

UMEÅ UNIVERSITY MEDICAL DISSERTATIONS
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**From the Department of Medicine,
University of Umeå, Umeå, Sweden**

**Skeletal Muscle Potassium and Magnesium
in Diuretic Treated Patients**

**Effects of Potassium - Sparing Diuretics
or Magnesium Supplementation**

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av

Lars Widman

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Key words: Long-term diuretic treatment, arterial hypertension, congestive heart failure, muscle biopsy, intracellular electrolytes (Mg, K), spironolactone, triamterene, amiloride, peroral magnesium aspartate - hydrochloride

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Dedication

To Kjerstin, Hanna, Viktoria, Ulrika and Henrik

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This thesis is based on the following publications, which will be referred to by their Roman numerals (I - V):

- I. Dyckner T, Wester P-O, Widman L:
Effect of Spironolactone on Serum and Muscle Electrolytes in Patients on Long-Term Diuretic Therapy for Congestive Heart Failure and/or Arterial Hypertension.
Eur J Clin Pharmacol 30:535-540, 1986.
- II. Widman L, Dyckner T, Wester P-O:
Effects of Triamterene on Serum and Skeletal Muscle Electrolytes in Diuretic Treated Patients.
Eur J Clin Pharmacol 33:577-579, 1988.
- III. Dyckner T, Wester P-O, Widman L:
Amiloride prevents Thiazide induced Intracellular Potassium and Magnesium losses. Accepted for publication.
- IV. Dyckner T, Wester P-O, Widman L:
Effects of Peroral Magnesium on Plasma and Skeletal Muscle Electrolytes in Patients on Long-Term Diuretic Therapy.
Int J Cardiol 19:81-87, 1988.
- V. Widman L, Dyckner T, Wester P-O:
Amiloride in the Correction of Diuretic induced Intracellular Potassium and Magnesium Depletion. Submitted for publication.

INTRODUCTION

Worldwide diuretics are the most frequently prescribed drugs and are a cornerstone in the therapy for arterial hypertension and cardiac heart failure.

By the age of 60 16% of females in Gothenburg were on diuretic treatment, and the corresponding figures at the age of 70 were 12% of males and 28% of females (1).

Chlorothiazide, the oldest of the diuretic agents, was introduced in 1958. In the same year Laragh et al. (2) reported increased serum uric acid levels during chlorothiazide therapy. In 1958 Oven (3) described attacks of gout during diuretic therapy. The increase in uric acid induced by the diuretic is due to a decrease in renal clearance of uric acid (4). The loop diuretics, etacrynic acid and furosemide introduced in 1965 and 1970 respectively, also increase uric acid levels, as do the potassium-retaining diuretics, triamterene (introduced 1965) and amiloride (introduced 1970).

Wilkins (5) had already observed two patients developing hyperglycemia and glukosuria during chlorothiazide therapy in 1959. In 1964 Wolf and Parmely (6) were able to confirm that diuretic treatment decreased glucose tolerance. In an experiment on hypophysectomized and demedullated rats, they could demonstrate hyperglycemia after administering benzodiathiazides, but there was no permanent diabetes.

Diuretics also increase serum cholesterol and triglycerides (7, 8, 9, 10) which, like the impairment of glucose tolerance, seem to be coupled to the potassium losses induced by the diuretic (6, 7, 11).

The effects on potassium metabolism were recognized soon after the introduction of chlorothiazide (2, 12). The diuretic-induced potassium losses are generally less than 200 mmol, which corresponds to 5% of the total amount of body potassium (13). The mean incidence of diuretic-induced hypokalemia (serum K < 3.5 mmol/l), however, is almost 50% (14).

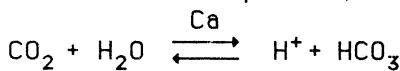
The hazards of hypokalemia is familiar to every clinician as well in the importance of maintaining a normal potassium balance when the diuretic treatment is combined with digitalis medication (15, 16, 17, 18, 19, 38, 44, 45). The diuretic induced magnesium losses and the close relationship between potassium and magnesium has until recently been ignored (23, 48, 56, 76, 77, 79, 80, 81, 85, 86, 90, 91, 92, 95, 96, 97, 98, 103, 105).

Tubular sites of action of diuretic agents and their effects on electrolytes

Fig. 1.

I. Carbonic anhydrase inhibitors

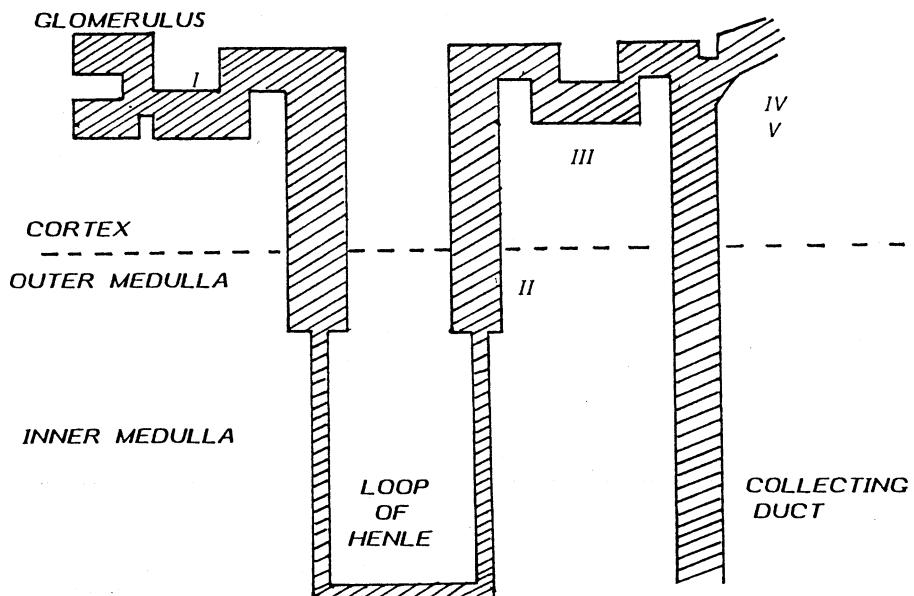
Carbonic anhydrase inhibitors exert their main effect in the proximal tubuli, although carbonic anhydrase has a widespread location along the nephron, 60-70 % of the filtered sodium is reabsorbed together with chloride and bicarbonate. Ninety per cent of the reabsorbed bicarbonate is coupled to hydrogen secretion, and carbonic anhydrase (Ca) is essential for this process, i.e.:



In the proximal tubuli carbonic anhydrase is located both in the luminal parts of the tubular cells and in the cytoplasm.

Carbonic anhydrase inhibitors cause an increased excretion of sodium potassium, water, chloride and bicarbonate. The losses of bicarbonate create a metabolic acidosis, reversing its diuretic action and in the end there is only a 5% increase in sodium output and a minimal increase in urinary magnesium losses.

Fig 1 Tubular sites of action of diuretic agents.



I Carbonic anhydrase inhibitors

II Loop diuretics

III Thiazides

IV Spirinolactone

V Amiloride and Triamterene

II. Loop diuretics

The loop diuretics (furosemide, etacrynic acid and bumetanide) exert their major effect in the ascending part of the loop of Henle. They block the $1\text{ Na}^+ - 1\text{ K}^+ - 2\text{ Cl}^-$ -cotransporter located in the luminal cell membrane (20). Furosemide also processes a mild carbonic anhydrase inhibiting action.

The loop diuretics are the most effective diuretic agents, since in the ascending loop of Henle 25% of the filtered load of solutes and water are reabsorbed. All filtered potassium is entirely reabsorbed in the proximal tubuli and in the loop of Henle.

Loop-diuretics act from the luminal site, and in the thick ascending part of the loop of Henle where 50-60 % of the filtered magnesium is reabsorbed. The urinary magnesium losses induced by the loop-diuretic can then be explained by a direct tubular effect.

III. Thiazides

The thiazide diuretic agents achieve their effect by inhibiting the electroneutral transport of sodium and chloride in the distal convoluted tubules, acting from the peritubular site. It is in this part of the nephron that 5-10 % of the load of sodium, chloride and water is reabsorbed.

Only 1-5 % of the filtered magnesium is reabsorbed in the distal convoluted tubules. Nevertheless long-term treatment with thiazides causes significant losses of magnesium (79, 81, 85, 103).

The reason may be that thiazide increases the tubular fluid volume and rates of flow, leading to increased urinary magnesium losses. The thiazides also induce a secondary hyperaldosteronism because of the extracellular volume contraction initiated by the diuretic.

Administering aldosterone has no acute effect on urinary magnesium losses (21), but the long-term effects may result in an increase in such losses (22). There have also been speculations that interactions between the thiazides and parathormone on the calcium metabolism may increase the urinary magnesium losses (23). The thiazide-induced bicarbonate losses are insignificant.

IV. Spironolactone

Under the influence of two transport systems the remaining sodium is reabsorbed in the distal convoluted tubule and the cortical part of the collecting duct.

The first is controlled by aldosterone, which also stimulates potassium and hydrogen ion secretion. Aldosterone secretion is stimulated by diminished effective blood volume, hyponatremia or hyperkalemia.

Under the influence of mineralocorticoids, mainly aldosterone, the luminal permeability of the cell to sodium and potassium is increased, as is the activity of the Na- K-ATP:ase. I.e. mineralocorticoids increase the synthesis of specific proteins - membrane channels and Na-K-ATP:ase.

Spirinolactone achieves its effect through a competitive binding to the receptors initiating the synthesis of these specific proteins (20).

The bioavailability of an oral dose of spirinolactone is about 90%. Spirinolactone is metabolised in the liver into many different metabolites, some of them active. Canrenone, the main active metabolite, is excreted in the urine. Spirinolactone conserves both potassium and magnesium (22, 24, 25).

V. Amiloride and Triamterene

The second transport system for reabsorbing sodium is not controlled by aldosterone and is located more distally.

In this part amiloride and also triamterene, which is pharmacologically similar to amiloride, exert their effect acting independently of the aldosterone levels.

Amiloride and triamterene probably act through the same mechanism. They exert their effect from the luminal part of the distal tubule and collecting ducts, blocking the entry of sodium into the cell and cancelling potassium secretion. Their reversible binding to the cell membrane blocks the transport of sodium into the cell through the sodium channels. The reduced sodium input causes decreased transepithelial potential difference leading to reduced potassium and hydrogen losses (26, 27, 28).

Following an oral dose about 25% of amiloride is absorbed and the drug is excreted unchanged in the urine. The amiloride induced sodium losses are small and the chloride excretion remains unchanged or is slightly increased during long-term therapy. This effect when used in combination with conventional diuretics can reduce the risk of hypochloremic alkaloses. On the other hand amiloride should be used with care in patients predisposed to metabolic or respiratory acidosis (29).

Amiloride and triamterene cause no significant losses of magnesium and only small losses of bicarbonate (30, 31, 32).

Since only 2% of the sodium and water is reabsorbed in this part of the nephron, spironolactone, amiloride and triamterene only exert a slight diuretic effect. Therefore their greatest benefit is gained in combination with either loop-diuretics or benzothiazides, by potentiating their diuretic effect and concomitantly, avoiding the potentially dangerous potassium losses.

Potassium

Potassium is the major intracellular ion. About 90% of the total amount of 3700 mmol potassium found in the body is located in the intracellular water; 1.5% is found in the extracellular water and 7.5% in the bone. Since the skeletal muscles contain most of the intracellular water, they also hold the majority of the potassium.

