

Effect of potassium salts in rats adapted to an acidogenic high-sulfur amino acid diet

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Low-grade metabolic acidosis, consecutive to excessive catabolism of sulfur amino acids and a high dietary Na:K ratio, is a common feature of Western food habits. This metabolic alteration may exert various adverse physiological effects, especially on bone, muscle and kidneys. To assess the actual effects of various K salts, a model of the Westernised diet has been developed in rats: slight protein excess (20% casein); cations provided as non-alkalinising salts; high Na:K ratio. This diet resulted in acidic urine (pH 5.5) together with a high rate of divalent cation excretion in urine, especially Mg. Compared with controls, K supplementation as KCl accentuated Ca excretion, whereas potassium bicarbonate or malate reduced Mg and Ca excretion and alkalinised urine pH (up to 8). In parallel, citraturia was strongly increased, together with 2-ketoglutarate excretion, by potassium bicarbonate or malate in the diet. Basal sulfate excretion, in the range of 1 mmol/d, was slightly enhanced in rats fed the potassium malate diet. The present model of low-grade metabolic acidosis indicates that potassium malate may be as effective as KHCO_3 to counteract urine acidification, to limit divalent cation excretion and to ensure high citrate concentration in urine.

Low-grade acidosis: Potassium: Magnesium: Malate: Sulfate

Western diets are generally excessive in protein and NaCl and also frequently deficient in fruit and vegetables. Protein sulfur amino acids catabolism results in SO_4 production, which is essentially eliminated through renal excretion (Bella & Stipanuk, 1995; Nakamura *et al.* 2002). Homeostatic systems may act to buffer excess dietary acid load, namely Ca mobilisation from bone (Barzel, 1995; Frassetto *et al.* 2001; New, 2002) or glutamine catabolism in kidneys, which yields HCO_3^- transferred to plasma together with NH_4^+ ions released in urine (Halperin *et al.* 1990). However, long-term periods of low-grade metabolic acidosis will lead to significant bone Ca wasting in rats and man (Won *et al.* 1996; Marangella *et al.* 2004), as well as to shift muscle protein metabolism towards proteolysis (Greiber & Mitch, 1992; Bushinsky, 1995). These effects contribute to worsen the propensity to osteoporosis and osteopenia; all the more since the kidney's ability to excrete a dietary acid load is impaired with ageing (Frassetto *et al.* 1996; New, 2002).

A substantial part of KHCO_3 used by the kidneys to neutralise fixed acidity arises from oxidation in tissues, especially in the splanchnic area, of K organic anions found in substantial amounts in fruits and vegetables. Citrate and malate anions are present in relatively similar amounts in portions of usually consumed fruits and vegetables, where they are partly neutralised by K and Mg; typically less than 50% in fruits (down to 5% in citrus) but 70–90% in vegetables (Demigné *et al.* 2004a). Relatively few studies have addressed the respective importance of K and organic anions

(such as citrate or malate) in the effects of plant foods on acid–base status and metabolism, especially mineral homeostasis, whereas there are reports supporting the interest of K itself as a protective element (He & McGregor, 2001; Demigné *et al.* 2004b).

To further investigate this domain, models of low-grade metabolic acidosis in experimental animals are still scarce and it must be noted that most of usual rat diets, especially those based on AIN93 recommendations, may be considered as overprotective in this respect (Ward *et al.* 2003). In fact, the composition of their mineral moiety (especially major cations) is more reminiscent of the ancestral human diet (high in K, poor in Na and rich in Ca and organic anions such as citrate) than of Western diets (with opposite characteristics). This type of rat diet leads to alkaline urine pH and moderate Ca and Mg excretion, and is a poorly suitable model to evaluate the effectiveness of high-K foods, such as fruits or vegetables. To address this problem, a model of a Westernised rat diet was designed and evaluated, and its responsiveness to supplementation with different K salts (chloride, bicarbonate or malate) was assessed.

Materials and methods

Preparation of rat diets

The control diet contained 200 g casein/kg (Louis François Cie, Saint-Maur, France) and 10 g methionine/kg, 100 g fat/kg (maize

Abbreviation: ACL, ATP citrate lyase.

