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Dietary protein and bone health

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The effects of dietary protein on bone health are paradoxical and need to be considered in context of the age, health status and usual diet of the population. Over the last 80 years numerous studies have demonstrated that a high protein intake increases urinary Ca excretion and that on average 1 mg Ca is lost in urine for every 1 g rise in dietary protein. This relationship is primarily attributable to metabolism of S amino acids present in animal and some vegetable proteins, resulting in a greater acid load and buffering response by the skeleton. However, many of these early studies that demonstrated the calciuric effects of protein were limited by low subject numbers, methodological errors and the use of high doses of purified forms of protein. Furthermore, the cross-cultural and population studies that showed a positive association between animal-protein intake and hip fracture risk did not consider other lifestyle or dietary factors that may protect or increase the risk of fracture. The effects of protein on bone appear to be biphasic and may also depend on intake of Ca- and alkali-rich foods, such as fruit and vegetables. At low protein intakes insulin-like growth factor production is reduced, which in turn has a negative effect on Ca and phosphate metabolism, bone formation and muscle cell synthesis. Although growth and skeletal development is impaired at very low protein intakes, it is not known whether variations in protein quality affect the achievement of optimal peak bone mass in adolescents and young adults. Prospective studies in the elderly in the USA have shown that the greatest bone losses occur in elderly men and women with an average protein intake of 16–50 g/d. Although a low protein intake may be indicative of a generally poorer diet and state of health, there is a need to evaluate whether there is a lower threshold for protein intake in the elderly in Europe that may result in increased bone loss and risk of osteoporotic fracture.

Dietary protein: Calcium metabolism: Insulin-like growth factor 1: Osteoporosis: Bone health

Over the last 80 years research into the importance of dietary protein for bone health has been dominated by a focus on the potential negative effects. Intervention studies have shown that a higher protein intake is associated with increased urinary Ca excretion, and cross-sectional and prospective population studies have demonstrated a link between a high protein intake and a greater risk of fracture. A paradox emerges with more recent evidence that a higher protein intake may actually benefit bone health by reducing bone loss and fracture risk in older adults. The present review will attempt to summarise and re-evaluate the earlier evidence for the negative effects of protein on bone health, and identify some of the issues that might affect the interpretation of the earlier findings. It will also address the more recent evidence on the positive effects of protein on bone health and identify some areas of research that may permit a clearer consensus to be reached.

Mechanistic basis for the calciuric effects of protein

Hepatic oxidation of the S-containing amino acids methionine and cysteine to H_2SO_4 and the consequent reduction in blood pH is thought to be the primary mechanism by which bone resorption is increased and urinary Ca losses occur in response to a higher dietary protein intake (Remer, 2000; Fig. 1). There is a misconception that animal protein (i.e. meat, eggs and dairy products) is the primary source of S amino acids, but nuts and cereals are also important sources (Paul *et al*. 1980; Oh, 2000; Table 1). The P and chloride content of the diet also determine the dietary acid load. However, the potential of the dietary acid load to increase bone resorption and urinary Ca excretion depends in part on the dietary alkali load (K, Na, Ca and Mg), which has been shown to neutralise the pH-lowering effects of a higher dietary acid load (Buclin *et al*. 2001). The dietary

Abbreviations: BMD, bone mineral density; IGF, insulin-like growth factor.

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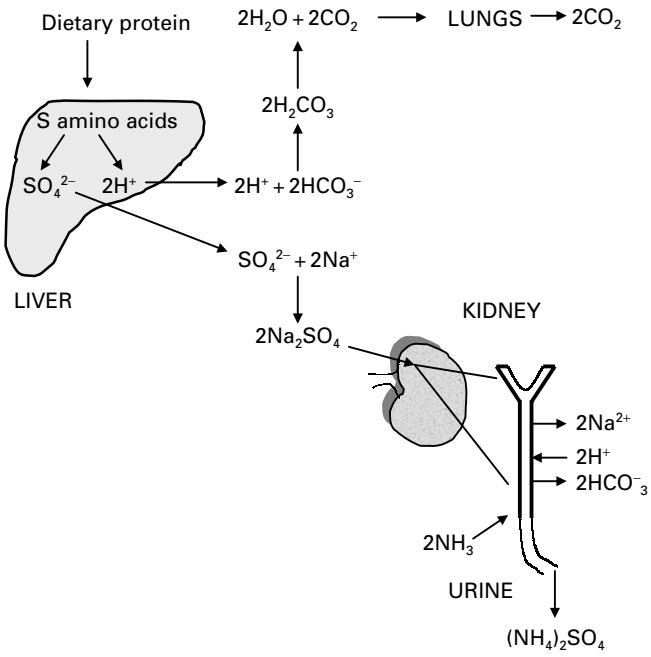

Fig. 1. The hepatic production, physiological buffering and renal excretion of acid equivalents. (Adapted from Remer, 2000.)

