Association of Haptoglobin with Sodium Sensitivity and Resistance of Blood Pressure

MYRON H. WEINBERGER, JUDY Z. MILLER, NAOMI S. FINEBERG, FRIEDRICH C. LUFT, CLARENCE E. GRIM, AND JOE C. CHRISTIAN

SUMMARY Sodium sensitivity and resistance of blood pressure were examined in 117 normotensive and 85 hypertensive subjects by means of a protocol using rapid extracellular fluid volume expansion with intravenously administered saline (2 L over 4 hours) followed by a day of low dietary sodium intake (10 mEq) and volume contraction induced by a diuretic (furosemide, 120 mg orally). Genetic markers were also examined. Both hypertensive and normotensive subjects with haptoglobin 1-1 phenotype were significantly more (p<0.05) likely to be sodium-sensitive than were those with 2-1 or 2-2 phenotypes, and subjects with 2-2 phenotypes were more apt to be sodium-resistant. A second population was examined in which both adults and children with haptoglobin 1-1 phenotype were found to have significantly (p<0.05) higher casual systolic and diastolic blood pressures. These two studies independently confirm a relationship between haptoglobin phenotypes and blood pressure and suggest an environmental factor (sodium) as well. (Hypertension 10: 445-446, 1987)

KEY WORDS • blood pressure • sodium sensitivity • sodium resistance • haptoglobin • hypertension

THE pathogenesis of human hypertension is complex and multifactorial. Substantial direct and indirect evidence implicates excessive dietary sodium intake as a factor in the development or maintenance of elevated blood pressure in some but not all persons.1-6 The incidence of hypertension is greatest in societies in which sodium intake is greater than 50 to 100 mEq/day and is virtually nonexistent in populations consuming less than 50 mEq/day.1-6 Furthermore, the age-related increase in blood pressure commonly observed in acculturated societies has also been linked to sodium intake. Reduction of dietary sodium intake or diuretic-enhanced excretion of sodium and water frequently, but not invariably, lowers blood pressure in hypertensive subjects and in some normotensive subjects.1, 7

The heterogeneity of blood pressure responsiveness to manipulation of sodium and extracellular fluid balance in hypertensive subjects has engendered the concept of sodium sensitivity and resistance of blood pressure.8 This variability of blood pressure responsiveness has recently been shown to occur in normotensive as well as hypertensive subjects.1, 9 In addition, normotensive subjects who are known to be at increased risk for the subsequent development of hypertension, such as older persons, blacks, or first-degree relatives of hypertensive subjects, have been shown to differ from other normal subjects in their ability to excrete a sodium load.10 One explanation for this variability in response to sodium could be the result of a gene-environment interaction. To explore this possibility, we examined the distribution of common genetic markers in normal and hypertensive subjects categorized with respect to sodium sensitivity or resistance of blood pressure.

Subjects and Methods
The studies were approved by the Human Use Committee of the Indiana University School of Medicine, and informed consent was obtained from all volunteer subjects after explanation of the procedures. The inpatient study population included 117 normotensive subjects and 85 with essential hypertension. The latter had...
received no antihypertensive drugs for at least 2 weeks before the study. The studies were conducted at the Clinical Research Center and employed a protocol (described previously\textsuperscript{5,11}) designed to accomplish rapid expansion of extracellular fluid volume and subsequent sodium and volume contraction.

After 1 day of acclimation to the Clinical Research Center, during which time subjects received a diet of 150 mEq of sodium and 70 mEq of potassium and a 24-hour urine sample was collected, blood pressure and blood samples were obtained at 0800 and a 4-hour urine collection was begun. Volume expansion was accomplished by intravenous infusion of 2 L of normal (0.9%) saline over a 4-hour period. At 1200, blood pressure was measured, blood sampled, the urine collection ended, and another was begun until bedtime. An additional urine collection encompassing the sleep period until 0800 completed the 24-hour collection. Day 2 was the volume expansion period during which subjects received 308 mEq of sodium. On Day 3, sodium and volume contraction were accomplished by ingestion of a diet containing 10 mEq of sodium, distilled water, 25 ml/kg, and furosemide, 40 mg given orally at 1000, 1400, and 1800. Blood pressure and blood samples were obtained after 2 hours of ambulation at 0800 on the following morning. Blood pressure was measured by certified observers, and the mean of two measurements at each time point was used for analysis. In addition to serum sodium, potassium, and creatinine determinations, made by autoanalyzer and flame photometric techniques, plasma renin activity and aldosterone concentrations were measured by radioimmunoassay techniques.\textsuperscript{11} Urinary excretion of sodium, potassium, and creatinine also was measured. Genetic markers were originally measured by the Department of Medical Genetics to examine associations with secondary forms of hypertension\textsuperscript{12} and included haptoglobin (Hp) phenotypes, ABO Rh, secretor, MNS, Kell, Duffy, Kidd, P, parotid double band protein, phosphoglucomutase-1 and acid phosphatase in a group of 202 normotensive and essential hypertensive subjects.