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oil–groundnut oil; 50:50, w/w), 150 g sucrose/kg, 15 g NaCl/kg, 16.8 g CaH(PO₄)₂(H₂O)/kg, 5 g MgSO₄(7H₂O)/kg, 4.8 g KCl/kg (Sigma, St Louis, MO, USA), 10 g trace element mix/kg (AIN-93M; Bethlehem, PA, USA), 10 g vitamin mix/kg (AIN-76A; Bethlehem, PA, USA) and wheat starch *quantum satis* to make up to 1000 g (here 478 g/kg). High-K diets were prepared on the same basis as the control diet, by adding either 38.2 g KCl/kg (KCl diet), 51.8 g KHCO₃/kg (potassium bicarbonate diet) or 50 g potassium malate/kg (potassium malate diet), at the expense of wheat starch. The concentrations of Na, Ca and Mg in all diets were 5.9, 4.0 and 0.5 g/kg, respectively. Dietary K concentration was either 2.5 g/kg (control diet) or 20 g/kg (high-K diets).

Animals and sampling procedures

Male Wistar rats (IFFA/CREDO, L'Arbresle, France) weighing approximately 180 g were randomly allocated to four groups of eight rats and fed one of the four semi-purified diets distributed as a moistened powder for 21 d. The rats were housed in wire-bottomed cages, and maintained in temperature-controlled rooms (22°C) with a dark period from 20.00 to 08.00 hours and free access to food during the dark period. Body weight was recorded on days 0, 7, 14, and 21 of the experiment; food intake determination and collection of urine were performed on four consecutive days at the end of the experiment. Rats were maintained and handled according to the recommendations of the Institut Nationale de la Recherche Agronomique Ethics Committee, in accordance with regional decree no. 87–848.

At the time of sampling (09.00 hours), rats were anaesthetised with sodium pentobarbital (40 mg/kg) and maintained on a plate at 37°C. Blood was drawn from the abdominal aorta into a heparinised syringe and plasma was obtained after centrifugation at 10 000 g for 2 min. Plasmas were stored at –20°C until analysis. After sampling, the rats were killed by an overdose of sodium pentobarbital.

Analytical procedures

Glucose, lactate, ammonia and urea were measured by enzymic procedures as previously described (Rémésy *et al.* 1978). Enzymes and coenzymes were purchased from Sigma (St Louis, MO, USA). Na and K in plasma and urine were quantified by flame photometry (PH90/ISA, Pouilly, France) and a PE560 atomic absorption spectrometer (Perkin Elmer, St-Quentin-en-Yvelines, France) was used for Ca and Mg measurements.

For analysis of urine anions, urine was 400-fold diluted with milli-Q water and analysed using a DX320 Dionex chromatograph (Dionex, Sunnyvale, CA, USA). Plasma samples were deproteinised by 4 volumes of methanol and then 50-fold diluted with milli-Q water. The anions were separated on a 4 × 250 mm AS 11 column/AG 11 pre-column (flow rate 1 ml/min). An EG40 eluent generator controlled elution by an OH[–] gradient (0.5 to 35 mmol/l in 20 min) and the conductivity detector was preceded by an ASRS self-regenerating suppressor. Peaks were identified and quantified by comparison with pure anion standards (Dionex).

Statistical analysis

Values are given as means with their standard errors and significance of differences ($P < 0.05$) between mean values was determined by ANOVA coupled with the Student's Newman–Keuls' test.

Results

Food intake, weight gain and urine parameters

Food intake was about 17 g/d except in the KCl diet group, which exhibited a lower intake, and hence a slightly lower energy intake (Table 1). Daily weight gain was significantly reduced in rats fed the K salts, especially in the KCl group, but when corrected for the energy density of the different diets (5% less than the basal diet with the K-salt diets) the differences were no longer significant. Daily urine excretion, 15.7 ml/d in controls, was markedly elevated in rats fed the KCl diet and, to a lesser extent, in those fed the KHCO₃ diet. Urine pH was acidic (about 5.5) in rats adapted to the control diet as well as to the KCl diet, whilst it was alkaline in rats fed the KHCO₃ or potassium malate diets (in the range of 7.7–8.05). Urine total N excretion (268 mg/d with the basal diet) was in the range of 300–360 mg/d in rats adapted to the K diets (NS).