A high intracellular potassium content is essential for a lot of cellular activities, including DNA and protein synthesis, the activation of certain enzyme systems and acid-base balance.

An adult human contains about 55 mmol potassium/kg body weight. With aging the body content of potassium declines parallel to the decrease in muscle mass. An elderly person contains about 20% less potassium than a young individual. Women contain more fat than men and as fat is short of potassium, female potassium content is about 75% that of males.

The maintenance of a normal gradient in potassium concentration across the cell membrane is essential for the normal excitability of muscles and nerve tissues. Changes in either intra- or extracellular potassium affects the resting membrane potential (see page 41). For the membrane potential the ratio of intra- to extracellular potassium itself is the critical point, rather than the actual concentration of potassium on either side of the cell membrane.

Sodium is important for potassium homeostasis and transport. Potassium and sodium concentrations are closely related to the movement of hydrogen ion out of and into the cell.

Serum potassium, apart from the actual acid-base status and intracellular potassium content, is dependent on the mineralocorticoid status, mainly aldosterone. Extracellular and serum potassium concentrations are kept stable within a small range by shifting out of and into the cellular compartments, potassium secretion by the epithelium of the colon and by the tubular handling mechanisms of the kidneys.

In the regulation of the potassium homeostasis the kidneys are most important. More than 90% of the ingested potassium is excreted in the urine. The tubular potassium reabsorption system is influenced by dietary potassium intake, sodium, mineralocorticoids, mainly aldosterone, current acid-base status and by the rate of flow and solute delivery of tubular fluid.

Potassium excretion by the kidneys is very efficient and exposure to large amounts of potassium does not lead to an increase in the total body potassium content. Total body potassium deficiency leads to a markedly reduced potassium excretion in the urine. When the deficit is as high as over 350 mmol, the excretion is kept as low as 1-5 mmol/day. Nevertheless when deficits of that magnitude exist the risk of developing illness is very high.

Potassium is filtered and reabsorbed together with sodium and water in the proximal tubules, independent of varying amounts in dietary intake.

The secretion of potassium in pars recta and the descending loop of Henle is connected with the potassium load. A total body potassium deficiency results in a reabsorption of potassium in the distal convoluted tubules and collecting duct. On the other hand when potassium excretion is necessary, K^+ ions are secreted by the distal convoluted tubules. Aldosterone exerts the greatest control over the daily potassium excretion and is also the major determinant of sodium excretion.

A decrease in serum potassium may be the result of a real total body potassium waste, but it may also be the consequence of a potassium shift from the extracellular to the intracellular compartment. In this context changes in pH are very important. In 1955 Schribner et al. (34) demonstrated that acidosis leads to an increase in extracellular potassium concentrations without any changes in total body potassium. The ratio between extra- and intracellular potassium varies inversely with pH of plasma (34, 35). I.e. hypokalemia in a patient with acidosis may be more dangerous than the same degree of hypokalemia in a patient with simultaneous alkalosis. In this matter, however, it must be pointed that the alkalosis per se leads to a further potassium loss from the body. This is due to the fact that in the alkalotic state potassium moves into the tubular cells. Burnell et al. 1956 (36) estimated after a clinical investigation that for every 0.1 unit change in extracellular pH there was an inverse change of serum potassium of

0.6 mmol/l. Spurr and Liu (37) reported a serum potassium change of 0.2 mmol/l for every 0.1 unit change in pH.

Total body potassium waste reflected by hypokalemia is most commonly caused by renal losses, usually after the administration of diuretic agents, although in part a reduction in serum potassium induced by diuretics is the result of a shift from external to internal compartments.

Thiazides and loop diuretics induce potassium losses in four ways, firstly by inhibition of sodium and chloride reabsorption in the ascending loop of Henle, secondly by increasing the amount of solute and water delivered to the distal segments of the nephron, thirdly by increasing the secretion of potassium in the distal tubule and fourthly by causing an extracellular metabolic alkalosis, resulting in an increased potassium secretion rate.

When serum potassium is above 3.0 mmol/l, it requires a change in total body potassium of 100-200 mmol to change serum potassium 1.0 mmol/l. If serum potassium falls below 3.0 mmol/l, a 1.0 mmol/l change in serum potassium then represents a total body waste of 200-400 mmol.

Thiazide and loop diuretic agents increase the potassium excretion, but the losses are generally less than 200 mmol, corresponding to 5% of the total body potassium content, muscle potassium also decreases by about 5% (14). Dyckner (39) reviewed 23 studies concerning potassium losses caused by diuretic treatment. All of them showed a numerical

decrease in the total body potassium amount, and in 11 investigations the decrease was significant.

In studies of long-term diuretic therapy of hypertension up to 1980 the incidence of hypokalemia (serum $K^+ < 3.5$ mmol/l) was almost 50%. Severe hypokalemia (serum $K^+ < 3.0$ mmol/l) was found in 7% of the patients (14).

Women are more prone to develop hypokalemia than men (40, 41) and it is independent of age, body weight, renal function, other drug medication or patient compliance.

Younger patients, treated with very small doses of diuretics because of an uncomplicated essential hypertension, seldom show any significant decrease in total body potassium (42).

In the study by Toner and Ramsay (40) consisting of 193 patients (91 men, mean age 56.5 and 102 women, mean age 54.5) taking 5 mg bendroflumethiazide/day, hypokalemia (serum $K^+ < 3.5$ mmol/l) was present in 19% and severe hypokalemia (serum $K^+ < 3.0$ mmol/l) was present in 1%.

The mean serum potassium value was significantly lower in women than in men ($p < 0.001$). Twenty-five per cent of the women and 12% of the men were hypokalemic ($p < 0.05$). The reason for the difference between the sexes is not known.

These results confirm those of earlier studies, among others, the study by Krakaer and Kauritzen (41). They studied 489 geriatric out-patients, 128 men, mean age 78.7 and 361 women, mean age 78.9 of whom 34% and 37% respectively were on diuretic therapy. 1/44 men (0.02%) was hypokalemic (serum $K^+ < 3.5$ mmol/l) compared to 25/134 women (19%).

The clinical implications of hypokalemia have been and still are being debated. Harrington et al. (43) conclude that diuretic-induced potassium losses are minimal among patients treated with diuretics for hypertension, potassium salts and potassium saving diuretics should not be prescribed routinely, unless the patient is receiving digitalis.

Kaplan, on the other hand, (44) points out the multiple hazards of diuretic-induced hypokalemia in the steadily growing older population - the increased risk of arrhythmias, especially in times of stress - the deleterious effects of hypokalemia in patients receiving digitalis - the direct correlation between hypokalemia and impaired glucose tolerance and rise in serum cholesterol - and also the possibility that hypokalemia raises blood pressure.

Hollifield (45) has demonstrated a significant correlation between the occurrence of ventricular ectopic beats/minute and the fall in serum potassium ($p < 0.001$).

Kaplan (44) focuses the interest on MRFIT (46) trials regarding patients with abnormal electrocardiograms at the beginning of the

studies. In these trials, diuretics were used in the first line. The doses were high, as much as 100 mg hydrochlorothiazide or chlorothalidone. Is hypokalemia the reason for the increased mortality from coronary heart disease in this group of patients?

In the Veterans Administration Cooperative Study (47) there were 8 sudden deaths in the group treated with diuretics compared to 4 in the group treated with placebo. However, the difference does not reach the level of significance (48).

Magnesium

Introduction

Magnesium is the second most abundant intracellular cation in the human body. About 600 mmol of the total amount of 1000 mmol magnesium is found in the skeleton and the majority is not biologically available. Twenty per cent of the body magnesium is found in the muscles and less than 1% in serum. Seventy per cent of the serum magnesium is filterable, the rest is proteinbound. The normal serum level is 0.70-1.00 mmol/l. The normal intracellular concentration is about 10 mmol/l of which only 0.1 mmol to 1 mmol/l is free. The rest is bound to different cellular structures. Thus there is no or a minimal magnesium gradient over the cell membrane (49, 50).

Magnesium is an essential activator for hundreds of enzymes involved in the transmembrane transport, neuromuscular excitability, muscle contractions, oxidative phosphorylation, glucose metabolism and the synthesis of proteins and nucleic acids.

Most of the magnesium is absorbed in the small intestines both in the upper and the lower parts (51). Magnesium absorption is dependent on the amount of magnesium in the diet. When there is a very low magnesium intake, 75% of the ingested magnesium is absorbed, while a magnesium rich diet resulted in a net absorption of 24% (52). Other factors influencing magnesium absorption are the current amount of calcium, vitamin D, phytate and proteins in the diet.

The National Research Council in the United States has set the RDA (Recommended Dietary Allowance) for Mg for adult men at 350 mg/day (~ 15 mmol) and for women at 300 mg/day (~ 13 mmol). In pregnancy and lactation an extra 150 mg (~ 7 mmol) is recommended (53, 54). These figures are also supported by Seelig (55) reviewing presented balance studies. Unfortunately, the magnesium content in the food in the western countries has declined during the last few decades, for example the hospital diet of patients with hypomagnesemia associated with diuretics was found to contain only nine mmol/day (56).

The kidneys are the major organ for maintaining a steady state magnesium plasma level. About 75 mmol magnesium is filtered through the kidneys every 24 hours, but usually only about 3% (3-5 mmol) is excreted (57, 58). Of the filtered magnesium 25-30 % is reabsorbed in the proximal convoluted tubule, 50% - 60% in the ascending part of the loop of Henles and about 5% in the distal part of the convoluted tubules.

Most of the dietary ingested magnesium leaves the body with the stools. During states of very low magnesium intake the kidneys are capable of saving magnesium very effectively and it can take months to develop magnesium deficiency (59, 60).

The percentage retention of i.v. administered magnesium is also used as a loading test for magnesium deficiency (61, 62).

Magnesium and hormones (Table 1)

The interactions between magnesium and the endocrine system are still not fully understood. Most hormones are able to influence magnesium metabolism, but generally the induced changes are small. There is no specific hormonal control of magnesium reabsorption identified, so far. The parathyroid hormone seems to play an important role in magnesium regulation. Other important hormones are mineralcorticoids, primarily aldosterone, cathecholamines and thyroid hormones.

Parathyroid hormone (PTH)

Magnesium has a modulating role in the secretion of the parathyroid hormone, but its exact extent during health and sickness is still under investigation. Magnesium is, however, essential for the release of PTH and for the action of PTH on bone, kidney and gut.

The administration of parathyroid hormone (PTH) to healthy people has produced conflicting results concerning magnesium excretion. Administration of PTH has led to decreased, unchanged and increased magnesium losses (63). Interactions between magnesium and calcium may be the explanation for the conflicting results.

Massry and Coburn (64) administered parathyroid hormone followed by infusion of magnesium chloride to dogs which resulted in reduced excretion of magnesium and calcium. Heaton F.W. (65), studying rats, demonstrated increased magnesium losses after PTH administration, but the rats were hypercalcemic. PTH given acutely to normocalcemic dogs caused decreased urinary calcium and magnesium excretion (66).

Table 1. Hormonal influence on normal magnesium handling

Hormone	Urinary magnesium	Magnesium /serum	Intracellular magnesium
Parathyroid hormone	↓	± 0	↑ (?)
Mineralcorticoids- /aldosterone	↑	↓	↓
Glucocorticoids	↑	↓	↓
Catecholamines	↑	↓	↓
Thyroid hormones	↑	↓	± 0
Insulin	↓	↓	↑ ↓
Calcitonin	↓	± 0 (?)	↑ (?)
Glucagon	↓	± 0 (?)	↑ (?)
ADH	↓	± 0 (?)	↑ (?)
Growth hormone	↑	↓ (?)	↓ (?)

