CORRELATION BETWEEN Na, K-ATPase ACTIVITY AND POTASSIUM AND MAGNESIUM CONTENTS IN SKELETAL MUSCLE OF RENAL STONE PATIENTS

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Abstract. Samples of external oblique muscles were surgically removed from 45 renal stone patients and analyzed for their K, Na and Mg content. The muscle samples were also measured for membrane Na, K-ATPase activity from the assay of its K^+-dependent 3-0-methyl fluorescein phosphatase (K^+-dependent 3-0-MFPase) activity. The results showed that the mean muscle contents ± SEM of K, Na and Mg were 65.2 ± 1.7 (range, 41.1 to 86.1), 45.5 ± 2.0 (range, 23.5 to 73.2) and 6.3 ± 1.0 (range, 4.1 to 8.5) μmol/g wet weight, respectively. The mean activity ± SEM of the K^+-dependent 3-0-MFPase or the Na, K-ATPase was calculated by subtracting the activity of the basal-form from that of the total-3-0-MFPase, which was 113 ± 21 (range, 11 to 177) nmol/g wet weight/minute. The activity of the Na, K-ATPase showed a significant correlation with muscle K-content ($r = 0.52$, $p<0.001$) and Mg content ($r = 0.45$, $p<0.002$). Though the external oblique muscles of renal stone patients in our study, as compared to data from other sources, had a considerably low concentration of K and Mg, they exhibited a good correlation with membrane-Na, K-ATPase activity. Our results, therefore, support previous observations made by other investigators.

INTRODUCTION

Signs of K-depletion, as reflected by hypokalemia, hypokaliurea, low K-intake, positive K-balance and low activity of Na, K-ATPase in the red blood cell membranes, are often encountered among both normal and stone forming rural residents of Northeast Thailand (Sriboonlue *et al*, 1991; 1998; 1999; Tosukhowong *et al*, 1992). These abnormalities are associated with renal stone disease as well as other clinical disorders frequently seen in the region (Sitprija *et al*, 1991). By direct measurement of K content in skeletal muscles, Bovornpadungkitti *et al* (2000) showed that the mean value of muscle-K concentration in renal stone formers was lower than those of other reports (Dyckner and Wester, 1978; Dorup *et al*, 1988b). Moreover, renal stone formers' muscles had a concurrently low level of Mg but a high level of Na. A number of studies have demonstrated that K-deficiency usually leads to a progressive and marked decrease in the activity of Na, K-ATPase, an enzyme in which Mg serves as a cofactor, in skeletal muscle. This response is seen in a variety of species, including human subjects suffering from K-deficiency induced by chronic diuretic treatment (Norgaad *et al*, 1981; Kjeldsen *et al*, 1984). Similarly, a rural northeast Thai population - having prevalent hypokalemia-demonstrated lower Na, K-ATPase activity in their red blood cell membranes than those in healthy urban subjects (Tosukhowong *et al*, 1992; 1996). One of the causes may be a down-regulated control of the enzyme by K-depletion. Our study was undertaken, in order to gain more understanding of the relationship between K- and Mg-content and Na, K-ATPase activity in the skeletal muscle of renal stone patients.

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MATERIALS AND METHODS

The protocol of this project was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Khon Kaen University. Written informed consent was obtained from each subject.

Subjects and muscle samples collection

Enrolled in our study were 45 male renal stone patients admitted to the Khon Kaen Regional Hospital for surgery between January and April, 1997. Included were those aged between 20 and 60 years, who had normal renal function (serum creatinine ≤ 2.3 mg/dl), no significant bacteriuria (bacterial count ≤ 10^5 colony forming units/ml of urine), and who were not on any drug treatment. During surgery, a small piece of external oblique skeletal muscle fiber-50 to 100 mg-was surgically removed from each subject and immediately stored at -80ºC. The muscle samples were used to analyze for K, Na and Mg contents, as well as to assay for the Na, K-ATPase activity.

Determination of K, Na and Mg content in muscle samples

About 20 mg of the muscle samples were used to determine K, Na and Mg contents by the trichloroacetic acid (TCA) method described by Dorup *et al* (1988a). The samples were homogenized in 2 ml of 5 g/l TCA using a hand glass homogenizer and centrifuged for 10 minutes at 600*g*. The clear supernatant was then used to analyze for Na, K and Mg using an atomic absorption spectrophotometer.

