TRACE ELEMENT NUTRITION AND BONE METABOLISM

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CONTENTS

INTRODUCTION

Many of the trace elements which have essential roles in animals, such as zinc (Zn), manganese (Mn) and copper (Cu), are required for the growth, development and maintenance of healthy bones (Rucker, 1988; Leach, 1988). Of the remaining trace or ultratrace elements, which by definition are normally present at low concentrations in animal tissues, some have a toxic effect on bone such as lead (Pb) and cadmium (Cd), and others have either low toxicity or have not been studied in any detail. At high concentrations, essential trace elements can also affect bone metabolism with harmful consequences but, rarely, a trace element can induce apparently beneficial effects at pharmacological doses. For example, fluoride (F) therapy increases bone density in patients with advanced osteoporosis. For some trace elements such as boron (B) and molybdenum (Mo), the evidence for a direct effect on bone, be it essential or toxic, is not conclusive at this time.

Although the essential trace elements are present in minute amounts, their influence on normal metabolic processes can be considerable and often amplified through interaction with or incorporation into proteins, particularly enzymes (Underwood & Mertz, 1987). Pharmacological and, usually, toxic effects, on the other hand, may involve enzyme inhibition by binding to the active site of an enzyme, displacement of essential metals from metal-dependent, bio-active molecules, direct interactions with metabolic intermediates or

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competitive interactions with major minerals. Both physiological and pharmacological effects, encompassing those which are beneficial and harmful, are reviewed here. The elements are discussed in order of their position in the periodic table, starting with group 2B, and those with similar chemical properties are grouped together. Several recent reviews and texts may be of help to those unfamiliar with bone biochemistry, growth and disease (Nimni, 1988; Riggs & Melton, 1988; Favus, 1990; Loveridge *et al.* 1992).

ZINC

Zn deficiency is associated with many kinds of skeletal abnormalities in fetal and postnatal development. Hurley (1981) has reviewed the teratogenic effects of Zn deficiency, which include abnormal development of ribs and vertebrae, agenesis of long bones, club foot, cleft palate and micrognathia (undersized mandible) in the rat. Offspring of Zn deficient rats also show impaired ossification (da Cunha Ferreira *et al.* 1989). Defects in skeletal development have also been reported in Zn deficient chicks, pigs, cows, rhesus monkeys and man (Hurley, 1981).

Leek *et al.* (1984, 1988) reported that marginal Zn deficiency in infant rhesus monkeys led to defective mineralization and delayed appearance of epiphyseal centres compared to pair-fed controls. The gross skeletal appearances bore many similarities to rickets: widening of growth plates, indistinct zones of provisional calcification, bowing of the long bones, and thin cortices. The similarity between these changes and those observed in hypophosphatasia (genetic absence of alkaline phosphatase, *EC* 3.1.3.1), may reflect the fact that alkaline phosphatase requires Zn as a cofactor (Adeniyi & Heaton, 1980). Congenital malformations have also been reported in acrodermatitis enteropathica in man (Hambidge *et al.* 1975). In Iranian schoolboys, Zn supplementation stimulated both skeletal growth and maturation (Ronaghy *et al.* 1974).

Many of the effects of Zn deficiency on bone metabolism may be related to a generalized impairment of nucleic acid and protein metabolism. Yamaguchi and co-workers have demonstrated Zn-related increases in protein synthesis, alkaline phosphatase activity and bone collagen content in bone tissue culture (Yamaguchi & Matsui, 1989; Yamaguchi *et al.* 1987). They also showed that oral β-alanyl-L-histidinato Zn (AHZ) significantly increased the Zn and calcium (Ca) content, alkaline phosphatase activity and DNA content of the femoral diaphysis in elderly rats (Yamaguchi & Ozaki, 1990*b*). Similar results were found in weanling rats (Yamaguchi & Ozaki, 1990*c*) and in rats subjected to hindlimb disuse (Yamaguchi *et al.* 1990). Yamaguchi & Miwa (1991) conclude that AHZ may be of value therapeutically in osteoporosis.

Haumont (1961) found bone Zn to be concentrated in the layer of osteoid prior to calcification, which corresponds to the greatest concentration of alkaline phosphatase. Although Zn is a cofactor for this enzyme, the effect of Zn deficiency on synthesis of the enzyme itself is more important (Adeniyi & Heaton, 1980). Bone alkaline phosphatase also requires magnesium (Mg) as a cofactor and excess Zn may inhibit alkaline phosphatase if magnesium is displaced (Ciancaglini *et al.* 1990).

Collagenase (*EC* 3.4.24.3) is another Zn dependent metalloenzyme, essential for bone resorption and remodelling (Swann *et al.* 1981). Enzyme activity declines with Zn deficiency in the chick (Starcher *et al.* 1980). Although bone carbonic anhydrase (*EC* 4.2.1.1) also requires Zn as a cofactor, Huber & Gershoff (1973) were unable to show any significant effect of Zn deficiency on this enzyme in the rat.

The skeleton contains a large proportion of the total body burden of Zn (Herzberg *et al.* 1990). The extent to which this Zn can be mobilized by conditions such as Ca deficiency

has been investigated in the pregnant rat (Hurley & Shyy-Hwa, 1972). Although early mobilization of maternal bone provided sufficient Zn to prevent fetal malformations, most fetal Zn was derived from maternal muscle mobilized later in the Ca deficient pregnancy (Masters *et al.* 1986). Bone appears to act as a Zn sink in the rat, as Zn released during skeletal breakdown is mostly reincorporated into the skeleton (Murray & Messer, 1981; Sherman *et al.* 1989). In man, the vertebral Ca/Zn ratio is inversely related to age, suggesting that skeletal Zn is conserved better than Ca in later life (Aitken, 1976).

Zn also occurs in the mineral component of bone, probably in hydroxyapatite (Murray & Messer, 1981; Sauer & Wuthier, 1990). It may complex with F, and both Zn and the Zn–F complex may improve the crystallinity of apatite (Lappalainen *et al.* 1983). Atik (1983) found osteoporotic patients to have lower levels of skeletal Zn than controls but this finding was not confirmed by others (Lappalainen *et al.* 1982; Reginster *et al.* 1988). However, Angus *et al.* (1988) found forearm bone mineral content to be correlated with Zn intake in pre-menopausal women.