Mineralcorticoids, especially aldosterone, cause increased renal magnesium losses (67). Chronic administration of aldosterone leads to increased urinary magnesium losses and is reversed by spironolactone (25, 67).

Secondary aldosteronism is prevalent in a variety of diseases, especially liver insufficiency, nephrotic syndrome and heart failure (68). Diuretic therapy is the most common cause of secondary aldosteronism (69).

The reduced circulating blood volume seen in heart failure and diuretic therapy activates the renin-angiotensin system since the body tries to maintain an effective arterial blood volume. The raised levels of angiotensin II lead to vasoconstriction and elevated levels of aldosterone, cause increased reabsorption of sodium and water, while urinary potassium and magnesium excretion are increased. Chronic administration of glucocorticoids leads to increased urinary magnesium losses (64). The reason may be the catabolic effect on bone, leading to magnesium losses or a raised glomerular filtration rate.

Acute administration of methylprednisolone to adrenal ectomized dogs caused no increase in magnesium excretion (70).

The cathecholamines, adrenaline and noradrenaline stimulate renin release through a β_1 -agonist action (71, 72). The raised aldosterone levels leads to decreased extra- and intracellular potassium and magnesium concentration. In sheep the cathecholamines have been found to stimulate lipolysis and increase uptake of magnesium in

adipocytes (73, 74). Rat hearts have been found to be depleted in magnesium after adrenaline infusions (73). Thus the effect of catecholamines on the magnesium metabolism is complex and seems to be both tissue and species specific.

Thyreotoxicotic patients have a decreased serum magnesium level and an increased urinary magnesium excretion. Hypothyreos on the other hand leads to a slight increase in serum magnesium and decreased urinary magnesium losses (64, 66).

Insulin increases magnesium uptake in muscle and liver, but reduces the uptake in the skeleton (75).

Calciotonin and glucagon act in a similar way as ADH in the loop of Henle, most likely through cyclic AMP and an analog of ADH has been found to decrease urinary magnesium excretion (66). Although cyclic AMP mediated hormonal regulation increases electrolyte absorption in the loop of Henle, this does not necessarily lead to increased electrolyte absorption since the electrolytes are influenced by other events occurring more distally in the nephron (66). Growth hormone probably increases urinary magnesium excretion (64).

MAGNESIUM DEFICIENCY

Magnesium deficiency is not infrequent, but primary magnesium depletion is extremely uncommon in an otherwise healthy person, since the kidneys have a great capacity to conserve magnesium, and generally the dietary intake of magnesium is sufficient.

The main group of patients at risk for developing potassium and magnesium depletion are elderly people on long-term diuretic treatment for hypertension and/or congestive heart failure. Among these patients there is a high incidence of secondary aldosteronism due to the heart disease and due to the diuretic treatment per se (76). Other complicating factors may be concomitant digitalis mechanism, low dietary intake of magnesium, malabsorption or a large intake of alcohol, which is known to increase urinary excretion of magnesium (77).

Table 2 presents factors and diseases that may lead to magnesium deficiency. Slight magnesium deficiency may be asymptomatic or the symptoms may be slight or uncharacteristic. Since magnesium deficiency is often combined with disturbance of electrolytes, such as hypokalemia and hypocalcemia, the symptomatology may be complex and related to disturbances of the other electrolytes.

The symptoms principally occur from the nervous system, the skeletal muscles, the gastro-intestinal tract and from the cardiovascular system (60).

Table 2. Causes of magnesium deficiency.**A. Gastrointestinal and nutritional causes**

1. Prolonged parenteral fluid administered without magnesium (beginning after 3 weeks)
2. Prolonged diarrhea, e.g. ulcerative colitis, Crohn's disease and chronic misuse of laxatives
3. Intestinal malabsorption
 - a) Idiopathic steatorrhea
 - b) Tropical and non-tropical sprue
 - c) Short bowel syndrome from any cause
4. Alcoholism
5. Acute recurrent pancreatitis
6. Starvation
7. Protein calorie malnutrition

B. Renal causes

1. Prolonged use of diuretics (especially loop diuretics)
2. Renal diseases
 - a) Renal tubular acidosis
 - b) Recovery from acute tubular necrosis (diuretic phase)
 - c) Chronic glomerulonephritis and pyelonephritis (rarely)
 - d) Aminoglycoside, cisplatin and cyclosporin induced renal injury

C. Endocrine and metabolic causes

1. Hyperparathyroidism
2. Hyperthyroidism
3. Primary and secondary aldosteronism
4. Diabetic ketoacidosis
5. Excessive lactation
6. Congenital hypoparathyroidism

Table 3 sums up the most important symptoms attributable to magnesium deficiency.

The consequences of magnesium deficiency are profound. Werner et al. (78) studying magnesium-deficient dogs found at autopsy degenerative vascular changes, especially in the heart, leading to myocardial necrosis.

The widespread use of diuretics is the most common cause of urinary magnesium losses, still frequently ignored. Ordinarily diuretic treatment increases the urinary magnesium losses by 25-400 % (79, 80, 81). In a short-term study by Hänze and Seyberth 1966 (82) loop-diuretics increased the urinary magnesium excretion 250-330 %.

In nine healthy volunteers 100 mg of chlorothiazide increased the 24-hour urinary magnesium excretion by 80%, compared to a control group (83). The corresponding figures for furosemide are a 50% increase in 24-hours of magnesium excretion (84, 85, 86).

The risk of developing magnesium depletion in a younger hypertensive individual without any obvious signs of cardiac disease treated with diuretics is generally low. Ljunghall et al. (87) found in patients who recurrently formed renal stones and were treated with thiazide that urinary magnesium excretion was initially raised, but that after two years, at follow-up, the values had normalized. After three years there was no magnesium deficiency noted in muscle biopsies.

Table 3. Symptoms attributable to magnesium deficiency.

1. Muscular twitchings and tremors
2. Muscular wastage
3. Muscular weakness
4. VEBs, VT, VF
5. Loss of appetite
6. Nausea
7. Diffuse abdominal pains
8. Diarrhoea or constipation
9. Numbness and tingling
10. Athetoid and choreiform movements
11. Vertigo, ataxia and nystagmus
12. Positive Chvostek sign
13. Spontaneous carpopedal spasm or tetany
14. Convulsions
15. Apathy, depression, asthenia
16. Difficulties in concentrating
17. Confusion, disorientation, hallucinations
18. Paranoia
19. Coma
20. Death

This agrees with a study by Cohen et al. (88). They studied 8 patients with normoreninemic hypertension for 3-5 years and 12 recurrent renal calcium oxalate stone-formers for 5-10 years. All were treated with 50 mg of hydrochlorothiazide/day together with potassium supplementation. None of the patients showed any decrease in muscle magnesium and all had normal i.v. magnesium loading test results.

Bergström et al. (89) could not demonstrate any significant decrease in either muscle magnesium or muscle potassium after five months of mefruside treatment in eight patients with essential hypertension.

However, for many patients diuretic-induced magnesium levels remain higher and thus after long-term of therapy will lead to magnesium deficiency. Dyckner and Wester reported that among 537 patients with congestive heart failure or arterial hypertension, receiving long-term diuretic therapy, tissue magnesium obtained by skeletal muscle biopsy was significantly reduced in 43% (90).

Seller et al. (91) reported that serum magnesium was significantly reduced in 13 patients with congestive heart failure compared to a healthy control group. All diuretic therapy had then been stopped for six weeks and the patients had been put on 100 mg of hydrochlorothiazide for 12 weeks but this did not result in any further decline in serum magnesium levels.

Serum magnesium, however, is not considered to be a sure way of diagnosing magnesium deficiency. Magnesium deficiency often exists in the presence of normal serum magnesium (92, 93, 94, 103).

Sheehan and White (56) conclude that "chronic low grade magnesium deficiency from diuretic treatment is more common than the published reports suggest". Over a period of 12 months they found clinically suspected hypomagnesemia in 21 patients. All had been treated either short-term or long-term with diuretic agents, some patients also had high intakes of alcohol. The mean age of the patients was 67 years. The principal diagnosis was heart failure; 13/21 had atrial fibrillation, and five suffered from digitalis intoxication. Fourteen of the 21 were on furosemide, the rest on thiazides.

In a subsample from the MRFIT participants (95) two serum Mg samples taken within four months were monitored. Most of the patients had been on diuretics, chlorthalidone or hydrochlorothiazide for at least four to five years. The serum magnesium levels were lower for the diuretic users than for the non-diuretic users, but the difference was not significant.

Whang et al. (96) reported a frequency hypomagnesemia and hypokalemia in ambulant hypertensive patients of 4.5% and 17% respectively.

In a retrospective investigation (97) of 963 paired potassium and magnesium measurements in 421 patients, 12% of the serum samples were hypokalemic ($\text{serum K}^+ < 3.5 \text{ mmol/l}$) and 26% hypomagnesemic ($\text{serum Mg}^{2+} < 0.75 \text{ mmol/l}$). The incidence of hypomagnesemia was significantly higher in the hypokalemic samples (38%) than in the normokalemic samples (25%), $p < 0.003$.

Whang et al. (98) found hypomagnesemia in 42% of hypokalemic patients (serum K \leq 3.5 mmol/l), in 23% of hyponatremic (serum Na⁺ \leq 130 mmol/l), and finally in 22% of the hypocalcemic patients (serum Ca²⁺ \leq 2.0 mmol/l). They claim that occurrence of these recently described electrolyte abnormalities should alert the clinician to the need to determine serum magnesium. It also seem wise to determine serum magnesium routinely in hospitalized patients.

Ryzen et al. (99) argue for regular monitoring of serum magnesium in critically ill patients as they found that 65% of such patients with normal or mildly abnormal renal function had hypomagnesemia (serum Mg²⁺ < 0.70 mmol/l). Even severely ill people with renal failure should be monitored for total body magnesium deficit, in spite of normomagnesemia. Alcohol and alcohol related diseases accounted for 50% of the admissions and half of the cases with hypomagnesemia.

Mg-K-interrelationship

In rats with induced depletions of potassium and magnesium (100) the potassium deficit will continue to exist in spite of large intakes of potassium. The potassium will be lost in the urine. Whang and Welt postulated that adequate magnesium levels are necessary for the maintenance of appropriate potassium levels after studying rat diaphragm muscle which contained less potassium when magnesium was absent from the bath.

Ryan and Hingerty 1967 (101) demonstrated that the time for restoring muscle potassium after potassium chloride infusion in two groups of

rats - one potassium deficient and the other potassium and magnesium deficient - was quite different. It took two hours in the former to resorte muscle potassium, but in the later muscle potassium was not restored until seven hours after the last injection. Corresponding figures for the sodium levels was five and a half hours in the potassium depleted group and after seven hours it had not reached normal levels in the doubly depleted rats.

Ryan et al. (102) made one group of rats potassium deficient, as a control group. They were compared to a group of rats made both potassium and magnesium depleted. The study showed that there was a significantly greater loss of potassium from skeletal and cardiac muscle in the doubly deficient rats than in the potassium depleted rats. There was also a relatively greater loss of potassium from skeletal muscle than from cardiac muscle.

