

Intake of fruit and vegetables: implications for bone health

Susan A. New

*Centre for Nutrition & Food Safety, School of Biomedical & Molecular Sciences, University of Surrey,
Guildford GU2 7XH, Surrey, UK*

*'Life is a struggle, not against sin, not against the money power, not against malicious animal
magnetism, but against hydrogen ions'
H. L. Mencken 1919*

'These famous words by Mencken in the early 20th century about the meaning of life and death, may also apply to the struggle of the healthy skeleton against the deleterious effects of retained acid!' (Kraut & Coburn, 1994). The health-related benefit of a high consumption of fruit and vegetables and the influence of this food group on a variety of diseases has been gaining increasing prominence in the literature over a number of years. Of considerable interest to the osteoporosis field is the role that bone plays in acid–base balance. Natural, pathological and experimental states of acid loading and acidosis have been associated with hypercalciuria and negative Ca balance, and more recently the detrimental effects of 'acid' from the diet on bone mineral have been demonstrated. Surprisingly, consideration of the skeleton as a source of 'buffer' contributing to both the preservation of the body's pH and defence of the system against acid–base disorders has been ongoing for over three decades. However, it is only more recently that the possibility of a positive link between a high consumption of fruit and vegetables and indices of bone health has been more fully explored. A number of population-based studies published in the last decade have demonstrated a beneficial effect of fruit and vegetable and K intake on axial and peripheral bone mass and bone metabolism in men and women across the age-ranges. Further support for a positive link between fruit and vegetable intake and bone health can be found in the results of the Dietary Approaches to Stopping Hypertension (DASH) and DASH-Sodium intervention trials. There is now an urgent requirement for the implementation of: (1) fruit and vegetable and alkali administration–bone health intervention trials, including fracture risk as an end point; (2) re-analysis of existing dietary–bone mass and metabolism datasets to look specifically at the impact of dietary 'acidity' on the skeleton.

Osteoporosis: Acid–base balance: Fruit and vegetables: Bone health: Alkali-forming foods

Our approach to examining the relationship between nutrition and bone health has been to focus on specific (or a variety of) nutrients commonly consumed in the human diet. Whilst this approach has enabled a greater understanding of the influence of the important bone minerals (i.e. Ca, P and Mg) on bone metabolism, there are still considerable gaps in our knowledge. An alternative approach to elucidating this complex relationship is the consideration of the 'foods' consumed rather than the nutrients contained within them. Since it is well known that site sensory characteristics and the direct hedonic pleasure derived from food are important determinants of food choice (Kearney & Gibney, 1998),

the use of a 'food'-orientated methodology is clearly a sensible way to investigate diet–disease relationships. It is noteworthy that there is a general international consensus about the proportions in which foods should be eaten. The message may be displayed in different formats (e.g. UK and Australia use a food plate, USA and Singapore use a food pyramid and Finland uses a food plate–food pyramid combination) but it is essentially the same (Ministry of Agriculture, Fisheries and Food, 1995; Fig. 1). There is general agreement that fruit and vegetable consumption should be adequate (at least five portions daily) for the optimisation of health.

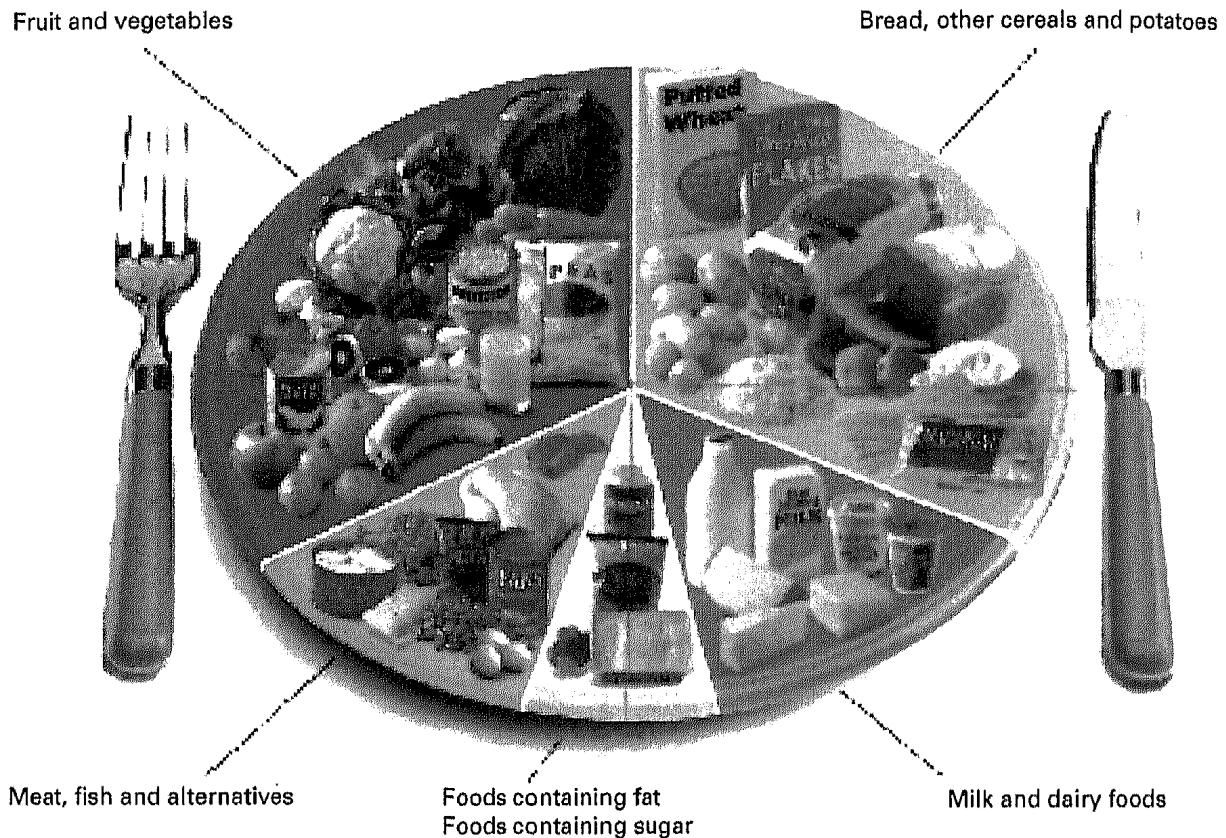


Fig. 1. Recommendations for the consumption of food groups: the UK plate model. (From Ministry of Agriculture, Fisheries and Food, 1995.)

