Effect of Dietary Sodium Reduction on Red Blood Cell Sodium Concentration and Sodium-Lithium Countertransport

RICHARD COOPER, MAURIZIO TREVISAN, LINDA VAN HORN, EMMANUEL LARBI, KIANG Liu, SERAPHIM NANAS, HIROTSUGU UESHIMA, CHRISTOPHER SEMPOS, DAVID OSTROW, AND JEREMIAH STAMLER

SUMMARY A randomized, crossover trial was carried out on the effect of moderate sodium reduction on red-blood-cell sodium metabolism. The participants were healthy high school students (mean age = 16 years, n = 33). Changes in sodium-lithium countertransport and intracellular sodium concentration were evaluated 24 days after a decrease in dietary sodium from approximately 110 to 40 mEq per day. Dietary sodium restriction had no significant effect on either sodium-lithium countertransport or intracellular sodium concentration.

(Hypertension 6: 731-735, 1984)

KEY WORDS • sodium • blood pressure • countertransport • randomized trial

Over the last several years, cation transport has become an important research focus in the field of hypertension. The principal areas that have been investigated relate to the relationship between cation transport and blood pressure (BP), and the influence of heredity. In addition, sex differences in some pathways for cation transport have been demonstrated, and several other factors have been linked to altered rates of exchange, including obesity, excess alcohol intake, and pregnancy.

If abnormal cation transport is in fact a marker and precursor of hypertension, it would be important to identify potentially modifiable environmental or lifestyle variables that might possibly be useful for preventive purposes. The study reported here was a randomized single-blind crossover trial on the effect of moderate sodium restriction on red-blood-cell (RBC) sodium concentration and sodium-stimulated lithium efflux.

Methods

Subjects

This experiment was performed as a part of a larger trial on sodium reduction in adolescents, which has been previously described. Briefly, 37 high school students attending a boarding school were recruited out of a group of 124 who volunteered for the larger study. The school was operated by the Seventh-Day Adventist Church and served lacto-ovo-vegetarian food. The school schedule required students to remain on the rural campus for 24-day periods, with a 5-day interval between each period. Informed consent was obtained from the students and their parents or guardians. Volunteers were then randomly assigned to either the regular institutional food (Group 1) or a diet estimated to be 50% reduced in sodium (Group 2). Of the 37 participants who had blood drawn at baseline, one did not complete the experimental diet and another left school. In addition, two individuals (one from each group) were found to have extremely high values of sodium-lithium countertransport (SLC), over two and one-half times the sample mean. Such extreme values usually occur in only 1% to 2% of the population. In our hands, the assay is not precise at this extreme range, and those two individuals were also not included in the final analyses. The descriptive characteristics of the two groups are presented in Table 1.
The RBC Na was determined by washing the packed cells three times with an isosmotic cold solution of 115 mM MgCl₂, and the amount of Na was expressed in mmol/liter of cells. The maximal rate of SLC was determined by the method of Canessa et al. after the cells were loaded with a solution containing 150 mM LiCl, glucose, and 10 mM TRIS-MOPS buffer, pH 7.4. The erythrocytes gained 6 mmol lithium/liter cells in 3 hours, which indicated adequate loading for measurement of maximum velocity of efflux. Lithium efflux (Li efflux) was measured in the magnesium and sodium media. The net efflux (SLC) was calculated by subtracting the efflux in magnesium from the efflux in sodium medium. Both methods have been described elsewhere. The rate constant of Li efflux into the Na-free medium was calculated as Li efflux into the MgCl₂ medium (Amol/liter cell/min) divided by the Li concentration (mmol/liter cells) of the loaded cells. This rate constant (min⁻¹) was taken as a good measurement of the diffusion leak pathway (Li leak), since Li does not have a sizable affinity for the Na-K cotransport under these conditions. Measurement of the maximal rate of SLC is possible under conditions of the assay, since these ensure saturation of the internal sites of Li (K_m = 0.5 mM) and external sites with Na (K_m = 25 mM). The hematocrit of the flux medium was maintained at 4% to obtain accurate measurement of the initial rate of Li efflux. A duplicate sample of every tenth specimen was submitted to the laboratory on a blind basis. Technical errors of the methods were 3.4% of the mean for RBC Na and 7.0% for SLC. Plasma Na and K were analyzed by flame photometry. Previous work has demonstrated the reliability of the storage procedure as well as consistency of the assay over time for the same individual. With each repeat examination, specimens from the same participants were handled in identical sequence. The within-batch technical error was 8.3% for SLC and 1.7% for RBC Na concentration based on 11 blind duplicates.

