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Symposium on 'Nutritional aspects of bone'

Markers of bone turnover in relation to bone health

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In recent years, there has been both an increasing awareness of, and interest in, bone health which has been combined with a recognition that nutritional factors may have an influential role in developing and maintaining bone status. In contrast to the situation approximately 15 years ago, when only 5 % of general practitioners acknowledged the occurrence of osteoporosis, this disorder of bone metabolism is now much more widely recognized. With 60 000 hip fractures resulting from osteoporosis each year in the UK alone, this debilitating disease has dramatic effects on society in both social and economic terms. It is important, therefore, that research defines more closely the mechanisms of bone loss that give rise to osteoporosis and the nutritional influences on these processes. The aim of the present paper is, first, to summarize the biochemical techniques currently available for assessing bone metabolism and, second, to review the current knowledge of nutritional influences on bone turnover using these markers.

BONE REMODELLING

Bone is an active tissue undergoing continual replacement of defective bone through the combined actions of osteoclasts and osteoblasts. This process may account for replacement of about 10 % of the adult skeleton each year. A third cell type, the osteocyte which is embedded in mineralized bone, may play an important role in detecting imperfections or microfractures and initiating a remodelling cycle. Each remodelling unit takes several months to complete the renewal process and, because resorption and formation at a particular site are separated in time, expressing bone balance as a simple ratio between the two will only be valid when the skeleton is in complete equilibrium (Parfitt *et al.* 1996). In many cases, changes in bone markers reflect primarily alterations in the number of remodelling sites, the activation frequency.

The purpose of the following sections is to provide a brief background to the main methods currently available for measuring bone formation and resorption rates, together with an assessment of their main advantages or disadvantages.

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BONE FORMATION MARKERS

Osteocalcin

Synthesized primarily by osteoblasts, osteocalcin (or bone Gla protein) appears to provide a good candidate as a bone marker and, following development of the first immunoassay in 1980 (Price & Nishimoto, 1980), various forms of assays for serum osteocalcin have been used extensively. This forty-nine-residue protein is characterized by the presence of up to three γ-carboxyglutamic acid (Gla) residues, formed post-ribosomally by a vitamin K-dependent enzymic carboxylation of glutamate residues. These Gla residues accentuate the Ca-binding properties, and most of the newly-synthesized protein is immediately bound to the bone mineral, with only a proportion being secreted directly into the blood. Most osteocalcin assays are now designed to measure this proportion of the intact protein in blood, and it is assumed that the proportion of total osteocalcin synthesis that this represents is consistent, although direct evidence is lacking. More recently, two-site immunoassays or assays using conformation-dependent antibodies that would eliminate possible cross-reaction with fragments of osteocalcin derived from bone matrix degradation (Garnero *et al.* 1992) have become commercially available. It is difficult, however, to compare assays directly, and measurements of osteocalcin should be assessed with respect to a reference population determined using the same assay in the same laboratory (Delmas *et al.* 1990).

Incomplete carboxylation of the susceptible glutamate residues presents another potential complication, although most assays react equally well with both carboxylated and non-carboxylated forms of the protein. Deficiencies in vitamin K, particularly in the elderly, would result in a larger population of under-carboxylated osteocalcin which has a lower affinity for the mineral and might contribute to an observed increase with age in osteocalcin concentrations (Knapen *et al.* 1989). Methods to distinguish between fully- and under-carboxylated forms of osteocalcin have been developed using hydroxyapatite *in vitro* (Knapen *et al.* 1989; Merle & Delmas, 1990), and the concentration of under-carboxylated form may be related to the risk of hip fracture (Szulc *et al.* 1993). A monoclonal antibody-based assay for determining under-carboxylated osteocalcin has recently been described (Vergnaud *et al.* 1997), but further studies are necessary to confirm the utility of these measurements.

In addition to the vitamin K dependence, osteocalcin synthesis is dependent on 1,25-dihydroxy cholecalciferol, having a vitamin D-receptor element in the promoter region of the gene. This property also contributes to the unique susceptibility of osteocalcin measurements to corticosteroid treatment which acts through interaction with the vitamin D-receptor element. Despite the many assumptions involved in applying osteocalcin measurements, the assay has been applied in a large number of clinical studies. The function of osteocalcin is unclear and its serum concentrations seem to reflect mainly the activity and number of osteoblasts rather than being directly related to bone mineralization. Recent evidence from gene knockout studies (Ducy *et al.* 1996) indicates that osteocalcin may play some inhibitory role in bone formation, since mice lacking the protein had larger and stronger bones than their osteocalcin-replete counterparts.