Importance of acid–base homeostasis to health

Acid–base homeostasis is absolutely critical to health. It is well documented that extracellular fluid pH remains between 7.35 and 7.45. Thus, it is a major requirement of our metabolic system to ensure that H^+ concentrations are maintained between 0.035 and 0.045 mEq/l (Green & Kleeman, 1991). It is essential for survival that H^+ concentrations are kept within these particularly narrow limits, and hence the body's adaptive response involves three specific mechanisms: (1) buffer systems; (2) exhalation of CO_2 ; (3) the renal system.

On a daily basis human subjects eat substances that both generate and consume H^+ , and as a net result adults consuming a normal Western diet generate approximately 1 mEq acid/kg body weight per d. The more acid precursors the diet contains, the greater the extent of systemic acidity (Kurtz *et al.* 1983). As individuals age there is a decline in their overall renal function, including their ability to excrete acid (Frassetto *et al.* 1996). Hence, with increasing age subjects become slightly, but significantly, more acidic (Frassetto & Sebastian, 1996). As shown in Fig. 2, blood H^+ concentrations increase with age and plasma HCO_3^- levels decrease (L Frassetto, personal communication).

Skeletal link to acid–base maintenance: key points

Three key points should be considered when examining a link between acid–base maintenance and the skeleton.

First, the theoretical considerations of the role that alkaline bone mineral may play in the defence against acidosis date back as far as the late 1880s–early 20th century (Goto, 1918; Irving & Chute, 1933; Albright & Reifstein, 1948). The fundamental concepts were established in the late 1960s–early 1970s. A number of studies published during this period provided evidence that in natural (e.g. starvation), pathological (e.g. diabetic acidosis) and experimental (e.g. NH_4Cl ingestion) states of acid loading and acidosis there is an association with both hypercalciuria and negative Ca balance (Gastineau *et al.* 1960; Reidenberg *et al.* 1966). The pioneering work of Lemann *et al.* (1966) and Barzel (1969) showed extensively the effects of 'acid' from the diet on bone mineral in both man and animals. As shown in Figs. 3 and 4, the studies of Barzel (1969) and Barzel & Jowsey (1969) demonstrate that long-term ingestion of NH_4Cl causes a decrease in bone substances and the development of osteoporosis in the rat; $KHCO_3$ prevents this bone loss.

At the first Conference on Osteoporosis in 1969 there was much debate on the possible role of the skeleton as a source of buffer, contributing to both the preservation of the body's pH and defence of the system against acid–base disorders (Barzel, 1970; Bernstein *et al.* 1970).

Second, the effect of dietary acidity on the skeleton only needs to be relatively small for there to be a large impact over time. In the 1960s Wachman & Bernstein (1968) put forward a hypothesis linking the daily diet to the development of osteoporosis based on the role of bone in

