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Abstract
Salt sensitivity and insulin resistance are correlated with higher cardiovascular risk. There is no information about changes in salt sensitivity (SS) and insulin sensitivity (IS) after a chronic salt overload in humans. The aim of this study was to evaluate these parameters in the elderly. Seventeen volunteers aged 70.5 ± 5.9 years followed a low-salt diet (LSD) for 1 week and a high-salt diet (HSD) for 13 weeks. We evaluated SS after one week (HSD1) and after 13 weeks (HSD13), and subjects' IS and lipids on their usual diet (UD) at HSD1, and at HSD13. Blood pressure (BP) was measured at each visit and ambulatory blood pressure monitoring (ABPM) was performed twice. SS was the same at HSD1 and HSD13. Systolic BP was lower on LSD than on UD (P = 0.01), HSD1 (P < 0.01) and HSD13 (P < 0.01). When systolic and diastolic BP were evaluated by ABPM, they were higher at HSD13 during the 24-h period (P = 0.03 and P < 0.01) and during the wakefulness period (P = 0.02 and P < 0.01) compared to the UD. Total cholesterol was higher (P = 0.04) at HSD13 than at HSD1. Glucose and homeostasis model assessment (HOMA) were lower at HSD1 (P = 0.02 and P = 0.01) than at HSD13. Concluding, the extension of HSD did not change the SS in an elderly group. The higher IS found at HSD1 did not persist after a longer HSD. A chronic HSD increased BP as assessed by ABPM. 

Key words: Salt sensitivity; Insulin sensitivity; Elderly; Ambulatory blood pressure monitoring; Blood pressure

Introduction
Salt intake is clearly associated with high blood pressure (BP) in epidemiologic studies (1,2). However, individuals are not homogeneous in their BP response to high salt intake. Salt overload increases (salt-sensitive individuals), maintains or sometimes even decreases BP (salt-resistant individuals) (3). It is known that salt sensitivity is correlated with higher cardiovascular risk (4,5) and that it is more prevalent with increasing age (6,7).

The most acceptable test to evaluate salt sensitivity is the oral test, in which the subject is submitted to a short period, usually of 1 week, of a low-salt diet (LSD) followed by the same period on a high-salt diet (HSD). Blood pressure is measured on the 7th day of each period (8). However, there are many differences among protocols regarding the intensity of salt restriction and salt overload. Salt restriction ranges from 9 (9) to 70 mEq (10) of sodium per day, and salt overload from 150 (11) to 345 mEq (10). There are also different classifications of salt sensitivity. Some investigators consider it necessary to have a mean BP increase of 5% (12), others of 10% (13) from LSD to HSD to classify someone as being salt sensitive. Other investigators prefer to consider absolute values, for example, a mean BP increase of 3 mmHg between diets (14). Finally, some investigators classify salt sensitivity in steps, ranging from the lowest to the highest (15).

In addition to all of these differences among protocols, one important question remains unanswered: is one week of salt overload sufficient to evaluate the salt effect? If a longer period of salt overload was provided, would the individual’s classification or the test result be changed?
Salt and insulin sensitivity in the elderly

HSD can change the metabolic status of animals and humans. Some previous studies have shown increased insulin sensitivity when salt overload is performed. Chronic HSD has been associated with higher insulin sensitivity in young (16,17) and old Wistar rats (18). One week of salt overload in healthy humans was also associated with higher insulin sensitivity when compared to salt restriction (19). On the other hand, studies on animals and humans have found lower insulin sensitivity during the use of HSD (20,21). These conflicting results can be attributed to differences in protocols and to associated diseases.

To the best of our knowledge, no previous study has evaluated the metabolic effect of prolonged salt overload in elderly humans.

Therefore, the aims of this study were to compare salt sensitivity measured after 1 week (the standard method) and after a prolonged salt overload in elderly people, and to evaluate the insulin sensitivity and lipid levels of these subjects on their usual diet and after 1 and 13 weeks of salt overload.

