Red Blood Cell Lithium-Sodium Countertransport in the Tecumseh Blood Pressure Study

Alan B. Weder, Nicholas J. Schork, Lisa Krause, and Stevo Julius

Human essential hypertension has more than one cause, but to dissect out subtypes, markers are required. The maximal activity of red blood cell lithium-sodium countertransport has been shown to be increased in hypertensive patients in case-control and population-based studies; in the latter, its distribution is a mixture of two overlapping but distinguishable subpopulations. In the present study, we classified 705 participants in the Tecumseh Blood Pressure Study as having either normal (mean, 0.234 mmol/l cells/hr; n=614) or high (mean, 0.463 mmol/l cells/hr; n=91) red blood cell lithium-sodium countertransport to determine if the red blood cell marker is associated with distinctive physiological characteristics. We found that subjects with elevated lithium-sodium countertransport have higher average blood pressure and a greater prevalence of hypertension than those with normal countertransport and that elevated blood pressure had been present since youth. Hemodynamically, the high countertransport group is characterized by elevated vascular resistance, whereas sympathetic nervous system activity appears to be slightly depressed. Subjects with increased lithium-sodium countertransport, compared with those with normal countertransport, have significantly lower average left ventricular mass index and only very infrequently demonstrate left ventricular hypertrophy. Our results support the usefulness of measurements of the maximal activity of red blood cell lithium-sodium countertransport as a way of distinguishing subgroups in the population. Our data are consistent with the idea that subjects with an elevated maximal activity for red blood cell lithium-sodium countertransport are a subset of the population with a genetic lesion that predisposes them to the development of essential hypertension.

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Human hypertension is a family of diseases defined by a single physical sign, elevated blood pressure. Clinicians have traditionally classified hypertension as primary, or essential, if its cause is unknown and secondary when it results from a definable endocrine, renal, or vascular abnormality. Furthermore, the suggestion that there are subtypes of essential hypertension has been forwarded; classifications have been proposed based on differences in the relation of renin levels to sodium excretion,\(^1\) on salt sensitivity of blood pressure,\(^2\) on hemodynamic characteristics,\(^3\) on differences of vascular and aldosterone responses to angiotensin II infusion,\(^4\) by the coexistence of dyslipidemias\(^5\) or obesity,\(^6\) and recently, by the association of hypertension with various measures of membrane transport.\(^7\)-\(^10\) Of the proposed subgrouping strategies, those using membrane transport markers are particularly appealing to workers investigating the genetic epidemiology of human hypertension since cation fluxes are mediated by intrinsic membrane proteins that are usually the products of single genes or gene families. One transport marker for hypertension, an elevated maximal activity (\(V_{\text{max}}\)) for red blood cell (RBC) lithium-sodium (\(\text{Li}^+\)-\(\text{Na}^+\)) countertransport,\(^7\) has several particularly appealing features: 1) the distribution of RBC \(\text{Li}^+\)-\(\text{Na}^+\) countertransport values is a mixture of two subpopulations at all ages and in both sexes;\(^11\)-\(^13\) 2) activity of the system is highly heritable;\(^14\)-\(^17\) and at least some families demonstrate major gene inheritance;\(^16\)-\(^18\); and 3) there are plausible physiological links between increased activity of the transporter and organ-level disorders that could promote the development of hypertension.\(^19\),\(^20\) We investigated the usefulness of elevated RBC \(\text{Li}^+\)-\(\text{Na}^+\) countertransport as a marker for hypertension-related characteristics by measuring the \(V_{\text{max}}\) for RBC...
Li\textsuperscript{+}-Na\textsuperscript{+} countertransport and various anthropometric, hemodynamic, and biochemical features of a population-based cohort of young normotensive and borderline hypertensive adults. The results indicate that elevated V\textsubscript{max} for RBC Li\textsuperscript{+}-Na\textsuperscript{+} countertransport is a marker delineating a subgroup of the population with distinctive pathophysiological features.

**Methods**

The subjects in this report are volunteer participants in the Tecumseh Blood Pressure Study who were recruited from a population of 18–42-year-old persons residing in Tecumseh, Mich., the site of a long-term epidemiological survey. None of the individuals in the present study were receiving antihypertensive treatment, and none of the women were known to be pregnant or taking birth control pills at the time of the study. All were white, and all were free of overt cardiovascular, renal, endocrine, and hepatic disease on physical and laboratory examinations. After a thorough explanation of the purposes of the study and the procedures involved, all signed a written, informed consent form previously approved by the institutional review board for human studies at the University of Michigan, Ann Arbor.

