Low Erythrocyte Na/K-Pump Activity and Number in Northeast Thailand Adults: Evidence Suggesting an Acquired Disorder

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Healthy northeastern Thais have a higher erythrocyte sodium concentration and a lower erythrocyte membrane Na,K-adenosine triphosphatase (ATPase) activity than central Thais. To elucidate whether the defect is hereditary or acquired, we studied plasma sodium and potassium and erythrocyte sodium, potassium, Na,K-ATPase activity, and ouabain-binding sites (OBS) in the following groups: healthy newborns of ethnic central Thais (group 1), healthy newborns of ethnic northeast Thais (group 2), healthy adults of central Thailand ethnicity who lived in the rural central region (group 3) or in Bangkok (group 4), healthy adults of northeast Thailand ethnicity who lived in the rural northeast region (group 5) or who migrated to work in Bangkok for at least 1 year (group 6). Erythrocyte Na was higher in group 2 than in group 1. Group 3 had lower erythrocyte Na,K-ATPase activity than group 4, and it was lower in group 5 than in group 6. Among all groups, group 5 had the highest erythrocyte Na (11.6 mmol/L, $F < 0.0001$) and the lowest Na,K-ATPase activity (63 mmol Pi/mg · h, $F < 0.0001$) and erythrocyte OBS (397 sites per cell, $F < 0.05$) than the other adult groups. There was a positive correlation between erythrocyte Na,K-ATPase and erythrocyte OBS ($r = .416$, $P < .0001$). Multiple regression analysis demonstrated a correlation between erythrocyte Na as a dependent variable and erythrocyte OBS, plasma potassium, erythrocyte potassium, and erythrocyte Na,K-ATPase ($r = .517$, $P < .0001$). The erythrocyte Na,K-ATPase/OBS ratio, an expression of Na,K-ATPase activity equalized for the number of Na,K-pump units, was lowest among rural adults of the central region (group 3) and the northeast region (group 5) ($F < 0.0002$). Our data suggest that rural dwellers in Thailand tend to have lower erythrocyte Na,K-ATPase activity than urban dwellers and that this is probably acquired after birth. It was more severe among those from the northeast versus the central region, and was less severe among those who migrated to an urban area. This defect in northeast rural dwellers was probably associated with low numbers of Na,K-pump units and a defect of the pump to express activity, whereas in central rural dwellers it was probably associated with the latter condition. We postulate that there might be circulating Na,K-pump inhibitors and metabolic disturbances that cause attenuation of Na,K-ATPase function and synthesis in the northeast Thailand rural population, and that such substances may have an environmental origin. There may be a relationship between these abnormalities and sudden unexpected deaths.

TRANSPORT OF SODIUM and potassium across cell membranes is an active process requiring an adenosine triphosphate (ATP)-dependent enzyme, sodium and potassium–activated ATPase (Na,K-ATPase). It moves three Na^+ ions outward and two K^+ ions inward for one ATP.[1] Erythrocytes have been widely used as a cell model for demonstrating enzymatic defects in man. Abnormalities in the function of Na,K-ATPase on cell membranes of erythrocytes have been reported in various diseases such as hypertension,[2,3] hyperthyroidism,[4] diabetes,[5] renal or liver disease,[6,7] and potassium depletion.[8] Race, age, and sex could also affect expression of Na,K-ATPase.[9,10]

Ouabain is a specific inhibitor of Na,K-ATPase. It binds the enzyme stoichiometrically at a molar ratio of 1:1. Radiolabeled ^{3}H-ouabain can be used to measure the number of Na,K-ATPase units (ouabain-binding capacity or pump units) on cell membranes.[11] Schmalzing et al[12] and Deluise and Flier[13] have demonstrated a constancy of the ouabain-binding capacity of erythrocyte membranes. A decreased number of ouabain-binding sites (OBS) indicates a reduction of Na,K-pump units. This correlates with decreased Na,K-ATPase activity or increased intracellular sodium concentration.[14]

