Early Alteration in Glomerular Reserve in Humans at Genetic Risk of Essential Hypertension
Mechanisms and Consequences

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Abstract—Essential hypertension has a familial predisposition, but the phenotype of elevated blood pressure has delayed penetrance. Because the kidney is a crucial determinant of blood pressure homeostasis, we studied early glomerular alterations in still-normotensive young subjects at genetic risk of hypertension. Thirty-nine normotensive adults (mean age 29 to 31 years), stratified by genetic risk (parental family history [FH]) of hypertension (26 with positive FH [FH+], 13 with negative FH [FH−]), underwent intravenous infusion of mixed amino acids. Before and during amino acid administration, we measured glomerular filtration rate (GFR), putative second messengers of amino acids (nitric oxide [NO] metabolites and cGMP), serum insulin and amino acid concentrations, and the FE Li+ as an index of renal proximal tubular reabsorption. The FH+ group had a blunted GFR rise in response to amino acids (2.43±8.16% versus 31.0±13.4% rise, P=0.0126). The amino acid–induced change in GFR correlated (r=0.786, P<0.01) with the change in urinary NO− metabolite excretion; a diminished rise in urinary NO− metabolite excretion in the FH+ group (P=0.0105) suggested a biochemical mechanism for the different GFR responses between FH groups: a relative inability to convert arginine to NO−. The FH+ group had a far lower initial cGMP excretion at baseline (261±21.1 versus 579±84.9 nmol·h−1/1.73 m2, P=0.001), although cGMP did not change during the amino acid infusion (P=0.703). FH status, baseline GFR, and baseline serum insulin jointly predicted GFR response to amino acids (P=0.0013), accounting for ≈45% of the variance in GFR response. Decline in FE Li+, an inverse index of proximal tubular reabsorption, paralleled increase in GFR (r=−0.506, P=0.01), suggesting differences in proximal tubular reabsorption during amino acids between the FH groups. GFR response to amino acid infusion was blunted in the FH+ group despite significantly higher serum concentrations of 6 amino acids (arginine, isoleucine, leucine, methionine, phenylalanine, and valine) in the FH+ group, suggesting a novel form of insulin resistance (to the amino acid–translocating action of insulin) in FH+ subjects. We conclude that blunted glomerular filtration reserve in response to amino acids is an early-penetrance phenotype seen even in still-normotensive subjects at genetic risk of hypertension and is linked to impaired formation of NO− in the kidney. Corresponding changes in GFR and fractional excretion of Li+ suggest that altered proximal tubular reabsorption after amino acids is an early pathophysiological mechanism. Resistance to the amino acid–translocating actions of insulin may play a role in the biological response to amino acids in this setting. This glomerular reserve phenotype may be useful in genetic studies of renal traits preceding or predisposing to hypertension. (Hypertension. 2001;37:898-906.)

Key Words: hypertension, essential ■ genetics ■ glomerular filtration rate ■ kidney

Heritability of essential hypertension is suggested by frequent positive hypertension family histories among hypertensives and has been demonstrated by twin and family studies, with estimates of genetic determination of blood pressure ranging from ≈30% to ≈70%.1,2 However, hypertension is an etiologically “complex trait,”3 with both genetic and environmental determinants. Lack of bimodality of blood pressure frequency distribution in the population suggests that its hereditary determination is also complex and non mendelian and perhaps polygenic.3 Indeed, allelic variation at several genetic loci is reportedly linked to or associated with common hypertension in the population.4

The phenotype of elevated blood pressure has delayed penetrance, typically occurring in the fourth, fifth, or sixth decades of life.1 Thus, phenotypes with earlier penetrance (“intermediate phenotypes”) are desirable in better under-
standing early, perhaps pathophysiological, alterations in the
course of hypertension.5–7 Such early-penetrance phenotypes
may also be valuable in genetic analyses of hereditary
predisposition to hypertension.5–7

The glomerular filtration increment in response to amino
cids, or “renal functional reserve,” is a general phenomenon
in mammals that occurs in species ranging from rodents to
humans.8 Patients with established essential hypertension
display diminished renal functional reserve,9 but the glomer-
ular response to amino acids is less well studied in normo-
tensive persons at genetic risk of hypertension,10 and the
mechanisms of the altered renal response to amino acid
infusion in hypertension are not clearly established.9 One
of the proposed mediators of the amino acid–induced renal
vasodilation is nitric oxide (NO).11 formed by the action of
the enzyme NO synthase (NOS) on its substrate arginine12; hence, the link between renal functional reserve and amino
acid infusion in general and to arginine in particular.

