Effect of various chloride salts on the utilization of phosphorus, calcium, and magnesium

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Only part of the effect of dietary protein on urinary calcium excretion can be ascribed to sulfur amino acids. We hypothesized that chloride, another factor often associated with isolated proteins, and another amino acid, lysine, affect utilization of calcium. The effects of supplemental dietary chloride, inorganic or organic, on calcium, phosphorus, and magnesium utilization were studied in two rat studies. Weanling Sprague-Dawley rats were fed semi-purified diets that contained moderate (1.8 mg Cl/g diet) or supplemental (15.5 mg Cl/g diet) chloride as sodium chloride, potassium chloride, or lysine monohydrochloride with or without calcium carbonate for 56 or 119 days. Rats fed supplemental sodium chloride or potassium chloride had higher urinary phosphorus excretion, more efficient phosphorus absorption, but unchanged tissue phosphorus levels after 7 and 16 weeks of dietary treatment as compared to rats fed moderate chloride. Rats fed supplemental sodium chloride or potassium chloride excreted more calcium in urine at 7 weeks and absorbed calcium less efficiently at 16 weeks. Tissue calcium concentrations were unaffected, but total tibia magnesium and plasma magnesium concentrations were lower in rats fed supplemental sodium chloride or potassium chloride than those fed moderate chloride. Lysine chloride with or without additional calcium elevated urinary calcium excretion even more than sodium chloride and potassium chloride ingestion. Rats fed lysine chloride with supplemental calcium had smaller apparent absorption and urinary losses of phosphorus and magnesium after 16 weeks and lower tibia and plasma magnesium concentrations than rats fed lysine chloride.

Keywords: chloride; calcium; magnesium; phosphorus; bioavailability; lysine

Introduction

It is well established that ingesting additional protein increases urinary calcium losses.[1,2] This effect is sometimes ascribed to the sulfur amino acid content of the protein.[3,4] However, Zemel et al.[4] showed that only 43% of increased urinary calcium losses caused by dietary protein could be attributed to the sulfur amino acid content. We hypothesized that some of the effect of proteinaceous foods on urinary calcium excretion could be due to the chloride anion often associated with protein, especially isolated protein, and/or to non-sulfur containing amino acids.

Previously, investigators have noted increased urinary calcium excretion when additional sodium chloride was fed,[5-10] and several of these investigators have ascribed the effect to sodium alone.[5-7] However, Whiting and Cole[11] reported the ingestion of additional chloride as various salts increased urinary calcium losses. The long-term effect of high chloride ingestion on mineral balance and tissue mineral levels needs further investigation in order to assess its practical significance.

Potassium chloride is substituted frequently for sodium chloride in low "salt" foods.[12] Although sodium and potassium are antagonistic in regard to their effects on blood pressure,[13] it cannot be assumed that this is true in regard to interactions with calcium. The impact of high levels of dietary potassium in conjunction with chloride on mineral utilization has not been thoroughly elucidated.

The purpose of our studies was to evaluate the effect supplemental chloride fed with an amino acid, lysine chloride, or an inorganic salt, sodium, or po-

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Materials and methods

Experimental design

Two studies were conducted. In both Study 1 and Study 2, 40 rats were fed one of five semi-purified diets: basal diet which contained 1.8 mg Cl/g (by analysis) (Basal Diet); basal diet supplemented with 0.4 mEq chloride as sodium chloride (Diet High Cl:Na); basal diet supplemented with 0.4 mEq chloride as potassium chloride (Diet High Cl:K); basal diet supplemented with 0.4 mEq chloride as lysine monohydrochloride (Diet High Cl:Lys); or with 0.4 mEq chloride as lysine monohydrochloride and 0.4 mEq calcium as calcium carbonate (Diet High Cl:Ca & Lys). The rats were fed for 56 days in Study 1, and for 119 days in Study 2.

Animals and diets

Weanling, male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) were used in both studies. All rats were housed individually in stainless steel, wire bottomed cages. The facilities met the requirements of the American Association for Accreditation of Laboratory Animal Care.

