ORIGINAL CONTRIBUTION

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Diet, evolution and aging

The pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet

■ **Summary** Theoretically, we humans should be better adapted physiologically to the diet our ancestors were exposed to during millions of years of hominid evolution than to the diet we have been eating since the agricultural revolution a mere 10,000 years ago, and since industrialization only 200 years ago. Among the many health problems resulting from this mismatch between our genetically determined nutritional requirements and our current diet, some might be a consequence in part of the deficiency of potassium alkali salts (K-base), which are amply present in the plant foods that our ancestors ate in abundance, and the exchange of those salts for sodium chloride (NaCl), which has been incorporated copiously into the contemporary diet, which at the same time is meager in K-base-rich plant foods.

Deficiency of K-base in the diet increases the net systemic acid load imposed by the diet. We know that clinically-recognized chronic metabolic acidosis has deleterious ef-

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fects on the body, including growth retardation in children, decreased muscle and bone mass in adults, and kidney stone formation, and that correction of acidosis can ameliorate those conditions. Is it possible that a lifetime of eating diets that deliver evolutionarily superphysiologic loads of acid to the body contribute to the decrease in bone and muscle mass, and growth hormone secretion, which occur normally with age? That is, are contemporary humans suffering from the consequences of chronic, diet-induced low-grade systemic metabolic acidosis?

Our group has shown that contemporary net acid-producing diets do indeed characteristically produce a low-grade systemic metabolic acidosis in otherwise healthy adult subjects, and that the degree of acidosis increases with age, in relation to the normally occurring age-related decline in renal functional capacity. We also found that neutralization of the diet net acid load with dietary supplements of potassium bicarbonate ($KHCO_3$) improved calcium and phosphorus balances, reduced bone resorption rates, improved nitrogen balance, and mitigated the normally occurring age-related decline in growth hormone secretion – all without restricting dietary NaCl. Moreover, we found that co-administration of an alkalinizing

salt of potassium (potassium citrate) with NaCl prevented NaCl from increasing urinary calcium excretion and bone resorption, as occurred with NaCl administration alone.

Earlier studies estimated dietary acid load from the amount of animal protein in the diet, inasmuch as protein metabolism yields sulfuric acid as an end-product. In cross-cultural epidemiologic studies, Abelow [1] found that hip fracture incidence in older women correlated with animal protein intake, and they suggested a causal relation to the acid load from protein. Those studies did not consider the effect of potential sources of base in the diet. We considered that estimating the *net* acid load of the diet (i. e., acid minus base) would require considering also the intake of plant foods, many of which are rich sources of K-base, or more precisely base precursors, substances like organic anions that the body metabolizes to bicarbonate. In following up the findings of Abelow et al., we found that plant food intake tended to be protective against hip fracture, and that hip fracture incidence among countries correlated inversely with the ratio of plant-to-animal food intake. These findings were confirmed in a more homogeneous population of white elderly women residents of the U. S.

These findings support affirma-

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tive answers to the questions we asked above.

Can we provide dietary guidelines for controlling dietary net acid loads to minimize or eliminate diet-induced and age-amplified chronic low-grade metabolic acidosis and its pathophysiological sequelae. We discuss the use of algorithms to predict the diet net acid and provide nutritionists and clinicians with relatively simple and reliable methods for determining and controlling the net acid load of the diet. A more difficult question is what level of acidosis is acceptable. We argue that any level of acidosis may be unacceptable from an evolutionarily perspective, and indeed, that a low-grade metabolic alkalosis may be the optimal acid-base state for humans.

Introduction

The nutritional requirements of humans were established by natural selection during millions of years of in which humans and their hominid ancestors consumed foods exclusively from a menu of wild animals and uncultivated plants [2, 3]. By contrast, the past 10,000 years – less than one percent of hominid evolutionary time – has afforded natural selection insufficient time to generate adaptations and eliminate maladaptations to the profound transformation of the human diet that occurred during that period consequent to the inventions of agriculture and animal husbandry, and more recently, to the development of modern food production and distribution technologies [2–5].

In comparison to the diet habitually ingested by pre-agricultural *Homo sapiens* living in the Upper Paleolithic period (40,000 to 10,000 years ago), also referred to as the Late Stone Age, the diet of contemporary *Homo sapiens* has an overabundance of fat, simple sugars, sodium and chloride, and a paucity of fiber, calcium and potassium [2]. From an evolutionary nutritional perspective, contemporary humans are Stone Agers habitually ingesting a diet discordant with their genetically determined metabolic machinery and integrated organ physiology [6]. This article discusses some of the potential consequences of these changes.

The modern dietary excess of NaCl and deficiency of K$^+$ and HCO$_3^-$ precursors

From extensive data on the diets of existing hunter-gatherer societies, and from inferences about the nature of the Paleolithic environment, Eaton and Konner analytically reconstructed the Paleolithic diet and estimated the probable daily nutrient intakes of Paleolithic humans [2]. In an estimated 3000 kilocalorie diet, meat constituted 35 percent of the diet by weight and plant foods, 65 percent. Total protein intake was estimated as 251 grams per day, of which animal protein was 191 grams, and plant proteins, 60 grams per day. By contrast, modern humans consume less than one-half that amount of animal protein, and only about one-third that amount of plant protein, per kilocalorie of diet consumed [7]. Sodium intake was estimated at about 29 meq per day, and potassium intake, in excess of 280 meq per day. By contrast, modern humans consume between 100–300 meq of sodium per day, and about 80 meq of potassium per day.

That is, in the switch to the modern diet, the K/Na ratio was reversed, from 1 to 10, to more than 3 to 1. Since food sodium is largely in the form of chloride salts, and food potassium largely in the form of bicarbonate-generating organic acid salts, the Cl/HCO$_3$ ratio of the diet has become correspondingly reversed. Further, the extent to which the dietary K/Na ratio is reversed increases with age [8], and presumably therefore also does the Cl/HCO$_3$ ratio. Yet, the biologic machinery that evolved to process these dietary electrolytes remains largely unchanged, genetically fixed in Paleolithic time [2]. Thus, the electrolyte mix of the modern diet is profoundly mismatched to its processing machinery and the extent of the mismatch increases with age. As a consequence of the diet-kidney mismatch, contemporary humans are not only overloaded with Na$^+$ and Cl$^-$ but also deficient in K$^+$ and HCO$_3^-$. Fig. 1 demonstrates this exchange of monovalent ions.

■ Adverse effects of excessive dietary sodium chloride

Excessive dietary sodium intake is mostly known to be associated with elevated blood pressure. Studies in indi-
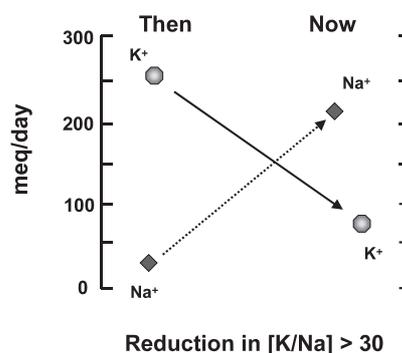

Fig. 1 Exchange of potassium intake for sodium (meq/day) in transition from pre-agricultural to modern diets.

viduals [9–11] as well as populations [12–15] have demonstrated correlations between dietary sodium intake and both systolic and diastolic blood pressure. Good blood pressure control has been linked with improvements in cardiac, cerebral and kidney function and in reductions in morbidity and mortality from cardiovascular and renal disease [16–19].

Dietary sodium is a less well-known determinant of urinary calcium excretion. Urinary excretion of calcium is well documented to vary directly with that of Na^+ [20]. Even a moderate reduction of dietary sodium, from 170 to 70 mmol/day, could attenuate not only hypertension but also hypercalciuria, and thereby prevent both kidney stones and osteoporosis. That the hypercalciuric effect of excessive dietary sodium may be a preventable cause of osteoporosis would seem supported by the results of recent studies in both post-menopausal women and adolescent girls [21, 22]. A bone-demineralizing effect of NaCl-induced hypercalciuria would also be in keeping with the many observations made by Nordin [23, 24] and Goulding and their associates [25, 26], in both humans and rats.

■ Lack of potassium in the diet

The evolutionarily recent increase in dietary sodium intake has been reciprocated by a decrease in dietary potassium intake. It has been estimated that our Paleolithic ancestors ate a diet containing in excess of 200 meq potassium daily [2]. What effects might this lack of potassium in the diet engender?

As early as 1928, Addison reported that potassium administration could lower elevated blood pressure in humans [27], and some 40 years later, Dahl et al. demonstrated that increasing the ratio of potassium to sodium in the diet of salt-sensitive hypertensive rats lowered blood pressure in a stepwise fashion [28].