Because the kidney is a crucial determinant of blood pres-
sure,13 this study focused on early renal alterations in still-
normotensive young subjects stratified by family history (genet-
ic risk) of hypertension.7,14 We therefore hypothesized that
impaired renal functional reserve is an intermediate, early-
penetrance phenotype in essential hypertension.5–7

Methods

Subjects

The institutional review board approved the protocol, all subjects
gave written informed consent, and procedures followed were in
accordance with institutional guidelines. Thirty-nine normotensive
individuals (24 men and 15 women, age range 22 to 46 years) with
no history of hypertension or kidney disease were evaluated. Blood
pressure readings were repeatedly normal (<135/85 mm Hg) in
each subject. All subjects were of European ancestry (with all 4
grandparents so identified). They were divided into 2 groups: on
the basis of positive family history (FH+; 26 subjects) or negative
family history (FH−; 13 subjects) of hypertension, as we previously
outlined.7,14 Seven of the 26 FH+ subjects were normotensive
members of pedigrees that we ascertained through a proband with
essential hypertension.15 A positive family history for hypertension
was defined as having a parent with hypertension before the age of
60 years, as evidenced by the parent’s elevated blood pressure
(>140/90 mm Hg), or by having a parent whose hypertension was
currently controlled with antihypertensive medication. The average
age of the parents of the FH+ group (at the time of the subject’s
study, or at the time of the parent’s death, if before the study) was
59.2 ± 1.5 years (range 44 to 80 years), whereas that of the FH−
group parents was 53.1 ± 1.1 years (range 47 to 64 years); the mean
parental ages thus differed in the 2 groups (Mann-Whitney U test,
U = 496, P = 0.013). Because only half of the subjects at genetic risk
of hypertension would be expected to inherit disease-predisposition
alleles, the FH+ group (n = 26) was designed to be larger than the
FH− group (n = 13), to allow detection of heterogeneity in the FH+
group. None of the subjects in this study were consuming medica-
tions (prescription or otherwise) that affect cardiovascular or renal
function. No dietary instruction was given.

Amino Acid Infusion and Renal
Clearance Protocol

Subjects ingested 600 mg (16.2 mmol) lithium carbonate16 by mouth
at 10 PM on the night before the test and then fasted after midnight.
Two intravenous access sites were placed (right and left forearms: 1
for infusions and the other for blood sampling), and an oral water
load of 1500 mL was given (to increase urine output and thereby
improve accuracy of clearance measurements).

Renal clearance of inulin (Cypros Pharmaceutical Corporation)
was used to measure glomerular filtration rate (GFR). After an initial
intravenous bolus of 1.4 g inulin, a continuous infusion of inulin was
started at 1 mL/min (1 g/100 mL in half-normal saline; ie, 10
mg/min) for 180 minutes.17 One hour after the start of the inulin
infusion, the subjects voided and urine was discarded, before the
baseline clearance period. In the next 30-minute (baseline) period,
blood was drawn for measurement of blood urea nitrogen, creatinine,
electrolytes, lithium, amino acids, inulin, glucose, and insulin, and a
timed urine test was collected for measurement of creatinine,
electrolytes, lithium, inulin, nitrate/nitrite (as an index of NO
production), and cGMP.

During the next 30 minutes, subjects received an intravenous
infusion of mixed amino acids without electrolytes (Travasol 10%
amino acids at pH 6.0; Baxter-Travenol) at a rate of 0.043 mL·kg−1 ·
min−1, and an acute urine collection allowed measurement of
creatinine, urea, sodium, potassium, chloride, lithium, nitrate/nitrite,
and inulin. At the midpoint of the amino acid infusion (15 minutes),
the blood collection was repeated; at the end of the amino acid
infusion, the timed (30 minute) urine collection was repeated.

Assays

Electrolytes, lithium, urea, and creatinine were measured spectro-
photometrically with an autoanalyzer. Inulin was measured accord-
ing to the alkali-stable spectrophotometric method, as we previously
described.17 Urinary cGMP was measured with radioimmunoassay
(DuPont-New England Nuclear). To measure urinary nitrate/nitrite
(NO3), nitrate (NO2) was first converted to nitrite (NO3) with
Escherichia coli nitrate reductase in vitro18; nitrite was then mea-
sured with the Griess spectrophotometric reaction (monitoring ab-
sorbance at 546 nm).19 After perchloric acid precipitation of serum
proteins, amino acids in the supernatant were measured with a
Beckman amino acid analyzer.20

