Decrease of Serum Paraoxonase Activity in Chronic Renal Failure

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Abstract. Paraoxonase is an esterase that hydrolyzes organophosphate compounds. The enzyme is associated with HDL and could protect LDL against peroxidation, which suggests a possible involvement of paraoxonase in the antiatherogenic properties of HDL. Paraoxonase activity has been shown to be low in patients with myocardial infarction, diabetes mellitus, or familial hypercholesterolemia. Because cardiovascular disease is the main cause of death in chronic renal failure, serum paraoxonase activity was measured by spectrophotometry using three synthetic substrates (phenyl acetate, paraoxon, and 4-nitrophenyl acetate) in 305 patients with kidney disease, including 47 patients with non-end-stage chronic renal failure, 104 patients treated with hemodialysis, 22 patients treated with peritoneal dialysis, and 132 renal transplant patients. Patients were compared with two groups of aged-matched control subjects (total number = 195). Especially with 4-nitrophenyl acetate, paraoxonase activity was lower in patients with some degree of renal insufficiency (chronic renal failure [P < 0.05], chronic hemodialysis [P < 10^{-4}], chronic peritoneal dialysis [P < 10^{-4}]) than in control subjects. In transplant patients, paraoxonase activity was not found to be different from that in control subjects. The decrease of paraoxonase activity and thus the reduction of its antiatherogenic properties in renal failure could be an essential factor of premature vascular aging, especially when dialysis is used. Renal transplantation seems to restore paraoxonase activity.

Cardiovascular disease is the main cause of death in chronic renal failure (CRF) patients (accounting for approximately 50% of cases; references 1 and 2). The relative risk of myocardial infarction is five times greater in patients on renal replacement therapy than in the general population (3). No clear explanation of this “accelerated” atherosclerosis has been found. Several factors, including increased prevalence of hypertension, diabetes mellitus, and lipid abnormalities, may contribute (4,5). After renal transplantation, atherosclerosis remains a major cause of mortality, and dyslipidemias are also frequently observed (6). Recently, several studies have shown a higher susceptibility of LDL to oxidation in CRF patients, suggesting that the protection of lipoproteins against oxidation might be impaired (7–9).

Because the oxidation hypothesis of atherosclerosis seems to be currently established (oxidized LDL promoting a number of processes leading to the formation of atherosclerotic plaques in the arterial wall: enhancement of macrophage uptake, cytotoxicity toward endothelial cells, immunogenicity, and production of oxysterols, cytokines, and growth factors) (10,11), several groups have studied the HDL-linked enzyme paraoxonase/arylesterase (12,13). Paraoxonase was first known for its protective activity against the toxicity of organophosphorus compounds (by hydrolyzing the toxic metabolite of parathion: paraoxon) and its ability to hydrolyze several aromatic carboxylic acid esters (such as phenyl acetate and 4-nitrophenyl acetate) (14). More recently, evidence has emerged linking human serum paraoxonase to atherosclerosis: It has been shown that in vitro purified paraoxonase decreased LDL lipid peroxidation (15) and that purified paraoxonase significantly reduced the ability of mildly oxidized LDL to induce monocyte–endothelial interactions (16). Thus, a protective role of paraoxonase against atherosclerosis has been suggested, as well as an explanation of the protective role of HDL lipoproteins against LDL oxidation. Other studies support this hypothesis by showing that a decreased activity was noticed in high cardiovascular risk patients with diabetes mellitus, heterozygous familial hypercholesterolemia, or myocardial infarction (17,18). In a previous study, we showed that paraoxonase activity decreased with age (19).

Caucasian populations display a triphasic distribution of serum paraoxonase activity toward paraoxon: Three phenotypes and genotypes (AA [low activity], AB [intermediate activity], and BB [high activity]) have been defined (20–22). Genetic studies have associated that variation with two paraoxonase isozymes whose difference is related to a single amino acid (glutamine or arginine 191) (23,24). A second polymorphism of the paraoxonase gene in position 54 is known; it has been shown to affect the concentration of paraoxonase, but not its specific activity (24–26). Recently,
two groups associated a higher coronary risk to the AB and BB phenotypes (27,28), whereas three other groups found no association between paraoxonase gene polymorphism and coronary heart disease (29–31).