In 1972 Lim and Jacob (103) presented a study consisting of 10 patients with valvular heart disease receiving diuretics because of heart failure. The mean duration of diuretic medication was 3.3 years. In 5/10 patients muscle magnesium was low, concomitantly muscle potassium was reduced, but only one was hypokalemic. 4/5 of these patients suffered from digitalis intoxication. After infusion of magnesium both muscle potassium and magnesium returned to normal levels, and the symptoms of digoxin poisoning were relieved.

These studies emphasise the importance of a normal magnesium balance for maintaining a high intracellular potassium level.

The normal ratio between intra- and extracellular potassium is essential for maintaining a normal cardiac rhythm and is maintained by $\text{Na}^+ - \text{K}^+$ -ATP:ase (the "sodium pump"), where sodium is actively transported out of the cell and potassium into the cell. Magnesium is essential for the optimal activity of the "sodium pump" (104).

In clinical magnesium deficiency the cell continuously loses potassium and sodium is accumulated on the inside of the cell, unless concomitantly magnesium deficiency is not corrected (100, 105). Although magnesium, as mentioned above, is necessary for the normal functioning of the sodium pump, no experiment to date has demonstrated a decline in sodium pump activity in conjunction with magnesium deficiency. Another attractive theory is that magnesium deficiency creates a "leaky membrane", which causes loss of potassium from the cell and a simultaneous accumulation of sodium on the inside of the cell.

In conclusion: The magnesium dependent $\text{Na}^+ - \text{K}^+$ -ATP:ase is responsible for maintaining a high intracellular potassium concentration. In a state of magnesium deficiency, the cell cannot maintain intracellular potassium concentration and potassium is continuously lost from the cell and sodium is accumulated on the inside of the cell leading to disturbances in the intra- to the extracellular potassium ratio. Changes in this ratio will cause changes in the resting membrane potential, potassium conductance and repolarisation favoring the occurrence of serious cardiac arrhythmias (106).

Magnesium and calcium interactions

Magnesium can induce arrhythmias through its interaction with calcium metabolism. Magnesium is considered a physiological calcium blocker (107). During the action potential magnesium has been shown to shorten the plateau phase and the duration of the action potential by blocking the inward current of calcium (108).

In patients with hypomagnesemia there will be some reduction in intracellular free magnesium, leading to an increased binding of calcium to troponie, i.e. a positive inotropic effect, enhanced release of calcium from the sarcoplasmatic recticulum and increased cellular calcium uptake. All these together result in raised intracellular calcium levels, favouring the induction transient afterpotentials and increasing the risk of cardiac arrhythmias (109).

The influence of magnesium on the development of muscle tension is expressed in the following formula (Shine):

$$\frac{\text{Ca}^{2+} \text{ (extracellular concentration)}}{\sqrt{\text{Mg}^{2+} \text{ (extracellular concentration)} + 0.7}} \quad (110)$$

Magnesium and digitalis interactions

Digitalis is a well-known inhibitor of magnesium dependent Na^+-K^+ -ATP:ase, which may explain the additional risk from digitalis medication in patients with simultaneous magnesium and potassium deficiency. The inhibition of Na^+-K^+ -ATP:ase cause increases intracellular sodium and calcium concentrations. A raised intracellular calcium concentration has a positive inotropic effect. The increased

efflux of calcium during the early diastole causes depolarising afterpotentials which can induce cardiac arrhythmias (109).

In states of hypokalemia there will be an increased binding of digoxin to cardiac muscle (111). Digoxin is partly eliminated by the kidneys, the mechanisms involved are glomerular filtration of non-protein bound digoxin from the plasma and tubular secretion. When serum potassium is reduced from 4.5 mmol/l to 2-3 mmol/l, the tubular secretion of digoxin is nearly blocked causing a prolonged digoxin half-life (111) and through reduced tubular reabsorption digitalis treatment causes increased renal losses of magnesium (112).

Zwillinger (113) was the first to notice, in 1935, the benefit of magnesium therapy as an effective antiarrhythmic agent in states of digitalis induced arrhythmias.

In animal experiments by Seller et al. (114) a statistically significantly reduced dose of strophanthidin ($p < 0.05$) was needed to produce arrhythmias when the serum magnesium concentration was reduced.

The anti-arrhythmic effect of magnesium in states of digitalis intoxication is not coupled to a reactivation of $\text{Na}^+ \text{-K}^+$ -ATP:ase, rather it may be linked to a direct membrane effect, preventing further loss of potassium from the cell (115).

Whang et al. (116) found in 136 patients receiving digitalis that 76 (56%) had an electrolyte abnormality, 21% were hyponatremic (serum

$\text{Na}^+ \leq 130 \text{ mmol/l}$) and 26 (19%) were hypomagnesemic (serum $\text{Mg}^{2+} < 0.63 \text{ mmol/l}$).

Dyckner and Wester reported in a study of long-term treatment with conventional diuretics and digitalis in 297 patients with coronary heart failure that 42% had hypokalemia, 37% hypomagnesemia and 12% hyponatremia. About half the patients had depletion of muscle potassium and magnesium and most of them had an excess of muscle sodium (90).

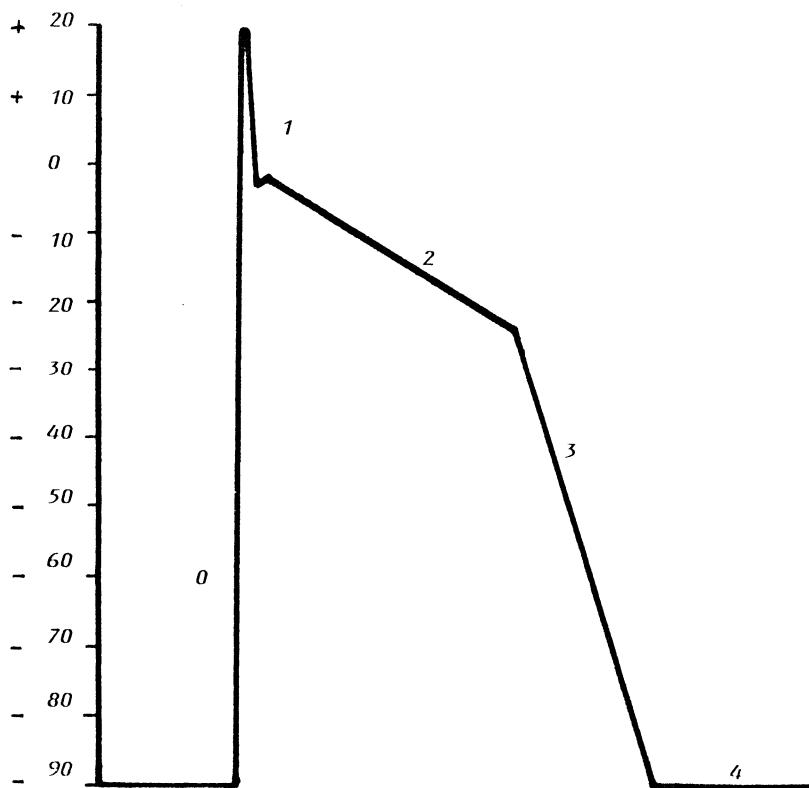
ELECTROPHYSIOLOGICAL ASPECTS

The resting membrane potential is about -90mV, negative inside. Under resting conditions, approximately, the resting membrane potential (RMP) is built up by the ratio between intracellular and extracellular potassium and only a minor role is played by Na^+ , Cl^- , Mg^{2+} and Ca^{2+} , i.e. according to Nernst's equation the

$$\text{RMP} = -61.5 \lg \frac{K_{ic}}{K_{ec}} \quad (117)$$

Looking at the different parts of the action potential of a non-pacemaker cell (Fig. 2).

Fig 2 Schematic illustration of the different AP phases in a non pacemaker cell.



Phase 0 represents a rapid sodium influx in sodium channels.

During Phase 1 there is a slowing of the sodium conductance, combined with an inward chloride current, which become successively inactivated parallel to the increasing RMP (less negative).

Phase 2 represents a slow inward Ca^{2+} current, and the net current flow is low. The threshold for the calcium current is -35 mV.

Phase 3 = repolarization, created by rapid loss of potassium.

Phase 4 corresponds to diastole and now the magnesium dependent Na-K-ATP:ase extrude sodium in exchange for potassium.

Disturbances in the intra/extracellular potassium ratio can cause cardiac arrhythmias. The electrolyte changes may be secondary to ischemic or damaged myocardial cells, acidosis or the use of diuretics, among others. Changes in the serum potassium concentration are more important than intracellular potassium changes since the percentual extracellular change is much more easily induced than the intracellular change.

Disturbances in extra- and intracellular potassium concentration and its effects on the cardiac rhythm

Hyperkalemia causes an initial increase, then a decreased automaticity and conduction, which is in part reversed by an increase in excitability (106, 118).

Hypokalemia results in a decrease of RMP (more negative), however the major effect of hypokalemia is on repolarisation. The relative refractory period is prolonged and enhanced automaticity will favour the occurrence of reentry phenomena. Clinically this can induce ventricular tachycardia or ventricular fibrillation (106, 118).

Changes in the intracellular potassium concentration is less studied. A decrease in the intracellular potassium concentration causes increased automaticity, slower conduction, and due to the fact that it will be closer to its threshold potential, the cell will be more excitable (119). Clinically a decrease in intracellular potassium concentration or hyperkalemia may give rise to intraventricular block in certain areas. This will favour the occurrence of serious arrhythmias such as ventricular tachycardia or ventricular fibrillation.

Disturbances in magnesium concentration and its effects on the cardiac rhythm

The influence of magnesium on the occurrence of cardiac arrhythmias is mediated in three ways: Firstly through direct action, secondly through an effect on potassium metabolism and lastly through its interactions with calcium metabolism.

Magnesium deficiency may also cause changes in ECG such as: widened QRS-complexes, peaked T-waves, the occurrence of U-waves, ST-T abnormalities and extended QT-intervals (120, 121, 122, 123). These changes, however, are not specific for magnesium deficiency, and hypomagnesemia is correlated to hypokalemia, hyponatremia and hypocalcemia (98).

Magnesium deficiency increases heart rate shortens the absolute and prolongs the relative refractory period (124, 125). The vulnerable phase of the heart is increased, leading to an increased risk of serious cardiac arrhythmias. On the other hand the administration of magnesium causes a slowed periodicity of the sino-atrial node, decreased intra-atrial and intraventricular conduction as well as prolongation of the atrio-ventricular transition time. The duration of the absolute refractory period is increased and there is a decrease in the relative refractory period (124, 125).

The risk of an abnormal impulse is decreased after magnesium administration, and the threshold for ventricular fibrillation is increased (115).

Clinical aspects of hypomagnesemia and magnesium as an antiarrhythmic agent

Dyckner (126) reported a higher incidence of serious ventricular ectopic beats, ventricular tachycardias or ventricular fibrillations in hypomagnesemic patients suffering from acute myocardial infarctions than in normomagnesemic patients not suffering from acute myocardial infarctions.

Kafka et al. (127) during a 13 month period reported a frequency of hypomagnesemia (serum $Mg^{2+} < 0.82 \text{ mmol/l}$) of 6% in patients suffering from acute myocardial infarction compared to the 46% incidence reported by Dyckner (126). According to Kafka et al. (127) the difference in incidence of hypomagnesemia between the two studies may be related to a variable magnesium water content in different

geographic areas.

