

*Original Article***Effect of an increase in the plasma potassium concentration on renal magnesium handling in healthy volunteers**

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**Abstract**

**Background.** Lower plasma magnesium concentrations are associated with clinical problems such as arrhythmias and hypertension. Plasma magnesium concentration is tightly controlled by the kidney. Modifying renal magnesium threshold may provide a means to increase the plasma magnesium concentration. Since evidence has been presented that potassium deficiency by itself may increase renal magnesium loss, the hypothesis that elevating plasma potassium would result in an increase in plasma magnesium concentration was tested in healthy volunteers.

**Methods.** Plasma potassium was raised in nine healthy volunteers by oral administration of 20 mg amiloride daily during 3 weeks. Magnesium metabolism was assessed before and after this period by plasma levels, urinary magnesium excretion and fractional magnesium excretion, and magnesium loading test (MLT). This MLT allows calculation of renal retention of a magnesium load.

**Results.** Basal plasma magnesium levels ( $0.84 \pm 0.07$  vs  $0.84 \pm 0.05$  mmol/l) as well as urinary magnesium excretion ( $4.37 \pm 1.73$  vs  $3.67 \pm 1.37$  mmol/day) and erythrocyte magnesium levels ( $1.72 \pm 0.16$  vs  $1.76 \pm 0.14$  mmol Mg/l red blood cells) were similar before and on amiloride. Plasma potassium rose significantly on amiloride ( $3.64 \pm 0.24$  vs  $4.07 \pm 0.54$  mmol/l,  $P < 0.05$ ). No change was observed in magnesium retention with the MLT:  $22.7 \pm 26.7$  vs  $29.2 \pm 20.6\%$  ( $P = 0.5$ ).

**Conclusions.** Despite an increased plasma potassium concentration, no change was observed in plasma magnesium levels, urinary magnesium excretion or renal magnesium retention of an intravenously administered magnesium load. This indicates that increasing plasma potassium within the normal range does not modify the renal magnesium threshold.

**Key words:** amiloride; magnesium; magnesium loading test; potassium

**Introduction**

Magnesium deficiency has been linked to various manifestations of cardiovascular disease. Evidence from population studies has accumulated that daily magnesium intake is inversely related to blood pressure [1–5]. A beneficial effect of oral magnesium supplementation has been observed in some studies with a randomized, placebo-controlled design [6–8]. It has also long been recognized that magnesium deficiency can be important in the pathogenesis of supraventricular and ventricular arrhythmias [9–11], also when associated with the use of digitalis [12]. Furthermore, magnesium deficiency leads to enhanced production of thromboxane and increased sensitivity to angiotensin II and vasopressin [13]. Insulin levels increase in normoglycaemic non-diabetic subjects with decreasing magnesium intake or plasma magnesium levels [5,14], indicating a link between magnesium and insulin sensitivity. The plasma magnesium concentration is well controlled and held within narrow limits [15]. The regulation of the plasma magnesium concentration takes place in the kidney by modifying reabsorption, mainly in the thick portion of the ascending loop of Henle [16]. Magnesium is thought to be reabsorbed along the transepithelial voltage gradient which is generated by the  $\text{Na}^+ - \text{K}^+ - \text{Cl}^-$  co-reabsorption, which in turn is driven by the basolateral  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  [16,17]. No hormonal system has been found to control renal magnesium homeostasis in humans; magnesium itself modifies magnesium reabsorption, magnesium reabsorption decreasing with increasing plasma magnesium concentrations and *vice versa* [15,16]. Since increasing plasma magnesium may have beneficial cardiovascular and metabolic effects [18–20], and since the plasma magnesium concentration is mainly controlled by renal magnesium handling, we looked for possibilities to increase the plasma magnesium concentration by modifying the renal magnesium threshold.

