers<sup>(41)</sup> between patients with IBD and control subjects. However, in addition to differences in the biochemical markers used, some caution is also warranted in comparing the results of some of these studies because of major differences in the various populations of patients with IBD that were studied, especially in relation to the type of disease (CD *v.* UC), corticosteroid usage and disease activity (active disease *v.* disease remission)<sup>(42)</sup>. For example, only three studies have compared the levels of bone turnover markers in patients with UC alone compared with controls<sup>(37,38,40)</sup>, and these have produced conflicting results. Similarly, only three studies have compared the levels of bone turnover markers in patients with quiescent CD, and not taking steroids, with those of controls<sup>(36,39,42)</sup>, and these studies also have produced conflicting results.

#### Aetiology of osteopenia and osteoporosis in inflammatory disease

The aetiology of bone loss in coeliac disease and IBD is multifactorial and complex. However, sex hormone deficiency, reduced physical activity, lifestyle factors (e.g. smoking), as well as the potential deleterious effects of circulating cytokines and other mediators (e.g. IL-1, IL-6 and TNF $\alpha$ ) released by the inflamed intestines have been suggested as contributory factors to bone loss associated with coeliac disease<sup>(6,20)</sup>. In addition, a deficiency of various nutrients, particularly of Ca and vitamin D, secondary to the coeliac disease has also been implicated in the pathogenesis of bone loss associated with coeliac disease<sup>(6,20)</sup>. It has been suggested that intestinal malabsorption (which can lead to Ca and vitamin D deficiency, general malnutrition and a reduced BMI) and the presence of inflammation are the two main pathological mechanisms underpinning decreased bone mass in coeliac disease<sup>(20)</sup>.

The pathogenesis of osteopenia and osteoporosis in IBD has been suggested to result from pathological rates of bone turnover also arising from multifactorial, but incompletely characterised, mechanisms that include sex hormone deficiency, reduced physical activity, prolonged corticosteroid therapy, bowel surgery, smoking and the potential deleterious effects of circulating IL-1, IL-6 and TNF $\alpha$  among other cytokines and mediators released by the inflamed intestines<sup>(43)</sup>. Another pathogenetic mechanism that has been implicated in the low BMD in IBD is the existence of one or more nutritional inadequacies



**Fig. 1.** Serum 25-hydroxyvitamin D (25 (OH) D) in patients with Crohn's disease (CD) and healthy control subjects, stratified by season and vitamin D-containing supplement use: (□), supplement users; (■), supplement non-users. Values are means and standard deviations represented by vertical bars for the number of subjects shown in parentheses. Mean values were significantly lower than those for late summer 2002: \* $P < 0.003$ . (Modified from McCarthy *et al.*<sup>(39)</sup>.)

secondary to the disease. Ca deficiency (as a result of either low intake or poor intestinal efficiency of absorption) has been reported in CD<sup>(4,44,45)</sup>. Lower vitamin D status has been reported in patients with UC and CD compared with either control subjects or a healthy population reference range<sup>(24,40,46–52)</sup>. Lower vitamin D status has recently been reported in patients with CD compared with age- and gender-matched controls during winter but also during summer (see Fig. 1). The lower vitamin D status in patients with CD compared with controls was found to be accompanied by elevated levels of markers of bone turnover<sup>(39)</sup>. Interestingly, when CD patients were stratified by supplement use, a significant seasonal variation in serum 25-hydroxyvitamin D was found in subjects not taking vitamin D-containing supplements ( $P < 0.003$ ), but no such seasonal variation was observed in supplement users (Fig. 1), suggesting the low-dose supplements (mean content 7.5 µg vitamin D) are to some extent protective of vitamin D status during winter<sup>(39)</sup>. It was also found in a separate study that vitamin D supplement use is a positive predictor of summer and winter serum 25-hydroxyvitamin D levels in Irish patients with CD<sup>(53)</sup>. Several reasons have been suggested for the lower vitamin D status of patients with IBD, including a reduced efficiency of intestinal absorption of vitamin D as a consequence of ileopathy, a disrupted enterohepatic circulation of vitamin D, renal insufficiency, reduced dietary intake and/or exposure to sunshine<sup>(39)</sup>.