Dyckner and Wester (105) demonstrated that in 34 patients suspected of being magnesium deficient, potassium infusions did not result in any change in the frequency of ventricular ectopic beats. The infusion of magnesium on the other hand caused a significantly reduced frequency of ventricular ectopic beats, simultaneously there was a significant increase in the muscle potassium levels.

Morton et al. 1984 (128) studied patients suffering from acute myocardial infarction hospitalized within eight hours of the onset of symptoms. The patients were randomly and blindly allocated to 36-hour infusion of magnesium sulphate or saline solution. The treatment group consisted of 40 patients and the control group of 36 patients. The groups were comparable except for the degree of heart failure on entry. There were 22 without heart failure and 18 with mild to moderate heart failure in the treatment groups. The corresponding figures for the control group were 30 and 6 respectively ($p < 0.01$). The results showed a significant reduction in the need for lidocain therapy ($p < 0.05$) and a tendency to fewer ventricular ectopic beats in the group treated with magnesium compared to the control group.

In 1986 Rasmussen et al. (129) reported the results of a study consisting of 273 patients with suspected myocardial infarction in which 130 patients with a proved myocardial infarction participated in a double-blind, placebo study. Fifty-six patients received magnesium infusions and 74 received placebo. After 4 weeks the mortality rate was 7% in the magnesium treatment group and 19% in the placebo group

($p \leq 0.045$). Antiarrhythmic therapy was needed by 21% in the magnesium treatment group and 47% in the placebo group ($p \leq 0.004$).

Smith and coworkers (130) presented a randomized blinded investigation consisting of patients suffering from acute myocardial infarction. There were 92 patients in the treatment group, who received an intravenous magnesium infusion while the control group of 93 patients has given an infusion of saline. The incidence of ventricular arrhythmias needing treatment was reduced by more than half in the magnesium treated group. There were two deaths in the "magnesium group" compared to seven in the control group. Intravenous magnesium infusion significantly ($p < 0.05$) reduced cardiac events (deaths and ventricular arrhythmias) compared to the control group.

Abraham et al. 1987 (131) in a randomized double-blind study studied 94 patients with acute myocardial infarction. Forty-eight patients received a magnesium infusion and 46 received placebo. There was a significant reduction in serious arrhythmias ($p < 0.05$) in the magnesium treated group compared to the placebo treated group. There was also a significant difference ($p < 0.001$), favouring the magnesium treated group, concerning the interval from the start of the infusion to the occurrence of arrhythmias; 14/16 patients in the placebo treated group had their first arrhythmias within two hours, while there were no serious ventricular arrhythmias in the magnesium treated group until about four hours after the start of the infusion.

There is also evidence in an huge amount of case reports that hypomagnesemia should be considered in the development of various

cardiac arrhythmias not induced by an acute myocardial infarction and that magnesium infusions can be beneficial in terminating these arrhythmias (132).

Magnesium infusion may even be of benefit in normomagnesemic patients. The reason could be that magnesium deficiency occurs in spite of normal serum magnesium (92, 93, 94, 103).

The use of magnesium infusions in states of digitalis intoxication has already been considered (see page 37).

In 1943 Boyd and Scherf (133) after administering intravenous magnesium sulphate succeeded in terminating paroxysmal atrial tachycardia in 7/7 non-digitalised patients.

Enselberg reported in 1950 (134) four patients, who were not taking digitalis, with ventricular ectopic beats where the frequency was greatly reduced after infusion of magnesium sulphate.

Chadda et al. (135) reported in 1973 the case of a patient with both hypokalemia and hypomagnesemia, suffering from recurrent supraventricular tachycardia, where the correction of hypokalemia failed, but infusion of magnesium sulphate succeeded and the patient returned to sinus rhythm.

Iseri et al. (136) reported that 7/8 patients with multifocal atrial tachycardia converted to sinus rhythm after infusion of magnesium sulphate; 6/8 patients were taking digoxin.

In 1986 Perticone et al. (137) reported the benefit of slow magnesium sulphate infusions in six patients with Torsade de Pointes.

According to Zwerling (138) there is still no definitive data to support a clear etiological relationship between hypomagnesemia and ventricular tachycardia in acute myocardial infarction. It is difficult to isolate hypomagnesemia from hypokalemia and other biochemical events occurring in states of acute myocardial infarctions.

Still he argues for a prophylactic intravenous magnesium infusion in patients with acute myocardial infarction, where one may suspect that the patient is magnesium depleted (e.g. alcoholism, congestive heart failure, loop diuretic treatment). When other drugs have failed or are relatively contraindicated in the setting of acute myocardial infarction or torsade de pointes, it also may be wise to use magnesium infusion.

The aims of the present study were to answer the following questions:

1. Can potassium-sparing diuretics also spare magnesium?
 - a) Spironolactone
 - b) Tiramterene
 - c) Amiloride
2. Can diuretic-induced potassium (K) and magnesium (Mg) disturbances be avoided giving Mg substitution orally?
3. Can amiloride correct diuretic-induced intracellular potassium and magnesium losses?

METHODS

At the beginning of each study the study blood samples, a chest X-ray, a 12 lead ECG and a skeletal muscle biopsy were performed. After 6 months a further set of samples was obtained from each patient.

The blood samples were analyzed for electrolytes by conventional autoanalyzer techniques (Na, K, Cl, CO₂, Ca, P, creatinine) and for Mg by atomic absorption spectroscopy.

The skeletal muscle biopsies were obtained from the lateral portion of the quadriceps muscle under local anaesthesia (139) and were analyzed for Na, K, Cl and Mg by atomic absorption spectroscopy.

The muscle tissue (20-40 mg wet weight) was placed on a piece of quartz glass and carefully dissected free from all visible fat and connective tissue. All traces of blood were wiped off by rolling the specimen on the piece of quartz glass. The remaining muscle tissue was immediately transferred to a preweighed platinum hook and repeatedly weighed on a Cahn 4700 electromagnetic balance. The original weight of the sample was obtained by extrapolation to zero time. The muscle tissue was then dried in an oven at 90 °C to constant weight and the water content was calculated by subtracting the dry weight from the extrapolated wet weight.

Neutral fat was extracted by placing the platinum hook with adhering dry muscle tissue in a quartz tube containing 10 ml redistilled petroleum ether for 2 h. The platinum hook with the muscle tissue was

reweighed after drying in the oven for a further 2 h, and the fat content was obtained by subtracting the fat free dry solids (FFDS) weight from the weight before extraction.

The electrolytes were extracted from the muscle tissue by treatment with 1N nitric acid for 15 h in quartz tubes. A Varian Techtron atomic absorption spectrophotometer was used to determine the content of sodium, potassium and magnesium. To minimize the interference by iron and phosphorus, an excess of those ions was added to the diluting solution for standards and samples, as described by Bergström (139).

Chloride was determined indirectly by atomic absorption spectrophotometry. An excess of silver nitrate was added to the electrolyte eluate, prepared according to Bergström et al. 1973 (89), which was then kept in a dark place for 12 h. After centrifugation, the silver content of the supernate was measured. The chloride content was obtained by fitting the measured silver value to a standard curve, prepared by precipitating known amounts of chloride with silver nitrate. Interference by binding of silver to protein was judged to be insignificant, since the protein concentration of the electrolyte eluate was negligible. The amounts of silver and halogens in muscle tissue were also considered unimportant in this respect.

The amounts of extra- and intracellular water were calculated by the chloride method (140). This method presupposes that chloride is freely diffusible across the cell membrane and is distributed according to Nernst's equation (117).

$$E = - \frac{RT}{F} \ln \frac{C_{le}}{C_{li}}$$

The measured resting membrane potential in normal humans is 87.2 mV (141) which, using Nernst's equation, gives a ratio of 26/l between the extra- and intracellular chloride concentrations.

The extracellular chloride concentration (C_{le}) was estimated from the plasma chloride concentration (C_l), using a correction for the Donnan factor and the plasma water content [H_2O_p] (142).

$$C_{le} = \frac{C_l \times 1000}{0.96 \times H_2O_p}$$

Knowing the total water content, the total amount of chloride, the extracellular chloride concentration and the ratio of the chloride concentrations between the extra- and the intracellular compartments, it is possible to calculate the water content in the extracellular space. The intracellular water content was obtained by subtracting the extracellular water content from the total water.

$$H_2O_{ec} = \frac{1000 \times C_{tot} - H_2O_{tot} \times C_{li}}{C_{le} - C_{li}}$$

$$H_2O_{ic} = H_2O_{tot} - H_2O_{ec}$$

Intracellular electrolyte concentrations were calculated by subtracting the amount in the extracellular space from the total quantity of the electrolyte and dividing the result by the amount of water in the intracellular space. The extracellular concentration of the

electrolyte has been assumed to equal its plasma concentration.

$$E_{ic} = \frac{1000 \times E_{tot} - H_2O_{ec} \times E_p}{H_2O_{ic}}$$

Statistical analysis

An unpaired t-test was performed on the mean changes in the treatment groups and the control groups to obtain a single probability for skeletal muscle, Mg and K and in paper IV also for serum-Mg. For comparisons within each groups before and after the study period, Student's t-test for paired observations was used. In the tables mean values \pm standard deviations are given, calculated by the formula for arithmetic means.

Study population - Normals

Serum muscle electrolytes (K, Mg) in healthy elderly people

To obtain normal values of skeletal muscle electrolytes in healthy elderly people, a study was designed, approved by the Etichal Committee of medical faulcty at the university of Umeå.

The selection of healthy people was done through a letter to every person above 60 years of age in the community of Umeå requesting their participation in the study.

After a telephone interview 31 healthy elderly normals were selected for further clinical investigation (16 men and 15 women). The volunteers were then hospitalized for one night.

After a personal interview, physical examination ECG, chest X-ray, blood pressure monitoring, routine chemical analysis per oral glucose tolerance test and blood samples for excluding thyroid or liver diseases 12 persons (7 men and 5 women) were accepted for a muscle biopsy (139) after a night's fast. The biopsies were handled and analyzed in the same way as described under methods (page 49), simultaneously blood sampling for as analyzing Na^+ , Cl^- , K^+ and Mg^{2+} was done.

The mean age for the men was 69.9 ± 21 and women 65.2 ± 3.2 respectively. The mean age for men and women together was 67.7 ± 3.3 (Table 4, 5).

Study population - Diuretic treated

Patients with atrial hypertension (AHT) and/or congestive heart failure (CHF) receiving conventional diuretic therapy for at least one year were recruited for a six months study period. Patients on other medicaiton known to interfere with potassium and/or magnesium metabolism were excluded with the exception of digitalis. Patients with reduced renal function were excluded (serum creatinine $> 140 \mu\text{mol/l}$). The patients were randomly assigned to unchanged therapy (controls) or to a treatment group in the four different studies (Table 4).

Study I. (Table 4)

Initially 48 patients were assigned to the study, 24 in each group. Due to laboratory errors one patient in the control group was excluded. In the treatment group there were three drop outs, two because of nausea after addition of spironolactone and one patient was excluded because of hyperclacemia found retrospectively in the prestudy blood samples.

Thus there were 44 patients included in the study, 23 (9 men, 14 women) in the control group and 21 (9 men, 12 women) in the treatment group. In the control group 12 patients had AHT (WHO Grade 1 in 2, Grade 2 in 8 and Grade 3 in 2, and 11 had CHF (5 NYHA II and 6 NYHA III). The mean age was 67.5 ± 5 years and the mean duration of prior diuretic therapy was 48 ± 39 months. The mean age in the treatment group was 68.5 ± 5 years, and the mean duration of prior diuretic therapy was 98 ± 72 months. Nine had CHF (4 NYHA II, 5 NYHA III) and 12 had AHT (WHO Grade 1 in 1, Grade 2 in 8, Grade 3 in 3).