A tendency to lower plasma magnesium concentrations in the presence of hypokalaemia and in association with increased urinary magnesium excretion or impaired renal magnesium conservation has been

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described in patients with primary hyperaldosteronism [21]. Since aldosterone itself has no effect on renal magnesium handling or the plasma magnesium concentration [22–24], it can be hypothesized that aldosterone-mediated low plasma potassium concentrations may impair renal magnesium reabsorption and consequently lower plasma magnesium concentrations. In animal experiments, dietary potassium deprivation and hypokalaemia have been shown to inhibit sodium, chloride, and water reabsorption in the ascending loop of Henle [25–27]. Low extracellular potassium levels are then associated with lower substrate-determined (potassium) activity of Na-K-ATPase with consequently less Na-K-Cl reabsorption and lower voltage difference, which secondarily affects magnesium reabsorption [25]. In human pathology, Bartter's syndrome is characterized by hypokalaemia and hypomagnesaemia in conjunction with renal potassium and magnesium wasting and hyperreninaemic hyperaldosteronism [28]. Inhibition of the renin–aldosterone axis by ACE inhibition significantly improves plasma potassium and magnesium concentrations [29]. Again, since aldosterone has no direct effect on renal magnesium handling, hypokalaemia as intermediary is suggested. We therefore hypothesized that the plasma magnesium concentration increases by a chronic rise in the plasma potassium concentration.

The aim of the present study was to assess the effect of sustained elevation of the plasma potassium concentrations for 3 weeks on plasma magnesium levels as well as on the magnesium loading test as a dynamic parameter of renal magnesium handling. Potassium concentrations were increased by oral administration of amiloride for 3 weeks.

## Subjects and methods

### Subjects

Nine healthy volunteers participated in the study; three males and six females; mean age 23.5 years (range: 20–26). At the inclusion visit, plasma magnesium, potassium, and creatinine were measured. In all subjects these were within normal limits; magnesium  $0.83 \pm 0.06$  mmol/l (range 0.73–0.94 mmol/l), potassium  $3.7 \pm 0.2$  mmol/l (range 3.4–3.9 mmol/l), creatinine  $79 \pm 12$   $\mu$ mol/l (range 68–107  $\mu$ mol/l).

### Methods

The duration of the intervention period was 3 weeks. Nutritional habits were unchanged. During this period, 20 mg amiloride was taken orally, 10 mg twice daily. Amiloride raises the plasma potassium concentration by decreasing potassium secretion in the late distal and collecting tubules because amiloride interferes with sodium reabsorption at this site with subsequent fall in the transepithelial voltage gradient which determines the potassium reabsorption [30].

Two similar sets of tests were performed before and after 3 weeks of amiloride. This test set consisted of 4 days of urine collection, that is 2 days before and 1 day immediately after a magnesium loading test (MLT). This MLT was

carried out according to Ryzen *et al.* [31]. The magnesium dose of the MLT was 0.1 mmol MgCl<sub>2</sub>/kg lean body mass and infused over a period of 4 h, dissolved in 100 ml glucose 5%, and administered through a cannula in a forearm vein. Blood samples were collected just before starting the MLT, after 120 min, and after 240 min which was the end of the MLT. The following day another sample was taken. Urine was collected during the 48 h before in separate periods of 24 h, and the 24 h after the start of the magnesium infusion. In case of the MLT at baseline, the amiloride was not started until the last urine sample was collected after the MLT. Similarly, with the MLT at the end of the intervention, medication was continued until the last urine was collected. Plasma magnesium, potassium, and creatinine together with erythrocyte magnesium levels were measured before and after the MLT.