### Statistical Analysis

Data were collected directly onto precoded forms and key punched. Statistical analysis was based on the difference between visits 2 and 3; these time points were at the end of either the experimental or control period based on the group assignment. Two-sided pooled t-tests were used to assess significance levels. Correlation analysis was also applied.

### Results

The two groups of participants were roughly comparable as a result of the randomization procedure (Table 1). More males, however, were in Group 2 than Group 1, and BP was slightly higher. The higher mean SLC in Group 2 can be attributed to the higher percentage of males.

The trial results are summarized in Tables 2 and 3. No significant change was seen for either group in BP, RBC Na concentration, or SLC. The BP dropped about 1 mm Hg, while SLC and RBC Na was unchanged.

#### Table 1. Baseline Characteristics of Participants in Broadview Sodium Restriction Trial

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (n = 16)</th>
<th>Group 2 (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>16.4 ± 1.5</td>
<td>16.3 ± 1.3</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/7</td>
<td>12/5</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>108.9 ± 12.2</td>
<td>110.9 ± 7.9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64.4 ± 9.8</td>
<td>66.1 ± 8.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.64 ± 0.10</td>
<td>1.64 ± 0.30</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.0 ± 9.7</td>
<td>63.0 ± 8.3</td>
</tr>
<tr>
<td>BMI (wt/ht²; kg/m)</td>
<td>22.5 ± 3.0</td>
<td>23.9 ± 2.6</td>
</tr>
<tr>
<td>Pulse rate (bpm)</td>
<td>79.8 ± 15.6</td>
<td>79.5 ± 9.0</td>
</tr>
<tr>
<td>SLC (m/mol/liter cells x min)</td>
<td>4.93 ± 1.33</td>
<td>5.98 ± 2.49</td>
</tr>
<tr>
<td>RBC [Na], (mEq/liter cells)</td>
<td>7.29 ± 1.01</td>
<td>7.12 ± 1.42</td>
</tr>
</tbody>
</table>

Values are means ± SD. M = male; F = female. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; wt = weight; ht = height; SLC = sodium-lithium countertransport; RBC = red blood cell.

### Protocol

Two food lines were created in the cafeteria, and a record was maintained of each meal. For Group 2, food items high in sodium were eliminated, and reduced sodium products were substituted when possible, as in the case of cheese, peanut butter, and margarine. Study nutritionists worked closely with the cafeteria staff to structure an experimental diet that was moderately reduced in sodium relative to the regular cafeteria. Participants were recruited on the basis of an agreement not to receive packages from home or eat meals away from school during the 24-day experimental period. A record was kept of attendance at meals. The sodium content of the diet during the trial was assessed by two independent methods. Randomly selected participants were asked to collect duplicate meals for a day at a time, and these were submitted for chemical analysis. Randomly assigned participants were also asked to collect overnight urine samples, which were analyzed for Na, K, and creatinine.

The first phase of the study lasted 24 days, with an intervening 5-day vacation, followed by a crossover of 24 days. On Days 1 and 24 of Phase I and Day 24 of Phase II, participants underwent a standard examination. Height and weight were measured in light indoor clothes, with shoes off. Blood pressure was measured after a 15-minute rest by a procedure previously described, and a 15 cc sample of blood was subsequently withdrawn with minimal hemostasis from the antecubital fossa. All examinations took place between 600 and 800. Blood was transported to the laboratory within 1 hour. The batch was divided in half, and an equal number of specimens was taken from each group and analyzed either immediately or placed in storage for analysis in 48 hours.
TABLE 2. Red-Blood-Cell Sodium Metabolism, Blood Pressure, and Related Variables During Sodium Reduction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1: control diet + experimental diet</th>
<th>Group 2: experimental diet + control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Control diet</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>108.9±12.2</td>
<td>-0.7±9.8</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>64.4±9.9</td>
<td>-3.8±13.0</td>
</tr>
<tr>
<td>SLC (*nmol/liter cells x min)</td>
<td>493±1.33</td>
<td>5.09±1.53</td>
</tr>
<tr>
<td>RBC [Na], (mEq/liter)</td>
<td>7.29±1.01</td>
<td>7.01±1.22</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.9±9.7</td>
<td>63.2±9.4</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>79.9±15.6</td>
<td>72.0±12.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. For abbreviations, see Table 1.