Study II. (Table 4)

The trial consisted of 39 patients, 20 controls (7 men, 13 women) and 19 (9 men, 10 women) in the treatment group since one patient in this group could not finish the study because of general malaise. In the control group 9 persons had AHT (2 WHO 1, 6 WHO 2 and 1 WHO 3). Eleven patients had congestive heart failure (8 NYHA II, 3 NYHA III). The mean age was 67 ± 4 years and the mean duration of earlier diuretic therapy was 58 ± 44 months. The treatment group included 14 patients with AHT (8 WHO 1 and 6 WHO 2) and 5 with CHF, all with NYHA II. The mean age was 60 ± 5.6 years and the mean duration of prior diuretic therapy was 64 ± 46 months.

Study III. (Table 4)

55 patients completed the study, 28 (14 men, 14 women) in the treatment group and 27 (13 men, 14 women) in the control group. There were three drop outs in the treatment group, one because of vertigo, one because of hypokalemia in conjunction with surgical therapy and one on account of development of a type II diabetes mellitus. In the control group 13 had AHT (4 WHO 1, 8 WHO 2 and 1 WHO 3) and 14 had CHF (8 NYHA II and 6 NYHA III). The mean age was 67 ± 4.1 years and the mean duration of prior diuretic was 67 ± 47 months. The treatment group included 14 patients with AHT (6 WHO 1, 8 WHO 2) and 14 patients with CHF (10 NYHA II, 4 NYHA III). The mean age of the patients and duration of earlier diuretic therapy was 66 ± 6.4 years and 62 ± 59 months respectively.

Study IV. (Table 4)

39 patients were assigned to the study, 19 in the control group (9 men, 10 women) and 20 in the treatment group (4 men, 16 women). In the control group 17 had AHT (2 WHO Grade 1, 15 WHO Grade 2). 5 patients had CHF (4 NYHA II and 1 NYHA III). 3 patients had both AHT (WHO grade 2) and CHF (1 NYHA 1 and 2 NYHA II). The mean age was 67.8 ± 4 years and the mean duration of prior diuretic therapy was 73 ± 54 months. In the treatment group 18 had AHT (6 WHO grade 1, 12 WHO grade 2) and 4 CHF (NYHA grade II). Two patients had both AHT and CHF (WHO grade 2 and NYHA grade II respectively). The mean age of the recruited patients was 62.2 ± 4.2 years and the mean duration of prior diuretic therapy was 100 ± 66 months.

Table 4. Study population.

Study		Number of participants			Mean age ± SD	Diagnosis CHF AHT	Mean duration of therapy (months ± SD)	Drop outs
		Total	Men	Women				
Normals		12	7	5	67.7±3.3	- -	- -	0
1. Spironolactone	C	23	9	14	67.5±5.0	11 12	48±39	1
	T	21	9	12	68.5±5.0	9 12	98±72	3
2. 37.5 mg Triamterene + 25 mg Hydrochlorothiazide	C	20	7	13	67.0±4.0	11 9	58±44	0
	T	19	9	10	60±5.6	5 14	64±46	1
3. 5 mg Amiloride + 50 mg Hydrochlorothiazide	C	27	13	14	67±4.1	14 13	67±47	0
	T	28	14	14	66±6.4	14 14	62±59	3
4. Magnesium aspartate HCl	C	19	9	10	67.8±4.0	5 17	73±54	0
	T	20	4	16	62.2±4.2	4 18	100±66	0
5. 5 mg Amiloride + 50 mg Hydrochlorothiazide								
		13	7	6	62.6±6.6	3 10	50±38	2

C = Control

T = Therapy

CHF = Congestive Heart Failure

AHT = Arterial Hypertension

Study V. (Table 4)

13 patients were included in the study (7 men and 6 women), mean age 62.6 ± 6.6 years. Two patients were excluded due to laboratory failure. 10 patients had arterial hypertension (5 WHO I, 5 WHO 2). 3 had congestive heart failure (2 NYHA II and 1 NYHA III). The mean duration of previous diuretic therapy was 50 ± 38 months. The previous diuretic therapy was during six months replaced with a combination of 5 mg amiloride + 50 mg hydrochlorothiazide.

Results. NormalsSerum electrolytes (Mg, K)

The mean serum K for men was 3.96 ± 0.22 mmol/l and for women 3.86 ± 0.46 mmol/l respectively. The mean serum K concentration for the whole group was 3.92 ± 0.32 mmol/l (Table 5 and 6).

The mean serum Mg level for men was 0.87 ± 0.07 mmol/l and for women 0.86 ± 0.11 mmol/l respectively. The mean serum Mg level for the whole group was 0.87 ± 0.08 mmol/l.

Muscle electrolytes (Mg, K)

The mean muscle Mg content for men was 4.50 ± 0.48 mmol/100 g FFDS and for women 4.53 ± 0.30 mmol/100 g FFDS respectively. For men and women together the mean muscle Mg content was 4.51 ± 0.40 mmol/100 g FFDS (Table 5 and 7).

The mean skeletal muscle K content was for men 43.1 ± 3.0 mmol/100 g fat free dry solids (FFDS) and for women 44.7 ± 3.2 mmol/100 g FFDS

respectively. For the whole group the mean skeletal muscle content was 43.7 ± 3.10 (Table 5 and 7).

Effects of spironolactone on serum and muscle electrolytes in patients on long-term diuretic therapy for congestive heart failure and/or arterial hypertension (Paper 1)

Serum electrolytes

There was no significant difference between the control and the treatment groups at the onset of the study for the mean values of serum K, Mg, Na, Cl, HCO_3 or PO_4 .

In the treatment group there was a significant rise of mean serum K from 3.62 ± 0.43 mmol/l to 3.90 ± 0.42 mmol/l ($p < 0.001$) after six months' study. The other electrolytes remained unchanged (Table 6).

Muscle electrolytes (Mg, K)

The mean muscle magnesium values were in both groups within normal borders at the onset of the study. After six months' study there was a decrease in the mean muscle Mg value in the control group from 4.44 ± 0.54 to 3.90 ± 0.67 mmol/100 g FFDS. On the other hand after administration 100 mg of spironolactone, the treatment group showed a significant increase compared to the control group from 4.38 ± 0.49 to 4.52 ± 0.52 mmol/l ($p < 0.01$) (Table 7).

TABLE 5

Normals

	Men (n = 7)	Women (n = 5)	Total (n = 12)
Mean age (\pm SD)	69.9 \pm 2.1	65.2 \pm 3.2	67.7 \pm 3.3
S-K mmol/l (mean value \pm SD)	3.96 \pm 0.22	3.86 \pm 0.46	3.92 \pm 0.32
S-Mg mmol/l (mean value \pm SD)	0.87 \pm 0.07	0.86 \pm 0.11	0.87 \pm 0.08
Skeletal-Mg mmol/100 g FFDS (mean value \pm SD)	4.50 \pm 0.48	4.53 \pm 0.30	4.51 \pm 0.40
Skeletal-K mmol/100 g FFDS (mean value \pm SD)	43.10 \pm 3.0	44.70 \pm 3.2	43.70 \pm 3.1

The mean muscle potassium in the two groups was somewhat low at the onset of the study, but after six months' study the treatment group showed a significant rise compared to the control group ($p < 0.001$) from 41.4 ± 4.8 mmol/100 g FFDS to 44.4 ± 4.5 mmol/100 g FFDS. The control group showed a further decrease from 41.4 ± 4.4 to 37.7 ± 6.1 mmol/100 g FFDS (Table 7).

Effects of triamterene on serum and skeletal muscle electrolytes in diuretic treated patients (Paper II)

Serum electrolytes

At the onset of the study there was no significant difference between the control group and the treatment group concerning serum Na, Cl, Ca, PO_4 , HCO_3 or K. After six months of unchanged therapy, there were no significant changes in these patients in the control group except for serum sodium, which had decreased from 142 mmol/l ± 2.3 to 140.6 ± 1.5 mmol/l ($p < 0.05$).

In the treatment group there was a significant decrease of serum Cl from 101.8 mmol/l ± 2.2 to 100.4 ± 1.6 mmol/l ($p < 0.05$). PO_4 increased significantly from 0.87 mmol/l ± 0.13 to 0.97 mmol/l ± 0.12 ($p < 0.05$). The other parameters including serum Mg and K remained essentially unchanged compared to the onset of the study.

At the beginning of the study the serum Mg values were significantly higher in the control group, 0.85 mmol/l ± 0.09 , compared to 0.77 mmol/l ± 0.08 in the treatment group ($p < 0.01$). There was no significant change in serum Mg during the study in any of the groups (Table 6).

Skeletal muscle electrolytes (Mg, K)

The mean muscle magnesium values were within normal limits at the onset of the study in both groups. But 25% of the patients in the control group and 46% in the treatment group has subnormal values for muscle Mg content.

After 6 months of unchanged therapy the control group demonstrated a significant decrease of their mean muscle Mg values from 4.37 ± 0.52 mmol/100 g FFDS to 3.84 ± 0.73 mmol/100 g FFDS, while the triamterene treated patients showed a significant increase in their mean muscle Mg values compared to the controls, from 4.03 ± 0.82 to 4.62 ± 0.44 mmol/100 g fat free dry solids ($p < 0.005$) (Table 7)

The mean muscle K was within normal values at the onset of the study. Only 10% of the patients in the triamterene treated group had subnormal levels at the beginning of the study. The corresponding figures in the control group was 32%. In the control group there was a drop in the mean muscle K values after 6 months on unchanged therapy from 41.6 ± 3.4 mmol/100 g FFDS to 38 ± 6.32 mmol/100 g FFDS, now 63% of the patients had subnormal values. The triamterene treated patients showed a significant increase of their mean muscle K values from 45.6 ± 5.46 mmol/100 g FFDS to 48 ± 3.83 mmol/100 g FFDS compared to the controls ($p < 0.05$) (Table 7).

Amiloride prevents thiazide induced intracellular potassium and magnesium losses (paper III)

Serum electrolytes

There was no significant difference between the control group and the treatment group at the onset of the study concerning plasma-Na, Cl, K, Mg, Ca, PO₄ or HCO₃. After six months of study there was a significant decrease of plasma sodium in both groups and an increase of plasma bicarbonate ($p < 0.05$) in the control group. All other serum electrolytes remained unchanged in the groups during the study period (Table 6).

Skeletal muscle electrolytes (Mg, K)

The mean muscle values were within normal limits at the onset of the study in both groups. But 21% of the patients in the treatment group and 33% of the controls had subnormal values. After six months of unchanged therapy there was a decrease of mean magnesium value from 4.25 ± 0.50 to 4.08 ± 0.46 mmol/l 100 g FFDS. The treatment group showed a significant increase in their mean values, compared to the control group, from 4.38 ± 0.58 to 4.66 ± 0.63 mmol/100 g FFDS ($p < 0.025$) (Table 7).

At the onset of the study the mean muscle K was subnormal in the control group 39.7 ± 4.16 mmol/100 g FFDS. 63% of the patients having subnormal values and borderline 40.5 ± 3.92 mmol/100 g FFDS, in the treatment group (50% of the patients having subnormal values).

