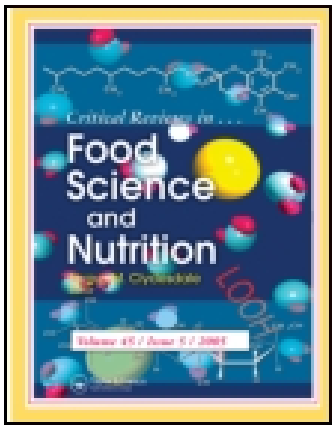

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Dietary Protein's and Dietary Acid Load's Influence on Bone Health

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A variety of genetic, mechano-response-related, endocrine-metabolic, and nutritional determinants impact bone health. Among the nutritional influences, protein intake and dietary acid load are two of the factors most controversially discussed. Although in the past high protein intake was often assumed to exert a primarily detrimental impact on bone mass and skeletal health, the majority of recent studies indicates the opposite and suggests a bone-anabolic influence. Studies examining the influence of alkalizing diets or alkalizing supplement provision on skeletal outcomes are less consistent, which raises doubts about the role of acid–base status in bone health. The present review critically evaluates relevant key issues such as acid–base terminology, influencing factors of intestinal calcium absorption, calcium balance, the endocrine-metabolic milieu related to metabolic acidosis, and some methodological aspects of dietary exposure and bone outcome examinations. It becomes apparent that for an adequate identification and characterization of either dietary acid load's or protein's impact on bone, the combined assessment of both nutritional influences is necessary.

Keywords Bone, Calcium Balance, Diet, Metabolic Acidosis, Net Acid Excretion, Potential Renal Acid Load, Protein Intake

INTRODUCTION

Bone health is influenced by a variety of factors. One important modifiable determinant is nutrition. The roles of dietary protein and dietary acid load in bone health, in particular, have given cause for discordant study interpretations and views. Although in the past high protein intake was often assumed to exert a primarily detrimental impact on bone mass and skeletal health, the majority of more recent studies indicate the opposite. In a systematic review and meta-analysis published in 2009, Darling et al. reported positive pooled correlations for the relation between total protein intake and bone mineral density (BMD) and bone mineral content (BMC) for nearly all bone sites (Darling et al., 2009). Less consistent than these later findings are the results of studies examining the influence of alkalizing diets or supplements on the skeletal system (Jehle et al., 2006; Macdonald et al., 2008; Cao et al., 2011; Fenton et al., 2011). In the present paper, we discuss some of the key issues and inconsistencies that have emerged regarding the above dietary influences on bone health, thereby also briefly focusing on problems of study design, measurements, and assessment methods.

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TERMINOLOGY OF NET ENDOGENOUS ACID PRODUCTION BY DIET

Usually, during normal metabolism an excess of hydrogen ions is produced daily by the body. Diet is an important determinant of daily net endogenous acid production implying that blood pH and bicarbonate buffer can be, at least mildly, nutritionally influenced. Conditions with modest pH and/or circulating bicarbonate reductions, still within the normal range, are termed subtle acidosis. Even these mild, nonclinical acidotic changes are assumed to adversely affect bone (Pizzorno et al., 2010). Accordingly, several (but not all) prospective observational studies reported bone-anabolic effects of alkalizing nutrients (Table 1).

In a number of papers published during the last few years on bone-relevant exposure and outcome variables, the term acid ash diet has been used when different aspects of net acid or base producing diets were addressed (Cloutier and Barr, 2003; Dargent-Molina et al., 2008; Fenton et al., 2009). The quantifying of acidity by titration after the ashing of diets, meals, or foods was applied decades ago to characterize the acidifying potential with the respective nutrition. However, nowadays this is an obsolete, exclusively chemically based concept that only considers the acidity or alkalinity of foods after chemical ashing but not the normal varying bioavailibility of those

Table 1 Prospective observational studies (epidemiological evidence level II) on dietary acid load exposures and bone densitometric outcomes

Study	Population	Dietary acidity-related exposures (assessment method)	Outcomes (assessment method)	Results	
				Dietary change	BMD
Tucker et al., 2001	615 men & women 69–97 years	Alkaliz. Nutrients,[1] protein (semiquantitative FFQ)	Four-year-BMD-change (DEXA)	Alkaliz. nutrients ↑ Protein ↑	+[2] +
Kaptoge et al., 2003	944 men & women 67–79 years	Alkaliz. nutrients, protein (seven-day food diary)	BMD-change per year after two–five years (DEXA)	Alkaliz. nutrients ↑ Protein ↑	↔[3] ↔
Macdonald et al., 2004	891 women 45–55 years (premenopausal)	Alkaliz. foods and nutrients (FFQ)	BMD-change per year follow-up BMD after five–seven year (DEXA)	Alkalizing foods ↑	+
Pedone et al., 2010, 2011	497 women 60–96 years	PRAL, protein (diet questionnaire)	Six-year-BMD-change[4] (pQCT), bone area[5] (pQCT)	PRAL ↓ Protein ↑ PRAL ↓	↔ +, +

Note: None of the studies assessed alkaline nutrients/PRAL and protein in the same model (adjusted for each other).
[1]Alkalizing minerals, primarily potassium, and magnesium.
[2]+, positive effect on BMD (i.e., reduction in BMD loss).
[3]↔, no effect.
[4]Initial analysis according to original publication.
[5]Reanalysis in response to Remer et al., 2011b.
BMD = bone mineral density, FFQ = food frequency questionnaire, DEXA = dual-energy X-ray absorptiometry, PRAL = potential renal acid load, PQCT = peripheral quantitative computed tomography.

individual cationic and anionic components responsible for the overall alkaline or acidic properties. Today's acid–base research considers intestinal absorption (bioavailibility) of the relevant nutrients (minerals, protein), metabolic release of the corresponding anions and cations into circulation (with subsequent renal elimination), the ion valence and the degree of ionic dissociation at the physiological pH of 7.4. In other words, it is not the body's total proton production that is estimated, but the net endogenous acid production (NEAP), i.e., the balance (difference) between anions and cations (mostly from diet) that have obligatorily to be eliminated via the kidney. This ion difference (in mEq) is characterized according to the stoichiometry of the renally excreted components of the urine ionogram and calculated as follows:

Total organic acids + chloride (mmol·1) + sulfate (mmol·2) + phosphate (mmol·1.8) − (sodium (mmol·1) + potassium (mmol·1) + magnesium (mmol·2) + calcium (mmol·2))

(Remer and Manz, 1994, 1995b).

If the difference is negative (Fig. 1a) and the individual is healthy, we have an alkalizing situation usually caused by a base-producing diet; if it is positive (Fig. 1b), a certain amount of proton excess has metabolically formed (exceeding the sum of bioavailable and renally excreted mineral cations). The above ion difference (without the organic acid fraction) can be clinically-chemically determined in 24-hour urine samples as a noninvasive direct biomarker of diet-dependant acid load, termed urinary potential renal acid load (urinary PRAL, Fig. 1). The difference can also be directly measured as titratable acid + ammonium – bicarbonate and is referred to as net acid excretion (NAE, Fig. 1) (including organic acids). NAE is considered as the best available biomarker to estimate NEAP (Frassetto et al., 2007a). One important advantage of analyzing NAE or urinary PRAL is that (at least in the steady state) these parameters reasonably reflect the difference of the anionic and cationic minerals of the urine ionogram after their intestinal absorption. Those anions and cations not absorbed, but eliminated via the gut, do, in fact, influence stool acidity but are for the most part metabolically unimportant.