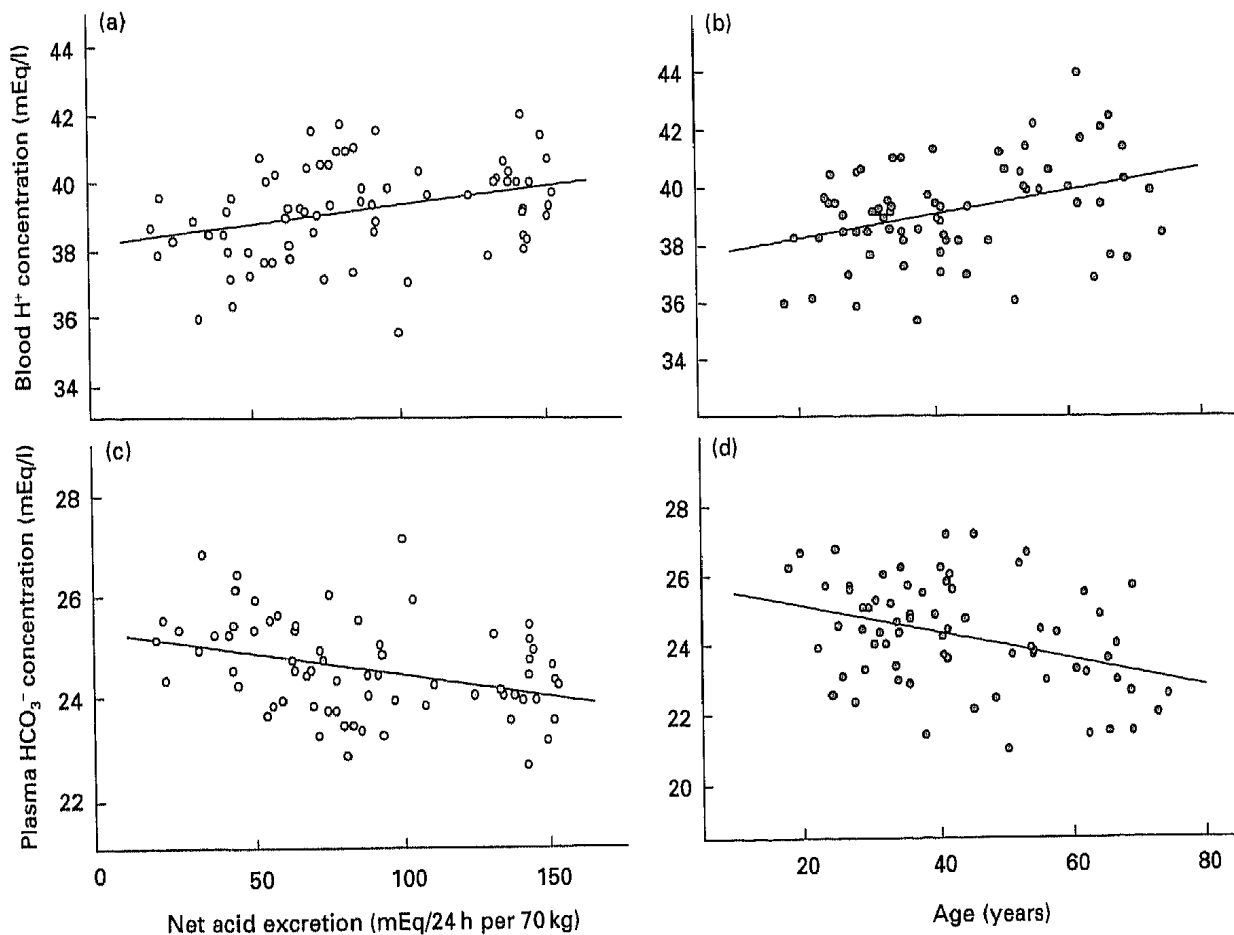


Fig. 2. Changes in acidity with age. Relationships between blood hydrogen ion concentration and (a) net acid excretion (NAE; r 0.34, $P < 0.005$) and (b) age (r 0.37, $P = 0.002$) and between plasma bicarbonate concentration and (c) NAE (r -0.36, $P < 0.005$) and (d) age (r -0.37, $P = 0.002$). (From Frassetto *et al.* 1996; reproduced with kind permission from Dr L. Frassetto.)

acid-base balance. These authors noted specifically that 'the increased incidence of osteoporosis with age may represent, in part, the results of a lifelong utilisation of the buffering capacity of the basic salts of bone for the constant assault against pH homeostasis'. The net production of acid is related to nutrition, and there is a gross quantitative relationship between the amount of acid produced (as reflected by urine pH) and the amount of acid-ash consumed in the diet. When considering the extent of loss, if 2 mEq Ca/kg body weight per d is required to buffer about 1 mEq fixed acid/kg body weight per d, over a period of 10 years (and assuming a total body Ca of approximately 1 kg) this interaction would account for a 15 % loss of inorganic bone mass in an average individual (Widdowson *et al.* 1951).

Third, there are clear mechanisms for a deleterious effect of acid on bone. In novel work in the 1980s Arnett & Dempster (1986, 1990) demonstrated a direct enhancement of osteoclastic activity following a reduction in extracellular pH. This effect was shown to be independent of the influence of parathyroid hormone (Arnett & Dempster, 1986, 1990; Arnett *et al.* 1994; Fig. 5). Furthermore, osteoclasts and osteoblasts appear to respond independently to small changes in pH in the culture media in which they are growing (Kreiger *et al.* 1992; Fig. 6). Work by Arnett &

Spowage (1996) and Bushinsky (1996) has shown evidence that a small drop in pH, close to the physiological range, causes a tremendous surge in bone resorption. Metabolic acidosis has also been shown to stimulate resorption by activating mature osteoclasts already present in calvarial bone rather than by inducing formation of new osteoclasts (Meghji *et al.* 2001). It is considered that almost all the bone mineral release that occurs in response to acidosis is due to osteoclast activation, which results in increased resorption pit formation in bone (with the organic matrix being destroyed at the same time; TR Arnett, personal communication). On the other hand, there is evidence that excess H^+ directly induce physico-chemical Ca release from bone (Bushinsky *et al.* 1994).

Vegetarianism and skeletal health: the potential renal acid load concept

The potentially-deleterious effect of specific foods on the skeleton has been a topic of recent debate (Fox, 2001; New *et al.* 2002a; New & Francis, 2003; Plant & Tidey, 2003). Remer & Manz (1995) examined the potential renal acid loads of a variety of foods and found that many grain products and some cheeses have a high potential renal acid load (Remer & Manz, 1995; Fig. 7). These foods, which are