The northeast region of Thailand is geographically different from the central region. It is an arid plateau where the soil is more sandy and less fertile. The central region is a plain—there is much more rainfall and the soil is more fertile. The northeast inhabitants are ethnically related more closely to the neighboring countries, eg, Laos and Cambodia, but the central population are ethnic central Thais or Thai-Chinese. Northeast Thailand has the lowest per capita income in the country. Northeast Thais, who are ethnic Thai-Lao, are known to have a high incidence of sudden death syndrome in young male adults, endemic distal renal tubular acidosis, and hypocitraturic renal stone disease.[15-18] The metabolic causes for this have been postulated.[19] However, the pathogenesis of these diseases has not been clearly elucidated. We have previously observed that northeast Thais have a significantly higher concentration of erythrocyte sodium and a lower activity of erythrocyte Na,K-ATPase than central Thais who are city dwellers and are from ethnic central Thai or Thai-Chinese backgrounds.[20] Erythrocyte sodium of both population groups correlated inversely with erythrocyte Na,K-ATPase.[20] The explanation for the erythrocyte membrane defect in resi-

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dents of the Northeast region remains unknown. It could be a hereditary or an acquired disorder. If it is a hereditary factor, erythrocytes of newborns and adults who are ethnic Northeast Thais should have similar defects. Moreover, the membrane enzyme abnormality should persist despite the migration of such subjects to other geographic areas. In contrast, if the defect is acquired by environmental factors, we expect Na,K-ATPase in erythrocyte membranes of rural dwellers from other regions to be decreased as well. Moreover, that of northeast Thais should be normal at birth, decreased during adulthood, and normalized again after migration to a different geographic region.

We therefore performed a cohort study in two infant groups whose parents were native residents of northeast and central Thailand, in three adult groups from the same geographic areas, and in a northeast Thai population who migrated to the central region. To learn whether decreased activity of membrane Na,K-ATPase in northeast Thais is due to a reduction in the number or in the function of the enzyme, we also determined the number of erythrocyte membrane Na,K-ATPase units. We were unable to find any other studies that simultaneously measured the activity and number of Na,K-ATPase units in different age groups from ethnically and geographically different populations.

SUBJECTS AND METHODS

Subjects

We studied six population groups. The study of groups 1, 4, and 6 was conducted at Bangkok, and that of group 3 was at Cholburi. Both cities are in central Thailand. The study of groups 2 and 5 was at Khon Kaen, about 400 km northeast of Bangkok. To rule out the effect of gender on the activity and number of membrane Na,K-ATPase units,[9,10] only healthy male subjects were recruited. Groups 1 and 2 consisted of 30 and 31 newborns with normal deliveries at Chulalongkorn University Hospital, Bangkok, and at Khon Kaen University Hospital, Khon Kaen, respectively. Parents of newborns in group 1 lived in Bangkok and were ethnic central Thai or Thai-Chinese. They denied ancestral migration from the northeast. Parents of group 2 newborns lived in the rural area around Khon Kaen. They were ethnic Thai-Lao and gave no history of migration from elsewhere. Group 3 consisted of 20 healthy rural dwellers in Cholburi Province (central rural dwellers), and group 4 of 30 healthy personnel from Chulalongkorn University Hospital (central urban dwellers). Group 3 and 4 subjects were ethnic central Thai. Group 5 consisted of 19 healthy rural dwellers from Khon Kaen Province, ethnic Thai-Lao (northeast rural dwellers). Group 6 consisted of 25 healthy male construction workers who were northeast Thai natives and had migrated to work in Bangkok for at least 1 year before the study. All subjects provided informed consent to participate in the study. The study protocol was approved by the Ethics Committees of the Departments of Medicine at Chulalongkorn University and Khon Kaen University Hospitals.