In addition, NEAP can be reasonably estimated from the diet (Frassetto et al., 2007a) either using the more accurate [if compared with real NAE titrations (Remer et al., 2003)] but also more elaborate dietary PRAL method (Remer and Manz, 1995b) or using the ratio of the two most important nutrients for the acid–base balance, i.e., protein and potassium intake (Frassetto et al., 1998). The latter protein:potassium ratio is a practical estimate of NEAP, if dietary intake data, especially for calcium, magnesium, and phosphorus (and possibly for sodium and chloride) are less reliable or not available. An acid ash diet hypothesis as phrased by Fenton et al. (Fenton et al., 2009; Fenton et al., 2011) no longer exists in modern nutritional acid–base research for the calculation of the diet's acid load.

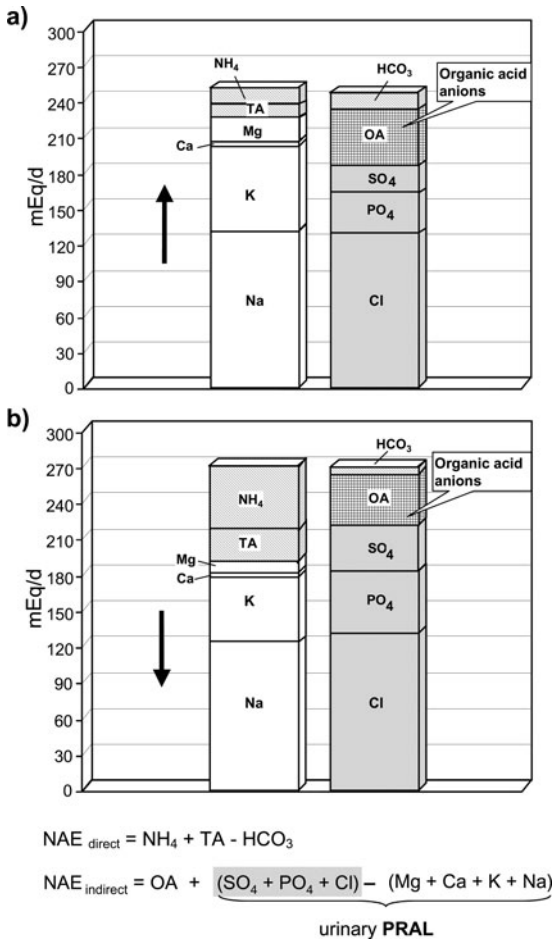

Figure 1 Urine ionogram (showing all quantitatively important urinary anions and cations) of a healthy subject, consuming (a) a fruit and vegetable-rich diet with greater sum of mineral cations (↑) than mineral anions or (b) a protein-rich diet with lower sum of mineral cations (↓) than mineral anions. Directly analyzed NAE $_{direct}$ = ammonium (NH_4) + titratable acid (TA) – bicarbonate (HCO_3); indirectly determined NAE $_{indirect}$ = (sulfate + phosphate + chloride) – (magnesium + calcium + potassium + sodium) + organic acids. Urinary potential renal acid load (PRAL) = NAE $_{indirect}$ – organic acids.

DIETARY PROTEIN'S INFLUENCE ON INTESTINAL CALCIUM ABSORPTION—ROLE OF OXALATE AND FIBER INTAKE

Increases in dietary protein intake in humans lead to elevations in urinary calcium excretion (Cao and Nielsen, 2010). Although there is a general agreement that protein-induced hypercalciuria is explained partly by an increased glomerular filtration rate (GFR) and a decreased renal calcium reabsorption (Schuette et al., 1980; Kerstetter et al., 1998), Kerstetter et al. have suggested that a better intestinal calcium absorption and retention during high-protein diets may add to the rise in urinary calcium excretion (Kerstetter et al., 2005). This notion of a beneficial effect of protein on intestinal calcium absorption can now be read in various current articles focusing on dietary protein and bone health, for example (Cao and Nielsen, 2010;

Thorpe and Evans, 2011). However, the majority of previous balance studies in this respect [summarized by Kerstetter et al. (2003)] as well as several isotopic studies [for references, see (Kerstetter et al., 2005)] did not find a positive relationship between protein intake and calcium absorption in the gut, indicating that the latter is not the primary cause of elevated urinary calcium losses. Also Ceglia et al. (2009), who examined particularly a high and a low protein intake level in older subjects, did not observe differences in fractional calcium absorption. It has recently been shown that caseinophosphopeptides, speculated to be involved in a possible mechanism of enhanced intestinal calcium absorption, do not raise the bioavailability of calcium (Teucher et al., 2006). In the diet experiments of Kerstetter et al. (2005) mentioned above, very similar diet compositions were used as in the intervention and control periods of a recently published study by Cao et al. (2011). When reevaluating the intervention study of Kerstetter et al. (2005) in the light of recent findings of Cao et al., a direct effect of higher protein intake on calcium absorption must be queried. Indeed, in one of these studies premenopausal (Kerstetter et al., 2005) and in the other postmenopausal (Cao et al., 2011) participants were examined; however, the corresponding difference in hormone status of the subjects should not relevantly affect protein's possible influence on intestinal calcium absorption.

In the randomized crossover study, Cao et al. examined postmenopausal women on two diets: one (LPLP) with a low protein intake and a low PRAL and one (HPHP) with high protein intake and higher PRAL. The authors found that high protein intake and higher PRAL led to increases in circulating insulin-like growth factor-1 (IGF-1) and renal calcium loss. They also observed that the latter was partially compensated by an increased fractional absorption of dietary calcium. Since no change in biomarkers of bone resorption or formation was seen, the authors concluded that a high-protein diet has no adverse effects on bone health, which is in line with most of the studies of the aforementioned systematic review by Darling et al. (2009). In addition, the well-performed and instructive feeding study of Cao et al. shed light on another important mechanism regarding calcium absorption and metabolism with high fruit and vegetable intake (as provided by LPLP), compared with a reduced fruit and vegetable intake (HPHP). The interventional HPHP diet yielded a lower total fiber content and a somewhat higher amount of phytate (due to the additional use of grain products) compared with LPLP, which all in all probably balanced out effects on calcium absorption. However, due to the higher fruit and vegetable intake, urinary 24-hour oxalate excretion was significantly elevated with LPLP. The underlying difference of oxalate (and fiber) intake can explain in a large part why calcium absorption was increased with low-fruit HPHP diet, since absorption losses via intestinal calcium oxalate formation (and binding to additional fiber components, e.g., cellulose) were probably reduced on HPHP. The higher calcium absorption loss with the high-fruit LPLP diet obviously led to a reduction in circulating ionic calcium for which a weak trend of reduced levels ($p = 0.11$) was still discernible in the feeding study, even after the compensatory increase in serum parathyroid hormone (PTH) had occurred (Cao et al., 2011). This effect of high-fruit (vegetable) diets, involving increases in PTH and thereby explaining a part of the observed elevation in renal calcium reabsorption with the LPLP diet, mirrors an important nutritional influence of fiber- and oxalic acid-rich plant foods on the "intestinal and renal PTH-calcium-balance axis." A similar metabolic constellation had already been reported for patients with jejunoileal bypass who showed a calcium malabsorption with consecutive intestinal oxalate hyperabsorption and hyperoxaluria and with (calcium deficit-related) increases in PTH and renal calcium reabsorption (Lindsjo, 1989).

