

Organic anions and potassium salts in nutrition and metabolism

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The present review examines the importance of dietary organic anions in preventive nutrition. Organic anions are chiefly supplied by plant foods, as partially neutralised K salts such as potassium citrate, potassium malate and, to a lesser extent, oxalate or tartrate salts. Animal products may also supply K anions, essentially as phosphate, but also as lactate as a result of fermentative or maturation processes, but these K salts have little alkalinising significance. Citrate and malate anions are absorbed in the upper digestive tract, while a substantial proportion is probably metabolised in the splanchnic area. Whatever their site of metabolism, these anions finally yield KHCO_3 which is used by the kidneys to neutralise fixed acidity. This acidity essentially reflects the oxidation of excess S amino acids to sulfate ions, which is mainly related to the dietary protein level. Failure to neutralise acidity leads to low-grade metabolic acidosis, with possible long-term deleterious effects on bone Ca status and on protein status. Furthermore, low-grade acidosis is liable to affect other metabolic processes, such as peroxidation of biological structures. These metabolic disturbances could be connected with the relatively high incidence of osteoporosis and muscle-protein wasting problems observed in ageing individuals in Europe and Northern America. Providing a sufficient supply of K organic anions through fruit and vegetable intake should be recommended, fostering the actual motivational campaigns ('five (or ten) per d') already launched to promote the intake of plant foods rich in complex carbohydrates and various micronutrients.

Organic anions: Potassium: Calcium: Metabolic acidosis: Osteoporosis

Introduction

Compared with the Stone Age diet, the modern human diet is excessive in salt and deficient in fruit and vegetables, which are rich in K^+ and organic anions such as citrate. The mismatch between the modern diet's makeup of these electrolytes and the still ancient renal capacity to process them gives rise to NaCl overload, low-grade K^+ deficiency and low-grade metabolic acidosis (Frassetto *et al.* 2001). The pH threshold under which food acidity is perceived as aggressive is relatively low, in the range of 3–4. In fact, this acid perception is conditional because it may be modulated by other constituents of the food, such as cations or simple sugars. This acceptance of acidic foods is probably an inherited feature and might reflect the fact that acidic foods are generally better preserved, from a nutritional as well as from a safety point of view, compared with foods showing neutral or even slightly alkaline characteristics. In most cases, a food's acidity reflects the presence of organic anions, such as malate, citrate or lactate, together with inorganic anions (for example, phosphate).

Organic anions are absorbed mainly in the digestive tract and metabolised in various tissues, especially those of the

splanchnic area (intestine, liver), and finally yield CO_2 and energy. Since they are present in partly neutralised forms, mainly as K salts, they may be considered as virtual precursors of KHCO_3 , an important alkalinising chemical species especially for kidney function.

In the present review we will document the various sources of organic anions liable to be absorbed with plant foods and therefore available in the digestive tract lumen, as well as the different possibilities of *in situ* generation of some anions from other diet constituents (especially carbohydrates). The metabolic impact of these various anions will be examined, particularly as regards the control of the acid–base equilibrium in the body, and the various physiological consequences that might arise from disturbances of this equilibrium.

Origin of the organic anions absorbed from the digestive tract

Anions from plant foods

Anions. Except for ripened cereals, intrinsically poor in organic anions, and to a certain extent legumes, most plant

Abbreviations: ACL, ATP citrate lyase; fw, fresh weight; PRAL, potential renal acid load.

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foods contain substantial quantities of organic anions; from 100 mg/100 g fresh weight (fw) up to 4000 mg/100 g fw, for the richest sources such as citrus fruits. In terms of molar concentrations, these values represent 0.5 to 20 mmol citrate units/100 g fw (Fig. 1). Malate and citrate are polycarboxylic anions which, at the acidic pH prevailing in plant products, are partially neutralised by cations (for example, malic acid in apples is virtually in a potassium malate¹⁻ form). These organic anions are frequently intermediate metabolites of the tricarboxylic cycle but, in some foods, the prevalent anion is not metabolisable, for example oxalate (red beet, spinach, rhubarb) or tartrate (grapes). Besides, relatively low concentrations of additional organic anions may be found, such as fumarate or succinate, as well as various phenolic acids (quinic, ferulic, caffeic, chlorogenic). Malate and citrate anions are frequently present simultaneously in a large variety of plant foods, but malate is the predominant anion in some fruits and vegetables (apples, cherries, plums, aubergines, cucumbers) whereas citrate is the major anion of citrus fruits, kiwi and members of the *Solanaceae* family (potatoes, tomatoes).

Some anions, generally present in small amounts, probably exert pharmacological effects; for example, glucarate is found in plant foods such as cabbages, tomatoes, grapefruits and apples. Glucarate is a precursor of D-glucuro-1,4-lactone, an inhibitor of β -glucuronidases, which could affect the detoxification of carcinogens and other promoters of carcinogenesis through a decreased hydrolysis of their glucuronides (Walaszek *et al.* 1997). Cholesterol-lowering properties have also been ascribed to D-glucuro-1,4-lactone (Yoshimi *et al.* 2000). Hydroxycitrate, present in some Far-Eastern fruits such as *Garcinia*, is known as an inhibitor of lipogenesis and has also been examined as a possible anorexigen; however, the trials were not conclusive (Heysfield *et al.* 1998).