Bone-specific alkaline phosphatase (EC *3.1.3.1*)

Measurement of total alkaline phosphatase represents a well-established technique in clinical chemistry which has been applied extensively in conditions where large increases in bone turnover occur, such as Paget's disease. For detecting more subtle changes in bone

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metabolism such as those occurring in osteoporosis, however, measurements of total enzyme activity in serum have proved less useful, and attention has been focused on measurements of the bone isoform of the enzyme. Of the four genes that code for alkaline phosphatase, giving rise to four true isoenzymes, the most abundant is the form expressed in many tissues including liver, bone and kidney. In practice, quantification of bone-specific alkaline phosphatase requires the ability to differentiate between liver and bone isoforms, the principal constituents of the enzyme activity in blood. These isoforms of tissue-non-specific alkaline phosphatase differ in their carbohydrate attachments and the degree of sialation. Until recently, the main methods available for separating and quantifying different isoforms of alkaline phosphatase were selective heat inactivation, often combined with chemical inhibition, and electrophoretic techniques (Price, 1993). These techniques are laborious to perform and lack precision, particularly when one isoform of the enzyme is in large excess. Lectin-precipitation methods provided a simpler technique in which alkaline phosphatase activity is measured before and after precipitation of the bone isoform by wheat-germ agglutinin.

Major advances in assay technology came with the development of antibodies specific for the bone isoform. An immunoradiometric assay based on two monoclonal antibodies provides a quantitative measurement of the amount of the enzyme protein in serum (Garnero & Delmas, 1993). An alternative approach has been to develop a microtitre plate assay based on a bone-specific monoclonal antibody attached to the plate to capture the alkaline phosphatase, the activity of which is measured colorimetrically in the plate (Gomez *et al.* 1995). The latter method has some advantage in expressing the results in activity units that can be compared directly with the results for total alkaline phosphatase measurements. Both immunoassays provide reasonable specificity for the bone isoform with only about 10 % cross-reactivity with the liver isoform.

Procollagen peptides

Collagen type I is synthesized as a precursor molecule approximately 50 % larger than the fibrillar form, with extension propeptides at both the N- and C-terminal ends which are released during collagen fibril formation and can be measured in the blood. As most of the organic matrix of bone is collagen type I, these metabolites have been suggested as markers of bone formation (Risteli & Risteli, 1993). Using antibodies raised against human propeptide, the C-terminal portion, procollagen type I C-propeptide (PICP), was shown to be present in the blood as a single 100 kDa species (Melkko *et al.* 1990) and this assay appeared to correlate with osteoblastic activity (Hassager *et al.* 1991). There are some uncertainties, however, regarding the normal physiological variations and metabolism of these peptides (Hassager *et al.* 1992) and the responses of the PICP assay to changes in bone formation rates are often rather limited (Alvarez *et al.* 1995).

Immunoassays for the N-terminal propeptide of procollagen I (PINP) have recently received renewed interest (Melkko *et al.* 1996; Orum *et al.* 1996). The preliminary clinical data using PINP assays are encouraging, but the fact the PINP and PICP assays can in certain clinical applications give different results emphasizes the importance of gaining further knowledge about the degradative pathways and clearance of these molecules (Risteli *et al.* 1995).

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BONE RESORPTION MARKERS

Until recently, the main method available for the assessment of bone resorption was urinary hydroxyproline. It was recognized, however, that this method lacked specificity and sensitivity for a number of reasons (Robins, 1982) and in considering the new methods that have recently been developed, it is essential to keep in mind the characteristics required for a valid assay and how well the drawbacks inherent in hydroxyproline measurements are overcome. A major problem with measuring hydroxyproline is the presence of this imino acid in all connective tissues as well as some rapidly-metabolized serum proteins such as the collagen-like subunit of the first component of complement, C1q. Use of the collagen cross-links, pyridinoline (Pyd) and deoxypyridinoline (Dpd), was a major step in providing tissue specificity because of the restricted distribution of these cross-links, and particularly their absence from skin. These cross-links are located within the collagen matrix and are released into the circulation following bone resorption. Because of its restricted tissue distribution, Dpd is proposed as a more specific bone marker than Pyd, even though the concentration of Dpd at other sites, such as in vascular tissue, is similar to that in bone collagen (Seibel *et al.* 1992). The concentration of the cross-link in different tissues is, however, only one relevant variable; the body pool size (total mass of tissue) and its turnover rate are also crucial considerations. On this basis, the contribution from vascular tissue to Dpd excretion is negligible in comparison with that from bone. Dpd, therefore, represents one of the most bone-specific markers currently available.

