Blood Pressure and Hormonal Responses to Short Whole Body Cold Exposure in Subjects with High Dietary Salt Intake

Olli Arjamaa1), Lauri Turunen1), Tero Mäkinen2), Jaana Laitinen2), Juhani Leppäläluoto3), Olli Vuolteenaho3) and Hannu Rintamäki2)

1) Institute of Arctic Medicine, University of Oulu
2) Oulu Regional Institute of Occupational Health
3) Department of Physiology, University of Oulu

Abstract. The objective of the present study was to test a hypothesis that a high dietary salt intake potentiates a cold induced increase in blood pressure in normotensive men. Male subjects (n=12) were given 7 g day⁻¹ sodium chloride during the cold months of the year, divided in 3–4 doses per day and dissolved in water, for 14 days additional to their normal diet which contained on the average 9.7 g sodium chloride per day. The same subjects, having their normal diet, served as controls. The resting blood pressure was measured on the fourteenth day seven times at the intervals of five minutes in a climatic chamber in thermoneutral conditions. Then the subjects, wearing a three-layer winter clothing, moved into a wind tunnel (−15 °C, air velocity 3.5 ms⁻¹) in which they stayed for fifteen minutes and the blood pressure was recorded at the intervals of three minutes. After the cold exposure, the subjects moved back into the climatic chamber for 30 min and the blood pressure was measured as before the cold exposure. Blood samples were drawn before and after the experiment for ion and hormone measurements. A 12 h urine sample was collected just prior to the cold exposure. A significant difference both in systolic (7 mmHg) and in diastolic (7 mmHg) blood pressure was found between a salt load group and control group under thermoneutral conditions, repeatedly measured over 30 min (paired Student’s t-test; p<0.05). During the whole body cold exposure, blood pressure significantly increased both with and without the extra salt load (repeated measures ANOVA, Student-Newman-Keuls; p<0.05). The level to which the mean arterial pressure increased during the exposure was independent of the salt intake and the profile of the mean arterial pressure curve was similar in both groups. The systolic pressure increased by 25 mmHg during the cold exposure. The increase in the diastolic pressure was significantly (paired Student’s t-test, p<0.05) higher in the high salt group (18 – 4 mmHg) than in the control group (12 – 3 mmHg) thus supporting partly our hypothesis. After the two-week high salt intake, serum Na⁺, K⁺, Cl⁻, Hct, and plasma Hb were at the similar level as before the extra salt intake. Plasma renin activity, NT-proANP, ANP, and serum aldosterone were not different between the groups, both before and after the cold exposure. The main findings are: 1) the mean arterial pressure increases to the same level and in the same manner independent of the salt load during a short whole body cold exposure and 2) in cold the diastolic blood pressure increases significantly more in people under a very high salt diet.

Keywords: whole body cold exposure, high salt diet, blood pressure, natriuretic hormones

Introduction

A large number of studies have been published on the effects of dietary salt intake on the resting blood pressure in humans. High salt intake appears to be associated with increased blood pressure with elevated risk of developing hypertension (for review, see Muntzel and Drüeke, 1992). Some subjects are, however, salt resistant which may have been a confounding factor in some epidemiological studies (Weinberger, 1996). Recently, a large population based study showed no relation of sodium intake to subsequent all-cause and cardiovascular-disease mortality in a general population (Alderman et al., 1998).

Low ambient temperature is a well-known environmental factor that increases blood pressure in man but its effects have not been studied as extensively as those of dietary salt intake. A previous report showed that sodium loading (10 g NaCl day⁻¹, additional to normal diet, for two weeks) significantly raised the diastolic blood pressure in the cold pressor test while there was no change in the systolic blood pressure (Ditto et al., 1993). In experimental studies, a cold pressor test simulating the effects of an acute cold stress on blood pressure has
been used in an attempt to identify normotensive subjects who might develop hypertension later in their life (Falkner et al., 1981; Dlin et al., 1983; Tanji et al., 1989). The results regarding the predictive value are, however, contradictory (Barnett et al., 1963; Harlan et al., 1964; Eich and Jacobsen 1967; Wood et al., 1984; Thomas et al., 1985). There are recent results showing that the hyperreactivity found in the cold pressor test during early life will later lead to hypertension (Kasagi et al., 1995; Loycke, 1995).