Table 1. Protein and sulphur amino acid contents (g/kg) of a selection of commonly-eaten foods (from Paul *et al*. 1980; Holland *et al*. 1991)

Food*	Protein	Methionine	Cysteine
Hard and soft cheeses	255	6–10	2–3·5
Lean beef steak	282	7·4	3·5
White fish (cod)	186	6·2	2·4
Oily fish (salmon)	168	5·8	2·4
Chicken breast	320	5·2	2·8
Peanuts	245	3·2	3·6
Boiled egg	125	3·9	2·2
Wheat breakfast cereal	107	2·0	3·0
Wholemeal bread	92	1·5	2·4
White bread	84	1·4	2·2
Fresh milk	32	0·94	0·31
Boiled spaghetti	36	0·74	1·2
Boiled rice	26	0·48	0·37
Peas	67	0·48	0·56
Potatoes	18	0·23	0·18
Onions	23	0·20	†
Oranges	11	0·12	0·10
Carrots	6	0·07	0.07
Apples	4	0·02	0·03

*All values are for cooked foods.
†Negligible amount.

acid–alkali balance has been quantified in different ways, including animal:vegetable protein (Frassetto *et al*. 2000; Sellmeyer *et al*. 2001), protein:K (Frassetto *et al*. 1998) and the potential renal acid load (Remer & Manz, 1995). When dietary alkali is insufficient there are several acute physiological response mechanisms, including the release of Na, carbonate and citrate from the hydration shell of bone and stimulation of bone resorption (Barzel, 1995; Barzel & Massey, 1998; Bushinsky *et al*. 2001). At the renal level (Fig. 1) the production of NH_3 by the proximal tubular cells is an important defence mechanism against acid load, as it combines with H^+ to form NH_4^+. This cation then combines with sulphate to form $(NH_4)_2SO_4$, which is excreted in urine, thus leading to the elimination of both ions (Remer, 2000).

Several other mechanisms by which a higher protein intake increases urinary Ca excretion have been postulated, including increased glomerular filtration rate and reduced tubular reabsorption of Ca. Glomerular filtration rate was increased by approximately 10 % and fractional Ca reabsorption was decreased by 1 % when six healthy adult males consumed 142 *v*. 47 g purified protein/d for 10 d (Kim & Linkswiler, 1979). On the basis that approximately 10 g Ca/d are filtered by the glomeruli, it was estimated that the increase in glomerular filtration rate might increase the filtered Ca load by 1 g/d and urinary Ca losses by 110 mg/d. Allen *et al*. (1979) suggested that the decrease in tubular reabsorption of Ca may be attributed to saturation of the renal transport process for Ca. Other researchers have suggested that reabsorption of Ca may be reduced when Ca is complexed to citrate, phosphate, sulphate, bicarbonate or ammonium ions (Lemann *et al*. 1966; Kim & Linkswiler, 1979). However, > 20 years after these theories were put forward there is still no definitive evidence for the mechanisms by which a higher protein intake might impair renal Ca transport. Furthermore, it is likely that the extent of impairment is dependent on the age and hormonal status of an individual.

Evidence from short-term intervention studies

Numerous short-term intervention studies have investigated the effects of increased protein intake on Ca excretion, and details of a number of these studies are outlined in Table 2. On the basis of these and other studies it has been estimated that there is a 1 mg rise in urinary Ca for each 1 g rise in dietary protein (Kerstetter & Allen, 1990). This empirical formula cannot be applied to all protein sources because many of the intervention studies utilised purified protein, including lactalbumin, wheat gluten and casein. The effects of meat protein have been found to be less exaggerated, which may be related to its higher P content (Hegsted *et al*. 1981; Heaney, 1993). Hegsted *et al*. (1981) showed that simultaneous increases in protein (from 50 g to 150 g) and P (from 1010 mg to 2525 mg) intakes caused a 28 % increase in urinary Ca, whereas an increase in protein intake alone caused a 115 % increase in urinary Ca. One of the most-widely quoted studies on the effects of meat intake on Ca excretion and balance (Spencer *et al*. 1978) showed, in a series of twenty-six studies of fourteen male patients over periods of 16–72 d, that increasing meat intake from an average of 200 g (approximately 83 g protein)/d to 300 g (approximately 140 g protein)/d did not increase urinary Ca excretion or modify intestinal Ca absorption (determined using ^{47}Ca). However, a criticism of this study is that the subjects had a variety of disorders, including hypothyroidism, psychoneurosis, osteoporosis, hypercalciuria and obesity, and so the findings cannot be generalised to the population. Draper *et al*. (1991) studied the effect of increasing the protein intake of postmenopausal women (*n* 8) from 58 g/d to 92 g/d for 15 d at each protein level. In