Because cardiovascular disease is the main cause of death in CRF, we measured paraoxonase activity in that population in which atherosclerosis incidence is high and premature. The relation between CRF and atherosclerosis is not well known.

Materials and Methods
Patients and Control Subjects
The control population (n) consisted of 195 (120 women, 75 men; mean age: 50.5 ± 24.5 yr [range, 17 to 105]) apparently healthy subjects who either attended a routine health check or were staff of the Dupuytren Hospital. Ninety-seven subjects were under age 65 and were not taking medication; 98 were older and were taking less than three medications. None had renal disease, familial hypercholesterolemia, diabetes, or myocardial infarction. The 305 CRF patients studied (132 women, 173 men) were either in- or outpatients of Dupuytren Nephrology Unit. There were 47 nondialyzed, non-end-stage renal disease (NESRD) patients (20 women, 27 men; mean age: 62.6 ± 17.6 yr [17 to 86 yr]; creatinine clearance: 22.1 ± 15.5 ml/min per 1.73 m² [range, 6 to 76]), 104 hemodialysis (HD) patients (40 women, 64 men; mean age: 58.7 ± 15.2 yr [20 to 83 yr]), 22 peritoneal dialysis (PD) patients (10 women, 12 men; mean age: 63.0 ± 18.7 yr [23 to 85 yr]), and 132 renal transplant (RT) patients (62 women, 70 men; mean age: 45.6 ± 12 yr [16 to 68 yr]; creatinine clearance: 48.4 ± 17.9 ml/min per 1.73 m² [range, 11 to 140]). All control subjects and patients were unrelated and Caucasian. Treatments were recorded. The study protocol was approved by the local ethics committee.

Methods
Serum paraoxonase activity was measured by spectrophotometry using three synthetic substrates: paraoxon (diethyl-4-nitrophenyl phosphate), phenyl acetate, and 4-nitrophenyl acetate.

The hydrolysis of paraoxon by the B isozyme was shown to be stimulated by a molar solution of NaCl, whereas the A isozyme was not. The hydrolysis of phenyl acetate was the same with both isozymes. This property allowed Eckerson et al. (20) to calculate the phenotype of a given subject by the following ratio:

\[ R = \frac{U(\text{paraoxon}) \times 10^3}{U(\text{phenyl acetate})} \]

(Enzyme activities are expressed in international units (U) per milliliter of serum: 1 U corresponds to the quantity of enzyme that hydrolyses 1 μmol of substrate per minute, at the given pH and temperature.) This ratio compares the hydrolysis of paraoxon in the presence of 1 M NaCl to the hydrolysis of phenyl acetate without NaCl and usually identifies three groups in Caucasian healthy populations. These groups match the three phenotypes: AA, AB, or BB. This “dual substrate method” correlates well with results obtained from genetic studies (25).

Venous blood was sampled (5 ml in a Vacutainer tube without additive) after an overnight fast, and serum was assayed on the same day or stored at −80°C until the assay. All reagents used in the different assays were supplied by Sigma (Paris, France). Analyses were performed at 25°C with an ultraviolet/visible spectrophotometer (UV 1205 Shimadzu, Courtaboeuf, France) equipped with a Peltier thermostated unit. Absorbance was measured automatically every 0.5 s or every second during 1 min. The amount of serum in the assay was determined so that the final absorbance did not exceed 1. Initial rates were computed by linear regression and expressed in U/ml. The within-day and day-to-day coefficients of variation were <5% (32).

Assays
Parnoxanase activity was measured with three synthetic esters: paraoxon (20), phenyl acetate (20), and 4-nitrophenyl acetate (33).