We attempted to test a hypothesis that increased dietary salt intake will affect the blood pressure response to an acute whole body cold exposure. We also followed plasma renin activity, aldosterone, ANP (atrial natriuretic peptide), and NT-proANP in order to characterize their roles in our experiments. A whole body cold exposure of subjects wearing a three-layer winter clothing was produced in a wind tunnel (\(-15\) C, wind 3.5 ms\(^{-1}\)) during a normal and a high salt diet.

**Methods**

**Subjects**

Twelve healthy, non-smoking males volunteered for the study. They were normotensive, the age ranged from 22 to 57 years (33 ± 11, mean ± SEM), weighing 76 – 13 kg, 177 – 7 cm in length, and whose body mass index was 24.0 – 3.3. The experimental protocol was explained to them and written consent was obtained from each subject before the study. The subjects were accustomed to measurements and experimental conditions and then they underwent a protocol before the study started.

**Protocol**

The study was performed in Oulu, Finland (65° northern latitude) during January to March. To avoid the effect of habituation to the measurement protocol, half (n=6) of the subjects were first tested without a salt load (group A) and the remaining half with a salt load (group B). After the cold exposure, there was a three-week wash-out period and then group A was tested with a salt load and group B without a salt load. Subjects without a salt load had their normal diet. The additional salt load consisted of 7 g NaCl day\(^{-1}\) in a form of crystalline table salt and it was divided in 3–4 doses and each dose was dissolved in a glass of water and taken for 14 days before the cold exposure. All the subjects kept a dietary record book for three days before the cold exposure. A 12-h urine was collected on the day before the cold exposure to determine the ion excretion.

Subjects were weighed and venous blood samples were drawn. Devices and electrodes which measured the blood pressure (ABPM- Meditech, Meditech KFT, Hungary), the temperature of the skin of forehead, chest, shoulder, brachium, back of the hand, anterior femur and calf, and rectal temperature (YSI 400-series thermistors, Yellow Springs Instruments, USA; data stored with Squirrel Datalogger 1200-series, Grant, UK) at 1–5 min intervals were attached. The mean skin temperature was calculated as an area-weighed average. The heart rate was also measured and stored automatically at 1 min intervals (Polar Sport Tester, Polar Electro, Finland).

The subjects wearing three-layer winter clothing were allowed to stabilize for 30 min at 18 C sitting in a chair in a climatic chamber. Clothing: shorts, t-shirt, trousers, long-sleeved shirt, Finnish military winter trousers and winter jacket, rubber boots with wool felt-linings, fibre pile inner gloves, nylon outer gloves, hat with earflaps, and scarf. The blood pressure was measured every 5 min and skin temperatures every 60 s. Then the subjects moved into a wind tunnel (\(-15\) C, wind speed 3.5 ms\(^{-1}\)) in which they remained in a sitting position, facing the wind and faces unprotected, for 15 min. In the cold, the heart rate was recorded every 5 s and the blood pressure was measured every 3 min. After the cold exposure, the subjects stayed an additional 30 min in the climatic chamber and in the same conditions as before the exposure. Recordings were made as during the first stabilization period. The subjects were asked to report their thermal sensations (ISO 10551 1995) before, during, and after the exposure to cold on a five point subjective scale. Another venous blood sample for hormonal measurements was drawn 40 min after the subjects had come out of the wind tunnel in order not to disturb the blood pressure recordings.

