Plasma Hydrogen Peroxide Production in Human Essential Hypertension
Role of Heredity, Gender, and Ethnicity

Fred Lacy, Mala T. Kailasam, Daniel T. O’Connor, Geert W. Schmid-Schönbein, Robert J. Parmer

Abstract—Oxygen free radicals, including hydrogen peroxide, may mediate oxidative stress in target organ tissues and contribute to cardiovascular complications in hypertension. To examine heritability of hydrogen peroxide production, we investigated this trait in a family-based cohort consisting of family members (n = 236) ascertained through probands (n = 57) with essential hypertension. Significant effects on hydrogen peroxide production were found for gender and ethnicity, with men having greater values than women (P < 0.001) and white subjects having greater values than black subjects (P = 0.025). Hydrogen peroxide production correlated directly with plasma renin activity (P = 0.015), suggesting an important interaction between circulating oxygen radicals and the renin-angiotensin system and a potential mechanism for lower hydrogen peroxide values observed in blacks. Heritability estimates from familial correlations revealed that approximately 20% to 35% of the observed variance in hydrogen peroxide production could be attributed to genetic factors, suggesting a substantial heritable component to the overall determination of this trait. Hydrogen peroxide production negatively correlated with cardiac contractility (r = −0.214, P = 0.001) and renal function (r = −0.194, P = 0.003). In conclusion, these results indicate that hydrogen peroxide production is heritable and is related to target organ function in essential hypertension. Genetic loci influencing hydrogen peroxide production may represent logical candidates to investigate as susceptibility genes for cardiovascular target organ injury. (Hypertension. 2000;36:878-884.)

Key Words: genetics, hypertension, essential, oxygen free radicals

There is increasing evidence that reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical may play a role in the development of organ damage associated with cardiovascular disease in general and hypertension in particular. We recently found evidence to suggest that hypertensive patients exhibit a significantly higher production of plasma peroxide than normotensive subjects.1 In addition, among still-normotensive subjects, those with a family history of hypertension have a higher production of plasma peroxide than those normotensives without a family history of hypertension. These findings suggest that there may be a genetic component that leads to elevated production of hydrogen peroxide. A number of recent studies in experimental models with genetic forms of hypertension (spontaneously hypertensive rats, salt-dependent Dahl hypertensive rats) have also demonstrated a significantly enhanced level of oxidative stress in the endothelial cells of arteriolar as well as venular segments of the circulation.2-5 These studies in genetic models of hypertension also suggest the hypothesis that a phenotype associated with an enhanced level of oxidative stress may ultimately have a heritable component in hypertension.

We therefore investigated hydrogen peroxide production in a family-based cohort, ascertained through probands with essential hypertension, to directly examine the role of heredity (as well as gender and ethnicity) in the overall determination of this trait. In addition, we examined potential relationships among hydrogen peroxide production, biochemical risk factors, and cardiovascular hemodynamic indices in this cohort. Our results demonstrate significant gender and race effects on this phenotype and document a substantial genetic component to the overall determination of this trait. In addition, hydrogen peroxide production correlates inversely with target organ function in these families. These results suggest that genetic loci operating through pathways that directly influence hydrogen peroxide production may contribute to blood pressure elevation as well as cardiovascular target organ effects in essential hypertension.

Methods

Subjects
Probands with essential hypertension and their first-degree family members were recruited for study. The following prospective ascer-
tainment criteria were applied; probands were identified from the Hypertension Clinics of the University of California and Veterans Affairs Medical Centers, San Diego, based on single ascertainment through a diagnosis of hypertension, defined as either a current history of antihypertensive medication or untreated diastolic blood pressure of >95 mm Hg on 3 separate outpatient determinations. Onset of hypertension in these probands was before the age of 60 years. We excluded secondary causes of hypertension by history, physical examination, and screening laboratory values. On the basis of these criteria, 57 probands with essential hypertension were enrolled in the study. A sequential sampling scheme\textsuperscript{6} was then applied to enroll all available first-degree relatives (ie, siblings, offspring, and parents) and spouses of the probands for study. Ethnic identity was assessed via self-identification and identification of parents and grandparents. White subjects claimed all 4 grandparents of European ancestry. Blacks claimed all 4 grandparents of sub-Saharan African ancestry.