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Table 2. Protein intervention studies examining the effects of increased consumption of purified sources of protein and meat on urinary calcium excretion

Reference	Subjects: *n*	Subjects: Gender	Subjects: Age (years)	Duration of dietary intervention (d)	Protein source	Protein doses (g/d)	P intake (mg/d)	Ca intake (mg/d)	Urinary Ca increase* (mg/g protein)	Apparent Ca absorption	Change in GFR (%)	Ca balance (mg/d)
Margen *et al.* (1974)	4–6	M	20–32	9–18	Egg albumin Soyabean protein Casein	75 *v.* 150, 300, 387 or 600	Not provided	900–2300	+0·3–0·8	–	–	–
Allen *et al.* (1979)	6	M	23–30	47 and 48	Amino acids Egg albumin	75 *v.* 225	2629 *v.* 2073	1392 *v.* 1451	+0·6	↔	+14	−137
Hegsted & Linkswiler (1981)	6	F	23–28	15 and 60	Soyabean protein Casein Lactalbumin Wheat gluten	46 *v.* 123	900	500	+1·25	↔	+12	−121
Pannemans *et al.* (1997)	19 10	M F	24–36 22–33	21	Egg white Wheat gluten Soyabean protein	69 *v.* 124	1519 *v.* 1841	1081 *v.* 1215	+0·7	↑(NS)	–	+13 (NS)
	17 9	M F	65–78 61–78		Meat Casein	61 *v.* 107	1250 *v.* 1501	873 *v.* 985	+0·8 (NS)	↑(NS)	–	−30 (NS)†
Kim & Linkswiler (1979)	6	M	21–29	10	Casein Lactalbumin Wheat gluten	47 *v.* 142	1110	515	+1·6	–	+10	−156
Hegsted *et al.* (1981)	8	M	19–25	12	Casein Lactalbumin	50 *v.* 150	1010 (low P)	500	+1·8	–	+16	−116
					Dried egg white	50 *v.* 150	2525 (high P)	500	+1·1	–	+8·4	−25
Block *et al.* (1980)	6	F	20–42	4 h	Wheat gluten Potassium co-precipitate of bovine milk S amino acids	15 *v.* 45 or 15 + S amino acids in 45 g diet	400	200	+1·7 μg/g per min (after 1·5 h)	–	↔	–
Lutz (1984)	6	F	38–62	10–16	Wheat gluten Casein Lactalbumin	44 *v.* 102	900	500	+1·6	↔	–	−68
Margen *et al.* (1974)	4–6	M	20–32	9–18	Beef and turkey	75 *v.* 150	2000	100	+ 0·82	–	–	–
Allen *et al.* (1979)	6	M	23–30	4 h	Cottage cheese	18 *v.* 54	642	400	+2·5 μg/g per min (after 2 h)	–	↔	–
Chu *et al.* (1975)	6	M	22–32	15	Beef and turkey	0 *v.* 75, 150 or 75 + 900 mg Ca	2000	100	+0·70	–	+24	−138 to −222
Spencer *et al.* (1978)	14	M	40–67	16–72								
Draper *et al.* (1991)	8	F	50–64	15	Meat	83 *v.* 140	800 *v.* 1300	200–2000	0–0·35 (NS)‡	↔	–	−82 to +238
					Meat Eggs Poultry Fish Cereal	55 *v.* 95	994 *v.* 1450	650	+0·50 (NS)	↔	–	−52 (NS)
Roughead *et al.* (2003)	15	F	50–75	56	Various meats	68 *v.* 117	617 *v.* 596	1266 *v.* 1679	0	↔	+14	+17·1 % of dose *v.* 15·6 % for low-protein diet

GFR, glomerular filtration rate; ↑, increase; ↔, no change; –, not determined.
*Effects on urinary Ca excretion were estimated from data provided from each study.
†A significant negative Ca balance was found during the lower-protein diet in the elderly subjects.
‡Data from two further studies consisting of one subject apiece were not included in the effect estimations

contrast to earlier studies a range of commonly-eaten high-protein foods (high-protein cereal, wholewheat bread, meat, eggs, protein, fish) was used to increase the protein content of the diet. The higher-protein diet did not have a significant effect on urinary Ca excretion or Ca balance and there was no change in serum parathyroid hormone. However, urinary cAMP was higher (indicating increased parathyroid hormone activity at the renal level), which led the authors to conclude that the 46 % increase in P intake on the high-protein diet may have stimulated parathyroid hormone renal reabsorption of Ca, thus partially offsetting the calciuric effects of the higher-protein diet.