Solute (x) excretions into the urine were normalized to time
(x/min), as well as to body surface area (1.73 m2 unit body surface
area) to account for interindividual differences in body size. Renal
clearance (Cx) of a solute (x) was computed as Cx = Ux/V/Px, where Cx
is clearance of x, Ux is urine concentration of x, V is urine volume
(for that time period), and Px is plasma concentration of x; for
example, Cmin = Umix/Vmix. Renal clearance values were normal-
ized to standardized body surface area (1.73 m2) as an index of body
size. Fractional excretion (FEx) of a solute (x), in percent, was
computed as:

\[
FE_x(\%) = \left( \frac{U_x}{P_x} \right) \cdot \left( \frac{V}{P_{mix}} \right) \cdot 100
\]

Statistics

Continuous variables (eg, GFR) are reported as the mean ±1 SEM
value. Dichotomous variables (eg, gender) are reported as propor-
tions. Differences or changes in continuous variables were evaluated
by nonparametric tests: the Mann-Whitney U test was used for inter-
group differences, and the Wilcoxon signed rank test was used for
changes in the same group under 2 circumstances. Differences in
dichotomous traits (proportions) were evaluated by \( \chi^2 \) test. Nonpara-
metric simple (bivariate) correlations were computed by the Spear-
man rank-order method. Two-tailed tests were typically used. Se-
quential changes were evaluated by 2-way repeated measures
ANOVA, with factoring for effects of group (family history), time
(baseline versus amino acid infusion), and group × time interaction.
When data were not normally distributed, ANOVAs were conducted
don log10-transformed data. Multivariate analyses (specifying several
independent variables with 1 dependent variable) were conducted by
stepwise multiple linear regression, with \( \alpha \) criteria for inclusion
(\( \alpha < 0.1 \)) or exclusion (\( \alpha > 0.1 \)) of an independent variable in the final
model. Frequency histograms were created in Microsoft Excel.
Bimodality (or mixture of distributions) was evaluated by the
maximum likelihood program ADMIX. Variances were compared
by Levene’s F test. Analyses were performed with the software
packages SPSS (Statistical Package for the Social Sciences), Mi-
Results

Family History Subject Groups: Baseline Characteristics (Demographic, Physiological, and Biochemical)

Demographic/Physical
The family history subject groups (Table 1) did not differ in mean age, gender, or size (body mass index and body surface area).

Physiological
Diastolic and mean blood pressures were similar, but the FH+ group had higher systolic blood pressure (by 9 mm Hg, \(P=0.049\); Table 1), although at 123 ± 2.3 mm Hg, the FH+ value was well within customary limits for normal systolic blood pressure.\(^1\) Baseline creatinine clearance was \(\approx 25\%\) higher in the FH+ group (\(P=0.014\)), reminiscent of previous findings on early elevation in GFR in subjects at genetic risk of hypertension,\(^2\) although baseline inulin clearances did not achieve a statistically significant difference (\(P=0.150\)).

Biochemical
Blood urea nitrogen, serum creatinine and electrolytes, serum glucose, arginine, and insulin values were similar at baseline, as was urinary sodium excretion (all \(P>0.05\)). Fractional excretion of sodium (FE\(\text{Na}^+\)) (\(P=0.004\)) was lower in the FH+ subjects, likely because baseline hyperfiltration in the FH+ subjects (see Ccreatinine, Table 1) coupled with similar sodium excretion rate obligates a lower FE\(\text{Na}^+\) to achieve balance at steady state. At baseline, the FH groups had similar fractional excretion of lithium (FE\(\text{Li}^+\)) (\(P=0.229\)) and renal excretion of NO metabolites (\(P=0.546\)), although the FH+ subjects had \(\approx 55\%\) lower cGMP excretion (\(P=0.001\)).

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TABLE 1. Baseline Variables in FH+ and FH− Normotensives