The importance of Ca and vitamin D intake are reflected in the British Society of Gastroenterology<sup>(4)</sup> guidelines for osteoporosis prevention in IBD and coeliac disease, which highlight the need to ensure adequate Ca (1500 mg/d) and to seek and treat vitamin D deficiency in both these at-risk groups. In the case of IBD they recommend 20 µg vitamin D/d during systemic steroid use.

Low vitamin K status may also be a causative factor in IBD-associated osteopenia. For example, evidence has been provided of low serum vitamin K concentrations in patients with longstanding CD (who were in remission and receiving no or very low doses of steroids), together with increased concentrations of undercarboxylated osteocalcin<sup>(42)</sup>, which is a biochemical index of vitamin K status as well as a predictor of hip fracture risk<sup>(54)</sup>. Similarly, Irish patients with longstanding CD (also in remission and receiving no or very low doses of steroids) have been shown to have increased concentrations of serum undercarboxylated osteocalcin<sup>(55)</sup>. Moreover, the increased concentrations of undercarboxylated osteocalcin (reflecting lower vitamin K status) in patients with CD in these studies appear to be positively associated with the rate of bone turnover<sup>(55)</sup> and inversely correlated with BMD at the lumbar spine<sup>(42)</sup>. In a more recent study it has been shown that patients with CD and patients with UC have significantly higher serum undercarboxylated osteocalcin levels (27% and 63% higher respectively) compared with their respective age- and gender-matched control

subjects<sup>(40)</sup>. Furthermore, serum undercarboxylated osteocalcin was found to be independently correlated with markers of bone resorption and bone formation, and thus the rate of bone turnover, in the group of patients with IBD (CD and UC), as well as in the patients with UC<sup>(40)</sup>. The reasons for the lower vitamin K status in patients with IBD, especially in patients with UC, are unclear. There may have been malabsorption of this fat-soluble vitamin in some patients as a consequence of ileopathy. However, there is also the intriguing possibility that differences in vitamin K status in patients with UC (as well as predominantly patients with colonic CD) may arise from altered bacterial flora that produce less vitamin K (menaquinone). However, menaquinone produced by colonic microflora is thought to be poorly absorbed, and therefore bacterial synthesis of menaquinone only represents a minor source of vitamin K in human nutrition (for review, see Institute of Medicine<sup>(56)</sup>). It is also possible that antibiotics often used in patients with IBD to treat their disease could kill vitamin K-producing flora. Clearly, the underlying reasons for low vitamin K status in patients with IBD require further investigation.

There has been no study, to the author's knowledge, of the association between vitamin K status and bone health in coeliac disease, an area also worthy of research. It is also worth noting that as the presence of gluten in the diet induces the gastrointestinal and systemic symptoms, including bone alterations in patients with coeliac disease, a gluten-free diet restores bone metabolism to an apparent normality. For example, a comparison of BMD and markers of bone turnover in patients with coeliac disease who were untreated and then treated with gluten-free diet for 12 months has shown an improvement in levels of bone turnover markers and BMD, as well as indices of nutritional status (including vitamin D and Ca)<sup>(22)</sup>. Moreover, an investigation of bone recovery in patients with coeliac disease during 5 years of a gluten-free diet has found that during the gluten-free diet period BMD increases or remains stable in a high proportion of adult patients<sup>(57)</sup>. The improvement in BMD was found to occur mostly within the first year after the establishment of a gluten-free diet<sup>(57)</sup>.