In the control group there was drops in the mean muscle potassium on unchanged therapy, from 39.7 ± 4.16 mmol/100 g FFDS to 38.2 ± 5.40

mmol/100 g FFDS. The treatment group showed a significant increase in their mean values, compared to the control group, from 40.5 ± 3.92 mmol/100 g FFDS to 45.7 ± 5.13 mmol/100 g FFDS ($p < 0.001$) (Table 7).

Effects of peroral magnesium on plasma and skeletal muscle electrolytes on long-term diuretic therapy (paper IV)

Serum electrolytes

The mean serum Mg concentration increased significantly in the treatment group compared to the control group, from 0.76 ± 0.08 mmol/l to 0.78 ± 0.06 mmol/l, the corresponding figures for the control group was 0.84 ± 0.12 mmol/l and 0.83 ± 0.07 mmol/l respectively ($p < 0.05$). Except for Mg, there were no significant change of serum electrolytes in the groups before and after the study period (Table 6).

Muscle electrolytes (Mg, K)

There was a significant rise within the magnesium treated group during the study period. In fact, mean muscle magnesium increased from 3.97 ± 0.61 mmol/100 g FFDS to 4.52 ± 0.70 mmol/100 g FFDS ($p < 0.02$). In the control group there was a decrease in mean muscle magnesium from 4.18 ± 0.47 mmol/100 g FFDS to 3.80 ± 0.62 mmol/l (n.s.). There was also a significant increase in the mean muscle Mg content compared to the control group ($p < 0.001$) (Table 7).

Concerning mean muscle potassium content it was a significant increase compared to the control group ($p < 0.05$). The actual figures in the treatment group were 42.4 ± 3.62 mmol/100 g FFDS at the onset of

the study, and six months later 43.2 ± 5.03 mmol/100 g FFDS. The corresponding figures in the control group were 40.1 ± 4.69 mmol/100 g FFDS and 37.7 ± 5.89 mmol/100 g FFDS respectively.

Amiloride in the correction of diuretic induced intracellular potassium and magnesium depletion. (Paper V)

Serum electrolytes

There was no significant change in the mean values of serum K, Mg, Na, Cl, Ca or PO_4 during the study.

Muscle electrolytes (Mg, K)

The mean skeletal muscle Mg value was low at the onset of the study, 3.90 ± 0.67 mmol/100 g FFDS, but at the end of the study the mean muscle Mg value was normalized, 4.77 mmol/100 g FFDS ($p < 0.005$). The mean skeletal muscle K was somewhat low at the beginning of the study, 40.7 ± 4.47 mmol/100 g FFDS, but after six months study it was a significant increase to 45.4 ± 4.27 mmol/100 g FFDS ($p < 0.005$).

Table 6. Results serum electrolytes (K, Mg)

Study		Number of participants	K/ se mmol/l \pm SD, mean values Total 0;	Mg/ se mmol/l \pm SD, mean values 6 months 0;	
Normals		12	3.92 \pm 0.32	-	0.87 \pm 0.08
I. Spironolactone	C	23	3.55 \pm 0.39	3.63 \pm 0.38	0.85 \pm 0.09
	T	21	3.62 \pm 0.43	p<0.001 3.90 \pm 0.42	0.86 \pm 0.09
II. 37.5 mg Triamterene + 25 mg Hydrochlorothiazide	C	20	3.54 \pm 0.38	3.51 \pm 0.31	0.85 \pm 0.09
	T	19	3.62 \pm 0.25	3.78 \pm 0.38	0.77 \pm 0.08
III. 5 mg Amiloride + 50 mg Hydrochlorothiazide	C	27	3.47 \pm 0.42	3.47 \pm 0.38	0.84 \pm 0.11
	T	28	3.59 \pm 0.37	3.58 \pm 0.33	0.82 \pm 0.08
IV. Magnesium aspartate HCl	C	19	3.42 \pm 0.38	3.47 \pm 0.39	0.84 \pm 0.12
	T	20	3.65 \pm 0.29	3.62 \pm 0.29	0.76 \pm 0.08
V. 5 mg Amiloride + 50 mg Hydrochlorothiazide		13	3.51 \pm 0.45	3.35 \pm 0.21	0.74 \pm 0.11
					0.71 \pm 0.06

C = Control
T = Therapy

Table 7. Skeletal muscle electrolytes (K, Mg)

Study		Number of participants Total	K/muscle ± SD, mean values; mmol/100 g FFDS		Mg/muscle ± SD, mean values; mmol/100 g FFDS 0; 6 months	
			0;	6 months	0;	6 months
Normals		12	43.70±3.1	-	4.51±0.40	-
I. Spironolactone	C	23	41.4±4.4	p<0.001	37.7±6.1	4.44±0.54
	T	21	41.4±4.8		44.4±4.5	4.38±0.49
II. 37.5 mg Triamterene + 25 mg Hydrochloro- thiazide (Triamtex®)	C	20	41.6±3.44	p<0.05	38±6.32	4.37±0.52
	T	19	45.6±5.46		48±3.83	4.03±0.82
III. 5 mg Amiloride + 50 mg Hydrochloro- thiazide (Moduretic®)	C	27	39.7±4.16	p<0.001	38.2±5.40	4.25±0.50
	T	28	40.5±3.92		45.7±5.13	4.38±0.58
IV. Magnesium aspartate HCl	C	19	40.1±4.69	p<0.05	37.7±5.89	4.18±0.47
	T	20	42.4±3.62		43.2±5.03	3.97±0.61
V. 5 mg Amiloride + 50 mg Hydrochloro- thiazide (Moduretic®)		13	40.7±4.47	p<0.005	45.4±4.27	3.90±0.67

C = Control

T = Therapy

DISCUSSION

The muscle biopsy technique elaborated by Bergström (139) is well established. The determination of intracellular values is calculated, presuming a normal resting membrane potential (141). However, as the sick cell has a low membrane potential (143), chloride may enter the cell in increased amounts. The use of the chloride method (140) may lead to an overestimation of the extracellular space. In that case the calculated intracellular space falsely becomes too small, causing too high an intracellular concentration.

Because of the possibilities outlined above the main conclusions from these studies are related to absolute values for skeletal muscle K and Mg related to fat-free dry solids (143). Concerning skeletal muscle K and Mg in healthy elderly people our results agree well with the results obtained in studies published earlier (Table 8).

The mean age for men in our study was 69.9 ± 2.1 years. The mean age for men in the study by Möller et al. (144) was 77.3 ± 2.49 years. The volunteers in the studies published by Sjögren et al. (145) and by Bergström et al. (144, 146) were younger. The mean age was 36 years in the study by Sjögren et al. (145) and the men participating in the study by Bergström et al. (144, 146) were between 20-36 years.

In our study the mean value for skeletal muscle K and Mg for men was 43.10 ± 3.0 mmol/100 g FFDS and 4.50 ± 0.48 respectively, compared to values obtained for men by Möller et al. (143) 44.78 ± 1.08 mmol/100 g FFDS and 4.27 ± 0.09 mmol/100 g FFDS respectively.

TABLE 8. Muscle content of Mg and K mean values mmol/100 g fat free dry solids (FFDS). Healthy individuals.

Study	Total n=12	Widman		Sjögren (145)		Möller (144)		Bergström et al (144, 146)
		Women n=5	Men n=7	Men n=20	Women n=10	Men n=10	Men n=24	
		mean age 67.7±3.3	mean age 66.2±3.2	mean age 69.9±2.1	mean age 36(19-64)	mean age 34(23-61)	mean age 77.3±2.49 (20-36)	
Mg	4.51±0.40	4.53±0.30	4.50±0.48	4.32±0.20	4.22±0.16	4.27±0.09	4.42±0.05	
K	43.70±3.10	44.70±3.2	43.10±3.0	46.40±3.47	44.20±3.71	44.78±1.08	45.68±0.34	

Regarding skeletal K Sjögren et al. (145) reported a mean value for men of 46.40 ± 3.47 compared to a mean value of 45.68 ± 0.34 mmol/100 g FFDS in Bergström et al. (144, 146). The corresponding figures for Mg was 4.32 ± 0.20 mmol/100 g FFDS and 4.42 ± 0.05 mmol/100 g FFDS respectively.

For women the mean values for skeletal muscle K and Mg are also in agreement with the data obtained by Sjögren et al. (145), although our volunteers were older. The mean age in our study was 66.2 ± 3.2 years compared to 34 years (23-61) in the study by Sjögren et al. (145). The actual figures for mean muscle K and Mg were 44.70 ± 3.2 mmol/100 g FFDS and 4.53 ± 0.30 mmol/100 g FFDS respectively in our study. The corresponding figures obtained by Sjögren et al. (145) were 44.20 ± 3.71 mmol/100 g FFDS and Mg 4.22 ± 0.16 mmol/100 g FFDS respectively.

The observation that the controls in the four different studies show a decrease in their skeletal muscle K and Mg values (Table 7), despite previous diuretic therapy for several years, probably indicates that the patients adhered more strictly to the prescribed regimen during the study period.

Patients at risk of developing potassium and magnesium depletion are often elderly patients on long-term diuretic treatment for hypertension and/or congestive heart failure. By the age of 60 diuretic drugs constitute one of the dominant groups of drugs prescribed (1). In this group of patients there is a high frequency of secondary aldosteronism due to heart disease together with a failing heart.

Secondary aldosteronism is also increased by the diuretic treatment per se (69). Other complicating factors that may increase the risk of potassium and magnesium depletion are simultaneous medication with digitalis and a low intake of magnesium (147, 56). According to Dyckner and Wester (92) elderly people on diuretic treatment for more than three continuous years show a decrease in their mean muscular values. The mean age in their investigated groups were 68.4 years. In the present studies the mean age ranged between 60.5 to 68.5 years, and the mean duration of diuretic therapy ranged between 48-98 months (Table 4).

The normal ratio between intra and extracellular potassium is essential for maintaining a normal membrane potential (117) and the ratio is maintained by Na-K-ATP:ase (the "sodium-pump"), where sodium is actively pumped out of the cell and potassium into the cell. Magnesium is essential for the optimal activity of the sodium pump (104). Changes in the intra- to extracellular potassium ratio will cause changes in the resting membrane potential, potassium conductance and repolarisation, favoring the occurrence of serious heart arrhythmias (106). However the action mechanism behind the magnesium effect on the potassium metabolism has not been definitively settled. In 1963 Whang & Welt (100) demonstrated in rats that normal magnesium levels were a presquite for the maintenance appropriate potassium levels. These results were then confirmed by Ryan and Hingerty (101). In double depleted rats (Mg and K depletion) there was a significantly greater loss of potassium from skeletal and cardial muscle than in single (potassium) depleted groups of rats. Clinically the close relationship between K and Mg has been demonstrated by Dyckner &

Wester (105) where a skeletal muscle potassium deficiency could not be corrected unless the magnesium deficiency was corrected. The magnesium infusions also caused a significant reduction in ventricular ectopic beats, while the potassium infusions did not.

Although magnesium is an essential co-factor for the optimal functioning of the "sodium pump" (104) and digoxin is a well-known inhibitor of the Na-K-ATP:ase, no experiment to date has demonstrated a decline in Na-K-ATPiase activity in magnesium deficiency. An other attractive theory is that magnesium deficiency creates a "leaky membrane", which causes loss of potassium from the cell, while simultaneously sodium is accumulated on the inside of the cell. The anti-arrhythmic effect of magnesium seems not to be coupled to reactivation of Na-K-ATP:ase, rather the effect may be linked to a direct membrane effect, preventing further loss of potassium (116).