<table>
<thead>
<tr>
<th>Variable</th>
<th>FH+</th>
<th>FH−</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>31.1 ± 1.5</td>
<td>29.5 ± 2.5</td>
<td>0.353</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>17/9</td>
<td>7/6</td>
<td>0.566</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>25.3 ± 0.83</td>
<td>23.8 ± 0.96</td>
<td>0.231</td>
</tr>
<tr>
<td>Body surface area, m(^2)</td>
<td>1.92 ± 0.048</td>
<td>1.80 ± 0.067</td>
<td>0.159</td>
</tr>
<tr>
<td><strong>Physiological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>123 ± 2.3</td>
<td>114 ± 3.7</td>
<td>0.049*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>70 ± 1.5</td>
<td>67 ± 2.0</td>
<td>0.151</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>88 ± 1.5</td>
<td>83 ± 2.3</td>
<td>0.071</td>
</tr>
<tr>
<td>Ccreatinine, mL (\cdot) min(^{-1}) /1.73 m(^2)</td>
<td>108 ± 5.64</td>
<td>93.2 ± 9.38</td>
<td>0.150</td>
</tr>
<tr>
<td>Ccreatinine, mL (\cdot) min(^{-1}) /1.73 m(^2)</td>
<td>115 ± 6.31</td>
<td>92.2 ± 5.94</td>
<td>0.014*</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>12.3 ± 0.66</td>
<td>11.6 ± 0.81</td>
<td>0.758</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.98 ± 0.043</td>
<td>0.96 ± 0.045</td>
<td>0.490</td>
</tr>
<tr>
<td>Serum glucose, mg/dL</td>
<td>82.0 ± 1.24</td>
<td>77.4 ± 2.13</td>
<td>0.091</td>
</tr>
<tr>
<td>Serum arginine, µmol/L</td>
<td>90.3 ± 5.22 (20)</td>
<td>85.4 ± 4.10 (10)</td>
<td>0.559</td>
</tr>
<tr>
<td>Serum insulin, µU/mL</td>
<td>12.6 ± 1.49</td>
<td>10.6 ± 0.93</td>
<td>0.808</td>
</tr>
<tr>
<td>sGlucose (\cdot) sInsulin (product)</td>
<td>1051 ± 135</td>
<td>834 ± 82</td>
<td>0.715</td>
</tr>
<tr>
<td>sArginine (\cdot) sInsulin (product)</td>
<td>1348 ± 225 (20)</td>
<td>887 ± 101 (10)</td>
<td>0.091</td>
</tr>
</tbody>
</table>

\(a\) indicates serum; \(u\), urine; Ccreatinine, creatinine clearance normalized to body surface area; Ccreatinine, inulin clearance normalized to body surface area; sGlucose, serum glucose; sInsulin, serum insulin; uNO\(_x\), urinary nitrate or nitrite; sArginine, serum arginine.

Differences in continuous (quantitative) variables were evaluated by nonparametric Mann-Whitney \(U\) test, whereas differences in dichotomous variables were evaluated by \(\chi^2\) test. *\(P<0.05\), \(n\) in parentheses for that observation (if less than the total \(n\) for that subgroup). To convert mg/dL to mg/L, multiply by 10.
Changes in Physiological Variables (Including GFR) During Amino Acid Infusion

FH+ subjects had substantial (~92% overall, *P=0.0126) blunting of the C\textsubscript{inulin} increment in response to amino acids (Table 2). Figure 1 shows the C\textsubscript{inulin} responses graphically, with 2-way (by FH) repeated measures ANOVA (Table 3): amino acid infusion elevated C\textsubscript{inulin} overall (time effect, *P=0.005). Change in urinary NO\textsubscript{x} excretion lithium; ∆ urine cGMP, change in urinary cGMP; ∆ urine microalbumin, change in microalbuminuria.

Changes in continuous (quantitative) variables were evaluated by non-parametric Mann-Whitney U test, ∗*P<0.05, n in parentheses for that observation (if less than the total n for that subgroup). For definitions of units, see legend to Table 1. To convert mg/dL to mg/L, multiply by 10.

## Mediators of Renal Hemodynamic Changes

There was a substantially greater increment in urinary NO\textsubscript{x} metabolite excretion in the FH− group (*P=0.0105, Table 2), and 2-way repeated measures ANOVA (Table 3, Figure 2) showed a significant interaction between family history and the amino acid effect on NO\textsubscript{x} excretion (*P=0.005). Change in

### Changes in Biochemical Variables During Amino Acid Infusion

**Electrolytes**

The fall in FE\textsubscript{Na\textsuperscript{+}} was greater in FH− subjects (*P=0.025, Table 2). By 2-way repeated measures ANOVA (Table 3), FE\textsubscript{Na\textsuperscript{+}} was influenced by both family history (*P=0.011) and amino acid infusion (*P<0.001), and the amino acid effect was more pronounced in FH− subjects (*P=0.025). During amino acid infusion, FE\textsubscript{Li\textsuperscript{+}} did not differ systematically between the FH groups (Tables 2 and 3). Change in urinary microalbumin excretion did not differ between the FH groups (Tables 2 and 3).