**Analyses**

Dietary recordings were analysed by Micro-Nutrica program (v. 2.0, 1993). Blood samples were collected both to 10 ml serum vacuum tubes (first kept at room temperature for 30 min) and to 3 ml EDTA tubes which were centrifuged and stored at - 80 C until assayed as were the urine samples. Hematocrit, hemoglobin, the number of red and white cells, and red cell indexes were immediately analysed with an automatic cell counter (Coulter T-540-series, Coulter Electronics LTD, UK). Na\(^+\), K\(^+\), and Cl\(^-\) of blood and urine samples were measured by the direct ion selective method (Microlyte 3+2 Ion Selective Analyzer, Kone Instruments, Finland).

**Radioimmunoassays**

ANP (atrial natriuretic peptide) was extracted from plasma using SepPak C\(_{18}\) cartridges (Vuolteenaho et al., 1992). NT-proANP (N-terminal fragment of proANP) was assayed directly from unextracted plasma. The radioimmunoassay protocols have been described previously for ANP (Vuolteenaho et al., 1985) and NT-proANP (Vuolteenaho et al., 1992). The sensitivities of the ANP and NT-proANP were 1.0 and 40 pmol/l plasma, respectively. The within and between assay coefficients of variation in each assay were <10% and <15%, respectively. Both of assays were specific for the...
particulate peptide. The assays, however, cross-reacted fully with proANP. With these methods, the following plasma levels (mean – SD) were detected in healthy adults aged 20–55 years: ANP 10.9 – 4.0 pmol l⁻¹ and NT-proANP 227 – 84 pmol l⁻¹.

Aldosterone (Oris Industrie) and renin activity (Pharmacia) measurements were performed using clinical radioimmunoassay kits according to the instructions provided by the manufacturers.

Statistics
Results are shown as means – SEM. Paired Student’s t-test or repeated measures ANOVA with Student-Newman-Keuls multicomparison test was used in the analyses of the results.

Results
According to the diet diary, the normal daily salt intake was 9.7 g day⁻¹ in the subjects before starting any experiments. When 7 g extra salt was added in the daily diet, divided in three doses and taken with water, the urinary sodium 12 h⁻¹ and the urinary volume 12 h⁻¹ were significantly (paired Student’s t-test; * = p<0.05) increased as shown in Table 1. The mean energy consumption was 2463 kcal day⁻¹ in the controls and 2495 kcal day⁻¹ during cold exposure as shown in Table 1. The mean energy consumption was significantly (paired Student’s t-test; * = p<0.05) increased as shown in Table 1.

Although the subject wore a three-layer winter clothing during the cold exposure all subjects experienced severe cold sensations during a stay in the wind tunnel as judged from the records of thermal sensations. The mean skin temperature was 33 C in both groups before the cold exposure and decreased significantly during the cold exposure (Fig. 1). During the salt load, the mean skin temperature decreased to 26 C while in the controls, the lowest temperature was 27 C as shown in Fig. 1. The difference was, however, statistically non-significant (paired Student’s t-test; p>0.05). 30 min after the cold exposure the mean skin temperature had slowly increased to 31 C in both groups when the recordings were stopped (Fig. 1).

All the subjects were normotensive and the resting systolic blood pressure was 124 – 3 mmHg and the diastolic 81 – 3 mmHg. After the salt load, a significant decrease of 7 mmHg in systolic blood pressure compared to the control group (paired Student’s t-test; * = p<0.05) was recorded just before the cold exposure under undisturbed thermoneutral conditions (Fig. 2). The difference in diastolic blood pressure between the control and high salt group, 7 mmHg, before the cold exposure was also statistically significant (paired Student’s t-test; * = p<0.05, Fig 2). There were, however, some interindividual variation in the response, as in 4 subjects there were either no response or the blood pressure increased slightly. Both in the control and high salt groups, the cold exposure increased the systolic and diastolic blood pressure (Fig. 2). However, the level to which blood pressures rose was similar in both groups and this finding is more clearly noted in Fig. 3 in which the mean arterial pressure (MAP) is shown. With respect to changes in the systolic pressure by cold exposure (about 25 mmHg) and by post-exposure no significant (paired Student’s t-test, p>0.05) differences were, however, found between the groups (Fig. 4A). The diastolic blood pressure increase during the cold exposure was 12 – 3 mmHg in the control group while in the high salt group the change was significantly larger: 18 – 4 mmHg (Fig. 4B, paired Student’s t-test; * = p<0.05).