Accordingly, an important point to be made is that it is not necessarily the higher protein intake (of higher PRAL diets) that improves calcium absorption as is commonly argued (Cao and Nielsen, 2010; Kerstetter et al., 2011). Instead, it is probably the lower fiber and oxalate content of most high-protein diets that eventually results in increased fractional calcium absorption.

NUTRITIONAL ACID LOAD: IMPACT ON CALCIUM BALANCE?

More than a decade ago, Sebastian et al. (1994) reported that the subchronic administration of potassium bicarbonate for 18 days, which markedly reduced NAE, increased calcium balance in postmenopausal women. Since then a number of researchers have suggested that high renal calcium losses, not compensated by intestinal calcium absorption, may be responsible for the potential bone-catabolic effects of high NEAP diets. In a recent meta-analysis involving only superior quality calcium balance studies, clear negative effects of nutritionally induced elevations in NAE on calcium retention could not be confirmed (Fenton et al., 2009). Although small chronic calcium losses with higher NAEs—accumulating over the years—may have escaped detection of the balance measurement techniques used, it can be concluded that a clear negative calcium balance does not primarily underlie the potentially deleterious long-term skeletal influences of diet-related acid production in healthy individuals. Accordingly, the frequently proposed assumption that the dietary acid load-induced hypercalciuria promotes in the first instance a negative calcium balance with a loss of bone mineral content (Marangella et al., 2004), is no longer a reasonable explanation for dietary acidity-related bone effects. From studies on clinical metabolic acidosis, it can be deduced that cellular and systemic endocrine changes (i.e., changes of the inner milieu) could be the relevant factors causally involved in the process of acidity-induced bone loss. However, it should be kept in mind that a specific effect of higher H^+ loads on renal calcium reabsorption exists, independent of the protein-associated partial increase in urinary calcium loss that is basically due to the GFR rise by protein ingestion. Marked increases in urinary calcium excretion have been observed after ammonium chloride (Bothor et al., 1989; Osther et al., 1994) and methionine (Remer, 2000) induced

acidification under otherwise constant nutrition (including constant protein intake). Although the exact tubular mechanism of the particular acid load-driven renal calcium loss has only been partly clarified (Yeh et al., 2006; Bushinsky, 2010), it can be assumed that a higher dietary acidity in the long-term may contribute to a negative calcium balance, e.g., discernible in bone mineral reductions (Alexy et al., 2005; Remer et al., 2011a).

NUTRITIONAL ACID LOAD AND METABOLIC ACIDOSIS: IMPACT ON SYSTEMIC H+ CONCENTRATION AND ENDOCRINE METABOLIC MILIEU

Clinical Metabolic Acidosis

Marked bone loss is known to occur during clinical metabolic acidosis (Mitch, 2006; Pereverzev et al., 2008) characterized by circulating bicarbonate reductions and blood pH levels below 7.35 (Louden et al., 1999). Various cellular and systemic endocrine changes due to the decreases in blood pH and circulating bicarbonate buffer have been shown likely to contribute to this bone catabolism. As a direct effect on cell function, extracellular acidification dramatically enhances osteoclastic bone resorption and osteoclast survival. The intracellular signaling leading to stimulated resorption by osteoclasts in an acidified milieu shares similarities with that of the proresorptive cytokine receptor activator of NF-kappaB ligand (RANKL) (Komarova et al., 2005).

Metabolic acidosis has direct effects on the growth hormone (GH)/IGF-1 endocrine axis (Goldberg et al., 2006). As in animals (Challa et al., 1993), the serum IGF-1 levels are decreased in response to metabolic acidosis also in humans (Brungger et al., 1997). In line herewith, higher IGF-1 serum concentrations have been reported in nonacidotic adults ingesting an alkalizing supplement in comparison with age- and nutritionally matched controls without supplementation (Ceglia et al., 2009). However, other studies failed to show this (Maurer et al., 2003). In children with renal-tubular acidosis, the acidosis-induced growth retardation has been shown to be reversible upon alkali administration (McSherry and Morris, 1978). Especially, the peripheral sensitivity of IGF-1 secretion to GH action (Brungger et al., 1997) and hepatic GH receptor expression (Kuemmerle et al., 1997) appear to be diminished during metabolic acidosis. In this condition, circulating GH levels may be elevated due to a reduced IGF-1 feedback signal within the GH-axis (Wiederkehr and Krapf, 2001; Wiederkehr et al., 2004). Several mineralization enhancing effects on bone cells have been reported for IGF-1 (Yakar et al., 2009).

Metabolic acidosis also increases glucocorticoid secretion, as has been demonstrated by elevated urinary 24-hour cortisol and tetrahydrocortisol excretion rates in healthy men ingesting NH_4Cl (Sicuro et al., 1998). Elevated glucocorticoids decrease osteoblast function and number and increase bone resorption by stimulating osteoclastogenesis (via increasing RANKL expression) and cartilage-degrading collagenase activity (Canalis and Delany, 2002). These changes in the endocrine-metabolic milieu are believed to be relevantly involved in the bone loss of manifest metabolic acidosis.

Moderately Elevated Systemic Proton Concentration

Potential bone loss by acid-producing diets as well as the underlying mechanisms is definitely more subtle than in clinical metabolic acidosis. But also subtle alterations (acting in the long run) require measurable changes in the inner milieu, i.e., in blood pH and/or circulating bicarbonate levels. Corresponding small, but significant blood pH changes (usually within the physiological range) have been reported after administration of alkalizing mineral supplements (Ball et al., 1996; Konig et al., 2009) and after dietary interventions (Giannini et al., 1999; Buclin et al., 2001). However, such changes in systemic acid–base status are frequently not discernible, especially if fasting blood samples are collected >10–12 hours after the last intervention-related supplement or diet ingestion. Significant relationships have been demonstrated between varying 24-hour NAE levels (with different diets) and plasma hydrogen ion concentrations (Kurtz et al., 1983; Frassetto et al., 2007b) showing that total daily acid load correlates with systemic proton concentrations. A substantially high sodium chloride ingestion (clearly exceeding average dietary salt intake) can also induce a moderate metabolic acidosis (Frassetto et al., 2007b) independent of the underlying dietary acid load. Conditions with such moderately elevated systemic proton concentration (values still within the normal range) are frequently termed subclinical, low-grade, or subtle metabolic acidosis (Arnett, 2003; Frassetto et al., 2007b).

Subtle Metabolic Acidosis

The endocrine-metabolic effects of a clinical metabolic acidosis on bone status do not abruptly stop at the low end of the normal range of blood pH and/or bicarbonate concentration. Rather, these influences operate over a broader range of free proton and bicarbonate buffer variation. It can thus be assumed that also milder reductions in blood pH and circulating buffer capacity, as inducible by diet modification, impair the inner (endocrine) milieu and hence contribute to a less favorable bone metabolism with consequences in the long run. Accordingly, alkalization of normal subjects on a usual western diet has been demonstrated to lead to a significant reduction in cortisol secretion (Maurer et al., 2003). An association between 24-hour urinary glucocorticoid metabolite and net acid excretion rates has also been observed in healthy adult females (Remer et al., 2008).