In states of magnesium deficiency a vicious circle might be said to exist. Magnesium deficiency may cause potassium deficiency and potassium depletion reduces tubular secretion of digoxin up to 50% (111). In congestive heart failure 35-45% of all deaths occur suddenly without any evidence of functional deterioration and these patients have a high frequency of ventricular arrhythmias (149). The high incidence of sudden death may be explained by the heart disease per se but other contributory factors are the activation of endocrine compensatory mechanisms; the activation of the renin-angiotensin system and increased levels of noradrenaline causing accumulation of sodium and increased losses of potassium and magnesium. As mentioned above diuretics per se increase secondary aldosteronism (69), contributing to

the disturbance of the potassium/ magnesium balance, favoring the occurrence of serious cardiac arrhythmias. In the setting of an acute myocardial infarction, diuretic treatment may also increase the risk of hypokalemia and hypomagnesemia leading to an increased risk of serious arrhythmias (15, 16, 17, 18, 19). In 40% of the hypokalemic patients there is also simultaneous hypomagnesemia (96). Hollifield (48) has demonstrated a significant correlation between ventricular ectopic beats and a decline in serum potassium and magnesium levels.

In subgroup analyses from some large scale hypertension studies there have been reports of increased sudden death among participants with pathological ECG (46, 47, 149, 150, 151). The principal treatment drugs in these trials were diuretics.

In the MRFIT-trial (46) mortality from coronary heart disease with pathological ECG (SI group) compared to the control group (UC) was 2.9% and 1.9% respectively. In the SI group there were 17 sudden deaths and in the control group 7. In the VA-study (47) there were 8 sudden deaths in the diuretic-treated group and 4 in the control group. In the Oslo study there were 6 sudden deaths in the treatment group and none in the control group (149). However subgroup analyses must be treated with great care and so far according to Berglund (152) there is no clear cut unambiguous scientific data to support the idea that diuretic treatment per se precipitates sudden death. In this context the findings of Morgan et al. (153) are interesting. In their study 172 men with mild hypertension (95-109 mm Hg) were randomly assigned to one of the following groups: no therapy, low sodium diet, chlorothiazide therapy 500 mg/day without potassium supplementation and the final group,

propranolol (80 mg-320 mg/day in divided doses). The patients were followed up for from six months to three years. In the thiazide group of men ($n = 55$) there was a higher mortality rate than expected but not in the other three groups. In the thiazide group there were 13 deaths (10/13 due to myocardial infarction/sudden death), compared to 13 deaths (8/13 due to myocardial infarction/sudden death) amongst the remaining 117 men. In a group of men with more severe hypertension (diastolic blood pressure > 110 mm Hg) randomly treated with chlorothiazide or propranolol, the thiazide-group showed no increased mortality rate compared to the propranolol group. Recently the results from the MAPHY-study have been published (154). The study included 3234 men aged 40-60 years. They were randomized to metoprolol ($n = 1609$) or to thiazides ($n = 1625$). After a median follow-up time of 4.2 years there was a significantly reduced total mortality in the metoprolol treated men compared to the thiazide treated men ($p = 0.28$), mainly due to a significantly reduced mortality from myocardial infarction or sudden coronary death ($p = 0.12$).

Our results in Paper I are in favor with earlier studies and confirms the magnesium and potassium sparing ability of spironolactone (22, 24, 25). Horton et al. (22) administered 1 g of spironolactone/day to two patients with primary aldosteronism and there was a marked reduction in urinary potassium- and magnesium excretion. Wheeler et al. (24) carried out total body potassium measurements in 19 patients with liver cirrhosis. Of 13 patients receiving spironolactone none had a total body potassium below the expected values. Lim and Jacob (25) infused a soluble spironolactone derivate to twelve men with liver cirrhosis resulting in a significant reduced 24-hour urinary magnesium

($p < 0.01$) and potassium ($p < 0.05$) excretion compared to a previous control sampling period.

1967 Hänze and Segberth (82) demonstrated a significant reduction in diuretic-induced urinary magnesium excretion when triamterene was added. Devane and Ryan (155) using saline load rats and Leary and Reyes (30) studies on healthy volunteers also demonstrated a reduction in magnesium urinary excretion when triamterene was added. Agreeing with these findings our results also indicate that triamterene is capable of maintaining normal cellular K and Mg content. The EWPHE (155) is one of the few large scale hypertension studies which has shown a significant reduction in cardiovascular death. Interestingly the principal treatment was a combination of hydrochlorothiazide and triamterene in this study. It might possibly be that the electrolyte (K, Mg) saving ability of triamterene contributed to the positive result. In the EWPHE-Study (156) there was a 60% reduction of the total number of myocardial infarctions. However only 35% of the original 850 participants were still receiving the randomized therapy when the trial was closed (152).

Amiloride according to our results can be considered as a magnesium-saving diuretic agent. In a short-term study (one week) Bergström 1975 (157) demonstrated that a combination of 15 mg amiloride + 150 mg hydrochlorothiazide did not cause any change in skeletal muscle magnesium. Devane and Ryan (155) studying rats, showed a significant reduction in urinary magnesium excretion compared to controls after administering a combination of amiloride

and furosemide. The administration of 5-10 mg of amiloride did not cause any significant increase in urinary Mg excretion after 24 hours not even when used in combination with 50 mg hydrochlorothiazide.

The results from Paper V even indicate that amiloride may possibly even correct diuretic-induced skeletal muscle K and Mg depletion.

The addition of orally administered magnesium aspartate HCl in combination with diuretic treatment caused a significant increase in skeletal muscle Mg and K. Sjögren (158) orally administered magnesium hydroxide to type I diabetics. At the onset of the study their muscle potassium and magnesium was low compared to controls. The treatment, as in our study, was well tolerated and after 21 weeks there was a significant rise in skeletal muscle K ($p < 0.001$) and Mg respectively ($p < 0.001$).

The Swedish National Board of Health and Welfare has recently made guidelines how to avoid electrolyte disturbances in diuretic treatment of heart failure (159) and mild hypertension (160):

In heart failure potassium saving diuretics amiloide or spironolactone (triamterene is not available on the Swedish market) is recommended together with a thiazide or loop diuretic agent. In mild hypertension, "a low dose of a betablocking drug, or a thiazide without fixed addition of potassium is advised as first line treatment. In general the thiazide is to be combined with spironolactone or amiloride in patients who are elderly or who show signs of cardiac disease". According to the present studies another possible alternative may be oral magnesium substitution.

Conclusions

The potassium-sparing diuretics (spironolactone, amiloride and triamterene) prevents the diuretic induced skeletal muscle depletion of Mg and K.

The addition of peroral magnesium aspartate to previous long-term (> 1 year) diuretic treated patients with arterial and/or congestive heart prevents the diuretic induced skeletal muscle depletion of K and Mg.

The addition of amiloride to previous long-term (> 1 year) diuretic treated patients with arterial hypertension and/or congestive heart failure seems to correct diuretic induced skeletal muscle depletion of K and Mg.

General summary: (Table 4, 6, 7)

1. Skeletal muscle electrolytes, K and Mg were obtained in 12 healthy elderly people (7 men and 5 women), mean age 67.7 ± 3.3 years. The mean values for skeletal muscle K and Mg were 43.1 ± 3.0 mmol/100 g FFDS and 4.50 ± 0.48 mmol/100 g FFDS respectively.
2. Three different potassium-sparing diuretics were added during a six month study period to prior long-term (> 1 year) diuretic treated patients with arterial hypertension and/or congestive heart failure:

a) Spironolactone (paper I).

The study group consisted of 21 patients (9 men and 12 women), mean age 68.5 ± 5.0 years. The control group consisted of 23 patients (9 men and 14 women), mean age 67.5 ± 5.0 years. The mean serum K in the treatment group increased significantly from 3.62 ± 0.43 mmol/l to 3.90 ± 0.42 mmol/l ($p < 0.001$). The mean skeletal muscle K and Mg level increased significantly compared to the control group from 41.4 ± 4.8 mmol/100 g FFDS to 44.4 ± 4.5 mmol/100 g FFDS ($p < 0.001$) and Mg from 4.38 ± 0.49 mmol/100 g FFDS to 4.52 ± 0.52 mmol/100 g FFDS respectively ($p < 0.01$).

b) Triamterene (paper II).

The study group consisted of 19 patients (9 men and 10 women), mean age 60 ± 5.6 years. The control group included 20 persons (7 men and 13 women), mean age 67.0 ± 4.0 years. The mean skeletal muscle K and Mg in the treatment group showed compared to the control group a significant increase from 45.6 ± 5.46 mmol/100 g FFDS to 48.0 ± 3.83 mmol/100 g FFDS ($p < 0.05$) and Mg 4.03 ± 0.82 mmol/100 g FFDS to 4.62 ± 0.44 mmol/100 g FFDS ($p < 0.005$), respectively.

c) Amiloride (paper III).

The study group consisted of 28 participants (14 men and 14 women), while the control group included 27 participants (14 men and 13 women). The mean age was 66.0 ± 6.4 years and 67 ± 4.1 years respectively.

In the study group, compared to the control group, there was a significant increase in the mean skeletal muscle K and Mg from $40.5 \pm$

3.92 mmol/100 g FFDS to 45.7 ± 5.13 mmol/100 g FFDS ($p < 0.001$) and Mg from 4.38 ± 0.58 mmol/100 g FFDS to 4.66 ± 0.63 mmol/100 g FFDS ($p < 0.025$) respectively.

3. Oral addition of Mg-aspartate HCl (15 mmol/day) to long-term (> 1 year) diuretic treated patients with arterial hypertension and/or congestive heart failure (paper IV).

The study group consisted of 20 patients (4 men and 16 women) and the control group included 19 participants (9 men and 10 women). The mean age was 62.2 ± 4.2 years and 67.8 ± 4.0 years respectively. The study group showed a significant increase in the mean serum Mg level compared to the control group from 0.76 ± 0.08 mmol/l to 0.78 ± 0.06 mmol/l ($p < 0.05$). The study group also showed a significantly increase in their mean skeletal K and Mg levels, compared to the control group from 42.4 ± 3.62 mmol/100 g FFDS to 43.2 ± 5.03 mmol/100 g FFDS ($p < 0.05$) and Mg from 3.97 ± 0.61 mmol/100 g FFDS to 4.52 ± 0.07 mmol/100 g FFDS ($p < 0.001$), respectively.

4. Addition of amiloride corrects diuretic induced skeletal muscle K and Mg depletion (paper V).

The study group consisted of 13 patients (7 men and 6 women) with long-term (> 1 year) diuretic treatment for arterial hypertension and/or congestive heart failure. The mean age was 62.6 ± 6.6 years.

The previous diuretic treatment was replaced with a combination of 50 mg hydrochlorothiazide + 5 mg amiloride. At the onset of the study the mean skeletal K value was somewhat low, 40.7 ± 4.47 mmol/100 g FFDS but at the end of the study, six months later, the mean skeletal

muscle values were well within normal values, 45.4 ± 4.27 mmol/100 g FFDS ($p < 0.005$). The mean skeletal muscle Mg value was low at the onset of the study, 3.90 ± 0.67 mmol/100 g FFDS, but at the end of the study the mean muscle Mg value was normalized, 4.77 ± 0.52 mmol/100 g FFDS ($p < 0.005$).

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