When the cold exposure was over the MAP remained at a significantly (repeated measures ANOVA, Student-

### Table 1 Urinary Na and volume after a normal and a two-week high dietary salt intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urinary Na mmol/12 h</th>
<th>Urinary volume ml/12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97 – 12</td>
<td>522 – 61</td>
</tr>
<tr>
<td>High salt intake</td>
<td>141 – 41*</td>
<td>804 – 110*</td>
</tr>
</tbody>
</table>

Means and SEM are given (n=12), paired Student’s t-test, * = p<0.05.

### Table 2 Serum Na⁺, K⁺, Cl⁻, Hct, and plasma Hb before and after a whole body cold exposure during a normal and a two-week high dietary salt intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na⁺ mmol l⁻¹</th>
<th>K⁺ mmol l⁻¹</th>
<th>Cl⁻ mmol l⁻¹</th>
<th>Hct ratio</th>
<th>Hb g l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Before cold</td>
<td>135 – 1</td>
<td>4.1 – 0.1</td>
<td>100 – 1</td>
<td>0.44 – 0.7</td>
<td>154 – 2</td>
</tr>
<tr>
<td>After cold</td>
<td>135 – 1</td>
<td>4.3 – 0.1</td>
<td>101 – 1</td>
<td>0.45 – 0.7</td>
<td>157 – 2</td>
</tr>
<tr>
<td>High salt intake</td>
<td>135 – 1</td>
<td>4.0 – 0.1</td>
<td>101 – 1</td>
<td>0.44 – 0.6</td>
<td>155 – 3</td>
</tr>
<tr>
<td>Before cold</td>
<td>136 – 1</td>
<td>4.4 – 0.1</td>
<td>102 – 1</td>
<td>0.45 – 0.6</td>
<td>157 – 3</td>
</tr>
</tbody>
</table>

Means and SEM are given (n=12), paired Student’s t-test, p>0.05.
Mean skin temperature, calculated as an area-weighted average, of twelve healthy male subjects during a whole body cold exposure under a normal (Control) and a high-salt diet (NaCl). The difference at 47 min is statistically non-significant (paired Student's $t$-test, $p>0.05$).

Blood pressure of twelve healthy male subjects during a whole body cold exposure under a normal (Control) and a high-salt diet (NaCl). The difference in systolic and diastolic blood pressures between the groups before the cold exposure are statistically significant (paired Student's $t$-test; $* = p<0.05$).

Mean arterial pressure of twelve healthy male subjects during a whole body cold exposure under a normal (Control) and a high-salt diet (NaCl). Difference between post-exposure and pre-exposure within the two groups are statistically significant (repeated measures ANOVA, Student-Newman-Keuls; $* = p<0.05$).

Blood pressure changes of twelve healthy male subjects during a whole body cold exposure under a normal (Control) and a high-salt diet (NaCl). Panel A: Change in systolic blood pressure during cold exposure (15 min, -15°C, wind 3.5 m/s) and post-exposure (30 min, +18°C). Panel B: Change in diastolic blood pressure during cold exposure and post-exposure (paired Student's $t$-test, $* = p<0.05$).
Newman-Keuls; * = p<0.05) higher level than before the exposure in both groups respectively (Fig. 3). The heart rate did not change during the salt load and the responses in cold and after the exposure were similar in both groups as shown in Fig. 5. The peaks in the heart rate noted in Fig. 5 are partly due to motor activity when the subjects moved from the climatic chamber into the wind tunnel and out of it.

As shown in Table 3, the plasma renin activity, ANP, NT-proANP, and serum aldosterone were not different (paired Student’s t-test; p>0.05) between the control and high salt intake subjects, both before and after the cold exposure. The urine aldosterone (ug 12 h⁻¹) collected prior the cold exposure was 4328 – 740 nmol l⁻¹ in the normal diet group and 3377 – 1023 nmol l⁻¹ in the high salt group (paired Student’s t-test, p>0.05).