In normotensive humans, Morris and colleagues recently demonstrated that increases in blood pressure induced by sodium loading could be progressively attenuated by increasing dietary potassium intake from 30 mmol/day to 120 mmol/day. In this study, potassium was given as the bicarbonate salt. Interestingly, this decline in blood pressure was significantly greater in the 24 African-American males than in the 14 Caucasian males in the study [29], suggesting not just a dietary, but a genetic component to the response of blood pressure to potassium bicarbonate ingestion.

In this same study, supplemental $KHCO_3$ can also override the hypercalciuric effect of dietary NaCl-loading, even though such supplementation further increases the urinary excretion of sodium. In a recently reported metabolic study of middle-aged normal men fed a diet marginally deficient in both K^+, 30 mmol/d, and calcium, 14 mmol/d, increasing dietary NaCl from 30 to

250 mmol/d induced a 50 % increase in urinary calcium that supplemental $KHCO_3$ either reversed or abolished, depending on whether it was supplemented to 70 or 120 mmol/d, mid- and high-normal intakes, respectively [29]. As an apparent consequence of its demonstrated natriuretic effect, supplemental $KHCO_3$ also reversed and abolished, respectively, NaCl-induced increases in blood pressure in these men with such normotensive "salt-sensitivity" (Fig. 2), a precursor of hypertension [30, 31]. In women fed a normal K^+ diet, supplemental K-citrate prevented not only the hypercalciuria induced by dietary NaCl-loading, but also prevented an increase in biochemical markers of bone resorption (Sellmeyer, D., et al, unpublished observations).

■ Specific adverse effects of excessive dietary chloride

Although much work has been done on the adverse effects of dietary sodium chloride on blood pressure, very little has been done to explore the specific role of excessive dietary chloride. And yet, the chloride content of the modern diet is at least as high as the sodium content [32]. Does the exchange of the bicarbonate we used to eat for the chloride that we presently eat have any adverse effects?

Morris and colleagues first demonstrated in uninephrectomized rats given deoxycorticosterone that while treatment with sodium as a combination of the bicarbonate and acetate salt raised blood pressure, treatment with sodium as the chloride salt raised blood pressure to a significantly higher level [33]. Luft et al. demonstrated that sodium as the chloride salt raised blood pressure in stroke-prone spontaneously hypertensive rats [34] and sodium as the bicarbonate salt lowered blood pressure in mildly hypertensive humans [35]. More recently, Morris et al. have done studies investigating the effects of KCl and KBC (potassiumbicar-
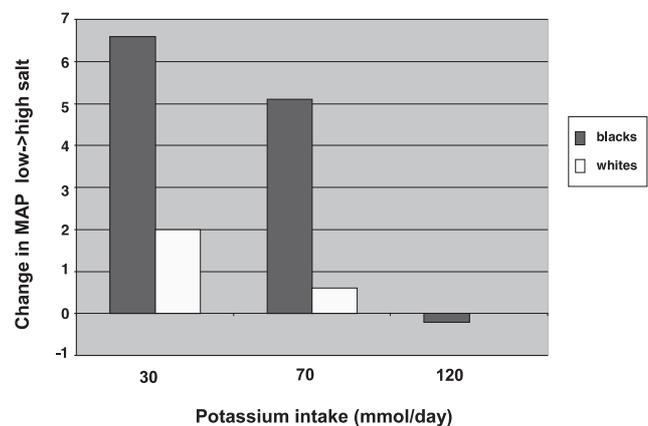

Fig. 2 Increasing dietary potassium decreases mean arterial pressure (MAP) even on high salt diets.

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bonate) on blood pressure, frequency of stroke and severity of the renal lesions in the SHRSP [36]. Rats treated with KCl had significantly higher PRA than rats treated with KBC. In each group and in all combined, the severity of hypertension was highly correlated with the levels of PRA (log transformed). KCl loading induced greater increases in BP than in control or KBC rats (Fig. 3).

The incidence of strokes was significantly higher with KCl than with KB/C (Table 1). In the KCl/KBC rates, strokes occurred only in animals with SBP > 248 mmHg and with PRA > 26.5 ng/ml/h (logPRA = 1.42).

Light microscopic examination of the kidneys revealed glomerular, tubular, interstitial, and vascular lesions (histologically ranked in combination) similar in quality but significantly more frequent and more severe with KCl supplementation than either KB/C or CTL [36]. Irrespective of dietary supplements, renal lesions were rare in rats with SBP < 200 mmHg. The overall severity of renal lesions was highly correlated with the level of PRA (log transformed) (R^2 = 0.67, p < 0.0001). Protein-uria was significantly greater with KCl than either KB/C or CTL (Table 1). Creatinine clearance was significantly greater in KB/C than in KCl or CTL (Table 1). Morris and colleagues concluded that the extent of renal damage and likelihood of stroke are determined by the severity of hypertension.

Diet and acid-base

In contrast to its excess chloride content, the modern diet lacks bicarbonate and anion precursors that generate bicarbonate on metabolism. As a consequence, the *net* acid load of the modern diet is higher than it would otherwise be. The rest of this article will discuss this bicarbonate-deficiency-mediated dietary acid excess.
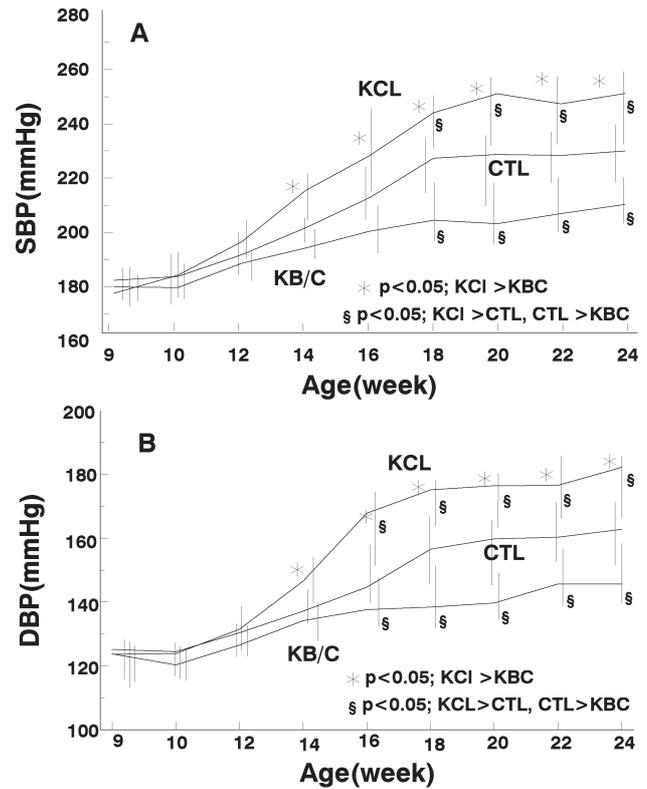

Fig. 3 Change in systolic (SBP) and diastolic blood pressure (DBP) with age in stroke prone spontaneously hypertensive rats (SPSHR) treated with a usual rat diet (CTL), or supplemented with KCl or potassium bicarbonate. Data are presented as median and 95 % CI.

■ Endogenous acid production

Endogenous acid production can be considered as comprising three components: 1) organic acids produced during metabolism that escape complete combustion to

Table 1 Effects of KCl vs. KB/C in SHRSP before and 15 Weeks after initiation of dietary supplements

	Age 9 Weeks (baseline)			Age 25 Weeks (15 weeks after assignment)		
	KCl	KB/C	CTL	KCl	KB/C	CTL
SBP (mmHg)	173 (169/185)	176 (173/181)	178 (174/184)	248 (230/258)*	204 (197/217)**	226 (212/235)
DBP (mmHg)	124 (115/130)	124 (117/129)	125 (118/132)	179 (167/186)*	144 (140/156)**	161 (149/171)
PRA (ng/ml/hr)				17.4 (8.6/30.8)[1,+]	6.2 (4.7/11.2)	13.6 (6.8/26.9)
Strokes total				6/17[+]	0/15	1/20
Renal lesions (overall rank)				37 (13)*	17 (13)	24 (13)
UV-protein (mg/d)	64 (53/70)	59 (51/66)	53 (51/62)	251 (179/301)*	108 (96/153)	147 (111/172)
Creatinine clearance (ml/min/100 g BW)				0.46 (0.13)	0.65 (0.19)**	0.48 (0.14)
UV-Na (mEq/d)	1.17 (0.54)	1.39 (0.56)	1.60 (0.55)	1.34 (0.45)	1.57 (0.22)	1.30 (0.30)
BW (g)	218 (23)	222 (22)	218 (21)	319 (24)	326 (18)	331 (13)

SBP, DBP, PRA, UV-Protein: median and (95 % C. I.)
Renal lesions, creatinine clearance, UV-Na, BW: mean(±SD)
[1] Data not available from 2 rats who had died of stroke.
*p < 0.05; KCl vs. either KB/C or CTL, **p < 0.05; KB/C vs. either KCl or CTL, +p < 0.05; KCl vs. KB/C.

carbon dioxide and water; 2) sulfuric acid (H_2SO_4) produced from the catabolism of methionine and cystine, the sulfur-containing amino acids in dietary proteins; and 3) potassium bicarbonate ($KHCO_3$) produced from the metabolism of the potassium salts of organic anions in the vegetable foods of the diet, for example potassium citrate and potassium malate. The potassium bicarbonate so produced titrates sulfuric and organic acid and thereby downregulates net endogenous acid production (NEAP).