**Anthropometrics and Blood Pressure**

Evaluations were performed at a field clinic in Tecumseh. Subjects were instructed to fast for at least 12 hours before their appointment. On presentation to the clinic, they underwent a brief history and physical examination, including measurements of height, weight (in street clothes without shoes), and triceps, biceps, subscapular, and suprailiac skinfold thicknesses (by caliper). To estimate obesity, the percentage over ideal body weight was calculated using Metropolitan Life Insurance tables appropriate to age and sex. After these initial examinations, subjects rested in a seated position for at least 2 minutes with the right arm comfortably supported at the level of the heart before blood pressure was measured and recorded twice, with the right arm comfortably supported at the level of the heart before blood pressure was measured. Systolic and diastolic blood pressures were reported below represent the average of two readings. Mean arterial pressure plus one third pulse pressure.

**Echocardiographic and Doppler Measurements**

All echocardiographic and Doppler studies were performed on an Advanced Technology Laboratories Ultramark IV (Advanced Technology Laboratories, Inc., Bellevue, Wash.) by an experienced technician (L.K.). Interventricular septal and posterior wall thicknesses and left ventricular internal diameter were measured from end-diastolic M-mode echocardiographic images by both the American Society of Echocardiography (ASE\textsuperscript{21}) and the Penn conventions. ASE measurements were used for all purposes except determination of left ventricular mass, which was calculated by the Penn-cube formula using Penn convention measurements\textsuperscript{22}:

\[
LVM=1.04[(IVS\textsubscript{d}+PW\textsubscript{d}+LVID\textsubscript{d})^3-(LVID\textsubscript{d})^3]-13.6
\]

where LVM is left ventricular mass, IVS is interventricular septal thickness, d is end diastolic, PW is posterior wall thickness, and LVID is left ventricular internal diameter. Left ventricular mass index (LVMI) was calculated by dividing left ventricular mass by body surface area in square meters. Relative wall thickness was calculated as 2PW\textsubscript{d}/LVID\textsubscript{d}.

For cardiac output measurements, two-dimensional images of the aortic root were recorded in the long axis view with a 2.25 mHz transducer, and the aortic cross-sectional area calculated from the aortic root diameter measured on the two-dimensional image at the level of the aortic leaflets during mid-systole. Doppler measurements were obtained after subjects had rested in the recumbent position for a period of 30 minutes. Aortic outflow was recorded from the suprasternal notch with a continuous wave Doppler transmitter, and cardiac output was calculated as the product of the velocity-time integral during systole and the aortic cross-sectional area. Cardiac index is cardiac output divided by body surface area in square meters. Vascular resistance (in arbitrary units) was calculated by dividing mean arterial blood pressure by cardiac output.

Resting forearm hemodynamics were assessed by mercury-in-Silastic strain gauge (Hokanson Instruments, Issaquah, Wash.) plethysmography using a technique we have described in detail elsewhere.\textsuperscript{23}

To obtain maximal flow for calculation of minimum forearm vascular resistance, forearm ischemia was induced by complete occlusion of forearm blood flow by a blood pressure cuff applied to the upper arm and inflated to above systolic pressure for 10 minutes while subjects rhythmically squeezed a slightly inflated folded sphygmomanometer cuff connected to a mercury manometer. A pediatric cuff encircling the wrist was then inflated above systolic pressure to exclude the hand circulation, and the upper arm cuff was released. Flow and blood pressure were measured during the ensuing maximal hyperemic phase, when the arterioles were completely relaxed and the resistance to flow reflected structural properties of the vessels. Under these circumstances vascular hypertrophy caused the wall of a thickened blood vessel to impinge on its lumen, resulting in an increased resistance to flow.\textsuperscript{24}

**Catecholamine, Renin, and Red Blood Cell Studies**

For plasma catecholamines, 7 ml whole blood was drawn through an indwelling intravenous catheter into a syringe and immediately transferred into an iced vacutainer containing ethyleneglycol-bis-(\(\beta\)-aminoethyl ether) N,N,N',N' -tetraacetic acid and glutathione. Baseline catecholamine levels were drawn after at least 20 minutes quiet supine rest, and a second catecholamine determination was performed
after 2 minutes of mental arithmetic (serial subtraction of 13 from 1,079). The details of the mental arithmetic protocol have been published. Plasma was separated within 30 minutes of blood collection, frozen on dry ice, and subsequently stored at -70°C until analysis by radioenzymatic assay.

