Optimal nutrition: calcium, magnesium and phosphorus

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In the past, a major challenge for nutrition research was in defining indicators of nutritional adequacy. More recently, the research base related to the role of nutrition in chronic disease has expanded sufficiently to permit moving beyond deficiency indicators to other indicators with broader functional significance. Thus, nutrition research is faced with the new challenge of defining 'optimal nutrition'. One definition of optimal nutrition with respect to any particular nutrient could be when a functional marker reaches an 'optimal value' or plateau beyond which it is no longer affected by intake or stores of the nutrient. A functional marker of nutrient status could be defined as a physiological or biochemical factor which (1) is related to function or effect of the nutrient in target tissue(s) and (2) is affected by dietary intake or stores of the nutrient (which may include markers of disease risk). Examples of such indicators or markers are those related to risk of chronic diseases such as osteoporosis, CHD, or hypertension. The present review focuses on the concept of optimal nutrition with respect to three nutrients, Ca, Mg and P. However, for P and Mg there are as yet no functional indicators which respond to dietary intake, and in such cases nutrient requirements are established using more traditional approaches, e.g. balance data. For Ca, there has been interest in using maximal Ca retention, which is based on balance data, bone mass measurements and biomarkers of bone turnover as useful functional indicators of the adequacy of Ca intake.

Calcium: Magnesium: Phosphorus: Functional markers

In the not so distant past, a major challenge for nutrition research was in defining indicators of nutritional adequacy. This research was usually limited to classical deficiency states. For example, the dietary allowance for vitamin C has traditionally been set at a level that will prevent scorbutic symptoms for several weeks on a diet lacking vitamin C (National Research Council, 1989*a*).

More recently, the research base related to the role of diet and nutrition in chronic disease has expanded sufficiently to permit moving beyond deficiency indicators to other indicators with broader functional significance. Thus, nutrition research is faced with the new challenge of defining 'optimal nutrition' with respect to the various essential nutrients.

One definition of optimal nutrition with respect to any particular nutrient could be when a functional marker or indicator reaches an 'optimal value' or plateau beyond which it is no longer affected by intake or stores of the nutrient. In this respect, a functional marker of nutrient status could be defined as a physiological and/or biochemical factor which (1) is related to function or effect of the

nutrient in target tissue(s) and (2) is affected by dietary intake or stores of the nutrient (which may include markers of disease risk). Examples of such indicators or markers are those related to risk of chronic diseases such as osteoporosis, CHD, or hypertension.

However, for many nutrients there are as yet no functional indicators which respond to dietary intake, and in such cases nutrient requirements are established using more traditional approaches, e.g. balance data.

The present review focuses on the concept of optimal nutrition with respect to three nutrients, Ca, Mg and P. While each of these nutrients has very diverse functional and/or structural roles in the body, all three nutrients play a key role in the development and maintenance of bone and other calcified tissues. Indeed, 99 % of the body Ca (National Research Council, 1989*a*), 85 % of the body P (National Research Council, 1989*a*), and 50–60 % of the body Mg (Elin, 1987) are found in bone and other calcified tissues. However, other biological roles for these nutrients and their possible relevance to human health and to decreasing risk of disease will also be considered.

Phosphorus

P makes up about 6·5–11 g/kg body weight in adults (Aloia *et al*. 1984). In the adult body 85 % of the P is in bone, with the remaining 15 % distributed within the soft tissues (Diem, 1970). Total P concentration in whole blood is 13 mmol/l, most of which is in the phospholipids of erythrocytes and plasma lipoproteins, with approximately 1 mmol/l present as inorganic phosphate (Pi). This Pi component, while constituting only a minute percentage of body P (< 0·1 %), is of critical importance. In adults this component makes up about 15 mmol in total and is located mainly in the blood and extracellular fluid. It is into this Pi compartment that phosphate is inserted on absorption from the diet and resorption from bone, and from this compartment that most urinary P and hydroxyapatite mineral P are derived. This compartment is also the primary source from which the cells of all tissues derive both structural and high-energy phosphate (Institute of Medicine, 1997).

Structurally, P occurs as hydroxyapatite in calcified tissues and as phospholipids, which are a major component of most biological membranes, and as nucleotides and nucleic acids. Other functional roles of P include: (1) the buffering of acid or alkali excesses, hence helping to maintain normal pH; (2) the temporary storage and transfer of the energy derived from metabolic fuels; (3) by phosphorylation, the activation of many catalytic proteins. Since phosphate is not irreversibly consumed in these processes and can be recycled indefinitely, the actual function of dietary P is first to support tissue growth (either during individual development or through pregnancy and lactation), and second to replace excretory and dermal losses. In both processes it is necessary to maintain a normal level of Pi in the extracellular fluid, which would otherwise be depleted of its P by growth and excretion (Institute of Medicine, 1997).