Accompanying cations. The K content of fruits is generally between 2.5 and 10 mEq/100 g fw and that of vegetables is usually higher, sometimes exceeding 15 mEq/100 g fw. In fact, the most salient feature which distinguishes fruits and vegetables is the K:organic acids concentration ratio (in mEq); this ratio is basically lower than 0.5 in most

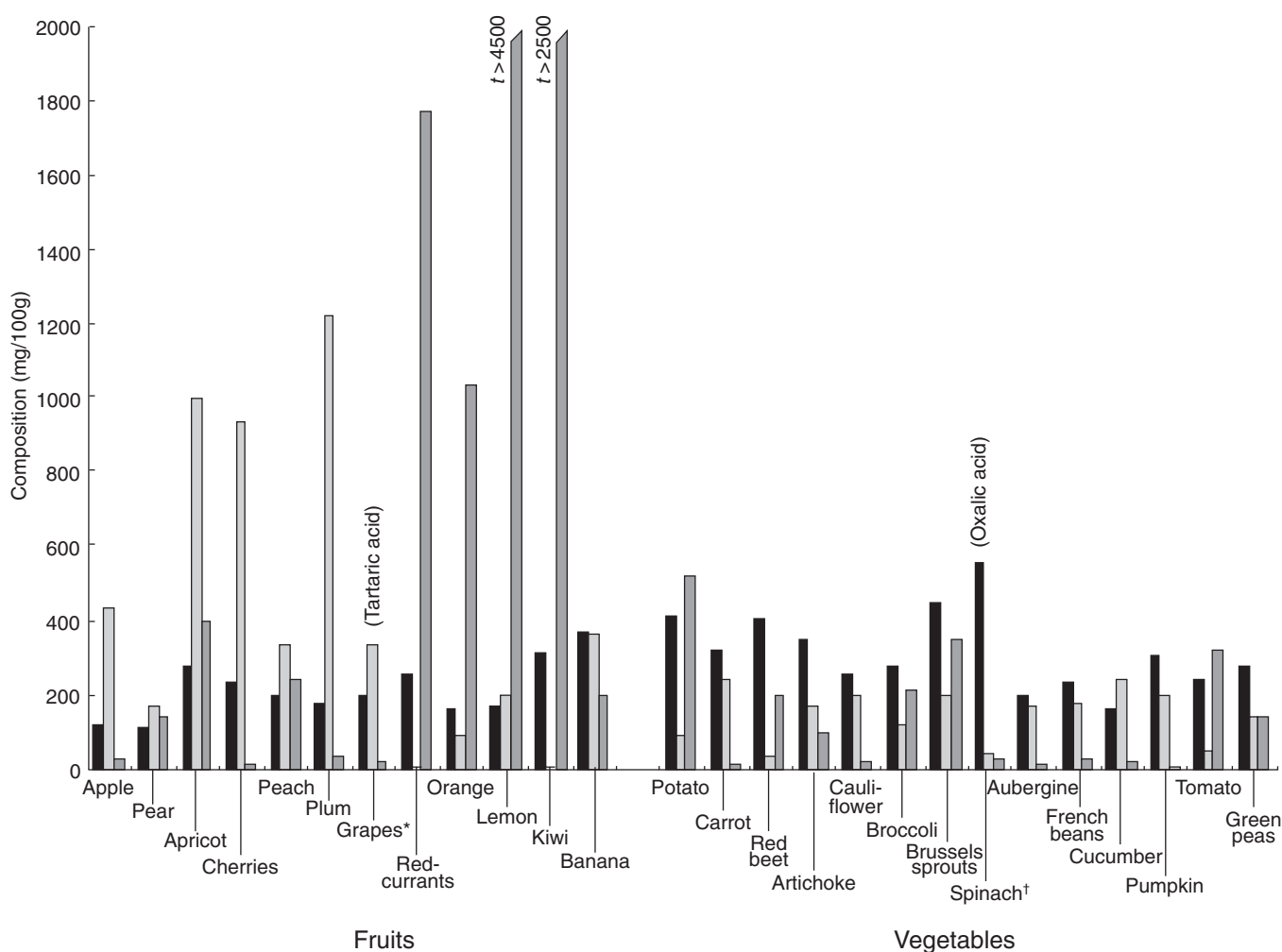


Fig 1. K (■), malic acid (■) and citric acid (■) composition of some examples of usual plant foods (mg/100 g fresh weight). * Tartaric acid content is shown for grapes instead of malic acid content. † Oxalic acid content is shown for spinach instead of K content.

fruits whilst it always exceeds 1 (up to 2.6 in pumpkins) in vegetables. In other terms, organic acidity of vegetables (frequently lower than in fruits) is more completely neutralised by K ions and vegetables exhibit a greater alkalinising potency. It must be noted that, within the fruit and vegetable groups, some foods show an atypical composition, which has some nutritional and culinary background. A fruit such as banana exhibits a K:organic acids concentration ratio > 1 (coherent with the possibility of using bananas as a vegetable) and this ratio is much lower than that of most of the other vegetables except for tomato, probably reflecting its possible utilisation as fruit (for example, for juice confectionery).