NEAP then is computed as the sum of organic acid production and sulfuric acid production minus the intestinally absorbed potassium salts of organic anions that are metabolized to potassium bicarbonate.

All foods contain sulfur-containing amino acids, although fruits in general contain very little; animal products and cereal grains contain very little or no potential base – this comes mainly from fruits and other non-grain plant foods. Organic acid production is driven in part by the quantity of base-precursors in the diet, so increasing dietary base precursors does not yield equivalent reductions in NEAP. The greater the quantity of organic and sulfuric acids produced from metabolism, and the lower the amounts of potassium salts metabolizable to bicarbonate, the greater the NEAP.

■ Estimating the diet net acid load

It is possible to quantify NEAP in normal subjects ingesting whole food diets by measurements of the quantity of the inorganic constituents of diet, urine and stool, and of the total organic anion content of the urine [37]. However, such studies are extremely time-consuming and labor-intensive. Kurtz et al. utilized renal net acid excretion (RNAE) as a quantitative index of NEAP, since under steady-state conditions there is a predictable relation between these two variables [37, 38], and since net acid excretion is more readily measured. Nearly 90 % of the variance in net acid excretion among the subjects was accounted for by differences in net endogenous acid production (Fig. 4).

Measuring RNAE to estimate NEAP of whole food diets was first used about 90 years ago [39]. Volunteers ate large amounts of one particular food item for approximately one week, while doing sequential 24-hour urine collections, which were then analyzed for ammonia, titratable acids and total carbon dioxide – the constituents of RNAE. This approach has a number of drawbacks; not only is it tedious and time-consuming, but as Blatherwick wrote in his article discussing the effects of a boiled cauliflower diet, "It became very distasteful after the third day, so that the experiment was discontinued."

Methods of estimating diet net acid load solely from dietary intake have also been developed. Remer and
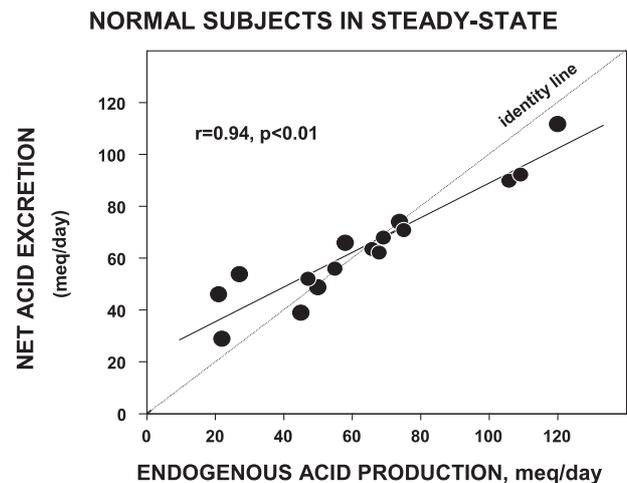

NORMAL SUBJECTS IN STEADY-STATE

Fig. 4 Close correspondence of endogenous acid production to renal net acid excretion in normal subjects (r=0.94, p < 0.01).

Manz developed an algorithm for calculating net acid excretion using a formula that estimated net intestinal absorption of cations and anions, organic acids and sulfate. In this study, RNAE as determined by the formula $\sum (Cl^- + P^{1.8-} + SO_4 + OA - Na^+ - K^+ - Ca^{2+} - Mg^{2+})$ correlated reasonably well with the measured NAE [40]. Using a similar formula, Remer and Manz also calculated the potential acid load for individual food items [41].

Frassetto et al. developed a somewhat less involved but nearly as precise method, using an algorithm to predict diet acid load from only two diet constituents: diet protein and potassium content [42]. Data from healthy subjects at steady-state, eating one of 20 whole food diets as part of metabolic balance studies that measured RNAE were analyzed; both dietary protein and dietary potassium intake were demonstrated to be independent predictors of RNAE, when evaluated by multiple regression analysis. Because protein and potassium were not correlated to each other, the ratio of dietary protein to potassium was evaluated. This ratio correlated significantly with the difference between the sulfur (i. e., potential acid) and potential base contents of the diets (Fig. 5 A & B), and accounted for 70–75 % of the variation in RNAE of the diets studied.

■ Acid-base balance in normal humans

Three factors have been found to be independent predictors of the set point for blood hydrogen ion and bicarbonate concentration; the partial pressure of carbon dioxide, NEAP and age. Madias et al. [43] were the first to propose that the interindividual differences in plasma acidity in normal subjects can be accounted for in part by corresponding differences in the level at which plasma PCO_2 is regulated by the respiratory system in

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Fig. 5 Comparison of the predictive ability of dietary sulfur minus potential base and the ratio of protein to potassium on steady-state renal net acid excretion (RNAE) for the 16 of 20 diets studied for which sulfur and potential base contents were known. Protein is expressed as g/day/2500 kcal; RNAE, sulfur, potential base and potassium are expressed as meq/day/2500 kcal.
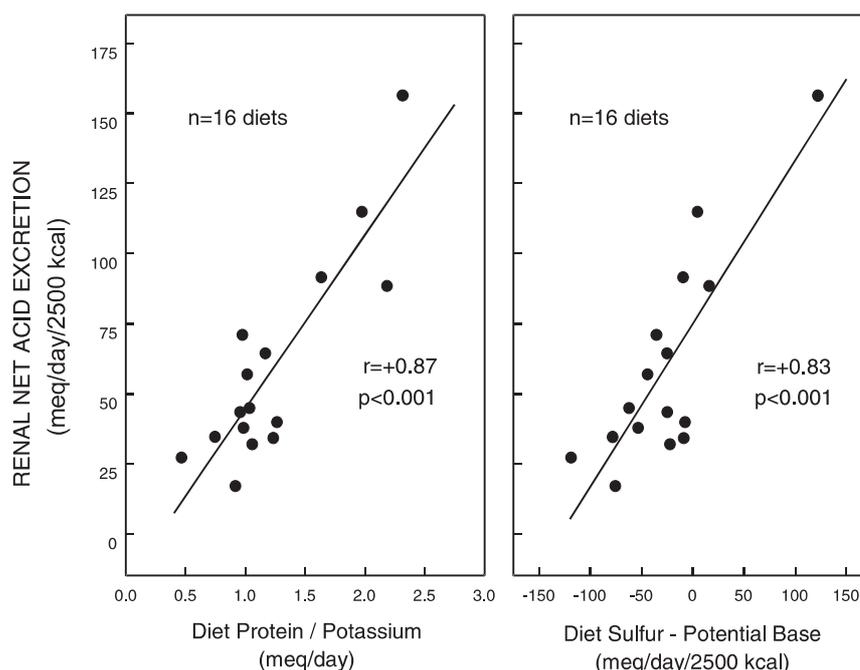
response to factors other than those entrained by changes in plasma acidity itself. In normal subjects they observed a positive correlation between plasma [H$^+$] and plasma PCO2 among subjects.

Kurtz et al. [44] were the first consider whether "metabolic" factors might also play a role in determining the interindividual differences in plasma acidity and plasma [HCO$_3^-$] in normal subjects. At steady-state, there was a significant direct relationship between plasma acidity and RNAE, and a significant inverse relationship between plasma [HCO$_3^-$] and RNAE, after adjusting for the effects of interindividual difference in the respiratory set-point for PCO$_2$. Subsequently, Frassetto et al. [45] extended these findings to a larger number of subjects and a wider variety of diets. Adding bicarbonate to the diet sufficient to reduce RNAE to nearly zero significantly reduced blood [H+] and increased plasma [HCO$_3^-$] [46].