Preparation of Blood Samples

All laboratory procedures were conducted by two of the researchers (P.T. and C.K.). A 20-mL heparinized blood sample was collected in an ice-cold syringe from an umbilical vein of group 1 and group 2 subjects at delivery and from peripheral veins of groups 3 to 6 in a fasting state. The blood was immediately centrifuged at 1,800 rpm at 4°C for 10 minutes. The plasma was analyzed for sodium and potassium content by flame photometry (model 480; Ciba-Corning, Medfield, MA). The buffy coat and the uppermost layer of erythrocytes were removed. The remaining erythrocytes were washed three times with 10 vol ice-cold 112-mmol/L $MgCl_2$ solution. An aliquot of washed erythrocytes was resuspended in an equal volume of ice-cold saline-histidine buffer (155 mmol/L NaCl and 3 mmol/L histidine, pH 7.5), kept at −20°C, and shipped to the Department of Biochemistry, Chulalongkorn University Hospital, for erythrocyte membrane studies. The remaining erythrocytes were analyzed for intraerythrocyte sodium and intraerythrocyte potassium by the method of Mayer and Starkey.[21] Erythrocyte OBS were analyzed by the method of Deluise et al.[22] However, the method was slightly modified by increasing the [^{3}H]-ouabain incubation time from 60 to 120 minutes to maximize ouabain binding.[10]

Erythrocyte ATPase Assay

Isolation of erythrocyte membranes was performed as previously described by Hanahan and Ekholm,[23] with a slight modification.[20] Frozen erythrocytes in saline-histidine buffer were thawed, and 0.1 mg/mL saponin in the same buffer was added. The membranes were separated by centrifugal washing at 23,500 × *g* for 20 minutes five times, and then resuspended in the same buffer. A 0.1-mL aliquot of membrane suspension was incubated in 0.4 mL ATPase assay medium (100 mmol/L NaCl, 50 mmol/L Tris hydrochloride, 15 mmol/L KCl, 5 mmol/L $MgCl_2$, 5 mmol/L ATP, and 1 mmol/L EGTA) at 37°C for 90 minutes. The reaction was stopped by addition of trichloroacetic acid. Membrane protein content was measured by the Lowry method,[24] and it ranged from 3 to 5 mg/mL. Phosphorus was analyzed by Lawrence's method.[25] ATPase activity was expressed as nanomoles inorganic phosphate released per milligram membrane protein per hour. Na,K-ATPase activity was the difference between P_i released by the action of erythrocyte membranes on ATP in the absence and presence of 1.0 mmol/L ouabain. Intraassay variation from the same membrane pool was less than 4.9%, and interassay variation when the frozen erythrocytes were kept for no longer than 5 days was less than 5%.

Erythrocyte OBS Assay

Ice-cold ouabain-binding buffer (140 mmol/L NaCl, 30 mmol/L HEPES, and 10 mmol/L dextrose, pH 7.4) was added to an aliquot of washed erythrocytes to produce a hematocrit level of 5% to 10%. Cells were counted with a hemocytometer. Triplicate 400-μL erythrocyte suspensions were mixed with 50 μL [^{3}H]-ouabain solution (specific gravity, 47 Ci/mmol/L; concentration, 1 mCi/mL; Amersham, Buckinghamshire, UK) with final concentrations ranging from 8 to 64 nmol/L. After incubation at 37°C for 120 minutes, the cell pellet was washed three times with ice-cold 140-mmol/L choline chloride. The reaction was stopped by addition of 5% trichloroacetic acid. Membrane-bound radioactivity was counted by a liquid scintillation spectrometer (Rack Beta 1219; LKB Wallacoy, Turku, Finland). Specific [^{3}H]-ouabain binding was determined as the concentration difference between bound ^{3}[H]-ouabain in the absence and in the presence of 0.1 mmol/L unlabeled ouabain. It was expressed as [^{3}H]-ouabain binding sites per cell from Scatchard plots as previously described.[26] Intraassay coefficient of variation from the same pool of erythrocytes obtained on the same day was less than 6.5%.

Statistical Analysis

Comparison of statistical differences was made by ANOVA and multiple logistic regression analysis using the SPSS/PC$^+$ program.[27] The data are expressed as the mean ± SEM.