The daily food supply of organic anions is obviously dependent on fruit and vegetable intake. It could be in the range of 1–2 g/d in low-plant-food consumers, and easily reaches 3–4 g/d in subjects consuming a diversified omnivorous diet and more than 5 g/d in vegetarian individuals. These values of intake are close to those for K, which is consistent with the fact that organic anions are mainly K salts, but it must be kept in mind that K is also present in food products of animal origin.

Influence of cultivar, maturity or food processing. The degree of maturity influences the concentrations of organic acids in plant foods, for example citric acid in citrus fruits accumulates during fruit development but tends to decline at the stage of maturity. Food processing such as boiling is probably a cause of organic salt losses, especially for vegetables since fruits are generally consumed uncooked. This point is still incompletely documented, but there is little doubt that foods that are steamed or briefly fried probably maintain greater concentrations of potassium malate or citrate than if cooked using procedures that lead to extensive leaching (Table 1).

Anions in animal foods

These foods are frequently poor in organic anions if consumed directly but, most frequently, they contain substantial quantities of organic anions resulting from maturation and/or fermentation processes. An exception is cows' milk, which contains noticeable amounts of citrate (5–8 mmol/l). On the other hand, fermented dairy products are rich in lactate anions, arising from lactose fermentation (by *Lactobacilli* and *Streptococci*). Yoghurts contain more than 100 mmol lactic acid/l; thus a standard serving of 125 g yoghurt supplies about 1.3 g lactic acid. Cheeses are also enriched in lactate, but its concentration may be very different according to the type of cheese.

Table 1. Influence of the cooking process on the potassium level in potatoes

Treatment	Concentration (mg/100 g food)
Uncooked	530
Boiled	330
Oven cooked	540
French fries	700
Chips	1190

The L-lactate anion is part of the anionic profile of muscles *in vivo* (contributing 5–10 mmol/kg fw, which is marginal). However, it accumulates in muscle tissues during the process of meat maturation through anaerobic glycolysis from glycogen stores and it can be present in substantial concentrations (50 to 80 mmol/kg).

It appears, therefore, that animal foods may represent a non-negligible source of organic anions, but in a markedly distinct pattern from that observed in plant foods. The organic anion present in animal products is typically lactate and this anion is produced secondarily (through post-mortem metabolism or fermentation) in matrixes where the unmetabolisable anions, chiefly phosphate, prevail. Therefore, the alkalinising potential of animal foods is quite limited even when foods contain noticeable K concentrations (Oh, 2000). The quantities of organic anions ingested with animal foods may be estimated at about 1.5–2.0 g/d and it seems probable that, in subjects consuming a high-meat and -dairy products diet, this supply will be greater than that from plant foods whereas it will be marginal in vegetarian individuals (Fig. 2).

Anions produced *in situ* in the digestive tract

There is a permanent generation of substantial quantities of organic anions in the large intestine, essentially SCFA (namely acetate, propionate and butyrate). SCFA are the endproducts of microbial symbiotic fermentation present in the distal part of the digestive tract, which uses as substrates dietarily unavailable carbohydrates (fibre, resistant starch) and endogenous sources (sloughed epithelial cells, digestive secretions). Besides SCFA, other organic anions (succinate, lactate, galacturonate) can be found in notice-

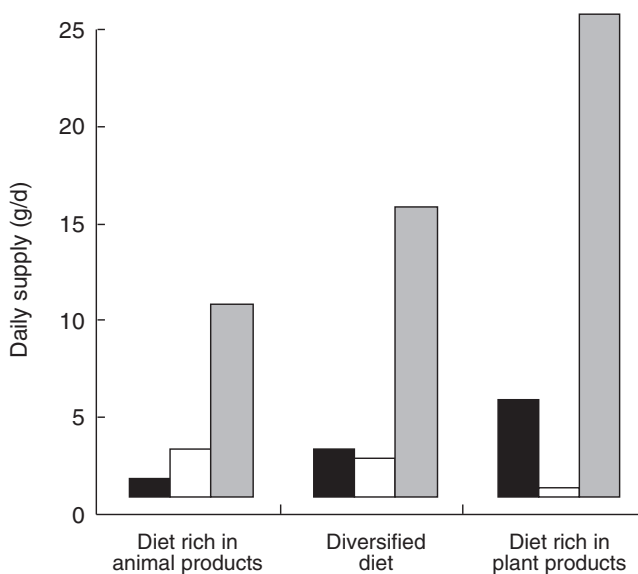


Fig 2. Estimation of the daily availability of organic anions from plant foods (chiefly malate and citrate; ■), from animal products (chiefly lactate; □) and from large-intestine fermentation (chiefly SCFA, i.e. acetate, propionate and butyrate; ▒) with various diet patterns.

able amounts, under specific conditions (acidic fermentation, pectin hydrolysis) (Demigné *et al.* 1999; Aprikian *et al.* 2003). The daily intake of fibre in Western countries is in the range of 15–20 g/d; thus, together with endogenous sources, a total substrate supply of 30 g/d for colonic fermentation is considered as probable. The overall yield of the colonic fermentation for SCFA production is roughly 50 %, which implies that this process generates around 15 g SCFA/d which are efficiently absorbed (>90 %) through the colonic mucosa (Cummings & Macfarlane, 1997). It appears therefore that the production of organic anions in the colon, which is closely dependent on nutritional conditions, is a major source of organic anions absorbed from the digestive tract.