Subjects and Methods

The study was performed in accordance with the Declaration of Helsinki (1989) and was approved by the Ethics Committee of the Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil. Written informed consent was obtained from all subjects. Twenty elderly individuals (60 years or older) with no previous use of anti-hypertensive or anti-diabetic drugs, selected from the Geriatric Service of the Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil, were evaluated. Exclusion criteria were: mean blood pressure higher than 160 (systolic) and 100 mmHg (diastolic), evidence of secondary hypertension, the diagnosis of cancer or other states of disability, use of drugs that potentially increase BP (non-steroidal and steroidal anti-inflammatory drugs, tricyclic antidepressants, vasoconstrictor drugs, etc.) and intake of more than 105 g of alcohol per week. Other medications in regular use were maintained until the end of the study. During the protocol, 3 patients dropped out of the study because of epigastric pain with the intake of the salt capsules. The symptoms stopped immediately after interruption. Seventeen patients concluded the protocol. The volunteers were aged 70.5 ± 5.9 years, 10 women, white, 4 with untreated hypertension (3 of them with white coat hypertension), 10 with high-normal BP and 3 normotensives.

Mean basal BP was obtained with an automatic device (Dixtal 2710, Brazil) in the Clinician’s office. Three measurements were obtained at the first visit (Visit 1) and three after 1 week (Visit 2). The diagnosis of hypertension (systolic BP of 140 mmHg or higher and/or diastolic BP of 90 mmHg or higher) and high-normal BP (systolic BP of 120 mmHg or higher and/or diastolic BP of 80 mmHg or higher) was made on the basis of the mean of these six measurements with the subjects on their usual diet. Body weight was measured at the first visit. A blood sample was drawn for the determination of fasting blood glucose (Glicose HK Liquiform, Labtest, Brazil), HDL-cholesterol (HDL Cholesterol monophasic AA plus, Wiener Lab., Argentina), total cholesterol (Colestat, Wiener Lab.), triglycerides (TG Color GPO/PAP AA, Wiener Lab.), and serum insulin (Coat-A-Count Insulin, Siemens, USA). Three 24-h urine specimens were collected for the determination of urinary sodium excretion (UNaV). Twenty-four-hour ambulatory blood pressure monitoring (ABPM) was performed on the non-dominant limb (Spacelabs-90207, USA) (22). The device was programmed to take measurements every 15 min from 7:00 am to 11:00 pm (awake period), and every 20 min from 11:00 pm to 7:00 am (asleep period). Recordings were accepted only if at least 80% of the readings could be successfully obtained. Data from the monitors were downloaded into a computer and rejected if systolic BP was lower than 80 mmHg or higher than 250 mmHg and if diastolic BP was lower than 40 mmHg or higher than 140 mmHg.

After Visit 2, LSD was started. Patients were instructed to use no salt for cooking and to add no salt to cooked or raw food. They were also asked to avoid industrialized food. During this period, volunteers received capsules of placebo to be taken three times a day, two capsules at each time, after breakfast, lunch and dinner. A 24-h urine specimen was collected on the 7th day and volunteers returned to the office to have their BP measured (six measurements at each visit, from Visit 3) and to be weighed. A volunteer was considered to have followed the LSD when the UNaV dropped at least 100 mmol from usual diet UNaV or became lower than 100 mmol/day. Fifteen volunteers reached that goal and only their results were considered to evaluate salt sensitivity at this point in the study. At that time, the volunteers were asked to continue preparing the diet as before but now with 6 g of salt added per day (two packages of 3 g each) and to take 6 capsules of 1 g of salt each following the same regimen employed with the placebo (total increase of 200 mmol sodium/day).

One week later, 24-h urine was collected, a second blood sample was drawn (biochemistry was repeated), and BP and body weight were determined (Visit 4).