A second major drawback with urinary hydroxyproline measurements that had to be overcome was the fact that this assay measures not only degradation of insoluble tissue collagen, but also release of hydroxyproline from biosynthetic intermediates, including degradation of precursors intracellularly. The latter process may account for more than 15 % of new collagen synthesis (Bienkowski, 1984). As the formation of pyridinium cross-links occurs extracellularly at the final stage of maturation of the collagen fibril, these compounds have unique advantages as markers in reporting the degradation only of mature collagen and not any biosynthetic intermediates.

With these advantages in terms of specificity and sensitivity, the measurement of urinary cross-links has been used widely in recent years. Their application is also facilitated by the lack of any requirements for dietary restrictions (Colwell *et al.* 1993).

Free, total and peptide cross-link markers

Following the development of an HPLC method applicable to measurements of pyridinium cross-links in urine (Black *et al.* 1988), automation of the system, including the use of an internal standard, greatly improved the precision of the assay (Pratt *et al.* 1992). Initially, measurements of total cross-links were performed after acid-hydrolysis of the urine and these techniques were able to confirm the validity of the cross-links as bone resorption markers (Robins *et al.* 1991). In particular, these assays were shown to correlate closely with bone histomorphometry (Delmas *et al.* 1991) and with radioisotopic methods (Eastell *et al.* 1997). The HPLC assay was used to show that about 40 % of the pyridinium cross-links were present in urine in the free form and could be measured without hydrolysis (Robins *et al.* 1990; Abbiati *et al.* 1993). The proportion of free cross-links was found to be relatively consistent and these observations led to the development of direct immunoassays that initially measured both cross-links and very small peptides (Seyedin *et al.* 1993) and, subsequently, of a monoclonal antibody-based assay for the bone-specific cross-link, Dpd (Robins *et al.* 1994*b*).

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Recently, a number of other assays have been developed based not on the cross-links themselves but on peptides derived from amino acid sequences in the vicinity of the cross-linking regions of collagen type I. The NTx assay (Hanson *et al.* 1992) is based on a monoclonal antibody recognizing a peptide comprising part of the N-terminal telopeptide. The assay procedure was standardized in terms of 'bone collagen equivalents' by reaction with peptides derived from a digest of a known quantity of bone (Hanson *et al.* 1992). More recent studies indicate, however, that the assay shows equivalent reaction with collagen type I peptides from other tissue sources (Robins, 1995).

Several assays based on the C-terminal telopeptide location of collagen cross-linking have been developed. Antibodies to a relatively large peptide (8·5 kDa) derived from collagenase (*EC* 3.4.24.8) digestion of bone collagen form the basis of the collagen type I cross-linked C-telopeptide assay (Risteli *et al.* 1993). As a serum assay, this method is probably not directly comparable with the other assays which at present are all urine-based. By raising polyclonal antibodies against a synthetic octapeptide comprising the cross-linking region of the C-terminal telopeptide of collagen type I, Bonde *et al.* (1994) developed an inhibition ELISA, known generically as a CTx assay, that showed statistically significant correlations with pyridinium cross-link measurements when applied to urine. Later studies have shown that this assay measures only peptides containing iso-aspartyl bonds (Fledelius *et al.* 1997), the formation of which represents an age-related process in extracellular proteins. The relevance of these factors on the application of CTx measurements to assess bone resorption has yet to be fully evaluated.

Galactosyl-hydroxylysine (Gal-Hyl), a component of collagenous proteins excreted in urine, has been shown to be a marker primarily of bone resorption (Krane *et al.* 1977) and has received renewed interest following the development of HPLC techniques for analysis (Moro *et al.* 1988). Because it is derived from an intracellular modification, Gal-Hyl may also reflect the degradation of newly-synthesized collagen. Nevertheless, the urinary excretion values obtained correlate well with the cross-link values (Bettica *et al.* 1992).