There are several difficulties in the interpretation of many of the protein intervention studies, including small numbers of subjects, short duration and wide variations in Ca and Na intake. The necessity of having complete faecal and urine collections for calculation of apparent Ca absorption and Ca balance can result in calculation errors. In addition, the use of creatinine for estimating glomerular filtration rate has limitations when the meat intake is increased, since meat contains a high level of creatine, which is readily converted to creatinine (Chu *et al*. 1975).

In the most-recently published study, in which post-menopausal women consumed either low- or high-meat diets (45 g *v*. 297 g, equivalent to 68 *v*. 117 g protein/d) for 8 weeks in a controlled crossover design, the high-meat diet was found to have no effect on bone and Ca metabolism markers, Ca retention or Ca absorption (Roughead *et al*. 2003). Urinary titratable acidity and pH were initially lower on the high-meat diet, but by 8 weeks the levels of both indicators were similar to those observed for the low-protein diet, suggesting that adaptation may have occurred. Although glomerular filtration rate was increased by approximately 14 % on the high-meat diet, the authors concluded that this difference was most likely to be a result of the higher creatine intake from meat. At 8 weeks, this study is the longest investigation of the effects of increased meat intake on Ca retention and other relevant bone measurements, and the findings suggest that adaptation may occur. It is therefore questionable whether the current estimations of the calciuric effects of protein are appropriate, particularly in the absence of estimates of the effects of protein in a broader age-range of subjects from different ethnic groups.

The protein and hip fracture controversy

The calciuric effect of dietary protein was also believed to provide the mechanistic basis for the association found between cross-cultural hip fracture incidence in women > 50 years of age and animal-protein intake (Abelow *et al*. 1992). The highest rate of hip fracture was found to occur in industrialised Western countries, which had animal-protein intakes *per capita* between 60 and 80 g/d. On the other hand, the lowest incidence occurred in indigenous Asian and African populations in which animal-protein intakes were considerably lower. Frasetto *et al*. (2000) extended the same cross-cultural analysis to thirty-three countries and reached a similar conclusion, with the additional finding that hip fracture incidence was inversely related to vegetable-protein intake. There are several obvious limitations to both studies, not least the poor applicability of population food consumption data to women > 50 years of age. Furthermore, Asian and African ethnic groups are recognised to have a reduced risk of osteoporotic fracture (Aspray *et al*. 1996; Yan *et al*. 1999), which may be attributable to a multiplicity of factors, including differences in bone structure, genotype and lifestyle. Frassetto *et al*. (2000) attempted to overcome this factor by limiting the analysis to predominantly Caucasian populations, and the positive association between hip fracture rate and animal-protein intake remained.

Whether vegetarians have a skeletal advantage in terms of better bone mineral status or reduced fracture incidence has not been established (Department of Health, 1998). Higher consumption of cereal grains and nuts could potentially provide a similar dietary acid load to that of animal protein (Table 1). However, this effect may be counteracted by higher consumption of alkali foods. Most of the studies examining differences between vegetarians and non-vegetarians have been conducted in very specific population groups (Marsh *et al*. 1980, 1983, 1988; Tylavsky & Anderson, 1988), which does not permit generalisation to the wider population.

Prospective evidence for a negative effect of protein on bone health

There are two prospective studies providing evidence that fracture incidence is related to higher protein intake (Feskanich *et al*. 1996; Meyer *et al*. 1997). The Nurse's Health Study (Feskanich *et al*. 1996) was a 12-year survey of 85 900 women aged 35–59 years. Protein intake was assessed by a mailed food-frequency questionnaire at three time-points (baseline, year 4 and year 6) and fracture incidence was self-reported biennially. Women who consumed > 95 g total protein (i.e. animal and vegetable protein)/d had a greater risk of forearm fracture compared with those who consumed < 68 g/d. A higher intake of vegetable protein alone was not associated with increased risk, but women consuming five or more servings of beef, pork or lamb had an increased risk of forearm fracture compared with women who consumed less than one serving per week. Meyer *et al*. (1997) found no association between non-dairy animal-protein intake and hip fracture incidence in a prospective study of 40 000 Norwegian men and women (aged 35–49 years at baseline) conducted over an average period of 11 (range 0·01–13·8) years. At one time point during the study subjects completed a food-frequency questionnaire, which they filled in at home and returned by post. Intake of non-dairy animal protein was not found to be associated with hip fracture, but women in the lowest quartile of Ca intake and highest quartile of non-dairy animal-protein intake (values not provided) had an elevated risk of fracture. This pattern was not observed with total protein intake. A major limitation of both studies was the use of a mailed food-frequency questionnaire on a limited number of occasions and limited evaluation of other lifestyle and dietary factors that may have contributed to fracture risk.