Inadequate P intake is expressed as hypophosphataemia. Only limited quantities of phosphate are stored within cells, and most tissues depend on extracellular fluid Pi for their metabolic phosphate. When extracellular fluid Pi levels are low, cellular dysfunction follows. At a whole organism level, the effects of hypophosphataemia include anorexia, anaemia, muscle weakness, bone pain, rickets and osteomalacia, general debility, increased susceptibility to infection, paraesthesia, ataxia, confusion, and even death (Lotz *et al*. 1968). The skeleton will exhibit either rickets in children or osteomalacia in adults. In both groups the disorder consists of a failure to mineralize forming growth plate cartilage or bone matrix, together with impairment of chondroblast and osteoblast function (Lotz *et al*. 1968). These severe manifestations are usually confined to situations in which extracellular fluid Pi falls below approximately 0·3 mmol/1. P is so ubiquitous in various foods that near total starvation is required to produce dietary P deficiency. Re-feeding of energy-depleted individuals, such as those recovering from alcoholic bouts or from diabetic ketoacidosis, either orally or parenterally without attention to supplying Pi can precipitate extreme, even fatal, hypophosphataemia (Knochel, 1977; Bushe, 1986; Dale *et al*. 1986). Al-containing antacids, by binding P in the gut, can, when consumed in high doses, produce hypophosphataemia in their own right, as well as aggravate phosphate deficiency related to other problems (Lotz *et al*. 1968).

Until recently, dietary P recommendations have been tied to Ca, usually on an equimass or equimolar basis (see Table 1), and this approach was used in the USA, EU and UK in establishing recommended dietary allowances, population reference intakes, and reference nutrient intakes respectively for P (National Research Council, 1989*a*; Department of Health, 1991; Scientific Committee for Food, 1993).

However, the US Food and Nutrition Board recently suggested that a Ca : P concept of defining P requirements is of severely limited value, in that there is little merit to having the ratio 'correct' if the absolute quantities of both nutrients are insufficient to support optimal growth. Furthermore, the intake ratio, by itself, fails to take into account both differing bioavailabilities and physiological adaptive responses of Ca and P (Institute of Medicine, 1997).

Although P balance might seem to be a logical indicator of nutritional adequacy, it is not an adequate criterion, since an adult can be in zero balance at an intake inadequate to maintain serum Pi within the normal range (Institute of Medicine, 1997). Even during growth balance will be positive in direct proportion to soft tissue and bony accumulation, but as long as plasma Pi is high enough the degree of positive balance will be limited either by the genetic programming or by availability of other nutrients (Institute of Medicine, 1997). During senescence, if there is loss of bone or soft tissue mass, P balance will be negative. However, as long as plasma Pi remains within normal limits, these balances will reflect other changes occurring in the body and will not be an indicator of the adequacy of dietary P (Institute of Medicine, 1997).

Since P intake directly affects serum Pi, and because both hypo- and hyperphosphataemia directly result in dysfunction or disease, the US Food and Nutrition Board considered that the most logical indicator of nutritional adequacy of P intake is serum Pi (Institute of Medicine, 1997). If serum Pi is above the lower limits of normal for age, P intake may be considered adequate to meet cellular and bone formation needs of healthy individuals. However, the relationship between Pi and P intake has been clearly established only for adults, and while the adverse effects of low serum Pi are well understood during growth, it is harder to define the critical values for P intake associated with the normal range of serum Pi values in infants and children. Thus, estimates of P requirements were based on a factorial approach in infants, children and adolescents and on serum Pi in adults (Institute of Medicine, 1997).

Although it is not likely that all levels of serum Pi within the normal range are equally beneficial for cell functioning, insufficient information exists to allow selection of any one value within the population normal range as superior to any other. The recommendation for P was based on the intake associated with maintenance of serum Pi at the lower end of the normal range. It was acknowledged that a limitation of this approach lies in the fact that available data on serum Pi apply mostly to the fasting state, whereas it is the integrated 24 h serum Pi that is most closely related to absorbed P intake and that constitutes the actual exposure that the tissues experience (Institute of Medicine, 1997). Moreover,

Table 1. Recommended phosphorus intakes in the USA and UK*

US RDA(1989)		UK RNI (1991)‡		US RDA (1997)§	
Age-group (years)	(mg/d)	Age-group (years)	(mg/d)	Age-group (years)	(mg/d)
0–0·5	300	0–1	400	0–0·5	100 ‖
0·5–1	500	1–3	270	0·5–1	275 ‖
1–3	800	4–6	350	1–3	460
4–6	800	7–10	450	4–8	500
7–10	800	11–14 M	775	9–13	1250
11–14 M	1200	15–18 M	775	14–18	1250
15–18 M	1200	11–14 F	625	19–30	700
19–24 M	1200	15–18 F	625	31–50	700
25–50 M	800	19–50	550	51–70	700
> 51	800	> 50	550	> 70	700
11–14 F	1200	Pregnanc	NI	Pregnancy	
15–18 F	1200	Lactation	+44	≤18	1250
19–24 F	1200			19–50	700
25–50 F	800			Lactation	
> 51	800			≤18	1250
Pregnancy	1200			19–50	700
Lactation					
0–0·5	1200				
0·5–1	1200				

RDA, recommended dietary allowance; RNI, reference nutrient intake; M, requirements for males; F, requirements for females; NI, no increment.