The committed processes for SCFA absorption in the large intestine are complex and diversified (Sellin, 1999); some of them encompass a concomitant absorption of cations (K^+ and/or Na^+) and might therefore afford alkalinising opportunities (Kunzelmann & Mall, 2002). However, the overall impact of colonic fermentation on the acid–base equilibrium is still poorly understood since, for example, some processes involved in SCFA absorption require an anionic exchange between an $SCFA^-$ and an HCO_3^- , transferred from the extracellular body fluids into the colonic lumen (Demigné *et al.* 1999). Butyrate is a major energetic fuel for the colonic mucosa and has been shown to be metabolised in priority compared with other substrates such as glucose or glutamine (Mortensen & Clausen, 1996). The glutamine-sparing effect could be relevant in terms of acid–base control since glutamine is an effective precursor of neutralising metabolites (HCO_3^- and NH_3) in the kidneys (Welbourne & Joshi, 1990). The colon–liver enterohepatic cycling of NH_3-N is also a process with an equivocal significance. This is because urea transfer in the large intestine lumen generates two NH_3 , which can act as a sink for H^+ through NH_4^+ formation, but a part of colonic NH_3 is also reabsorbed and further detoxified to urea in the liver, a purported HCO_3^- -consuming process (Häussinger, 1997).

These considerations indicate that the actual influence of intestinal fermentation on the acid–base equilibrium is difficult to evaluate. Furthermore, such an influence (if any) is probably quite different when colonic fermentation is poorly active, in practically neutral pH conditions, compared with when substrate availability allows the development of acidic fermentation (De Groot *et al.* 1995), establishing an H^+ concentration gradient between the bulk colonic medium and extracellular fluid ranging from 10 to 50.

Absorption and metabolism

Absorption

Most of the organic anions from foods are effectively absorbed in the small intestine, or even in the stomach by diffusion if the protonated form is lipophilic (for example, acetate or butyrate). Citrate and other intermediates of the tricarboxylic cycle (succinate, α -ketoglutarate and probably malate) are transferred into the enterocytes through a secondary active transport requiring Na^+ movements across

the plasma membrane (Na-dependent dicarboxylate transporter). Various transporters of this type have been identified in the digestive tract, including the colon; these transporters differ in their K_m for substrates, from about 1 mmol/l to about only 10 μ mol/l (Pajor, 1999). The coupling of di- or tricarboxylate anions with that of Na^+ has been used in oral rehydrating formulations designed for the treatment of diarrhoea. Other dietary anions such as lactate are also readily absorbed, through a relatively specific process, since absorption of the L-isomer is markedly faster than that of its D-counterpart.

The other organic anions (oxalate, tartrate, phenolic acids) in the diet are generally less absorbable than malate, citrate or lactate, they are poorly metabolised in the body's tissues and the absorbed fraction is essentially channelled towards excretion by the kidneys.

Oxalic acid is abundant in a limited group of plant foods (red beet, spinach and rhubarb). Its absorption exhibits some particularities. Oxalate absorption is: (i) dependent on the dietary Ca availability; (ii) favoured in the colon by acidic fermentation conditions, together with possibilities of complete breakdown by the host microflora; (iii) possibly balanced by an opposite secretion of oxalate into the intestinal lumen (Hatch & Freel, 1995; Albiñ & Savage, 2001). Hautmann (1993) has also reported that the stomach could be a critical site for intestinal oxalate absorption.

Tartrate is essentially found, in its natural L(+) form, in grapes. This anion is partly absorbed from the digestive tract; it has been shown that colonic bacteria metabolise the bulk of ingested tartrate in human subjects, and only 14 % of ingested tartrate appears unchanged in the urine (Chadwick *et al.* 1978).

The other organic anions present in lesser amounts in plant foods (quinic, ferrulate, caffeate, chlorogenic) are part of the phenolic acids group. Phenolic acids may be absorbed at different levels of the digestive tract (small intestine, colon) and are then excreted by the kidneys in various conjugated forms.

Metabolism

The intestine itself is a site of metabolism for malate and citrate (Wolffram *et al.* 1994), yielding CO_2 or other intermediate metabolites such as amino acids (alanine, proline, etc) or lactate. This domain is, in fact, still incompletely known and certainly merits further investigation.

The liver is a major site for organic anions metabolism and the removal of circulating dicarboxylates involves at least two transport systems; an Na^+ -dicarboxylate cotransport and a system of anion exchange (Moseley *et al.* 1992; Zimmerli *et al.* 1992). The transferred anions are then channelled into various metabolic pathways, either towards complete oxidation, incorporation into glucose and liver glycogen or use for glutamine synthesis in perivenous hepatocytes (Stoll *et al.* 1991). This last point is noteworthy because it suggests that organic anions might exert an alkalinising effect not only through HCO_3^- generation, but also through glutamine production (by being used in the kidneys for HCO_3^- and NH_4^+ formation). In fact, there are arguments supporting the view that a sub-population of liver cells (5–7 %) is particularly adapted for coupling organic

anions and glutamine metabolism, through the predominance or exclusive presence of various processes. These processes involve: glutamine synthase; Na-dependent glutamate transport; enriched plasma membrane SN1 (amino acid transporter); malate (and closely related anions) membrane transport; less acidic cytoplasm (Häussinger *et al.* 1989).