However, direct in vivo evidence for low-grade acidosis-induced negative endocrine-metabolic impacts on bone mass and size is scarce. Only a few intervention studies performed in special population groups (without particular

endocrine-metabolic focus) reported bone catabolic effects due to an acidogenic diet regimen (Bergqvist et al., 2008) or bone-anabolic effects after ingestion of an alkalizing potassium salt (Jehle et al., 2006). In an intervention study in healthy postmenopausal women, Macdonald et al. (2008) observed no such positive skeletal influences of potassium citrate supplementation or increased fruit and vegetable intake.

Yet, some sophisticated bone culture experiments have yielded interesting results with regard to the bone tissue's response to metabolically realistic changes in its microenvironment. These studies broadly mimic in vivo conditions of subtle metabolic acidosis. One study demonstrated a doubling of bone resorption pits with modest interstitial pH reductions of < 0.05 units (Arnett, 2003) [as attainable by nutrition (Frassetto et al., 2007b)]. Another reported a higher bone volume density (determined by microcomputed tomography) with 24 mM of extracellular bicarbonate concentration than with 12 mM in the culture medium (Geng et al., 2009). In this context, it is important to consider that the buffer capacity of the interstitium is lower than that of plasma, so that a given amount of acid will affect the interstitial pH and bicarbonate level (i.e., the osteoblasts', osteoclasts', and osteocyte's microenvironment) to a greater extent than the blood's proton and buffer level (Street et al., 2005). The above is in contrast to the recent suggestions that dietary induced shifts in systemic pH may not be large enough to impair bone function (Fenton et al., 2011; Kerstetter et al., 2011). At present, it is not possible to clearly state what the physiological interstitial pH and bicarbonate range of bone tissue is, but due to the lower buffering compared with the intravasal system, it is very probably below 7.4 and 24 mM, respectively. Accordingly, it cannot be argued that the cited in vitro bone experiments are physiologically implausible (Fenton et al., 2011).

Altogether, a positive calcium balance due to an alkalization-dependent reduction in renal calcium loss is obviously not the principal mover of an enhanced bone health in healthy subjects. The endocrine-metabolic improvement of the bone cells' microenvironment (with an altered interstitial proton concentration and buffering) appears to be the primary mechanism responsible for the long-term bone-anabolic influence of diets with a high alkalizing potential. Even nutritionally induced modest increases in skeletal interstitial buffering, if regularly achieved (particularly at habitually higher dietary protein intakes), may move bone anabolism toward an optimum with positive preventive medical consequences.

INTERRELATED EFFECTS OF PROTEIN INTAKE AND ACID LOAD ON BONE: REQUIREMENT FOR A COMBINED DIETARY EVALUATION

Evidence has strongly grown that a higher protein intake can strengthen bone health. In fact, a few studies found no bone–diet protein associations (Nieves et al., 1995) and some even reported adverse associations of bone parameters with total protein (Metz et al., 1993; Kim et al., 2008) or animal protein (Dargent-Molina et al., 2008) intake. However, the recent systematic review of Darling et al. (2009)—including 28 eligible studies—confirmed a small beneficial overall association between total protein intake and BMD and BMC. The underlying mechanism of this protein-induced bone anabolism is believed to act primarily via an increase in circulating IGF-1 concentration, a peptide hormone synthesized in the liver as well as locally in all target tissues including bone. Increasing protein intake increases blood level of IGF-1, which constitutes a major determinant of bone growth and mineral acquisition (Yakar et al., 2002). IGF-1 may also improve muscularity, in turn improving bone strength. In addition, elevations in protein intake stimulate glomerular filtration rate and hereby raise renal net acid excretion capacity (Remer and Manz, 1995a; Jehle et al., 2000). This capacity increase relevantly encourages the kidney's elimination of surplus protons and hence attenuates potentially bone-catabolic effects of a higher acid production. Accordingly, if endogenous net acid production is only moderately high for a given high protein intake, the bone-anabolic protein effects will prevail. This has obviously been the case in the diet study of Cao et al. (2011) (already described above), in which apart from the high protein ingestion with HPHP, the potassium intake was also relatively high, whereas the phosphorus intake was rather low. At a protein intake of 120 g/d with HPHP (Cao et al., 2011), the respective NAE and PRAL amounted to only about 60 and 30 mE/d, respectively, and that corresponded to NAE and PRAL levels of an average US diet with a clearly lower protein intake of about 80 g/d (Remer and Manz, 2003). In a previous diet experiment using natural foods, we measured a clearly higher NAE of 121.6 mEq/d and calculated a PRAL of 72.5 mEq/d with a protein intake of 120 g per day (Remer and Manz, 1994).

The results of the study of Cao and colleagues strongly support the notion that if the functional improvement of renal processes (increased glomerular filtration rate associated with higher acid excretion capacity) through high protein intake is accompanied by an appropriate—but not necessarily very strong—alkalization (e.g., higher potassium, lower phosphorus intake, reduced PRAL), then no adverse acute or subacute effects on bone metabolism must be feared.

Thus, it has to be emphasized that—depending on the food choices—a high protein intake does not necessarily imply a concurrent high dietary acid load. In case of only a moderately high acid load for a given high (in principle bone-anabolic) protein intake, e.g., at a protein intake of 100–120 g/d and an NAE level <70 mEq/d (PRAL ≤ 30) (Cao et al., 2011), the interventional alkalization with fruit and vegetables along with a concurrent protein reduction (diet LPLP) cannot be expected to provide further clearly discernible bone-metabolic benefits. From studies like that (Cao et al., 2011) it cannot be concluded that high protein intakes have invariably no adverse effects on bone health, because respective results of specific controlled studies with both high protein intake (>100 g/d) and high NAE (>100 mEq/d), i.e., without an at least partially neutralized acid load, are lacking.

On the other hand, if the study focus is particularly on the dietary acid load's influence on bone health, the absolute NAE (or PRAL) level alone is not determining, but the respective NEAP level for the given protein intake is relevant. Hence, in epidemiological studies the protein intake has to be adjusted. The higher the NAE for a given (bone-anabolic) protein intake in subjects with normal kidney function, the bigger the chance that the protein anabolism will be leveled out or that the catabolic influence of net endogenous acid production may prevail [possibly in elderly with reduced acid excretion capacity (Berkemeyer et al., 2008)]. To our knowledge, corresponding prospective and mutually dependant results have hitherto been only reported for healthy children (Alexy et al., 2005).

Unfortunately, the interrelated dependence of the skeletal system on both protein intake and dietary acid load is frequently disregarded and that might be one reason why in several cross-sectional surveys and randomized placebo-controlled trials no significant association between protein intake and bone parameters has been observed. It might also explain why in an additional meta-analysis of Darling et al (2009), no association of higher dietary protein with lower relative risk of hip fractures could be detected. At present, it can only be speculated whether a long-term preventive role of the combination of net alkali producing diets and appropriately high protein intakes exists with regard to certain fracture risks.

All in all, it should not be ignored that for bone outcomes, confounding effects of protein intake may be present if dietary acid loads (NEAP) are the predictors of interest and vice versa. The nonconsideration of one may result in a less clear (or non-) detection of the influence of the other and may partly explain inconsistencies of study results like those recently reported from the Framingham Osteoporosis Study (McLean et al., 2011). The problem that a negative association with acid load may be masked by a positive association with protein (and vice versa) has been recognized and is currently under discussion (Jesudason and Clifton, 2011; McLean et al., 2011). This underlines the necessity for the use of suitable assessment methods, specific enough to separate moderate nutritional anabolic and catabolic effects.