Effects of protein intake on calcium recommendations for developing countries

Although the impact of protein intake on Ca requirements and bone health has not been established conclusively, the recently revised dietary Ca recommendations for developing countries (Food and Agriculture Organization/World Health Organization Expert Consultation, 2002) accounted for the lower protein intake (20–40 g/d) of developing countries in their estimations of theoretical Ca requirements. By using the estimated 1 mg increment in Ca for every 1 g protein intake, the recommendation for adults was calculated to be 750 mg/d, as compared with the 1000 mg/d recommendation that was based on Western European, American and Canadian data. Although this downward adjustment attempts to account for ethnic differences in Ca requirements, it is not known whether a higher protein intake results in calciuria in non-Caucasian individuals. As with the cross-cultural associations found between protein intake and hip fracture, there are likely to be numerous other factors that influence bone health and Ca requirements in developing countries.

Protein supplementation and reduced bone loss

In parallel with the controversy that grew over the negative effects of protein on bone health, there were also studies that suggested that certain segments of the population could benefit from increasing their protein intake. Geinoz *et al*. (1993) observed that patients with higher protein intakes during their hospital stay had higher femoral neck and lumbar spine bone mineral densities (BMD). After 4 weeks in hospital women with a higher protein intake had better muscle strength and stair-climbing performance. Such findings do not suggest a specific effect of protein intake *per se*, but may reflect better general health status and thus better appetite in these patients. However, two further studies substantiated the observational evidence by demonstrating that protein supplementation for 5 weeks reduced the medical complication rate and duration of hip fracture in patients with a recent hip fracture (Delmi *et al*. 1990; Tkatch *et al*. 1992). Further convincing evidence of the benefits of protein supplementation in this age-group was provided by Schurch *et al*. (1998) in a randomised double-blind protein supplementation trial with elderly Swiss patients with hip fracture (thirty-seven women and four men, aged 81·1 (SD 7·4) years) who had a baseline protein intake of 45·0 (SD 15·2) g/d. All patients received one oral dose of cholecalciferol (5 mg) to correct any possible vitamin D deficiency and were randomised to receive either a protein supplement (containing 900 g milk proteins/kg and providing (/d) 300 µg vitamin A, 30 µg vitamin K, 20 mg vitamin C, 550 mg Ca, 91 mg Mg, 429 mg P) or an isoenergetic placebo containing maltodextrins, but not the multinutrients. After 6 months of supplementation the protein-supplemented group were found to have higher levels of insulin like-growth factor 1 (IGF-1) and reduced proximal femur bone loss compared with the placebo group. Hospital stay was also reduced by 21 d in the protein-supplemented group. Much emphasis has been placed on the independent effects of protein in this study, but it is also possible that they may be a result of the combined or additive effects of protein and the various nutrients contained in the supplement. No other intervention studies have been conducted in this age-group and there is an immediate need for longer-term studies to determine whether increasing protein intake would be an effective means of reducing fracture-related morbidity and maintaining bone health in elderly men and women. Identification of the upper intake limit and interactions with other nutrients in relation to bone is also essential.

Bone loss is reduced in elderly men and women with high animal-protein intake

Two large prospective studies have shown that higher animal-protein intake is associated with reduced bone loss over a 4-year period in elderly men and women who were participants of the Framingham Osteoporosis Cohort (Hannan *et al*. 2000) and The Rancho Bernardo Heart and Chronic Disease Study (Promislow *et al*. 2002). In a third study of elderly women from the Iowa Women's Health Study (Munger *et al*. 1999) those who had the highest animal-protein intake had a decreased risk of hip fracture. In the Framingham cohort the mean total protein intake for the men (*n* 224) was 69·3 (SD 23·9) g/d and that for the women (*n* 392) was 68·0 (SD 23·5) g/d. After adjustment for all potential confounders, including age, height, weight and weight changes, total energy intake, smoking, physical activity, those in the lowest quartile of percentage protein intake showed the greatest BMD losses at the femur and spine sites and lower percentage animal protein was related to greater bone loss at the femur and spine. There were similar findings in Rancho Bernardo Study (Promislow *et al*. 2002), with a high animal-protein intake also appearing to have a protective effect against bone loss. It was surprising, however, that the greatest bone losses occurred in women with the highest vegetable-protein intake and a similar non-significant trend was found in men. The Iowa Women's Health Study (Munger *et al*. 1999) also reported an increase in age-adjusted hip fracture risk with increasing quartile of vegetable-protein consumption. This finding is in conflict with the evidence that higher fruit and vegetable intake has a positive effect on bone (New *et al*. 1997, 2000; Muhlbauer & Li, 1999; Tucker *et al*. 1999). It is difficult to explain the greater bone loss with the higher-vegetable-protein diet, because the components of the diet were not described in either study, nor were the associated lifestyle factors discussed, although animal-protein intake was shown to be positively associated with vegetable-protein intake in the Rancho Bernardo Study. Further research is needed in order to elucidate the interactions between animal and vegetable protein and their relative importance for maintaining bone health in the elderly.