In fact, whatever the pathway for the metabolism of organic anions, their C is finally oxidised to CO₂ (in equilibrium with circulating HCO₃⁻ in extracellular fluids) in the shorter or longer term. Insofar as these anions are present in foods as K⁺ salts, which are highly digestible, it can be assumed that the endproduct of their metabolism is KCO₃⁻.

Nutrition and acid–base equilibrium

Acidifying characteristics of different types of foods

During their complete oxidation, carbohydrates or lipids do not generate unmetabolisable acidity, even if their partial oxidation (into lactic acid or ketone bodies, respectively) may result in metabolic acidosis when excessive. In contrast, proteins contain various amino acids whose catabolism is liable to affect the acid–base equilibrium. The impact of the oxidation of proteins on this equilibrium is not unequivocal. Basic amino acids ('cationic', such as lysine, histidine or arginine) yield various metabolites together with H⁺, and S amino acids (methionine, cysteine) are also acidogenic because they yield sulfate anions (unmetabolisable acidity). However, oxidation of the dicarboxylic amino acids ('anionic', such as glutamate or aspartate) 'consumes' acidity. Even if some proteins (for example, rich in neutral and anionic amino acids) could theoretically be alkalinising, the fact remains that most of the dietary proteins (especially those well balanced for their amino acid composition) are acidifying (Remer & Manz, 1995).

Evaluation of the acidifying potential of various foods

Basically, a food or a diet which provides an excess of inorganic anions (fixed anions) such as Cl⁻, H_nPO₄⁽³⁻ⁿ⁾⁻ or SO₄²⁻, compared with inorganic cations (Na⁺, K⁺, Ca²⁺, Mg²⁺) will be ascribed acidifying properties, in keeping with the classical 'dietary ash hypothesis'. The net excretion of acidity by kidneys is referred to as:

$$(\text{Cl} + \text{P} + \text{SO}_4 + \text{organic anions}) - (\text{Na} + \text{K} + \text{Ca} + \text{Mg}).$$

The urinary excretion of SO₄ and all the other ions may be estimated from the data of food intake and food composition tables, using specific absorption coefficients, which are, for example: proteins, 75 %; P, 63 %; Cl, 95 %; Na, 95 %; K, 80 %; Ca, 25 %; Mg, 23 %. In addition, the valences (2 for Ca and Mg) and the ionic charge of PO₄ at physiological pH (1.8) are also taken into account as well as, for proteins, an average percentage of methionine (2.4 %) and cysteine (2.0 %) (Remer, 2000). Practically, the above calculation may be simplified by considering that Na and Cl are essentially provided as NaCl in most foods.

According to these assumptions, it is possible to estimate a potential renal acid load (PRAL) for various foods, which

ranges between 34 mEq/100 g (Parmesan) and -21 mEq/100 g (grapes). As a general rule, fruits and vegetables display negative PRAL values, milk and yoghurt are close to 0 and meat, fish, poultry and cheese (and some cereal products) exhibit positive PRAL values (Remer, 2000) (Fig. 3). At the scale of a complex diet fed during several days, renal acidity excretion may change from about 10 mEq/d up to 60–70 mEq/d during the transition from a lacto-ovo-vegetarian diet to an omnivorous diet moderately rich in proteins. Only minute differences are to be found between the measured values and values obtained through the calculation of the PRAL of consumed foods. In parallel the urinary pH (6.7 in initial conditions) may drop down to 5.9 (Remer & Manz, 1995). These urinary balances also depend on the rate of organic anions excretion (especially citrate), which is relatively constant for each individual.

Excessive dietary proteins from food with high PRAL adversely affect bone (each additional 1 g dietary protein results in an additional loss of about 1.75 mg Ca/d), unless buffered by the consumption of alkali-rich foods or supplements (Barzel & Massey, 1998; Heaney, 2001). Abelow *et al.* (1992) reported that the incidence of hip fractures in women aged 50 years and older correlates positively with a country's average annual per capita consumption of animal protein. Data from Frassetto *et al.* (2000) and Sellmeyer *et al.* (2001) strongly strengthen the generalisation of a worldwide association of hip fractures in women with animal protein consumption. In fact, the decisive risk factor for hip fracture would not be the rate of production of fixed acid from animal protein but the net rate of endogenous acid production, when all sources of dietary acid and base are considered. Thus, a vegan diet with protein derived equally from grains and legumes would deliver at least as many mmol S/g protein as would a meat-based diet (Heaney, 2001), but it yields significantly lower rates of net endogenous acid production than do mixed animal and vegetable