Plasma renin activity was measured by radioimmunoassay of angiotensin I generated by a 60-minute incubation of plasma in the presence of excess substrate at pH 6.0.

For RBC studies, 20 ml whole blood was drawn into a heparinized syringe. One milliliter whole blood was set aside for gravimetric determination of cell water content as previously described; the remainder was transferred to a 50 ml screwtop polyethylene tube and centrifuged at 1,000 g for 15 minutes, and the supernatant and buffy coat was then removed. One milliliter of the packed RBCs was transferred to a 13 ml plastic tube and washed three times with an ice-cold solution consisting of (mM) MgCl₂ 75, sucrose 85, and Tris-MOPS 10 (pH 7.4 at 4°C).

After the last wash, the supernatant was removed and the cell pellet was thoroughly mixed. The hematocrit of the RBC suspension was determined in duplicate and a 200 μl aliquot mixed with 10 ml 5% trichloroacetic acid (TCA). The Na⁺ concentration of the supernatant was subsequently determined by atomic absorption spectrophotometry (Perkin-Elmer 2380 Atomic Absorption Spectrophotometer, Perkin-Elmer Corp., Norwalk, Conn.) using bracketed TCA standards. The Na⁺ content of fresh RBCs was calculated from the supernatant Na⁺ concentration corrected for hematocrit and dilution factors. The remaining RBCs were mixed with a holding solution consisting of (mM) KCl 140, NaCl 15, glucose 10, and TRIS-MOPS 10 (pH 7.4 at 4°C) and were placed on ice for subsequent transport to Ann Arbor. At the main laboratory, RBC Li⁺-Na⁺ countertransport was measured within 48 hours by the method of Canessa et al as Na⁺-stimulated Li⁺ efflux from RBC loaded with at least 4 mmol Li⁺/l cells. Efflux media were MgCl₂- and NaCl-based as in our earlier description.

**Childhood Data**

Many of the subjects in the present study had previously participated in a Tecumseh Health Study examination as children. During this earlier examination, blood pressure and heart rate readings were recorded by trained observers after the subjects had rested for a minimum of 2 minutes in the sitting position. In addition, the children were weighed and measurements of height and subscapular skinfold thickness were obtained.

**Statistical Analysis**

Univariate mixture analysis was used to determine whether there were subgroups in the distribution of V_max for RBC Li⁺-Na⁺ countertransport in the population. As we have previously described, countertransport values were first adjusted for the effects of gender and for the first-, second-, and third-order power effects of age, weight, and height and for interaction effects of these variables. The resulting adjusted distributions were then examined for the presence of a mixture with simultaneous adjustment for skewness. The optimal number of mixed distributions within the population was determined by χ² testing. Individuals were then classified as "high" or "normal" countertransport by comparing their weighted average probabilities of belonging to each distribution.

Comparisons between groups were performed with Student’s unpaired t test and by χ² test, and bivariate relations were analyzed by least-squares regression. In all cases, significance was accepted at the p<0.05 level. All data are expressed as mean±SD.

**Results**

Seven hundred and five participants in the Tecumseh Blood Pressure Study had measurements of RBC Li⁺-Na⁺ countertransport. As shown in Figure 1, univariate mixture analysis revealed that the distribution of RBC Li⁺-Na⁺ countertransport is composed of two groups (p<0.00001 versus one or three groups), which is consistent with our earlier findings. The larger subgroup (n=614, 87.1%) has a mean V_max for Li⁺-Na⁺ countertransport activity (Table 1) similar to those that we and others have reported for normotensive subjects in case-control and population-based studies and is subsequently referred to as the "normal countertransport" group. The smaller subgroup (n=91, 12.9%) has a mean V_max for Li⁺-Na⁺ countertransport activity (Table 1) similar to that observed in hypertensive subjects and is referred to below as the "high countertransport" group. Both groups had a similar proportion of males (56.1% versus 54.6%, high versus normal countertransport, p=0.64 by χ²), so that no adjustment for gender differences was necessary in the subgroup comparisons.