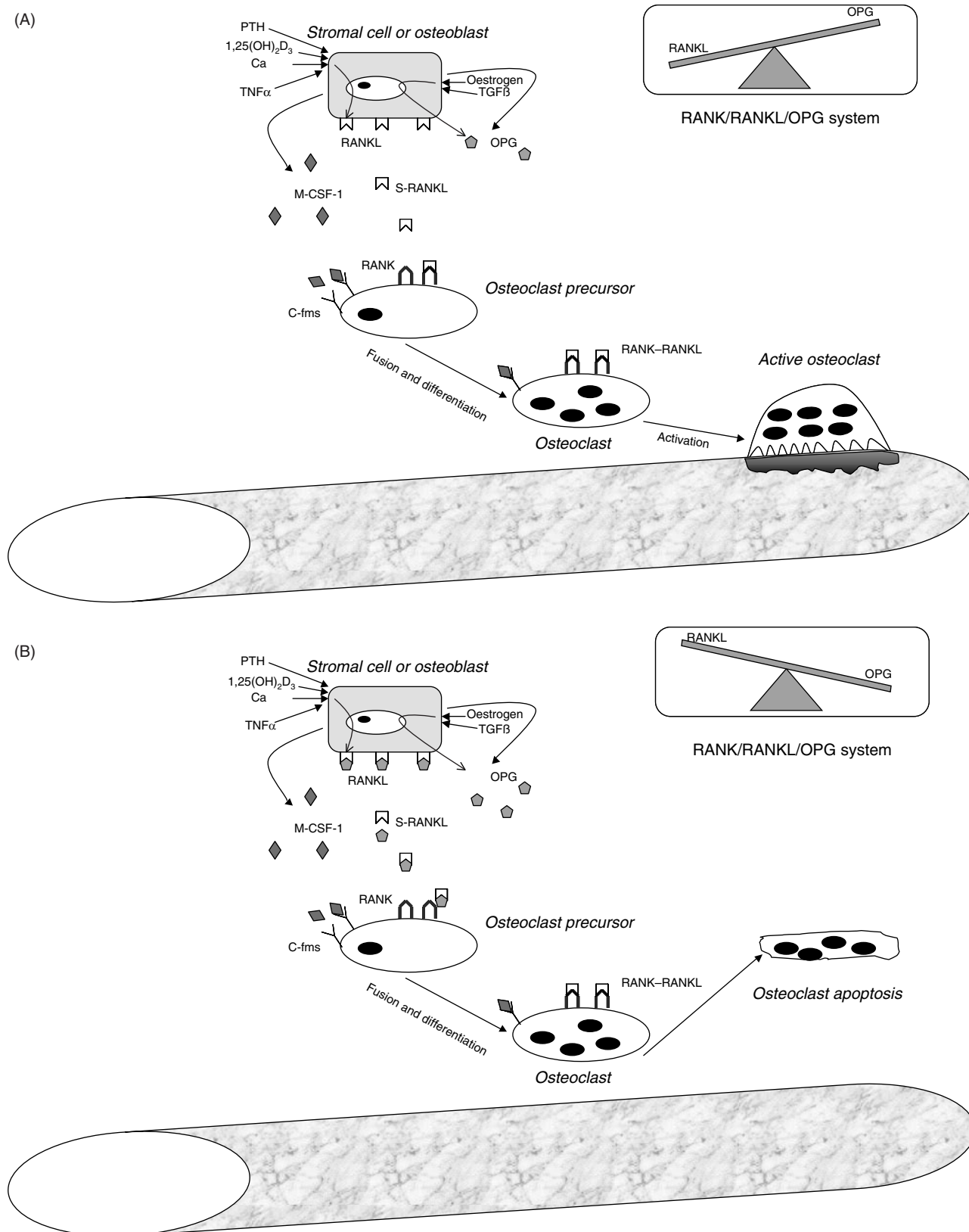
### The effect of inflammation on skeletal metabolism and its regulation

There is a common origin of osteoclasts (bone-resorbing cells) and certain immune cells, the haematopoietic stem cells in the bone marrow<sup>(58)</sup>. Osteoclasts develop from precursors in the mononuclear monocyte-macrophage cell line after stimulation by two cytokines, macrophage colony-stimulating factor-1 and the receptor for activated NF- $\kappa$ B (RANK) ligand (RANKL), both of which are produced by marrow stromal cells and their derivative osteoblasts<sup>(59)</sup> (see Fig. 2). Osteoblasts (bone-forming cells) are of mesenchymal origin and share a common precursor cell with adipocytes<sup>(58)</sup>. Bone metabolism (and the inherent communication system between osteoclasts and osteoblasts) has to be tightly regulated in order to bring about coordinated changes in bone mass throughout