Bedrest in healthy adult males induced negative Zn balance (Krebs *et al.* 1988), possibly because of bone atrophy, but this was partly prevented with the addition of F to the diet. In post-menopausal women urinary Zn has been suggested as a marker of bone resorption, since women with osteoporosis excreted more than 800 μg Zn g^{-1} creatinine in urine (Herzberg *et al.* 1990).

Saltman & Strause (1991) reported that supplements of trace minerals, with or without Ca, in post-menopausal women had beneficial effects on bone density. As Ca supplementation is being widely advocated for post-menopausal women, it is important to assess the effect on trace element status, particularly as Ca complexes with Zn and phytate to prevent Zn absorption (Sandström & Lönnerdal, 1989).

Other trace elements may interact with Zn and thereby affect bone metabolism. Thus high Zn intakes may exacerbate the bony lesions induced by low Cu diets (Pond *et al.* 1990), but ameliorate the toxic skeletal effects of Cd (Suzuki *et al.* 1990; Kaji *et al.* 1990), vanadium (V), germanium (Ge), selenium (Se) and aluminium (Al) (Yamaguchi *et al.* 1989; Yamaguchi & Uchiyama, 1987; Yamaguchi & Ozaki, 1990*a*).

Zn may possibly modify hormonal influences on bone metabolism. For example, short term Zn infusions depressed calcitonin levels in man without affecting ionized Ca levels (Nishiyama *et al.* 1991). Yamaguchi & Oishi (1989) demonstrated that Zn enhanced the ability of 1,25-dihydroxycholecalciferol (1,25-DHCC) to increase alkaline phosphatase activity and DNA content in rat calvaria. Decreased somatomedin activity has been shown in Zn deficient rats (Bolze *et al.* 1987), associated with decreased glycosaminoglycan formation in rib epiphyses. These changes were greater than those seen in pair fed animals, suggesting that differences were not solely due to decreased food intake.

CADMIUM

Cd and Ca, which have similar chemical characteristics, are mutually antagonistic and the toxic effects of Cd are therefore enhanced in conditions of Ca deficiency (Webb, 1979). Vitamin D and protein deficiencies in combination with Ca deficiency further exacerbate the toxicity of Cd and such a combination of factors has been implicated in the aetiology of itai-itai disease, a crippling bone condition found in a limited area of the Toyama Prefecture, Japan (Lauwerys, 1979). Identified predominantly in post-menopausal women with a history of multiple childbirths, the disease was characterized by a combination of osteoporosis and osteomalacia resulting in extreme fragility and multiple fractures of bones. Thus factors which affect bone Ca reserves, such as pregnancy and lactation

(Bhattacharyya *et al.* 1988*a*) and low oestrogen status, e.g. in response to ovariectomy (Bhattacharyya *et al.* 1988*b*), are likely to exacerbate the effects of Cd on bone.

Larsson & Piscator (1971) proposed that Cd-induced renal tubular dysfunction and subsequent loss of Ca to urine was the principal cause of osteomalacia in itai-itai disease. This idea was subsequently disputed because some itai-itai patients had severe osteoporosis but no marked renal tubulopathy. In addition, Yoshiki *et al.* (1975) demonstrated that Cd-treated animals developed osteoporosis before developing renal tubular dysfunction. However, Kido *et al.* (1990) pointed out that the Cd exposure levels used in this study were much higher than those of people living in Cd contaminated environments. No significant association was found between the severity of osteopenia, as determined by a microdensitometric method, and urinary or blood Cd levels in elderly women from a Cd-contaminated area in the Kakehashi River basin, Ishikawa Prefecture, Japan (Kido *et al.* 1990). There were, however, significant negative correlations between bone density and the severity of renal dysfunction, which was assessed using several renal function tests.

Nogawa *et al.* (1987) demonstrated that Cd-exposed men and women have lower levels of serum 1,25-DHCC and higher levels of serum parathyroid hormone (PTH) than non-exposed control subjects. PTH normally stimulates the hydroxylation of 25-hydroxycholecalciferol (25-HCC) to its biologically active form in the kidney, and Nogawa and colleagues proposed that the lower levels of circulating 1,25-DHCC resulting from renal damage and impaired hydroxylase activity reduce intestinal Ca absorption efficiency whereas the higher concentrations of PTH enhance bone resorption. In a series of experiments in which crab-eating monkeys were fed Cd-contaminated rice or a diet containing 3 mg Cd kg^{-1} for 6 years, Kawashima *et al.* (1988) found no effect of Cd on serum vitamin D metabolite levels including 1,25-DHCC. In a second series of studies lasting 9 years, Kawashima *et al.* (1988) fed rhesus monkeys on a diet containing 3, 10, 30 or 100 mg Cd kg^{-1} and again found no effect of Cd on serum vitamin D metabolite levels. They did however observe a suppression of renal 25-HCC-1-hydroxylase (*EC* 1.14.13.13) activity at the two highest levels of Cd although no skeletal abnormalities were evident.

The structural and biochemical changes in bone relating to Cd intake are well documented. Kido *et al.* (1991) noted that elderly men and women living in a Cd-polluted environment had higher levels of serum osteocalcin than non-exposed subjects, indicating a higher rate of bone turnover. Histopathological examination of iliac bone from 62 autopsy cases of itai-itai disease revealed a reduction in bone density and in the presence of osteoblasts, marked osteoid accumulation with impaired mineralization and an increase in resorption surface area (Noda & Kitagawa, 1990). Godowicz & Godowicz (1990) compared bone repair in Cd-treated mice and control animals and found that 6 weeks after fracture the bones of control animals were fully healed, whereas with Cd treatment the bone fragments were interconnected by soft tissue, classified microscopically as fibrocartilaginous callus. Although earlier studies indicated that bone does not accumulate high levels of Cd when animals are treated with Cd in the diet or by injection (see Webb, 1979), Krishnan *et al.* (1990) have noted that the Cd content of bones of rats dosed with Cd in their drinking water increased in a dose-dependent manner. Ogoshi *et al.* (1989) found increased concentrations of Cd in the femurs of young rats given Cd in their drinking water for 4 weeks. They also showed that although Cd adversely affects the mechanical strength of femurs in young rats, it did not affect femur strength of adult and old rats given the same Cd supplements. From studies of embryonic chick bone in culture, Kaji *et al.* (1988*a*) concluded that Cd has a detrimental effect on periosteum and osteoblasts, resulting in a striking decrease in collagen content. Since this effect on collagen was much greater, and occurred at lower Cd concentrations, than a decrease in bone Ca, they proposed that the primary effect of Cd on bone formation is an inhibition of bone matrix formation.