**Blood Pressure**

As shown in Table 1, the high countertransport subgroup had significantly higher systolic and diastolic blood pressures than those with normal countertransport, although the average values were still within the normotensive range. Both systolic and diastolic blood pressures correlated significantly with the V_max for RBC Li⁺-Na⁺ countertransport for the entire group and for both subgroups (Table 2). The prevalence of hypertension in the high and normal countertransport groups was determined by classifying subjects as hypertensive if their average blood pressure exceeded 90 mm Hg diastolic or 140 mm Hg systolic and normotensive if both systolic and diastolic blood pressures were below these limits. Ninety-one participants, 12.9% of the entire group, were hypertensive, 71 in the normal countertransport group and 20 in the high countertransport group. Thus, the prevalence of hypertension was almost twofold greater in the high, compared with the normal, countertransport group (20 of 91 or 22.0%...
versus 71 of 614 or 11.6%, respectively, \( p < 0.006 \), by \( \chi^2 \). Normotensive subjects with high countertransport had significantly higher diastolic blood pressure than normotensive subjects with normal countertransport (77.9 ± 7.1 versus 75.1 ± 8.2 mm Hg, \( p < 0.01 \)). Systolic blood pressure was also higher in

### TABLE 1. Univariate Mixture Analysis: Anthropometric, Hemodynamic, and Biochemical Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal countertransport</th>
<th>High countertransport</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>614</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Male:female</td>
<td>335:279</td>
<td>52:39</td>
<td>0.64†</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31.4±4.5</td>
<td>30.9±4.8</td>
<td>0.36</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.6±17.8</td>
<td>77.2±16.0</td>
<td>0.72</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.9±9.8</td>
<td>170.7±9.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Body surface area (m(^2))</td>
<td>1.88±0.23</td>
<td>1.89±0.22</td>
<td>0.81</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>54.3±12.5</td>
<td>53.2±12.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Overweight (%)</td>
<td>16.0±22.0</td>
<td>17.0±19.7</td>
<td>0.68</td>
</tr>
<tr>
<td>Sum skinfolds (mm)†</td>
<td>74.6±31.3 (571)‡</td>
<td>80.8±33.6 (84)</td>
<td>0.09</td>
</tr>
<tr>
<td>RBC Li(^+)-Na(^+) countertransport (mmol Li(^+)/l cells-hr)</td>
<td>0.234±0.073</td>
<td>0.463±0.094</td>
<td>0.00001</td>
</tr>
<tr>
<td>RBC Na(^+) (mmol/l cells)</td>
<td>7.04±2.16 (552)</td>
<td>7.12±1.91 (86)</td>
<td>0.74</td>
</tr>
<tr>
<td>RBC H(_2)O (%)</td>
<td>66.7±1.7 (560)</td>
<td>66.4±1.7 (79)</td>
<td>0.08</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>114.8±11.9</td>
<td>117.9±13</td>
<td>0.02</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>77.4±10.1</td>
<td>81.2±9.0</td>
<td>0.0007</td>
</tr>
<tr>
<td>Heart rate (min(^{-1}))</td>
<td>69.1±9.4</td>
<td>69.8±10.8</td>
<td>0.55</td>
</tr>
<tr>
<td>Vascular resistance (units)</td>
<td>17.8±3.5 (536)</td>
<td>18.7±3.8 (80)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cardiac index (L/min/m(^2))</td>
<td>2.77±0.49 (541)</td>
<td>2.79±0.57 (80)</td>
<td>0.77</td>
</tr>
<tr>
<td>Stroke index (ml/m(^2))</td>
<td>45.0±7.5 (541)</td>
<td>44.3±8.9 (80)</td>
<td>0.43</td>
</tr>
<tr>
<td>Forearm blood flow (ml/min/100 g)</td>
<td>3.90±1.82 (476)</td>
<td>3.85±1.49 (74)</td>
<td>0.82</td>
</tr>
<tr>
<td>Forearm vascular resistance (units)</td>
<td>26.8±12.4 (476)</td>
<td>26.5±11.0 (74)</td>
<td>0.84</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>237±108 (520)</td>
<td>211±107 (70)</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>57±37 (520)</td>
<td>54±39 (70)</td>
<td>0.59</td>
</tr>
<tr>
<td>Plasma renin activity (ng Ang I/ml/hr)</td>
<td>2.0±1.6 (567)</td>
<td>2.0±0.9 (85)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Values are mean±SD. Numbers in parentheses are the number of observations if less than \( n \) for group. RBC, red blood cells; Na\(^+\), intracellular sodium; Ang I, angiotensin I.