Mechanisms by which protein positively affects bone health

Albright *et al*. (1941) wrote: 'a diet inadequate in protein might lead to a negative nitrogen balance and this in turn might make it impossible for the osteoblasts to lay down the necessary organic matrix, which is the first step in the

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formation of bone. We believe that some of the osteopathies which have been attributed to a lack of calcium and phosphorus in the diet are really due to protein starvation.' Since this statement was made, the understanding of the mechanisms of action of dietary protein has expanded to encompass a regulatory role in growth hormone and IGF-1 metabolism.

IGF-1 is an essential mediator of tissue anabolism, stimulating growth of multiple cell types, transport of amino acids and protein synthesis in muscle and skeletal tissues (Clemmons & Underwood, 1991). During growth it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate (Wang *et al*. 1999) and it is thus an essential factor for longitudinal growth. IGF-1 has been shown *in vitro* to increase osteoblast activity (Mohan *et al*. 1992; Langdahl *et al*. 1998) and production of type I collagen (McCarthy *et al*. 1989) and to act as a coupling factor for bone resorption and bone formation (Rubin *et al*. 2002). IGF-1 also has an important role in the regulation of Ca and P metabolism by stimulating renal transport of inorganic phosphate and kidney production of 1,25-dihydroxycholecalciferol (Caverzasio & Bonjour, 1989). This function may be of paramount importance during growth, when the high rate of collagen synthesis and mineralisation results in high requirements for Ca and phosphate. More recently, it has been shown that selective knock-out of the IGF-1 receptor gene in mouse osteoblasts results in mice with normal bone size and weight but a decrease in the rate of mineralisation of osteoid (Zhang *et al*. 2002). The authors suggested that osteoblast-derived IGF-1 might be essential for coupling collagen synthesis to sustained mineralisation.

Regulation of insulin-like growth factor 1 production by dietary protein

Given its essential role in growth and protein synthesis it is not surprising that hepatic IGF-1 production, plasma IGF-1 concentration and the proportion of free or active IGF-1 (i.e. the portion not bound to its principal binding protein IGF-binding protein 3) is regulated by dietary protein intake (Clemmons & Underwood, 1991; Thissen *et al*. 1994; Fig. 2). In animal studies protein fasting has been shown to induce a decrease in hepatic growth hormone-binding sites (Maiter *et al*. 1989) and protein restriction results in growth hormone-receptor defects (Thissen *et al*. 1992). The consequences of this effect include decreased hepatic production of IGF-1 and a lowering of the circulating concentration. Elevated production of IGF-binding protein 3 exacerbates the effect by decreasing the proportion of free IGF-1, thus decreasing its anabolic capacity (Clemmons & Underwood, 1991) and increasing IGF-1 clearance (Thissen *et al*. 1992). In protein-restricted rats normalisation of plasma IGF-1 by infusion failed to promote growth (Thissen *et al*. 1991), indicating that in the absence of an adequate protein supply end-organ resistance occurs. Several animal studies have shown that the IGF-1 deficit caused by protein restriction has adverse consequences for bone, including osteoporosis, impaired cortical bone formation and osteoblast resistance (Bourrin *et al*. 2000*a,b*).