Plasma metabolites and minerals

As shown in Table 2, arterial plasma concentrations of glucose, lactate, Na, K and Mg were not significantly altered by the diet conditions. There was a trend towards a slightly higher calcaemia in rats fed the alkalinising K diets, especially the potassium malate diet ($P < 0.01$). Plasma urea was significantly higher in rats fed the K diets than in controls, but there was no significant difference between the K diet groups. Plasma sulfate

Table 1. Daily food intake, weight gain and urinary parameters (eight rats per group) (Mean values with their standard errors)

	Control diet		KCl diet		KHCO ₃ diet		Potassium malate diet	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Food intake (g/d)	17.34	0.54	15.51	0.35	17.65	0.61	16.66	0.40
Weight gain (g/d)	6.04	1.34	5.14	1.40	5.54	1.33	5.35	1.29
FCE	0.35		0.33		0.31		0.32	
Urine excretion (ml/d)	15.7	2.1	27.9*	3.2	21.9	2.2	23.5*	2.1
Urine N excretion (mg/d)	268	30	363	42	300	26	364	35
Urine pH	5.50	0.09	5.44	0.10	8.05*	0.08	7.71*	0.16

FCE, food conversion efficiency (g weight gain/g food intake).

Mean value was significantly different from that for the control condition (* $P < 0.05$).

Table 2. Arterial plasma concentration of glucose, lactate, urea and minerals (eight rats per group) (Mean values with their standard errors)

	Control diet		KCl diet		KHCO ₃ diet		Potassium malate diet	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glucose (mmol/l)	9.34	0.58	9.05	0.40	9.03	0.10	9.34	0.24
L-Lactate (mmol/l)	2.51	0.28	2.42	0.14	2.53	0.12	2.28	0.08
Urea (mmol/l)	6.51	0.30	8.79*	0.70	9.95*	0.48	9.15*	0.64
Sulfate (mmol/l)	1.35	0.36	1.13	0.29	1.37	0.40	1.61	0.38
Na (mmol/l)	143	5	148	6	142	5	146	3
K (mmol/l)	4.17	0.29	4.61	0.26	3.73	0.15	4.13	0.12
Ca (mmol/l)	2.36	0.07	2.48	0.03	2.56*	0.03	2.59*	0.03
Mg (mmol/l)	1.35	0.04	1.29	0.02	1.21	0.06	1.36	0.03

Mean value was significantly different from that for the control condition (* $P < 0.05$).

concentration was in the range of 1.1–1.6 mmol/l and was not significantly altered by diet conditions.

Urinary cation and anion excretion

The results of cation analysis are shown in Figs. 1 and 2. Na excretion was about 4 mmol/d with all the diets whereas K excretion was practically 10-fold greater in rats fed the high-K diets than in controls accounting for the near-totality of K intake in rats fed the high-K diets. In contrast, there was a difference between K intake and K excretion in rats fed the control diet (excreted 0.85 mmol/d *v.* 1.1 mmol/d ingested). Urine Mg excretion in rats fed the control or the KCl diet was much greater (0.16–0.18 mmol/d) than in rats fed the alkalinising K diets (0.02–0.04 mmol/d). As a result, Mg excretion represented about 50% of the daily intake in rats fed the control or KCl diets, compared with 6–12% in those fed the alkalinising diets. Ca excretion in control rats was 0.36 mmol/d, much lower than that observed in rats fed the KCl diet (mean 0.93 mmol/d, with individual values peaking at 1.4 mmol/d). Ca excretion was reduced by the KHCO₃ and potassium malate diets, the KHCO₃ diet appearing more effective in this respect. It must be noted that urine Ca excretion represents a minute fraction of Ca intake, 2% on average, except in rats fed the KCl diet (about 6%). Calculated H⁺ excretion (see Equation 1) was about 50 μmol/d in controls and 100 μmol/d in rats fed the KCl diet, compared with less than 1 μmol/d in rats fed the KHCO₃ or potassium malate diets. In parallel, ammonium excretion was maximal in rats fed the control diet, and still noticeable in those adapted to the KCl diet, whereas it was strongly reduced in rats fed the KHCO₃ and potassium malate diets.

$$(10^{(-\text{pH})} \times 10^6) \times (\text{urine flow in ml/24 h}) \quad (1)$$