Frassetto et al. subsequently carried out a systematic analysis of measurements of blood [H$^+$] and PCO$_2$, plasma [HCO$_3^-$], RNAE and glomerular filtration rate (GFR, estimated as 24-hour creatinine clearance) in 64 healthy adult men and women over a wide range of ages, at steady-state on a controlled diet while residing in a clinical research center [45]. Those studies identified age as a significant determinant of the blood acid-base composition in adult humans. From young adulthood to old age (17–74 years), otherwise healthy men and women develop a progressive increase in blood acidity and decrease in plasma [HCO$_3^-$], indicative of an increasingly worsening low-grade metabolic acidosis (Fig. 6 A & B).

The age effect was significant even when the effects of

differences among subjects in diet net acid load were adjusted for. Indeed, age and diet net acid load (reflected in steady-state RNAE) were independent co-determinants of the degree of metabolic acidosis. In comparing the relative impact of age and diet net acid load, over their respective ranges (17–74 years, 15–150 meq/day), age had ~1.6 times greater effect on blood [H$^+$] and plasma [HCO$_3^-$] than diet net acid load. Increasing age therefore substantially amplifies the chronic low-grade metabolic acidosis induced by diet.

Age and GFR were highly correlated, and were not independent predictors of blood acidity or plasma bicarbonate. One explanation may be that renal acid-base regulatory function tends to decline with increasing age [47, 48]. Thus, as we age, renal acid-base regulatory function declines and the degree of diet-induced metabolic acidosis increases.

Pathophysiologic consequences of severe metabolic acidosis in humans

Before discussing the possible effects of the mild metabolic acidosis produced by age and diet, let us briefly review some of the effects on the body of more severe metabolic acidosis, such as that associated with advanced renal failure or in experimental loading studies with ammonium chloride. It is well recognized that severe metabolic acidosis can cause pathophysiologic consequences in humans. Long term increases in acid loads have been shown to affect multiple systems.

Fig. 6 Steady-state blood hydrogen ion content increases and plasma bicarbonate concentration decreases independently with increasing age and renal net acid excretion.
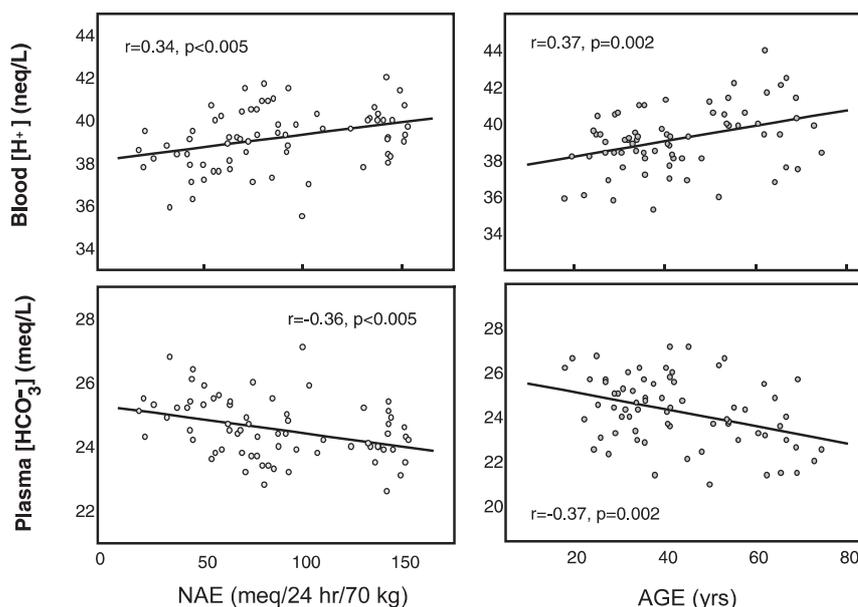
Chronic acidosis and bone

Loss of bone substance is a well-known pathophysiologic consequence of severe metabolic acidosis [49, 50]. Bone is a large reservoir of base in the form of alkaline salts of calcium (phosphates, carbonates), and those salts are mobilized and released into the systemic circulation in response to increased loads of acid [51–54]. The liberated base mitigates the severity of the attendant systemic acidosis, contributing to systemic acid-base homeostasis. The liberated calcium and phosphorus are lost in the urine, without compensatory increase in gastrointestinal absorption, and reduce bone mineral content [51, 53, 55, 56]. Reduction of bone mineral content occurs as an unavoidable disadvantage of the participation of bone in the body's normal acid-base homeostatic response to the acid load.

The response of bone to acute acidosis has been studied most extensively by Bushinsky and coworkers using a variety of in vitro models. Acute metabolic acidosis promptly results in buffering of hydrogen by bone carbonate, with attendant release of sodium, potassium and calcium [57–59].

When acid loading continues over days to weeks, bone continues to participate in systemic acid-base homeostasis, slowing the acidward shift in systemic acid-base equilibrium, to its own detriment [51–53]. Net external acid balance remains positive, indicating continuing internal buffering of the net acid load. Mobilization of bone base persists, and the bone minerals (calcium and phosphorus) accompanying that base continue to be wasted in the urine, without compensatory increases in intestinal absorption [60]. With chronicity of the acidosis, bone mineral content and bone mass progressively decline [61, 62] and osteoporosis develops [61, 63, 64].

The destructive process is not only a passive physicochemical dissolution of bone mineral by acidic extracellular fluid, but also an active process involving cell-mediated bone resorption and formation signaled by increased extracellular fluid $[H^+]$ and decreased $[HCO_3^-]$[1] [65–67]. Extracellular acidification increases the activity of osteoclasts, the cells that mediate bone resorption [65–67], and suppresses the activity of osteoblasts, the cells that mediate bone formation [65].

Not only the mineral phase, but also the organic phase of bone, is lost during chronic acidosis. Release of bone mineral by osteoclasts is accompanied by osteoclastic degradation of bone matrix [50, 61, 63, 64]. In chronic acid loading studies in humans, urinary hydroxyproline excretion increases [46, 51], and serum osteocalcin levels decrease [46], suggesting that matrix resorption increased and formation decreased.

Chronic acidosis and calcium excretion

Even mild reductions of plasma $[HCO_3^-]$ and arterial pH to values still within their normal range also induce an increase in urinary calcium, negative calcium balance [46] and a reduction in urinary citrate [68]. It has been suggested that a reduced urinary excretion of citrate may be useful in identifying such low-grade metabolic

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1 Chronic respiratory acidosis, in which acidemia but not hypobicarbonatemia occurs, is not accompanied by increased urinary excretion of calcium and phosphorus [100].

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acidosis (vide infra). In fact, supplemental $KHCO_3$ in amounts that can be predicted to induce only a modest increase in the plasma bicarbonate concentration, but one still attenuating of low-grade metabolic acidosis (vide infra) [46], can both induce a positive calcium balance, by reducing the urinary excretion of calcium, and reduce the formation of kidney stones, apparently by also correcting hypocitraturia [69]. Supplemental K-citrate and $KHCO_3$ are effective in reducing the urinary excretion of calcium and in increasing the urinary excretion of citrate presumably because both alkaline salts induce equal increases in plasma bicarbonate [68].

In patients with classic RTA, bicarbonate therapy that sustains correction of frank metabolic acidosis not only reduces the formation of calcium-containing kidney stones, but also induces a positive calcium balance [70, 71] and can induce healing of osteomalacia [72].

Furthermore, with bicarbonate therapy in children with classic RTA, can correct hypercalciuria and improve somatic growth, even when severe stunting has already occurred [73, 74]. Bicarbonate therapy has been found to induce these effects only when provided in sufficient amounts to maintain the plasma bicarbonate at concentrations well within the normal range. These amounts must be great enough both to offset the renal bicarbonate wasting that characterizes classic RTA in rapidly growing children, and to titrate their endogenously produced non-volatile acid [73].

Chronic acidosis and skeletal muscle nitrogen metabolism and renal nitrogen excretion

In disorders that cause chronic metabolic acidosis, protein degradation in skeletal muscle is accelerated [75–77], which increases the production of nitrogen end-products that are eliminated in the urine, thereby inducing negative nitrogen balance [77]. This disturbance of nitrogen metabolism apparently results directly from the acidosis, not its cause, nor from other sequelae of the underlying acidosis-producing disorder, because it occurs with widely differing acidosis-producing conditions [77–79] and because it is reversible by administration of alkali [80–82], which corrects the acidosis but not its cause.

Acidosis-induced proteolysis appears to be an acid-base homeostatic mechanism. By releasing increased amounts of amino acids, including glutamine and amino acids that the liver can convert to glutamine, which is the major nitrogen source used by the kidney for synthesis of ammonia, the kidney can increase the excretion of acid (as ammonium ion) in the urine, thereby mitigating the severity of the acidosis [76, 77, 83].

Metabolic acidosis induces nitrogen wasting in part by directly increasing the rate of protein degradation in skeletal muscle, without commensurately increasing the rate of protein synthesis [75, 76].