Observation of hydroxyapatite crystal growth and dissolution in vitro in the presence or absence of up to 5 μM Cd showed that although the metal had been incorporated into the crystal structure and did not affect crystal growth, the rate of dissolution was considerably reduced when the crystals contained Cd (Christoffersen *et al.* 1988). However, fetal rat limb bones pre-labelled with ^{45}Ca and incubated in culture medium released the Ca isotope at a much greater rate in the presence of 10 nM Cd than in the absence of the metal (Bhattacharyya *et al.* 1988*b*). A similar dose-dependent increase in bone resorption was found by Suzuki *et al.* (1989*a*) who incubated fetal mouse calvaria in a medium containing Cd. They also noted a Cd-induced stimulation of prostaglandin E_2 (PGE_2) production which, along with resorption, was inhibited by indomethacin. However, the rate of resorption was restored by exogenous addition of PGE_2 which suggests a role for PGE_2 in the stimulation of bone resorption by Cd. In further studies with an osteoblast-enriched cell culture, the stimulation of PGE_2 by Cd was inhibited by the presence of Zn (Suzuki *et al.* 1990).

Like Ca, Zn is antagonistic in its interaction with Cd. In studies with embryonic chick bones in culture, a Cd-induced inhibition in collagen synthesis was prevented by Zn (Miyahara *et al.* 1983). In similar culture conditions, Kaji *et al.* (1988*b*) detected toxic effects of Cd on bone growth and, specifically, mesenchymal cell proliferation, differentiation and also osteoid formation in embryonic chick femurs. All of these effects were reversible by the addition of Zn. Cd concentrations in osteoblasts (Bawden & Hammerström, 1975), and treatment of osteoblast-like cells (MC3T3-E1) in culture with Cd decreased cellular Zn content (Suzuki *et al.* 1989*b*). Mineralization by these cells was also inhibited by Cd, an effect that was reversed by the addition of Zn. The inhibition of bone alkaline phosphatase, an enzyme requiring Zn for activity, by Cd and the prevention of this inhibition by Zn has been noted on a number of occasions (Bonner *et al.* 1980; Kaji *et al.* 1988*a*; Suzuki *et al.* 1989*b*). However, Kaji *et al.* (1988*b*, 1990) found that, in contrast to Zn, Cd did not counteract the increase in diaphysis Ca content normally observed in cultures of embryonic chick femur.

Interactions of Cd with Cu have been observed although in contrast to Zn, Cu appears to enhance the toxic effects of Cd. For example, Cu enhanced the inhibition of collagen synthesis by Cd (Kaji *et al.* 1986). Iguchi & Sano (1982) demonstrated that the activity of bone lysyl oxidase (*EC* 2.3.2.3), a Cu-dependent enzyme which promotes crosslinking and therefore stabilization of collagen molecules, was inhibited when rats were fed a diet containing 50 mg Cd kg^{-1} and adequate Cu. Displacement experiments in vitro and further studies in vivo (Iguchi *et al.* 1990) indicate that the replacement of Cu by Cd at the active site of the enzyme is a possible mechanism of inhibition. Concerning itai-itai disease, Kaji *et al.* (1990, 1991) point out that pollution of the Jintsu river basin by Cu, Zn and other metals such as Pb and iron (Fe), in addition to Cd, may cause interactive effects on the bones of affected individuals and that attempts to characterize Cd toxicity on the basis of itai-itai osteopathology may be misleading.

COPPER

As discussed in several reviews (Fell, 1987; Davis & Mertz, 1987; Dollwet & Sorenson, 1988), Cu deficiency inhibits bone growth and promotes pathological changes characteristic of osteoporosis. There have been many reports of abnormal growth and bone development in animals which are Cu-deficient although it appears that children and some species such as chicks, pigs, dogs, horses and rabbits are more sensitive than others, for example sheep and rats. The teratogenic effects of Cu deficiency are also well documented (see Hurley, 1981). Osteoporosis is also associated with the Cu deficiency which results from genetically

determined malabsorption of Cu, such as in Menkes' disease and the mottled mutant mouse (Danks, 1987).

Elderly patients with fractures of the femoral neck were found to have significantly lower serum Cu levels than age and sex matched controls (Conlan *et al.* 1990). Howard *et al.* (1990) reported that post-menopausal women with a high dietary Ca intake combined with a high serum Cu level had a greater lumbar bone density than women with low Ca intake and low serum Cu. In a 2-year double-blind, placebo controlled study, Saltman & Strause (1991) have shown that bone loss in post-menopausal women given combined Ca (1000 mg d^{-1}) and trace mineral (2·5 mg Cu, 5·0 mg Mn and 15 mg Zn) supplements was significantly less than in the placebo group and in groups taking the trace mineral or Ca alone. The Cu content of bone was negatively correlated with bone Ca, bone density and collagen content in ageing mice (Massie *et al.* 1990), whereas it appears that bone Cu levels in human subjects with osteoporosis are the same or possibly slightly higher than in 'normal' individuals of the same age (Reginster *et al.* 1988; Baslé *et al.* 1990).

The bones and cartilage of Cu deficient animals show increased defects and fragility (Rucker *et al.* 1975; Bridges & Moffitt, 1990; Knight *et al.* 1990) and contain an enhanced proportion of soluble collagen (Carnes, 1971). This indicates a reduction in the amount of collagen crosslinking, a process which is essential for the maintenance of tensile strength. Since Cu is a cofactor of lysyl oxidase, an enzyme which is involved in the initiation and regulation of collagen and elastin crosslinking (Rucker & Murray, 1978; Tinker *et al.* 1988), and the activity of this enzyme in bone is greatly reduced in Cu deficiency (Siegel *et al.* 1970), it is presumed that this mechanism is of primary importance in Cu deficiency-related osteopathogenesis. Indeed, Robins *et al.* (1985) and Farquharson *et al.* (1989) have shown a significant reduction in pyridinium cross link concentration in the femoral diaphysis of Cu-deficient rats.