Results of anion analysis in Figs. 3 and 4 show that chloride excretion was in the range of 5–6 mmol/d in control rats or rats fed the KHCO₃ or potassium malate diet, and about 13 mmol/d in rats adapted to the KCl diet. Due to a relatively high provision of sulfur amino acids, there was a substantial excretion of sulfate in urine (about 0.9 mmol/d); sulfate excretion was significantly higher (1.3 mmol/d) in rats fed the potassium malate diet. Phosphate excretion in the present experiment was not particularly responsive to alkalinising conditions (maximal in rats fed the KCl diet). Citrate and 2-ketoglutarate excretion proved extremely sensitive to acid–base conditions. Both anions were undetectable in urine under acidic pH conditions; they were excreted at a

substantial rate in rats fed the KHCO₃ and potassium malate diets but 2-ketoglutarate excretion (on a molar basis) was lower than that of citrate. Oxalate excretion was in the μmol/d range; in contrast to citrate or 2-ketoglutarate, it was maximal in control conditions and markedly depressed with the high-K diets, especially the potassium malate diet.

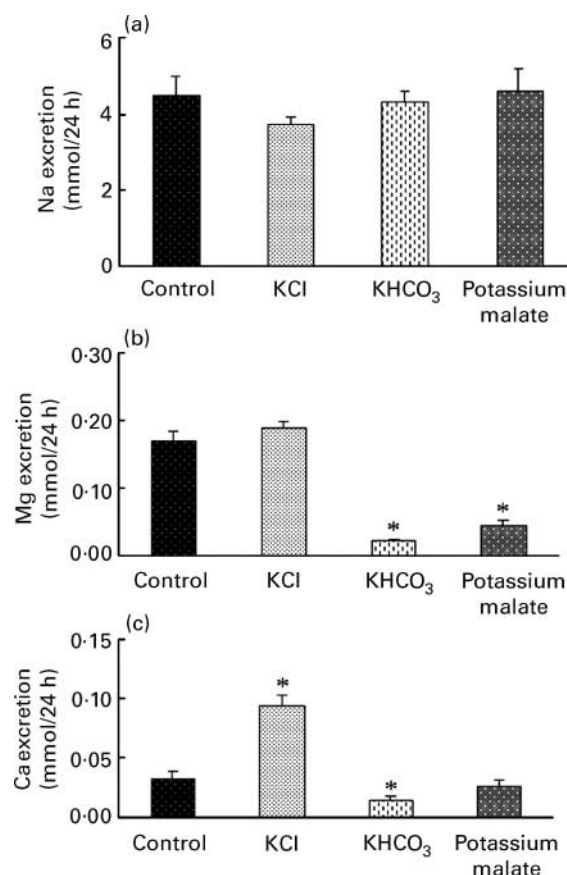


Fig. 1. Urinary excretion of Na (a), Mg (b) and Ca (c) in rats adapted to a control acidogenic diet or diets supplemented with various K salts (Cl, HCO₃ or malate). Values are means for eight rats per group (mmol/24 h), with their standard errors represented by vertical bars. Urine was collected over four consecutive days before rat slaughter and pooled. Mean value was significantly different from that for the control condition (* $P < 0.05$).