Brachial arterial systolic (SBP) and diastolic blood pressures (DBP) were measured (in triplicate) by indirect sphygmomanometry (Dinamap; Critikon) in seated subjects before blood collection; triplicate values were averaged. Cardiac output, stroke volume, and cardiac contractility were noninvasively assessed with the use of thoracic impedance cardiography (NCCOM-3; Bomed). The mean arterial pressure (MAP) was computed as DBP + 1/3(SBP – DBP).

Phenotypic information was obtained on 236 subjects from 57 families ascertained by the aforementioned criteria. All hypertensive subjects were off medications for at least 3 days when measurements were performed. All subjects gave written informed consent, and the study was approved by the Human Subjects Committee at the University of California, San Diego.

Blood Plasma Collection and Preparation

Blood samples (for all parameters) were drawn in the morning after an overnight fast via a single venipuncture. Venous blood was drawn from an antecubital vein into a heparinized evacuated tube (approximately 10 mL), and the plasma was removed from the blood cells by centrifugation (500 g, 10 minutes). The plasma sample was frozen at −75°C and stored until the measurements were made. The plasma samples were prepared for measurement by warming the frozen plasma to room temperature, and 2 aliquots of 100 μL were used for measurements.

Pilot studies were performed (n = 3 plasma samples) to compare plasma peroxide production in fresh versus frozen samples. There was no significant difference between the peroxide production between the 2 sample preparations.

Plasma Hydrogen Peroxide Measurement

The electrochemical measurement procedure and calibration using a modified Clark electrode as well as reagent preparation were previously described in detail.\textsuperscript{1,7} Briefly, measurements were performed by mixing an aliquot of 100 μL from the same plasma sample either with sodium azide (2.5 μL of a 2 mol/L stock solution), to block all breakdown of hydrogen peroxide, or with catalase (2.5 μL containing 0.03125 mg of the enzyme), to eliminate all hydrogen peroxide in the plasma. The electrode was placed into the 2 plasma samples, and its output was recorded for 10 minutes.

The difference in electrode current between the plasma azide and catalase samples was determined. Since this measurement reflects the ability of the plasma to produce hydrogen peroxide, we refer to it as hydrogen peroxide production.\textsuperscript{1} Between measurements the electrode was cleaned in a saline solution until the steady state baseline signal was obtained. The electrode was calibrated by addition of selected concentrations of hydrogen peroxide to buffer and to plasma with sodium azide. The electrode reading was further cross-checked with an independent technique to measure hydrogen peroxide levels with phenol red.\textsuperscript{1} The minimum sensitivity of the electrode is approximately 0.2 μmol/L.

Plasma peroxide measurements were performed blindly with respect to the identity of the subjects. Numbers were assigned to pedigree members, and the laboratory personnel knew only the plasma sample and corresponding pedigree numbers until completion of all peroxide measurements presented in this study.

Other Biochemical Assays and Measurements

Plasma renin activity was determined by radioimmunoassay (Incastar Corporation); serum creatinine and uric acid were determined by autoanalyzer. Total serum cholesterol and triglyceride levels were measured by enzymatic techniques with an ABA-200 apparatus (Abbott Biochromatic Analyzer, Abbott Laboratories). LDL and HDL cholesterol levels were measured according to the standardized procedures of the Lipid Research Clinics Program.\textsuperscript{9}

Glomerular filtration rate was estimated by the Cockcroft-Gault algorithm:\textsuperscript{10}

\[
(140 - \text{age in years}) \times (\text{weight in kg}) \\
72 \times (\text{serum creatinine in mg/dL})
\]

The result of this formula was multiplied by 1 for men and 0.85 for women\textsuperscript{11} and normalized for body surface area (m²).