Histological and biochemical studies of bones from Cu-deficient animals suggest that osteoblast function is inhibited whereas osteoclast activity is unaffected; the net result is inhibition of bone formation or loss of bone. In a 12-month study using ectopic subcutaneous implants of devitalized, demineralized bone powder to assess bone formation and mineral-containing bone particles to measure resorption, Strause *et al.* (1987) reported that a low Cu, low Mn diet inhibited both osteoblast and osteoclast activity in rats. However, osteoblast activity was impaired more than osteoclast activity.

Cu overload can also interfere with bone metabolism as shown, for example, by generalized loss of bone density, rickets and anomalous osteophytes in Wilson's disease patients (Seymour, 1987). Incubation of embryonic chick femurs with 2·5 μM Cu or more decreased collagen content of both diaphysis and epiphysis, mainly due to inhibition of collagen synthesis (Kaji *et al.* 1988*a*). Studies using the same culture system showed that Cu-induced inhibition of mineral and matrix formation was unaffected by Zn (Kaji *et al.* 1990) but was enhanced in the presence of Cd (Kaji *et al.* 1991). Cu also interacts with V to reduce vanadate-induced growth depression (Hill, 1990*a*: see p. 173).

IRON

Ferrous Fe is a loosely bound non-haem component of the enzymes procollagen proline hydroxylase (*EC* 1.14.11.2) and procollagen lysine hydroxylase (*EC* 1.14.11.4) which are essential for the hydroxylation of proline and lysine residues respectively in biosynthetic precursors of collagen (Prockop, 1971). Removal of Fe with chelators inactivates these enzymes; activity is restored by the addition of Fe^{2+} or Fe^{3+} but not of Ca^{2+}, Mg^{2+}, Zn^{2+}, cobalt (Co^{2+}) or Cu^{2+}. To the authors' knowledge, effects of Fe deficiency on bone metabolism have not been reported.

High dietary Fe intakes can reduce bone mineral content, as observed in chicks given 5000 mg Fe kg^{-1} diet (Baker & Halpin, 1991). The accumulation of Fe in bone is greatly increased in some patients undergoing dialysis (Phelps *et al.* 1988; McCarthy *et al.* 1991); in the latter study, deposition of Fe in bone occurred mainly in the younger patients, who also tended to have higher serum ferritin and lower serum immunoreactive PTH levels. Studies with ^{59}Fe suggested that non-ferritin bound Fe preferentially accumulated at osteoid seams but was also rapidly mobilized from this site (Huser *et al.* 1988). Post mortem examination of bones from itai-itai patients (see p. 169) showed accumulation of non-ferritin Fe at mineralization fronts and it was suggested that Fe and Cd act synergically in disrupting the process of mineralization (Noda *et al.* 1991). However, when rats were treated with ferric nitrilotriacetate, Fe was deposited not at the interface between osteoid and mineralized bone, as found for Al in rats treated with Al nitrilotriacetate, but in the osteoblasts and osteoclasts (Ebina *et al.* 1991).

MANGANESE

Mn is a cofactor for glycosyltransferases, which catalyse the transfer of a sugar from a nucleotide-diphosphate sugar to an acceptor molecule (Leach, 1971). Therefore Mn is essential for several stages in the formation of the glycosaminoglycan chondroitin sulphate. This is deficient in the epiphyseal cartilage and bone matrix in Mn deficient chicks (Leach & Muenster, 1962). A wide range of skeletal abnormalities is found in animals deprived of Mn in the pre-natal and early post-natal period (Hurley, 1981). These include chondrodystrophy in chick embryos, and thickened long bones, dysproportion of the skull, dysplasia of the tibial epiphysis and defects in the inner ear in the rat. More recently, Strause *et al.* (1987) reported defects in chondrogenesis, osteogenesis and bone resorption in Mn deficient rats.

Prolonged Mn deficiency has been associated with osteoporosis in man (Asling & Hurley, 1963), and low serum Mn levels have been found in osteoporotic subjects (Reginster *et al.* 1988). A preliminary report by Saltman & Strause (1991) suggested that a trace element supplement containing Cu, Zn and Mn may add to the beneficial effect of Ca supplementation on bone mineral density in post-menopausal women.

VANADIUM

Although there have been claims that V is an essential trace element, definitive proof is still lacking (Nielsen, 1988*a*). High dietary V intakes can be toxic and depress growth (Hill, 1990*c*) but they may also have beneficial pharmacological effects on bone. The stimulation by V of DNA and collagen synthesis and of alkaline phosphatase activity in cultures of 21-day old fetal rat calvaria indicated that V may enhance bone formation (Canalis, 1985). Lau *et al.* (1988) found similar results when treating cultured chicken calvarial cells, enriched with osteoblasts and osteoblast precursors, with 5–15 μM orthovanadate. The treatment also inhibited osteoblastic acid phosphatase (*EC* 3.1.3.2) activity and stimulated bone cell proliferation in the same way as F. The effects of V on DNA synthesis, collagen synthesis and alkaline phosphatase activity have subsequently been confirmed in vivo in studies with weanling rats (Yamaguchi *et al.* 1989). Bone acid phosphatase activity was not altered significantly in this study and V was found to stimulate bone formation within a narrow dose range.

The inhibitory effects of V on growth have been ameliorated by dietary supplementation with Zn (Yamaguchi *et al.* 1989), Cu (Hill, 1990*a*), chloride (Cl) (Hill, 1990*c*) and even

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mercury (Hg) (Hill, 1985, 1990*b*). Hill (1985) proposed that Hg may stimulate the conversion of vanadate to the less toxic form vanadyl.