Semi-purified diets were formulated according to the guidelines of the American Institute of Nutrition.[14] The basal diets contained 50% sucrose, 20% lactalbumin (Teklad Test Diets, Madison, WI), 15% cornstarch, 5% cellulose (Teklad Test Diets), 5% corn oil, 3.5% AIN-76 mineral mix, 1% AIN-76 vitamin mixture (Teklad Test Diets), 0.3% dl-methionine, and 0.23% choline dihydrogen citrate. Chloride salts were added to the respective diets with cornstarch being varied to balance the diets. The diets supplemented with chloride were determined to contain 15.5 mg Cl/g diet. The diets were analyzed to contain 5.06 mg Ca/g diet (except, Diet High Cl:Ca & Lys which contained 13.7 mg Ca/g diet), 0.5 mg Mg/g diet, and 4.40 mg P/g diet.

Deionized water was offered ad libitum. Feed consumption was recorded daily. Rats were weighed once a week.

Sample collection and analyses

Urine was collected during week 7 of Study 1, and during weeks 7 and 16 of Study 2. Urine was acidified, diluted, and frozen. Fecal samples were collected during weeks 7 and 16 in Study 2. Feces were dried to a constant weight, cleaned of foreign adhering matter, and ground to a fine powder.

Rats were fasted overnight, anesthetized, and killed by exsanguination at the conclusion of each study. Blood was collected via cardiac puncture in heparinized tubes. Kidneys and tibias were excised, cleaned, and weighed. All tissue samples and plasma were placed in acid-washed containers and frozen until analyses were done.

Diets, tissues, plasma, urine, and fecal samples were analyzed for calcium and magnesium by atomic absorption spectroscopy[15] and for phosphorus[16] by colorimetric procedures. Milk standard (SRM #1549) obtained from the National Institute of Standards and Technology (NISI) (Gaithersburg, MD) was analyzed with each batch of experimental samples. Milk standards were determined to contain 96% (N = 40), 97% (N = 34), and 96% (N = 38) of the certified NIST value for calcium, magnesium, and phosphorus.

Percent apparent absorption of minerals was calculated by the formula: [(intake − fecal loss) ÷ intake)] × 100. Apparent retention of minerals (mg/d) was calculated by the formula: (intake − fecal loss − urine loss) ÷ days analyzed.

Statistical analysis

The effects of dietary treatments were evaluated within the framework of general linear models for analysis of variance.[17] Tests for orthogonal contrasts were used to differentiate among means for variables that had been found to be affected significantly by the treatments. The contrasts reflected the purposes of the study: to determine the effects of inorganic chloride salts (Diets Basal vs High Cl:Na and High Cl:K), to determine the effect of amino acid salts (Diets High Cl:Lys and High Cl:Lys & Ca vs other diets), to compare the effects of NaCl and KCl (Diets High Cl:Na vs High Cl:K), and to assess the affect of added calcium in reversing the effects of lysine chloride (Diets High Cl:Lysine vs High Cl:Ca & Lys).

Results

Phosphorus utilization

In the first study after 7 weeks of dietary treatment, the ingestion of supplemental chloride as NaCl or KCl significantly increased urinary phosphorus excretion (*Table 1*). There was no difference in the effect of NaCl and KCl.

In the second study as in the first study, the ingestion of supplemental chloride as NaCl or KCl significantly increased urinary phosphorus excretion at 7 weeks (*Table 2*). The effect was sustained at 16 weeks. However, apparent absorption of phosphorus was also increased significantly; thus overall phosphorus balance was not affected by the ingestion of supplemental inorganic chloride.

Similarly, the ingestion of lysine chloride significantly affected urinary phosphorus excretion and apparent absorption of phosphorus after 7 (Studies 1 and 2) and 16 (Study 2) weeks. Rats ingesting supplemental calcium with lysine chloride had depressed urinary phosphorus excretion and apparent absorption of phosphorus as compared to rats fed just supplemental lysine chloride throughout the study. After 7 weeks, apparent retention of phosphorus as determined by balance techniques was less in rats fed lysine chloride than in rats fed the other diets.