Statistical Analysis

Values are presented as mean±1 SE. Group comparisons were analyzed by 2-factor ANOVA, factoring for the effects of ethnicity and gender. Univariate correlations were assessed by linear least-squares regression analysis. Simultaneous model multiple linear regression analysis was performed to assess the effect of several independent variables on plasma hydrogen peroxide concentrations. Maximum likelihood calculations for modality of plasma hydrogen peroxide concentrations (unimodal versus bimodal best fit models) were performed with the use of the ADMIX program (Steven C. Hunt, University of Utah\textsuperscript{12}). Histograms (frequency distributions) of hydrogen peroxide production were plotted with the number of bins (intervals into which the data are divided) approximating the square root of n subjects.\textsuperscript{12} Familial correlations were determined with the Familial Correlations module (FCOR) in the Statistical Analyses for Genetic Epidemiology (S.A.G.E.) program package (version 3.1, 1997), which calculates parent-offspring, intraclass sibling-sibling, and spousal correlations, with equal weight given to pairs, pedigrees, or nuclear families, separately. These familial correlations were used to estimate the heritability of plasma hydrogen peroxide generation. In this context, heritability is the proportion of the total variance in a quantitative trait that is due to additive genetic factors (also referred to as narrow-sense heritability (h\textsuperscript{2})). It is estimated by doubling the parent-offspring correlation (h\textsuperscript{2} = 2r\text{parent-offspring}).\textsuperscript{12} Typically, a heritability estimate of zero suggests little or no genetic effect, whereas an estimate of 1 suggests a very large genetic effect with little or no environmental effect. Intermediate heritability estimates reflect varying contributions of both environmental and genetic factors.

Results

Table 1 lists the demographic and clinical data from the hypertensive patients and their family members together with results of 2-way ANOVA, factoring for race and gender, on the data from the subjects. Black subjects were slightly younger than white subjects (P = 0.012 for race effect) but did not differ from white subjects in blood pressure, heart rate, weight, or body mass index (BMI) (P = NS for race effect). With respect to gender, SBP and MAP were elevated in men (P = 0.001, P = 0.014, respectively), while heart rate was slightly elevated in women (P = 0.047). DBP was not significantly different when we compared men and women or blacks and whites. Although men tended to be heavier than women (P < 0.001), there were no significant differences in BMI among the 4 groups.

The levels of hydrogen peroxide production were measured in the plasma of 236 subjects. After stratifying each member by blood pressure status, we found that hyperten-
sives had significantly higher plasma peroxide production than normotensives \( (3.358 \pm 0.145 \text{ mol/L}, n=74 \text{ versus } 3.000 \pm 0.087 \text{ mol/L}, n=162; P<0.027; \text{Figure 1A}) \). Linear regression analysis also revealed a weak but significant relationship between hydrogen peroxide production and SBP \((r=0.133, P=0.041; \text{Figure 1B})\) in this family-based cohort ascertained through probands with essential hypertension. Plasma peroxide production was significantly elevated in men compared with women \((P<0.001; \text{Figure 2})\). Blacks had slightly lower peroxide levels compared with whites \((P=0.025; \text{Figure 2})\). White men had on average a 29% higher plasma peroxide production than white women. The plasma peroxide production of white men was approximately 9% higher than that of black men. Similarly, black men had on average a 35% higher plasma peroxide production than black women, and white women had about a 14% higher plasma peroxide production than black women.

Multiple linear regression analysis (Table 2) revealed that even after controlling for age and BMI, both race and gender remained significant unique predictors of hydrogen peroxide production \((P=0.016 \text{ and } P<10^{-7}, \text{respectively})\). Hence, the difference in plasma peroxide production between white and black subjects in this study was not due to differences in age. Inspection of the standardized coefficients from the multiple regression analysis revealed that gender was the strongest unique predictor of all variables tested.

Figure 3 shows the frequency distribution of hydrogen peroxide production in this cohort of subjects. The histogram suggested a bimodal distribution, and this was confirmed by maximum likelihood analysis, which yielded discrete frequency peaks corresponding to hydrogen peroxide production of 2.69±0.85 and 4.56±0.85 \text{ mol/L}, with proportions of