BORON

Until recently there was no evidence to suggest an essential role for B in animals. However, since the early 1980s, Nielsen, Hunt and colleagues have reported beneficial effects of dietary B on bone in rats and chicks and bone-related hormones and minerals in both animals and humans. Nevertheless, B deprivation where nutrition is otherwise adequate would appear to have limited effect and B is reported to have its greatest influence in combination with other metabolic or nutritional stress factors, for example vitamin D and/or Mg deficiency (Nielsen, 1990). It has been suggested that B could be an important nutritional factor determining the incidence of osteoporosis (Nielsen *et al.* 1987; Nielsen & Hunt, 1989). Remedies for osteoporosis which contain B are being sold to the public by some supplement companies. B is found mainly in foods of plant origin, particularly fruit and vegetables, and the mean human dietary intake of B in the UK and USA is approximately 1–3 mg d^{-1}.

In one study, Nielsen *et al.* (1987, 1988*a*) gave healthy post-menopausal women a low B diet (0·25 mg B d^{-1}) for an acclimation period of 24 d followed by 4 consecutive periods of 24 d to investigate the effects of Mg and Al deprivation/supplementation. Twelve of the women continued the study for 2 more B-supplemented periods each of 24 d when they were given 3·25 mg B d^{-1}. This increased plasma concentrations of oestradiol-17β from 21 to 41 pg ml^{-1} and those of testosterone from 0·31 to 0·83 ng ml^{-1} and decreased urinary Ca excretion (0·117 to 0·065 g d^{-1}) when the dietary Mg intake was low (200 mg d^{-1}).

In contrast, Beattie & Peace (1992) found no significant changes in plasma testosterone, mineral balance and urinary Ca and pyridinium cross link excretion in healthy post-menopausal women when their daily B intake was increased from 0·33 to 3·33 mg. However, all the women were hypercalciuric throughout this 6-week study and in positive Ca balance, which indicates that there was a B-independent stimulatory effect of the low-B (low vegetable) diet on Ca absorption. Such an effect may have masked any influence of the B supplement. Nielsen (see Peace & Beattie, 1991), suggested that the 21-d period of B depletion in this study may have been too short to elicit an effect on subsequent supplementation. Care needs to be taken in the interpretation of apparent changes in post-menopausal plasma oestradiol levels when these are measured by commercial assay kits (Beattie & Peace, 1992).

Beattie & Weersink (1992) have demonstrated that borates form a complex with 2-hydroxyoestrone in vitro, thereby inhibiting methylation of the catechol oestrogen by catechol-*O*-methyltransferase; this mechanism may be responsible for the B-related perturbations of oestrogen metabolism observed by Nielsen and colleagues. However, further studies showed that a dietary B supplement of 40 mg kg^{-1} had no effect on the metabolic fate of tritiated oestradiol in ovariectomized rats (Beattie, 1992).

In a further human study, Nielsen *et al.* (1990) reported that supplementation of a low Mg, low B basal diet with 3 mg B d^{-1} significantly increased plasma concentrations of ionized Ca in women, decreased plasma calcitonin but did not significantly affect serum 1,25-DHCC or osteocalcin levels. They noted that B and oestrogen therapy tended to have similar biochemical effects and argued that any beneficial effects of B on bone may be mediated through a similar mechanism. However, in the absence of bone density measurements, the assertion that B influences bone metabolism in human subjects remains speculative.

The most consistent effect of B in rats and chicks, whose nutrition was otherwise adequate, has been to increase body weight (Nielsen, 1988*b*). Nevertheless, significant effects of B supplementation on major mineral metabolism have been noted, particularly when Mg and/or vitamin D are deficient in the diet. For example, Hegsted *et al.* (1991) found a higher apparent balance of Ca and P in rats fed a B-supplemented diet (2·72 mg kg^{-1}) which was also deficient in vitamin D. In mineral balance studies with sheep, Brown *et al.* (1989) showed that the apparent absorption of Ca was increased when the B intake increased from 30 to 75 or 200 mg d^{-1}. Bock *et al.* (1990) noted that urinary Ca loss in female rats fed a low B diet (0 mg B kg^{-1}) tended to be greater than that in rats given a B-supplemented diet (0·12 mg B kg^{-1}). B may either increase or decrease plasma Ca levels, depending on the influence of other dietary factors (Hunt *et al.* 1983; Nielsen *et al.* 1988*b*; Hunt, 1989).

Plasma total alkaline phosphatase has been determined in several B studies because borate inhibits this enzyme. However, the effect of dietary B on bone alkaline phosphatase has yet to be unequivocably established. Increasing dietary B intake of chickens from 0·28 mg d^{-1} to 3·00 mg d^{-1} partly offset the increase in plasma alkaline phosphatase caused by increasing dietary content of vitamin D from deficient level of 125 i.u. kg^{-1} to a superadequate level of 2500 i.u. kg^{-1} (Hunt & Nielsen, 1982). However, when a similar protocol was used but chickens were given a diet containing 20 g Ca kg^{-1} and 500 mg Mg kg^{-1}, B had no modifying effect on plasma alkaline phosphatase in chickens with a low vitamin D intake (Hunt *et al.* 1983). Similarly, Hunt (1989) found no significant effect of B when Mg deficiency (300 mg Mg kg^{-1}) was associated with vitamin D deficiency (125 i.u. kg^{-1}) at a dietary Ca intake of 10 g kg^{-1} or indeed when Mg intake was adequate (500 mg kg^{-1}).

Changes in bone-related hormones and mineral levels would be expected to induce morphological and/or biochemical changes in bone which can be measured directly in animal studies. Although several significant effects of B have been reported, they have yet to be substantiated. It is also questionable whether some of the effects which have been reported are beneficial. In a series of studies with rats, Nielsen and colleagues (Nielsen *et al.* 1988*b*, *c*; Shuler & Nielsen, 1988) found that a dietary B level of 3 mg kg^{-1} tended to depress bone Ca and Mg concentrations although the presence of 10 g arginine kg^{-1} diet reversed this effect (Nielsen *et al.* 1988*c*). Beattie & Macdonald (1991) also found a lower concentration of Ca, Mg and phosphorus (P) in the femurs of female rats fed a diet containing 40 mg B kg^{-1} for 12 weeks from weaning. This phenomenon, which was more apparent in Mg-deficient, ovariectomized animals, was probably related to an increase in the bone organic matter since the total bone mineral content was unaffected. King *et al.* (1991), who injected up to 1 mg B into turkey eggs, also found no effect of B on the mineral content of the hatched chick tibiae but observed a B-related reduction in lipid-free bone dry weight suggesting a decrease in organic matrix. Hegsted *et al.* (1991) gave rats a vitamin D deficient diet with or without a B supplement of 3 mg kg^{-1} for 12 weeks and while they noted a significant B-related increase in bone Mg concentration, there was no effect on Ca or P levels.