The results of the MLT are expressed as magnesium retention which is defined according to Ryzen:  $(1 - ((\text{Post-load Mg} - \text{Pre-load Mg}) / \text{Dose infused})) * 100\%$  [31]. This means that retention is zero when all the infused magnesium is excreted and that retention is 100% when post-MLT urinary excretion equals pre-MLT urinary excretion. Baseline (pre-MLT) magnesium excretion is calculated by multiplying pre-MLT urinary magnesium/creatinine ratio with total post-MLT urinary creatinine. In this way, possible inadequate collection is corrected for with the post-MLT collection as yardstick. Retention is also calculated according to the method of Johansson:  $\text{retention} = ((\text{Total load} - \text{Excretion post-load}) / \text{Total load}) * 100\%$  [32]. Total load is defined as the infused dose together with the magnesium in the diet, which is presumed equal to the baseline urinary excretion. In both calculations, the urinary magnesium excretion in the 24 h immediately before the MLT is taken as baseline excretion. Additionally, fractional magnesium excretion is calculated which is defined as  $((\text{magnesium clearance} / \text{creatinine clearance}) * 100\%)$  and given as percentage. Fractional potassium excretion is also calculated.

### Laboratory analysis

Magnesium, potassium, and creatinine in plasma and urine were determined using standard laboratory techniques. Erythrocyte magnesium levels were calculated using the plasma magnesium concentration, magnesium concentration in whole blood and the haematocrit, using the formula:

Erythrocyte Mg = Mg in whole blood – (Mg in plasma \* (1 – Ht)) / Ht, expressed as mmol Mg/l red blood cells (RBC) [33].

### Statistical analysis

Results are given as mean and standard deviation, in case of a skewed distribution as median and 95th percentile. Means were compared with the paired Student *t* test, medians with the sign-rank test. Associations between two variables were determined using univariate regression analysis; Spearman test provided the correlation coefficients. The level of significance was set at 0.05.

If a statistically significant difference was found, the mean difference is given with a 95% confidence interval (95% CI).

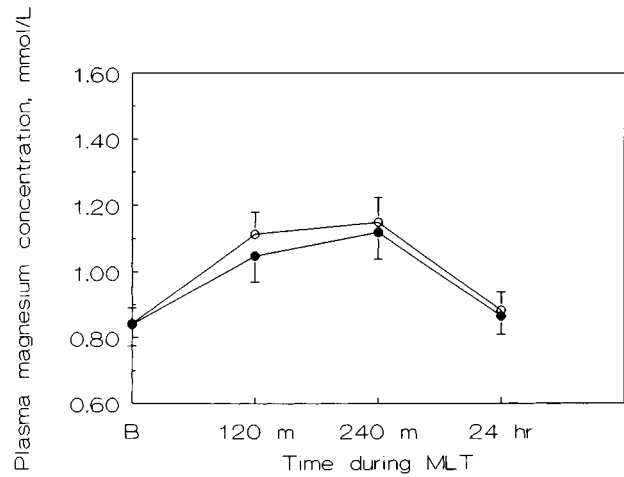
## Results

### Parameters before the loading tests

The plasma magnesium concentration before and after 3 weeks of 10 mg amiloride b.i.d. were similar:  $0.836 \pm 0.063$  and  $0.840 \pm 0.044$  mmol/l ( $P=0.9$ ), Table 1. In contrast, the plasma potassium concentration had increased from  $3.64 \pm 0.24$  mmol/l to  $4.07 \pm 0.54$  ( $P<0.05$ ); mean difference  $0.422$  mmol/l; 95% CI  $0.003-0.841$  mmol/l. There was no correlation between the plasma magnesium and potassium concentrations either at baseline ( $P=0.7$ ) or after the study period ( $P=0.8$ ). Also when the change in plasma potassium and magnesium between baseline and end of the study were plotted, no correlation emerged ( $P=0.3$ ). Amiloride intake was satisfactory, estimated by both pill counting and by the rise in the plasma potassium concentration. No significant difference was observed in the total daily urinary magnesium and potassium excretion before the first (without medication) and the second (on amiloride) MLT, Table 1. The plasma creatinine concentration before and after amiloride treatment was similar ( $82.1 \pm 11.3$  and  $84.2 \pm 10.3$   $\mu\text{mol/l}$ , respectively,  $P=0.4$ ), making a major volume contraction unlikely.