TABLE 3. Changes in Red-Blood-Cell Sodium Metabolism, Blood Pressure, and Related Variables during Sodium Restriction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Treatment effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>-0.7±9.8</td>
<td>-0.9±6.7</td>
</tr>
<tr>
<td></td>
<td>DBP (mm Hg)</td>
<td>-3.8±13.0</td>
<td>+0.6±12.0</td>
</tr>
<tr>
<td></td>
<td>SLC (*nmol/liter cells x min)</td>
<td>-0.28±0.70</td>
<td>+0.77±0.90</td>
</tr>
<tr>
<td></td>
<td>RBC [Na], (mEq/liter cells)</td>
<td>+0.09±0.51</td>
<td>-0.13±0.80</td>
</tr>
<tr>
<td></td>
<td>Weight (kg)</td>
<td>-0.4±1.2</td>
<td>-1.0±0.9</td>
</tr>
<tr>
<td></td>
<td>Pulse rate (bpm)</td>
<td>+1.4±5.1</td>
<td>+0.4±4.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. For abbreviations, see Table 1.

*Average change for both groups.

Weight declined modestly, but the change was not statistically significant. Analysis by subgroups, based on initial weight, BP, SLC, or sex, also yielded no significant trends.

In an effort to confirm previous findings of an association between SLc and BP, the two groups were combined and examined at baseline. SLC was significantly correlated with systolic BP (r = 0.306; p = 0.0126), while for Group 1 this relationship was weaker (r = 0.239; p = 0.090). For Group 2 alone, a significant relationship was observed between change in SLC and change in BP (r = 0.430, p = 0.0321). Change in SLC was significantly correlated with change in RBC sodium concentration (r = 0.304, p = 0.043, both groups combined).

Mean dietary Na reduction for both phases during the experimental diet was 67% based on randomly assigned overnight urine samples (Table 4). From previous extensive work with urine collection, we estimated these values represented a 24-hour excretion of 110 and 40 mEq of Na during the control and experimental diets, respectively. Potassium was stable throughout. Food analyses from the trial as a whole demonstrated similar Na and K contents of the sample meals (110 and 44 mEq, respectively).

Discussion

The role of habitual high sodium intake in the pathogenesis of hypertension remains incompletely elucidated. Recent work demonstrating an association between levels of active cation transport and BP has provided the basis for a new look at mechanisms relating dietary Na and BP. Two previous studies reported changes in RBC Na metabolism with reduction in salt intake. In the investigation reported here, however...
ever, minimal nonsignificant changes in RBC Na concentration and SLC were observed after a reduction in dietary sodium from approximately 110 to 40 mEq/24 hr for 3 weeks. This study had a power of 0.80 to detect a difference in SLC of 0.8 4amol (1 standard deviation) at a significance level of 0.05. Only a small nonsignificant change in BP occurred over the experimental period, and weight was reduced modestly. At least as assessed by the cation transport pathway measured here, moderate changes in dietary intake do not have a short-term effect on RBC exchange rates or concentration, despite a cross-sectional relationship between SLC and BP. However, some evidence did emerge from this trial that dynamic relationships exist between Na metabolism and BP. In one group, change in SLC correlated with change in BP. Change in SLC also correlated significantly with change in RBC Na concentration.

Although extreme salt restriction has a clear-cut effect on the BP of hypertensive adults, it is difficult to maintain, even for very sick individuals. Such a regimen is not practical as a preventive strategy. Experience with moderate salt restriction has been limited, but recent studies have indicated that it results in modest reduction in BP of hypertensive adults. The value of such dietary recommendations must also be tested in the normotensive population.