With paraoxon, 500 μl of 2/5 prediluted serum was added to 2 ml of glycine buffer (50 mM, pH 10.05, 25°C) containing 1.0 M NaCl, 1.0 mM CaCl₂, and 1.25 mM paraoxon (O,O-diethyl-O-4-nitrophenylphosphate). The rate of generation of 4-nitrophenol was determined at 412 nm \( (ε_{412} = 17,000 \text{ M}^{-1} \cdot \text{cm}^{-1} \) at pH 10.05). In addition, the hydrolysis of paraoxon was followed over 15 min in a small group of 20 subjects (five of each category) with a measurement wavelength set at 450 nm \( (ε_{450} = 3500 \text{ M}^{-1} \cdot \text{cm}^{-1}) \) to avoid excessive absorbances.

With phenyl acetate, 500 μl of 1/100 prediluted serum was added to 2 ml of Tris-HCl buffer (9 mM, pH 8.00, 25°C) containing 0.9 mM CaCl₂, and 1.25 mM phenyl acetate. The rate of generation of phenol was determined at 270 nm \( (ε_{270} = 1306 \text{ M}^{-1} \cdot \text{cm}^{-1}) \). With 4-nitrophenyl acetate, 250 μl of 1/20 prediluted serum was added to 2 ml of Tris-HCl buffer (25 mM, pH 7.4, 25°C) containing 1.0 mM CaCl₂, 2.5% methanol, and 0.625 mM 4-nitrophenyl acetate. The rate of generation of 4-nitrophenol was determined at 402 nm \( (ε_{402} = 14,000 \text{ M}^{-1} \cdot \text{cm}^{-1}) \) at pH 7.4.

With each substrate, the kinetic curves were linear for at least 1 min. There was no evidence of pre-steady-state kinetics.

Statistical Analyses
The S-Plus software (34) was used. The statistical distribution of enzyme activity was studied by the Kolmogorov–Smirnov test. Case-control difference in continuous variables was studied by t test or Mann–Whitney test. The χ² test was used to compare phenotypic distributions.

Two methods were used to compare the enzyme activities of the patients and the control subjects. The first method involved the selection, within the control population, of a subgroup whose age matched the patients’ ages (i.e., no significant difference between the mean ages of the control and patient groups). This analysis needed one control subgroup (n = 156) for the NESRD, HD, and PD patients (whose ages were similar), and another one (n = 185) for the RT patients (whose age was significantly lower).

A second method used with the whole set of data (patients and control subjects, without previous selection) involved a multiple regression analysis, taking into account the influence of age, sex, and renal disease on enzyme activity, as well as the interaction between age and renal disease. The regression model was expressed as:

\[ \log(\text{Activity}) = a_0 + a_1 \times \text{Sex} + a_2 \times \text{Age} + a_3 \times \text{NESRD} + a_4 \times \text{HD} + a_5 \times \text{PD} + a_6 \times \text{RT} + b_1 \times \text{Age} \times \text{NESRD} + b_2 \times \text{Age} \times \text{HD} + b_3 \times \text{Age} \times \text{PD} + b_4 \times \text{Age} \times \text{RT} \] (with Sex = 1 for male and 0 for female; NESRD = 1 for NESRD patients or 0, HD = 1 for hemodialysis patients or 0, PD = 1 for peritoneal dialysis patients or 0, and RT = 1 for renal transplant patients or 0). This model was used in the total population (500 subjects), including control subjects (for whom NESRD = HD = PD = RT = 0).

Results
Figure 1 shows an example of kinetic curves obtained with paraoxon in four NESRD patients. There was no evidence of pre-steady-state kinetics (i.e., exponential decrease of hydrolysis rate toward a constant steady-state value), even when the
hydrolysis was followed for 15 min. The enzyme activities observed in our subjects are summarized in Tables 1 and 2. The hydrolysis rates of paraoxon exhibited wide variations in the control population as in CRF patients. With phenyl acetate and 4-nitrophenyl acetate, the variations (in both control and CRF groups) were high but smaller than with paraoxon. Wide dispersion was also noticed within the different phenotype groups (data not shown). Paraoxonase activity showed very different levels with the substrate used: When compared to the activity with paraoxon, phenyl acetate hydrolysis was almost 500-fold higher and 4-nitrophenyl acetate hydrolysis about 10-fold higher. This was observed in every group of subjects. The Kolmogorov–Smirnov test did not show significant departure from the log-normal distribution in each group.