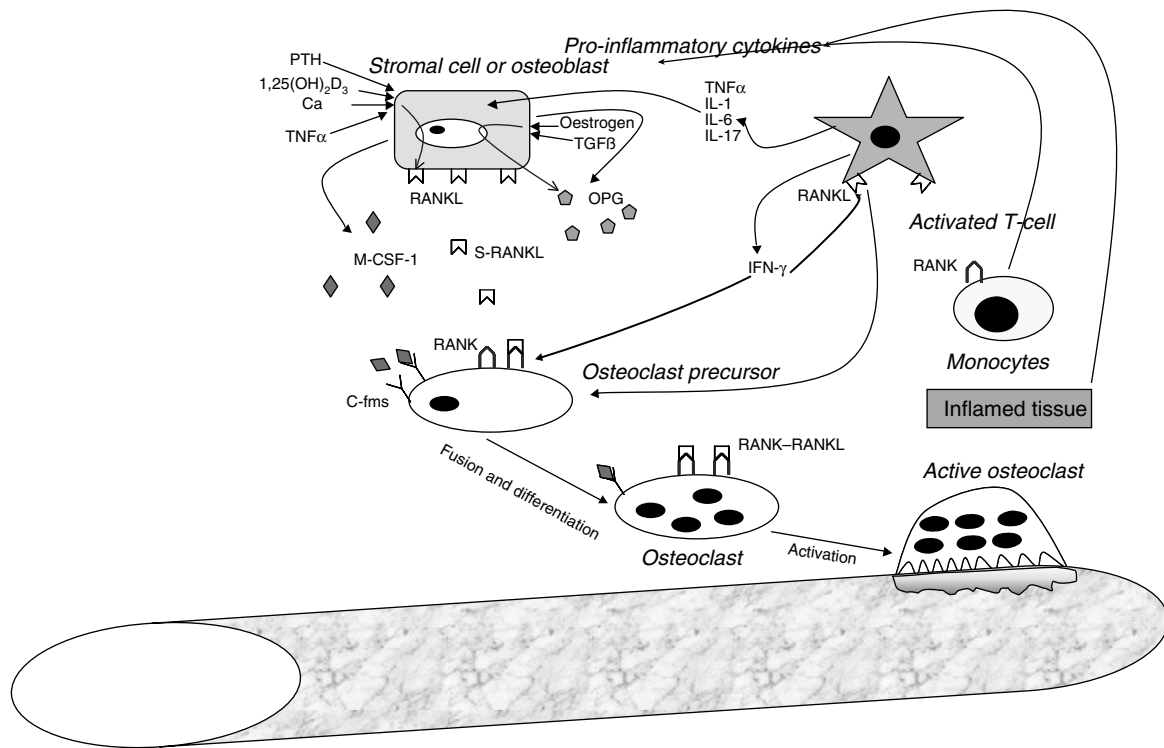
life. The cellular events in the normal process of bone remodelling, or turnover, have been described elsewhere<sup>(1)</sup> and are beyond the scope of the present review. Although calcitropic and sex hormones (especially oestrogen) have a key role in the regulation of bone metabolism, another primary regulatory system for bone remodelling exists, the RANK/RANKL/osteoprotegerin (OPG) system. This system is an osteo-immunological system and it has an important regulatory role in maintaining the coupling between bone resorption and bone formation<sup>(58)</sup>, a prerequisite for normal remodelling of bone, while an uncoupling of remodelling can result in bone loss and fragility. The RANK/RANKL/OPG regulatory system has been expertly reviewed in detail elsewhere<sup>(58–61)</sup>. In brief, during normal bone remodelling marrow stromal cells and osteoblasts produce RANKL, which binds to the transmembrane receptor RANK in osteoclast precursors and induces differentiation and activation (see Fig. 2(A)). This process occurs through the transcription factor NF- $\kappa$ B. Osteoblasts also produce OPG, a soluble decoy receptor that blocks RANKL and maintains control of the remodelling process. OPG, which binds to RANKL with high affinity, is vital to the success of the RANK/RANKL/OPG regulatory system of bone metabolism. By impeding the RANK-RANKL complex formation OPG, when in sufficient amounts relative to RANKL, can drive the osteoclastogenesis pathway towards apoptosis (Fig. 2(B)).

The RANK/RANKL/OPG regulatory system is influenced by many cytokines and mediators<sup>(58–61)</sup>. Osteoclast precursors as well as mature osteoclasts express receptors for pro-inflammatory cytokines IL-1, IL-6 and TNF $\alpha$ <sup>(58)</sup>. In addition, pro-inflammatory cytokines suppress OPG expression while simultaneously enhancing that of RANKL, with the net effect being a marked increase in osteoclast formation and function<sup>(59)</sup>. The modulation of the bone resorptive activity of RANKL by circulating OPG may help to explain the increased bone loss in clinical situations accompanied by increased levels of TNF $\alpha$ , IL-1 or parathyroid hormone<sup>(59)</sup>. TNF antibodies or a soluble TNF receptor-IgG fusion protein potently suppress the bone loss in disorders of inflammatory osteolysis, such as in rheumatoid arthritis<sup>(62)</sup>.