### Table 1. Subject Demographic and Physical Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>White Male</th>
<th>White Female</th>
<th>Black Male</th>
<th>Black Female</th>
<th>Race</th>
<th>Gender</th>
<th>Race×Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>62</td>
<td>71</td>
<td>46</td>
<td>57</td>
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<tr>
<td>Age, y</td>
<td>43.6±1.9</td>
<td>41.1±1.5</td>
<td>37.4±2.2</td>
<td>37.8±1.6</td>
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</tr>
<tr>
<td>SBP, mm Hg</td>
<td>139±2</td>
<td>126±3</td>
<td>134±3</td>
<td>127±3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>80±2</td>
<td>75±1</td>
<td>77±2</td>
<td>76±2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>99±2</td>
<td>92±2</td>
<td>96±3</td>
<td>93±2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>73±1</td>
<td>74±1</td>
<td>72±2</td>
<td>76±2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>93.6±2.3</td>
<td>75.5±2.1</td>
<td>90.9±3.0</td>
<td>79.0±2.8</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.5±0.7</td>
<td>29.5±0.8</td>
<td>29.9±0.9</td>
<td>29.8±1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma renin activity, ng/(mL·h)</td>
<td>2.43±0.24</td>
<td>2.09±0.23</td>
<td>1.72±0.28</td>
<td>1.31±0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\*\(P<0.05\).

**Figure 1.** A. Plasma hydrogen peroxide production in subjects stratified by blood pressure status. Bar graph shows results of plasma hydrogen production in normotensive \((n=162)\) vs hypertensive \((n=74)\) members of this cohort. Hypertensive subjects had greater hydrogen peroxide levels than normotensive subjects \("P=0.027\). B. Scatterplot and linear regression analysis of the relationship between hydrogen peroxide production and SBP \((n=236)\).

**Figure 2.** Plasma hydrogen peroxide production in family members ascertained through probands with essential hypertension. Bar graph shows results of determination of hydrogen peroxide production stratified by ethnicity and gender. Values are mean±SEM. Two-way ANOVA revealed significant effects on hydrogen peroxide production for race \((F=5.098, P=0.025)\) and gender \((F=36.744, P<0.001)\) and no significant race×gender interaction \((F=0.032, P=0.859)\).
TABLE 2. Multiple Linear Regression Analysis: Dependent Variable: Plasma Hydrogen Peroxide Production

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Coefficient</th>
<th>Standardized Coefficient</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.002</td>
<td>-0.025</td>
<td>-0.414</td>
<td>0.679</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.855</td>
<td>-0.368</td>
<td>-6.187</td>
<td>&lt;10^-7</td>
</tr>
<tr>
<td>BMI</td>
<td>0.030</td>
<td>0.172</td>
<td>2.384</td>
<td>0.005</td>
</tr>
<tr>
<td>Race</td>
<td>-0.341</td>
<td>-0.146</td>
<td>-2.424</td>
<td>0.016</td>
</tr>
</tbody>
</table>

This model predicts plasma hydrogen peroxide production with multiple R=0.429, R²=0.184, adjusted R²=0.170, F=13.05, P<10^-8.

77% and 23%, respectively (χ²=11.787, P=0.0028, for bimodal versus unimodal distribution). Tramoidal models were not superior to bimodal models (by χ², all P>0.179).

Results of familial correlations for plasma hydrogen peroxide production are shown in Table 3, with the r values for parent-offspring correlations, intraclass sibling-sibling correlations, and spousal correlations calculated, giving equal weight to pairs, pedigrees, or nuclear families, separately. Within each of the 3 separate analyses, for either pairs, pedigrees, or nuclear families (reading columnwise in Table 3), values for parent-offspring correlations and sibling-sibling correlations were consistently greater than those for spousal correlations. The spousal correlations were, in turn, near zero, a pattern consistent with a substantial genetic component to the overall determination of this trait.

To estimate the heritability of plasma hydrogen peroxide production, we used the formula h² = 2×r-parent-offspring, yielded heritability estimates of 0.352, 0.234, and 0.208, using the r-parent-offspring data from Table 3, for pairs, pedigrees, or nuclear families, respectively. These results suggest that approximately 20% to 35% of the observed variance in this trait can be attributed to genetic factors.

Table 4 shows the univariate linear correlations of biochemical and biophysical parameters with hydrogen peroxide production. SBP, pulse pressure, weight, and BMI correlated positively with increasing plasma peroxide, while stroke volume index and contractility correlated negatively with increasing plasma peroxide. The probability value ranged from 0.041 for SBP to <0.001 for body weight. The biochemical parameters plasma triglycerides (P=0.004) and plasma renin activity (P=0.015) correlated positively with increasing plasma peroxide. In addition, renal function correlated negatively with increasing plasma hydrogen peroxide production (r=-0.194, P=0.003, for glomerular filtration rate; r=0.296, P<0.001, for serum creatinine). Because of this significant relationship between renal function and hydrogen peroxide levels, we performed additional multiple regression analyses and found that even after controlling for age, BMI, and renal function, the effects of race and gender on hydrogen peroxide production were still highly significant (P=0.003 and P<0.001, respectively).