Blood pressure sensitivity or resistance to sodium was defined on the basis of the change in mean arterial pressure (MAP) after sodium and volume depletion compared with that observed after sodium loading, as previously described.\textsuperscript{3} Those subjects demonstrating a decrease in MAP of 10 mm Hg or more were considered sodium-sensitive, while those in whom MAP decreased less than 5 mm Hg, including subjects in whom a rise in MAP was observed after sodium and volume depletion, were defined as sodium-resistant. Changes between 5 and 10 mm Hg were classified as indeterminate with respect to response to sodium. For continuous variables, comparisons among the three phenotypes were made using analysis of variance with Duncan's multiple range test to identify significant group differences. Sodium sensitivity and resistance in the homozygous phenotypes were analyzed by a 2 × 2 contingency table and Fisher's exact test.

Genetic markers were also obtained in a larger outpatient study of cardiovascular risk factors.\textsuperscript{13} Sitting blood pressures were obtained in 738 persons (adults and children) during a 4-hour clinic visit that included medical, psychological, and anthropometric examinations. In the present analysis, individual blood pressures were used, since this larger sample size included many partial family structures. The purpose was to include the largest sample possible in order to detect differences in blood pressure in the phenotypic groups. Analyses of variance were performed as described for the inpatient study.

Results

Demographic information regarding the subjects studied at the Clinical Research Center and characterized on the basis of their responsiveness to sodium is presented in Table 1. Sodium sensitivity was observed in 26% of normotensive subjects and 51% of hypertensive subjects, while resistance was seen in 58 and 33%, respectively.\textsuperscript{3} The sodium-sensitive subjects of both groups were significantly (p < 0.05) older than those resistant to the effects of sodium on blood pressure. We have previously reported that sodium-sensitive subjects of both groups have significantly (p < 0.05) lower levels of plasma renin activity than do the sodium-resistant group but that renin levels alone are not an accurate predictor of sensitivity or resistance.\textsuperscript{3}

The results of haptoglobin phenotype characterization are shown in Table 2. When all subjects, normotensive and hypertensive of both races, were combined, a significant (p < 0.05) difference in phenotype

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<th>Table 1. Demographic Characteristics of the Subjects</th>
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Age values are means ± SEM. Race and sex values are numbers of subjects.

<table>
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<th>Table 2. Haptoglobin Phenotype Measurements</th>
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<td>Number of subjects</td>
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distribution was observed among sensitive and resistant subjects (Figure 1). Those with Hp1-1 were more likely to be sodium-sensitive, while those with Hp2-2 were more apt to be resistant. Calculating an odds ratio on the data from Table 2 in hypertensive and normotensive subjects, we estimated that Hp1-1 subjects are 2.5 times more likely to be sodium-sensitive ($\chi^2 = 4.29, p<0.05$).

The heterozygotic phenotype Hp2-1 was composed of similar numbers of sensitive and resistant subjects. The mean ages of subjects in each of the phenotypic groups were not significantly different. When the subjects in each of the phenotypic groups were compared for blood pressure responsiveness, a significant ($p<0.05$) difference was seen. The average decrease in MAP after sodium and volume contraction was $10.8 \pm 2.6$ (SE) in Hp1-1, $8.6 \pm 1.3$ in Hp2-1, and $5.2 \pm 1.2$ mm Hg in Hp2-2. Within the sodium-sensitive group of subjects, the mean age of each phenotypic group was found to be significantly ($p<0.05$) different (Hp1-1, $33.8 \pm 3.7$; Hp2-1, $41.3 \pm 2.8$; Hp2-2, $46.6 \pm 3.5$ years). This finding suggests that sodium sensitivity is manifested earlier in those with Hp1-1 phenotype than in the other groups. No such age differences were observed among the sodium-resistant phenotypes (Hp1-1, $27.8 \pm 3.6$; Hp2-1, $30.0 \pm 2.7$; Hp2-2, $25.6 \pm 1.9$ years).

Table 3 presents the results of outpatient blood pressures in the haptoglobin phenotypic groups among the families of identical twins. These results suggest that persons with the Hp1-1 phenotype have significantly higher blood pressure, a result that is not explained by differences in body size or age between the groups. This result was seen in both adults (>18 years of age) and children (age range, 4–18 years).