RANKL is also produced by activated T-cells<sup>(58,59)</sup> (Fig. 3). With chronic or recurrent immune activation of either systemic or gastrointestinal origin there may be a reduction in the body's ability to limit the production of RANKL<sup>(58)</sup>. This effect results in increased osteoclast activation through a diversion of osteoprogenitor cell differentiation away from monocyte-macrophage cell development and towards osteoclastogenesis. Osteoclastic activity, induced by pro-inflammatory cytokines and activated T-cell-induced RANKL, is thought to be modulated by the action of IFN- $\gamma$  on TNF receptor-associated factor 6<sup>(63)</sup>. TNF receptor-associated factor 6 is a RANK adaptor protein that mediates NF- $\kappa$ B activation. When there is chronic elevated antigenic load or excessive oxidative stress, which increases pro-inflammatory cytokine-induced RANKL, the activation of osteoprogenitor cell differentiation towards osteoclastogenesis may adversely affect the regulation of bone remodelling<sup>(58)</sup>. It is in this abnormal



**Fig. 2.** A diagrammatic representation of the receptor for activated NF- $\kappa$ B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) regulatory system. (A) RANKL binds to RANK expressed by osteoclast precursors and induces differentiation and activation. This process is also driven by the binding of macrophage colony-stimulating factor-1 (M-CSF-1) to its receptor (C-fms). (B) OPG acts as a decoy receptor by binding to RANKL and thus blocking RANKL and inhibiting osteoclast formation. The balance between RANKL and OPG plays an important role in controlling the bone remodelling process. PTH, parathyroid hormone; 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1,25-dihydroxycholecalciferol; TGF $\beta$ , transforming growth factor  $\beta$ ; S-RANKL, soluble RANKL.



**Fig. 3.** The receptor for activated NF- $\kappa$ B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) regulatory system is also influenced by activated T-cells, monocytes and inflamed tissue. Pro-inflammatory cytokines arising from activated T-cells and monocytes and from inflamed tissue, as well as direct production of RANKL by activated T-cells, lead to osteoclast activation. PTH, parathyroid hormone; 1,25(OH) $_2$ D $_3$ , 1,25-dihydroxycholecalciferol; TGF $\beta$ , transforming growth factor  $\beta$ ; S-RANKL, soluble RANKL; IFN- $\gamma$ , interferon- $\gamma$ .

state that chronic immune activation may alter interferon- $\gamma$  modulating capacity. This uncoupling of the remodelling process results in bone loss.

Thus, the normal regulatory control of bone metabolism may be adversely affected by pro-inflammatory cytokines released from inflamed intestines as well as from mature T-cells in patients with IBD and coeliac disease (Fig. 3). Interestingly, while one study has reported an increased RANKL:OPG ratio in patients with untreated coeliac disease<sup>(64)</sup>, another study has shown that serum OPG and RANKL levels are higher, and the OPG:RANKL ratio is lower, in female patients with coeliac disease on long-term treatment with gluten-free diet compared with age-matched controls<sup>(65)</sup>. Furthermore, plasma OPG have been shown to be elevated in CD and UC whereas soluble RANKL levels are not significantly different in patients with IBD compared with healthy controls<sup>(66)</sup>. The treatment of patients with CD with infliximab (a TNF blocker) has been shown to decrease OPG concentration and serum crosslaps (a marker of bone resorption)<sup>(67)</sup>. The authors have suggested that elevated OPG in CD could be a counter-regulatory response to inflammatory cytokines or may reflect T-cell activation. Inhibition of RANKL has been suggested as a promising future strategy for inhibiting inflammatory bone loss in patients with chronic inflammatory arthritis<sup>(68)</sup>.