![Figure 1. Change in inulin clearance during intravenous amino acid infusion in normotensive subjects stratified by family history (FH, genetic risk) of essential hypertension. Results are analyzed by 2-way repeated measures ANOVA. Amino acid infusion elevated C\textsubscript{inulin} overall (time effect, *P=0.028), with an interaction (P=0.014) between FH category and time (infusion). Because C\textsubscript{inulin} data were not normally distributed (see Results), the ANOVA was performed on log\textsubscript{10} transformed data.](image)

### Table 2. Changes in Variables (Physiological or Biochemical) During Amino Acid Infusion

| Variable Change | FH+ (n=26) | FH− (n=13) | *P*
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Physiological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆ C\textsubscript{inulin} mL · min\textsuperscript{-1} · 1.73 m\textsuperscript{2}</td>
<td>2.43±8.16</td>
<td>31.0±13.4</td>
<td>0.0126*</td>
</tr>
<tr>
<td>∆ C\textsubscript{creatinine} mL · min\textsuperscript{-1} · 1.73 m\textsuperscript{2}</td>
<td>13.3±13.2</td>
<td>37.4±18.4</td>
<td>0.134</td>
</tr>
<tr>
<td><strong>Biochemical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆ serum glucose, mg/dL</td>
<td>4.95±1.16</td>
<td>5.20±2.42</td>
<td>0.879</td>
</tr>
<tr>
<td>∆ serum arginine, μmol/L</td>
<td>150±12.8 (20)</td>
<td>87.4±11.7 (10)</td>
<td>0.0008*</td>
</tr>
<tr>
<td>∆ serum insulin, μU/mL</td>
<td>17.5±4.00</td>
<td>9.3±3.66</td>
<td>0.059</td>
</tr>
<tr>
<td>∆ sGlucose · sInsulin, product</td>
<td>1666±398</td>
<td>842±323</td>
<td>0.074</td>
</tr>
<tr>
<td>∆ sArginine · sInsulin, product</td>
<td>7291±1807 (20)</td>
<td>3135±1074 (10)</td>
<td>0.091</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>∆ FE\textsubscript{Na\textsuperscript{+}}, %</td>
<td>-0.119±0.049</td>
<td>-0.351±0.100</td>
<td>0.025*</td>
</tr>
<tr>
<td>∆ FE\textsubscript{Li\textsuperscript{+}}, %</td>
<td>3.09±1.96 (20)</td>
<td>-1.35±1.09 (10)</td>
<td>0.244</td>
</tr>
<tr>
<td>∆ urine NO\textsubscript{x}, μmol · h\textsuperscript{-1} · 1.73 m\textsuperscript{2}</td>
<td>-5.90±2.93 (20)</td>
<td>14.8±7.49 (10)</td>
<td>0.0105*</td>
</tr>
<tr>
<td>∆ serum arginine/urine</td>
<td>4.97±0.715</td>
<td>1.51±0.502</td>
<td>0.002*</td>
</tr>
<tr>
<td>NO\textsubscript{x}, μmol/L · μmol\textsuperscript{-1} · h\textsuperscript{-1} · 1.73 m\textsuperscript{2}</td>
<td>-57.8±46.2</td>
<td>58.5±40.5</td>
<td>0.141</td>
</tr>
<tr>
<td>∆ urine cGMP, nmol · h\textsuperscript{-1} · 1.73 m\textsuperscript{2}</td>
<td>9.07±34.0 (20)</td>
<td>-32.2±79.7 (10)</td>
<td>0.563</td>
</tr>
<tr>
<td>∆ urine microalbumin, μg · h\textsuperscript{-1} · 1.73 m\textsuperscript{2}</td>
<td>57.8±46.2</td>
<td>58.5±40.5</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Δ C\textsubscript{creatinine}, change in creatinine clearance; ∆ C\textsubscript{inulin}, change in inulin clearance; ∆ sinsulin, change in serum insulin; ∆ sArginine, change in serum arginine; ∆ FE\textsubscript{Na\textsuperscript{+}}, change in fractional excretion of sodium; ∆ FE\textsubscript{Li\textsuperscript{+}}, change in fractional excretion lithium; ∆ urine NO\textsubscript{x}, change in urinary nitrate or nitrite; ∆ urine cGMP, change in urinary cGMP; ∆ urine microalbumin, change in microalbuminuria.
GFR correlated with change in urinary NOx excretion ($r=0.786, P<0.001$). Although urinary cGMP excretion differed substantially between the FH groups throughout the study ($P<0.001$, Table 3), it did not change acutely in response to amino acid infusion ($P=0.703$).

### Amino Acids, Insulin, and Glucose

During amino acid infusion, serum arginine concentration rose substantially higher in the FH+ subjects ($P=0.0008$, Table 2), even though the FH groups received identical amino acid infusion doses, standardized to kilograms of body weight (see Methods), and their body weights were similar (Table 1). By ANOVA (Table 3, Figure 3), not only did amino acid infusion increase serum arginine ($P<0.001$), but also arginine concentration was consistently higher in the FH+ group ($P=0.01$) and the FH+ group had a greater arginine increment during amino acid infusion ($P=0.003$).