*Significance testing by \( t \) test.
†Significance testing by \( \chi^2 \) test.
‡Triceps, biceps, subscapular, and suprailliac.
the high countertransport normotensive subjects, but the difference was not significant (113.9±10.3 versus 112.8±10.4 mm Hg, p=0.41).

**Body Size, Obesity, and Metabolism**

The high countertransport group was slightly heavier, with thicker skinfolds and greater obesity, although none of the differences reached statistical significance (Table 1). As shown in Table 2, there were highly significant correlations between V^max for RBC Li^+*-Na^+ countertransport and weight, height, body surface area, lean body mass, and percent overweight for the entire group and within each subgroup.

**Systemic and Forearm Hemodynamics**

The higher blood pressure observed in the group with an elevated V^max for RBC Li^+*-Na^+ countertransport was associated with a higher average vascular resistance; cardiac index and stroke volume index did not differ significantly between the high and normal countertransport groups (Table 1). Measurements of forearm hemodynamics at rest also did not differ between the groups.

**Vasoactive Hormones**

High countertransport subjects had significantly lower basal plasma norepinephrine levels than normal countertransport subjects (Table 1), and norepinephrine correlated inversely with V^max for RBC Li^+*-Na^+ countertransport (Table 2). Plasma levels of epinephrine were similar in normal and high countertransport subjects at baseline (Table 1), but during mental arithmetic, plasma epinephrine rose 13±36 pg/ml in the normal countertransport subgroup, an average increase of 23% over baseline but only 4±39 pg/ml (7% increase over baseline) in the high countertransport subgroup, and the difference in epinephrine responsiveness was of borderline significance (p=0.07). During mental arithmetic, the average change in norepinephrine was trivial in both groups, with a rise of 3 pg/ml in the normal countertransport subgroup and a fall of 3 pg/ml in the high countertransport group; the difference between the groups was not significant.

Renin activity was not significantly different between the groups (Table 1), and plasma renin activity was not significantly correlated with the V^max for RBC Li^+*-Na^+ countertransport (Table 2).

**Cardiovascular Structural Measurements**

Even though systolic and diastolic blood pressures were significantly higher in the high countertransport group, as displayed in Figure 2, LVMI was significantly lower (93.1±17.6 g/m², n=78, versus 98.5±19.9 g/m², n=543, high versus normal countertransport, p=0.02). Of the 53 subjects (22 men and 31 women) with left ventricular hypertrophy (LVMI more than 134 g/m² for men and more than 110 g/m² for women), 49 (92%) were in the low countertransport group. In the normotensive subjects, the trend...
FIGURE 2. Graph showing comparison of left ventricular mass index in 621 subjects. Group with high maximal activity (V_max) for red blood cell (RBC) Li^+-Na^+ countertransport (n=78) has a significantly (p=0.02) lower left ventricular mass index compared with group with normal countertransport (n=543). Standard error bars are shown as heavy lines above the bars. Individual values for men (x) and women (o) with left ventricular hypertrophy (left ventricular mass index more than 134 g/m^2 for men and more than 110 g/m^2 for women) are shown for the four of 78 high countertransport and the 49 of 543 normal countertransport subjects.

Table 3. Echocardiographic Measures of Left Ventricular Diastolic Size (American Society of Echocardiography Convention) in Normal and High Countertransport Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal countertransport</th>
<th>High countertransport</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>480</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Interventricular septal thickness (mm)</td>
<td>10.2±0.4</td>
<td>10.3±0.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Posterior wall thickness (mm)</td>
<td>10.2±0.4</td>
<td>10.3±0.4</td>
<td>0.48</td>
</tr>
<tr>
<td>Left ventricular internal diameter (mm)</td>
<td>47.5±1.7</td>
<td>45.8±1.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.43±0.06</td>
<td>0.45±0.08</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*p*Significance by t test.