The dual substrate method showed a trimodal repartition in our Caucasian control population (Figure 2), as in the pathologic CRF populations with a ratio \( R < 1.6 \) for homozygous AA, \( 1.6 \leq R < 4 \) for heterozygous AB, and \( R \geq 4 \) for homozygous BB subjects. According to the \( \chi^2 \) test, the apparent phenotype distributions were not significantly different in

**Table 1.** Paraoxonase activity (U/ml) assayed with the substrates 4-nitrophenyl acetate, paraoxon, and phenyl acetate in the serum of age-matched control subjects, patients with non-end-stage chronic renal disease, hemodialysis, and peritoneal dialysis.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>4-Nitrophenyl Acetate</th>
<th>Paraoxon</th>
<th>Phenyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (( n = 156 ))</td>
<td>1.64 ± 0.66</td>
<td>0.20 ± 0.14</td>
<td>94 ± 32</td>
</tr>
<tr>
<td></td>
<td>[0.45; 4.78]</td>
<td>[0.003; 0.804]</td>
<td>[37; 211]</td>
</tr>
<tr>
<td></td>
<td>1.18</td>
<td>0.09</td>
<td>70</td>
</tr>
<tr>
<td>NESRD (( n = 47 ))</td>
<td>1.42 ± 0.60(^b)</td>
<td>0.19 ± 0.16</td>
<td>95 ± 37</td>
</tr>
<tr>
<td></td>
<td>[0.63; 3.78]</td>
<td>[0.02; 0.98]</td>
<td>[42; 197]</td>
</tr>
<tr>
<td></td>
<td>1.27</td>
<td>0.15</td>
<td>84</td>
</tr>
<tr>
<td>HD (( n = 104 ))</td>
<td>1.07 ± 0.37(^c)</td>
<td>0.14 ± 0.07(^c)</td>
<td>74 ± 20(^c)</td>
</tr>
<tr>
<td></td>
<td>[0.11; 2.28]</td>
<td>[0.01; 0.34]</td>
<td>[25; 128]</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.13</td>
<td>71</td>
</tr>
<tr>
<td>PD (( n = 22 ))</td>
<td>1.12 ± 0.48(^c)</td>
<td>0.13 ± 0.08(^b)</td>
<td>95 ± 25</td>
</tr>
<tr>
<td></td>
<td>[0.53; 2.40]</td>
<td>[0.01; 0.32]</td>
<td>[54; 164]</td>
</tr>
<tr>
<td></td>
<td>0.98</td>
<td>0.12</td>
<td>92</td>
</tr>
</tbody>
</table>

\(^a\) Comparisons were performed with the \( t \) test. Results are given as mean ± SD, [minimum; maximum], and median. NESRD, non-end-stage renal disease; HD, hemodialysis; PD, peritoneal dialysis.

\(^b\) \( P < 0.05 \).

\(^c\) \( P < 0.001 \).
**Table 2.** Paraoxonase activity (U/ml) assayed with the substrates 4-nitrophenyl acetate, paraoxon, and phenyl acetate in the serum of age-matched control subjects and renal transplant patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>4-Nitrophenyl Acetate</th>
<th>Paraoxon</th>
<th>Phenyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (n = 185)</td>
<td>1.89 ± 0.92</td>
<td>0.22 ± 0.17</td>
<td>100 ± 33</td>
</tr>
<tr>
<td></td>
<td>[0.45; 6.18]</td>
<td>[0.016; 0.84]</td>
<td>[37; 252]</td>
</tr>
<tr>
<td></td>
<td>1.67</td>
<td>0.17</td>
<td>97</td>
</tr>
<tr>
<td>RT (n = 132)</td>
<td>1.80 ± 0.68</td>
<td>0.20 ± 0.12</td>
<td>106 ± 25</td>
</tr>
<tr>
<td></td>
<td>[0.33; 4.16]</td>
<td>[0.03; 0.69]</td>
<td>[34; 203]</td>
</tr>
<tr>
<td></td>
<td>1.66</td>
<td>0.15</td>
<td>106</td>
</tr>
</tbody>
</table>

* Comparisons were performed with the t test. Results are given as mean ± SD, [minimum; maximum], and median. No significant differences were found between patients and controls. RT, renal transplant.