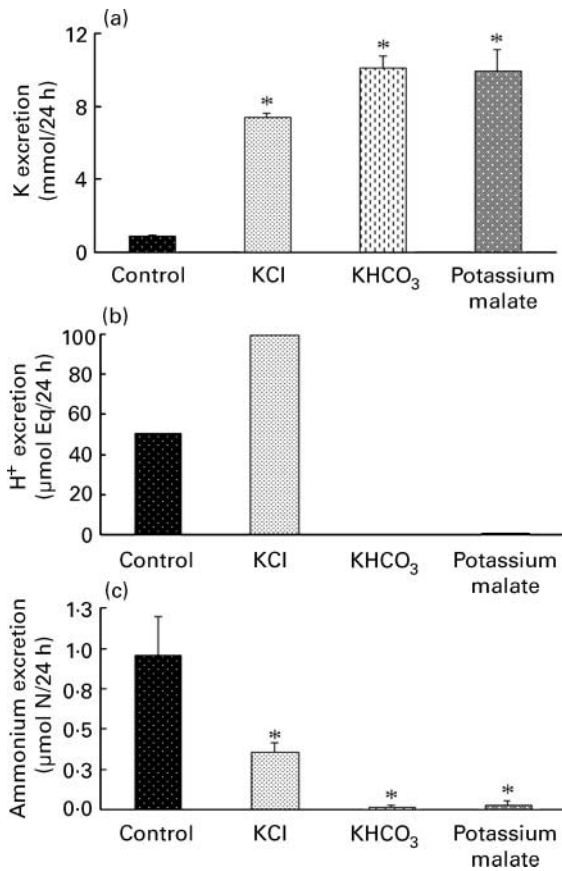


Fig. 2. Urinary excretion of K (a) (mmol/24 h), H⁺ (b) and ammonium (c) (μmol/24 h) in rats adapted to a control acidogenic diet or diets supplemented with various K salts (Cl, HCO₃ or malate). Values are means for eight rats per group, with their standard errors represented by vertical bars. Urine was collected over four consecutive days before rat slaughter and pooled. Mean value was significantly different from that for the control condition (**P* < 0.05).

Discussion

The characteristics of the present acidogenic diet are typical of the so-called Western diet and probably provide a more physiological model of latent acidosis than, for example, addition of NH₄Cl in drinking water, which is effective in providing an overload of hydrogen ions but also profoundly disturbs N metabolism (Cheema-Dhadli *et al.* 1987). Dietary protein intake in excess of maintenance requirements generates a substantial fixed acidity, namely SO₄²⁻, as a result of sulfur amino acid catabolism (Remer, 2000). Methionine supplementation itself is an effective mean to increase urinary net acid excretion as SO₄²⁻ (Remer & Manz, 1994). The present results suggest that increasing dietary K is not effective against acidosis in the form of the Cl salt, as previously reported (Morris *et al.* 1999). In fact, acidosis may even be more pronounced with KCl in the diet, as reflected by the very high rate of Mg, Ca or H⁺ excretion. This point is noteworthy since KCl is frequently proposed as a substitute of NaCl in dietetic interventions aiming at lowering blood pressure and the risk of stroke (Gillera *et al.* 1996). Some previous studies support the view that KCl loading could decrease acid excretion, but to a lesser extent than KHCO₃, and an indirect effect of K on H⁺ excretion has been postulated (van Buren *et al.* 1992). Nevertheless, it must be kept in mind that

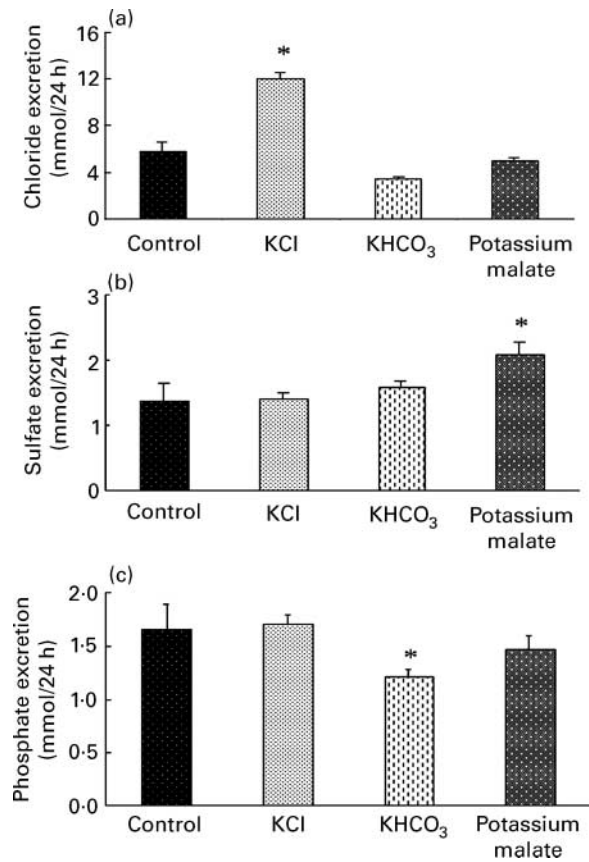


Fig. 3. Urinary excretion of chloride (a), sulfate (b) and phosphate (c) in rats adapted to a control acidogenic diet or diets supplemented with various K salts (Cl, HCO₃ or malate). Values are means for eight rats per group (mmol/24 h), with their standard errors represented by vertical bars. Urine was collected over four consecutive days before rat slaughter and pooled. Mean value was significantly different from that for the control condition (**P* < 0.05).

investigations in this domain have seldom been carried out in acidotic models.