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nephine after mental arithmetic (r=0.12, p<0.01, n=504), but not at baseline (r=0.03, p=0.42, n=590). Plasma renin activity did not correlate significantly with LVMI (r=-0.01, p=0.76, n=652).

Forearm vascular resistance after 10 minutes of ischemic exercise, a measure of structural vascular hypertrophy, was not significantly different for the high versus normal countertransport subgroups for the entire population (2.07±0.55 versus 2.09±0.74 arbitrary units, respectively, p=0.19) or for the normotensive subjects (2.02±0.55 versus 2.07±0.73 arbitrary units, respectively, p=0.64).

Characteristics in Youth

Five hundred and forty-five (77.3%) of the subjects of our present study had participated as young children in a screening examination for the Tecumseh Health Study. Shown in Table 4 are the characteristics in youth of subjects currently belonging to the high and normal countertransport groups. In addition to its association with higher blood pressure in youth, as children those subjects currently classified in the high countertransport group had a marginally higher heart rate, a known predictor of risk for future hypertension, but they were not overweight or obese, as assessed by subcapular skinfold thickness. Interestingly, the V_max for RBC Li^+-Na^+ countertransport determined in the present study correlated significantly with diastolic blood pressure in youth (r=0.09, p=0.04).

Discussion

The results of the present study confirm our earlier observation of the usefulness of mixture analysis in the delineation of a subgroup of the population characterized by elevated RBC Li^+-Na^+ countertransport and high blood pressure. The prevalence of high countertransport is lower (12.9%) in the present population-based sample than in our earlier study (27%), which undoubtedly reflects an overrepresentation of hypertensive subjects in the earlier sample.

Previous studies of the Tecumseh community demonstrate that mean systolic and diastolic blood pressures rise until age 50. It therefore seems clear that many of the participants in the current study may yet develop essential hypertension, and we interpret our findings as suggesting that high countertransport represents an important marker for a tendency to...
develop hypertension. First, although our subjects are on average early in the fourth decade of life, almost twice as many high countertransport individuals have already developed hypertension as those with normal countertransport (22.0% versus 11.6%). Second, normotensive members of the high countertransport group presently show significant increases in diastolic blood pressure compared with normotensive subjects with normal countertransport. Finally, in our retrospective analysis we show that this high countertransport subgroup had a significantly elevated blood pressure as early as age 6. Thus, it seems very likely that an elevated $V_{\text{max}}$ for RBC Li$^+$-Na$^+$ countertransport is associated with a predisposition to future hypertension, and in addition, it seems to be a risk factor that is largely independent of other risk factors such as obesity. Longitudinal follow-up of the current study participants is planned, and although we cannot be certain how many in the high countertransport group will eventually develop hypertension, we predict that the incidence of new onset hypertension in the normotensive subjects identified as at risk based on their assignment to the high countertransport group will be greater than in that in the low countertransport group.

The hemodynamic characteristics associated with elevated RBC Li$^+$-Na$^+$ countertransport are those we have reported earlier: normal cardiac index and elevated vascular resistance. The basis of this increased vascular resistance remains unknown, but does not appear attributable to activity of the SNS or the renin-angiotensin system in the present study. Although plasma norepinephrine is only an indirect measure of SNS activity, it would be difficult to reconcile our observation of significantly lower levels of plasma norepinephrine in the high countertransport group with increased SNS discharge. Indeed, the sum of the evidence, which includes a normal forearm vascular structural component and an LVMI that is inappropriately low for the level of blood pressure, taken together with the finding of a low basal plasma norepinephrine concentration and sluggish epinephrine reactivity during mental arithmetic, suggests that the activity of the SNS is suppressed during the earliest stages of evolution of hypertension in patients with high countertransport.

Although suppressed SNS activity in early hypertension may seem incongruous, it is actually what might be expected if the central nervous system has a pressure-seeking function. In this theoretical construct, the central nervous system is thought to have the capability of sensing blood pressure and regulating SNS outflow as necessary to maintain blood pressure homeostasis. Thus, unlike hyperkinetic borderline hypertension, where there is good evidence that increased activation of sympathetic outflow contributes to increased cardiac index and inappropriately normal vascular resistance, elevated countertransport may raise blood pressure by some as yet unknown mechanism that is independent of direct activation of the SNS, and the nervous system may respond by decreasing autonomic output.