### Parameters related to the magnesium loading test

The plasma magnesium concentrations before, during, and after MLT are shown in Figure 1. With the MLT employed in this study, a very mild hypermagnesaemia was achieved. During the first MLT, plasma magnesium concentration increased significantly from baseline to 120 min with a further increase from 120 to 240 min (end of infusion) and returned to baseline level after the end of the infusion. Plasma magnesium concentration showed a similar pattern during the second MLT. Comparing plasma magnesium concentrations during the two tests reveals that the plasma magnesium concentration after 120 min is higher on amiloride than at baseline:  $1.113 \pm 0.067$  vs  $1.048 \pm 0.079$  mmol/l,  $P=0.049$ ; mean difference  $0.065$  mmol/l, 95% CI  $0.007-0.115$  mmol/l. Similarly, the increase in plasma magnesium concentration (120 min – baseline) is signi-



**Fig. 1.** Plasma magnesium concentration during the first (baseline) and second (on amiloride) magnesium loading test. MLT, magnesium loading test; B, baseline; m, min; hr, hours.

ficantly higher on amiloride compared with baseline:  $0.265 \pm 0.032$  vs  $0.220 \pm 0.044$  mmol/l,  $P=0.044$ ; mean difference  $0.045$  mmol/l, 95% CI  $0.011-0.044$  mmol/l. Erythrocyte magnesium levels did not change during the two loading tests and the levels before the test were similar:  $1.72 \pm 0.16$  vs  $1.76 \pm 0.14$  mmol Mg/l RBC,  $P=0.6$ .

### Magnesium retention

The basal magnesium retention using the calculation of Ryzen was  $22.7 \pm 26.7\%$ , magnesium retention on amiloride  $29.2 \pm 20.6\%$ ,  $P=0.5$  [31]. No correlation was found between magnesium retention before and on amiloride ( $P=0.5$ ) nor between change in plasma potassium and change in percentage retention. Individual values are shown in Table 2. The calculation of Johansson *et al.* [32] showed a baseline retention of  $14.6 \pm 17.7\%$  and a retention on amiloride of  $16.6 \pm 12.7\%$  ( $P=0.8$ ) [32]. No association was found between retention before and on amiloride or between change in plasma potassium level and change in magnesium retention.

**Table 1.** Plasma magnesium concentration before and after 3 weeks of amiloride dosage

	Baseline			Amiloride		
	Day -2	Day -1	Day of MLT	Day -1	Day -2	Day of MLT
Urine magnesium (mmol/day)	$3.90 \pm 1.31$	$4.37 \pm 1.73$	$7.74 \pm 1.75$	$3.92 \pm 1.35$	$3.67 \pm 1.37$	$8.06 \pm 2.31$
Urine potassium (mmol/day)	$85.0 \pm 28.0$	$60.6 \pm 23.6$	$59.1 \pm 7.4$	$71.2 \pm 48.7$	$69.5 \pm 27.7$	$68.9 \pm 13.6$
Urine creatinine ( $\mu\text{mol/day}$ )	$10.1 \pm 2.7$	$9.7 \pm 2.2$	$9.7 \pm 1.8$	$9.4 \pm 1.9$	$8.8 \pm 1.6$	$10.3 \pm 2.4$
Plasma magnesium (mmol/l)			$0.84 \pm 0.07$			$0.84 \pm 0.05$
Plasma potassium (mmol/l)			$3.64 \pm 0.24$			$4.07 \pm 0.54^1$
Fractional magnesium excretion (%)			$4.09 \pm 1.28$			$4.02 \pm 1.52$
Fractional potassium excretion (%)			$13.7 \pm 4.8$			$16.2 \pm 6.3$

MLT, Magnesium loading test; Day -2, 48–24 h before start of MLT; Day -1, 24–0 h before start of MLT; Day of MLT, The 24 h from start of MLT infusion; <sup>1</sup> $P<0.05$  amiloride vs baseline.