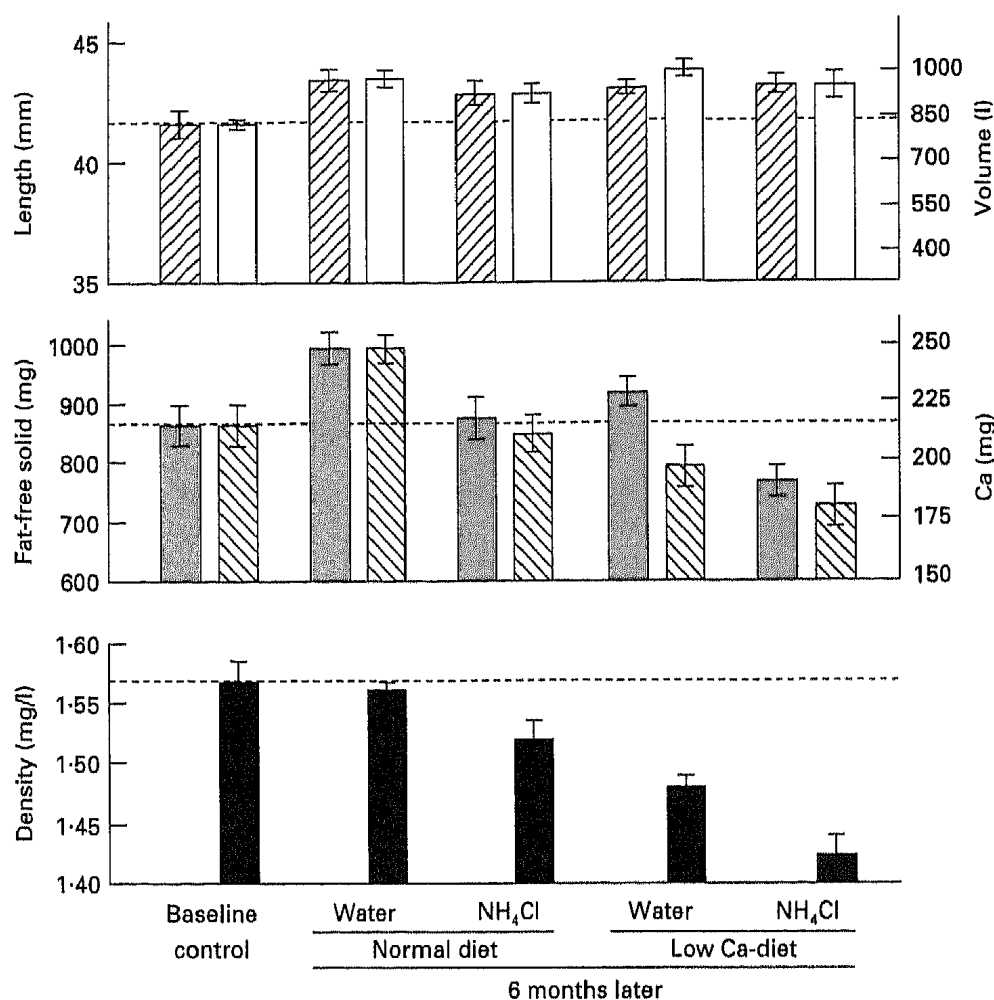


Fig. 3. Long-term ingestion of ammonium chloride (2 g/l) caused a decrease in bone characteristics and the development of osteoporosis in the rat. (▨), Length; (□), volume; (■), fat-free solid; (▨), calcium; (■), density; (---), baseline level. Values are means with their standard errors represented.

likely to be consumed in large quantities by lacto-ovo vegetarians, may provide an explanation for the lack of a positive effect on bone health indices in studies in which vegetarians and omnivores are compared (New, 2002).

Few studies investigating bone health have focused attention on populations consuming a diet highly dependent on animal foods, particularly meat (Hammond & Storey, 1970). Mazess & Mather (1974) examined the bone mineral content of forearm bones in a sample of 217 children, eighty-nine adults and 107 elderly Eskimo natives of the north coast of Alaska (Mazess & Mather, 1974). After the age of 40 years the Eskimos of both genders were found to have a deficit of bone mineral of between 1 and 15 % relative to standards developed for the Caucasian population. An even greater bone loss with ageing was found in Canadian Eskimos (Mazess & Mather, 1975). These findings are of considerable interest in relation to the interaction between diet and bone in the regulation of systemic acid-base balance, and further work in this area is clearly warranted (New, 2003).

Evidence for a beneficial effect of fruit and vegetables and alkali on indices of bone health

Observational studies

A variety of population-based studies published in the latter part of the 20th century, and more recently between 2001 and 2003, have demonstrated a beneficial effect of fruit and vegetable and K intake on indices of bone health in young boys and girls (Jones *et al.* 2001; Tylvasky *et al.* 2002), premenopausal (Michaelsson *et al.* 1995; New *et al.* 1997, 2000; Macdonald *et al.* 2001; Whiting *et al.* 2002), perimenopausal (New *et al.* 2000; Macdonald *et al.* 2001) and post-menopausal women, and elderly men and women (Eaton-Evans *et al.* 1993; Michaelsson *et al.* 1995; Tucker *et al.* 1999; Chen *et al.* 2001; Miller *et al.* 2001; New *et al.* 2002b; Stone *et al.* 2001; Fig. 8).

In order to clarify the extent of the effect of fruit and vegetables and K intake on bone health a systematic review of nine relevant observational studies, involving 4824 subjects, was recently undertaken. The analysis suggested that a small (approximately 0.9 % risk of a low BMD