Atherosclerosis also has an inflammatory component and in recent years there has been a growing recognition of the common link between vascular calcification and bone loss. For example, it has been shown that the mean total

coronary Ca score in women with osteoporosis is significantly higher than that of women with normal BMD status (221.7 v. 41.9)<sup>(69)</sup>. There are a number of lines of evidence to suggest commonality in the mechanisms underpinning Ca metabolism in bone and the vasculature. Amongst the various lines of evidence proposing a link between osteoporosis and atherosclerosis are the suggestion that there may be osteoblast-like and osteoclast-like cells in vasculature, the presence of bone signalling molecules and transcription factors in the arterial wall, a role of the LDL receptor gene in bone mass, as well as a role of leptin and adiponectin, all of which have been reviewed elsewhere<sup>(70,71)</sup>. In recent years high-sensitivity C-reactive protein (hs-CRP), a marker of low-grade inflammation, has been used as a risk marker for atherosclerosis. It has been shown recently that subjects in the highest quartile of hs-CRP, compared with those in the lowest quartile, have a higher relative risk of fracture, even though hs-CRP is unrelated to BMD (as assessed by ultrasound)<sup>(72)</sup>. Furthermore, this study has shown that hs-CRP is inversely associated with bone turnover markers<sup>(72)</sup>, which is similar to recent preliminary findings in overweight young adult men and women<sup>(73)</sup>. In contrast, a recent study of healthy pre- and post-menopausal women has shown that subjects in the highest quartile of hs-CRP, compared with those in the lowest quartile, have significantly higher levels of markers of bone turnover ( $P = 0.001$ )<sup>(74)</sup>. Furthermore, hs-CRP increases linearly from normal patients to patients with osteopenia to patients with osteoporosis<sup>(74)</sup>. It has

been suggested that arterial mineral metabolism is normally in balance; however, a pro-inflammatory environment may lead to uncoupling<sup>(70)</sup>, suggestive of a possible involvement of the RANK/RANKL/OPG regulatory system in the vasculature.

### Direct role for nutrition in suppressing inflammation-induced bone loss

There are many nutrients and food components that are believed to possess anti-inflammatory potential, including marine-based *n*-3 fatty acids, some vitamins, some minerals (such as Se), many phytochemicals and probiotics. By suppressing or dampening down inflammation and the release of pro-inflammatory cytokines and mediators such anti-inflammatory food components may help ameliorate the altered bone metabolism arising directly (RANK/RANKL/OPG driven) and/or indirectly (nutrient deficiency secondary to inflammatory disease) in some diseases with an inflammatory component. However, this aspect has not received much attention experimentally in human studies. The possibility that suppressing inflammation in patients with CD, by supplementation with fish oils (and antioxidants) for 24 weeks, would down regulate the rate of bone turnover in these patients has been investigated<sup>(75)</sup>. However, while fish oil supplementation was found to downregulate interferon- $\gamma$  production from mitogen-stimulated peripheral blood mononuclear cells, no significant effect on markers of bone turnover was found.

Interestingly, over the last few years there have been some emerging data suggestive of an interaction between certain nutrients and the RANKL/OPG system. For example, in a randomised double-blind intervention trial genistein (a soyabean phyto-oestrogenic compound) supplementation (54 mg/d) for 24 months has been shown to increase serum OPG in post-menopausal women<sup>(76)</sup>, which may explain, at least in part, the suggested bone-sparing effect of genistein<sup>(77)</sup>. In recent animal studies dietary Mg deficiency in rats has been reported to lead to decreased OPG and increased RANKL (typical of osteoclastogenesis) accompanied by bone loss<sup>(78)</sup>, while in a rheumatoid arthritis mouse model an *n*-3 fatty acid-rich diet has been shown to decrease RANKL mRNA and enhance OPG mRNA in lymph nodes, associated with greater BMD<sup>(79)</sup>. The causal relationship between nutrient intake and RANKL and/or OPG remains to be investigated, but should these important regulatory compounds be amenable to modification by dietary means it could offer great scope for nutritional therapy of inflammatory disease-associated bone loss.

### Conclusion

It is clear that altered bone turnover as well as bone loss are common among patients with inflammatory-related diseases. Nutrition may have an aetiological role as well as treatment potential for this bone loss. While intriguing, more research is needed to understand the impact of nutrition on the RANK/RANKL/OPG regulatory system,

especially in the context of inflammatory disease. This research may pay dividends not only in relation to prevention of osteoporosis, but also atherosclerosis.

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