METHODOLOGICAL ISSUES IN STUDYING DIETARY INFLUENCES ON BONE

In a longitudinal examination in healthy children and adolescents, we previously observed both positive associations of protein intake and negative associations of dietary PRAL with diaphyseal bone parameters (Alexy et al., 2005). The use of repeated three-day weighed dietary records and of peripheral quantitative computed tomography (pQCT) for bone analysis were the strengths of our study, the results of which have now been confirmed by expanded 24-hour urinary biomarker measurements (Remer et al., 2011a). The weighed diet record that we utilized is considered to be one of the most reliable dietary assessment tools available, and pQCT represents a method for bone analysis that can quantify not only mass but also structural bone characteristics.

Bone Measurement

As useful as dual-energy X-ray absorptiometry (DEXA) analysis has become in the assessment of body composition and overall bone status, it does have notable limitations. Densitometry by DEXA yields a two-dimensional projection of the bone, like a shadow image (Rauch and Schoenau, 2005; de Bono et al., 2010). Thus, no true volumetric BMD, but a so-called areal density is obtained (de Bono et al., 2010). This is strongly size dependent and does not separate cortical and trabecular bone. BMD assessed by DEXA rather integrates bone properties like true volumetric density and bone thickness into a single number (Rauch et al., 2002), not allowing specific determination of bone geometry, volumetric density, or microarchitecture. This relative nonspecificity of areal BMD could be one reason for inconsistent results of different DEXA studies. In addition, DEXA is very technician dependent, i.e., if a repeat scan is not done in exactly the same position as the first scan, the results may substantially vary. Volumetric BMD itself is no good indicator of bone strength (Rauch and Schoenau, 2008; Wang et al., 2009). Cross-sectional area, periosteal circumference, or strength strain index better reflect long bone's bone strength (Rauch and Schoenau, 2008; de Bono et al., 2010). BMD of long bones can physiologically decrease if cross-sectional area increases along with a normal or even improved bending strength (Wang et al., 2009). Hence, it cannot be excluded that the non-response observed by Macdonald et al. (2008) for DEXA-BMD measurements in postmenopausal women, supplemented with potassium citrate (or ingesting increased amounts of fruits and vegetables), might not reflect an ineffectiveness of the alkali provision but could be the result of a densitometric technique that did not analyze appropriately enough. In line herewith, it has been criticized that DEXA can miss much of the changes in bone strength induced by potent antiresorptive therapy with the bisphosphonate alendroate or the strongly bone-anabolic hormone PTH (Keaveny et al., 2008). Due to the fact that higher BMDs (by standard DEXA) lack architectural bone specificity and accordingly can even be associated with a higher fracture incidence, e.g., in patients with type 2 diabetes (Burghardt et al., 2010), the future use of more specific high-resolution imaging techniques appears necessary to understand the physiological architectural responses of bone to various (nutritional) treatments. With such measurements, it should be more likely possible to identify whether a substantial increase or decrease in dietary PRAL (for a given protein intake) may have a significant bone strength-reducing or improving effect. It cannot be excluded that such future studies will perhaps confirm what the recent DEXA findings of the randomized controlled trial in postmenopausal women of Mcdonald et al (2008) have suggested that potassium citrate supplementation or increased fruit and vegetable intake in healthy subjects, already ingesting a low-PRAL diet of about

0 mEq/d at baseline, has no additional long-term benefit on bone.

Dietary Assessment

Limitations of bone analyses are not the only contributions to conflicting results in acid base studies on bone health. Dietary assessment methods can be too inaccurate for an appropriate differentiation between different nutrient effects. Compared with the more detailed dietary recording by a seven-day diet diary, higher intercorrelations of estimated nutrient intakes occur (including of those most relevant for acid–base calculations) if nutrition assessment is based on food frequencies questionnaire (Day et al., 2001). These higher correlations still prevail after correction for energy intake (Michels et al., 2004) and are probably due to more correlated errors with the use of food frequencies questionnaires (Michels et al., 2004). PRAL-values obtained with a diet history questionnaire showed only modest agreement with a 16-day-weighed dietary record (Murakami et al., 2008).

In line with these findings, a single 24-hour-dietary recall is not only too imprecise to estimate daily salt intake [considered as additional contributor to metabolic acidosis (Frassetto et al., 2007b) and important determinant of urinary calcium excretion] but obviously also too imprecise to identify a direct protein-anabolic influence on bone parameters (Thorpe et al., 2008). In the latter study, the positive association of lumbar spine BMD with dietary protein could only be observed after adjustment for the estimated amounts of sulfur derived from the recorded protein intake. The authors concluded that the higher sulfur originating from certain proteins may particularly suppress bone anabolism. Although this is probably partly true, it should be kept in mind that those animal protein sources with higher methionine and cysteine content like milk, cheese, and meat also show the strongest (bone-anabolic) IGF-1-increases. Accordingly, the detrimental impact of metabolically generated sulfate from methionine- and cysteine-rich protein is in part compensated by the stimulating effect on IGF-1. The other relevant nutrient is potassium and to a lesser extent also magnesium, calcium, and phosphorus. If both protein and potassium intake data are obtained only by a lower quality assessment method [with higher intercorrelation between the recorded nutrients and presumably higher correlated measurement errors (Day et al., 2001)], then their difference (considerably determining the acid load) cannot be expected to become reliably calculated. It is intelligible that the most reasonable estimate of a dietary acid load will be obtained if the assessment method not only estimates protein and potassium but also the other acid–base nutrients (especially phosphorus, magnesium, calcium) with an acceptable accuracy. Here, appropriate biomarker measurements, e.g, of NAE or urinary PRAL would be particularly helpful. However, such biomarker measurements have limitations as well. As a minimum prerequisite they require 24-hour urine collections and ideally repeat measurements on the same individuals. Spot urine samples or short-term second void urines collected for a few hours under fasting conditions (Fenton et al., 2010) do in no way allow assessment of diet-dependent acid loads.

CONCLUDING REMARKS

Raising protein intake appears to be one of the strongest dietary bone-anabolic measures in children and adults who have adequate micronutrient nutrition, provided the concurrent ingestion of potential base-rich foods, i.e., fruits and vegetables, is appropriate to neutralize acid equivalents generated through hydrolysis of phosphoproteins and degradation of sulfur-containing amino acids. The underlying cause for this protein effect is not an ameliorated calcium absorption but probably a higher IGF-1 activity after protein ingestion. An improvement of the endocrine metabolic milieu with enhanced GH and IGF-1 signaling may also play a role when the bone cells' microenvironment is modestly, yet habitually influenced by alkalizing low PRAL (not necessarily low protein) diets. In healthy subjects, calcium balance is not relevantly affected by higher or lower dietary acid loads. The clear interdependency between protein intake and endogenous acid production strongly suggests that studies on protein or PRAL effects do require a combined assessment of both dietary changes. Unfortunately, most observational studies have so far analyzed protein intake- and dietary acidity-related exposures separately, which might explain (apart from methodological issues) some of the observed inconsistent results. Despite this, in three of the four hitherto published prospective observational studies in adults (epidemiological evidence level II) on dietary acid load exposures and bone densitometric outcomes, positive trends have been observed with regard to an alkalizing nutrition (Table 1). This appears remarkable, although it has been criticized that some of these findings could have been confounded by unconsidered osteoporosis risk factors (Fenton et al., 2011).