Assay of Na, K-ATPase activity in muscle samples

The activity of K^+-dependent 3-0-methylfluorescein phosphatase (K^+-dependent 3-0-MFPase) is always associated with that of the Na, K-ATPase (Albers and Koval, 1996). Therefore, the activity of Na, K-ATPase can be indirectly assessed from the assay of the K^+-dependent 3-0-MFPase. We chose the fluorimetric assay of this enzyme because it can be done with high sensitivity using only small amount of crude tissue homogenate (Huang and Askari, 1976; Norgaard *et al*, 1984).

Crude homogenate preparation: Crude homogenate, described by Norgaad *et al* (1984), was prepared by homogenizing about 50 mg of muscle sample in 450 μl of homogenate buffer containing 30 mmol/l histidine, 2 mmol/l ethylenediaminetetraacetic acid (EDTA), and 250 mmol/l sucrose, pH 7.2 at 0ºC. The latent ATPase activity was then demasked by the transferring of 100 μl of homogenate to 900 μl of buffer containing 20 mmol/l imidazole, 2 mmol/l EDTA, 250 mmol/l sucrose, and 0.08 g/l sodium deoxycholate pH 7.0, then suspended for 30 minutes at 25ºC.

Determination of Na, K-ATPase activity from K-dependent 3-0-MFPase activity: The enzyme activity was measured by the method described by Norgaard *et al* (1984). The assay medium of this enzyme contained 19.5 mmol/l 3-0-methylfluorescein phosphate (3-0-MFP), 4 mmol/l $MgCl_2$, 1 mmol/l EDTA, 80 mmol/l Tris, pH 7.6 and 10 μl of the muscle homogenate in a final volume of 2,600 μl. The activity of K dependent-3-0-MFPase was measured after the addition of 2 mol/l KCl to give a final K concentration of 10 mmol/l. The mixture was then allowed to incubate for 20 minutes at 37ºC. The amount of fluorescence-due to the formation of 3-0-methylfluorescein (3-0-MF)-was measured by a fluorescence spectrophotometer (Model 650-40, Hitachi). The excitation and emission wavelengths were operated at 475 and 515 nm, respectively, and the slit width was set at 5 nm using 3-0-MF (0.2 mmol/l) as the standard.

Chemicals

All chemicals were of analytical grade. The EDTA, 3-0-MFP, 3-0-MF, sodium deoxycholate, and Tris were from Sigma Chemicals, St Louis, MO, USA. All the other chemicals were from Merck Co, Darmstadt, Germany.

RESULTS

The results of external oblique muscle analysis are shown in Table 1. The mean K,

Table 1
K, Na and Mg contents and activities of basal, total and K-dependent 3-0-methyl fluorescein phosphatase (K-dependent 3-0-MFPase) of external oblique skeletal muscles obtained from 45 stone patients.

	X ± SE	Range
Muscle contents (μmol/g wet weight)		
K	65.2±1.7	41.1-86.1
Na	45.5±2.0	23.5-73.2
Mg	6.3±1.0	4.1-8.5
Activity of 3-0-MFPase (nmol/g wet weight/minute)		
Basal activity	789±20	460-1,102
Total activity	902±22	509-1,256
K^+-dependent activity	113±21	11-177

Na and Mg contents were 65.2 ± 1.7, 45.5 ± 2.0 and 6.3 ± 1.0 μmol/g wet weight, respectively. These values were similar to our previous study using the same muscle fiber. (Bovornpadungkitti *et al*, 2000). The activity of Na, K-ATPase in crude homogenate was assessed indirectly from the assay of K^+-dependent 3-0-MFPase activity. While the total 3-0-MFPase representing all ATPase activities was 902 ± 22 nmol/g wet weight/minute, the basal 3-0-MFPase-representing all ATPase activities in the absence of K-was 789 ± 20 nmol/g wet weight/minute. Therefore, the activity of Na, K-ATPase or K^+-dependent 3-0-MFPase is calculated from the difference between the total and the basal 3-0-MFPase, which was 113 ± 21 nmol/g wet weight/minute.

The statistical analysis showed that the activity of Na, K-ATPase (K^+-dependent 3-0-MFPase) exhibited a significantly positive correlation with both K and Mg contents of the muscles (Figs 1 and 2).

