Changes in Erythrocyte Contents of Potassium, Sodium and Magnesium and Na, K-pump Activity after the Administration of Potassium and Magnesium Salts

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Low potassium and magnesium status and decreased Na, K-pump activity is an endemic condition among rural Northeast Thais. The authors examined the effect of supplementing potassium and magnesium on erythrocyte potassium, sodium and magnesium content and on Na, K-pump activity. Rural Northeast Thai renal stone patients (62) were recruited, divided into four groups and supplemented for one month with potassium chloride (Group 1, n = 16), potassium-sodium citrate (Group 2, n = 15), chelated magnesium (Group 3, n = 16) and potassium-magnesium citrate (Group 4, n = 15) in order to achieve 40 mmol potassium, 10 mmol magnesium and 60 mmol citrate daily. After supplementation with potassium (Groups 1, 2 and 4), plasma potassium and Na, K-pump activity rose significantly in Groups 1, 2 and 4, but erythrocyte potassium rose only in Groups 2 and 4. When supplementing elemental magnesium (Groups 3 and 4), the chelated magnesium caused a significant increase in plasma potassium, erythrocyte potassium, sodium and magnesium without a significant increase in Na, K-pump activity. By contrast, potassium-magnesium citrate caused a significant increase in erythrocyte potassium and magnesium and Na, K-pump activity, but depressed erythrocyte sodium. These results suggest the forms of potassium and /or magnesium salts being supplemented should be considered because they affect erythrocyte potassium, sodium and magnesium content and Na, K-pump activity differently.

Keywords : Erythrocyte Na, K-pump, Potassium supplementation, Magnesium supplementation, Renal stone

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sium are interrelated, depletion of both cations frequently coexists\textsuperscript{6,15,16}. In patients with combined magnesium and potassium deficiency, due to long-term treatment with diuretics, oral supplementation of magnesium alone normalized both potassium and magnesium status and Na, K-pump activity in skeletal muscles\textsuperscript{16}.

The authors' aim was to measure the effect of supplementing potassium and magnesium on Na, K-pump activity and on the cellular potassium, magnesium, sodium content in rural Northeast Thai renal stone subjects. Relatedly, the authors evaluated whether or not the forms of supplemented potassium and magnesium salts gave variable results. Due to the convenience in obtaining specimens, erythrocytes were chosen as the model cells.

**Material and Method**

The present research was approved by the Ethics Committee for Human Experiments, Khon Kaen University, and written informed consent was obtained from all participating subjects.

**Subjects and experimental design**

The authors recruited 62 rural Northeast Thai renal stone subjects (30 males and 32 females). The form of stones was identified by a mobile ultrasound unit from the Faculty of Medicine, Khon Kaen University. Subjects were randomly allocated into four groups according to the treatment regimens. Group 1 (n =16; 10 males and 6 females); Group 2 (n =15; 5 males and 10 females); Group 3 (n =16; 8 males and 8 females); and, Group 4 (n =15; 7 males and 8 females) were treated for one month with potassium chloride, potassium-sodium citrate, chelated magnesium (buffered amino acid chelate, product of Albion Laboratories, Inc., Utah, USA), and potassium-magnesium citrate, respectively. The treatments supplied each subject about 40 mmol potassium in Group 1, 40 mmol potassium and 60 mmol citrate in Group 2, 10 mmol magnesium in Group 3 and 40 mmol potassium, 10 mmol magnesium and 60 mmol citrate in Group 4.

The study protocol was community-based, so participants traveled to health centers near their home to get their supplements. Village health workers dispensed the supplements morning and evening. The supplementation regime lasted a month.

**Statistical analysis**

Descriptive statistics (range, mean ± SD) were used to describe the continuous data.

**Blood collection and analysis**

All four groups of subjects were organized to have blood taken by four mobile research teams on the same days both before and after supplementation. Ten milliliters of fasting heparinized blood was obtained from each subject both before and after supplementation. Group 2 provided another blood sample one month after supplementation was stopped. After 10 min centrifugation at 1800 rpm in a refrigerated centrifuge, the plasma was removed and analyzed for electrolytes using an autoanalyzer, and the magnesium by atomic absorption spectrophotometer. After removing the buffy coat and the uppermost layer of erythrocytes, the remaining erythrocytes were washed three times with about 10 volumes of ice-cold MgCl\textsubscript{2} solution (112 mmol/L). An aliquot of washed erythrocyte was used to analyze for the erythrocyte content of sodium, potassium and magnesium by the Mayer and Starkey method\textsuperscript{17}. The rest of the erythrocytes were used to assay for Na, K-pump activity.