**Discussion**

A substantial body of direct and indirect evidence exists to link sodium intake and blood pressure. Despite this evidence, considerable controversy about the value of modification of dietary sodium intake in the prevention or treatment of hypertension continues. The skeptics point out the inconsistency of blood pressure responses to sodium restriction among hypertensive persons as an argument against the sodium hypothesis. In addition, they emphasize that the normotensive majority does not appear to suffer adverse consequences of a long period of high dietary sodium intake. Only recently has the heterogeneity of blood pressure responsiveness to manipulation of sodium intake been substantiated and confirmed.13,37 We now accept the fact that not all humans respond to a sodium load with similar changes in blood pressure.

Some studies have suggested that these differences in blood pressure responsiveness to sodium may be due to alterations in renal function, modulation of aldosterone production by angiotensin II, aging, or racial, familial, environmental, or genetic factors.14-16 Complex interactions probably occur between blood pressure control systems that influence sodium sensitivity and resistance. To our knowledge, the present observations are the first to identify a genetic marker associated with sodium sensitivity of blood pressure. Such an observation, if confirmed, has broad implications for the treatment and primary prevention of hypertension.

We found that there was a greater likelihood of sodium sensitivity among Hp1-1 subjects and of sodium resistance among Hp2-2 subjects and that the heterozygotic group was equally composed of both response types. The differences in responsiveness to sodium were not related to age, since no overall age bias was observed among the three phenotypic groups. Additional confirmation of the validity of these observations can be seen in the blood pressure responses of the three phenotypic groups. The decrease in MAP was greatest in those homozygous Hp1-1 subjects, with the heterozygotic group being intermediate and the Hp2-2 subjects less responsive. Among the sodium-sensitive subjects, those with the Hp1-1 phenotype were significantly younger than subjects in the other two groups.
suggested that increased blood pressure is more apt to occur at a younger age in Hp1-1 subjects than in those with the Hp2-1 or Hp2-2 phenotype. No such age-related differences were observed among the sodium-resistant subjects. Although this observation does not exclude the possibility of sodium sensitivity of blood pressure in those subjects with Hp2-1 or Hp2-2 phenotype, it suggests that those subjects with the Hp1-1 phenotype are more likely to show an increase in blood pressure at a younger age, thus enabling primary intervention studies in a shorter time than might otherwise be required. On the other hand, sodium sensitivity of blood pressure and an age-related blood pressure increase might be anticipated at a later age in Hp2-1 or Hp2-2 subjects.

The outpatient data obtained from the study of families of identical twins support this hypothesis. Even though the blood pressures were within the normal range, subjects with the homozygous Hp2-2 phenotype had lower blood pressures than did those with other phenotypes, suggesting that the presence of the Hp1 gene is a predisposing factor for increases in blood pressure. It remains to be shown whether dietary intervention has a differential blood pressure effect in the groups, a finding that would further strengthen the relationship between haptoglobin phenotype and sodium sensitivity of blood pressure.

Studies conducted in uncharacterized hypertensive and normotensive subjects in Germany and Australia reported an increased frequency of the Hp1-1 phenotype among hypertensive subjects as compared with normotensive subjects. Those studies did not examine specific characteristics of hypertension. The Hp1-1 phenotype has been found to be associated with non-insulin-dependent diabetes mellitus: Those having the Hp1-1 phenotype were twice as likely, and those with the Hp1-2 phenotype were 1.5 times as likely, to have non-insulin-dependent diabetes mellitus as were those with the Hp2-2 phenotype. There is no obvious rationale for a mechanistic relationship between haptoglobin phenotype and blood pressure; the correlation may have been observed by chance. The observation of a dose-response relationship with blood pressure, and the independent confirmation from two different populations of the current study as well as two other reports make this possibility unlikely. In addition, the association may not be causal. The observation from the present study that the Hp1 phenotype is linked to sodium sensitivity of blood pressure makes it important to study this association further. Finally, the haptoglobin phenotype may provide a marker for a hypertension-related gene. If so, identification of such a gene or relationship will provide an important new approach in the prevention and study of hypertension.

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References

2. Tobian L. Dietary salt (sodium) and hypertension. Am J Clin Nutr 1979;32:2659-2667
4. McGregor GA. Sodium is more important than calcium in essential hypertension. Hypertension 1985;7:628-637
Association of haptoglobin with sodium sensitivity and resistance of blood pressure.
M H Weinberger, J Z Miller, N S Fineberg, F C Luft, C E Grim and J C Christian

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