RESULTS

Newborn Period (groups 1 and 2)

There was no difference between group 1 and group 2 with respect to plasma sodium, plasma potassium, erythrocyte potassium, erythrocyte Na,K-ATPase activity, the ratio of erythrocyte Na,K-ATPase to total ATPase activity, and erythrocyte OBS (Table 1). Erythrocyte Na of group 2 subjects (11.8 ± 0.2 mmol/L) was significantly higher than that of group 1 subjects (9.8 ± 0.4 mmol/L, $P < .001$). The erythrocyte Na,K-ATPase/OBS ratio was also comparable between these two groups.

Adult Period (groups 3 to 5)

There was no significant difference in age among group 3 (37 ± 3 years), group 4 (40 ± 2 years), group 5 (36 ± 2 years), and group 6 (35 ± 2 years). Group 5 subjects had lower plasma sodium (135 ± 2 mmol/L) than any other group ($F < 0.005$). Compared with the first two newborn groups, plasma potassium in any adult group was significantly lower but within normal limits ($F < 0.001$). Erythrocyte Na of group 5 subjects (11.6 ± 0.9 mmol/L) was higher than that of newborns in group 1 or of adults in groups 3 and 4, but was comparable to that of newborns in group 2 ($F < 0.0001$). The erythrocyte Na,K-ATPase activity of group 3 (85 ± 3 nmol P_i/mg · h) was lower than that of group 4 (118 ± 7 nmol P_i/mg · h) but higher than that of group 5 (63 ± 4 nmol P_i/mg · h) ($F < 0.0001$). Erythrocyte Na,K-ATPase activity in group 5 was significantly lower than in any other group ($F < 0.0001$). Similarly, the erythrocyte Na,K-ATPase to total ATPase activity ratio in group 3 (0.51 ± 0.02) was significantly lower than in group 4 (0.59 ± 0.02) but higher than in group 5 (0.35 ± 0.02). The ratio in group 5 was also the lowest among the four adult populations ($F < 0.0001$). Erythrocyte OBS did not differ markedly among groups, except that group 5 subjects had significantly lower erythrocyte OBS than group 4 subjects (397 ± 16 *v* 484 ± 14 sites per cell, $P < .05$). Erythrocyte Na,K-ATPase/OBS ratios of group 3 (0.18 ± 0.01) and group 5 (0.16 ± 0.01) were lower than in group 2 and group 4 ($F < 0.0001$).

Northeast Thai Migrant Workers (group 6)

In group 6, plasma sodium, plasma potassium, and erythrocyte potassium levels were comparable to those in the other adult groups. Erythrocyte Na was lower than in groups 1, 2, and 5 ($F < 0.0001$). Erythrocyte K in this group (94 ± 1 mmol/L) was lower than in group 2 ($P < .02$). Both erythrocyte Na,K-ATPase activity (91 ± 8 nmol P_i/mg · h) and the erythrocyte Na,K-ATPase to total ATPase activity ratio (0.44 ± 0.02) in group 6 were significantly lower than in group 4 but higher than in group 5 ($F < 0.0001$). Erythrocyte OBS (443 ± 23 sites per cell) and the erythrocyte Na,K-ATPase to OBS ratio (0.21 ± 0.01) in this group tended to be higher than in group 5, but did not reach statistical significance.

Multiple Logistic Correlation

When the data from all groups were combined, erythrocyte Na,K-ATPase activity correlated positively with erythrocyte OBS ($r = .416$, $P < .0001$; Fig 1). In addition, erythrocyte Na correlated inversely with erythrocyte OBS and erythrocyte Na,K-ATPase activity and positively with erythrocyte K and plasma K ($r = .517$, $P = .0001$; Table 2). Erythrocyte K also correlated weakly but significantly with erythrocyte OBS ($r = .291$, $P < .002$).