**Erythrocyte membrane preparation and assay for Na, K-pump activity**

The method described by Hanahan and Ekholm\textsuperscript{18} with slight modifications\textsuperscript{2} was used in the preparation of an erythrocyte membrane. After washing with MgCl\textsubscript{2} solution, the erythrocytes were re-suspended in an equal volume of ice-cold saline-histidine buffer (155 mmol/L NaCl and 3 mmol/L histidine, pH 7.5) with an added 0.1 mg/mL of saponin in the same buffer. The membrane was separated using centrifugal washing five times at 23,500xg for 20 min then re-suspended in the same saline-histidine buffer. To the assay for Na, K-pump activity, a 100 μL aliquot of membrane suspension was incubated at 37°C for 90 min in 400 μL of ATPase-assay medium containing 100 mmol/L NaCl, 50 mmol/L Tris HCl, 15 mmol/L KCl, 5 mmol/L MgCl\textsubscript{2}, 5 mmol/L ATP, and 1 mmol/L EGTA. The reaction was stopped by adding trichloroacetic acid. The protein and phosphorus contents of the membrane in the medium were measured using methods developed by Lowry et al\textsuperscript{19} and Lawrence\textsuperscript{20}, respectively. Na, K-pump activity was expressed as nanomoles of inorganic phosphate released per milligram membrane protein per hour (nmolP/mg protein/hr). Na, K-pump activity was, thus, the difference between the inorganic phosphate released by the action of the erythrocyte membranes on ATP in the absence and presence of 1.0 mmol/L of ouabain.
Results

Baseline parameters for plasma and erythrocytes

The baseline values of plasma electrolytes, erythrocyte content and Na, K-pump activity are shown in Table 1. The mean (± SD) of plasma electrolytes of all four groups of the subjects were within normal ranges. Erythrocyte contents of potassium, sodium and magnesium and its Na, K-pump activity were not significantly different among the groups.

Statistical analysis of plasma and erythrocyte parameters after supplementation

Table 2 shows percentage change in plasma and erythrocyte content and Na, K-pump activity after supplementation. Significant increases in plasma parameter were observed only for potassium in Groups 1, 2 and 3 and a significant decrease of magnesium in Group 1. In the case of the erythrocyte content, significant increases were observed for potassium in Groups 2, 3 and 4, for magnesium in Groups 3 and 4, and a significant decrease of sodium in Group 4.

Fig. 1 shows the changes in erythrocyte Na, K-pump activity after supplementation for a period of one month. Those supplemented with salts containing elemental potassium (i.e. Groups 1, 2 and 4) experienced significant, though variable, increases in Na, K-pump activity. The supplementation of chelated magnesium in Group 3, however, resulted in only slightly increased Na, K-pump activity. The change of Na, K-pump activity after the supplementation was also shown in Table 2 as percentage.

Na, K-pump activity after withdrawal of supplementation

One month after stopping potassium-sodium citrate supplementation, Group 2 subjects were re-examined for erythrocyte Na, K-pump activity (Fig. 2). Mean Na, K-pump activity decreased significantly close to baseline.

Correlations between erythrocyte Na, K-pump activity and its contents

After the supplementing regimens, only Group 4 had significant changes to all parameters related to Na, K-pump activity (i.e. increased erythrocyte potassium and magnesium but decreased sodium content) (Table 2). These changes also exhibited a good correlation with the corresponding changes in Na, K-pump activities (Fig. 3).
Discussion
Supplementation with an equivalent amount of elemental potassium (40 mEq) but in three different forms (potassium chloride, potassium-sodium citrate and potassium-magnesium citrate) variously affected erythrocyte Na, K-pump activity and contents of potassium, sodium and magnesium. Increased potassium status up-regulated Na, K-pump activity as expected, but potassium-sodium citrate and potassium-magnesium citrate caused the greatest increase—significant increases in intracellular potassium occurred only in Groups 2 and 4. These observations are probably related to the low potassium status among rural Northeast Thai renal stone patients(6).

