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.
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 -80°C. 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 600g. The clear supernatant was then used to analyze for K, Na 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 Norgaard 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 0°C. 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 25°C.

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 37°C. 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,
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.

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**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.

<table>
<thead>
<tr>
<th>Muscle contents (µmol/g wet weight)</th>
<th>X ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>65.2±1.7</td>
<td>41.1-86.1</td>
</tr>
<tr>
<td>Na</td>
<td>45.5±2.0</td>
<td>23.5-73.2</td>
</tr>
<tr>
<td>Mg</td>
<td>6.3±1.0</td>
<td>4.1-8.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activity of 3-0-MFPase (nmol/g wet weight/minute)</th>
<th>X ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal activity</td>
<td>789±20</td>
<td>460-1,102</td>
</tr>
<tr>
<td>Total activity</td>
<td>902±22</td>
<td>509-1,256</td>
</tr>
<tr>
<td>K⁺-dependent activity</td>
<td>113±21</td>
<td>11-177</td>
</tr>
</tbody>
</table>

650
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 exchange of Na for K.

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.
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).

ACKNOWLEDGEMENTS

Financial support was provided by the National Research Council of Thailand and the Faculty of Medicine, Khon Kaen University. The authors thank Mr Bryan Roderick Hamman for assistance with the English language presentation of the paper.

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