The ingestion of inorganic chloride for 8 weeks in

Table 1 Urinary excretion of calcium, magnesium, and phosphorus by rats fed various chloride salts in Study 1

Dietary treatment	Urine P	Urine Ca	Urine MG
	(mg/day)		
Basal	15.2 ± 1.1[b]	1.4 ± 0.2[a,b]	2.6 ± 0.3
High Cl:Na	19.8 ± 1.3	3.5 ± 0.5	2.7 ± 0.2
High Cl:K	18.3 ± 1.4	2.6 ± 0.3	2.3 ± 0.2
High Cl:Lys	17.8 ± 1.0	4.1 ± 0.4	1.9 ± 0.2
High Cl:Ca & Lys	1.2 ± 0.2	8.7 ± 1.1	2.0 ± 0.1
Orthogonal contrasts of treatments	Probability values		
Basal vs. High Cl:Na & High Cl:K	0.1	0.05	NS[c]
High Cl:Lys & High Ca & Lys vs. others	0.0001	0.0001	0.01
High Cl:Na & High Cl:K	NS[c]	NS[c]	NS[c]
High Cl:Lys vs. High Cl:Ca & Lys	0.0001	0.0001	NS[c]

[a] Values are means ± SEM; N = 8 rats/treatment. [b] Treatments in column differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal controls are shown below. [c] Not significant.

Table 2 Phosphorus utilization of rats fed various chloride salts in Study 2

Dietary treatment	Urine P		Apparent absorption P[a]		Apparent retention P[b]	
	7 weeks	16 weeks	7 weeks	16 weeks	7 weeks	16 weeks
	(mg/d)		(%)		(mg/d)	
Moderate Cl	16.3 ± 1.1[c,d]	20.0 ± 1.1[d]	69 ± 2[d]	51 ± 2[d]	29 ± 1[d]	14 ± 2
High Cl:Na	20.8 ± 0.7	26.8 ± 1.1	74 ± 1	57 ± 2	27 ± 1	12 ± 2
High Cl:K	20.3 ± 1.0	29.1 ± 2.2	73 ± 2	55 ± 2	26 ± 1	8 ± 2
High Cl:Lys	21.8 ± 0.9	32.0 ± 2.9	74 ± 1	57 ± 2	24 ± 1	5 ± 3
High Cl:Ca & Lys	1.4 ± 0.3	2.7 ± 0.8	42 ± 2	21 ± 5	25 ± 1	10 ± 3
Orthogonal contrasts of treatments	Probability values					
Basal vs. High Cl:Na & High Cl:K	0.0001	0.005	0.05	0.05	NS[e]	NS[e]
High Cl:Lys & High Ca & Lys vs. others	0.0001	0.0001	0.0001	0.0001	0.005	NS[e]
High Cl:Na & High Cl:K	NS[e]	NS[e]	NS[e]	NS[e]	NS[e]	NS[e]
High Cl:Lys vs. High Cl:Ca & Lys	0.0001	0.0001	0.0001	0.0001	NS[e]	NS[e]

[a] Apparent absorption (%) = [(intake − feces) ÷ intake] × 100. [b] Apparent retention (mg/d) = (intake − feces − urine) ÷ number of days studied. [c] Values are means ± SEM: N = 8 rats/treatment. [d] Treatments in column differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal contrasts are shown below. [e] Not significant.

Study 1, or for 17 weeks in Study 2, had no significant effect on tibia phosphorus concentrations (*Table 3*). However, the ingestion of lysine chloride resulted in elevated concentrations of phosphorus but lower total amounts of phosphorus in tibias in both studies. These data reflect the fact that in both studies the rats fed lysine Cl were smaller than the rats fed the other diets.

None of the dietary changes affected kidney or plasma phosphorus concentrations. To conserve space, only kidney and plasma phosphorus concentrations after 17 weeks of treatment are reported in Table 3.