Variability of biochemical markers

For all the biochemical markers of bone turnover, the analytical variabilities have been established by detailed studies and these are generally in the range 5–10%. In using markers to monitor changes induced by certain dietary components or by drug therapies, it is clearly also important to have some knowledge of the biological variability. A study of the effects of 6-month hormone-replacement therapy using several different markers showed that the changes in resorption indices measured in urine were greater than those for the serum formation markers, but that the variability was also greater (Hannon *et al.* 1995). Taking into account both the analytical and biological variabilities, the calculated least significant change for each marker was of the same order as the change induced by oestrogen. Thus, in order to minimize variability, it may be necessary to perform several measurements in individuals to off-set the effects of day-to-day variations.

OESTROGENS AND BONE METABOLISM

The important role played by oestrogens in bone metabolism is well recognized, although the mechanisms of action at the cellular level are less well understood. The effects of oestrogen withdrawal around the time of the menopause can be readily detected with biochemical markers, particularly those for bone resorption. For example, a preliminary study of the excretion of Dpd in 225 women for which the menopausal status had been well

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defined showed significant increases in both the peri- and post-menopausal groups in comparison with the premenopausal controls (Robins *et al.* 1994*a*). Evidence has recently been obtained that, even in elderly women, oestrogen deficiency contributes significantly to the high level of bone resorption (McKane *et al.* 1997). Treatment of a group of women having a mean age of about 74 years with oestrogen resulted in lower bone resorption markers than those for an age-matched, untreated group, and these changes were accompanied by a corresponding decline in serum parathyroid hormone levels; dietary Ca was similar in both study groups.

The new biochemical marker techniques are ideally suited, therefore, to monitoring the effects of oestrogen on bone and are currently being used, for example, to assess compliance for hormone-replacement therapy. With the wide distribution of plant oestrogens or phyto-oestrogens in the diet (Knight & Eden, 1995), there is now considerable interest in the possibility of using the techniques described in the present review to investigate the potential beneficial effects of phyto-oestrogens.

NUTRITIONAL INFLUENCES ON BONE METABOLISM

The influence of nutritional factors on bone metabolism has received little attention compared with that given to the effects of these same factors on bone density. Consequently, our knowledge of the role that dietary intake has to play on the development and maintenance of bone health still remains largely undefined. The following section will review current knowledge on the influence of several key nutrients on bone metabolism, i.e. P, K, Na, Mg and vitamin K. This list is necessarily selective because of the limited length of the review; Ca and vitamin D have been discussed elsewhere in the symposium.

Phosphorus

This nutrient is as important for bone health as Ca since it makes up about half the weight of bone mineral and, hence, must be available in adequate amounts in the diet to mineralize and maintain the skeleton. It is present in relatively adequate amounts in the diet and attention, therefore, has tended to focus on the potentially harmful effects of excessive amounts and the characteristics of a low Ca : P value.

In contrast to protein, P has a hypocalciuric effect, thus negating the hypercalciuric influence of protein as these nutrients naturally appear together in many foods. Increased P intake has been shown to depress ionized Ca, which in turn leads to an increase in parathyroid hormone and increased hydroxyproline excretion (Calvo *et al.* 1988) which may last for at least 4 weeks (Calvo *et al.* 1990). Similar results have also been obtained when both Ca and P intakes were lowered, suggesting that the effects are not necessarily due specifically to increased P intake (Barger-Lux & Heaney, 1993). High intakes are also known to suppress the renal synthesis of calcitriol, which in turn could lead to a decrease in Ca absorption. However, there is some evidence to show that increased P intake reduces Ca loss, increasing Ca absorption and, thus, total body Ca is not affected (Heaney, 1993).

Very few studies have found harmful effects of excess P intake (and Ca : P values) on bone health in human subjects. Heaney & Recker (1982) examined the influence of varying P intakes on Ca balance and failed to show a deleterious effect. In human volunteers, few studies have assessed directly the effects of dietary P intake on the markers of bone formation and resorption. In our own cross-sectional study of sixty-five healthy pre- and peri-menopausal women, we found that low intakes of P were significantly associated with

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higher Pyd and Dpd excretion and this relationship remained significant after adjustment for many of the important confounding factors (New *et al.* 1996).