Potassium deficiency has been shown to cause intracellular acidosis(21) leading to increased renal tubular citrate oxidation and re-absorption and finally decreased urinary citrate excretion(22). Since rural Northeast Thai renal stone subjects have a low potassium status associated with hypocitraturia(23), they may also have intracellular acidosis. Sakhaee et al(24) demonstrated that supplementation to renal stone patients with three different forms of potassium, only potassium citrate and potassium bicarbonate, not potassium chloride, clearly resulted in an increase in urinary pH and citrate excretion. These effects are due to the pre-existing intracellular acidosis had been corrected and largely accountable for by the provision of an alkali load from their citrate and bicarbonate components. Supplementation with potassium chloride, on the other hand, did not provide such alkali load and, thus, had no effect on acid-base status of the cells. Despite the provision of an equal substrate potassium, but in different forms, the present results also demonstrated that the acid-base status of cells had some effect on Na, K-pump activity as observed by others(25).

Potassium and magnesium deficiencies always coexist(6,15,16), so supplementation with extra potassium alone, cannot correct for potassium, whereas supplementation with magnesium can(16). This is partly attributed to magnesium being an essential cofactor for the Na, K-pump activity(1). For example, animals fed magnesium-deficient fodder had concomitant reduced muscle potassium and magnesium, but unchanged Na, K-pump activity(26). These magnesium-depleted muscles showed a significant increase in potassium efflux, so when treated with magnesium a graded decrease was observed. Thus loss of potassium
from skeletal muscles, seen in moderate magnesium deficiency, cannot readily be attributed to reduced potassium influx from the reduction of Na, K-pump activity, rather to the increased efflux of potassium through magnesium-sensitive potassium channels. Similarly, Flattman and Lew(27) demonstrated that Na, K-pump activity of human erythrocyte was not modified by acute alterations in extracellular magnesium. The authors also observed supplementation of chelated magnesium (Group 3) caused a non-significant increase in Na, K-pump activity. The lack of change in Na, K-pump activity may also be attributed to pre-existing intracellular acidosis similar to that observed when supplementing with potassium chloride. However, after chelated magnesium supplementation, the erythrocyte content increased in magnesium and both potassium and sodium perhaps because the increase in magnesium content decreased potassium efflux as observed in skeletal muscles(26). Increased intracellular magnesium may affect membrane properties causing not only a decreased potassium efflux but also increased sodium influx just as Elloy et al(29) showed that a reduction in erythrocyte magnesium content with EDTA and ionophore A23187 caused a reduction in potassium and sodium contents via the decline in Na-K cotransporter activity. Thus, an increase in the erythrocyte contents of magnesium, potassium and sodium, after supplementation with chelated magnesium, could be accounted for by the magnesium loading effect and the increase in cellular magnesium content causing a further increase in Na-K cotransporter activity.

Potassium-magnesium citrate is a new prescription drug used successfully in the treatment of renal stone disease(29). It improves both potassium and magnesium status and increases urinary excretions of citrate and magnesium, both stone inhibitors(29). This mechanism was observed in the Group 4 subjects, who were treated with potassium-magnesium citrate, and experienced a significant increase in both potassium and magnesium status, Na, K-pump activity, erythrocyte contents of both potassium and magnesium and a significant decrease in erythrocyte sodium. These changes in erythrocyte contents were closely correlated with the change in Na, K-pump activity. The effects of supplementations, however, are not longlasting. One month after potassium-sodium citrate supplementation was withdrawn, Na, K-pump activity in Group 2 subjects had returned to near baseline values. Rural Northeast Thai stone subjects likely suffer chronic potassium and magnesium depletion and thus need longer or periodic supplementation just as patients on long-term diuretic therapy (viz. magnesium aspartate) where it needs as long as six months to gain a significant increase in muscle potassium and magnesium content(31). Since the main factor causing low potassium status among the presented subjects is low intake(5), long-term stability requires a change in eating habits to foods high in these two essential minerals.