**Table 2.** Magnesium retention test (%) at baseline and on amiloride according to Ryzen (A, [31]) and Johansson (B, [32])

A			B	
Subject	Baseline	On amiloride	Baseline	On amiloride
1	41.1	36.8	18.6	16.8
2	53.5	53.0	29.7	25.8
3	20.8	15.0	10.3	7.1
4	-9.2	58.2	-5.8	40.5
5	-5.5	28.3	-3.7	21.3
6	24.1	12.4	44.1	6.7
7	21.5	-4.1	12.1	-2.3
8	-6.7	18.8	-4.4	11.7
9	64.5	44.1	30.3	22.1
Mean	22.7	29.2	14.6	16.6
SD	26.7	20.6	17.7	12.7

## Discussion

Renal magnesium handling is the most important factor in the regulation of the magnesium status of an individual. Raising magnesium levels in the long term therefore implies changing the renal magnesium threshold. Such a rise would be of importance in view of reports indicating a beneficial effect of chronically raising the magnesium levels. However, the present study in normokalaemic volunteers showed that increasing the plasma potassium concentration is not a method to change renal magnesium handling, since 3 weeks oral amiloride accompanied by a rise in the plasma potassium concentration did not change the steady-state plasma magnesium levels nor the renal handling of an intravenous magnesium load.

Renal magnesium and potassium handling are very different. Potassium is reabsorbed almost completely in the proximal tubule and potassium excretion in the late distal tubule and collecting ducts is responsible for the urinary potassium excretion [34]. Magnesium is partly reabsorbed in the proximal ( $\pm 25\%$ ) and distal ( $\pm 5\%$ ) tubule, but mainly ( $\pm 65\%$ ) in the thick ascending part of the loop of Henle [15,16]. The latter site is therefore the main site of regulation of renal magnesium reabsorption concentration. This difference in renal handling between potassium and magnesium indicates that if potassium modifies renal magnesium handling, this is the result of potassium interfering with the tubular processes involved in magnesium reabsorption and not by luminal interaction between magnesium and potassium at a common tubular site. Madler and Iseri [35] have suggested that low plasma potassium has a direct negative effect on magnesium reabsorption. The present study was aimed at testing the reverse hypothesis, that raising plasma potassium augments renal magnesium reabsorption.

In contrast to our hypothesis, we did not find that (modest) elevation of the plasma potassium concentration increased the plasma magnesium concentration or delayed the excretion of a magnesium load. Before concluding upon its possible physiological significance,

we should discuss whether this negative finding was the result of an inherent pitfall of the protocol. In particular, we should consider whether amiloride stimulates magnesium excretion directly, since that would oppose an indirect inhibitory effect of the increase in the plasma potassium concentration. However, this is very unlikely. Earlier studies have shown that amiloride, in the dosages used presently, has no acute effects on urinary magnesium excretion in healthy humans [36,37]. In rats, high dosages of amiloride do in fact the opposite, i.e. inhibition of magnesium excretion [38-42]. Thus it is questionable that small elevations of the plasma potassium concentration within the normal range, as induced presently, are indeed relevant as determinants for the magnesium balance. Whether this is different for changes in the plasma potassium concentration in the hypokalaemic range is uncertain. Amiloride causes a simultaneous rise in plasma potassium and magnesium levels in patients with heart failure treated with loop diuretics, supporting the hypothesis that increasing plasma potassium can increase plasma magnesium [43]. A similar simultaneous rise in plasma potassium and magnesium was observed in patients with Bartter's syndrome treated with a ACE inhibitor [23]. Primary potassium depletion has been shown to be able to cause reversible tubular dysfunction affecting several ions, resulting in for example hyperphosphaturic hypophosphataemia [44-46].