* Estimates of P requirements refer to males and females unless stated otherwise.
† RDA based on Ca:P approach (National Research Council, 1989a).
‡ RNI based on Ca:P approach (Department of Health, 1991).
§ RDA based principally on factorial approach and on serum inorganic phosphate levels (Institute of Medicine, 1997).
‖ Adequate intake based principally on human milk content (0–0·5 years) or human milk plus solids (0·5–1·0 years).

fasting serum Pi is only weakly correlated with current P intake (Portale *et al*. 1987).

Magnesium

Total body Mg content in the normal adult is approximately 25 g (1000 m mol), of which 50–6 0% resides in bone. One-third of skeletal Mg is exchangeable, and it is this fraction that may serve as a reservoir for maintaining normal extra-cellular Mg concentration (Elin, 1987). Extracellular Mg accounts for about 1 % of the total body Mg.

The physiological role of Mg is principally related to enzyme activity, with over 300 enzyme systems dependent on the presence of this cation (Wacker & Parisi, 1968a,b,c). All enzyme systems utilizing ATP require Mg for sub-strate formation. Intracellular free Mg $^{2+}$ also acts as an allosteric activator of enzyme action, including critical enzyme systems such as adenylate cyclase (*EC* 4.6.1.1), phospholipase C (*EC* 3.1.4.3), and Na$^+$/K$^+$-transporting ATPase (*EC* 3.6.1.37; Rude, 1998). Transport of other ions such as K and Ca across the plasma membrane may also require the presence of Mg (Rude, 1998). Mg has been termed 'nature's physiological Ca channel blocker' (Iseri & French, 1984; White & Hartzell, 1989). During Mg depletion intracellular K decreases while Ca and Na increase (Iseri & French, 1984; Whang *et al*. 1985; White & Hartzell, 1989; Dorup & Clausen, 1993). Mg is critical for a number of cellular functions, including oxidative phosphorylation, glycolysis, DNA transcription and protein synthesis (Rude, 1998).

Severe Mg depletion leads to specific biochemical abnormalities and clinical manifestations that can easily be detected. Hypocalcaemia is a prominent manifestation of moderate to severe Mg deficiency in human subjects (Rude *et al*. 1976). Mg is also important in vitamin D metabolism and/or action. For example, vitamin D resistance has been reported in Mg-depleted human subjects (Medalle & Waterhouse, 1973). Neuromuscular hyperexcitability is the initial problem observed in individuals who have or are developing Mg deficiency (Rude & Singer, 1980).

There is emerging evidence that habitually low intakes of Mg and resulting abnormal Mg metabolism are associated with aetiological factors in various metabolic diseases, including cardiovascular disease, hypertension, disorders of skeletal growth and osteoporosis, neuromuscular disorders and diabetes mellitus (Rude, 1998). However, the data currently available are insufficient to permit an estimate of optimal intake of Mg for health (Institute of Medicine, 1997).

Several potential indicators for estimating daily Mg requirement have been suggested (Rude, 1998). While Mg is an abundant cation in the body, over 90 % is located either intracellularly or in the skeleton (Elin, 1987). Serum Mg concentration is the most commonly used test to assess Mg status, but total serum Mg concentration appears to be held within a tight range (17–22 m g/l) (Lowenstein & Stanton, 1986). A serum concentration of less than 17mg/l usually indicates some degree of Mg depletion (Wong *et al*. 1983; Quamme, 1993). However, the measurement of serum Mg concentration may not reflect the true total body Mg content, and low intracellular Mg has been documented in patients with serum levels above 1 7mg/l (Ryan *et al*. 1981; Ryzen *et al*. 1985, 1986; Rude *et al*. 1991; Nadler *et al*. 1992; Rude & Olerich, 1996). Intracellular Mg levels in

muscle, erythrocytes and lymphocytes, and bone Mg appear to provide a more accurate assessment of body Mg status, but such measurements are not readily available for clinical use (Dyckner & Wester, 1978; Ryan *et al.* 1981; Ryzen *et al.* 1985, 1986; Rude *et al.* 1991). Recently, ion-specific electrodes have become available for determining ionized Mg in plasma. Early results suggest that this measurement may be a better index of Mg status than total serum Mg concentration, but further evaluation is necessary (Rude, 1998). The Mg load, or tolerance, test has been used for many years as a method of assessing Mg status, but it has been reserved usually for subjects who are deemed to be at risk of Mg deficiency but have normal serum levels of Mg (Rude, 1998). This test relies on evaluating the amount of Mg excreted in urine following an intravenous infusion of Mg. However, the Mg load test requires normal renal handling of Mg. Age may also be a confounding variable, since older subjects have been reported to retain more Mg than younger subjects despite comparable dietary Mg intake (Gullestad *et al.* 1994).