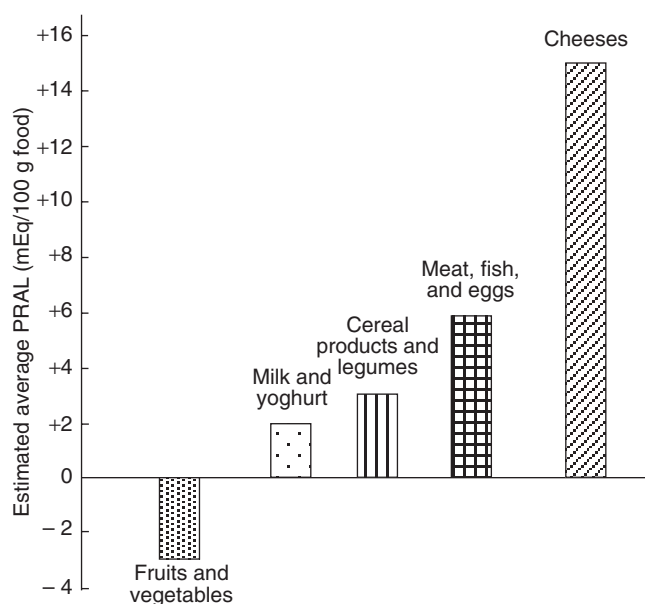


Fig 3. Average potential acid renal load (PRAL) values for the major categories of foods. (Adapted from Remer & Manz, 1995; Remer, 2000.)

diets. However, although protein was associated with an increased risk of forearm fracture for women who consumed more than 95 g/d, Feskanich *et al.* (1996) did not find any association between adult protein intake and the incidence of hip fracture. Also, it has to be said that a low protein intake may also compromise bone quality, especially in the elderly (Bonjour *et al.* 1997). In fact, the influence of dietary protein on Ca retention is complex, possibly transitory (Roughead *et al.* 2003), and liable to be modified by other nutrients in the diet (Massey, 2003).

Role of potassium and sodium

The addition of exogenous buffers, as chemical salts or as fruits and vegetables, to a high-protein diet results in a reduction in net acid excretion and decreased calciuria (Barzel & Massey, 1998). Metabolic balance studies have shown that restoring intakes of K^+ and HCO_3^- to levels approaching those in the diet of our prehistoric forebears with either supplemental $KHCO_3$ or with fruits and vegetables can alleviate hypertension, prevent kidney stones and protect against the occurrence of osteoporosis (Morris *et al.* 2000). Accordingly, low K intakes increase daily and fasting urinary Ca excretion rates (Lemann, 1999). This Ca-sparing effect occurs through reduced bone resorption and increased formation (Sebastian *et al.* 1994), whether the K salt is citrate or bicarbonate. Indeed, commonly complex dietary proteins with a high P content (Spencer *et al.* 1988) or consumed together with $KHCO_3$ supplementation (Sebastian *et al.* 1994) do not cause Ca loss in adults. In the study of Lutz (1984), subjects had a similar Ca balance when consuming either the higher-protein diet (102 g) plus bicarbonates or a moderate-protein diet (44 g).

The human body has a very low need for salt but we become addicted to the taste of salt as a consequence of its widespread and essential use as a food preservative. There is evidence that the range of usual dietary intakes is associated with urinary Ca loss, leading to adverse effects on bone metabolism because renal clearance of both minerals is linked in the proximal renal tube (Shortt & Flynn, 1990). Even if Ca homeostasis is generally well regulated (increased Ca loss triggers a better Ca absorption from the gut), the duration of this adaptive process may not be sufficient. This is why only 3% of the variance in urinary Ca excretion could be explained by Ca intake (Matkovic *et al.* 1995). It has been estimated that a 100 mmol increment in daily Na intake is associated with an average Ca loss in urine of approximately 1 mmol in free-living normocalciuric healthy populations (Nordin *et al.* 1993). Thus, if uncorrected, excess Na would lead to an annual skeletal loss of about 1% (Weaver *et al.* 1999). However, the limited studies which have investigated such an association with bone mineral density in human subjects have produced conflicting results. Actually, Ginty *et al.* (1998) have shown that adaptation of absorption may compensate for increased urinary Ca loss, at least in healthy young women. Results from Greendale *et al.* (1994) did not support a detrimental effect (prospective study from the Rancho-Bernado cohort). Nevertheless, other studies in adults implicated Na as a risk factor for urinary Ca loss (Massey & Whiting, 1996), bone loss (Devine *et al.* 1995) and high bone resorption (Jones *et al.* 1997).

Anti-acidosis mechanisms operating in kidneys

Under steady-state conditions, the urinary output of electrolytes matches gastrointestinal electrolyte absorption. The kidney sustains normal acid–base homeostasis by reabsorbing an appropriate amount of the filtered bicarbonate and excreting a quantity of acid in urine that corresponds with the endogenously generated acid load.

Phosphate is the major buffer system in urine and its excretion is increased during acidosis, probably as a result of a decrease of the preferentially transported form (HPO_4^{2-}) together with a direct effect of pH on the apical phosphate carrier in the proximal tubule. Citrate transport is frequently analysed owing to its sensitivity to systemic pH; changes in citrate excretion are altered by changes in the transported chemical form, namely citrate²⁻, and in kidney metabolism (Brennan *et al.* 1988). It is now well established that acidosis promotes hypocitraturia and the tubular reabsorption of citrate. The renal excretion of citrate is normally dependent on the net absorption of alkali from the digestive tract (Sakhaee *et al.* 1993), but this relationship is less tight in subjects suffering distal renal tubular acidosis. In general, lithiasic subjects who have no renal tubular acidosis frequently exhibit hypocitraturia, which is chiefly of digestive origin and probably consecutive to an insufficient intake of alkalinising agents (fruits and vegetables). In this view, Hess *et al.* (1994) proposed that ‘low vegetable-fibre intake and low urine volume’ could be added to the list of risk factors for low urine citrate.