The present study was performed in healthy volunteers with a normal plasma potassium concentration. It is conceivable that the influence of plasma potassium on renal magnesium handling holds only for the hypokalaemic range but not for the normokalaemic range. This implies that the kalipenic effects as suggested by Nadler and Iseri reflects some kind of functional tubular damage [35], with the rise in plasma magnesium the result of correcting hypokalaemia. The study by Gutsche *et al.* [25] showed that a linear relation exists between the degree of dysfunction of the thick ascending loop of Henle, assessed by sodium and water reabsorption, and the plasma potassium level in the hypokalaemic range. It may be that the relation becomes flattened out with higher plasma potassium concentration with less to no effect of changing the plasma potassium concentration on tubular function. However, dietary potassium deprivation of Dahl rats led to an increased magnesium excretion with only a modest reduction in mean plasma potassium concentration from 4.7 to 3.5 mmol/l in salt-sensitive and from 4.8 to 3.8 mmol/l in salt-resistant strains with no change in plasma magnesium concentrations [47].

Erythrocyte magnesium levels are thought to reflect extracellular magnesium at the time of erythropoiesis with a small, gradual loss of intracellular magnesium during their life span [48]. It is therefore not surprising that 3 weeks amiloride did not cause a change in erythrocyte magnesium levels. During MLT, with increased plasma magnesium concentrations, no change was in erythrocyte magnesium levels were observed either. Substances such as catecholamines

and insuline induce an acute increase in erythrocyte magnesium levels [49,50]. This indicates that it requires substances which alter membrane properties to change erythrocyte magnesium levels.

MLT was performed as an index of dynamic renal magnesium handling. We chose the low dose infusion (0.1 mmol Mg per kg body-weight) as described by Ryzen *et al.* [31], because higher doses used in other studies led to marked hypermagnesaemia that can make interpretation of the results doubtful [51]. As expected, the MLT employed in this study led to minimal hypermagnesaemia, always below 1.15 mmol/l. Two hours after start of loading, the plasma magnesium concentration was slightly but significantly higher on amiloride than at baseline. One may hypothesize that the enhanced renal potassium transport induced by amiloride with magnesium transport passively linked to it, leads to a diminished excretory capacity of magnesium loads without effect on the long-term levels.

The search for ways to lower the renal magnesium threshold is hampered by the lack of knowledge on the processes governing magnesium reabsorption. Magnesium may be reabsorbed paracellularly or transcellularly along the transepithelial voltage or electrochemical gradient [52,53]. The luminal  $\text{Na}^+\text{-K}^+\text{-Cl}^-$  co-transport system is quintessential in the voltage dependent reabsorption of magnesium but additional sodium-dependent and sodium-independent transport systems have been postulated [15–17,52]. Mechanisms involved in the intracellular handling and transport to the interstitium are unknown. Animal experiments by House and Bird [54] have shown that oral potassium loading can stimulate cellular magnesium uptake by increasing  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity. Renal  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity decreases *in vitro* with decreasing extracellular potassium concentration [55], while animal experiments have shown this kalipenic effect mainly to take place in the cortical collecting duct [56]. Chronic potassium loading in experimental animals has been shown to increase  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity in the late distal tubule and collecting ducts as well as to a lesser degree in the thick ascending part of the loop of Henle [57,58]. However, experimental evidence linking  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity, located at the basolateral membrane, directly to magnesium reabsorption is lacking. The link between magnesium reabsorption and basolateral  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity would be theoretically attractive since basolateral  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity determines transepithelial voltage difference through  $\text{Na}^+\text{-K}^+\text{-Cl}^-$  co-transport system and the transepithelial voltage difference is instrumental in magnesium reabsorption.

In conclusion, a rise in the plasma potassium concentration does not result in higher plasma or erythrocyte magnesium levels nor in a change in the handling of a magnesium load, indicating that raising the plasma potassium concentration is not a method to improve magnesium status in normokalaemic subjects.

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