Figure 2. Control subject phenotypes, determined by the dual substrate method. ●, A; ○, AB; ■, B.

control subjects (AA: 46%; AB: 42%; BB: 12%), in nontransplant CRF patients (AA: 49.7%; AB: 49.3%; BB: 6.4%), and in RT patients (AA: 49.2%; AB: 49.3%; BB: 6.4%). However, the calculated distributions in the pathologic groups may be doubtful, because we observed a significant increase of the activating effect of NaCl in the HD and TR groups (but not in the NESRD patients) (35).

Creatinine clearances of NESRD patients were significantly lower than creatinine clearances of RT patients (P < 0.0001). No significant correlation between paraoxonase activity and creatinine clearance was found.

Comparison of patients with age-matched control groups showed that the mean paraoxonase activity was diminished in NESRD, HD, and PD patients (Table 1). The highest modification was noticed in HD patients, whatever the substrate. In PD patients, the decrease was found mainly with paraoxon and 4-nitrophenyl acetate. In NESRD patients, a decrease was observed only with 4-nitrophenyl acetate, and was smaller than in HD or PD patients. The activity in RT patients did not differ significantly from the control subjects (Table 2). It is interesting to note that, among the three substrates, 4-nitrophenyl acetate was the only one that showed a modified activity in most patient groups.

Multiple regression analysis (Table 3) confirmed these results and, in addition, showed that paraoxonase activity decreased with age. This phenomenon was observed with all three substrates, and its magnitude was similar in control subjects, NESRD, and PD patients (due to the absence of significant interaction terms for these groups in the equation).

**Table 3.** Paraoxonase activity (U/ml) according to different substrates and factors

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Factor</th>
<th>4-Nitrophenyl Acetate</th>
<th>Paraoxon</th>
<th>Phenyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>a_0</td>
<td>Gender</td>
<td>0.3903^b</td>
<td>−0.5766^b</td>
<td>2.0856^b</td>
</tr>
<tr>
<td>a_2</td>
<td>Age</td>
<td>−0.0033^b</td>
<td>−0.0040^b</td>
<td>−0.0022^b</td>
</tr>
<tr>
<td>a_3</td>
<td>NESRD</td>
<td>−0.0621^c</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>a_4</td>
<td>HD</td>
<td>−0.4490^b</td>
<td>−0.3720^d</td>
<td>−0.1027^b</td>
</tr>
<tr>
<td>a_5</td>
<td>PD</td>
<td>−0.1698^b</td>
<td>−0.1678^c</td>
<td>NS</td>
</tr>
<tr>
<td>b_4</td>
<td>Age × HD</td>
<td>0.0043^b</td>
<td>0.0043^c</td>
<td>NS</td>
</tr>
<tr>
<td>r^2</td>
<td></td>
<td>0.57</td>
<td>0.32</td>
<td>0.47</td>
</tr>
</tbody>
</table>

^a Log (Activity) = a_0 + a_1 × Sex + a_2 × Age + a_3 × NESRD + a_4 × HD + a_5 × PD + a_6 × RT + b_1 × Age × NESRD + b_4 × Age × HD + b_5 × Age × PD + b_6 × Age × RT. Abbreviations as in Tables 1 and 2.

^b P < 0.001.

^c P < 0.05.

^d P < 0.01.
However, in HD patients, with 4-nitrophenyl acetate and paraoxon, the interaction term ($\beta_2$, Table 3) nearly compensated the coefficient of age ($\alpha_2$), thus making the influence of age negligible in this category of patients, with these substrates.