After 8 weeks, rats in Study 1 fed the basal diet and diets supplemented with NaCl, KCl, lysine chloride, and lysine chloride with calcium weighed: 277 ± 6 (mean ± SEM), 213 ± 9, 262 ± 14, 222 ± 9, and 223 ± 8 g, respectively. In the second study after 17 weeks, the rats fed the basal diet and the diets supplemented with NaCl, KCl, lysine chloride without and with additional calcium weighed: 356 ± 7, 352 ± 7, 348 ± 6, 317 ± 7, and 319 ± 6 g, respectively. The reduced weights of rats fed lysine chloride were not due to decreased feed intake by rats fed lysine chloride, because the feed intakes of rats in the other treatments in both studies were limited to the intakes of rats fed lysine chloride. The restriction in feed intake was small; the average feed intake of rats fed the basal diet and the diets with supplemental NaCl, KCl, lysine Cl without and with additional calcium were: 12.5 ± 0.4, 12.8 ± 0.3, 12.7 ± 0.4, 12.5 ± 0.4, and 12.6 ± 0.4 g/day in Study 1, respectively, and 12.9 ± 0.3, 12.9 ± 0.5, 13.2 ± 0.3, 13.1 ± 0.3, and 13.2 ± 0.3 g/day in Study 2, respectively.

Calcium utilization

After 7 weeks of dietary treatment, the ingestion of supplemental chloride as NaCl or KCl significantly increased urinary calcium excretion in Study 1 ($P < 0.05$: *Table 1*) and in Study 2 ($P < 0.051$: *Table 4*).

Table 3 Tissue phosphorus concentrations in rats fed various chloride salts for 8 weeks (Study 1) and 17 weeks (Study 2)

Dietary treatment	Tibia P				Kidney P	Plasma P
	8 weeks		17 weeks		17 weeks	17 weeks
	(mg/g)	(mg/tibia)	(mg/g)	(mg/g tibia)	(mg/g)	(μg/ml)
Basal	78 ± 1[a,b]	37 ± 1[b]	88 ± 1[b]	55 ± 1[b]	2.57 ± 0.07	78 ± 7
High Cl:Na	79 ± 1	35 ± 2	88 ± 1	55 ± 2	2.57 ± 0.04	85 ± 6
High Cl:K	79 ± 1	36 ± 1	87 ± 1	56 ± 1	2.50 ± 0.08	79 ± 5
High Cl:Lys	82 ± 2	31 ± 1	90 ± 1	51 ± 1	2.55 ± 0.03	86 ± 5
High Cl:Ca & Lys	80 ± 1	30 ± 1	90 ± 1	53 ± 1	2.60 ± 0.04	89 ± 6
Orthogonal contrasts of treatments	Probability values					
Basal vs. High Cl:Na & High Cl:K	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]
High Cl:Lys & High Cl:Ca & Lys vs. others	0.05	0.0001	0.005	0.05	NS[c]	NS[c]
High Cl:Na vs. High Cl:K	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]
High Cl:Lys vs. High Cl:Ca & Lys	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]

[a] Values are means ± SEM; N = 8 rats/treatment. [b] Treatments in columns differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal contrasts are shown below. [c] Not significant.

Table 4 Calcium utilization of rats fed various chloride salts in Study 2

Dietary treatment	Urine Ca		Apparent absorption Ca[a]		Apparent retention Ca[b]	
	7 weeks	16 weeks	7 weeks	16 weeks	7 weeks	16 weeks
	(mg/d)		%		(mg/d)	
Basal	2.2 ± 0.4[c,d]	2.7 ± 0.4[d]	53 ± 2[d]	19 ± 4[d]	37 ± 1[d]	12 ± 4
High Cl:Na	3.2 ± 0.3	3.6 ± 0.5	57 ± 2	22 ± 4	42 ± 2	14 ± 3
High Cl:K	3.5 ± 0.5	3.6 ± 0.6	55 ± 2	22 ± 3	38 ± 1	14 ± 3
High Cl:Lys	5.9 ± 0.6	6.5 ± 0.6	50 ± 2	25 ± 3	32 ± 1	12 ± 2
High Cl:Ca & Lys	11.0 ± 0.9	11.1 ± 1.0	29 ± 1	15 ± 5	41 ± 2	17 ± 9
Orthogonal contrasts of treatments	Probability values					
Basal vs. High Cl:Na & High Cl:K	0.051	NS[e]	NS[e]	0.05	NS[e]	NS[e]
High Cl:Lys & High Cl:Ca & Lys vs. others	0.0001	0.0001	0.0001	NS[e]	NS[e]	NS[e]
High Cl:Na vs. High Cl:K	NS[e]	NS[e]	NS[e]	NS[e]	NS[e]	NS[e]
High Cl:Lys vs. High Cl:Ca & Lys	0.001	0.0001	0.0001	0.005	0.0001	NS[e]

[a] Apparent absorption (%) = [(intake − feces) ÷ feces] × 100. [b] Apparent retention (mg/d) = (intake − feces − urine) ÷ number of days analyzed. [c] Values are means ± SEM; N = 8 rats/treatment. [d] Treatments in column differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal contrasts are shown below. [e] Not significant.