High salt intake was maintained until the end of the study. Twenty-four-hour urine was collected every 14 days in order to confirm the maintenance of the high salt intake (Visits 5 to 10). At each visit the number of salt and placebo...
capsules was counted so that the researcher could also check compliance by counting the capsules. At the last visit, after 13 weeks of salt overload, a second ABPM was performed, body weight was measured and a third blood sample was drawn for biochemical tests.

If systolic BP increased at least 10 mmHg from LSD to HSD, patients were considered to be salt sensitive.

The homeostasis model assessment (HOMA) was determined by the following formula: insulin resistance = plasma insulin (pmol/L) x blood glucose (mmol/L) / 135 (23).

Statistical analysis

The results obtained with the patients on their usual diet, LSD, after 1 week of HSD (HSD1), and after 13 weeks of HSD (HSD13) were compared with a unified approach to mixed linear models (24,25) (SAS/STAT®, Version 9, USA: SAS Institute Inc., 2002-2003). The mean BPs obtained by ABPM during the basal period and after 13 weeks of HSD were compared by the Student paired test. The McNemar test was used to compare salt sensitivity after 1 week and after 13 weeks of salt overload (26). Results are reported as means ± SD.

Results

The usual UNaV was 215.24 ± 133.08 mmol/day (N = 17). After 1 week of LSD, UNaV was 70.03 ± 57.97 mmol/day (N = 15; P < 0.01, LSD vs usual, HSD1 and HSD13). After 1 week of salt overload, UNaV was 297.75 ± 112.75 mmol/day (N = 17; P = 0.01 vs usual), and these levels were maintained up to the 13th week (294.71 ± 104.83 mmol/day, N = 17; P = 0.01 vs usual).

Ten of 15 subjects were classified as salt sensitive after 1 week and 13 weeks of salt overload. Three volunteers classified as salt resistant after 1 week of salt overload were classified as salt sensitive after 13 weeks and three volunteers initially classified as salt sensitive were classified as salt resistant after 13 weeks. The group salt sensitivity was the same after one or 13 weeks of salt overload (P > 0.05).

Systolic BP was lower on LSD than on the usual diet (P = 0.01), HSD1 (P < 0.01) and HSD13 (P < 0.01; Figure 1A). There was no difference (P = 0.13) between usual diet systolic BP and HSD13 BP. However, when systolic BP was evaluated by ABPM, it was higher after 13 weeks of salt overload during the 24-h period (P = 0.03) and during the awake period only (P = 0.02) compared with the usual diet (Table 1).

Diastolic BP was lower on LSD than on the usual diet (P = 0.03) and HSD13 (P = 0.01; Figure 1B). There was no difference (P = 0.51) between usual diet diastolic BP and HSD13, though diastolic BP assessed by ABPM was higher during the 24-h period (P < 0.01) and during the awake period (P < 0.01) after chronic overload than on the usual diet (Table 1).

Body weight did not differ among usual diet (66 ± 15

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Figure 1. Systolic (A) and diastolic (B) blood pressures of subjects on different salt diets. Box plots indicating the median (thick line), the mean (filled lozenges), and range of blood pressure of subjects on their usual diet (215.24 ± 133.08 mmol sodium/day), on a low-salt diet (LSD, 70.03 ± 57.97 mmol sodium/day), and after 1 week (HSD1, 297.75 ± 112.75 mmol sodium/day) and 13 weeks (HSD13, 294.71 ± 104.83 mmol sodium/day) on a high-salt diet. The boundaries of the box indicate the lower and upper quartiles. Open lozenges: Outliers with values between 1.5 and 3 box lengths from the boundaries of the box. A: *P = 0.01 vs usual, and P < 0.01 vs HSD1 and HSD13 (mixed linear model test). B: *P = 0.03 vs usual, and P = 0.01 vs HSD13 (mixed linear model test).
kg), LSD (65 ± 17 kg), HSD1 (67 ± 17 kg), and HSD13 (67 ± 15 kg; P > 0.05).

Total cholesterol was higher (P = 0.04) at HSD13 than at HSD1. HDL-cholesterol and triglycerides were similar on all diets (Table 2).