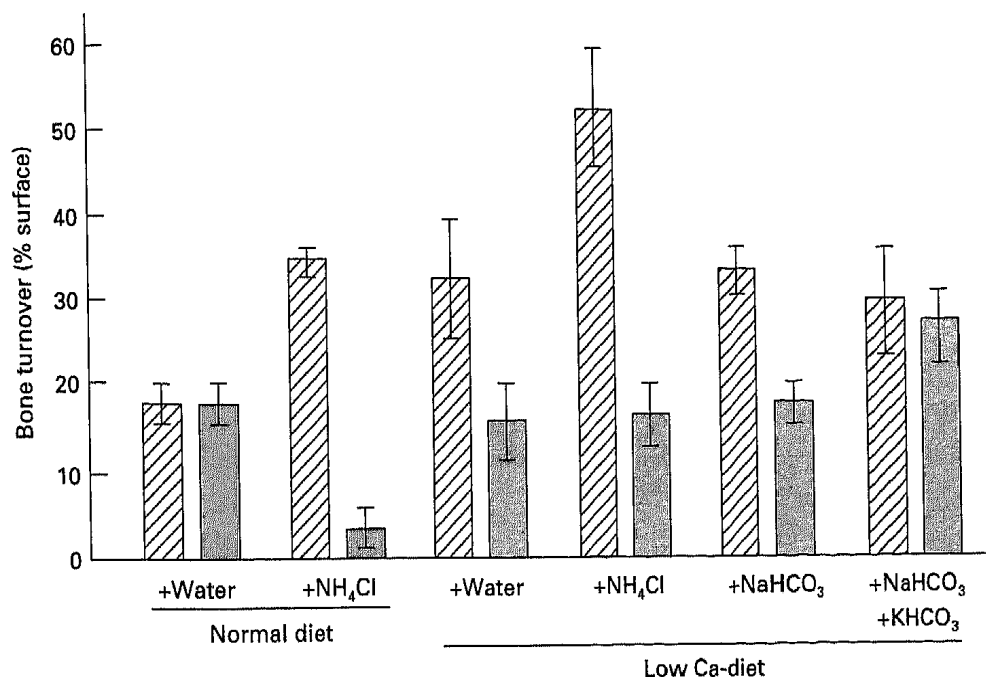


Fig. 4. Bone turnover changes in response to the intake of a normal diet with water or ammonium chloride (1.5 g/l), or a low-calcium diet with water, sodium bicarbonate (0.5 g/l) or sodium bicarbonate (0.25 g/l) and potassium bicarbonate (0.3 g/l). Values are means with their standard errors represented by vertical bars. Ingestion of ammonium chloride increased bone resorption and both sodium bicarbonate and potassium bicarbonate prevented the loss of bone. (From Barzel & Jowsey, 1969.)

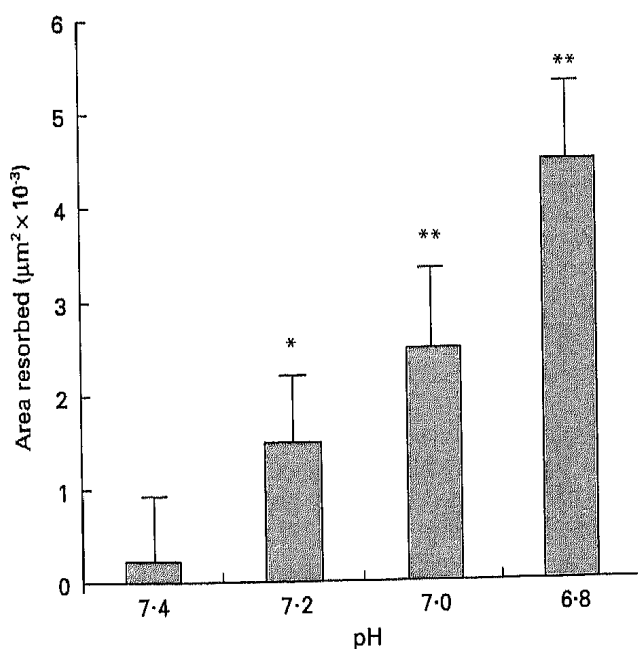


Fig. 5. Increase in osteoclastic activity with a reduction in intracellular pH. Values are means with their standard errors represented by vertical bars. Mean values were significantly different for those at pH 7.4: * $P < 0.05$, ** $P < 0.01$. (From Arnett & Dempster, 1986.)

attributable to K intake), but nonetheless significant ($r = 0.098$, $r^2 = 0.009$), effect on bone health (SA New and DJ Torgerson, unpublished results). There is now an urgent need for a much more detailed analysis of which types of fruit and vegetables have the most direct impact on the skeleton and whether potatoes should be included in the

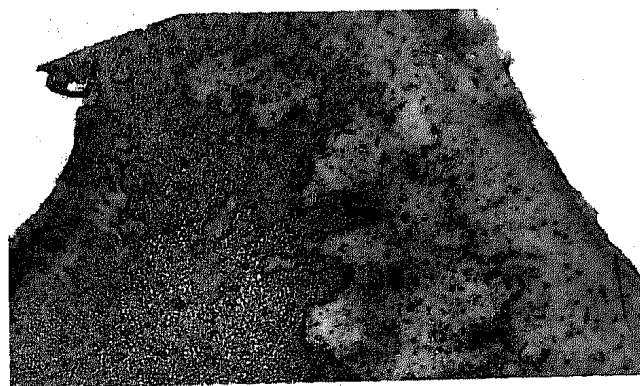
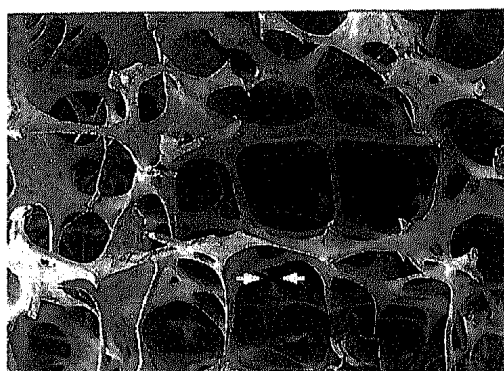


Fig. 6. Resorption of mouse calvarial bone stimulated by bicarbonate acidosis. (From Meghji *et al.* 2001; reproduced with kind permission of Dr T. R. Arnett.)

calculations for many of these observational studies. Potatoes have a potential renal acid load of -4.0 mmol/100 g edible portion but are categorised into different food groups in different countries. For example, in the UK Balance of Health programme potatoes are included in the breads, rice and other starch food group, whereas in Denmark they are included in the fruit and vegetable group. Furthermore, in the UK potatoes are not included in the 'five portions a day' recommendation, whereas in other European countries they are included.

Intervention studies

Further support for a positive link between fruit and vegetable intake and bone health can be found in the results of



Acid-etched holes in osteoporotic bone?