The actual mechanisms of the control of citraturia are now well known. In rats, in parallel with hypocitraturia, chronic acidosis increases the cortical activity of ATP citrate lyase (ACL). Abundance of the ACL protein in the renal cortex is dramatically enhanced within a few days, but there is little change in ACL mRNA (Melnick *et al.* 1998). Hypokaliemia also promotes hypocitraturia and a rise in ACL activity. Aconitase activity in the renal cortex mitochondria is affected by changes in the acid–base equilibrium (Melnick *et al.* 1998). In the rat, chronic metabolic acidosis and a K deficit result in a rise of aconitase activity, concomitant with a greater abundance of aconitase protein. Conversely, the administration of alkalinising agents in the diet may slightly depress aconitase activity. Activity of the Na^+ –citrate co-transporter in the apical membrane is enhanced by metabolic acidosis, but is not altered by the addition of alkalinising agents in the diets. Aruga *et al.* (2000) have shown that the abundance of Na^+ –dicarboxylate-1 co-transporter protein is enhanced by chronic acidosis, in parallel with the corresponding mRNA. Likewise, it has been observed that several members of the Na^+ – HCO_3^- co-transporter family are up regulated in rats with chronic metabolic acidosis (Kwon *et al.* 2002).

Physiopathological aspects

Acid–base status and calcium metabolism

Because the urinary excretion of acid is insufficient, other homeostatic systems such as bone are required to buffer the excess dietary acid load (Barzel, 1995; Bushinsky, 2001; New, 2002). The skeleton serves as a substantial reservoir of labile base in the form of alkaline Ca salts, which can be

mobilised to defend blood pH. Indeed, 80 % of total body carbonate is in the hydration shell, the water surrounding bone, as are 80 % of citrate and 35 % of Na (Green & Kleeman, 1991). Osteoclasts and osteoblasts respond independently to small changes in pH, a slight drop in pH causing a burst in bone resorption (Kriegger *et al.* 1992; Arnett & Sakhaee, 1996). Further metabolic acidosis even blunts the capacity of growth hormone to stimulate the systemic production of insulin-like growth factor 1, which along with growth hormone is a major determinant of bone mass (Green & Maor, 2000) (Table 2). Bone also plays a major role in the storage of phosphate, the phosphate buffer system being very important to relieve fixed acid loads and to enable the maintenance of pH within restricted limits (Pautard, 1961).

Thus, a negative Ca balance implies the depletion of bone mineral. Over 25 years ago, Wachman & Bernstein (1968) suggested that lifetime buffering of the low-level acid loading and metabolic acidosis that occur in individuals eating ordinary diets was sufficient to impose a chronic demand for base of skeletal origin (Lennon *et al.* 1966). If bone is mobilised to buffer only 1 mEq acid/d, 15 % of the total body Ca in an average individual is lost in a decade (Barzel & Massey, 1998). This is why Nordin *et al.* (1987) suggested that age-related bone loss may be more attributable to excessive Ca loss than to inadequate Ca intake.

Chronic metabolic acidosis due to excessive intakes of sulfate and chloride anions increases with age at constant endogenous acid production, apparently due to the normal age-related decline of renal function (Frassetto *et al.* 1996). Thus, though mild, it leads to high losses of Ca in the urine and has been implicated in the pathogenesis of the physiological disturbances and degenerative diseases characteristic of ageing such as osteoporosis (through enhanced bone resorption) (Lemann *et al.* 1986; New, 2002). Indeed, experimentally induced chronic metabolic acidosis by acid loading induced the loss of bone mass (Barzel & Jowsey, 1969). *In vitro*, short-term metabolic acidosis (3 h culture) elicits a Ca efflux, secondary to physiochemical bone

mineral dissolution, while over 24 h it results from cell-mediated bone resorption (Bushinsky, 1995) because acidosis causes a greater release of K than Ca and osteoclastic function is necessary to support this phenomenon (Bushinsky, 2001).

Acid–base status and protein metabolism

Metabolic acidosis induces N wasting and depresses protein metabolism in human subjects. This may be particularly critical in aged individuals, more prone to renal dysfunction, and could promote muscle dystrophy and an aggravation of the consequences of Ca losses, through alterations of the protein matrix of bones. The committed mechanisms involved in the connection between acid–base equilibrium and protein metabolism are certainly complex and still incompletely understood. Nevertheless, there is little doubt that glutamine is a key factor, as a precursor of ammonium and bicarbonate in kidneys (Welbourne & Joshi, 1990) as well as an effector of cellular metabolism, as shown in the liver through glutamine-induced p38^{MAPK} activation mediating the inhibition of autophagic proteolysis at the level of autophagosome formation (Häussinger *et al.* 2001). In the same way, even small corrections of serum bicarbonate in acidotic subjects apparently promote the down regulation of branched-chain amino acid degradation (Rodriguez-Bayona & Peragon, 1998) and muscle proteolysis via the ubiquitin–proteasome system which plays a central role in the control of the proteolytic pathway (Combaret *et al.* 2001; Pickering *et al.* 2002).