We cannot confirm the observation of Yap et al that a high $V_{\text{max}}$ for RBC Li$^+$-Na$^+$ countertransport is associated with a tendency to develop left ventricular hypertrophy; our high countertransport subjects had significantly lower LVMI than those with normal countertransport, and both groups had similar minimum forearm vascular resistances, suggesting that there was also no excessive vascular hypertrophy. However, we wish to emphasize the important methodological differences between the earlier study and ours. Yap et al measured Li$^+$ efflux, whereas we measured Na$^+$-stimulated Li$^+$ efflux. Although the same countertransporter can mediate Li$^+$ efflux and influx, it seems that the regulatory defect causing an increased $V_{\text{max}}$ for Li$^+$-Na$^+$ countertransport in essential hypertension is intracellular, and it has been suggested that measurements of Li$^+$ influx and efflux may not be congruent.

In addition, our patients were younger, had milder hypertension and presumably had less severe left ventricular hypertrophy, since we used echocardiographic measurements while Yap et al applied electrocardiographic criteria. Thus, the two studies may not be strictly comparable, and although it appears that in our study, in patients at the earliest stage of hypertension, an elevated $V_{\text{max}}$ for RBC Li$^+$-Na$^+$ countertransport is not associated with left ventricular hypertrophy, it remains possible that as blood pressure rises, the course of progression of hypertrophy may be different. Our high countertrans-

### Table 4. Characteristics of High and Normal Countertransport Groups in Youth

<table>
<thead>
<tr>
<th>Variable</th>
<th>High countertransport</th>
<th>Normal countertransport</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>65</td>
<td>480</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>6.2±3.5</td>
<td>6.4±3.2</td>
<td>0.73</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>23.4±12.6</td>
<td>24.6±12.3</td>
<td>0.45</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>117.4±25.4</td>
<td>117.8±22.0</td>
<td>0.90</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>114.8±11.1</td>
<td>110.2±12.2</td>
<td>0.006</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72.1±9.2</td>
<td>68.3±9.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Heart rate (min$^{-1}$)</td>
<td>101.6±23.4</td>
<td>97.4±19.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Subscapular skinfold (mm)</td>
<td>6.9±3.2</td>
<td>7.4±4.6</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Values are mean±SD. Significance by $t$ test.
port subjects did demonstrate concentric remodeling of the left ventricle, and it is of interest that Devereux et al (personal communication) have recently shown that structural remodeling, even without concomitant left ventricular hypertrophy, increases cardiovascular risk. In our planned longitudinal studies it will be of interest to determine if structural remodeling progresses to left ventricular hypertrophy and if left ventricular hypertrophy progresses at a faster pace in our high countertransport subjects than in those with normal countertransport as blood pressure increases.

Finally, we wish to comment on several previously reported observations of correlations of biochemical and cellular factors with RBC Li\(^+\)-Na\(^+\) countertransport activity. We find no relation between plasma renin activity and RBC Li\(^+\)-Na\(^+\) countertransport, confirming our earlier report, which called into question the relation noted by Brugnara et al. In the present study, we found the \(V_{\text{max}}\) for RBC Li\(^+\)-Na\(^+\) countertransport to be significantly inversely correlated with RBC H\(_2\)O content (Table 2), which is consistent with previous reports. RBC Na\(^+\) content correlated positively with the \(V_{\text{max}}\) for RBC Li\(^+\)-Na\(^+\) countertransport only in the high countertransport group.

In summary, our findings confirm the importance of a high \(V_{\text{max}}\) for RBC Li\(^+\)-Na\(^+\) countertransport as a marker for risk for essential hypertension. Our physiological measurements suggest that the marker is associated with SNS characteristics that are distinctly different from those seen in hyperkinetic borderline hypertension, another state with an increased risk for future hypertension. A tendency toward elevated blood pressure in the high countertransport group seems to be lifelong, since blood pressure was elevated in this group as early as age 6. Finally, we find no evidence to suggest that a high \(V_{\text{max}}\) for RBC Li\(^+\)-Na\(^+\) countertransport is associated with a tendency toward cardiac or vascular hypertrophy.

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**KEY WORDS** • essential hypertension • lithium-sodium countertransport • erythrocytes • hemodynamics • ventricular wall mass • catecholamines
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