Dietary B supplements had no significant effects on rat bone density (Hegsted *et al.* 1991; Beattie & Macdonald, 1991) or on the mechanical properties of femurs (McCoy *et al.* 1990; Beattie & Wytch, unpublished observations). However McCoy *et al.* (1990) reported an increase in load to break force in compression tests of vertebrae from B-treated, Ca-deficient rats. Hunt (1989) has noted an accumulation of osteoclasts on marrow sprouts of the proximal tibial epiphysial plate of vitamin D-deficient, B-treated chicks. In this respect, B has some similarity with gallium (Ga) which increases the chemical resistance of calcified tissue to resorption, thereby stimulating osteoclast recruitment (see p. 176).

ALUMINIUM

The toxic effects of aluminium have become a major concern in recent years and there is a wealth of evidence which shows various osteopathological effects of Al (Malluche & Faugère, 1988; Klein, 1990). Al is found as a contaminant in dialysis and total parenteral nutrition (TPN) solutions and is therefore a hazard for long term TPN and dialysis patients (Monteagudo *et al.* 1989). Absorption from dietary sources is generally regarded to be low ($< 3\%$) and is $< 1\%$ for Al-containing antacids (Malluche & Faugère, 1988; Klein, 1990). Nevertheless, accumulation of Al in bone may become significant with prolonged ingestion of antacids or contaminated food and water. In addition, other nutrients can influence Al absorption and deposition in bone. For example, the presence of citrate in addition to Al chloride in the drinking water of rabbits enhanced Al absorption and its accumulation in bone whereas ascorbate prevented bone Al accumulation and enhanced its excretion (Fulton & Jeffrey, 1990). Al appears to inhibit bone formation by reducing osteoblast activity, osteoid mineralization and matrix formation (Drüeke *et al.* 1988; Rodriguez *et al.* 1990). For further information on Al, readers are referred to the above mentioned comprehensive reviews of the recent literature.

GALLIUM

The effects of dietary Ga on bone metabolism have not been reported and there is little information concerning the Ga content of foods. Although most of the literature concerns the administration of pharmacological doses of this element, usually by injection, or tissue culture work, the possibility that dietary Ga could affect bone metabolism should be addressed.

The discovery that Ga inhibits bone resorption was a fortuitous consequence of studies on the use of $GaNO_3$ to treat certain human tumours (Warrell *et al.* 1983). A transient hypocalcaemia, which was not attributable to increased urinary Ca excretion, was noted in patients undergoing Ga infusion. Subsequent studies with bone explants in culture showed that pre-incubation with $GaNO_3$ significantly inhibited the bone resorption stimulated by PTH or a lymphokine preparation (Warrell *et al.* 1984). Examination of trace element distribution in bone by synchrotron X-ray microscopy showed a preferential accumulation of Ga at sites of new bone formation and a related decrease in Zn and Fe (Bockman *et al.* 1990*a*, *b*).

In an attempt to elucidate the mechanism, Hall & Chambers (1990) examined the effect of $GaNO_3$ on the activity of isolated osteoclasts cultured on cortical bone slices and found that it inhibited resorption in a concentration-dependent manner between about 0·005 and 50 μg Ga ml^{-1}. Pre-incubation of the bone slices with 100 μg $GaNO_3$ ml^{-1} for 18 h at 37 °C, followed by extensive washing before culture of the osteoclasts, greatly inhibited resorption. Donnelly *et al.* (1991) noted that in spite of an increase in osteoclast numbers, resorption of Ga-containing bone particle implants in rats was less than that of Ga-free implants. Devitalized bone powder from Ga-treated rats was less soluble in acetate buffer and less readily absorbed by monocytes (Repo *et al.* 1988). It appears that incorporation of Ga on to or into calcified bone causes some chemical change which inhibits osteoclastic bone resorption. Cournot-Witmer *et al.* (1987) injected rats with almost 12 mg Ga kg^{-1} every other day for 16 or 30 d and found increased numbers of osteoclasts, decreased serum Ca and elevated immunoreactive PTH. In the light of subsequent research it can be deduced that the chemical resistance of Ga-exposed bone to resorption by osteoclasts led to a fall in circulating Ca which in turn stimulated the release of PTH causing an increase in osteoclast recruitment.

SILICON

Although Si is the second most abundant element in the earth's crust, its absorption and retention by animals is sufficiently low for it to be classified as a trace element. It is essential for normal bone matrix formation and probably also for bone mineralization (Carlisle, 1986, 1988). Signs of Si deficiency include depressed growth, gross abnormalities of skull bone architecture, leg bone abnormalities including reduced circumference, thinner cortex and reduced flexibility. These effects are independent of vitamin D-induced bone abnormalities.

Studies of embryonic bone cartilage in culture showed significant Si-related increases in dry weight, in collagen (as indicated by elevated hydroxyproline content), and also in matrix glycosaminoglycans (Carlisle, 1985, 1988). Bone Si is located mainly in osteoblast mitochondria, which supports the view that it is required for connective tissue matrix formation. Optimal activity of proline hydroxylase, which is required for collagen biosynthesis, appears to depend on the presence of adequate Si (Carlisle *et al.* 1981). Connective tissue Si is a component of animal glycosaminoglycans and their protein complexes and Si may therefore also have a structural function (Carlisle, 1988). Dietary requirements of Si may be influenced by interaction with Mo and Al, which inhibit tissue accumulation of Si (Carlisle, 1979; Carlisle & Curran, 1988).