Potassium

Recently, attention has focused on the influence of K on bone health. The skeleton may play an important role in acid–base homeostasis by the mobilization of skeletal salts to balance the endogenous acid generated from acid-producing foods. $KHCO_3$ administration is known to be associated with a decreased urinary Ca excretion (Lemann *et al.* 1989, 1991) and a general improvement in Ca balance (Lemann *et al.* 1993). More recently, it has been shown to improve mineral equilibrium and skeletal metabolism in healthy post-menopausal women (Sebastian *et al.* 1994). Serum osteocalcin concentrations increased from 5·5 (SD 2·8) to 6·1 (SD 2·8) ng/ml and urinary hydroxyproline excretion decreased from 28·9 (SD 12·3) to 26·7 (SD 10·8) mg/d after $KHCO_3$ was given orally for 18 d in doses of 60–120 mmol/d.

These findings suggest that either increased plasma acidity or decreased plasma bicarbonate concentrations may stimulate bone resorption directly by the process of mineral dissolution and indirectly by reducing the pH and bicarbonate concentrations of osteoclasts (Bushinsky & Sessler, 1992). This in turn promotes the adhesion of osteoclast cells to their bone resorptive sites and the secretion of H^+ ions into the bone resorbing fluid compartments (Arnett & Dempster, 1986; Teti *et al.* 1989). Acidosis may also inhibit osteoblast function and consequently limit bone formation (Krieger *et al.* 1992).

Our own investigations showed significant associations between dietary intakes of K and markers of bone resorption but not bone formation (New *et al.* 1996). Women who were in the lowest quartile for K intake had a significantly higher bone resorption. K was found to explain 10 % of the variation in Pyd excretion and 28 % of the variation in Dpd excretion, both analyses being independent of other non-dietary factors. Furthermore, follow-up subjects at 1 year showed a trend for women who gained bone to have higher intakes of K and Mg (New, 1995). In one other study which examined directly the relationship between dietary K intake and bone formation (osteocalcin) in women aged 28–74 years, no significant association between the two variables was found (Michaelsson *et al.* 1995).

Sodium

There is much evidence supporting a positive relationship between urinary Na and Ca excretion in young and adult free-living individuals consuming a normal diet (Shortt & Flynn, 1990). Re-absorption of Ca and Na in the proximal tubule and loop of Henle are known to be linked. Thus, a reduction in renal Na re-absorption (such as that induced by a high salt intake) also leads to a reduction in Ca absorption and increased Ca urinary loss. Hypercalciuria, caused by high Na intakes, is well established in both human subjects (Chan *et al.* 1992) and animals (Chan *et al.* 1993). Increased Ca excretion in human subjects may also be accompanied by increased urinary hydroxyproline excretion, suggesting an elevated bone resorption (Need *et al.* 1991). Few studies have examined directly the relationship between 24 h urinary excretion of Na (which is the best indicator of dietary intake) and bone resorption using pyridinium cross-links, although this technique has been used recently to demonstrate a significant relationship between Dpd excretion and the increased urinary Ca resulting from a high Na intake (Lietz *et al.* 1997).

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Magnesium

This nutrient is considered by many to be extremely important in skeletal metabolism (Thomas, 1988; Trimmer, 1988). In animal studies, Mg deficiency causes cessation of bone growth, decreased osteoblastic and osteoclastic activity, osteopenia and increased bone fragility, and the development of a form of 'aplastic bone disease' (Schwartz & Reddi, 1979). The ability of Mg deficiency to induce osteoporosis may be explained by the impairment of a skeletal ATPase (H^+/K^+-transporting ATPase; *EC* 3.6.1.36) responsible for transporting K^+ into the skeletal interstitium in exchange for H^+ extrusion. The resulting decrease in the pH of the skeletal interstitium may cause a slow, but relentless, dissolution of bone independently of other direct effects of Mg deficiency on bone or the indirect effects on parathyroid hormone secretion and action (Driessens *et al.* 1987).

The significant relationships which we found between dietary intake of Mg and bone resorption have not been previously reported. Dietary Mg was found to account for 8 % of the variation in Pyd excretion and 27 % of the variation in Dpd excretion, both analyses being independent of other non-dietary factors (New *et al.* 1996).