DISCUSSION

The values for erythrocyte Na,K-ATPase activity and the number of erythrocyte OBS in newborns from the central region (group 1) and the northeast region (group 2) and in Bangkok dwellers (group 4) were comparable to data from healthy controls in previous reports.[2-5,14,28-30] Erythrocyte Na,K-ATPase activity also correlates with the number of erythrocyte OBS (Fig 1), a finding previously reported.[12,13,31] Interassay and intraassay variations for Na,K-ATPase activity and erythrocyte OBS in this study were minimal, indicating that our analytical methods are accurate and reliable. However, erythrocyte Na,K-ATPase activities of groups 1 and 2 were threefold less than those reported by Sigstrom et al,[32] possibly due to different techniques. We

Table 1. Plasma Sodium and Potassium, Erythrocyte Sodium and Potassium, Erythrocyte Na,K-ATPase Activity, Erythrocyte Na,K-ATPase/Total ATPase Activity Ratio, Erythrocyte OBS, and Erythrocyte Na,K-ATPase/OBS in Different Thai Populations

	Group						
	1 (n = 30)	2 (n = 31	3 (n = 20)	4 (n = 30)	5 (n = 19)	6 (n = 25)	F
Plasma							
Sodium (mmol/L)	138 ± 0.8	140 ± 0.8	143 ± 1[1,2]	143 ± 0.5[1,2]	135 ± 1.8[1,2,3,4,6]	139 ± 1[3,4]	<0.0001
Potassium (mmol/L)	4.6 ± 0.13	4.8 ± 0.14	4.0 ± 0.06[1,2]	3.9 ± 0.04[1,2]	4.0 ± 0.09[1,2]	3.9 ± 0.05[1,2]	<0.0001
Erythrocyte							
Sodium (mmol/L)	9.8 ± 0.35	11.8 ± 0.24[1]	8.0 ± 0.36[1,2]	7.9 ± 0.35[1,2]	11.6 ± 0.88[1,3,4,6]	8.4 ± 0.43[1,2]	<0.0001
Potassium (mmol/L)	97 ± 1	101 ± 3	100 ± 2	95 ± 2	94 ± 2	94 ± 1[2]	<0.02
Na,K-ATPase (nmol P_i/mg · h)	100 ± 5	106 ± 8	85 ± 3[4,6]	118 ± 7	63 ± 4[1,2,3,4,6]	93 ± 8[4]	<0.0001
Na,K-ATPase/total ATPase activity ratio	0.44 ± 0.62	0.45 ± 0.04	0.51 ± 0.02[4]	0.59 ± 0.02[1,2]	0.35 ± 0.02[2,3,4,6]	0.44 ± 0.02[4]	<0.0001
OBS (sites per cell)	441 ± 11	445 ± 21	468 ± 11	484 ± 14	397 ± 16[4]	443 ± 23	<0.05
Na,K-ATPase activity/OBS	0.23 ± 0.01	0.25 ± 0.02	0.18 ± 0.01[2,4]	0.24 ± 0.01	0.16 ± 0.01[1,2,4]	0.21 ± 0.01	<0.0001

NOTE. Group 1, central newborns; group 2, northeast newborns; group 3, central rural dwellers; group 4, Bangkok city dwellers; group 5, northeast rural dwellers; group 6, northeast migrant workers. The superscript number indicates the population group that is significantly different at $P < .05$.