We designed this trial as a short-term study to explore the effects of reducing Na in the diet of adolescents, and we chose RBC Na metabolism as a marker. We had previously demonstrated that persons in the age range of 18 to 28 years with high-normal BP and/or a family history of hypertension have higher mean levels of SLC. We also found a correlation between SLC and BP in black children aged 11 to 14 years, although these findings were not replicated in a second study of white children of the same age. Baseline data from the present study further demonstrate this cross-sectional association. It seems reasonable to assume, therefore, given the finding of higher SLC in frankly hypertensive adults, that cation transport may be a marker for risk of future hypertension.

There are a number of potential explanations for the negative outcome of this experiment. It is possible that no relationship exists between dietary Na and RBC Na metabolism. It is further possible that the Na intake was not reduced to a sufficiently low level. Although a relationship between RBC Na metabolism and BP exists at this age, and since BP is still normal by adult standards, the potentially abnormal response to a high salt intake may not have emerged as yet. By the same token, there may not have been enough high risk individuals in this study to demonstrate an effect. Examination of two subgroups potentially at higher risk — those above the median for body mass index (BMI) and SLC at baseline — was also negative, however.

Changes in maximal rate of Na exchange by the countercurrent transport pathway could be influenced by circulating hormones or structural changes in the cell membrane. In the latter case, one would have to wait until new cells are released into the circulation in sufficient numbers to demonstrate a change; given a mean RBC survival of 90 days, this trial may have been too short. Recent work has demonstrated an extreme short-term sensitivity of SLC to a dialyzable factor in plasma, although its relationship to control of this pathway under physiological conditions is unknown. Finally, it is possible that the participants did not adhere adequately to the experimental regime. Based on urine collections and careful monitoring of behavior, that last possibility seems unlikely, however.

Morgan, et al. reported a significant reduction in the rate constant for Na efflux from RBCs after dietary intake was reduced from 200 to 100 mEq/day. The eight participants all had severe hypertension, and the experimental period lasted 2 weeks. SLC accounts for less than 5% of sodium transport and does not contribute to net exchange. It may well be that efflux, as measured by Morgan, et al., and SLC respond in different ways to changes in Na balance. Working with lymphocytes, Ambrosioni et al. found a significant decrease in Na concentration when a diet containing 6 to 10 g NaCl/day was switched to a diet supplying 3 to 5 g. The five participants were borderline hypertensives aged 14 to 31 years; the experimental period lasted 6 weeks. No change in BP was observed. In addition to changes in Na concentration, an important finding from this study was a very high correlation between intralymphocytic Na concentration and change in BP during provocative maneuvers (arithmetic, hand grip, and exercise). Since lymphocytes contain a nucleus, they may respond differently to changes in Na intake than RBCs. This trial also lasted 3 weeks longer than ours, and participants were specifically chosen for being at above-average risk for hypertension. In a brief report, Canessa, et al. noted a 10% increase in the maximum velocity of SLC in a group of eight hypertensive patients when a 200 mEq sodium diet was altered to contain 10 mEq. A smaller increase was observed in a group of seven normotensive subjects. Neither change was statistically significant. The experiment lasted 6 days.

A popular current theory relating Na intake to BP argues for a circulating factor that alters Na transport across the cell wall. Although most of the data are derived from studies with RBCs, a generalized defect is being proposed, one including smooth muscle in the vascular wall. Alterations in Na transport and concentration are hypothesized to affect calcium balance, and thereby contractility. If dietary Na indeed initiates this process, it will be important for this theory to demonstrate a response to experimental alteration of intake. Such a change does not appear to occur in adolescents after a 3-week reduction from 110 to 40 mEq/day.

Acknowledgments

We gratefully acknowledge the enthusiastic support this project has received from the students and staff of Broadview Academy, LaFox, Illinois. We are especially indebted to Jean Thiry for the long hours spent in preparing the experimental diet. We thank Irma J. Robinson for her help in preparing the manuscript.
EFFECT OF REDUCED Na INTAKE ON Na METABOLISM

References

Effect of dietary sodium reduction on red blood cell sodium concentration and sodium-lithium countertransport.
R Cooper, M Trevisan, L Van Horn, E Larbi, K Liu, S Nanas, H Ueshima, C Sempos, D Ostrow and J Stamler

Hypertension. 1984;6:731-735
doi: 10.1161/01.HYP.6.5.731

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/6/5/731

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/