DISCUSSION

Both normal and stone forming rural dwellers of Northeast Thailand are likely to be K-depleted (Sriboonlue *et al,* 1991; 1998; 1999; Tosukhowong *et al,* 1992). Recently, Bovornpadungkitti *et al* (2000) demonstrated that most of the muscle samples obtained from renal stone subjects residing in this region were low in both K and Mg compared to others (Dorup *et al,* 1988a,b). Our results support the observation made by Bovornpadungkitti *et al* (2000).

The values of K and Mg concentration in the muscle samples obtained from renal stone formers were similar to those of patients receiving long-term diuretic treatment for arterial hypertension or congestive heart failure (Dyckner and Wester, 1987; Dorup *et al,* 1993; 1988b). Since none of the subjects had gastrointestinal or renal dysfunctions, the main causes of their K- and Mg- depletion should be a low K-intake and an excessive loss of K in the sweat (Sriboonlue *et al*, 1998). The possibility of the presence of inhibitors for K-transport (Na, K-ATPase) in their plasma, both endogenous and/or exogenous in origin, might be another contributing factor to their low K and Mg status (Sitprija *et al,* 1990).

In both experimental animals fed on low K and Mg diets and patients on long-term diuretic treatments, a decrease in both the number and activity of Na, K-ATPase of the skeletal muscle was observed (Norgaard *et al,* 1981; Kjeldsen *et al,* 1984; Dorup *et al,* 1988b). In turn, this reduction in Na, K-ATPase is closely correlated with a reduction in muscle-K and Mg concentration (Kjeldsen *et al,* 1984; Kjeldsen and Norgaard, 1987). This is supported by our finding that the mean values of muscle-K and Mg contents were lower than other reports and exhibited a good correlation

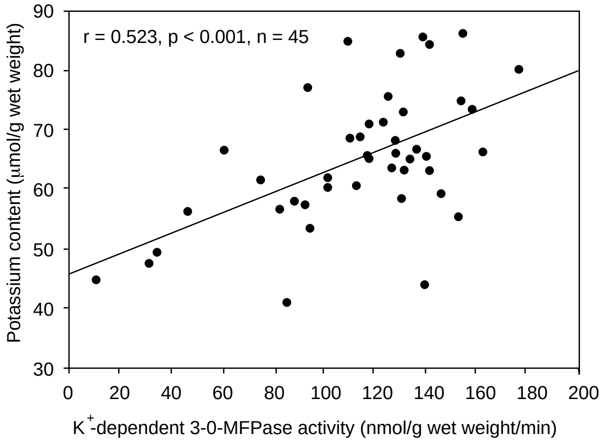

Fig 1–Correlation between potassium content and K-dependent 3-0-methyl fluorescein phosphatase (K-dependent 3-0-MFPase or Na, K-ATPase) activity of the external oblique muscles obtained from 45 stone patients.

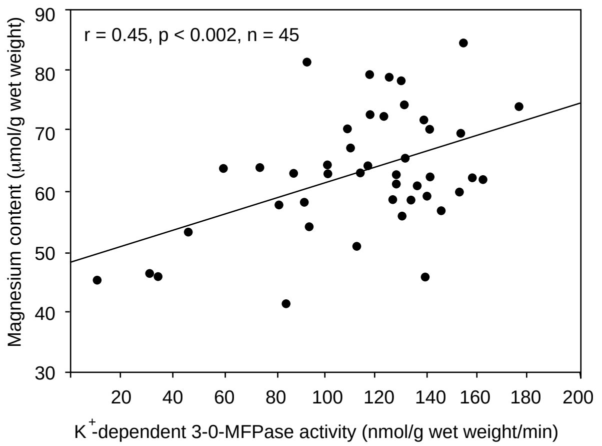

Fig 2–Correlation between magnesium content and K-dependent 3-0-methylfluorescein phosphatase (K-dependent 3-0-MFPase or Na, K-ATPase) activity of the external oblique muscles obtained from 45 stone patients.

with Na, K-ATPase activity in the muscle. It has been proposed that K-depletion could induce a down-regulation of Na, K-ATPase.

Four isoforms of Na, K-ATPase have been described in muscle tissue (Thompson and McDonough, 1996) and their existence is believed to provide the potential for differential function and regulation. The down-regulation of Na, K-ATPase by K-depletion may be regulated at the level of expression of these isoforms, by both the amount and type of the isoforms. Another possible mechanism of the down-regulation is the presence of inhibitor(s), which are ouabain-like, for Na, K-ATPase (Valdes, 1985); its levels in the plasma may be also regulated by the K-status where it increases when K is depleted.