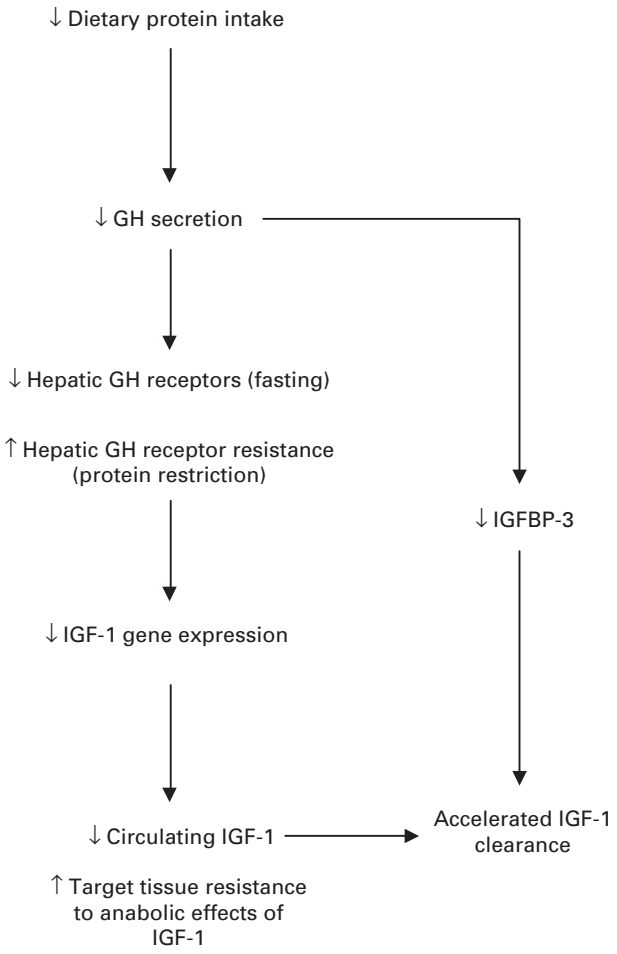

Fig. 2. The effects of protein on insulin-like growth factor (IGF) production and plasma concentration. GH, growth hormone; IGFBP, IGF-binding protein; ↓, decreased; ↑, increased. (Adapted from Thissen *et al*. 1994.)

In human subjects cross-sectional studies in older women have shown a positive association between plasma IGF-1 and BMD (Langlois *et al*. 1998) and muscle strength and mobility (Cappola *et al*. 2001). It has also been shown that serum concentrations of IGF-1 and IGF-binding protein 3 are lower in osteoporotic patients with spinal fractures compared with those without fractures (Sugimoto *et al*. 1997). However, with the exception of the study described earlier by Schurch *et al*. (1998), which showed that protein supplementation increased the IGF-1 concentration in elderly patients with hip fracture, there are no other human intervention studies that have evaluated the effects of modifying protein intake from different sources on IGF-1 in this age-group. IGF-1 production is also influenced by sex steroid hormone status, and the relative importance of the age-related decline in oestrogen and androgen status compared with the influence of protein intake remains undetermined in man.

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Calcium balance

Although many of the early studies focused on the negative effects of a high protein intake on Ca balance, more recent evidence suggests that a low protein intake has a negative effect on Ca metabolism and balance. Short-term intervention trials in healthy women have shown that a low protein intake (0·7 g/kg) is associated with an increase in serum parathyroid hormone and a reduction in both urinary Ca excretion and Ca absorption. The latter finding was not anticipated, as it was expected that increased parathyroid hormone would be associated with greater production of 1,25-dihydroxyvitamin D and increased Ca absorption (Kerstetter *et al*. 1997, 2000, 2003). These findings led the authors to suggest that intestinal and/or skeletal handling of Ca is altered by a low-protein diet. Although the duration of these studies was too short (4 d) to indicate whether adaptation could occur, it is possible that the negative effects of a low protein intake on bone loss in the elderly (Hannan *et al*. 2000; Promislow *et al*. 2002) could be mediated by similar perturbations in Ca metabolism and absorption. However, this mechanism remains to be proven. It has been suggested that the essential amino acid lysine may play a role in Ca metabolism. Studies in animals and human subjects have shown that Ca absorption is higher when lysine intake is increased (Wolinsky & Fosmire, 1982; Civitelli *et al*. 1992; Civitelli, 1993), but the underlying mechanism has not been identified.

Protein–calcium interaction

In recent years there has been a focus on the possible interaction between dietary Ca and protein intake. Although the possible negative effects of a higher protein intake may be compensated by a high Ca intake or exacerbated by a low Ca intake, the potential anabolic effects of protein may be maximised by a higher Ca intake. Evidence in favour of the former theory has been suggested in the Nurse's Health Study (Feskanich *et al*. 1996), which found that women with a high-total-protein (> 95 g/d) high-Ca diet (> 827 mg/d) had a lower risk of fracture than women with a high-total-protein low-Ca diet (< 531 mg/d). Limited evidence is also available from the study by Meyer *et al*. (1997), which showed that women with a low Ca and high non-dairy animal-protein intake had a greater risk of fracture.