The ratio of the NOS substrate arginine to the NOS product NOx metabolites NO2 and NO3 (arginine/NOx) rose far higher in FH+ than in FH− subjects during amino acid infusion ($P=0.002$, Table 2 and Figure 4). By ANOVA (Table 3), the ratio rose overall during amino acid infusion ($P<0.001$) but far more in FH+ subjects ($P=0.003$).

The plasma concentration of each of the natural amino acids rose substantially during amino acid infusion ($P<0.001$; time effect on ANOVA); 2- to 3-fold increments were noted for each amino acid. Although the FH groups received identical amino acid infusion doses (standardized to kilograms of body weight), the FH+ subjects had higher overall serum concentrations of 6 amino acids (arginine [P=0.01], isoleucine [P=0.023], leucine [0.014], methionine [P=0.014], phenylalanine [P=0.014], and valine [P=0.031]) and showed greater increments in the plasma concentration of 3 amino acids during infusion (FH×time effect on ANOVA), including not only arginine (P=0.004) but also methionine (P=0.014) and phenylalanine (P=0.014).

The FH groups did not differ in amino acid effects on serum glucose or insulin (Table 2). The amino acid infusion caused an overall (time effect on ANOVA; Table 3) increase in serum insulin ($P<0.001$). Despite the rise in serum insulin and the lack of glucose in the intravenous infusate, serum glucose rose during the amino acid infusion ($P<0.001$);
stimulation of glucagon release by amino acids (especially arginine and alanine)\(^2\) may contribute to the rise in glucose in this setting. Indeed, serum glucose was greater in FH+ subjects during the entire study period (\(P=0.049\), Table 3).

**Predictors of Amino Acid–Induced Change in GFR**

We evaluated whether any of 14 likely independent variables at baseline (demographic/physical [FH, age, gender, body mass index], physiological [mean arterial pressure, \(C_{\text{inulin}}\)], or biochemical [serum insulin, serum glucose, serum arginine, \(\text{FE}_{\text{Li}}\), \(\text{FE}_{\text{Na}}\), urinary sodium excretion, urinary cGMP excretion, urinary NOx excretion]) could predict a change in GFR (\(C_{\text{inulin}}\)) during amino acid infusion. Although this 14-variable model was not significant (\(F=2.06, P=0.109\)), stepwise regression produced a significant (multiple \(R^{2}=0.670, F=7.04, P=0.0013\)) model in which 3 independent variables (FH, baseline \(C_{\text{inulin}}\), and baseline serum insulin) predicted \(\approx 45\%\) (adjusted \(R^{2}=0.449\)) of the variance in GFR response to amino acids; of these 3 independent variables, the most significant were hypertension FH (\(T=2.48, P=0.019\)) and baseline \(C_{\text{inulin}}\) (\(T=2.70, P=0.012\)). In simple (bivariate) analyses, change in GFR correlated with initial insulin clearance (\(r=−0.488, n=39, P=0.002\)), serum insulin (\(r=−0.338, n=38, P=0.038\)), and FH (\(r=0.435, n=39, P=0.006\)).

Thus, a positive family history for hypertension and higher initial GFR and serum insulin each predicts a blunted GFR response to amino acids.

**Covariates (Correlates) of Amino Acid–Induced Change in GFR**

We also evaluated whether amino acid–induced change in several biochemical independent variables (urinary cGMP excretion, urinary NOx excretion, serum insulin, serum arginine, \(\text{FE}_{\text{Li}}\), or \(\text{FE}_{\text{Na}}\) correlated with change in GFR (\(C_{\text{inulin}}\)) during amino acid infusion. Stepwise regression created a significant model (multiple \(R^{2}=0.803\), adjusted \(R^{2}=0.610, F=18.2, P<0.0001\)) in which change in urinary NOx excretion (\(T=4.24, P=0.0004\)) and change in \(\text{FE}_{\text{Li}}\) (\(T=−2.10, P=0.049\)) jointly predicted \(\approx 61\%\) of the GFR response to amino acids. On simple (bivariate) analyses, change in GFR correlated with change in both urinary NOx excretion (\(r=0.786, n=29, P<0.001\)) and \(\text{FE}_{\text{Li}}\) (\(r=−0.506, n=29, P=0.01\)). Thus, the amino acid–induced increment in GFR correlated with both an increment in urinary NOx excretion and a decrement in \(\text{FE}_{\text{Li}}\).