**Discussion**

Paraoxonase activity in CRF was investigated with three substrates: phenyl acetate, paraoxon, and 4-nitrophenyl acetate. The kinetic curves were always linear in our experimental conditions. Some authors (36,37) reported a pre-steady-state phase with paraoxon in some samples, but their conditions (substrate and enzyme concentrations, pH, solvent) differed widely from ours. It has been suggested that the reaction of paraoxon with serum cholinesterase could explain the initial "burst" of 4-nitrophenol observed in some experiments (36). Because paraoxon is an irreversible inhibitor of cholinesterase, the amount of 4-nitrophenol released by this reaction cannot exceed the molar concentration of the enzyme, which is approximately 50 nM in the average patient (O. Lockridge, personal communication). It is very unlikely that this reaction could have influenced our results, because the serum was diluted 2.5 times and the amount of 4-nitrophenol released was in the range 10 to 100 $\mu$M.

As shown previously in other high cardiovascular risk populations (17,18), this study showed that paraoxonase activity was lower in patients suffering from CRF than in control subjects, especially when HD or PD was required. In transplant patients, paraoxonase activity was not different from that in control subjects.

The decrease of paraoxonase activity was marked and significant in dialysis patients, leading to very low activities whatever the substrate. In NESRD patients, results were less significant with paraoxon than with 4-nitrophenyl acetate. Even if the latter substrate was never used when the relationship between paraoxonase activity and atherosclerosis was investigated, 4-nitrophenyl acetate has already been used in several studies for measuring paraoxonase activity and has been shown to be rather specific for this enzyme; it also has been shown to be a “discriminating” substrate (like paraoxon but unlike phenyl acetate), useful to phenotype paraoxonase (33). For the first time, this study suggests that 4-nitrophenyl acetate may be very sensitive for detecting variations in paraoxonase activity, perhaps because it provides higher activities within narrower ranges and thus probably diminishes analytical errors. Moreover, 4-nitrophenyl acetate is a nontoxic ester, unlike paraoxon, and thus would be more suitable for routine determinations.

As we noticed previously (19), paraoxonase activity decreased with age in control subjects. Considering the suspected protective role of paraoxonase in atherosclerosis, it is interesting to note that aging is accompanied by this (albeit modest) reduction in enzyme activity. Paraoxonase activity diminishing with time could be a non-negligible factor of increased atherosclerosis development in elderly people. Age also decreased paraoxonase activity in NESRD and PD patients but not in HD patients.

The reduced activity measured in patients did not seem to be related to phenotype differences (i.e., no excess of AA phenotypes in the patient population). In HD patients, the decrease in enzyme activity was detected with all three substrates, including phenyl acetate, which is not sensitive to the phenotype. In addition, this study did not demonstrate a significant difference between the phenotype distribution in the patient and control groups, or between these groups and literature data (38,39). It is not possible to completely dismiss a methodologic bias, because it has been shown that the stimulation of paraoxonase by NaCl, which is the basis of the phenotyping method used here, is more important in HD and RT patients, but not in NESRD patients (37). The direct determination of the genotype was not available to us. However, it seems justified to admit that, at least for NESRD patients, the decrease in enzyme activities is not related to the phenotype. In addition, the recent results of Hasselwander et al. (40) have shown that the amount of enzyme protein is not modified in patients with CRF, so that the observed alterations represent a decrease in the specific activity of the enzyme. These last results also seem to rule out the possible implication of a second polymorphism (Met/Leu) in position 54, since this polymorphism has a strong effect on enzyme concentration (26). We were not able to check these findings in our patients, however, because neither the determination of the genotype nor the immunossay of the enzyme was available to us.

In an Italian population, Schiavon et al. also found that paraoxonase activity (toward paraoxon) was significantly lower ($P < 0.01$) in 60 HD patients than in 64 healthy subjects (41). Mean paraoxonase activity was approximately 1.5 times higher in our French groups than in the Italian groups. Higher activities in our group could be explained by different assay conditions (buffers, pH, calcium concentrations) or by unknown environmental or population differences. But paraoxonase activity variations between non-HD and HD patients in each population were about the same (decrease ratio: 0.636 in our study versus 0.620 in Schiavon’s study), which corroborates the notion that dialysis or uremia probably induces changes in the enzyme even if the studied population or the paraoxonase activity assay conditions change. Schiavon et al. did not explore NESRD or PD patients.