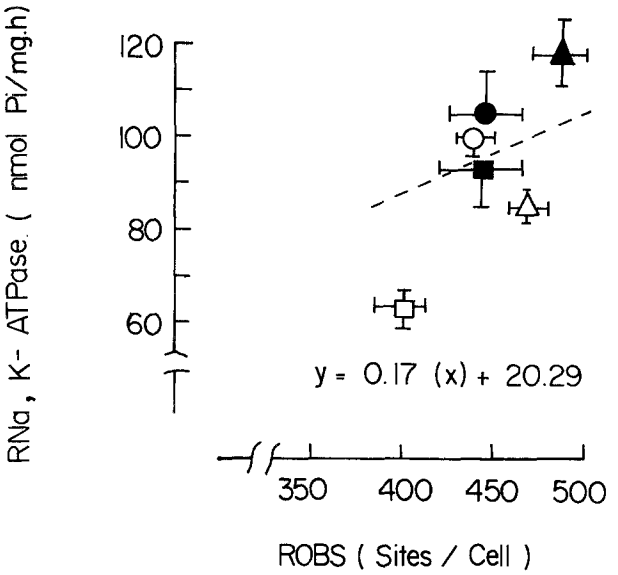

Fig 1. Correlation between erythrocyte Na,K-ATPase (RNa,K-ATPase) and erythrocyte OBS (ROBS) among the 6 populations ($y = 0.17x + 20.29$, $r = .416$, $P < .0001$). Data are the mean ± SEM of the values in group 1 (○), group 2 (●), group 3 (△), group 4 (▲), group 5 (□), and group 6 (■).

could find no other reports of erythrocyte OBS in newborns.

We demonstrated in this study that erythrocyte Na,K-ATPase activity and erythrocyte OBS among newborns in groups 1 and 2 and in Bangkok City dwellers (group 4) were not different. In contrast, erythrocyte Na,K-ATPase activity in central rural dwellers (group 3) and northeast rural dwellers (group 5) was lower than in group 4. Indeed, this and the erythrocyte Na,K-ATPase to total ATPase activity ratio and erythrocyte OBS were lowest in group 5. Northeast Thai migrant workers (group 6) had higher a erythrocyte Na,K-ATPase activity and erythrocyte Na,K-ATPase to total ATPase activity ratio than group 5, but still lower than those of group 4. Erythrocyte OBS in group 6 tended to be higher than in group 5, but this did not reach statistical significance. The results can be summarized as follows. (1) Newborns from both geographic regions had comparable biochemistry values. (2) Rural dwellers from both regions had lower erythrocyte Na,K-ATPase activity than urban dwellers; however, the difference was greater among northeast than among central Thais. (3) Low erythrocyte Na,K-ATPase activity in central rural dwellers was not accompanied by low erythrocyte OBS or high erythrocyte Na, whereas in northeast rural dwellers it was. (4) There was a decrease in erythrocyte Na and an increase in erythrocyte Na,K-ATPase activity and the erythrocyte Na,K to total ATPase activity ratio among northeast rural villagers who migrated to the central region.

Table 2. Multiple Regression Analysis

Dependent Variable	Independent Variable	β Coefficient	*P*	*r*
RNa	ROBS	−0.342	.0001	.528
	RK	0.263	.0008	
	BK	0.205	.0068	
	RNa,K-ATPase	−0.214	.0111	
RK	ROBS		<.002	.291

Abbreviations: BK, plasma potassium; RK, erythrocyte potassium; RNa, erythrocyte sodium; RNa,K-ATPase, erythrocyte Na,K-ATPase activity; ROBS, erythrocyte OBS.

Although low activity of erythrocyte Na,K-ATPase was present in rural dwellers of both regions (groups 3 and 5) and the activity of erythrocyte Na,K-ATPase of all population groups correlated positively with the number of erythrocyte OBS (Fig 1), it was only in group 5 that low numbers of Na,K-pump units (OBS) were present. It is not known from this study why erythrocyte OBS was diminished in group 5. Diminution in the number of membrane Na,K-pump units could be caused by genetic, acquired, or a combination of both factors. Inherited defects include the lack of a normal or the presence of an abnormal gene encoding for the α- or β-subunit of the enzyme, a translational or transcriptional defect in subunit mRNA production, defective subunit synthesis or incorporation into the whole enzyme, or impaired insertion of the synthesized enzyme into cell membranes.[33] Erythrocyte OBS was comparable in the two newborn groups, and it was higher in group 6 than in group 5. This seems to weaken the argument for a genetic role of the low number of erythrocyte Na,K-pump units in northeast rural Thais; however, it does not absolutely negate such a genetic cause. The random selection of study subjects in groups 5 and 6 might be erroneously biased and confound the difference in Na,K-ATPase activity or unit number observed. Moreover, the genetic defect of Na,K-ATPase in group 6 subjects might not be expressed after migration to a new environment possibly lacking critical factors essential for phenotype expression. Nevertheless, it is highly plausible that the defects are acquired by one or more environmental factors existing in northeast Thailand, and that these factors can be extinguished partially or completely after subjects migrate to a different environment.