In contrast to KCl, KHCO₃ or potassium malate were effective in alkalinising urine and counteracting various adverse effects of low-grade acidosis such as excessive Ca and Mg elimination, or hypocitraturia. Various Na⁺-dependent dicarboxylate transporters (for succinate, 2-ketoglutarate and probably malate) have been identified in the digestive tract, with K_m for substrates ranging from about 1 mmol/l down to less than 100 μmol/l (Pajor, 1999). The intestine plays a major role for malate or citrate absorption and metabolism (Wolffram *et al.* 1994; Pajor, 1999), which yields CO₂ or other intermediate metabolites such as amino acids (alanine, proline, etc) or lactate. In the liver the removal of circulating dicarboxylates involves at least two transport systems; a Na⁺-dicarboxylate symport and a system of anion exchange (Zimmerli *et al.* 1992). Organic anions are then channelled in different metabolic pathways: (i) direct oxidation to CO₂; (ii) incorporation into glucose and liver glycogen; or (iii) use for glutamine synthesis in perivenous hepatocytes (Stoll *et al.* 1991).

In control or KCl-fed rats, the percentage of ingested Mg excreted by the kidneys was high (about 50%). Considering that the percentage of Mg absorption in normal rats is about 70 (Coudray *et al.* 2002), there was certainly a very poor Mg retention in acidotic rats in contrast to rats fed alkalinising K diets. It is

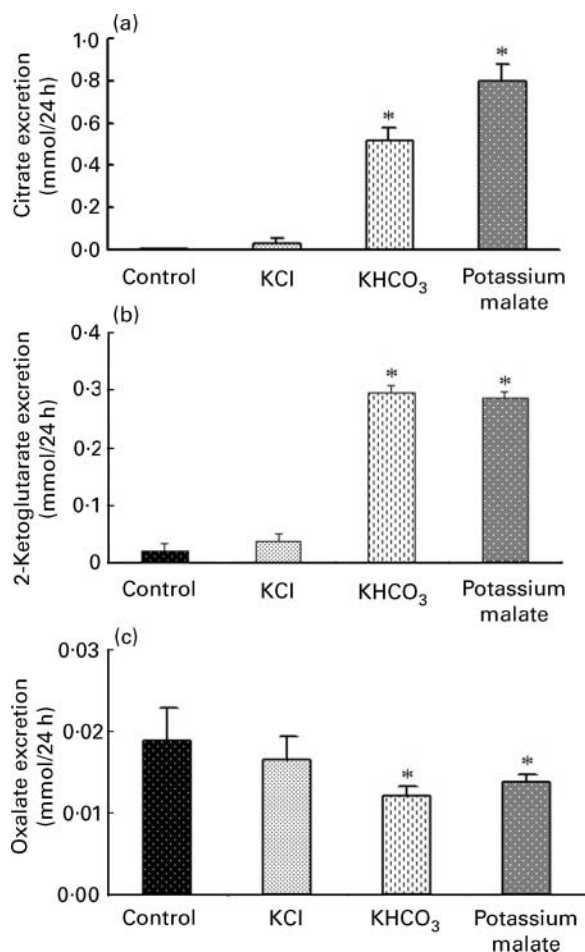


Fig. 4. Urinary excretion of citrate (a), 2-ketoglutarate (b) and oxalate (c) in rats adapted to a control acidogenic diet or diets supplemented with various K salts (Cl, HCO₃ or malate). Values are means for eight rats per group (mmol/24 h), with their standard errors represented by vertical bars. Urine was collected over four consecutive days before rat slaughter and pooled. Mean value was significantly different from that for the control condition (* $P < 0.05$).

well established that metabolic acidosis is associated with urinary Mg wasting, possibly due to a direct effect of H on distal Mg transport (Dai *et al.* 1997; Quamme, 1997). In spite of the large fluctuations of renal Mg excretion, plasma Mg concentration was extremely constant. However, since extracellular Mg represents less than 1% of the total Mg pool, it remains to be assessed if the changes observed in the present study had any significant influence on the intracellular Mg activity (Ryan, 1993). Ca excretion represents a minute percentage of Ca supplied by the diets (presently 1–2%) except in rats fed the KCl diet (about 6%). Rat diets are frequently rich in Ca (4 g/kg in the present experiment), and since Ca absorption is subjected to a tight regulation, the observed excretion of Ca could represent a relatively substantial part of absorbed Ca. In this view, calcaemia was significantly lower in rats fed the control or KCl diets than in rats fed the alkalinising diets. Conceivably, rats fed these last diets had a normal calcaemia whereas acidotic rats were relatively hypocalcaemic. The comparison between Ca and Mg excretion in the present experiment suggests that Mg may play a substantial role, besides Ca, in the compensation of low-grade metabolic acidosis (in the short term at least).