On the basis of the univariate results (Table 4) showing a significant negative correlation for cardiac contractility and plasma hydrogen peroxide production, we further analyzed this in a multiple regression model (Table 5). After we controlled for a variety of variables, including age, gender, and BMI, the effect of hydrogen peroxide production on cardiac contractility was still significant (P=0.044). Similarly, after we controlled for the same set of variables, the

TABLE 3. Familial Correlations for Plasma Hydrogen Peroxide Production

<table>
<thead>
<tr>
<th>Correlation</th>
<th>No. of Pairs</th>
<th>Pairs</th>
<th>Pedigrees</th>
<th>Nuclear families</th>
</tr>
</thead>
<tbody>
<tr>
<td>r (parent-offspring)</td>
<td>121</td>
<td>0.176</td>
<td>0.117</td>
<td>0.104</td>
</tr>
<tr>
<td>r (siblings)</td>
<td>131</td>
<td>0.117</td>
<td>0.071</td>
<td>0.141</td>
</tr>
<tr>
<td>r (spousal)</td>
<td>23</td>
<td>0.076</td>
<td>-0.005</td>
<td>0.076</td>
</tr>
</tbody>
</table>

TABLE 4. Linear Correlations With Plasma Hydrogen Peroxide Production

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.045</td>
<td>0.492</td>
</tr>
<tr>
<td>SBP</td>
<td>0.133</td>
<td>0.041*</td>
</tr>
<tr>
<td>DBP</td>
<td>0.059</td>
<td>0.372</td>
</tr>
<tr>
<td>Heart rate</td>
<td>-0.097</td>
<td>0.141</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>0.159</td>
<td>0.015*</td>
</tr>
<tr>
<td>Stroke volume index</td>
<td>-0.138</td>
<td>0.037*</td>
</tr>
<tr>
<td>Contractility</td>
<td>-0.214</td>
<td>0.001*</td>
</tr>
<tr>
<td>BMI</td>
<td>0.161</td>
<td>0.013*</td>
</tr>
<tr>
<td>Body weight</td>
<td>0.268</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GFR</td>
<td>-0.194</td>
<td>0.003*</td>
</tr>
<tr>
<td>Biochemical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.036</td>
<td>0.606</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.072</td>
<td>0.270</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.188</td>
<td>0.004*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.043</td>
<td>0.515</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.091</td>
<td>0.165</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.296</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Plasma renin activity</td>
<td>0.161</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

GFR indicates glomerular filtration rate.
*P<0.05.
effect of hydrogen peroxide production on glomerular filtration rate was still significant ($P=0.014$) (Table 6).

### Discussion

The specific mechanisms that predispose individuals with elevated blood pressure to the development of target organ injury are incompletely understood. Both environmental and genetic factors may play a role in this process. Oxygen free radicals may mediate oxidative stress in target organ tissues and contribute to cardiovascular complications observed in hypertension, including enhanced arteriolar tone and elevation of arterial resistance and pressure. In this study we investigated hydrogen peroxide production in a family-based cohort consisting of family members ascertained through probands with essential hypertension. We determined familial correlations for hydrogen peroxide production as a quantitative trait in this cohort and used these results to estimate the heritability of this trait. Heritability estimates revealed that approximately 20% to 35% of the observed variance in hydrogen peroxide production could be attributed to genetic factors, suggesting a substantial heritable component to the overall determination of this trait.

Previous studies have demonstrated that blood pressure analyzed as a quantitative trait is heritable. The values for parent-offspring and sibling-sibling correlations observed in our study for hydrogen peroxide production are similar in magnitude to those values that have been previously reported for SBP and DBP. For example, $r_{parent-offspring}$ values of 0.120 were obtained for both SBP and DBP in the Tecumseh study, one of the largest cohorts to investigate familial aggregation of blood pressure.$^{13}$ Thus, the hydrogen peroxide production phenotype exhibits a heritable component that is similar to or perhaps even slightly greater in magnitude than that of blood pressure as a quantitative trait.