Increased erythrocyte Na is generally known as a prime regulator for synthesis of membrane Na,K-ATPase. When the level is high, membrane Na,K-ATPase should be upregulated.[34-36] An inverse correlation between erythrocyte Na and erythrocyte Na,K-ATPase or erythrocyte OBS (Table 2) could imply that membrane Na,K-pump function or number are decreased and erythrocyte Na is increased reciprocally. This indicates a lack of this stimulating effect of high erythrocyte Na on the membrane Na,K pump in group 5 subjects. It could be due to a defect in Na,K-pump synthesis, in assembly of the Na,K pump into the membranes, or in the rate of internalization of the pump into the cell, or to the presence of a Na,K-pump inhibitor. Increased erythrocyte Na, despite a normal activity and number of membrane Na,K-pump units, in group 2 subjects suggests that erythrocyte Na in this group could be retained by mechanisms other than a Na,K-pump defect.

The low erythrocyte Na,K-ATPase activity and erythrocyte Na,K-ATPase to OBS ratio in groups 3 and 5 suggest that the activity of Na,K-ATPase is defective despite being normalized to an equal amount of the Na,K-pump unit. Since the activity and the ratio in groups 4 and 6 were

improved, this might indicate the presence of a digitalis-like inhibitor acting on the Na,K-transporting enzyme or the presence of different enzyme expression among different population groups. If this is true, this inhibitor must be present in both rural populations of this study. Vanadium, an inhibitor of the Na,K pump, is abundant in the water and soil of northeast Thailand, and it might play a causal role in depressed Na,K-pump activity in northeast Thais.[37] Further studies of the role of vanadium in these subjects are needed. It is known that different isoforms of the α-subunit of Na,K-ATPase have a different affinity for substrate and sodium transport,[33] but it is not known whether the Na,K pump of human erythrocytes could contain different α-isoforms under different circumstances. We interpret the results to indicate that group 3 subjects had low erythrocyte Na,K-ATPase activity secondary to the presence of a circulating Na,K-pump inhibitor, whereas group 5 subjects had low activity secondary to both the inhibitor and diminution of Na,K-pump units.

Hypokalemia might lead to diminution of membrane Na,K-pump activity.[38-39] However, changes in erythrocyte Na,K-pump number and activity in this study could hardly be explained by changes in plasma potassium level alone. There is no correlation between erythrocyte Na,K-ATPase activity and plasma potassium. Plasma potassium levels in all adult populations (groups 3 to 6) were lower than in their respective newborn counterparts. Yet the erythrocyte Na,K-pump activity and number in these adult groups changed in the opposite direction, ie, increased in group 4 and decreased in groups 3 and 5.

The increased intracellular sodium since birth and decreased Na,K-ATPase number and function secondarily acquired at a later age in ethnic northeast Thais could have important clinical consequences. Decreased Na,K-pump activity could lead to increased cytosolic calcium and decreased intracellular pH. Chronic alteration of intracellular sodium, calcium, and pH could have several pathophysiological consequences that may be detrimental to health and may be causally related to various metabolic disorders common in northeast Thailand.[40] These include the sudden unexplained death syndrome,[15] hypokaliuric hypocitraturia associated with renal stone disease,[17,18] hypokalemic periodic muscular paralysis, and renal tubular acidosis.[16] All these conditions are more prevalent in northeast than in central Thailand. Further investigation is needed to understand the relationship between this membrane transporting-enzyme defect and those disorders.

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