Of the two randomized controlled trials that used changes in BMD as the outcome measure (Jehle et al., 2006; Macdonald et al., 2008), only one found significant increases in BMD in osteopenic but otherwise healthy postmenopausal women (Jehle et al., 2006). For reasons not clearly understandable, this latter trial has been downgraded as lower quality study in a recently published review and assessment article on dietary acid load and bone disease (Fenton et al., 2011). However, a particular strength of the trial with osteopenic females was that it specifically examined the effect of endogenous alkali production by controlling for the administered amount of potassium (potassium citrate vs. potassium chloride). In the aforementioned review paper, the authors included publications with various differing outcomes and various differing exposures for their assessment of randomized intervention studies and prospective observational studies, respectively. This, however, yielded a rather biased picture of the available data. Taken together, future studies using improved dietary assessment and bone measurement techniques may probably bring out more clearly the potential of alkalinizing dietary

measures for the strengthening of bone at given levels of protein intake in eligible population groups.

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ABBREVIATIONS

BMC	Bone mineral content
BMD	Bone mineral density
DEXA	Dual-energy X-ray absorptiometry
GFR	Glomerular filtration rate
GH	Growth hormone
HPHP	High protein-higher PRAL
IGF-1	Insulin-like growth factor-1
LPLP	Low protein-low PRAL
NAE	Net acid excretion
NEAP	Net endogenous acid production
pQCT	Peripheral quantitative computed tomography
PRAL	Potential renal acid load
PTH	Parathyroid hormone
RANKL	Receptor activator of NF-kappaB ligand

REFERENCES

Alexy, U., Remer, T., Manz, F., Neu, C. M. and Schoenau, E. (2005). Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am. J. Clin. Nutr.* **82**(5):1107–1114.

Arnett, T. (2003). Regulation of bone cell function by acid-base balance. *Proc. Nutr. Soc*. **62**(2):511–520.

Ball, D., Greenhaff, P. L. and Maughan, R. J. (1996). The acute reversal of a diet-induced metabolic acidosis does not restore endurance capacity during high-intensity exercise in man. *Eur. J. Appl. Physiol. Occup. Physiol*. **73**(1–2):105–112.

Bergqvist, A. G., Schall, J. I., Stallings, V. A. and Zemel, B. S. (2008). Progressive bone mineral content loss in children with intractable epilepsy treated with the ketogenic diet. *Am. J. Clin. Nutr.* **88**(6):1678–1684.

Berkemeyer, S., Vormann, J., Gunther, A. L., Rylander, R., Frassetto, L. A. and Remer, T. (2008). Renal net acid excretion capacity is comparable in prepubescence, adolescence, and young adulthood but falls with aging. *J. Am. Geriatr. Soc*. **56**(8):1442–1448.

Bothor, C., Berg, W., Donner, M. and Pirlich, W. (1989). Calcium and citrate excretion by patients with calculi and healthy probands during induced acidosis. *Urologe. A*. **28**(2):112–115.

Brungger, M., Hulter, H. N. and Krapf, R. (1997). Effect of chronic metabolic acidosis on the growth hormone/IGF-1 endocrine axis: New cause of growth hormone insensitivity in humans. *Kidney Int*. **51**(1):216–221.

Buclin, T., Cosma, M., Appenzeller, M., Jacquet, A. F., Decosterd, L. A., Biollaz, J. and Burckhardt, P. (2001). Diet acids and alkalis influence calcium retention in bone. *Osteoporos Int*. **12**(6):493–499.

Burghardt, A. J., Issever, A. S., Schwartz, A. V., Davis, K. A., Masharani, U., Majumdar, S. and Link, T. M. (2010). High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* **95**(11):5045–5055.

Bushinsky, D. A. (2010). Contribution of intestine, bone, kidney, and dialysis to extracellular fluid calcium content. *Clin. J. Am. Soc. Nephrol*. **5**(Suppl 1):S12–S22.

Canalis, E. and Delany, A. M. (2002). Mechanisms of glucocorticoid action in bone. *Ann. NY Acad. Sci*. **966**:73–81.

Cao, J. J., Johnson, L. K. and Hunt, J. R. (2011). A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. *J. Nutr.* **141**(3):391–397.

Cao, J. J. and Nielsen, F. H. (2010). Acid diet (high-meat protein) effects on calcium metabolism and bone health. *Curr. Opin. Clin. Nutr. Metab. Care*. **13**(6):698–702.

Ceglia, L., Harris, S. S., Abrams, S. A., Rasmussen, H. M., Dallal, G. E. and Dawson-Hughes, B. (2009). Potassium bicarbonate attenuates the urinary nitrogen excretion that accompanies an increase in dietary protein and may promote calcium absorption. *J. Clin. Endocrinol. Metab.* **94**(2): 645–653.

Challa, A., Chan, W., Krieg, R. J. Jr., Thabet, M. A., Liu, F., Hintz, R. L. and Chan, J. C. (1993). Effect of metabolic acidosis on the expression of insulin-like growth factor and growth hormone receptor. *Kidney Int*. **44**(6):1224–1227.

Cloutier, G. R. and Barr, S. I. (2003). Protein and bone health: Literature review and counselling implications. *Can. J. Diet. Pract. Res*. **64**(1):5–11.

Dargent-Molina, P., Sabia, S., Touvier, M., Kesse, E., Breart, G., Clavel-Chapelon, F. and Boutron-Ruault, M. C. (2008). Proteins, dietary acid load, and calcium and risk of postmenopausal fractures in the E3N French women prospective study. *J. Bone. Miner. Res*. **23**(12):1915–1922.

Darling, A. L., Millward, D. J., Torgerson, D. J., Hewitt, C. E. and Lanham-New, S. A. (2009). Dietary protein and bone health: A systematic review and meta-analysis. *Am. J. Clin. Nutr.* **90**(6):1674–1692.

Day, N., McKeown, N., Wong, M., Welch, A. and Bingham, S. (2001). Epidemiological assessment of diet: A comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. *Int. J. Epidemiol*. **30**(2):309–317.

de Bono, S., Schoenmakers, I., Ceesay, M., Mendy, M., Laskey, M. A., Cole, T. J. and Prentice, A. (2010). Birth weight predicts bone size in young adulthood at cortical sites in men and trabecular sites in women from The Gambia. *Bone*. **46**(5):1316–1321.

Fenton, T. R., Eliasziw, M., Tough, S. C., Lyon, A. W., Brown, J. P. and Hanley, D. A. (2010). Low urine pH and acid excretion do not predict bone fractures or the loss of bone mineral density: A prospective cohort study. *BMC Musculoskelet Disord*. **11**:88.

Fenton, T. R., Lyon, A. W., Eliasziw, M., Tough, S. C. and Hanley, D. A. (2009). Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance. *J. Bone. Miner. Res*. **24**(11):1835–1840.

Fenton, T. R., Tough, S. C., Lyon, A. W., Eliasziw, M. and Hanley, D. A. (2011). Causal assessment of dietary acid load and bone disease: A systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. *Nutr. J*. **10**:41.

Frassetto, L. A., Lanham-New, S. A., Macdonald, H. M., Remer, T., Sebastian, A., Tucker, K. L. and Tylavsky, F. A. (2007a). Standardizing terminology for estimating the diet-dependent net acid load to the metabolic system. *J. Nutr.* **137**(6):1491–1492.

Frassetto, L. A., Morris, R. C. Jr. and Sebastian, A. (2007b). Dietary sodium chloride intake independently predicts the degree of hyperchloremic metabolic acidosis in healthy humans consuming a net acid-producing diet. *Am. J. Physiol. Renal. Physiol*. **293**(2):F521–525.