**Change in GFR: Variance, Frequency Distribution, and Maximum Likelihood Analysis for Modality**

By maximum likelihood analysis, the change in GFR in these 39 subjects after amino acids best fit a model of 2 distributions rather than 1 distribution (\(\chi^{2}=29.6\), 4 degrees of freedom, \(P<0.001\)), and a model of three distributions was not superior (\(\chi^{2}=0.474, 2 df, P=0.78\)).

A frequency histogram of the GFR responses in the 2 FH groups (plot not shown) revealed a clear shift in peak frequency between the FH− (peak at 0% to 25% change) and FH+ (peak at 25% to 50% change) groups; however, the FH+ group did not display clear-cut evidence of bimodality in GFR response, and the variance (SD\(^2\)) of GFR response to amino acids did not differ in the FH+ and FH− groups (\(F_{\text{max}}=0.365, P=0.964\)).

**Discussion**

Renal functional reserve is impaired in a variety of renal disease states, including systemic hypertension.\(^8,9\) The absence of renal functional reserve is associated with hyperfiltration, which may represent a pathogenic process that contributes to glomerulosclerosis and hence to the progression of renal insufficiency.\(^8,9\)

Here, we investigated renal functional reserve in young (average age \(\approx 30\) years) normotensive adults stratified by genetic risk (family history) of hypertension and explored potential mechanisms underlying group differences. In con-
firmation of the observations of Grunfeld et al.\textsuperscript{10} we found that renal functional reserve was already blunted in still-normotensive subjects at genetic risk of hypertension. Of note, FH\textsuperscript{+} subjects had somewhat higher GFR values at baseline when measured by $C_{\text{creatinine}}$ (by $\approx 25\%$, $P=0.014$) although not when measured by $C_{\text{inulin}}$ ($P=0.150$); early elevation in GFR in normotensive subjects at genetic risk of hypertension has been observed previously\textsuperscript{22} and may in itself represent a form of hyperfiltration even in the absence of an exogenous amino acid challenge. Indeed, in a multivariate analysis of baseline predictors of renal functional reserve in this study, not only hypertension family history status ($T=2.48$, $P=0.020$) but also baseline GFR (as $C_{\text{inulin}}$, $T=-2.70$, $P=0.012$) were significant predictors of the GFR increment in response to amino acids, with higher baseline GFR predicting smaller GFR response. Perhaps subjects with higher initial GFR values cannot respond with full increments in GFR after amino acids, because their GFR values are already closer to a putative “ceiling” for the amino acid effect.

Thus, blunted renal functional reserve after amino acid infusion is an early-penetrance phenotype\textsuperscript{5–7} in the course of development of human genetic hypertension, because it is already found even in the still-normotensive offspring (FH\textsuperscript{−}) of essential hypertensives. The early appearance of this phenotype in still-normotensive FH\textsuperscript{+} subjects suggests a pathophysiological role for this renal phenotype in the later development of hypertension, rather than simply a late response to high blood pressure. Indeed, the FH\textsuperscript{+} subjects displayed baseline GFR values well within normal limits, although their baseline microalbumin excretion rates were already higher than those of FH\textsuperscript{−} controls. A decline in renal functional reserve in the FH\textsuperscript{+} group was heterogeneous in frequency distribution, although not clearly bimodal on inspection of the histogram (plot not shown), weighing against a major gene (or mendelian) effect on this phenotype.\textsuperscript{7} Nevertheless, discrete subgroups may have contributed to the FH difference in GFR response, because on maximum likelihood analysis, the response best fit a model of 2 response distributions rather than 1 ($\chi^2=29.6$, $P<0.001$). It should be noted that familial resemblance (in FH\textsuperscript{+} subjects) does not necessarily indicate gene action, because families may share and transmit environmental and behavioral factors\textsuperscript{1–3}; nonetheless, we did study these individuals under controlled conditions, and the demographic and physical characteristics of the FH groups were similar.

What mediated the changes in GFR after amino acids in this study, and why were the GFR responses blunted in the FH\textsuperscript{+} subjects? One proposed explanation for the effect of amino acids on GFR is that the NOS substrate arginine is maintained, a finding similar to that observed in FH\textsuperscript{−} individuals in this study. In contrast, reductions in proximal tubular reabsorption during glycine administration characterize the conditions associated with absence of a vasodilatory response.\textsuperscript{11,24} The reduction in proximal tubular reabsorption during glycine is likely to activate the tubuloglomerular feedback system by increasing distal delivery of sodium chloride.\textsuperscript{25} Activation of this system in turn limits glomerular arteriolar vasodilation and the increase in nephron filtration rate. Therefore, one could postulate that reduced proximal tubular transport in FH\textsuperscript{+} may constitute an early penetrance phenotype whose expression can be unmasked by an amino acid infusion. The mechanism responsible for the reduction in proximal reabsorption in the FH\textsuperscript{+} remains to be defined.