Chronic acidosis and growth hormone

More severe forms of metabolic acidosis from renal tubular acidosis and chronic renal failure in children are associated with low levels of growth hormone, and their height and weight are often below the 5th percentile for age. In 6 pediatric subjects with chronic renal failure and 6 subjects with renal tubular acidosis, Caldas and colleagues demonstrated that treatment with enough bicarbonate to correct the pH and plasma bicarbonate levels to normal causes both 24-hour mean growth hormone and IGF-1, a growth-related hormone, to double, from 2.7 ± 0.2 to 4.8 ± 0.2 and 156 ± 17 to 271 ± 19 respectively ($p < 0.001$) [84]. As mentioned above, treating children with RTA with potassium citrate, who are short and have weak bones, causes them to start growing at a normal rate and to attain normal stature [73]. Brunnger et al. [85] report that experimentally induced, chronic metabolic acidosis in humans results in hepatocellular resistance to growth hormone and consequent reduction in serum IGF-1 levels, and Mahlbacher subsequently showed that driving IGF-1 production with exogenous growth hormone could correct acidosis-induced nitrogen wasting [86].

Pathophysiologic consequences of diet-induced, age-amplified chronic low-grade metabolic acidosis in humans

It is understandably difficult to think "metabolic acidosis" when the values for plasma acid-base composition are in the range traditionally considered normal, though clinicians are accustomed to considering metabolic acidosis under those circumstances in the context of diagnosing "mixed" acid-base disorders. The term "metabolic acidosis" implies pathophysiologic sequelae. If such sequelae were not present with normal diet net acid loads, one might remain skeptical about the appropriateness of the term. But, as discussed in this next section, such acidosis-induced pathophysiological conditions as negative calcium and phosphorus balance, accelerated bone resorption, and renal nitrogen wasting appear to be consequences of the normal diet acid load, as they are significantly improved by "normalizing" blood acid-base composition by neutralizing the diet net acid load with small amounts of exogenous base [46, 87, 88].

Although the degree of diet-induced, age-amplified metabolic acidosis may be mild as judged by the degree of perturbation of blood acid-base equilibrium from currently accepted norms, its pathophysiologic significance cannot be judged exclusively from the degree of

that perturbation. Adaptations of the skeleton, skeletal muscle, kidney and endocrine systems that serve to mitigate the degree of that perturbation impose a cost in cumulative organ damage that the body pays out over decades of adult life [89, 90].

■ Evidence that diet-induced metabolic acidosis mobilizes skeletal base

In the studies referred to in the previous section, the effects of metabolic acidosis were studied in response to large exogenous acid loads. What is the evidence that bone contributes to acid-base homeostasis in subjects with the chronic low-grade metabolic acidosis that results from eating a normal, net acid-producing diet?

For any level of acid loading, if bone is contributing to acid-base homeostasis, even though blood acid-base equilibrium appears to be stable, not all of the daily net acid load should be recoverable in the urine [51, 52], i. e., acid should appear to be accumulating in the body on a daily basis. As discussed earlier, continued acid retention in normal subjects has been demonstrated at diet net acid loads within the normal range. The stability of the blood acid-base equilibrium is de facto evidence of the existence of an internal reservoir of base that continually delivers base to the systemic circulation in an amount equal to the fraction of the net acid load that the kidneys fail to excrete. Bone is the major such internal reservoir of base known to exist.

Another way to test whether persisting bone loss occurs in response to chronic low-level diet-induced metabolic acidosis is to examine the effect of neutralizing the diet net acid load by addition of exogenous base. Such studies have been carried out in postmenopausal women [46]. Potassium bicarbonate, when administered in doses that nearly completely neutralize the diet net acid load, reduces urinary wasting of calcium and phosphorus, improves preexisting negative balances of calcium and phosphorus, and as indicated by biochemical markers, reduces the rate of bone resorption and stimulates the rate of bone formation [46]. Lemann [91] likewise demonstrated significant improvement in calcium and phosphorus balances when the diet net acid load was neutralized during potassium bicarbonate administration in humans.

Thus, two lines of evidence indicate that chronic low-level diet-induced acidosis imposes a chronic drain on bone: a) stability of blood acid-base equilibrium in the face of continuing retention of acid, and, b) amelioration of negative calcium and phosphorus balances, reduction of bone resorption and stimulation of bone formation attendant to neutralization of the dietary acid load.

■ Evidence that diet-induced metabolic acidosis is a factor in the pathogenesis of clinical osteoporosis

If chronic low-level diet-induced metabolic acidosis imposes a chronic, clinically significant drain on bone mass, it might be possible to account in part for differences in bone mass among individuals by differences in the net acid load from their habitual diets. Unfortunately, the measurements of net acid production or excretion rates needed to test that possibility directly are not currently available. It is possible, however, to obtain indirect but still realistic estimates of the differences in diet net acid load among select groups of individuals, and to relate those to differences in rate of bone mass among those groups.

Specifically, it is possible to estimate the differences in diet net acid load among the residents of different countries. That can be accomplished using international food consumption data compiled by the United Nation's Food and Agricultural Organization (FAO). For each of some 130 countries, FAO tables report consumption of vegetable and animal foods in units of daily per capita vegetable and animal protein consumed. Many vegetable foods are rich in potassium salts of organic anions [92] that can be metabolized to the base, bicarbonate, which in turn reduces the net rate of endogenous acid production for a given rate of acid production from animal foods [39, 93, 94]. Animal foods have a relatively lower content of potassium and organic anions. Per unit protein, the potassium content of many vegetable foods exceeds that of animal foods by more than an order of magnitude. Because organic anion content of foods parallels that of potassium, the content of base precursors also is substantially greater in those vegetable foods than in animal foods. For a given total protein intake, therefore, the ratio of vegetable-to-animal protein consumed can provide a rough index for comparison of the base-to-acid-generating potential of the diet among the differing countries.

It is also possible to approximate differences in bone mass among countries, based on published reports of the incidence of hip fractures in women over the age of 50 years. Hip fracture incidence is a good index of bone mass because bone mass is a major determinant of the incidence of fractures of bone in older individuals. So, if chronic low-level diet-induced metabolic acidosis imposes a chronic, clinically significant drain on bone mass, it might be possible to account in part for differences in hip fracture incidence among countries by differences in the ratio of vegetable-to-animal protein consumed.

Fig. 7 depicts the results of such an analyses for the 33 countries in which both hip fracture incidence and per capita food consumption data were available as of 1999 [95]. Note that there is a strong nonlinear relation between fracture incidence and ratio of vegetable-to-animal protein consumed. Over two-thirds ($r^2=0.70$) of the
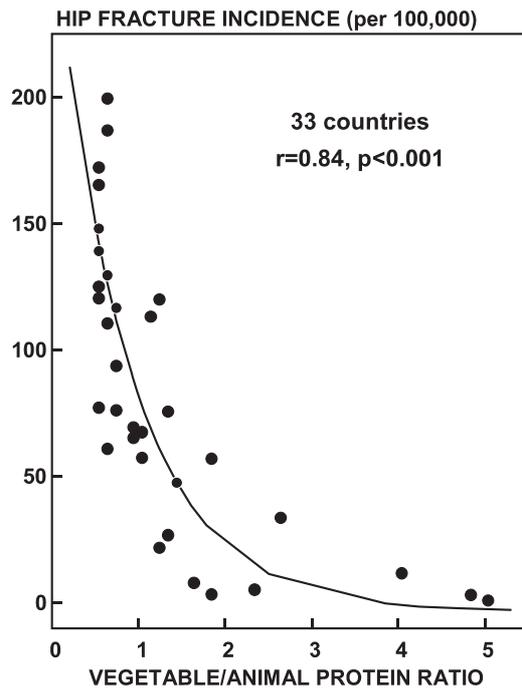
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HIP FRACTURE INCIDENCE (per 100,000)

**33 countries
r=0.84, p<0.001**

VEGETABLE/ANIMAL PROTEIN RATIO

Fig. 7 Age-adjusted hip fracture incidence in women in 33 countries decreases as the ratio of vegetable to animal foods in the diet increases (p < 0.001).

total variability in hip fracture incidence among countries can be accounted for by its correlation with the ratio of base-generating (vegetable) to acid-generating (animal) foods consumed. Countries with the lowest ratio of vegetable-to-animal protein intake have the highest incidence of hip fracture, and vice versa. This finding provides evidence that dietary base deficiency relative to acid load is a factor in the pathogenesis of the decline in bone mass that occurs with age. Given that low-grade metabolic acidosis of severity proportionate to the diet net acid load is to be expected, this finding supports the hypothesis that diet-induced chronic low-grade metabolic acidosis is a factor in the pathogenesis of clinical osteoporosis.