In addition, consistent with our previous results,$^{1}$ we found that hypertensive patients had significantly greater hydrogen peroxide production than their normotensive counterparts. Additionally, in previous studies of unrelated individuals, we found that normotensive subjects with a genetic risk of hypertension (positive family history of hypertension) had significantly greater hydrogen peroxide production than blood pressure–matched normotensives without a family history of hypertension.$^{1}$ Taken together with the current results, these data suggest that hydrogen peroxide production is a heritable trait that may have earlier penetrance than elevated blood pressure and may perhaps predict subsequent development of hypertension, as well as contribute to target organ damage in susceptible individuals.

Linear regression analysis also revealed a weak but significant relationship between hydrogen peroxide production and SBP ($r=0.133$, $P=0.041$; Figure 1B) in this family-based cohort ascertained through probands with essential hypertension. As discussed above, in our previous studies involving unrelated individuals,$^{1}$ even normotensive subjects with a positive family history of hypertension had increased values for hydrogen peroxide production (compared with normotensive subjects who were family history negative for hypertension). Thus, these results obtained for the present family-based cohort (which includes subjects who are predominantly family history positive for hypertension) may underestimate the strength of the association between hydrogen peroxide production and blood pressure. Indeed, in our previous studies of unrelated individuals, the correlation for hydrogen peroxide production and blood pressure was much stronger ($r=0.70$, $P<0.001$).$^{1}$

Examination of the frequency distribution for hydrogen peroxide production in this cohort suggested a bimodal distribution for this trait (Figure 3). Maximum likelihood analysis also suggested that the data fit a mixture of 2 distributions ($\chi^2=11.787$, $P=0.0028$ for bimodal versus unimodal distribution). Such a bimodal distribution may suggest, or is at least consistent with, the possibility of a major gene ( mendelian, single locus) effect on heritability of this phenotype. It should be noted, however, that mendelian (major gene or single locus) inheritance of a phenotype can only be formally demonstrated by showing that clear segregation occurs in families and that there are consistent linkage results across families$^{14}$; furthermore, major gene effects need not always underlie bimodally distributed traits.$^{15}$ Additionally, even traits controlled by a single genetic locus need not display bimodality, the appearance of which is dependent on there being a sufficiently large difference between 2 subgroups in trait means, coupled with nonoverlapping (ie, sufficiently small) variances around each mean.$^{11}$

Although the precise sources of hydrogen peroxide in humans are unknown, several pathways may contribute to the generation of hydrogen peroxide in plasma and tissues. Hydrogen peroxide may be generated through the action of xanthine oxidase, and studies in the spontaneously hypertensive rat and the Dahl hypertensive rat suggest that endothelial xanthine oxidase may make an important contribution to

### Table 6. Multiple Linear Regression Analysis: Dependent Variable: Glomerular Filtration Rate

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Coefficient</th>
<th>Standardized Coefficient</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-1.303</td>
<td>-0.561</td>
<td>-13.352</td>
<td>&lt;10^-19</td>
</tr>
<tr>
<td>Gender</td>
<td>22.911</td>
<td>0.356</td>
<td>6.051</td>
<td>&lt;10^-13</td>
</tr>
<tr>
<td>BMI</td>
<td>2.273</td>
<td>0.468</td>
<td>11.033</td>
<td>&lt;10^-19</td>
</tr>
<tr>
<td>H2O2 production</td>
<td>-3.109</td>
<td>-0.110</td>
<td>-2.472</td>
<td>0.014</td>
</tr>
</tbody>
</table>

This model predicts glomerular filtration rate with multiple $R=0.783$, $R^2=0.613$, adjusted $R^2=0.606$, $F=90.325$, $P<10^{-16}$.
vascular oxidative stress.\textsuperscript{3,16} Our own previous studies suggest that xanthine oxidase in plasma can make a direct contribution to the hydrogen peroxide signal detected with the electrode technique used in this study.\textsuperscript{7} Alternatively, hydrogen peroxide can be produced by the dismutation of superoxide radicals through the activity of superoxide dismutase.\textsuperscript{17} Hydrogen peroxide levels may also be influenced and regulated by the action of antioxidants, including the enzyme catalase, which converts hydrogen peroxide to water, as well as by the scavenger effect of glutathione.\textsuperscript{18} In addition, recent studies suggest that hydrogen peroxide may be produced through the activity of NADH/NADPH oxidase.\textsuperscript{19} Thus, the measured hydrogen peroxide concentrations in plasma may reflect the combined activities of several contributing pathways. The present measurements were performed by determining hydrogen peroxide levels during catalase inhibition and catalase excess; thus, such measurements reflect hydrogen peroxide production by oxidase (or superoxide dismutase) rather than catalase activity. There is clearly a need for a broader exploration of all enzyme systems involved in the production of reactive oxygen species in hypertensives.