**Table 3** Plasma renin activity, ANP, NT-proANP, and serum aldosterone before and after a whole body cold exposure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Renin activity ug l⁻¹ h⁻¹</th>
<th>Aldosterone nmol l⁻¹</th>
<th>ANP pmol l⁻¹</th>
<th>NT-proANP pmol l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before cold</td>
<td>1.5 – 0.3</td>
<td>32 – 13</td>
<td>11 – 3</td>
<td>91 – 26</td>
</tr>
<tr>
<td>After cold</td>
<td>1.9 – 0.8</td>
<td>15 – 6</td>
<td>9 – 2</td>
<td>83 – 24</td>
</tr>
<tr>
<td>High salt intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before cold</td>
<td>1.0 – 0.2</td>
<td>10 – 2</td>
<td>11 – 3</td>
<td>113 – 33</td>
</tr>
<tr>
<td>After cold</td>
<td>1.0 – 0.1</td>
<td>25 – 10</td>
<td>9 – 2</td>
<td>83 – 24</td>
</tr>
</tbody>
</table>

Control: normal diet; High salt intake: two-week high dietary salt intake. Means and SEM are given (n=11), paired Student’s t-test, p>0.05.

**Discussion**

This study sought to determine the combined effects of high dietary salt intake and acute cold exposure on blood pressure in normal healthy subjects. It is well known that a cold exposure increases blood pressure and that the effect is directly related to the duration and the amplitude of the exposure. It is also known that high dietary salt intake will lead to elevated blood pressure, at least in salt sensitive subjects (Muntzel and Drüke, 1992). In the present study, calculated from a dietary record book and analyzed with Micro-Nutrice software, the mean salt intake in the normal daily diet of the subjects was 9.7 g day⁻¹ which corresponds to the average salt intake in Finland. Judged from a urine sample, a total volume collected for 12 h before the cold exposure, the salt load increased significantly the sodium and water excretion of the subjects. The serum levels of Na⁺, K⁺ and Cl⁻ ions were, however, similar in both groups.

We chose a whole body cold exposure, short and rigorous, as to a stimulus which simulated the true conditions experienced during outdoor activities in northern Finland in winter. Other cold tests, like the cold pressor test which is widely used describe the responses of a restricted body surface area to cold which is clearly inferior to the whole body cold exposure.

Both the systolic and the diastolic blood pressure were significantly lower in the high salt group than in the control group before the cold exposure, measured several times in conditions which were thermoneutral and undisturbed (Fig. 2). As the mean arterial pressure (MAP) decreased during high salt intake the subjects in these experiments were so called counterregulators (Overlack et al., 1993; Ruppert et al., 1993; Overlack et al., 1995) although the intervention in our study was from a normal to a high salt diet. The mechanisms behind the counterregulation are not known but the divergent blood pressure responses to varying salt intake may depend on other hemodynamic and hormonal factors (Overlack et al., 1993; Overlack et al., 1995; Luft and Weinberger 1997). It is worth of noting that...
when Luft et al. (1979) subjected normotensive black and white male volunteers to a wide range of sodium intakes for two weeks (stepwise increase to 1500 mmol day⁻¹, compared to 294 mmol day⁻¹ in our study) several subjects experienced blood pressure decrease during salt loading. In summary, salt loading in normotensive subjects results in various pressure levels depending on the protocol, duration and amount of the loading and the mechanisms behind these diverse findings have multiple explanations (Muntzel and Drüeke, 1992).