After 16 weeks of dietary treatment, rats fed inorganic chloride did not ($P < 0.59$) excrete more calcium in urine than animals fed the basal diet but apparently absorbed calcium more efficiently ($P < 0.05$).

The ingestion of supplemental lysine without additional calcium more than doubled urinary calcium excretion, and the ingestion of supplemental lysine with calcium more than quadrupled urinary calcium excretion as compared to rats fed the basal diet in Studies 1 and 2. Ingestion of the supplemental calcium caused a significant reduction in the efficiency of calcium absorption at 8 and 16 weeks in Study 2; this is the typical response to elevated calcium intake. However, calcium balance was greater among rats fed lysine chloride with additional calcium than among those fed lysine without the additional calcium at 7 weeks.

The tissue levels of calcium reflected the excretion and balance data (*Table 5*). The ingestion of inorganic chloride did not affect tibia, kidney, or plasma calcium concentrations. However, the rats that ingested the lysine chloride had significantly less calcium in their tibias than other rats. This reflected their smaller body sizes. Plasma calcium concentrations averaged 108 ± 2 μg/ml in Study 1, and 110 ± 1 μg/ml in Study 2. There were no significant differences in plasma calcium concentrations among treatments after 8 weeks, so these data are not shown in *Table 5*.

Magnesium utilization

The ingestion of supplemental chloride as NaCl or KCl in Study 1 (*Table 1*) did not affect urinary excretion of magnesium, but in Study 2 (*Table 6*) it increased urinary excretion of magnesium slightly but significantly. Rats ingesting lysine chloride differed from the other rats in regard to urinary excretion, apparent absorption, and apparent retention of magnesium after 7 but not 16 weeks. Overall, the greatest effect of the dietary treatments on magnesium utilization occurred when rats were fed Diet High Cl:Ca & Lys. Those rats ab-

Table 5 Tissue calcium levels of rats fed various chloride salts for 8 weeks (Study 1) and 17 weeks (Study 2)

Dietary treatment	Tibia Ca				Kidney Ca		Plasma Ca
	8 weeks		17 weeks		8 weeks	17 weeks	17 weeks
	(mg/g)	(mg/tibia)	(mg/g)	(mg/tibia)	(μg/g wet wt)		(μg/ml)
Basal	160 ± 2[a]	76 ± 2[b]	192 ± 2	120 ± 3[b]	41.2 ± 1.3[b]	65.4 ± 2.7[b]	112 ± 2[b]
High Cl:Na	162 ± 3	71 ± 4	194 ± 3	122 ± 6	40.3 ± 1.3	61.4 ± 1.7	112 ± 2
High Cl:K	163 ± 1	74 ± 2	197 ± 4	127 ± 4	39.0 ± 1.3	61.1 ± 1.8	111 ± 2
High Cl:Lys	162 ± 2	62 ± 2	193 ± 3	109 ± 2	40.2 ± 1.4	58.7 ± 0.7	107 ± 1
High Cl:Ca & Lys	163 ± 2	61 ± 2	201 ± 1	118 ± 2	56.1 ± 5.1	66.6 ± 2.5	110 ± 2
Orthogonal contrasts of treatments	Probability values						
Basal vs. High Cl:Na & High Cl:K	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]
High Cl:Lys & High Cl:Ca & Lys vs. others	NS[c]	0.0001	NS[c]	0.01	0.01	NS[c]	0.05
High Cl:Na vs. High Cl:K	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]
High Cl:Lys vs. High Cl:Ca & Lys	NS[c]	NS[c]	NS[c]	NS[c]	0.0005	0.005	NS[c]

[a] Values are means ± SEM; N = 8 rats/treatment. [b] Treatments in column differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal contrasts are shown below. [c] Not significant.