Thus, it is not surprising that the principal method of estimating dietary Mg requirements in the past has been the dietary balance study (Hunt & Schofield, 1969; Greger & Baier, 1983; Mahalko *et al.* 1983; Schwartz *et al.* 1984). The US, EU and UK authorities have used Mg balance data in establishing recommended dietary allowances, population reference intakes, and reference nutrient intakes respectively for Mg (National Research Council, 1989*a*; Department of

Health, 1991; Scientific Committee for Food, 1993; Institute of Medicine, 1997; See Table 2). There are still significant errors associated with the use of balance data for Mg. The US Food and Nutrition Board set out minimum criteria which needed to be fulfilled for balance studies to be included for the development of the new recommended dietary allowances (Institute of Medicine, 1997). These criteria included either an adaptation period of at least 12 d or a determination of balance while subjects consumed self-selected diets.

Calcium

Ca, which accounts for 10–20 g/kg body weight in adults, is a major component of mineralized tissues, with over 99 % of the total body Ca found in these tissues. Ca is required for normal growth and development of the skeleton (National Research Council, 1989*a*). The remainder is present in blood, extracellular fluid, muscle and other tissues, where it plays a role in mediating vascular contraction and vasodilation, muscle contraction, nerve transmission and glandular secretion (Institute of Medicine, 1997).

The concentration of Ca in blood is tightly regulated (2·3–2·75 mmol/l; British Nutrition Foundation, 1989). The regulation of plasma Ca concentration is achieved through a complex physiological system comprising the interaction of the calcitropic hormones, such as parathyroid hormone

Table 2. Recommended magnesium intakes in the USA and UK*

US RDA (1989)†		UK RNI (1991)‡		US RDA (1997)§	
Age-group (years)	(mg/d)	Age-group (years)	(mg/d)	Age-group (years)	(mg/d)
0–0·5	40	0–1	55–80 ||	0–0·5	30 ¶
0·5–1	60	1–3	85	0·5–1	75 ¶
1–3	80	4–6	120	1–3	80
4–6	120	7–10	200	4–8	130
7–10	170	11–14	280	9–13	240
11–14 M	270	15–18	300	14–18 M	410
15–18 M	400	19–50 M	300	19–30 M	400
19–24 M	350	>50 M	300	31–50 M	420
25–50 M	350	19–50 F	270	51–70 M	420
>51 M	350	>50 F	270	>70 M	420
11–14 F	280	Pregnancy	NI	14–18 F	360
15–18 F	300	Lactation	+50	19–30 F	310
19–24 F	280			31–50 F	320
25–50 F	280			51–70 F	320
>51 F	280			>70 F	320
Pregnancy	300			Pregnancy	
Lactation				≤18	400
0–0·5	355			19–30	350
0·5–1	340			31–50	360
				Lactation	
				≤18	360
				19–30	310
				31–50	320

RDA, recommended dietary allowance; RNI, reference nutrient intake; M, requirements for males; F, requirements for females; NI, no increment.
* Estimates of Mg requirements refer to males and females unless stated otherwise.
† RDA based on factorial approach (National Research Council, 1989*a*).
‡ RNI based on factorial approach (Department of Health, 1991).
§ RDA based principally on balance data (Institute of Medicine, 1997).
\|\| 55 mg Mg/d for 0–3 months, 60 mg Mg/d for 4–6 months, 75 mg Mg/d for 7–9 months, 80 mg Mg/d for 10–12 months.
¶ Adequate intake based principally on Mg content of human milk or human milk plus solids.

(PTH), 1,25-hydroxycholecalciferol (1,25(OH)$_2$D$_3$) and calcitonin, with specific target tissues (kidney, bone and intestine) which serve to increase or to decrease the entry of Ca into the extracellular space. Only in extreme circumstances, such as severe malnutrition or hyperparathyroidism, is the serum ionized Ca concentration below or above the normal range. The secretion of these hormones is governed wholly or in part by the plasma concentration of ionized Ca, thus forming a negative feedback system. PTH and 1,25(OH)$_2$D$_3$ are secreted when plasma Ca is low, while calcitonin is secreted when plasma Ca is high (British Nutrition Foundation, 1989).

Given the high proportion of body Ca and the importance of bone as the major reservoir for Ca, development and maintenance of bone is the major determinant of Ca needs. Ca requirements vary throughout an individual's life, with greater needs during the periods of rapid growth in childhood and adolescence, during pregnancy and lactation, and in later life.

Chronic Ca deficiency resulting from inadequate intake or poor intestinal absorption gives rise to a PTH-mediated increase in bone resorption and is one of several important causes of reduced bone mass and osteoporosis (National Research Council, 1989b; National Institutes of Health, 1994; Institute of Medicine, 1997). Osteoporosis is characterized by a reduced bone mass (bone mineral density (BMD) more than 2·5SD below the mean for young adult females), increased bone fragility, and increased risk of fracture (World Health Organization, 1994). There has been a large increase in the incidence of osteoporosis in many countries in recent years (Riggs & Melton, 1995).