Glucose and HOMA were lower (P = 0.02 and P = 0.01, respectively) at HSD1 than at HSD13 (Table 2). There was no difference in insulin sensitivity (P = 0.3) between usual diet and HSD13.

Discussion

It is known that changes in salt intake can modify BP

Table 1. Ambulatory 24-h blood pressure monitoring during the awake and asleep periods of elderly subjects on their usual diet and after a long-term (13 weeks) high-salt diet.

<table>
<thead>
<tr>
<th>Period</th>
<th>Usual diet</th>
<th>High-salt diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>120 ± 12</td>
<td>127 ± 11*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>70 ± 8</td>
<td>74 ± 7*</td>
</tr>
<tr>
<td>Awake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>121 ± 13</td>
<td>128 ± 11*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>71 ± 8</td>
<td>75 ± 7*</td>
</tr>
<tr>
<td>Asleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>116 ± 12</td>
<td>123 ± 12</td>
</tr>
<tr>
<td>Diastolic</td>
<td>65 ± 10</td>
<td>70 ± 9</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD for N = 17 subjects. Usual diet: 215.24 ± 133.08 mmol sodium/day; high-salt diet: 294.71 ± 104.83 mmol sodium/day. Blood pressure was measured by 24-h ambulatory blood pressure monitoring. *P < 0.05 compared to usual diet (Student paired test).

Table 2. Comparison of metabolic factors for subjects on different diets.

<table>
<thead>
<tr>
<th></th>
<th>Usual diet</th>
<th>High-salt diet for 1 week</th>
<th>High-salt diet for 13 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Chol (mmol/L)</td>
<td>5.4 ± 0.8</td>
<td>5.3 ± 1.0</td>
<td>5.6 ± 0.8*</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.4 ± 0.8</td>
<td>5.1 ± 0.4</td>
<td>5.6 ± 0.9*</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>69.5 ± 41.7</td>
<td>55.6 ± 34.7</td>
<td>62.5 ± 34.7</td>
</tr>
<tr>
<td>HOMA (pmol/L mmol/L)</td>
<td>2.72 ± 1.73</td>
<td>1.87 ± 1.27</td>
<td>3.06 ± 1.89*</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD for N = 17 subjects. T-Chol = total cholesterol; HDL = high-density lipoprotein; HOMA = homeostasis model assessment. Usual diet: 215.24 ± 133.08 mmol sodium/day; high-salt diet: 297.75 ± 112.75 mmol sodium/day (after 1 week) and 294.71 ± 104.83 mmol sodium/day (after 13 weeks). *P < 0.05 compared to high-salt diet for 1 week (mixed linear models).
a normal-salt diet (0.5% sodium), of LSD (0.06% sodium) or HSD (3.12% sodium), found that insulin sensitivity was higher in rats on HSD than on a normal-salt diet, with higher insulin-regulated glucose transporter (GLUT4) expression, enhanced insulin signaling, and GLUT4 translocation.

Severe salt restriction (20 to 60 mmol sodium/day) has been associated with higher cholesterol levels (37,38); however, moderate salt restriction of 85 mmol sodium/day does not modify lipid concentration (37). The present study showed a modest increase of cholesterol levels after prolonged high salt intake. A salt- rich diet for 4 or 5 weeks also increased cholesterol levels in lean and obese Zucker rats (39) but the mechanism responsible for this effect has not been elucidated.

As the extension of salt overload period after one week of low-salt diet did not change the salt sensitivity in this group, we can determine it using the traditional shorter method. Since there was no higher insulin sensitivity after a prolonged salt overload, which was previously shown in animal models, and higher BP and cholesterol levels were detected, the results point to a potential harmful effect of an increase in sodium intake, even though the usual diet already had a high salt content. The findings of the present study may not be applicable to young or middle-aged individuals, since these groups were not submitted to prolonged high-salt diet before and the numerous differences between groups, with higher salt sensitivity and lower insulin sensitivity being characteristic of the elderly.

Acknowledgments

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References

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