Another unexpected finding in this study was the difference in plasma amino acids levels displayed by FH\textsuperscript{+} individuals compared with FH\textsuperscript{−} individuals (Tables 2 and 3, Figure 2). During the amino acid infusion, FH\textsuperscript{+} subjects displayed greater increments (FH\textsuperscript{+}×time effect) in serum insulin, methionine, and phenylalanine even though the same amino acid dose was administered to each individual (per kg body wt). Thus, the FH\textsuperscript{+} subjects experienced even greater serum amino acid stimuli to hyperfiltration, excluding a simple pharmacokinetic explanation for blunted renal functional reserve in the FH\textsuperscript{+} subjects.

The mechanism responsible for impaired disposition of the amino acid load in FH\textsuperscript{+} remains ill defined. Insulin plays a crucial role in translocation of not only glucose but also amino acids from the extracellular to the intracellular space\textsuperscript{26}; specific insulin effects on the transport of cationic amino acids (eg, arginine) are especially well established.\textsuperscript{22,27,28,29} The FH\textsuperscript{+} subjects displayed multiple signs of insulin resistance, including (1) higher overall serum glucose concentrations, (2) higher overall serum concentrations of several amino acids (arginine, isoleucine, leucine, methionine, phenylalanine, and valine), and (3) greater increments in 3 amino acids during amino acid infusion (arginine, methionine, and phenylalanine), despite increments of serum insulin at least as great as those seen in the FH\textsuperscript{−} group. Thus, a relative
impairment in the ability to drive amino acids into cells may be a manifestation of insulin resistance in persons at genetic risk of hypertension. If amino acid resistance to the effects of insulin is a general feature of insulin-resistant states (eg, hypertension, obesity, type II diabetes, and renal failure), then our results could have general implications that complicate the interpretation of renal functional reserve studies in such states; namely, a given amino acid dose is likely to achieve a higher plasma concentration of that amino acid in an insulin-resistant state. Thus, resistance to the physiological (GFR) actions of amino acids is all the more remarkable in such states. Indeed, resistance to the GFR-elevating actions of amino acids has been described in several insulin-resistant states, including hypertension,9,24 experimental diabetes,29 and renal failure.29 If intracellular translocation of amino acids is required for elevation of GFR, then perhaps it is not surprising that GFR responses to amino acids are blunted in such insulin-resistant states. Indeed, because NOS is a cytosolic enzyme, the most common mechanistic model for amino acid effects on GFR (arginine-NOS-NO·-cGMP) is likely to involve NOS action only on intracellular arginine. Hence, our results suggest a plausible, novel explanation for blunted GFR in insulin-resistant states: diminished substrate (arginine) delivery to NOS. This explanation is also compatible with frequent observations of improved glomerular reserve after ACE inhibition,29 because some ACE inhibitors may actually improve (decrease) insulin resistance in hypertension.30

An assignment of family (parental) history of hypertension (Table 1) is not a matter of certainty, especially because the age-dependent penetrance of hypertension is variable and may not occur until well into the sixth decade of life.1,5,6 Indeed, the parents of our FH– individuals were, on average, younger than the parents of the FH+ group (53.1 ± 1.1 versus 59.2 ± 1.5 years, P = 0.013), increasing the possibility of miscategorization of some members of the FH– subject group; however, such misclassification would tend to abolish (rather than create) the numerous biochemical and physiological distinctions we observed between the FH groups (Tables 1 to 3, Figures 1 to 4).

We studied only individuals of European ancestry here (Table 1). Because hypertensives of sub-Saharan African ancestry display differences in autoregulation of glomerular filtration,41 it will be of particular interest to investigate such subjects with similar protocols.

In conclusion, blunted glomerular reserve in response to amino acid infusion in still-normotensive young adults with a positive family history of hypertension is an early-penetration phenotype in the natural history of hypertension. Our results suggest several pathogenic features that may be at work in creating this phenotype, including insulin resistance to the amino acid–translocating effects of this hormone, baseline hyperfiltration, and decreased proximal tubular reabsorption during amino acid infusion. Phenotypes with earlier penetrance,5–7 such as diminished renal functional reserve, may be especially useful in genetic analyses of hypertension. Coupled with the pathophysiological importance of renal functional reserve or hyperfiltration,8 the further investigation of such phenotypes becomes increasingly attractive in genetic linkage or association studies probing the role of particular genes governing renal involvement in the development or consequences of human essential hypertension.

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