Prominent risk factors associated with cardiovascular disease include male sex,\textsuperscript{20–22} obesity,\textsuperscript{23} and age,\textsuperscript{24,25} and these have been shown to be associated with decreased antioxidant activity in the kidney,\textsuperscript{21,24} brain,\textsuperscript{23,24} and heart.\textsuperscript{24,25} In this study we found a highly significant gender effect on hydrogen peroxide production, with men having significantly greater levels than women (Figure 2). Recent studies in experimental animals have demonstrated gender differences in the vascular generation of superoxide anions,\textsuperscript{26} and human studies have suggested gender differences in the circulating activities of superoxide dismutase, catalase, and glutathione peroxidase.\textsuperscript{27} Premenopausal women have a lower risk of cardiovascular disease than postmenopausal women, and estrogen replacement therapy reduces risk of cardiovascular disease in postmenopausal women,\textsuperscript{28–30} suggesting an important role for sex hormones in mediating this effect. Recent studies suggest a direct role for estrogen in decreasing oxidative stress.\textsuperscript{31–33} Thus, our results may suggest a specific mechanism through which gender differences in target organ injury could, at least in part, be mediated.

We also noted a small but statistically significant effect for ethnicity on hydrogen peroxide production, with lower values for hydrogen peroxide production observed in black subjects compared with white subjects (Figure 2). Recent studies suggest a role for the NADH/NADPH oxidase system in the generation of hydrogen peroxide.\textsuperscript{19} In addition, NADH/ NADPH oxidase is directly influenced by the renin-angiotensin system, and angiotensin II induces the formation of hydrogen peroxide generation through activation of NADH/ NADPH oxidase. Indeed, we previously observed that angiotensin II receptor blockade diminishes plasma hydrogen peroxide production in hypertension.\textsuperscript{1} Consistent with previous studies of the renin-angiotensin system,\textsuperscript{34,35} we observed lower plasma renin activity in blacks than in white members of our cohort (\(P=0.004\)) (Table 1). In addition, we found that plasma hydrogen peroxide production directly correlated with plasma renin activity (\(P=0.015\)) (Table 4). Thus, the observed ethnic differences in hydrogen peroxide production may be the result of ethnic differences in the activity of the renin-angiotensin system, perhaps through its effect on NADH/NADPH oxidase.\textsuperscript{19} At the same time, these results may also suggest that NADH/NADPH oxidase is a source of circulating levels of hydrogen peroxide in humans. In addition, since target organ damage may be more severe in blacks than in whites,\textsuperscript{36} these results suggest that additional factors (ie, the ability of target organs to scavenge oxygen radicals) may be responsible for the observed ethnic differences in hypertensive target organ injury.

Finally, oxidative stress, as a result of an imbalance between oxidants and antioxidants, may develop over an extended period of time into frank organ dysfunction and failure. In this study we noted that increases in hydrogen peroxide production were significantly associated with decreases in cardiac contractility and renal function (Table 4), consistent with this hypothesis. Moreover, the effects of hydrogen peroxide production on cardiac contractility and renal function were observed even after controlling for a variety of potentially confounding variables, such as age, gender, and BMI (Tables 5 and 6). Thus, hydrogen peroxide production was a significant unique predictor of renal and cardiac function in this cohort.

In summary, these studies suggest that hydrogen peroxide production is heritable in families ascertained through probands with essential hypertension. In addition, hydrogen peroxide production correlates inversely with renal and cardiac function in these individuals. Thus, taken together, these results suggest that genetic loci operating through pathways that influence hydrogen peroxide production may contribute to cardiovascular target organ effects in essential hypertension. Loci influencing hydrogen peroxide production thus represent logical candidates to investigate as susceptibility genes for cardiovascular target organ injury.

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