The more traditional method of estimating daily Ca requirements is by a factorial approach, in which an estimate of the average physiological requirement for absorbed Ca derived from balance data is adjusted for incomplete utilization and variation both in requirements among individuals and in the bioavailability of Ca among food sources. This approach is subject to considerable uncertainty, as is evident from the wide variation in estimates of daily Ca requirements made by different expert authorities. For example, the US and UK authorities have established very different recommendations for Ca intake (National Research Council, 1989a; Department of Health, 1991; see Table 3). Much of this divergence arose due to different interpretations of available data, e.g. different absorption efficiencies and estimates of obligatory losses of Ca were used by the two authorities.

Given the importance of dietary Ca for bone health there is a clear need for better measures of Ca adequacy, and recent research suggests that improved assessment of Ca adequacy may be made using a number of approaches.

Maximal calcium retention

Recently maximal Ca retention has been considered in the USA as a functional indicator of Ca requirement (Institute of Medicine, 1997; Jackman et al. 1997). To maximize skeletal strength it is important to develop and maintain a Ca reserve in the skeleton. Genetics or heredity to a large extent determines the upper limits of both skeletal size and density (Institute of Medicine, 1997), thus providing a maximum size for the Ca reserve. An adequate Ca intake is needed to achieve and preserve these upper limits. In contrast, an inadequate Ca intake, among other factors, limits the development and preservation of the maximal reserve. It follows that Ca intake requirement for each age-group is the intake

Table 3. Recommended calcium intakes in the USA and UK*

US RDA (1989)†		UK RNI (1991)‡		US AI (1997)§	
Age-group (years)	(mg/d)	Age-group (years)	(mg/d)	Age-group (years)	(mg/d)
0–0·5	400	0–1	525	0–0·5	210
0·5–1	600	1–3	350	0·5–1	270
1–3	800	4–6	450	1–3	500
4–6	800	7–10	550	4–8	800
7–10	800	11–14 M	1000	9–13	1300
11–14 M	1200	15–18 M	1000	14–18	1300
15–18 M	1200	11–14 F	800	19–30	1000
19–24 M	1200	15–18 F	800	31–50	1000
25–50 M	800	19–50	700	51–70	1200
>51 M	800	>50	700	>70	1200
11–14 F	1200	Pregnancy	NI	Pregnancy	
15–18 F	1200	Lactation	+550	≤18	1300
19–24 F	1200			19–50	1000
25–50 F	800			Lactation	
>51 F	800			≤18	1300
Pregnancy	1200			19–50	1000
Lactation					
0–0·5	1200				
0·5–1	1200				

RDA, recommended dietary allowance; RNI, recommended nutrient intake; AI, adequate intake; M, requirements for males; F, requirements for females; NI, no increment.
* Estimates of Ca requirements refer to males and females unless stated otherwise.
† RDA based on factorial approach (National Research Council, 1989a).
‡ RNI based on factorial approach (Department of Health, 1991).
§ AI based principally on maximal Ca retention (Institute of Medicine, 1997).

needed to achieve maximal Ca retention in that age-group. Thus, maximal Ca retention is considered by some researchers to be a good functional indicator because (1) its relationship to maximal skeletal Ca and the associated decrease in the component of fracture risk that can be affected by the diet, (2) there is a plateau intake, at least in children, who are in positive Ca balance, above which additional Ca will cause no further gains in retention, and by extension it is the lowest intake at which dietary Ca dependent bone accretion is maximized (Institute of Medicine, 1997). At intakes above this plateau intake more Ca is absorbed, but instead of being retained in the skeleton it is excreted in the urine. In the case of older adults, who typically are in negative Ca balance, their plateau intake, as for children, is that above which Ca retention cannot be further increased with dietary Ca. Although adults may continue to lose bone at the plateau intake (for reasons such as oestrogen deficiency, smoking and sedentary lifestyle etc.), this bone loss is not a consequence of inadequate Ca intake and cannot be prevented with dietary Ca (Institute of Medicine, 1997).

Jackman *et al.* (1997) used Ca intake data from balance studies to develop a non-linear regression model to predict the lowest value of intake at which mean maximal Ca retention is attained. This approach was used by the US Food and Nutrition Board which established recommendations for daily Ca intake for different population groups (Institute of Medicine, 1997). A major limitation of the method is the limited balance data available for some population groups. For example, it was not possible to estimate requirements for males and females separately in certain age-groups, and bone mineral mass data was used to estimate the requirements for pregnant or lactating females (Table 3).