Recently Sellmeyer et al. have reexamined the relationship between the ratio of vegetable-to-animal food intake and hip fracture rates in a more homogeneous population (white elderly women residents of the U. S), and found a similar result [96]. In addition, they found, with repeated measures of hip bone mineral density, that the rate of bone loss in the subjects was greatest in those with the lowest vegetable-to-animal food intake ratio. Those studies are significant because they eliminate the confounding effects of racial and cultural factors on hip fracture risk unavoidable in the cross-cultural study [95], and support the hypothesis that chronic low-grade diet-induced metabolic acidosis accelerates bone loss rates in humans.

Using a less indirect index of diet net acid load,

namely the ratio of dietary protein-to-potassium [42], New and associates recently reported their observations on bone health in elderly Scottish women to include estimates of dietary net acid load [97]. The values for lumbar spine mass were lower and the values of urinary excretion of bone resorption markers were higher in those women in the highest quartile of net acid load, compared to those in the lowest quartile. Further, the net acid load was significantly higher in the group of subjects who had sustained fractures during the observation period, compared to those who had not.

■ Evidence that diet-induced metabolic acidosis effects renal nitrogen excretion in humans

Frassetto and coworkers also explored the possibility that nitrogen wasting might occur even with the low-grade "tonic" background metabolic acidosis that accompanies eating a typical net acid-producing diet [87]. In postmenopausal women, correcting their diet-induced low grade metabolic with potassium bicarbonate in amounts that just neutralized their daily diet net acid load, reduced in urinary ammonium excretion, which returned to control when the acidosis was allowed to recur by discontinuing the $KHCO_3$ supplement (Fig. 8). But, in addition to the reduction in ammonia nitrogen excretion during $KHCO_3$ administration, a sustained reduction in urea nitrogen excretion also occurred, suggesting that the higher pre-treatment urea nitrogen excretion rates were contributing to the acidosis-induced nitrogen wasting (Fig. 8). The reductions in urea and ammonia excretion contributed about equally to the nitrogen sparing effect.

The most straightforward interpretation of these findings is that $KHCO_3$ administration reduced NEAP and corrected the pre-existing low-grade metabolic acidosis, reducing the total rate of renal ammonia production and, by raising urine pH, reducing intraluminal trapping of ammonium ion. As a consequence, both the excretion of ammonia in the urine and the delivery of ammonia to the systemic circulation via the renal vein decreased. The reduction in urine ammonia contributed directly to improvement in nitrogen balance. The reduction in ammonia delivery to the systemic circulation via the renal vein contributed indirectly to improvement in nitrogen balance by limiting substrate (viz., ammonia) availability for hepatic urea production [98], thereby reducing external loss of nitrogen as urinary urea. And by correcting the pre-existing low-grade metabolic acidosis, $KHCO_3$ decreased the pre-treatment rate of muscle proteolysis, further contributing to the improvement in nitrogen balance. The magnitude of the $KHCO_3$-induced nitrogen sparing effect was potentially sufficient to both prevent continuing loss of muscle mass and to restore previously accrued deficits.
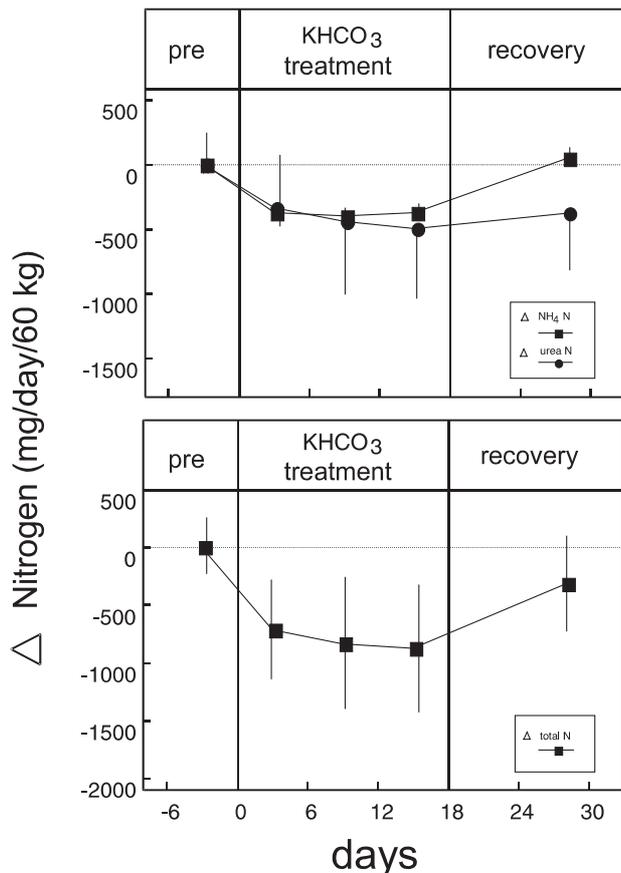
Fig. 8 Decrease in urinary nitrogen excretion only during the period when the diet in these normal postmenopausal women is supplemented with sufficient base to lower their net acid excretion to near zero.

■ Evidence that diet-induced metabolic acidosis effects growth hormone excretion in humans

Frassetto and coworkers also explored the possibility that growth hormone excretion might be affected by this same low-grade "tonic" background metabolic acidosis [87]. In postmenopausal women, correcting their diet-induced low grade metabolic with potassium bicarbonate in amounts that just neutralized their daily diet net acid load, was accompanied by an increase in 24-hour mean growth hormone secretion. The average total serum GH secretion, calculated as the 24-hour integrated serum GH concentration, increased from 826 ± 548 pg/ml before $KHCO_3$ to 915 ± 631 pg/ml after $KHCO_3$ supplementation ($p < 0.05$), approximately an 11 % increase over baseline. Was this physiologically significant? Consistent with the effect of growth hormone on bone metabolism, osteocalcin levels also rose in nearly every subject after $KHCO_3$ supplementation, and were higher at nearly all time points. The 24-hour mean osteocalcin level rose from 7.0 ± 0.9 to 8.3 ± 1.2 ng/ml after $KHCO_3$ treatment ($p < 0.005$).

Stone age diets for the 21st century?

Increasingly, nutritionists are directing attention to the potential detrimental health effects of the major transformation of the human diet that occurred relatively recently in evolutionary time [99], viewing them as the effects of a conflict of the encounter of old genes with new fuels [3]. Our group is emphasizing the potential conflict between our old genes and new levels of K-base and NaCl in our diet, an insufficiency of the former and oversufficiency of the latter. In this effort, much remains to be understood, and many interesting questions can be formulated. The subtitle above is one such question. What is the optimal NaCl intake for humans under ordinary circumstances? Does adding NaCl to the diet really make much difference if K-base intakes are optimal? What are optimal K-base intakes? Was the Paleolithic diet net base-producing? Is the optimal systemic acid-base status of humans a low-grade diet-induced chronic metabolic alkalosis without potassium deficiency? Should we increase our protein intakes and balance the acid effects with increased K-base?

Prospects

Based on the studies and arguments reviewed here, it seems reasonable to expend further effort to investigate the extent of the modulating effect of dietary NaCl and K-base on the expression of osteoporosis, age-related decline in muscle mass, kidney stones, and perhaps age-related decline in renal function. Re-exchanging the NaCl in our present diet for the K-base that our ancestral Homo and pre-Homo hominid species ate in abundance can be shown to correct diet-induced low-grade metabolic acidosis, and the consequent biochemical evidences of decreased growth hormone secretion, increased bone resorption with decreased bone formation and increased protein catabolism. Beyond that, the supplementation of the diet with K-base can override the effects of NaCl loading on blood pressure and urinary calcium excretion. Thus, increasing dietary K-base to levels approaching those of our stone-age forebears, either with fruits and non-grain plant foods, or with supplemental K-base, would seem to hold particular promise for preventing or delaying expression of these age- and diet-related diseases and their consequences.