The acidosis-induced alterations in protein metabolism involve various endocrine systems, including a decrease in insulin-like growth factor 1 levels (due to a loss of peripheral growth hormone sensitivity), moderate hyperthyroidism and hyperglucorticoidism (Greiber & Mitch, 1992; Wiederkehr & Krapf, 2001). In addition, various metabolic disturbances liable to be accompanied by acidosis (diabetes, sepsis) result in a decrease in insulin action on protein synthesis or degradation but, in contrast, the

Table 2. Chronic effects of metabolic acidosis

Function	Process	Organ or tissue
General cellular effects	K (and Mg) efflux from the cells	Muscles
Protein metabolism	Tissue protein degradation ↑	Muscles
	BCAA oxidation ↑	
	Albumin synthesis ↓	Liver
Ca homeostasis	Ca mobilisation ↑	Bone
	Active vitamin D synthesis ↓	Several organs
Glucose metabolism	Gluconeogenesis and lactate utilisation ↓	Liver
	Gluconeogenesis and Gln utilisation ↑	Kidney
Excretory function	Ca and P excretion ↑	Kidney
	Citrate excretion ↓	
	Lithiasis risk ↑	
Oxidative status	Free Fe and Cu ↑ Others ?	Blood plasma
Endocrine functions	GH secretion ↓	Hypophyse
	GH receptor expression ↓	Liver
	IGF-1 expression ↓	Chondrocytes

↑, Increase; BCAA, branched-chain amino acids; ↓, decrease; Gln, glutamine; GH, growth hormone; IGF, insulin-like growth factor.

antiproteolytic action of insulin could be improved during hyperthyroidism (Grizard *et al.* 1999).

Acidosis and peroxidation of biological structures

An acidification of extracellular pH may enhance LDL oxidation, by shortening the 'lag phase' and accelerating hydroperoxides and thiobarbituric acid-reactive substances formation. These alterations themselves promote their uptake by macrophages (Morgan & Leake, 1995). One of the mechanisms whereby variations of acid–base equilibrium might affect the processes of peroxidation is by changes in bioavailability of cations such as Fe or Cu (Leake, 1997; Salovaara *et al.* 2002). Fe is carried by transferrin on specific clusters of amino acids and an acidification of plasma pH can protonate these amino acids and release Fe from its binding sites. However, this last effect is significant only for definitely acidic and unphysiological pH, sometimes lower than 6. Effects on Cu availability (Cu is considered as more potent than Fe to initiate LDL oxidation) through a release of Cu from caeruloplasmin are obtained with less drastic acidification (pH 7). It must be noted that these studies were generally carried out on *ex vivo* systems, which explains why severely disturbing or even potentially lethal pH values could be studied. Nevertheless, some authors consider that, in the presence of metabolic acidosis (ketoacidosis, renal deficiency) even effectively compensated with modest change in blood pH, the buffering capacity of interstitial fluid may be severely depressed, thus allowing the emergence of local sites of severe acidosis (for example, near atheromatous lesions where LDL oxidation chiefly takes place). Deleterious effects of a prolonged period of systemic acidosis could also affect the internal structures of tissues and cells, through various mechanisms such as intracellular K and Mg losses and Ca movements between its various subcellular pools.

Conclusions on the role of fruit and vegetables

Clearly, dietary factors affecting the amount of Ca lost in the urine have a major influence on Ca balance and may even be more important than those that modulate the intestinal availability of Ca. This is why the inevitable loss of Ca in the urine is greater for Western-type diets rich in factors such as animal proteins, sulfate, Na or coffee (Heaney & Recker, 1982; Guéguen & Pointillart, 2000). The critical determinant of hip-fracture risk in relation to the acid–base effects of diet is the net load of acid in the diet. Thus, it is worthwhile to consider decreasing the rate of bone attrition by the use of a diet favouring alkaline ash, which leads to the promotion of fruit and vegetable intake (Remer & Manz, 1995; Frassetto *et al.* 1996). New *et al.* (1997, 2000) report that fruit consumption predicted greater bone density at all four bone sites measured in postmenopausal women and, when K was considered, Ca intake was no longer significantly related to bone mass. Tucker *et al.* (1999) have confirmed, in a cross-sectional and longitudinal study, that alkaline-producing dietary components such as K contribute to the maintenance of bone density. An increase in fruit and vegetable intake from 3.6 to 9.5

daily servings decreased urinary Ca from 157 (SD 7) to 110 (SD 7) mg/d *v.* a drop of 14 ± 6 in controls (Appel *et al.* 1997). In conclusion, greater attention needs to be paid to the causes of Ca loss, which should lower the Ca requirement. Indeed, urinary losses remain an important and inadequately appreciated aspect of Ca nutrition. A diet may be inadequate in Ca not simply because it is intrinsically Ca-poor but also because it is insufficient to offset excretory losses.

From a practical point of view, a supply of K in the range of 2.5–3.5 g/d as fruits and vegetables supposes a substantial intake of these plant foods, namely 0.6–0.8 kg/d (assuming a mean concentration of 3 g/kg fw). Such a supply would be possible in the framework of 'ten per d' recommendations for fruit and vegetable consumption, with servings of an average 80–100 g weight. These recommendations could seem high and not realistic, but it must be kept in mind that they are already fulfilled by a noticeable percentage of the population, including not only vegetarians but also a fraction of omnivorous subjects.

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