Bone mass

Measures of bone mass may also be used as indicators of Ca adequacy. These measures include bone mineral content (BMC; which is the amount of mineral at a particular skeletal site such as the femoral neck, lumbar spine or total body) and BMD (which is BMC divided by the area of the scanned region). In addition to their relationship to bone mass and strength, recent studies have indicated that BMD and BMC are strong predictors of fracture risk (Black *et al.* 1992; Cummings *et al.* 1993; Melton *et al.* 1993). In adults, a decrease of 1SD in femoral neck and lumbar spine BMD is associated with a 2·5-fold and 2-fold increase in risk of hip and vertebral fracture respectively (Cummings *et al.* 1993).

Cross-sectional studies that relate dietary Ca intake to BMD are of modest value in establishing the Ca requirement. Limitations of such studies include: inaccuracies in measuring Ca intake; Ca intake at one point in time may not reflect lifetime Ca intake; bone mass at a single time point results from the lifelong influence of many confounding variables that are not measured (Institute of Medicine, 1997). In contrast, randomized placebo-controlled Ca intervention studies that measure change in BMD provide valuable evidence for the intake requirement for Ca. The findings of many of these controlled Ca intervention trials

have been reviewed by Dawson-Hughes (1991), Institute of Medicine (1997) and Prentice (1997). For example, supplementation studies in post-menopausal women show that an increase in Ca intake reduces bone loss at the hip (Dawson-Hughes *et al.* 1990; Nelson *et al.* 1991; Reid *et al.* 1993; Chevalley *et al.* 1994; Prince *et al.* 1995), especially for subjects with low habitual Ca intake (Dawson-Hughes *et al.* 1990). However, increases in Ca intake have little effect on spinal-bone mineral in older women (Nelson *et al.* 1991; Chevalley *et al.* 1994; Prince *et al.* 1995). There have also been a number of published studies of Ca supplementation in children and adolescents that have examined bone mineral status (Johnston *et al.* 1992; Lloyd *et al.* 1993; Andon *et al.* 1994; Lee *et al.* 1994, 1995; Nowson *et al.* 1995; Dibba *et al.* 1998, 1999). The emerging picture is that particularly in prepubertal children, an increased Ca intake is associated with higher bone mineral status of approximately 1–5 %, depending on the skeletal site (for review, see Prentice, 1997). Taken together, the findings of such studies demonstrate that BMD responds to Ca intake and, thus, may be a useful functional marker of Ca adequacy. A major strength of such longitudinal studies is that the increment in Ca intake is known. In addition, due to relatively large sample sizes and subject randomization, the influence of confounding factors that might affect bone mass is greatly reduced. Limitations of such studies include: the need for relatively large sample sizes; expense; most of the studies carried out to date have used only one or two dietary levels of Ca (Institute of Medicine, 1997).

An important consideration in the interpretation of longitudinal Ca intervention studies is the phenomenon of the 'bone remodelling transient', a term used to describe the suppression of bone remodelling which is seen with Ca supplementation and which results in a one-time initial gain in bone mass over the first 3–12 months after increasing Ca intake (Frost, 1973). It is thought to reflect completion of osteoblast activity after the inhibition of osteoclastic activity by the increased Ca intake (Slemenda *et al.* 1997). It is transient to the extent that much if not all the gain in bone mass appears to be sustained only for the duration of Ca supplementation. This has been observed on follow-up of some Ca supplementation trials in children, which showed that differences in bone mass between subjects and controls disappeared after supplementation ceased (Lee *et al.* 1996, 1997; Slemenda *et al.* 1997), although not all studies show this (Bonjour *et al.* 1997). However, this one-time gain in bone mass resulting from higher Ca intake may result in a higher peak bone mass in young adults or in a decrease in bone loss in later life, if the higher Ca intake is maintained (Institute of Medicine, 1997).

Although the available data relating dietary Ca intake to BMD is still limited, the US Food and Nutrition Board used BMD and BMC data as additional evidence to support the recommendations based on maximal Ca retention (Institute of Medicine, 1997). Notably, where data was sufficient, as was the case for pregnancy and lactation, bone mass measurements, (particularly BMD) were used to establish the estimates of Ca intake requirements (Institute of Medicine, 1997).

Biochemical indicators

While it has been suggested that there is as yet no biochemical indicator that reflects Ca nutritional status (Institute of Medicine, 1997), recent research suggests that biochemical markers of bone turnover predict bone mass changes and fracture risk, and respond to dietary Ca intake.

Bone turnover is central to maintenance of the structural function of the skeleton. It is a cyclical process whereby the skeleton undergoes continual renewal by a phased sequence of bone resorption and bone formation, which are coupled, but separated in time (Kanis, 1991). Osteoclasts, the cells responsible for bone resorption, dissolve away a small discrete portion of the surface. The resulting resorption cavity is refilled by the action of osteoblasts, the bone-forming cells. Osteoblasts lay down bone matrix (osteoid) which gradually becomes mineralized to form new bone. There is a strict chronological sequence of events, with recruitment of osteoblasts occurring approximately 6–7 weeks after resorption (Eriksen et al. 1984), and with newly-formed bone mineralizing rapidly in the initial stages but more slowly thereafter (Prentice, 1997).