LEAD

Most body Pb resides in the skeleton, where until recently it has been considered to be of little importance. As lead readily crosses the placenta, it can begin to accumulate in the skeleton of the fetus (Pounds *et al.* 1991), and continues to accumulate until old age. It is estimated that at the age of twenty 78% of total body Pb in humans is stored in bone, rising to 96% at the age of eighty (Saltzman *et al.* 1990). Bone Pb may therefore provide the best marker of chronic Pb exposure, which may be measured in vivo by X-ray fluorescence techniques (Nordberg *et al.* 1991). However, bone Pb levels vary with skeletal site, and may be affected by disease processes (Milachowski, 1988). Blood Pb is probably the best marker of acute Pb exposure, but correlates poorly with bone Pb.

Mobilization of Pb from skeletal stores may result from any condition which increases bone turnover, such as pregnancy, lactation, post-menopausal bone loss and osteoporosis (Silbergeld *et al.* 1988; Silbergeld, 1991). In the absence of such conditions, the half-lives of cortical and trabecular bone Pb are 10–20 and 5 years respectively.

High dietary phosphate intakes may decrease body Pb burdens by increasing intraluminal Pb precipitation in the gut, thus limiting Pb absorption (Barton & Conrad, 1981). Low Ca intakes and the administration of vitamin D may enhance Pb absorption, suggesting that Pb and Ca share common mechanisms for absorption (Shields & Mitchell, 1941; Smith *et al.* 1978). However, Pb may also inhibit renal 25(OH)CC 1-hydroxylase (*EC* 1.14.13.13) activity (Edelstein *et al.* 1984). Pb exposed children may have reduced plasma 1,25-DHCC levels, which return to normal after Pb chelation therapy (Rosen *et al.* 1980; Mahaffey *et al.* 1982). This finding has not been confirmed by others (Laraque *et al.* 1990; Koo *et al.* 1991). There are case reports of rickets in children associated with Pb poisoning (Caffey, 1938; Vico & Dessy, 1988). The second National Health and Nutrition Examination Survey (NHANES II) found a highly significantly association between height, weight and chest circumference of United States children and their blood Pb levels, even in the so-called 'normal' range for blood Pb levels. However Pb may be acting as a composite marker of nutritional and socio-economic variables (Schwartz *et al.* 1986). The ability of

dietary Pb to inhibit intestinal Ca absorption when dietary Ca intakes are low may be relevant to these findings (Smith *et al.* 1981; Fullmer & Rosen, 1990).

Reduced plasma levels of osteocalcin have been found in Pb poisoned children (Markowitz *et al.* 1988). Pb chelation therapy in these children resulted in elevation of osteocalcin levels, suggesting increased mineralization rates. Pb also partly prevents the increase in osteocalcin levels in osteosarcoma cells treated with insulin-like growth factor I and vitamin D (Long *et al.* 1990*a*; Angle *et al.* 1990). It has been suggested that Pb displaces Zn from the 1,25-DHCC receptor, changing receptor–DNA binding. Pb may also displace Ca from osteocalcin itself, impairing binding of this protein to hydroxyapatite.

Impairment of skeletal development has been reported in livestock grazing on Pb-contaminated pastures (Butler *et al.* 1957; Clegg & Rylands, 1966). Since Pb poisoning also affects renal, neurological and hormonal functions, it is difficult to examine specific effects of Pb on bone in these animals. Using only a small number of dogs, Anderson & Danylchuk (1977) demonstrated decreased bone formation in response to chronic low dose Pb intoxication. Other workers have shown Pb to inhibit bone matrix formation in rabbits (Hass *et al.* 1967), possibly as a result of inhibition of proline hydroxylation, with cellular accumulation of incompletely hydroxylated procollagen (Vistica *et al.* 1977), and defective cross linking of collagen due to interference with the Cu cofactor of lysyl oxidase (Ellender & Ham, 1987). Klein *et al.* (1991) have also shown that Pb reduces procollagen formation by ROS 17/2.8 osteosarcoma cells, which appear to be good models of the effect of Pb on osteoblasts (Long *et al.* 1990*b*). Osteoclasts resorbing Pb-containing bone develop Pb inclusion bodies in the cytoplasm and nuclei (Bonucci *et al.* 1983). From microscopic examination, Pb appears to be more toxic to osteoclasts than osteoblasts (Bonucci & Silvestrini, 1988), although much of osteoclastic function is controlled by osteoblastic signalling. Pb may bind to the active site of osteoclastic carbonic anhydrase (*EC* 4.2.1.1), inhibiting the proton production necessary for bone resorption (Calhoun *et al.* 1985).

Pb has the potential to displace Ca in numerous biological systems. It readily displaces Ca in hydroxyapatite crystals. In soft tissue, Pb may actually induce ectopic bone formation for reasons which are obscure (Pounds *et al.* 1991). The non-collagenous matrix proteins osteonectin and bone sialoproteins I and II have Ca binding sites which could be influenced by Pb (Sauk & Somerman, 1991). Pb also perturbs intracellular Ca (Schanne *et al.* 1989, 1990), but the significance of these events for the skeleton is not known.

FLUORINE

The ability of F to affect the biological function of bone cells, as well as the physicochemical properties of bone crystals, has been much studied in relation to industrial and endemic fluorosis and the treatment of osteoporosis. F readily substitutes for the hydroxyl ion in bone hydroxyapatite, creating a more stable crystal, which is less acid soluble (Grynpas, 1990). It does not diffuse into formed bone, but becomes incorporated during bone formation. Normally, hydroxyapatite crystals run parallel to collagen fibres in bone, whereas fluoroapatite crystals run perpendicular to the fibres (Posner, 1967). F may also reduce the amount of protein bound to bone mineral (Grynpas, 1990).

The skeleton responds to F by increasing osteoblast number and subsequently bone formation. F increases osteoprogenitor cell proliferation in vitro by enhancing the activity of bone cell mitogens (Farley *et al.* 1990). This may result from a direct inhibition of osteoblastic acid phosphatase/phosphotyrosyl protein phosphatase (*EC* 3.1.3.16) activity, leading to an increase in cellular tyrosyl phosphorylation and bone cell proliferation (Lau *et al.* 1989).