It is noteworthy that the alkalinising diets slightly enhanced SO₄²⁻ excretion. Metabolic acidosis has been shown to reduce SO₄²⁻ reabsorption in the kidneys (Puttappathi *et al.* 1999) as well as chronic renal failure, which depresses Na⁺/SO₄²⁻ co-transport (NaSi) and SO₄²⁻/oxalateⁿ⁻-HCO₃⁻ exchanger (Sat-1) (Fernandes *et al.* 2001). On this basis, alkalinising agents might restore SO₄²⁻ reabsorption through a reversal of transporter down regulation by acidosis and possibly through provision of HCO₃⁻ for the SO₄²⁻/anion exchanger. In fact, the greater rate of SO₄²⁻ excretion in rats fed the alkalinising diets suggests that its reabsorption, whether activated or not, could not match an increased generation of SO₄²⁻. Other sulfur metabolism endproducts have not been measured, such as taurine, but they generally represent a minor part of total sulfur catabolism (Nakamura *et al.* 2002).

Phosphate is the major buffer system in urine and its excretion is generally increased during acidosis, as a result of a decrease of the preferentially transported form (HPO₄²⁻), together with a direct effect of pH on the apical phosphate carrier in the proximal tubule (Ambuhl *et al.* 1998). This response to acidosis was not apparent in the present experiment, possibly a reflection of the high level of phosphate in the rat diets (also a feature of Western diets). Citrate transport is also frequently analysed owing to its sensitivity to systemic pH; citrate excretion is altered by changes in the transported chemical form, namely citrate²⁻, and in kidney metabolism (Brennan *et al.* 1986). It is now well established that acidosis promotes hypocitraturia and the tubular reabsorption of citrate. In rats, in parallel to hypocitraturia, chronic acidosis increases the cortical activity of ATP citrate lyase (ACL); abundance of the ACL protein in the renal cortex may be dramatically enhanced within a few days, but with little change in ACL mRNA (Melnick *et al.* 1996). Hypokalaemia also promotes hypocitraturia and a rise of ACL activity. In the rat, chronic metabolic acidosis and K deficiency also raises aconitase activity and, conversely, alkalinising agents in the diet may slightly depress its activity (Melnick *et al.* 1998). In parallel, activity of the apical Na⁺/citrate co-transporter is enhanced by metabolic acidosis in the kidneys, but seems unresponsive to the addition of alkalinising agents in the diet (Aruga *et al.* 2000). 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 this view, it has been proposed that 'low vegetable fibre intake and low urine volume' could be added to the list of risk factors for low urine citrate (Hess *et al.* 1994). 2-Ketoglutarate also represents a noticeable contribution to anion excretion, although less than that of citrate. Kidneys exhibit substantial capacities of 2-ketoglutarate metabolism and transport (Burckhardt & Burckhardt, 2003) and it has been shown that acute metabolic acidosis has little effect on renal handling of 2-ketoglutarate, whereas alkalosis results in the addition of 2-ketoglutarate by the renal cells both to blood and luminal fluid (Martin *et al.* 1989).

In conclusion, the present study proposes a model of low-grade metabolic acidosis of dietary origin in the rat. This model is responsive to dietary K manipulations and it establishes that, for example, potassium malate is practically as potent as potassium bicarbonate in exerting alkalinising effects. The present experiment raises questions about the actual effects of K organic salts on protein metabolism, since effects of potassium malate on blood urea are in line with a role of hepatic urea synthesis as a pathway for the removal of metabolically generated bicarbonate (Häussinger, 1997) but are less consistent with the purported inhibition of proteolysis by alkalinising agents (Greiber & Mitch,

1992). Another noticeable observation is the fact that Mg shows the same responsiveness to urine acidification or alkalisation as Ca and it would be interesting to assess whether Mg, abundant in plant foods together with K, is liable to spare Ca in case of metabolic acidosis.

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