The rate of bone formation and bone resorption, the balance of which ultimately determines bone mass, may be assessed by measurement of biochemical markers (Eastell et al. 1993).

Biochemical markers for monitoring bone turnover all rely on the measurement in serum or urine of enzymes or matrix proteins synthesized by osteoblasts or osteoclasts that spill over into body fluids, or of osteoclast-generated degradation products of the bone matrix itself. Serum levels of skeletal alkaline phosphatase (EC 3.1.3.1) and osteocalcin are currently the most convincing markers of bone formation, while the most useful markers of bone resorption are all products of collagen degradation that currently are best measured in urine (Eyre, 1996). These newer markers have been reported to have improved specificity and responsiveness to bone cell activity over more traditional markers, such as urinary hydroxyproline and serum total alkaline phosphatase (Delmas, 1992; Eyre, 1992; Eriksen et al. 1995), which have a number of well-documented limitations (Robins, 1982; Delmas, 1992).

Produced primarily by osteoblasts, most of the newly-synthesized osteocalcin is immediately bound to the bone mineral, with only a proportion being secreted directly into blood (Gundberg et al. 1983). It is measured in serum by an immunoassay, and appears to be a good marker of the rate of bone formation (Eyre, 1996). Total alkaline phosphatase activity in serum is still the most used index of bone formation in clinical use, especially in conditions where large increases in bone turnover occur, such as Paget's disease (Robins & New, 1997). However, it is less useful for detecting more subtle changes in bone metabolism, and bone-specific bone alkaline phosphatase measured in serum using specific monoclonal antibodies is now widely used as a biochemical marker of bone formation (Eyre, 1996).

It has been consistently shown that the most specific and sensitive markers of bone resorption are urinary markers of bone resorption (Eyre, 1996). Urinary levels of the pyridinium cross-links of collagen, pyridinoline (Pyr) and deoxypyridinoline (Dpyr), have been the most studied markers of bone resorption in the last decade (Eyre, 1996). Pyr and Dpyr function as mature cross-links in collagen of most connective tissues other than skin. Since bone is such a major reservoir of type I collagen in the body and turns over faster than most major connective tissues, Pyr and Dpyr cross-links in urine are primarily derived from bone resorption (Eyre, 1996). This conclusion is supported by the molar ratio Pyr:Dpyr in urine which is usually similar to that of bone (between 3 and 4), compared with most non-osseous connective tissue in which Dpyr is usually present at less than 10 % of the content of Pyr (Eyre, 1992). Theoretically, therefore, urinary Dpyr is more specific than Pyr as a marker of bone resorption. These complex amino acids appear not to be metabolized after tissue collagen is degraded, and are excreted in urine where they can be measured by HPLC using their natural fluorescence for detection (Eyre et al. 1984). In addition, determination of free cross-link content in urine has been facilitated by the development of direct immunoassays which implement monoclonal antibody technology (Robins et al. 1994).

Recently, a number of other urinary 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 (Robins & New, 1997). These assays include those that use monoclonal antibodies recognizing peptides comprising part of either the N- or C-terminal telopeptides, known generically as the urine-based NTx assay (Hanson et al. 1992) and the serum-based CTx assay (Risteli et al. 1993) respectively.

Recently, it has been recognized that an increased rate of bone turnover in adults may be a risk factor for fracture (Riggs et al. 1996), because it exacerbates bone loss (Hansen et al. 1991). Conversely, a reduced rate of bone turnover may lower risk of fracture by maximizing development of peak bone mass in young adults (Slemenda et al. 1997) and slowing the rate of bone loss in later life (Riggs et al. 1996). High rates of bone turnover are also associated with a disruption of the trabecular network, leading to a loss of connectivity which is not necessarily reflected in a decrease of bone mass (Parfitt, 1984).

The availability of these newer markers of bone turnover has widened the scope for investigation of subtle changes in bone metabolism such as those brought about by dietary influences. Although the available data are still limited, they do indicate that these biochemical markers of Ca and bone metabolism are influenced by dietary Ca intake and, thus, might be of value in defining optimal Ca intakes. For example, Riggs et al. (1998) carried out a 4-year randomized controlled Ca intervention trial (1600 mg Ca/d) in post-menopausal women who had a mean usual Ca intake of 714 mg/d. Ca supplementation significantly reduced serum osteocalcin and urinary Pyr levels as well as bone loss at the femur and total body. Similar findings of reduced bone turnover have been reported in a number of other Ca supplementation studies conducted over periods of 2–4 years in peri- or post-menopausal women (Elders et al. 1994; Prince et al. 1995; McKane et al. 1996). Such observations are not limited to older age-groups, but have been made also in children and adolescents. For example, Johnston et al. (1992) showed that Ca supplementation (1000 mg/d) for 3 years significantly reduced serum levels of osteocalcin in

prepubertal children with a mean usual Ca intake of 900 mg/d. The reductions in serum osteocalcin levels with Ca supplementation were accompanied by greater increases in BMD. Similarly, Dibba *et al.* (1998) showed that Ca supplementation (714 mg/d) for 12 months of 10-year-old rural Gambian children accustomed to a low Ca intake (300–400 mg/d) significantly lowered serum levels of osteocalcin and increased bone mineral status ($P \leq 0.001$; Dibba *et al.* 1999) over that time. However, Cadogan *et al.* (1997) did not observe any change in serum osteocalcin or a number of other indicators of bone turnover in adolescent girls whose mean usual Ca intake of 746 mg/d was supplemented with an additional 568 ml milk/d for 18 months, although BMC and BMD were reported to increase.