Mg depletion may also contribute to these regulatory mechanisms. The low levels of K and Mg found in our renal stone subjects corroborate the lower activity of measured Na, K-ATPase found in the external oblique muscle. These results are consistent with previous findings of low Na, ATPase activity in the red blood cells of rural dwellers in Northeast Thailand (Tosukhowong *et al*, 1992; 1996).

The functional importance of the down-regulation of Na, K-ATPase by K-deficiency presumably confers greater survival (Clausen *et al*, 1987) because the reduction in the capacity to remove K from the extracellular phase into the muscles thus limits the risk of developing further hypokalemia, thereby lowering the risk of cardiac arrhythmias or muscle paralysis-other conditions often seen in Northeast Thailand (Sitprija *et al*, 1991). K-depletion leading to the inhibition of Na, K-ATPase has been proposed as an important causal factor of renal stone disease in Northeast Thailand (Sitprija *et al*, 1991).

In renal tubular cells, the inhibition of Na, K-ATPase could lead to a decrease in intracellular K with a concomitant increased intracellular Na. This would further exacerbate the increase in intracellular H^+-partly through the suppression of Na-H antiport by the high level of intracellular Na. It is well recognized that cellular acidosis and K-depletion will reduce citrate concentrations by a decrease in activity of citrate synthesizing enzymes (Nissim *et al*, 1990) and at the same time accelerate citrate oxidation by an increase in both the activity of mitochondrial aconitase (Melnick *et al*, 1998) and cytosolic ATP-citrate lyase (Melnick *et al*, 1996). This decrease in cellular citrate levels would subsequently drive the Na-citrate cotransporter to move citrate intracellularly at a more rapid rate (Adler *et al*, 1974). In turn this process could cause hypocitraturia, by an increased renal citrate reabsorption (Levi *et al*, 1991). This proposed metabolic pathway is consistent with our own previous reports, where we demonstrated an unusually high prevalence of hypocitraturia among renal stone formers (Sriboonlue *et al*, 1991; 1996), and the mechanism proved to be an increased reabsorption of citrate (Sriboonlue *et al*, 1996).

Renal stone disease is prevalent in Northeast Thailand as are K and Mg depletion. Further study is needed to understand the roles of K and Mg related to the pathogenesis of the stone forming disease: particularly, studies into the supplementation of these two minerals, either directly (as potassium citrate, potassium magnesium citrate, etc) or through the diet (in foods high in K and Mg).

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REFERENCES

Adler S, Zett B, Anderson B. Renal citrate in the potassium deficient rat: role of potassium and chloride ions. *J Lab Clin Med* 1974; 84: 307-16.

Albers RW, Koval GJ. Sodium-potassium-activated adenosine triphosphatase of electrophorus electric organ. *J Biol Chem* 1996; 241: 1896-8.

Bovornpadungkitti S, Sriboonlue P, Tavichakorntrakool, *et al.* Potassium, sodium and magnesium contents in skeletal muscle of renal stone-formers: A study in an area of low potassium intake. *J Med Assoc Thai* 2000; 83: 756-63.

Clausen T, Everts ME, Kjeldsen K. Quantification of the maximum capacity for active sodium-potassium transport in rat skeletal muscle. *J Physiol* 1987; 388: 163-81.

Dyckner T, Wester PO. Plasma and skeletal muscle electrolytes in patients on long-term diuretic therapy for arterial hypertension and/or congestive heart failure. *Acta Med Scand* 1987; 222: 231-6.

Dyckner T, Wester PO. The relation between extra-and intracellular electrolytes in patients with hypokalemia and/or diuretic treatment. *Acta Med Scand* 1978; 204: 269-82.

Dorup I, Skajaa K, Clausen T. A simple and rapid method for the determination of the concentrations of magnesium, sodium, potassium and sodium, potassium pumps in human skeletal muscle. *Clin Sci* 1988a; 74: 241-8.

Dorup I, Skajaa K, Clausen T, Kjeldsen K. Reduced concentrations of potassium, magnesium, and sodium-potassium pumps in human skeletal muscle during treatment with diuretics. *Br Med J* 1988b; 296: 455-8.

Dorup I, Skajaa K, Thybo NK. Oral magnesium supplementation restores the concentrations of magnesium, potassium and sodium-potassium pumps in skeletal muscle of patients receiving diuretic treatment. *J Int Med* 1993; 233: 117-23.