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References

1. Abelow BJ, Holford TR, Insogna KL (1992) Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. Calcif Tissue Int 50:14–18

2. Eaton SB, Konner M (1985) Paleolithic nutrition. A consideration of its nature and current implications. N Engl J Med 312:283–289

3. Neel JV (1999) When some fine old genes meet a 'new' environment. In: Simopoulos A (ed) Evolutionary aspects of nutrition and health. Karger, Basel, pp 1–15

4. Eaton SB, Cordain L (1997) Evolutionary aspects of diet: old genes, new fuels. Nutritional changes since agriculture. World Rev Nutr Diet 81:26–37

5. Cordain L (1999) Cereal grains: humanity's double-edged sword. In: Simopoulos A (ed) Evolutionary aspects of nutrition and health. Karger, Basel, pp 19–73

6. Eaton SB, Konner M, Shostak M (1988) Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 84:739–749

7. Smit E, Nieto FJ, Crespo CJ, Mitchell P (1999) Estimates of animal and plant protein intake in US adults: results from the Third National Health and Nutrition Examination Survey, 1988–1991. J Am Diet Assoc 99:813–820

8. Frisancho AR, Leonard WR (1984) Blood pressure in blacks and whites and its relationship to dietary sodium and potassium intake. J Chron Dis 37:515–519

9. Dahl LK (1972) Salt and hypertension. Am J Clin Nutr 25:231–244

10. Kawasaki T, Delea CS, Bartter FC, Smith H (1978) The effect of high-sodium low-sodium intakes on blood pressure and other related variables in human subjects with idiopathic hypertension. Am J Med 64:193–198

11. Myers JB, Morgan TO (1984) Effect of alteration in sodium chloride intake on blood pressure of normotensive subjects. J Cardiovasc Pharmacol 6: S204–S209

12. Chalmers J, Morgan T, Doyle A, Dickson B, Hopper J, Mathews J, Matthews G, Moulds R, Myers J, Nowson C, Scoggins B, Stebbing M (1986) Australian National Health and Medical Research Council Dietary salt study in mild hypertension. J Hypertens 4 (suppl 6): s629–s637

13. Gleibermann L (1973) Blood pressure and dietary salt in human populations. Ecol Food Nutr 2:143–156

14. Oliver WJ, Cohen EL, Neel JV (1975) Blood pressure, sodium intake, and sodium related hormones in the Yanomamo Indians, a "no-salt" culture. Circulation 52:146–151

15. Poulter N, Khaw KT, Hopwood BE, Mugambi M, Peart WS, Sever PS (1984) Salt and blood pressure in various populations. J Cardiovasc Pharmacol 6 (Suppl 1):S197–S203

16. Australian Natl Hlth Res Council (1989) Fall in blood pressure with modest reduction in dietary salt intake in mild hypertension. Lancet 1:399–402

17. Fodor JG, Whitmore B, Leenen F, Larochelle P (1999) Lifestyle modifications to prevent and control hypertension. 5. Recommendations on dietary salt. Canadian Hypertension Society, Canadian Coalition for High Blood Pressure Prevention and Control, Laboratory Centre for Disease Control at Health Canada, Heart and Stroke Foundation of Canada. CMAJ 160:S29–S34

18. Yamori Y, Nara Y, Mizushima S, Sawamura M, Horie R (1994) Nutritional factors for stroke and major cardiovascular diseases: international epidemiological comparison of dietary prevention [see comments]. Health Rep 6: 22–27

19. Peterson JC, Adler S, Burkart JM, Greene T, Hebert LA, Hunsicker LG, King AJ, Klahr S, Massry SG, Seifter JL (1995) Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. Ann Intern Med 123: 754–762

20. Burtis WJ, Gay L, Insogna KL, Ellison A, Broadus AE (1994) Dietary hypercalciuria in patients with calcium oxalate kidney stones. Am J Clin Nutr 60: 424–429

21. Cappuccio FP, Meilahn E, Zmuda JM, Cauley JA (1999) High blood pressure and bone-mineral loss in elderly white women: a prospective study. Study of Osteoporotic Fractures Research Group. Lancet 354:971–975

22. Devine A, Criddle RA, Dick IM, Kerr DA, Prince RL (1995) A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. Am J Clin Nutr 62:740–745

23. Nordin BEC, Polley KJ (1987) Metabolic consequences of the menopause. Calcif Tissue Int 41:S1–S59

24. Nordin BEC, Need AG, Morris HA, Horowitz M (1992) Sodium, calcium and osteoporosis. In: Burkhardt P, Heaney R (eds) Nutritional aspects of osteoporosis. Raven Press, New York, pp 279–295

25. Goulding A (1990) Osteoporosis: why consuming less sodium chloride helps to conserve bone. N Z Med J March 18:120–122

26. McParland BE, Goulding A, Campbell AJ (1989) Dietary salt affects biochemical markers of resorption and formation of bone in elderly women. Br Med J 299:834–835

27. Addison WLT (1928) The use of sodium chloride, potassium chloride, sodium bromide, and potassium bromide in cases of arterial hypertension which are amenable to potassium chloride. Can Med Assoc J 18:281–285

28. Dahl LK, Leitl G, Heine M (1972) Influence of dietary potassium and sodium/potassium molar ratios on the development of salt hypertension. J Exp Med 136:318–330

29. Morris RC, Jr., Sebastian A, Forman A, Tanaka M, Schmidlin O (1999) Normotensive salt sensitivity: effects of race and dietary potassium. Hypertension 33:18–23

30. Sullivan JM (1991) Salt sensitivity: definition, conception, methodology, and long-term issues. Hypertension (17 Suppl):I61–I68

31. Luft FC, Miller JZ, Grim CE, Fineberg NS, Christian JC, Daugherty SA, Weinberger MH (1991) Salt sensitivity and resistance of blood pressure: age and race as factors in physiological responses. Hypertension (17 Suppl): I102–I108

32. Al-Bander SY, Nix L, Katz R, Korn M, Sebastian A (1988) Food chloride distribution in nature and its relation to sodium content. J Am Diet Assoc 4: 472–475

33. Kurtz TW, Morris RC, Jr. (1983) Dietary chloride as a determinant of sodium-dependent hypertension. Science 22: 1139–1141

34. Luft FC, Steinberg H, Ganten U, Meyer D, Gless KH, Lang RE, Fineberg NS, Rascher W, Unger T, Ganten D (1988) Effect of sodium chloride and sodium bicarbonate on blood pressure in stroke-prone spontaneously hypertensive rats. Clin Sci 74:577–585

35. Luft FC, Zemel MB, Sowers JA, Fineberg NS, Weinberger MH (1990) Sodium bicarbonate and sodium chloride: effects on blood pressure and electroyte homeostasis in normal and hypertensive man. J Hypertens 8:663–670

36. Tanaka M, Schmidlin O, Olson JL, Yi SL, Morris RC (2001) Chloride-sensitive renal microangiopathy in the stroke-prone spontaneously hypertensive rat. Kidney Int 59:1066–1076

37. Lennon EJ, Lemann J, Jr., Litzow JR (1966) The effect of diet and stool composition on the net external acid balance of normal subjects. J Clin Invest 45:1601–1607

38. Relman AS, Lennon EJ, Lemann J, Jr. (1961) Endogenous production of fixed acid and the measurement of net balance of acid in normal subjects. J Clin Invest 40:1621–1630

39. Blatherwick NR (1914) The specific role of foods in relation to the composition of the urine. Arch Int Med 14:409–450

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

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

42. Frassetto LA, Todd KM, Morris RC, Jr., Sebastian A (1998) Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. Am J Clin Nutr 68:576–583

43. Madias NE, Adrogue HJ, Horowitz GL, Cohen JJ, Schwartz WB (1979) A redefinition of normal acid-base equilibrium in man: carbon dioxide as a key determinant of normal plasma bicarbonate concentration. Kidney Int 16:612–618

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

45. Frassetto L, Morris RC, Jr., Sebastian A (1996) Effect of age on blood acid-base composition in adult humans: role of age-related renal functional decline. Am J Physiol 271:1114–1122

46. Sebastian A, Harris ST, Ottaway JH, Todd KM, Morris RC, Jr. (1994) Improved mineral balance and skeletal metabolism in postmenopausal women treated with potassium bicarbonate [see comments]. N Engl J Med 330: 1776–1781

47. Lindeman RD (1986) Anatomic and physiologic age changes in the kidney. Exp Gerontol 21:379–406

48. Davies DF, Shock NW (1950) Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males. J Clin Invest 29:496–507

49. Barzel US (1995) The skeleton as an ion exchange system: implicaitons for the role of acid-base imbalance in the genesis of osteoporosis. J Bone Miner Res 10:1431–1436

50. Kraut JA, Mishler DR, Singer FR, Goodman WG (1986) The effects of metabolic acidosis on bone formation and bone resorption in the rat. Kidney Int 30:694–700

51. Lemann J, Jr., Litzow JR, Lennon EJ (1966) The effects of chronic acid loads in normal man: further evidence for participation of bone mineral in the defense against chronic metabolic acidosis. J Clin Invest 45:1608–1614

52. Lemann J, Jr., Lennon EJ, Goodman AD, Litzow JR, Relman AS (1965) The net balance of acid in subjects given large loads of acid or alkali. J Clin Invest 44:507–517

53. Litzow JR, Lemann J, Jr., Lennon EJ (1967) The effect of treatment of acidosis on calcium balance in patients with chronic azotemic renal disease. J Clin Invest 46:280–286