Table 6 Magnesium utilization of rats fed various chloride salts in Study 2

Dietary treatment	Urine Mg		Apparent absorption Mg[a]		Apparent retention Mg[b]	
	7 weeks	16 weeks	7 weeks	16 weeks	7 weeks	16 weeks
	(mg/d)		%		(mg/d)	
Moderate Cl	3.0 ± 0.2[c,d]	3.1 ± 0.2[d]	73 ± 2[d]	59 ± 2[d]	2.1 ± 0.1[d]	1.2 ± 0.2
High Cl:Na	3.5 ± 0.2	3.7 ± 0.2	75 ± 1	63 ± 2	1.8 ± 0.1	0.8 ± 0.2
High Cl:K	3.2 ± 0.2	3.7 ± 0.2	72 ± 2	60 ± 2	1.8 ± 0.2	0.6 ± 0.2
High Cl:Lysine	3.2 ± 0.2	4.0 ± 0.1	73 ± 1	54 ± 2	1.4 ± 0.4	0.5 ± 0.2
High Cl:Ca & Lysine	2.5 ± 0.1	2.9 ± 0.1	60 ± 2	51 ± 4	1.5 ± 0.1	0.6 ± 0.2
Orthogonal contrasts of treatments	Probability values					
Basal vs. High Cl:Na & High Cl:K	0.05	0.05	NS[e]	NS[e]	NS[e]	NS[e]
High Cl:Lys & High Ca & Lys vs. others	0.0005	NS[e]	0.0001	NS[e]	0.05	NS[e]
High Cl:Na & High Cl:K	NS[e]	NS[e]	NS	NS[e]	NS[e]	NS[e]
High Cl:Lys vs. High Cl:Ca & Lys	0.0001	0.0001	0.0001	0.0001	NS[e]	NS[e]

[a] Apparent absorption (%) = [(intake − feces) ÷ intake] × 100. [b] Apparent retention = (intake − feces − urine) ÷ number of days studied. [c] Values are means ± SEM; N = 8 rats/treatment. [d] Treatments in column differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal contrasts are shown below. [e] Not significant.

sorbed magnesium less efficiently and lost less in urine.

Rats ingesting of supplemental chloride as NaCl or KCl had less magnesium per tibia and lower plasma concentrations of magnesium at 8 weeks in Study 1, and had lower concentration of magnesium in tibias after 17 weeks in Study 2 than rats fed the basal diet. The ingestion of supplemental calcium depressed tissue magnesim levels; rats ingesting Diet High Cl:Ca & Lys rather than Diet High Cl:Lys had depressed concentration of magnesium in bone (after 8 and 17 weeks) and plasma (after 8 weeks). Kidney magnesium concentrations were not affected by the dietary treatments and are not shown in *Table 7*.

Discussion

Previously, Calvo et al.[18] demonstrated that urinary calcium excretion was increased by variable amounts when diets were supplemented with various proteins. They hypothesized that the differences were related to differences in the excretion of sulfate from sulfur amino acids and perhaps sodium. But, Zemel et al.[4] found that the sulfur amino acid content of protein accounted for only 43% of the effect of the protein-rich dry ingredients on urinary calcium excretion.

We hypothesized that other differences among proteins could be important. For example, we determined by chemical analysis that the isolated proteins contained widely different levels of chloride (e.g., casein, 0.25 mg Cl/g; lactalbumin, 0.51 mg Cl/g; soy, 2.49 mg Cl/g; egg white solids, 12.2 mg Cl/g; and nonfat dry milk, 11.2 mg Cl/g).