Bone 'dissolving' foods:

Parmesan cheese	34.1
Turkey breast	9.9
Spaghetti noodles	6.5
Milk chocolate	2.4

Bone 'sparing' foods:

Oranges	-2.7
Tomatoes	-3.1
Bananas	-5.5
Spinach	-14.1

Fig. 7. The potential renal acid loads of different foods and their effect on bone. (Data from Remer & Mantz, 1994; Fox, 2001.)

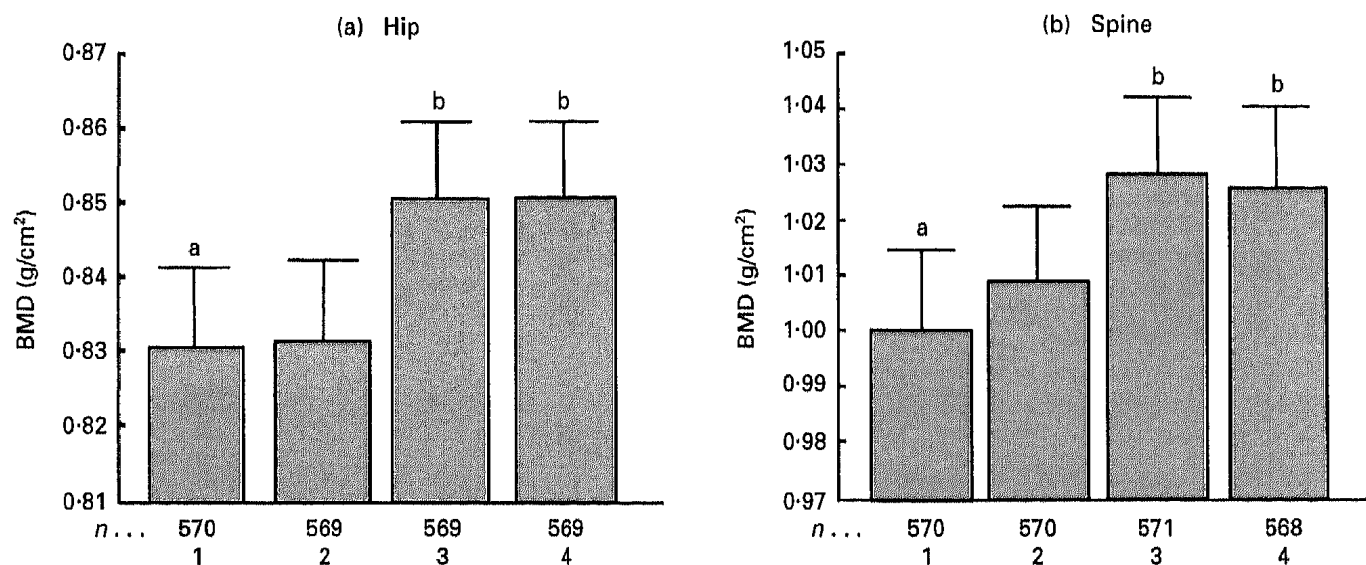


Fig. 8. Quartiles of fruit intake and bone mineral density (BMD) of the hip (a) and spine (b) for women participating in the Aberdeen Prospective Osteoporosis Screening Study (longitudinal data). Values are means with their standard errors represented by vertical bars. ^{a,b}, Values with unlike superscript letters were significantly different ($P < 0.001$). (From Macdonald *et al.* 2001; reproduced with kind permission of Dr H. Macdonald.)

the Dietary Approaches to Stopping Hypertension (DASH) and DASH-Sodium intervention trials. In the DASH trial, diets rich in fruit and vegetables were associated with a significant fall in blood pressure compared with baseline measurements. However, of particular interest to the bone field was the finding that increasing fruit and vegetable intake from 3.6 to 9.5 daily servings decreased the urinary Ca excretion from 157 mmol/d to 110 mg/d (Appel *et al.* 1997). The authors suggested this was due to the 'high fibre content of the diet possibly impeding Ca absorption'. However, a more likely explanation, put forward by Barzel (1997), was a reduction in the 'acid load' with the fruit and vegetable diet compared with the control diet. This study was the first population-based fruit and vegetable intervention trial showing a positive effect on Ca economy (albeit a secondary finding).

More recently, Lin *et al.* (2001) have reported the findings of the DASH-Sodium trial. The impact of two dietary patterns on indices of bone metabolism was examined. The DASH diet emphasises fruits, vegetables and

low-fat dairy products and has reduced levels of red meats, and in the DASH-Sodium trial three levels of Na intake were investigated (50, 100 and 150 mmol/l). Subjects consumed the control diet at a Na intake of 150 mmol/d for 2 weeks. They were then randomly assigned to eat the DASH diet or the control diet at each of the three levels of Na intake for a further 4 weeks in random order. When compared with the control diet, the DASH diet was found to reduce both bone formation (by measurement of the marker osteocalcin) by 8–10 % and bone resorption (by measurement of the marker cross-linked C-terminal telopeptides) by 16–18 % (F Ginty, personal communication). Interestingly, Na intake did not significantly affect the markers of bone metabolism. This intervention study is important as it shows a clear benefit of the high intake of fruit and vegetables on markers of bone metabolism. Research is now required to determine the long-term clinical impact of the DASH diet on bone health and fracture risk, and to clarify the exact mechanisms involved in the protective effect of this diet on the skeleton.