While it takes at least 1 year to obtain a measurable response of bone mass to changes in Ca intake, responses of biochemical indicators of bone metabolism occur over a shorter period. Indicators of bone resorption respond within hours to days, while indicators of bone formation respond over months. There are a number of short-term studies which show a response of biochemical indicators of bone resorption to changes in dietary Ca. For example, Ginty *et al.* (1998) examined the effect of increased Ca intake on biochemical markers of bone turnover in healthy young adults (mean age 23 years) over a 2-week period. The results showed that supplementation of the usual diet (22 mmol (882 mg) Ca/d) with additional Ca (20 mmol (800 mg)/d) for a 2-week period resulted in a significant reduction in the urinary excretion of Pyr and Dpyr, indicating a reduction in the rate of bone resorption. Similar findings have been reported in a number of other short-term studies which examined the influence of Ca supplementation on biochemical markers of bone turnover (Horowitz *et al.* 1984, 1994; Need *et al.* 1987; Shapses *et al.* 1995; Rubinacci *et al.* 1996; Scopacasa *et al.* 1998).

Evidence from longer-term Ca intervention studies (1–4 years; Johnston *et al.* 1992; Elders *et al.* 1994; Prince *et al.* 1995; McKane *et al.* 1996; Dibba *et al.* 1998, 1999; Riggs *et al.* 1998) indicates that the changes in bone metabolism observed in short-term studies are sustained while the Ca intervention is maintained, and thus such short-term studies may be useful indicators of Ca adequacy. However, such short-term studies should be of a minimum of 2 weeks duration, as Dawson-Hughes *et al.* (1993) has shown that Ca-regulating hormone levels and fractional Ca retention require up to 2 weeks to stabilize after a change in dietary Ca intake. Thus, once sufficient time is allowed for adaptation to new dietary Ca intake, studies which use biochemical markers of bone turnover may be useful in predicting the long-term effects of Ca on bone turnover and bone mass, and thus may be of use in defining optimal Ca intakes.

The effect of Ca supplementation on bone turnover in the previously mentioned studies is due to the increased Ca intake raising plasma Ca, leading to a suppression of plasma PTH and consequently the renal production of $1,25(OH)_2D_3$. Reduced serum levels of PTH and $1,25(OH)_2D_3$ reduce the stimulus for osteoclastic bone resorption (Rubinacci *et al.* 1996).

Evidence for this PTH-mediated mechanism is provided by the significant reduction in serum PTH in response to Ca supplementation observed in a number of these studies (McKane *et al.* 1996; Riggs *et al.* 1998). For example, McKane *et al.* (1996) showed that serum PTH was reduced by 40 % and urinary Dpyr by 35 % in post-menopausal women who were maintained on a supplemented Ca intake (60·2 mmol (2414 mg)/d) for up to 3 years compared with age-matched subjects on their usual Ca intakes (20·4 mmol (815 mg)/d). Riggs *et al.* (1998) showed that serum PTH and bone loss were reduced in post-menopausal women supplemented with an additional 1600 mg Ca/d for 4 years compared with unsupplemented age-matched subjects whose habitual Ca intake was 714 mg/d. Indeed, the responsiveness of serum PTH to dietary Ca suggests that it too could be considered as a potential indicator of Ca adequacy.

Conclusions

At present, serum Pi seems to be the best marker for estimating adequacy of P intake in adults. However, the model that relates dietary intake to serum Pi requires further investigation in infants, children and adolescents. There are, as yet, no functional indicators of P status. The dietary balance approach is considered to be the best available method for estimating Mg requirements. While the Mg tolerance test is a useful indicator of Mg status in the clinical setting, intracellular Mg content (e.g. erythrocyte or lymphocyte Mg) is a more practical approach to assessing status. The lack of functional markers of Mg status is a significant disadvantage for studies relating Mg intake or status to health outcomes such as hypertension, cardiovascular disease and osteoporosis. Maximal Ca retention (which is based on balance data) and bone mass measurements are useful functional indicators of the adequacy of Ca intake and have been used by the US Food and Nutrition Board in estimating the 'adequate intake' of Ca for different population groups (Institute of Medicine, 1997). Biochemical markers of bone turnover have been shown to respond to dietary Ca in short-term (weeks) and long-term (years) studies and are predictive of changes in bone mass and fracture risk. Thus, the response of these markers to Ca intake may be useful for estimating dietary Ca requirements.

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