Frassetto, L. A., Todd, K. M., Morris, R. C. Jr. and Sebastian, A. (1998). Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am. J. Clin. Nutr.* **68**(3):576–583.

Geng, W., Hill, K., Zerwekh, J. E., Kohler, T., Muller, R. and Moe, O. W. (2009). Inhibition of osteoclast formation and function by bicarbonate: Role of soluble adenylyl cyclase. *J. Cell Physiol*. **220**(2):332–340.

Giannini, S., Nobile, M., Sartori, L., Dalle Carbonare, L., Ciuffreda, M., Corro, P., D'Angelo, A., Calo, L. and Crepaldi, G. (1999). Acute effects of moderate dietary protein restriction in patients with idiopathic hypercalciuria and calcium nephrolithiasis. *Am. J. Clin. Nutr.* **69**(2):267–271.

Goldberg, R., Reshef-Bankai, E., Coleman, R., Green, J. and Maor, G. (2006). Chronic acidosis-induced growth retardation is mediated by proton-induced expression of Gs protein. *J. Bone. Miner. Res.* **21**(5):703–713.

Jehle, S., Hulter, H. N. and Krapf, R. (2000). On the mechanism of growth hormone-induced stimulation of renal acidification in humans: Effect of dietary NaCl. *Clin. Sci. (Lond).* **99**(1):47–56.

Jehle, S., Zanetti, A., Muser, J., Hulter, H. N. and Krapf, R. (2006). Partial neutralization of the acidogenic Western diet with potassium citrate increases bone mass in postmenopausal women with osteopenia. *J. Am. Soc. Nephrol.* **17**(11):3213–3222.

Jesudason, D. and Clifton, P. (2011). The interaction between dietary protein and bone health. *J. Bone Miner. Metab.* **29**(1):1–14.

Kaptoge, S., Welch, A., McTaggart, A., Mulligan, A., Dalzell, N., Day, N. E., Bingham, S., Khaw, K. T. and Reeve, J. (2003). Effects of dietary nutrients and food groups on bone loss from the proximal femur in men and women in the 7th and 8th decades of age. *Osteoporos Int.* **14**(5):418–428.

Keaveny, T. M., Hoffmann, P. F., Singh, M., Palermo, L., Bilezikian, J. P., Greenspan, S. L. and Black, D. M. (2008). Femoral bone strength and its relation to cortical and trabecular changes after treatment with PTH, alendronate, and their combination as assessed by finite element analysis of quantitative CT scans. *J. Bone Miner. Res.* **23**(12):1974–1982.

Kerstetter, J. E., Kenny, A. M. and Insogna, K. L. (2011). Dietary protein and skeletal health: A review of recent human research. *Curr. Opin. Lipidol.* **22**(1):16–20.

Kerstetter, J. E., O'Brien, K. O., Caseria, D. M., Wall, D. E. and Insogna, K. L. (2005). The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *J. Clin. Endocrinol. Metab.* **90**(1):26–31.

Kerstetter, J. E., O'Brien, K. O. and Insogna, K. L. (1998). Dietary protein affects intestinal calcium absorption. *Am. J. Clin. Nutr.* **68**(4):859–865.

Kerstetter, J. E., O'Brien, K. O. and Insogna, K. L. (2003). Low protein intake: The impact on calcium and bone homeostasis in humans. *J. Nutr.* **133**(3):855S–861S.

Kim, J., Lim, S. Y. and Kim, J. H. (2008). Nutrient intake risk factors of osteoporosis in postmenopausal women. *Asia Pac. J. Clin. Nutr.* **17**(2):270–275.

Komarova, S. V., Pereverzev, A., Shum, J. W., Sims, S. M. and Dixon, S. J. (2005). Convergent signaling by acidosis and receptor activator of NF-kappaB ligand (RANKL) on the calcium/calcineurin/NFAT pathway in osteoclasts. *Proc. Natl. Acad. Sci. USA.* **102**(7):2643–2648.

Konig, D., Muser, K., Dickhuth, H. H., Berg, A. and Deibert, P. (2009). Effect of a supplement rich in alkaline minerals on acid-base balance in humans. *Nutr. J.* **8**:23.

Kuemmerle, N., Krieg, R. J. Jr., LattaK, Challa, A., Hanna, J. D. and Chan, J. C. (1997). Growth hormone and insulin-like growth factor in non-uremic acidosis and uremic acidosis. *Kidney Int. Suppl.* **58**:S102–105.

Kurtz, I., Maher, T., Hulter, H. N., Schambelan, M. and Sebastian, A. (1983). Effect of diet on plasma acid-base composition in normal humans. *Kidney Int.* **24**(5):670–680.

Lindsjo, M. (1989). Oxalate metabolism in renal stone disease with special reference to calcium metabolism and intestinal absorption. *Scand. J. Urol. Nephrol. Suppl.* **119**:1–53.

Louden, J. D., Roberts, R. R. and Goodship, T. H. (1999). Acidosis and nutrition. *Kidney Int. Suppl.* **73**:S85–S88.

Macdonald, H. M., Black, A. J., Aucott, L., Duthie, G., Duthie, S., Sandison, R., Hardcastle, A. C., Lanham New, S. A., Fraser, W. D. and Reid, D. M. (2008). Effect of potassium citrate supplementation or increased fruit and vegetable intake on bone metabolism in healthy postmenopausal women: A randomized controlled trial. *Am. J. Clin. Nutr.* **88**(2):465–474.

Macdonald, H. M., New, S. A., Golden, M. H., Campbell, M. K. and Reid, D. M. (2004). Nutritional associations with bone loss during the menopausal transition: Evidence of a beneficial effect of calcium, alcohol, and fruit and vegetable nutrients and of a detrimental effect of fatty acids. *Am. J. Clin. Nutr.* **79**(1):155–165.

Marangella, M., Di Stefano, M., Casalis, S., Berutti, S., D'Amelio, P. and Isaia, G. C. (2004). Effects of potassium citrate supplementation on bone metabolism. *Calcif. Tissue Int.* **74**(4):330–335.

Maurer, M., Riesen, W., Muser, J., Hulter, H. N. and Krapf, R. (2003). Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. *Am. J. Physiol. Renal. Physiol.* **284**(1):F32–40.

McLean, R. R., Qiao, N., Broe, K. E., Tucker, K. L., Casey, V., Cupples, L. A., Kiel, D. P. and Hannan, M. T. (2011). Dietary acid load is not associated with lower bone mineral density except in older men. *J Nutr. Endocrinol. Metab.* **96**(9):2861–2868.

McSherry, E. and Morris, R. C. Jr. (1978). Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis. *J. Clin. Invest.* **61**(2):509–527.

Metz, J. A., Anderson, J. J. and Gallagher, P. N. (1993). Intakes of calcium, phosphorus, and protein, and physical-activity level are related to radial bone mass in young adult women. *Am. J. Clin. Nutr.* **58**(4):537–542.

Michels, K. B., Bingham, S. A., Luben, R., Welch, A. A. and Day, N. E. (2004). The effect of correlated measurement error in multivariate models of diet. *Am. J. Epidemiol.* **160**(1):59–67.

Mitch, W. E. (2006). Metabolic and clinical consequences of metabolic acidosis. *J. Nephrol.* **19**(Suppl 9):S70–S75.

Murakami, K., Sasaki, S., Takahashi, Y. and Uenishi, K. (2008). Association between dietary acid-base load and cardiometabolic risk factors in young Japanese women. *Br. J. Nutr.* **100**(3):642–651.