Clinical studies

The clinical application of the effect of normal endogenous acid production on bone is of considerable interest, with extensive work in this area at the whole-body level by Lemann and co-workers (Lemann *et al.* 1967, 1979, 1986, 1989, 1991) and at the cellular level by Bushinsky and co-workers (Bushinsky *et al.* 1983, 1993, 1994, 1997; Bushinsky & Sessler, 1992; Bushinsky, 1997). As shown in Table 1, Sebastian *et al.* (1994) demonstrated that KHCO_3 administration resulted in a decrease in urinary Ca and P, with overall Ca balance becoming less negative (or more positive). Changes were also seen in markers of bone metabolism, with a reduction in urinary excretion of hydroxyproline (bone resorption marker) and an increased excretion of serum osteocalcin (bone formation marker). Concern has been raised that the level of protein consumed by the women in the study was higher than the typical intake for American women in this age-group, and a call has been made for further studies to be undertaken with dietary protein being consumed at a more reasonable level (Wood, 1994). More recently, Buclin *et al.* (2001) have examined the effect of dietary modification on Ca and bone metabolism. The 'acid-forming' diet increased urinary Ca

excretion by 74 % and bone resorption (as measured by C-terminal peptide excretion) by 19 % in comparison with the alkali-forming diet, both at baseline and after an oral Ca load.

The study by Sebastian *et al.* (1994) is of clinical importance and may have valued implications for the prevention and treatment of post-menopausal osteoporosis (Morris, 2001). Long-term studies of the effect of alkali administration on bone loss with age are now urgently required.

Dietary balance and the skeleton: the concept of net endogenous acid production

Determination of the acid-base content of diets consumed by individuals and populations is a useful way to quantify the link between acid-base balance and skeletal health. Every day individuals eat substances that both generate and consume H^+ , and the net result is that consumption of a normal Western diet is associated with chronic low-grade metabolic acidosis (Kurtz *et al.* 1983). The severity of the associated metabolic acidosis is determined, in part, by the net rate of endogenous (non-carbonic) acid production (NEAP), which varies with diet. Since 24 h urine

Table 1. Effect of potassium bicarbonate supplementation on mineral metabolism and skeletal balance in post-menopausal women (from Sebastian *et al.* 1994)

	Before KHCO_3		During KHCO_3		Change	
	Mean	SE	Mean	SE	Mean	SE
Ca balance (mg/d per 60 kg)	-180	124	-124	76	+56	76
P balance (mg/d per 60 kg)	-208	127	-161	92	+47	64
Serum osteocalcin (ng/ml)	5.5	2.8	6.1	2.8	+0.6	0.48
Urinary hydroxyproline (mg/d)	28.9	12.3	26.7	10.8	-2.2	*
NRAE (mmol/d)	70.9	10.1	12.8	21.8	-58.1	*

NRAE, net renal acid excretion.

The mean change was significant: * $P < 0.05$.

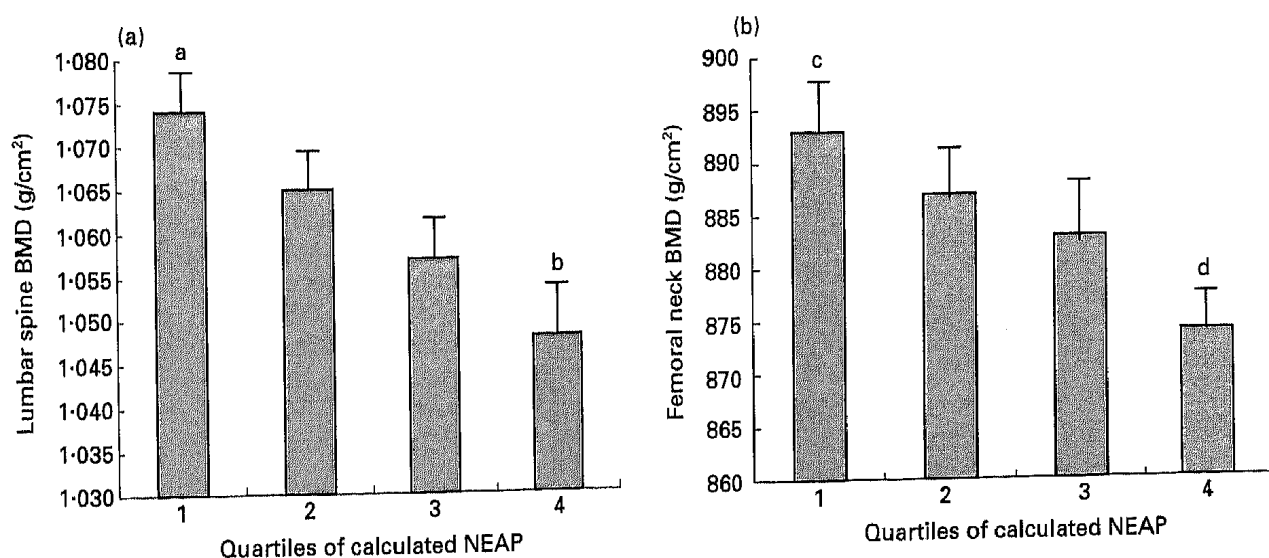


Fig. 9. The association between net endogenous non-carbonic acid production (NEAP) and bone mass (bone mineral density; BMD) of the lumbar spine (a) and femoral neck (b) for 994 Scottish women participating in the Aberdeen Prospective Osteoporosis Screening Study (baseline data). Values are means with their standard errors represented by vertical bars. ^{a,b,c,d} Values with unlike superscript letters were significantly different: ^{a,b} $P < 0.04$; ^{c,d} $P < 0.08$. (From New *et al.* 2001.)

collections are impractical for population-based studies, an alternative is to examine the net acid content of the diet. Frassetto *et al.* (1998) have found that protein:K predicts net acid excretion and, in turn, net renal acid excretion predicts Ca excretion. They proposed a simple algorithm to determine the net rate of NEAP from considerations of the acidifying effect of protein (via sulfate excretion) and the alkalisising effect of K (via provision of salts of weak organic acids).