54. Bushinsky DA, Chabala JM, Gavrilov KL, Levi-Setti R (1999) Effects of in vivo metabolic acidosis on midcortical bone ion composition. Am J Physiol 277: F813–F819

55. Breslau NA, Brinkley L, Hill KD, Pak CYC (1988) Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J Clin Endocrinol Metab 66:140–146

56. Gafter U, Kraut JA, Lee DBN, Silis V, Walling MW, Kurokawa K, Haussler MR, Coburn JW (1980) Effect of metabolic acidosis on intestinal absorption of calcium and phosphorus. Am J Physiol 239:G480–G484

57. Bushinsky DA, Wolbach W, Sessler NE, Mogilevsky R, Levi-Setti R (1993) Physicochemical effects of acidosis on bone calcium flux and surface ion composition. J Bone Miner Res 8:93–102

58. Bushinsky DA, Lam BC, Nespeca R, Sessler NE, Grynpas MD (1993) Decreased bone carbonate content in response to metabolic, but not respiratory, acidosis. Am J Physiol 265: F530–F536

59. Bushinsky DA, Lechleider RJ (1987) Mechanism of proton-induced bone calcium release: calcium carbonate dissolution. Am J Physiol 253:F998–F1005

60. Lemann J, Jr., Litzow JR, Lennon EJ (1967) Studies of the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. J Clin Invest 46:1318–1328

61. Barzel US, Jowsey J (1969) The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin Sci 36:517–524

62. Burnell JM (1971) Changes in bone sodium and carbonate in metabolic acidosis and alkalosis in the dog. J Clin Invest 50:327–331

63. Upton PK, L'Estrange JL (1977) Effects of chronic hydrochloric and lactic acid administrations on food intake, blood acid-base balance and bone composition of the rat. Quart J Exp Physiol 62:223–235

64. Newell GK, Beauchene RE (1975) Effects of dietary calcium level, acid stress, and age on renal, serum, and bone responses of rats. J Nutr 105:1039–1047

65. Krieger NS, Sessler NE, Bushinsky DA (1992) Acidosis inhibits osteoblastic and stimulates osteoclastic activity in vitro. Am J Physiol 262:F442–F448

66. Arnett TR, Dempster DW (1986) Effect of pH on bone resorption by rat osteoclasts in vitro. Endocrinology 119: 119–124

67. Teti A, Blair HC, Schlesinger P, Grano M, Zambonin-Zallone A, Kahn AJ, Teitelbaum SL, Hruska KA (1989) Extracellular protons acidify osteoclasts, reduce cytosolic calcium, and promote expression of cell-matrix attachment structures. J Clin Invest 84:773–780

68. Simpson DP (1983) Citrate excretion: a window on renal metabolism. Am J Physiol 244:F223–F234

69. Sakhaee K, Alpern R, Jacobson HR, Pak CYC (1991) Contrasting effects of various potassium salts on renal citrate excretion. J Clin Endocrinol Metab 72: 396–400

70. Albright F, Burnett CH, Parson W, Reifenstein EC, Roos A (1946) Osteomalacia and late rickets: the various etiologies net in the United States with emphasis on that resulting from a specific form of renal acidosis, the therapeutic indications for each etiological sub-group, and the relationship between osteomalacia and Milkman's syndrome. Medicine 25:399–479

71. Greenberg AJ, McNamara H, McCrory WW (1966) Metabolic balance studies in primary renal tubular acidosis: effects of acidosis on external calcium and phosphorus balances. J Pediatr 69: 610–618

72. Richards P, Chamberlain MJ, Wrong OM (1972) Treatment of osteomalacia of renal tubular acidosis by sodium bicarbonate alone. Lancet 2:994–997

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

74. Caldas A, Broyer M, Dechaux M, Kleinknecht C (1992) Primary distal renal tubular acidosis in childhood: clinical study and long-term follow-up of 28 patients. J Pediatr 121:233–241

75. Garibotto G, Russo R, Sofia A, Sala MR, Sabatino C, Moscatelli P, Deferrari G, Tizianello A (1996) Muscle protein turnover in chronic renal failure patients with metabolic acidosis or normal acid-base balance. Miner Electrolyte Metab 22:58–61

76. May RC, Kelly RA, Mitch WE (1986) Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. J Clin Invest 77:614–621

77. Williams B, Layward E, Walls J (1991) Skeletal muscle degradation and nitrogen wasting in rats with chronic metabolic acidosis. Clin Sci 80:457–462

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78. Bell JD, Margen S, Calloway DH (1969) Ketosis, weight loss, uric acid, and nitrogen balance in obese women fed single nutrients at low caloric levels. Metabolism 18:193–208

79. May RC, Kelly RA, Mitch WE (1987) Mechanisms for defects in muscle protein metabolism in rats with chronic uremia. Influence of metabolic acidosis. J Clin Invest 79:1099–1103

80. Papadoyannakis NJ, Stefanidis CJ, McGeown M (1984) The effect of the correction of metabolic acidosis on nitrogen and potassium balance of patients with chronic renal failure. Am J Clin Nutr 40:423–627

81. Hannaford MC, Leiter LA, Josse RG, Goldstein MB, Marliss EB, Halperin ML (1982) Protein wasting due to acidosis of prolonged fasting. Am J Physiol 243:E251–E256

82. Gougeon-Reyburn R, Lariviere F, Marliss EB (1991) Effects of bicarbonate supplementation on urinary mineral excretion during very low energy diets. Am J Med Sci 302:67–74

83. Cersosimo E, Williams PE, Radosevich PM, Hoxworth BT, Lacy WW, Abumrad NN (1986) Role of glutamine in adaptations in nitrogen metabolism during fasting. Am J Physiol 250:E622–E628

84. Caldas A, Fontoura M (1993) Effects of chronic metabolic acidosis (CMA) in 24-hour growth hormone secretion. J Am Soc Nephrol 4:828–828

85. Brungger M, Hulter HN, 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: 216–221

86. Mahlbacher K, Sicuro A, Gerber H, Hulter HN, Krapf R (1999) Growth hormone corrects acidosis-induced renal nitrogen wasting and renal phosphate depletion and attenuates renal magnesium wasting in humans. Metabolism 48:763–770

87. Frassetto L, Morris RC, Jr., Sebastian A (1997) Potassium bicarbonate reduces urinary nitrogen excretion in postmenopausal women. J Clin Endocrinol Metab 82:254–259

88. Frassetto L, Morris RC, Jr., Sebastian A (1996) Potassium bicarbonate increases serum growth hormone concentrations in postmenopausal women. J Am Soc Nephrol 7:1349

89. Alpern RJ (1995) Trade-offs in the adaptation to acidosis. Kidney Int 47:1205–1215

90. Alpern RJ, Sakhaee S (1997) The clinical spectrum of chronic metabolic acidosis: homeostatic mechanisms produce significant morbidity. Am J Kid Dis 29: 291–302

91. Lemann J, Jr., Gray RW, Pleuss JA (1989) Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. Kidney Int 35: 688–695

92. Souci SW, Fachmann W, Kraut H (1986) Food Composition and Nutrition Tables. Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp 1–1032

93. Hu J-F, Zhao X-H, Parpia B, Campbell TC (1993) Dietary intakes and urinary excretion of calcium and acids: a cross-sectional study of women in China. Am J Clin Nutr 58:398–406

94. Halperin ML (1982) Metabolism and acid-base physiology. Artif Organs 6: 357–362

95. Frassetto LA, Todd KM, Morris RC, Jr., Sebastian A (2000) Worldwide incidence of hip fracture in elderly women: relation to consumption of animal and vegetable foods. J Gerontol A Biol Sci Med Sci 55:M585–M592

96. Sellmeyer DE, Stone KL, Sebastian A, Cummings SR (2001) A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women. Am J Clin Nutr 73:118–122

97. New S, Macdonald HM, Grubb DA, Reid DM (2001) Positive association between net endogenous noncarbonic acid production (NEAP) and bone health: further support for the importance of the skeleton to acid-base balance. Bone 28 (Suppl 5):594

98. Cheema-Dhadli S, Jungas RL, Halperin ML (1987) Regulation of urea synthesis by acid-base balance in vivo: role of NH3 concentration. Am J Physiol 252:F221–F225

99. Simopoulos AP (1999) Evolutionary Aspects of Nutrition and Health: Diet, Exercise, Genetics and Chronic Disease. Karger, Basel, pp 1–145

100. Lau K, Nichols R, Tannen RL (1987) Renal excretion of divalent ions in response to chronic acidosis: evidence that systemic pH is not the controlling variable. J Lab Clin Med 109:27–33