When the both groups were exposed to -15 C in a wind tunnel (3.5 ms⁻¹) the MAP responded immediately with an increase in all subjects. The increase was rapid and the MAP reached the same level in both groups during the cold exposure. The starting level was lower in the high salt group but the steepness of the change and the level to which the MAP increased were similar in both groups. It appeared as if the response of vasculature was similar and regulated by other factors than NaCl loading. It is known that cutaneous vasoconstriction increases blood pressure in cold without an equivalent increase in cardiac output (Epstein et al., 1969; Raven et al., 1970). The autonomous nervous system may modulate this pressure response as an acute cooling augments alpha₂- but not alpha₁- adrenergic contractile responses (Flavahan et al., 1985) while beta receptors have been shown to play a minor role in a cold-induced vasoconstriction (Reed et al., 1991). In our study, it appeared as if the salt load did not change the responsiveness of the peripheral vessel wall to cold in normotensive subjects.

Ditto et al. (1993) made cold pressor tests after 2 weeks of dietary sodium loading (an extra 10 g day⁻¹) and after 2 weeks of maintaining a normal diet in 18 healthy normotensive caucasians. The extra sodium diet did not have any overall effect on resting cardiovascular activity but the diastolic blood pressure reactivity increased in the cold pressor test. However, the amount of NaCl in their normal diet was not reported. During the cold exposure in our study, the systolic increase was about 25 mmHg in both groups (Fig. 4A) while in the diastolic pressure (4B), the change was significantly higher in the high salt group than in the control (18 vs. 12 mmHg, see also Fig. 3) suggesting an increased diastolic blood pressure reactivity during the cold exposure.

When the cold exposure was over and the subjects had moved to the climatic chamber out of the wind tunnel the blood pressure remained at a significant higher level in both groups than before the cold exposure (Fig. 3). When the recordings were ceased 40 min after the cold exposure and the blood pressure had returned about to the pre-exposure level the plasma ANP and NT-proANP were also at the same level as before the cold exposure (Table 3). There are two possible explanations for the findings. The first one would be that the plasma ANP is not directly associated with sodium intake but rather is regulated by the actual pressure changes. Plasma NT-proANP that has a longer half-life in plasma than ANP did not either change in response to cold exposure during different salt diets giving further support to the first explanation proposed. The second possibility would be that the plasma ANP responds to a salt overload at the early stages of adaptation and some other mechanisms or substances than ANP become later more important in the control of salt balance. As to renin and aldosterone, they might not be involved, since we did not observe any changes in their plasma levels during our experiments. It should also be noted that an earlier study from our laboratory showed that plasma ANP increased slightly in response to a whole body cold exposure as late as 2 h (Hassi et al., 1991).

Taken together, these findings show that a heavy salt load, an additional 7 g day⁻¹ for two weeks to the normal dietary salt intake of 9.7 g day⁻¹ in healthy male subjects who were normotensive did not change the blood pressure response during a whole body cold exposure. The level to which MAP reached in cold and the profile of the MAP curve were similar independent of the salt load. The extra salt load caused under our experimental protocol a counterregulation response in which the mean arterial blood pressure, measured before cold exposure, was significantly lower compared with the situation when the subjects were on their normal diet. During an acute whole body cold exposure, the increase in the diastolic blood pressure was significantly higher during the salt load than during the normal diet. Mechanisms and mediating substances behind these phenomena, other than renin, aldosterone, ANP, and NT-proANP, remain to be studied.

References


Arjamaa, O et al. 209


Harlan WR Jr, Osborne RK, Graybiel CA (1964) Prognostic value of the cold pressor test and the basal blood pressure based on an eighteen-year follow-up study. Am J Cardiol 13: 683-687


Muntzel M, Drüeke T (1992) A comprehensive review of the salt and blood pressure relationship. AJH 5: 15-42S


Overlack A, Ruppert M, Kolloch R, Kraft K, Stumpe KO (1995) Age is a major determinant of the divergent blood pressure responses to varying salt intake in essential hypertension. AJH 8: 829-836


Received: June 18, 1999
Accepted: August 19, 1999
Correspondence to: Olli Arjamaa, Institute of Arctic Medicine, University of Oulu, P. BOX 5000, 90401 Oulu, Finland

E-mail: olli.arjamaa@oulu.fi