Following F therapy in osteoporosis there is an increase in unmineralized osteoid and a delay in mineralization (Kragstrup *et al.* 1989). Since F is preferentially deposited at sites of new bone formation, the osteoblasts involved in remodelling are exposed to particularly high levels of F (Wiers *et al.* 1990). The bone matrix also has a woven rather than lamellar appearance. Histologically, the picture resembles osteomalacia, such that vitamin D and Ca have been administered with F in many osteoporotic patients (Jowsey *et al.* 1972). The mineralization defect relates partly to the duration of therapy and also to the dose of F employed. F has a narrow therapeutic index of probably 30–50 mg NaF d^{-1} (Kleerekoper & Balena, 1991); if higher doses are used or if treatment is prolonged beyond 2 years mineralization defects are likely to occur.

Ca and vitamin D do not necessarily prevent histological abnormalities in osteoporosis treated with F (Compston *et al.* 1980). Although Ca is thought to suppress the secondary hyperparathyroidism which may occur in response to F, this was not confirmed by Stamp *et al.* (1990). However, in areas of endemic fluorosis high Ca intakes may reduce the prevalence of hyperparathyroidism (Mithal *et al.* 1991), possibly in part because Ca inhibits F absorption (Briançon *et al.* 1990). Krishnamachari (1987) has reviewed the various clinical presentations of endemic fluorosis. Particularly in adolescents and young adults, decreased bone mass in the peripheral skeleton is associated with *genu valgum* and *varum* (knock-knee and bow-leg), sabre (curved) tibia and secondary hyperparathyroidism. Low Cu intakes are also thought to modify the appearance of fluorosis (Krishnamachari, 1987).

F preferentially leads to new bone formation in the axial skeleton, which is mostly trabecular bone. Although there is a marked increase in individual trabecular thickness, it is thought that trabecular connectivity is not restored, thus limiting the ability of F to decrease the risk of fracture in osteoporosis (Aaron *et al.* 1991). In fact F may accumulate in calluses of trabecular microfractures, possibly compromising the healing of these microfractures (Boivin *et al.* 1991).

Unless accompanied by secondary hyperparathyroidism, F does not seem to increase osteoclastic bone resorption. In the concentrations used therapeutically in osteoporosis, F may lead to fewer resorption lacunae and decreased area resorbed per osteoclast (Okuda *et al.* 1990). This may be because F is directly toxic to the osteoclast or because fluoroapatite is less soluble. Consequently, there is uncoupling of bone turnover with bone formation outstripping bone resorption.

Little is known of the overall effect of F on the mechanical properties of bone. Wolinsky *et al.* (1972) found that femurs of F-treated rats had a lower breaking stress and a decrease in the limit and modulus of elasticity. However, Henrikson *et al.* (1970) were unable to demonstrate any effect in the femurs of beagles fed up to 1 mg F kg^{-1} d^{-1}. It appears that significant increases in trabecular volume may be required to overcome the adverse effects of F on the physical characteristics of bone mineral (Carter & Beaupré, 1990). Simple increases in axial bone mass in response to F therapy may not result in an increase in bone strength.

Based on the results of four prospective placebo-controlled trials on the effect of NaF on vertebral fracture rate in osteoporosis (Dambacher *et al.* 1986; Mamelle *et al.* 1988; Kleerekoper *et al.* 1989; Riggs *et al.* 1990), Kleerekoper & Balena (1991) concluded that NaF is no more effective than placebo. Indeed, there is also considerable concern that F may actually increase the fracture rate in the peripheral skeleton, possibly by decreasing the bone mass. The 'painful lower extremity syndrome' which occurs as a side effect of F therapy for osteoporosis has many characteristics of a stress fracture. In none of the above trials did F significantly increase hip fracture rate, but Riggs *et al.* (1990) found a statistically significant increase in overall peripheral fracture rate, although the dose of NaF used was probably above the therapeutic range.

The best epidemiological study of F exposure and risk of fracture took into account some of the confounding variables which may influence bone mass (Sowers *et al.* 1991). Residence in the high F community (4 mg F l^{-1} drinking water) was associated with lower radial bone mass in pre- and post-menopausal women, an increased rate of radial bone mass loss in pre-menopausal women, and significantly more fractures among post-menopausal women.

The extent to which fluoridation of the water supply to prevent dental caries may influence bone metabolism is unknown, as dosages are markedly lower than those ingested in areas of endemic fluorosis or used in the treatment of osteoporosis. Very high levels of F ingestion have been associated with osteosarcoma in rats, but no evidence of increased risk has been found in man (Hrudey *et al.* 1990; McGuire *et al.* 1991; Mahoney *et al.* 1991).

CONCLUSIONS

Trace elements have a major effect on bone metabolism and deficiencies of essential minerals such as Cu or excess intake of toxic elements, for example Cd, cause debilitating bone diseases. The list of inorganic nutrients or dietary contaminants which influence bone metabolism is gradually increasing as attention turns to some of the less well studied elements in the periodic table. The difficulty in assessing the importance of these elements in human diets in particular is compounded by a lack of food composition data for most of these minerals. Nevertheless, there is evidence for effects on bone of trace elements not included in this review such as molybdenum (Spence *et al.* 1980), Se (Dong-Xu, 1987), Ge (Yamaguchi & Uchiyama, 1987) and bismuth (Bradley *et al.* 1989).

The significance of trace element interactions and also interactions with other nutrients on bone metabolism and osteopathology is clear from the literature discussed in this review. For example, dietary advice for women at risk of developing osteoporosis, or indeed those women or men diagnosed as osteoporotic, often stresses the importance of a high Ca intake, but high levels of Ca supplementation may inhibit absorption of essential trace elements and the advantages of this rationale are therefore questionable. Supplementation with the elements Zn, Mn and Cu in addition to Ca may therefore be of benefit in reducing bone loss in post-menopausal women.

Element interactions can be additive, synergic or antagonistic and such information is crucial in the assessment of dietary requirements and element toxicity. Essential elements such as Zn tend to ameliorate the toxic effects of, for example, Cd although, rarely, an antagonistic interaction between toxic elements suppresses the pathological changes associated with one or the other element (e.g. V and Hg). With the current state of knowledge concerning individual element effects and two-element inter-relationships, attempts to identify three or more nutrient interactions simultaneously are frequently more confusing than illuminating. Nevertheless, an appreciation of the importance of multi-element and multi-nutrient interactions is essential to understanding the role of trace elements in the aetiology of bone disease.

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