Vitamin K

As indicated earlier, this nutrient may also be important to bone health since the synthesis of the principal non-collagenous protein of bone (osteocalcin) and matrix Gla protein is dependent on vitamin K. However, the evidence is rather confusing as significantly reduced circulating levels of vitamin K have been reported, not only in women suffering osteoporotic fractures of the spine and hip (Hodges *et al.* 1993), but also in elderly women with good bone health (Hodges *et al.* 1990). Under-carboxylation of osteocalcin has been reported in osteoporotic women (Knapen *et al.* 1989) and although supplementation with vitamin K has been shown to correct this defect (Douglas *et al.* 1995), there are considerable technical problems associated with the measurement of osteocalcin, as shown by many conflicting reports in the literature (Delmas *et al.* 1990). Furthermore, osteocalcin is also dependent on vitamin D for its synthesis and there are some data to suggest that supplementation with vitamin D alone can also return carboxylation of osteocalcin to normal levels (Szulc *et al.* 1993).

To date, no studies have addressed directly the relationship between dietary intake of vitamin K and bone density or bone metabolism due to the lack of a dietary database relating to vitamin K content of foods. This has recently been developed in the USA (Booth *et al.* 1995) and a UK database will shortly be available (Price *et al.* 1996).

Proposed areas for future research

In summary, although the relationship between nutritional factors and bone health has received considerable attention in the last decade, our understanding of the exact mechanisms involved and the key nutrients required for optimum bone health still remains largely undefined. Areas of particular interest include the potential roles of dietary K, Mg and vitamin K in developing and maintaining bone health. Furthermore, antioxidant vitamins and minerals may also have a role to play in the development and subsequent maintenance of bone health, as vitamin C is essential for the hydroxylation of proline and lysine for the cross-linkage required for normal collagen fibre formation.

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BONE TURNOVER AND RISK OF OSTEOPOROSIS

Until very recently, the risk of osteoporosis has been assessed mainly by measurements of bone mineral density (BMD). These continue to provide valuable information on bone status, particularly as recent Consensus Conferences (Consensus Development Conference, 1993) and the World Health Organization (1994) have now defined osteoporosis in terms of BMD values. Two large prospective studies reported recently, however, have provided evidence that the rate of bone turnover may itself be a risk factor. In the Rotterdam study (van Daele *et al.* 1996), over 10 000 individuals over 54 years of age were followed for about 2 years, and the urinary bone markers and lifestyle characteristics of seventeen women suffering a fracture during that time, were each compared with three aged-matched controls. The results showed that high urinary Dpd cross-link concentrations were a significant risk factor for future hip fracture. This study also emphasized the importance of adequate mobility for bone health. The EPIDOS prospective study comprising about 7500 women used a similar protocol but, as the cohort was much older (more than 75 years of age), a larger proportion sustained a hip fracture during the 22-month follow-up period (Garnero *et al.* 1996). The data showed that low BMD was a risk factor with an odds ratio of 2·7 for the increased likelihood of hip fracture, but that high bone resorption, as indicated by urinary marker concentrations above the premenopausal range, was also predictive with an odds ratio of about 2. Interestingly, combining BMD with bone markers markedly increased the prediction of hip fracture (Garnero *et al.* 1996). These results indicate, therefore, that increased bone resorption measured using the recently-developed biochemical markers could reflect increased bone fragility independently of bone mass.

CONCLUDING REMARKS

There have been major advances in recent years, both technically and conceptually, in the development of biochemical bone markers. The methods of choice for bone resorption are those based on measuring collagen cross-links in urine and, for bone formation, serum bone-specific alkaline phosphatase as well as improved osteocalcin assays are now available. The increased sensitivity and specificity of these markers over their predecessors, urinary hydroxyproline and serum total alkaline phosphatase, has widened the scope for investigations of subtle changes in bone metabolism such as those brought about by dietary influences. Such studies are leading to an improved understanding of the relationship between nutrient intakes and bone health. In terms of the risk of developing osteoporosis, high bone turnover has been shown to be a significant factor that should be considered together with many others, including bone density, bone quality as indicated by ultrasound, lifestyle, nutrition and perhaps genetic markers. In individuals, any one of these factors will give an incomplete and possibly false assessment of the risk of future bone fracture. The biochemical bone markers, however, are likely to form one of a panel of factors used to assess the risk of osteoporotic fracture.

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