Huang W, Askari A. (Na^+ + K^+) - Activated adenosine triphosphatase: fluorimetric determination of the associated K^+-dependent 3-0-methylfluorescein phosphatase and its use for the assay of enzyme samples with low activities. *Anal Biochem* 1975; 66: 265-71.

Kjeldsen K, Norgaard A, Clausen T. Effect of K-depletion on ^{3}H-ouabain binding and Na-K-contents in mammalian skeletal muscle. *Acta Physiol Scand* 1984; 122: 103-17.

Kjeldsen K, Norgaard A, Norgaad A. Effect of Mg-depletion on ^{3}H - ouabain binding site concentration in rat skeletal muscle. *Magnesium* 1987; 6: 55-60.

Levi M, McDonald LA, Pseising A, Alpern RJ. Chronic K-depletion stimulates rat renal brush-border membrane Na-citrate cotransporter. *Am J Physiol* 1991; 261: F767-73.

Melnick JZ, Srere PA, Elshourbagy NA, Moe OW, Preisig PA, Alpern RJ. Adenosine triphosphate citrate lyase mediates hypocitraturia in rats. *J Clin Invest* 1996; 98: 2381-7.

Melnick JZ, Preisig PA, Moe OW, Srere P, Alpern RJ. Renal cortical mitochondrial aconitase is regulated in hypo- and hypercitraturia. *Kidney Int* 1998; 54: 160-5.

Nissim I, NissimI, Yudkoff M. Carbon flux through tricarboxylic acid cycle in rat renal tubules. *Biochim Biophys Acta* 1990; 1033: 194-200.

Norgaard A, Kjeldsen K, Clausen T. Potassium depletion decreases the number of ^{3}H-ouabain binding sites and the active Na-K transport in skeletal muscle. *Nature* 1981; 293: 739-41.

Norgaard A, Kjeldsen K, Hansen O. ($Na^+ + K^+$) - ATPase activity of crude homogenates of rat skeletal muscle as estimated from their K^+-dependent 3-0-methylfluorescein phosphatase activity. *Biochim Biophys Acta* 1984; 770: 203-9.

Sitprija V, Tungsanga K, Eiam-Ong S, *et al*. Renal tubular acidosis, vanadium and buffaloes. *Nephron* 1990; 54: 97-8.

Sitprija V, Tungsanga K, Eiam-Ong S, *et al.* Metabolic syndromes caused by decreased activity of ATPases. *Semin Nephrol* 1991; 11: 249-52.

Sriboonlue P, Prasongwatana V, Tungsanga K, *et al*. Blood and urinary aggregator and inhibitor composition in controls and renal-stone patients from northeastern Thailand. *Nephron* 1991; 59: 591-6.

Sriboonlue P, Prasongwatana V, Bovornpadoongkitti S, Suwantrai S. Some aspects of citrate metabolism in renal-stone patients from northeastern Thailand. *J Med Assoc Thai* 1996; 79: 737-43.

Sriboonlue P, Prasongwatana V, Suwantrai S, Bovornpadungkitti S, Tungsanga K, Tosukhowong P. Nutritional potassium status of healthy adult males residing in the rural northeast Thailand. *J Med Assoc Thai* 1998; 81: 223-32.

Sriboonlue P, Prasongwatana V, Bovornpadungkitti S, Tungsanga K, Tosukhowong P. Potassium needed for maintaining its balance in healthy male subjects residing in an area of low potassium intake and with a high environmental temperature. *J Med Assoc Thai* 1999; 82: 690-9.

Tompson CB, McDonough AA. Skeletal muscle Na, K-ATPase alpha and beta subunit protein levels respond to hypokalemic challenge with isoform and muscle type specificity. *J Biol Chem* 1996; 271: 32653-8.

Tosukhowong P, Chotikasatit C, Tungsanga K, *et al*. Abnormal erythrocyte Na-, K-ATPase activity in a northeastern Thai population. *Southeast Asean J Trop Med Public Health* 1992; 23: 526-30.

Tosukhowong P, Tungsanga K, Kittinantavorakoon C, *et al*. Low erythrocyte Na/K-pump activity and number in northeast Thailand adults:evidence suggesting an acquired disorder. *Metabolism* 1996; 45: 804-9.

Valdes R Jr. Endogenous digoxin-like immunoreactive factors: impact on digoxin measurements and potential physiological implications [review]. *Clin Chim* 1985; 31: 155-32.