This work confirms our previous observation in humans[19] and that of Whiting and Cole[11,20] in rats, that ingestion of supplemental chloride will increase urinary calcium losses even if sodium intakes are not elevated. Furthermore, our data suggest that potassium does not counter the effect of chloride on calcium and phosphorus utilization, but rather KCl and NaCl

Table 7 Tissue magnesium concentrations in rats fed various chloride salts for 8 weeks (Study 1) and 17 weeks (Study 2)

Dietary treatment	Tibia Mg				Plasma Mg	
	8 weeks		17 weeks		8 weeks	17 weeks
	(mg/g)	(mg/tibia)	(mg/g)	(mg/tibia)	μg/ml)	
Moderate Cl	2.68 ± 0.03[a,b]	1.27 ± 0.05[b]	2.86 ± 0.03[b]	1.70 ± 0.08	21.1 ± 0.9[b]	19.6 ± 0.7
High Cl:Na	2.54 ± 0.05	1.11 ± 0.05	2.64 ± 0.03	1.66 ± 0.07	19.7 ± 0.7	19.8 ± 0.8
High Cl:K	2.60 ± 0.04	1.18 ± 0.03	2.66 ± 0.02	1.71 ± 0.03	19.2 ± 0.8	19.1 ± 0.5
High Cl:Lys	2.96 ± 0.03	1.13 ± 0.03	3.02 ± 0.03	1.71 ± 0.03	24.1 ± 1.0	21.5 ± 1.1
High Cl:Ca & Lys	2.35 ± 0.07	0.87 ± 0.02	2.71 ± 0.04	1.59 ± 0.03	21.1 ± 0.8	20.4 ± 0.9
Orthogonal contrasts of treatments	Probability values					
Basal vs. High Cl:Na & High Cl:K	NS[c]	0.0001	0.05	NS[c]	0.05	NS[c]
High Cl:Lys & High Ca & Lys vs. others	NS[c]	0.0005	0.0001	NS[c]	0.005	NS[c]
High Cl:Na & High Cl:K	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]	NS[c]
High Cl:Lys vs. High Cl:Ca & Lys	0.001	0.0001	0.0001	NS[c]	0.01	NS[c]

[a] Values are means ± SEM; N = 8 rats/treatment. [b] Treatments in columns differ significantly as determined by analysis of variance. Differences among means as determined by orthogonal contrasts are shown below. [c] Not significant.

produce similar responses. This is contrary to Lemann et al.'s[21] suggestion that potassium may have an independent effect that leads to conservation of calcium by the kidney.

Although we demonstrated that ingestion of supplemental chloride, with sodium or potassium, initially tended to elevate urinary phosphorus and calcium excretion, we could not demonstrate differences in tissue levels of phosphorus and calcium. In contrast, Goulding and associates[22,23] observed that rats fed supplemental NaCl with low levels of calcium not only excreted more calcium and phosphorus in urine but also had decreased bone retention of these elements. They observed no compensation in the gut (i.e., increased apparent absorption) for the increased urinary losses of calcium and phosphorus as we did. This may reflect that they fed low (100 or 1000 μg Ca/g diet) levels of calcium; we fed an adequate (5000 μg Ca/g diet) level of calcium. They used 3-month-old rats[22]; we used weanling rats. These differences in the studies may be important in practical situations (e.g., when postmenopausal women are consuming less than optimal levels of calcium and high levels of NaCl). Moreover, tibia and plasma magnesium concentrations were sensitive to the effects of dietary chloride in our studies.

We hypothesized that lysine Cl could affect calcium utilization more than inorganic chloride salts. Lysine would be degraded, unlike sodium and potassium. Thus, the kidneys would have a fixed anion, chloride to excrete without a fixed cation supplied with the chloride. This could lead potentially to greater excretion of other cations, such as calcium. Moreover, Raven et al.[24] observed that although lysine promoted absorption of Ca-45 from ligated gut sections, lysine did not promote improved calcium balance in a feeding study. One potential explanation of these previous results would be increased urinary excretion of calcium.

Our observation was consistent with our hypothesis. Rats fed lysine Cl, with or without supplemental calcium, excreted more calcium in urine than rats fed the other diets. However, we did not observe improved absorption of calcium by rats fed lysine Cl. Moreover, the depression in the total calcium content, but not calcium concentration, of tibias of rats fed lysine Cl appeared to reflect, at least partially, the effect of lysine on total growth of the rats. Previously, Fico et al.[25] reported that ingestion of 2.9% lysine induced orotic aciduria and growth depression in rats.

This work demonstrates that dietary chloride, with or without sodium, can affect phosphorus, calcium, and magnesium utilization. The importance of these interactions are not clear but do deserve study because of the potentially wide, but generally unrecognized, variations in chloride intake of humans.

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