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References
การเปลี่ยนแปลงของปริมาณโพแทสเซียมโซเดียมและแมกนีเซียมและอัตราการทำงานของโซเดียม
บั้มในเม็ดเลือดแดง หลังจากการเสริมด้วยเกลือต่าง ๆ ของโพแทสเซียมและแมกนีเซียม

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การมีภาวะโพแทสเซียมและแมกนีเซียมต่ำของร่างกายรวมทั้งภาวะการทำงานลดลงของโซเดียมบั้ม จะพบได้บ่อย ๆ ในกลุ่มประชากรที่เป็นชาวชนบทของภาคตะวันออกเฉียงเหนือ ในรายงานนี้ขึ้นวิจัยโดยศึกษาผลของการเสริมโพแทสเซียมและแมกนีเซียมที่มีต่อปริมาณโพแทสเซียมโซเดียม และแมกนีเซียม รวมทั้งอัตราการทำงานของโซเดียมบั้มของเม็ดเลือดแดง ผู้เข้าร่วมโครงการได้แก่ กลุ่มนูญ (62 คน) โดยแบ่งออกเป็นกลุ่มทดลอง แล้วทำการเสริมด้วยกลุ่มเป็นระยะเวลาย่อยขึ้นต่อมาเรื่อยมาโพแทสเซียมคลอไรด์ (กลุ่มที่ 1,16 คน) สารละลายโพแทสเซียม-โซเดียม ซิเตรต (กลุ่มที่ 2, 15 คน) ยาเม็ดคีเลตแมกนีเซียม (กลุ่มที่ 3, 16 คน) และสารละลายโพแทสเซียม-แมกนีเซียมซิเตรต (กลุ่มที่ 4, 15 คน) เพื่อที่จะให้ได้รับโพแทสเซียมแมกนีเซียมและโซเดียมต่อวัน 40, 10 และ 60 มิลลิโมลต่อวัน ตามลำดับ ผลจากการเสริมด้วยโพแทสเซียม (กลุ่มที่ 1,2 และ 4) พบว่าการเสริมโพแทสเซียมในทดสอบ และอัตราการทำงานของโซเดียมบั้มเพิ่มขึ้นกว่าเดิมอย่างมีนัยสำคัญ ในกลุ่มที่ 1, 2 และ 4 ในขณะที่ปริมาณโพแทสเซียมในเม็ดเลือดแดงมีการเพิ่มขึ้นอย่างมีนัยสำคัญเฉพาะกลุ่มที่ 2 และ 4 สำหรับผลของการเสริมด้วยแมกนีเซียม (กลุ่มที่ 3 และ 4) พบว่ายาเม็ดคีเลตแมกนีเซียมมีผลทำให้เกิดการเพิ่มขึ้นอย่างมีนัยสำคัญทั้งระดับโพแทสเซียมในทดสอบ ปริมาณโพแทสเซียมโซเดียม และแมกนีเซียมในเม็ดเลือดแดง แต่ไม่มีผลต่อการทำงานของโซเดียมบั้มในเนื้อเยื่อต่าง ๆ ในขณะที่การเสริมด้วยโพแทสเซียมแมกนีเซียม ซิเตรต มีผลทำให้เกิดการเพิ่มขึ้นอย่างมีนัยสำคัญ สำหรับแมกนีเซียมในทดสอบ ปริมาณโพแทสเซียมโซเดียม และแมกนีเซียมในเม็ดเลือดแดง แต่ไม่มีผลต่อการทำงานของโซเดียมบั้มในเนื้อเยื่อต่าง ๆ รวมทั้งลดปริมาณโพแทสเซียมในเนื้อเยื่อต่าง ๆ แต่ไม่ปรากฏการรักษาเรื่อย ๆ ในการพิจารณาดูผลของการเสริมโพแทสเซียมแมกนีเซียม แมกนีเซียมจะต้องคำนวณให้ถูกต้องก็จะมีมากิวต์ในเนื้อเยื่อต่าง ๆ เนื่องจากและรูปไข่ไม่แตกต่างกันต่อปริมาณโพแทสเซียมโซเดียมและแมกนีเซียมรวมทั้งอัตราการทำงานของโซเดียมบั้มในเนื้อเยื่อต่าง ๆ