Nieves, J. W., Golden, A. L., Siris, E., Kelsey, J. L. and Lindsay, R. (1995). Teenage and current calcium intake are related to bone mineral density of the hip and forearm in women aged 30–39 years. *Am. J. Epidemiol.* **141**(4):342–351.

Osther, P. J., Mathiasen, H., Hansen, A. B. and Nissen, H. M. (1994). Urinary acidification and urinary excretion of calcium and citrate in women with bilateral medullary sponge kidney. *Urol. Int.* **52**(3):126–130.

Pedone, C., Napoli, N., Pozzilli, P., Lauretani, F., Bandinelli, S., Ferrucci, L. and Antonelli-Incalzi, R. (2010). Quality of diet and potential renal acid load as risk factors for reduced bone density in elderly women. *Bone.* **46**(4):1063–1067.

Pedone, C., Napoli, N., Pozzilli, P., Lauretani, F., Bandinelli, S., Ferrucci, L. and Antonelli-Incalzi, R. (2011). Author reply—Quality of diet and potential renal acid load as risk factors for reduced bone density in elderly women. *Bone.* **48**(2):416.

Pereverzev, A., Komarova, S. V., Korcok, J., Armstrong, S., Tremblay, G. B., Dixon, S. J. and Sims, S. M. (2008). Extracellular acidification enhances osteoclast survival through an NFAT-independent, protein kinase C-dependent pathway. *Bone.* **42**(1):150–161.

Pizzorno, J., Frassetto, L. A. and Katzinger, J. (2010). Diet-induced acidosis: Is it real and clinically relevant? *Br. J. Nutr.* **103**(8):1185–1194.

Rauch, F. and Schoenau, E. (2005). Peripheral quantitative computed tomography of the distal radius in young subjects—new reference data and interpretation of results. *J. Musculoskelet. Neuronal. Interact.* **5**(2): 119–126.

Rauch, F. and Schoenau, E. (2008). Peripheral quantitative computed tomography of the proximal radius in young subjects–new reference data and interpretation of results. *J. Musculoskelet. Neuronal. Interact.* **8**(3): 217–226.

Rauch, F., Tutlewski, B. and Schonau, E. (2002). The bone behind a low areal bone mineral density: Peripheral quantitative computed tomographic analysis in a woman with osteogenesis imperfecta. *J. Musculoskelet. Neuronal. Interact.* **2**(4):306–308.

Remer, T. (2000). Influence of diet on acid-base balance. *Semin. Dial.* **13**(4):221–226.

Remer, T., Dimitriou, T. and Manz, F. (2003). Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am. J. Clin. Nutr.* **77**(5):1255–1260.

Remer, T., Dimitriou, T. and Maser-Gluth, C. (2008). Renal net acid excretion and plasma leptin are associated with potentially bioactive free glucocorticoids in healthy lean women. *J. Nutr.* **138**(2):426S–430S.

Remer, T. and Manz, F. (1994). Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am. J. Clin. Nutr.* **59**(6):1356–1361.

Remer, T. and Manz, F. (1995a). Dietary protein as a modulator of the renal net acid excretion capacity: Evidence that an increased protein intake improves the capability of the kidney to excrete ammonium. *Nutr. Biochem.* **6**:431–437.

Remer, T. and Manz, F. (1995b). Potential renal acid load of foods and its influence on urine pH. *J. Am. Diet. Assoc.* **95**(7):791–797.

Remer, T. and Manz, F. (2003). Paleolithic diet, sweet potato eaters, and potential renal acid load. *Am. J. Clin. Nutr.* **78**(4):802–803; author reply 803–804.

Remer, T., Manz, F., Alexy, U., Schoenau, E., Wudy, S. A. and Shi, L. (2011a). Long-term high urinary potential renal acid load and low nitrogen excretion predict reduced diaphyseal bone mass and bone size in children. *J. Clin. Endocrinol. Metab.* **96**(9):2861–2868.

Remer, T., Shi, L. and Alexy, U. (2011b). Potential renal acid load may more strongly affect bone size and mass than volumetric bone mineral density. *Bone.* **48**(2):414–415; author reply 416.

Schuette, S. A., Zemel, M. B. and Linkswiler, H. M. (1980). Studies on the mechanism of protein-induced hypercalciuria in older men and women. *J. Nutr.* **110**(2):305–315.

Sebastian, A., Harris, S. T., Ottaway, J. H., Todd, K. M. and Morris, R. C. Jr. (1994). Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate. *N. Engl. J. Med.* **330**(25):1776–1781.

Sicuro, A., Mahlbacher, K., Hulter, H. N. and Krapf, R. (1998). Effect of growth hormone on renal and systemic acid-base homeostasis in humans. *Am. J. Physiol.* **274**(4 Pt 2):F650–657.

Street, D., Nielsen, J. J., Bangsbo, J. and Juel, C. (2005). Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *J. Physiol.* **566**(Pt 2):481–489.

Teucher, B., Majsak-Newman, G., Dainty, J. R., McDonagh, D., FitzGerald, R. J. and Fairweather-Tait, S. J. (2006). Calcium absorption is not increased by caseinophosphopeptides. *Am. J. Clin. Nutr.* **84**(1):162–166.

Thorpe, M. P. and Evans, E. M. (2011). Dietary protein and bone health: Harmonizing conflicting theories. *Nutr. Rev.* **69**(4):215–230.

Thorpe, M., Mojtahedi, M. C., Chapman-Novakofski, K., McAuley, E. and Evans, E. M. (2008). A positive association of lumbar spine bone mineral density with dietary protein is suppressed by a negative association with protein sulfur. *J. Nutr.* **138**(1):80–85.

Tucker, K. L., Hannan, M. T. and Kiel, D. P. (2001). The acid-base hypothesis: Diet and bone in the Framingham Osteoporosis Study. *Eur. J. Nutr.* **40**(5):231–237.

Wang, Q., Cheng, S., Alen, M. and Seeman, E. (2009). Bone's structural diversity in adult females is established before puberty. *J. Clin. Endocrinol. Metab.* **94**(5):1555–1561.

Wiederkehr, M. R., Kalogiros, J. and Krapf, R. (2004). Correction of metabolic acidosis improves thyroid and growth hormone axes in haemodialysis patients. *Nephrol. Dial. Transplant.* **19**(5):1190–1197.

Wiederkehr, M. and Krapf, R. (2001). Metabolic and endocrine effects of metabolic acidosis in humans. *Swiss. Med. Wkly.* **131**(9–10):127–132.

Yakar, S., Canalis, E., Sun, H., Mejia, W., Kawashima, Y., Nasser, P., Courtland, H. W., Williams, V., Bouxsein, M., Rosen, C. and Jepsen, K. J. (2009). Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. *J. Bone. Miner. Res.* **24**(8):1481–1492.

Yakar, S., Rosen, C. J., Beamer, W. G., Ackert-Bicknell, C. L., Wu, Y., Liu, J. L., Ooi, G. T., Setser, J., Frystyk, J., Boisclair, Y. R. and LeRoith, D. (2002). Circulating levels of IGF-1 directly regulate bone growth and density. *J. Clin. Invest.* **110**(6):771–781.

Yeh, B. I., Yoon, J. and Huang, C. L. (2006). On the role of pore helix in regulation of TRPV5 by extracellular protons. *J. Membr. Biol.* **212**(3):191–198.