To examine this theory further estimates of NEAP were calculated from the baseline and longitudinal datasets from the Aberdeen Prospective Osteoporosis Screening Study. In the baseline dataset women with the lowest estimate of NEAP were found to have higher lumbar spine and femoral neck BMD (Fig. 9) and significantly lower urinary pyridinium cross-link excretion (New *et al.* 2001). In the regression analysis the calculated NEAP explained 0.5 % of the variation in lumbar spine BMD ($P < 0.001$). Partial correlation coefficients were significant for weight, height and NEAP ($P < 0.001$, $P < 0.001$ and $P < 0.025$ respectively). Using the calculated regression equation, holding weight and height constant (using the mean values for the group) and looking at the difference in lumbar spine BMD between the minimum and maximum intakes of NEAP estimate, an 8 % reduction in lumbar spine BMD was found. Absolute values were 0.923 g/cm² for the highest intake of NEAP estimate and 0.999 g/cm² for the lowest intake of NEAP, a difference of 0.076 g/cm². At the forearm NEAP estimates were highlighted as an independent predictor of both peripheral cortical forearm and peripheral total BMD forearm bone mass, accounting for 0.7 and 0.5 % of the variation ($P < 0.001$).

Findings for bone resorption were mirrored in the longitudinal database from the Aberdeen Prospective Osteoporosis Screening Study. In approximately 2800 women, adjusting for the key confounding factors, those with the lowest estimate of NEAP were found to have significantly lower pyridinoline and deoxypyridinoline excretion (Macdonald *et al.* 2002).

These data do not suggest that dietary protein is detrimental to bone health, since even women in the lowest quartile of protein intake were consuming levels well above the UK estimated average requirement (mean intake 82.5 g/d). Rather, these data indicate that dietary K (as the denominator in the NEAP algorithm) is the critical component, i.e. diets that are characterised by less dietary acid (i.e. closer to neutral) are associated with better indices of bone health. Although the variation in factors, including weight and height, account for most of the variation in BMD among subjects, estimates of NEAP still accounted for a significant proportion of the BMD variation among subjects. Shifting from the top quartile to the lowest quartile of 'dietary acidity' as a group, or from the top intake to the lowest intake of 'dietary acidity' as an individual, resulted in a better bone mass. These findings concerning NEAP are critical, since in adults after the age of 30 years (approximately) both weight and height remain relatively stable; the majority of their influence in 'setting' bone mass is complete by this age. Thus, in post-menopausal and/or elderly women with one-time measure of BMD there are strong among-subjects influences of weight and height on BMD. However, it can be postulated that NEAP continues to 'wear' away bone gradually and indefinitely after the age

of 30 years, accelerating as the glomerular filtration rate falls with age (A Sebastian, personal communication). In other words, weight and height differences among subjects would not be likely to transfer their influence on BMD in each individual subject over time, but NEAP, as an ongoing 'dynamic' influence on bone, presumably does continue its influence on bone mass in each individual subject over time.

An additional benefit of calcium supplements on skeletal integrity?

Of growing interest in the literature is the recognition that Ca plays a critical role in the relationship between the balance of a beneficial v. detrimental effect of protein on the skeleton (Heaney, 1998, 2002; Dawson-Hughes & Harris, 2002). It is possible that Ca supplements may be favourable to bone, not just through the additional mineral that they supply, but also through their provision of additional alkali salts (New & Millward, 2003). Since the relationship between dietary protein and skeletal health remains controversial, it might be prudent to suggest re-analysis of existing nutrition and bone health datasets to focus specifically on the effect of protein-K and protein-Ca on indices of bone health. It may be that in the absence of sufficient dietary alkali to neutralise the protein-derived acid net Ca loss ensues and the anabolic drive of dietary protein on the bone matrix is ineffective in maintaining BMD.

Areas for further research

It should not be forgotten that it is generally believed that our modern diet is vastly different from the diet consumed by early man (Eaton & Konner, 1985). Estimates of the dietary content of pre-agricultural man indicate that Na intakes were approximately 600 mg/d and those of K approximately 7000 mg/d compared with corresponding present-day intakes of approximately 4000 and 2500 mg in the UK, USA and Australia (Gregory *et al.* 1990; Patterson *et al.* 1990).

It is also important to note that the positive associations found between fruit and vegetable consumption and bone may be a result of some other, yet unidentified, 'dietary' component rather than an effect of alkali excess (Oh & Uribarri, 1996). Convincing work by Muhlbauer *et al.* (2002, 2003) suggests that vegetables, herbs and salads commonly consumed in the human diet affect bone resorption in the rat by a mechanism that is not mediated by their base excess (Muhlbauer *et al.* 2002), but possibly through pharmacologically-active compounds that are currently being explored (Muhlbauer *et al.* 2003). Furthermore, it is important to consider that available mineral waters that have been shown to be beneficial to bone health are an important alkali source (in addition to supplying substantial mineral intakes; Burckhardt, 2003).

Future research should focus attention on longer-term intervention trials centred specifically on fruit and vegetable intake and alkali administration as the supplementation vehicle and should assess a wide range of bone health indices, including fracture risk. In addition, experimental studies (at both the cellular, animal and human level) should be conducted to determine whether there are other aspects of

fruit and vegetables that are beneficial to bone metabolism. It would also be helpful when re-analysing existing dietary-bone mass and metabolism datasets to look particularly at the impact of 'dietary acidity' on the skeleton, to further define the relationship between NEAP and skeletal integrity, and to investigate specifically whether very high protein intakes are detrimental to the skeleton in the absence of Ca and alkali-forming foods.

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