In CRF patients, an increased effect of oxidative stress on LDL lipoproteins in vitro has been reported (7–9), as it had been noticed in patients with familial hypercholesterolemia (42), diabetes mellitus (43), or coronary heart disease (44). This work sustains the relationship between low paraoxonase activity and diseases with low antioxidant defense and excessive lipid peroxidation. Paraoxonase may play its protective role in atherogenesis by hydrolyzing some products of lipid peroxidation and consequently by limiting LDL oxidation and foam cell synthesis (16). This allows us to hypothesize that during uremia, loss of that protection could represent a very important factor in atherosclerosis. In a previous study, we found that in HD patients, HDL prevented LDL oxidation but with a significantly reduced effect when compared with control subjects (8). We have completed this study by measuring serum paraoxonase activity and HDL paraoxonase activity in five HD patients and in five control subjects. Results showed
that both serum and HDL paraoxonase activity were significantly lower in the five HD patients (data to be published). This could represent additional proof that a decrease in paraoxonase activity may lead to failure in the protection of LDL oxidation in renal failure.

In this cross-sectional study, we measured serum lipids in a sample of 32 healthy subjects and 40 CRF patients. Total cholesterol, triglyceride, and HDL cholesterol levels were not significantly different between patients and control subjects (data not shown). Several studies that tried to show a relationship between paraoxonase activity and apolipoproteins, serum cholesterol, or triglyceride levels gave somewhat contradictory results (45–47). Schiavon et al. (41) found no significant difference between lipid profiles (total cholesterol, triglycerides, HDL cholesterol, apo A-1, apo B levels, HDL/apo A-1 ratio) of HD patients and control subjects, but they showed that the paraoxonase activity/HDL cholesterol ratio was significantly lower in uremic patients than in control subjects without significant correlation between paraoxonase activity and HDL cholesterol level or apo A1 level. The lack of prevention of LDL oxidation by HDL could be explained by this qualitative alteration of HDL in renal failure.

In transplant patients, paraoxonase activity seems to be restored to normal levels. Paraoxonase activity was higher in RT patients whose creatinine clearances were also significantly higher than creatinine clearances of the NESRD patients. Paraoxonase metabolism is still unknown. The DNA coding for paraoxonase has been found in liver cells (14), but no study has explored paraoxonase in kidneys. Decreased activity during CRF, especially when renal replacement therapy is required (HD and PD), and restoration of this activity after renal transplantation suggest, for the first time, that the kidneys play an important role in paraoxonase metabolism. During the pretransplantation evaluation, the patients with the most severe and widespread atherosclerosis lesions were excluded. Consequently, a bias may have been introduced. However, in five patients in whom paraoxonase activity was measured both before and after transplantation, values during the HD period (which were low) did not differ from those in other HD patients; the values doubled after transplantation and still did not differ from those in other RT patients (preliminary work to be published).

During renal failure, we suggest that within the environment of the arterial wall, the accumulation of nitrogen-derived products could decrease paraoxonase activity by directly inhibiting the enzyme or by modifying its synthesis/secretion. The recent study of Hasselwander et al. seems to confirm the former mechanism by showing that the protein concentration was not decreased in HD patients compared with control subjects (40).

In conclusion, this preliminary epidemiologic study opens a new research area in the understanding of atherogenesis mechanisms in CRF. The involvement of paraoxonase in the excessive LDL lipoperoxidation, noticed in uremia, and its physiologic substrates need to be determined. This study presents a potential explanation of accelerated atherosclerosis in CRF. The decrease of paraoxonase activity and thus the decrease of its antiatherogenic properties in renal failure could be an important factor in premature vascular aging, especially when dialysis is used.

Acknowledgments
We thank Mr. J. Comte for technical support and Mrs. C. Frugier for the preparation of the manuscript.

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