Dawson-Hughes & Harris (2002) showed that Ca-supplemented older men and women (≥ 65 years of age) in the highest tertile of protein intake (as % energy) had the greatest increases in whole-body and femoral neck BMD compared with those in the lowest tertile. No association was found between protein intake and bone change in the placebo group. Most recently, Rapuri *et al*. (2003) examined the effects of protein intake (also as % energy) on baseline BMD and rate of subsequent bone loss in 65–77-year old women. The highest quartile of protein intake was associated with higher BMD at the spine, mid radius and whole body. Among women in the lowest quartile of Ca intake (< 480 mg/d) no significant association was found between spine BMD and protein intake. However, in the upper two quartiles of Ca intake an association was observed between spine BMD and protein intake. A similar association was also seen at the whole body level. Longitudinally, bone loss did not differ by quartile of protein intake and Ca intake showed no effect. A point worth noting from this study was that dietary vitamin D increased with increasing protein intake, suggesting that the benefits of a higher protein intake may also be explained by other factors.

For the purposes of the present review a preliminary analysis of our own data from the Cambridge Bone Studies was carried out to examine whether there was an interaction between Ca supplementation and baseline protein intake in 16–18-year-old boys (*n* 110) and girls (*n* 101) who participated in two separate Ca intervention studies (Prentice *et al*. 2002; Stear *et al*. 2003). In these studies Ca supplementation (1000 mg Ca as $CaCO_3$/d) increased bone mineral content of the whole body, hip and spine in boys supplemented for 12·7 (SD 0·5) months. Similar results were observed in girls with higher compliance supplemented for 15·5 (SD 0·7) months. No significant interaction was found between baseline dietary protein intake (as % energy) and Ca supplementation in relation to bone mineral content change in either the boys or the girls, and there was no significant effect of protein intake on bone changes in the placebo group (F Ginty and A Prentice, unpublished results). However, as with the studies described earlier, the interpretation is limited by the *post hoc* nature of the analysis. It is necessary to conduct studies that specifically set out to address the mechanistic basis for an interaction between protein and Ca in different age-groups.

Effects of protein on peak bone mass

Although an adequate intake of protein is essential for growth, it is not known whether variations in protein intake and quality contribute to variations in bone size, mineral content and ultimately the achievement of optimal peak bone mass. Studies have shown a positive association between protein intake and bone mineral status in children (Hoppe *et al*. 2000), adolescents (Rizzoli, 1998) and young women aged 18–31 years (Teegarden *et al*. 1998). However, the findings in the younger subjects do not necessarily indicate a causal relationship, since protein intake is likely to be driven by growth requirements. Furthermore, BMD is not independent of size, and such associations may be artefacts (Prentice *et al*. 1994). Cadogan *et al*. (1998) found that supplementation of 12-year-old girls with 568 ml (1 pint) milk daily for 18 months was associated with an increase in plasma IGF-1 and bone mineral status compared with control subjects. It was proposed that the higher protein content of milk mediated a rise in plasma IGF-1 that, in turn, may have had a stimulatory affect on osteoblast activity, or may have promoted bone mineralisation, as suggested recently by Zhang *et al*. (2002). Bone growth is site-specific and varies with the stage of puberty, and it has been hypothesised that disruptions to growth through illness, poor diet etc. may result in site-specific deficits in bone mineral status and quality (Bass *et al*. 1999; Seeman *et al*. 2000). IGF-1 is a major determinant of bone growth and mineral content (Yakar *et al*. 2002), and plasma concentrations are

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approximately four to five times higher in adolescents than in adult (Juul *et al*. 1994). However, there are no data on the extent to which IGF-1 production is regulated by protein intake during puberty and whether variations in IGF-1 as a result of lower protein intake result in site-specific deficits, thus increasing risk of fragility later in life.

Summary and conclusions

Although there is much supporting evidence and a mechanistic basis for the calciuric effects of a high protein intake, there is a lack of evidence from long-term studies in different age-groups of continued urinary Ca losses and bone loss. Further information is required on the compensatory effects of a Ca- and/or alkali-rich diet. It is important in terms of public health to determine whether a low protein intake in the elderly predisposes them to a greater rate of bone loss. It is also necessary to identify the protective aspects of a higher-protein diet and whether there are other underlying dietary or lifestyle characteristics that may also explain the lower rate of bone loss observed in prospective studies. The finding that protein, multimineral and vitamin supplementation of elderly patients with hip fracture increases recovery time and reduces bone loss is also important. However, more studies are needed to support these findings and to determine whether it is an effect of protein *per se*, or a multi-nutrient effect. Although there is no doubt that an adequate protein intake during childhood and adolescence is essential to support normal growth and skeletal development, more research is needed to evaluate the role of protein quality and protein-diet interactions in the achievement of optimal peak bone mass. The available evidence appears to suggest that protein may have a biphasic effect on bone health. However, the upper and lower thresholds are difficult to define without taking overall diet, health status and age into account.

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