Increased Oxidative Stress and Platelet Activation in Patients With Hypertension and Renovascular Disease

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Background—Hypertensive patients with renovascular disease (RVD) may be exposed to increased oxidative stress, possibly related to activation of the renin-angiotensin system.

Methods and Results—We measured the urinary excretion of 8-iso-prostaglandin (PG) F$_{2\alpha}$ and 11-dehydro-thromboxane (TX) B$_2$ as indexes of in vivo lipid peroxidation and platelet activation, respectively, in 25 patients with RVD, 25 patients with essential hypertension, and 25 healthy subjects. Plasma renin activity in peripheral and renal veins, angiotensin II in renal veins, cholesterol, glucose, triglycerides, homocysteine, and antioxidant vitamins A, C, and E were also determined. Patients were also studied 6 months after a technically successful angioplasty of the stenotic renal arteries. Urinary 8-iso-PGF$_{2\alpha}$ was significantly higher in patients with RVD (median, 305 pg/mg creatinine; range, 124 to 1224 pg/mg creatinine) than in patients with essential hypertension (median, 176 pg/mg creatinine; range, 48 to 384 pg/mg creatinine) or in healthy subjects (median, 123 pg/mg creatinine; range, 58 to 385 pg/mg creatinine). Urinary 11-dehydro-TXB$_2$ was also significantly higher in RVD patients compared with healthy subjects. In RVD patients, urinary 8-iso-PGF$_{2\alpha}$ correlated with 11-dehydro-TXB$_2$ ($r_s=0.48; P<0.05$) and renal vein renin ($r_s=0.67; P<0.005$) and angiotensin II ($r_s=0.65; P=0.005$) ratios. A reduction in 8-iso-PGF$_{2\alpha}$ after angioplasty was observed in RVD patients with high baseline levels of lipid peroxidation. Changes in 8-iso-PGF$_{2\alpha}$ were related to baseline lipid peroxidation ($r_s=-0.73; P<0.001$), renal vein angiotensin II ($r_s=-0.70; P<0.01$) and renin ($r_s=-0.63; P<0.05$) ratios.

Conclusions—Lipid peroxidation is markedly enhanced in hypertensive patients with RVD and is related to activation of the renin-angiotensin system. Moreover, persistent platelet activation triggered or amplified by bioactive isoprostanes may contribute to the progression of cardiovascular and renal damage in this setting. (Circulation. 2002;106:2800-2805.)

Key Words: thromboxane • stenosis • hypertension • renin • angiotensin

Renovascular disease (RVD) represents a relatively rare form of secondary hypertension, which in most cases is associated with activation of the renin-angiotensin system as a result of the fall in renal blood flow and perfusion pressure due to renal artery stenosis.\(^1\) RVD is associated with increased cardiovascular mortality and is a major and increasingly prevalent cause of end-stage renal failure.\(^1\)–\(^3\) This may be related to increased angiotensin II activity, resulting in vasoconstriction, increased endothelin release, vascular remodeling, extracellular matrix deposition, and accelerated atherogenesis and glomerulosclerosis.\(^4\) These effects may contribute to the progression of cardiovascular and renal damage well beyond the effects of high blood pressure per se. Increased oxidative stress may occur as a consequence of renal artery stenosis and may play a role in the progression of RVD. Experimental evidence supports this hypothesis and suggests that increased oxidative stress may be related to activation of the renin-angiotensin system.\(^5\)–\(^6\) In patients with hypertension and RVD, the pro-oxidant effects of angiotensin II may amplify those due to coexisting metabolic disorders or long-standing hypertension.\(^1\)–\(^7\)

In the present study, we investigated whether oxidative stress is enhanced in RVD by measuring the urinary excretion of 8-iso-prostaglandin (PG) F$_{2\alpha}$, a noninvasive index of in vivo lipid peroxidation; 8-iso-PGF$_{2\alpha}$, which is also referred to as iPF$_{2\alpha}$-III, is a member of the F$_{2\alpha}$-isoprostanes, PG isomers that are formed nonenzymatically through free radical–catalyzed peroxidation of esterified arachidonate in cell membranes and circulating lipoproteins.\(^8\) Enhanced formation of F$_{2\alpha}$-isoprostanes has been reported in association with several
cardiovascular risk factors and has been found to correlate with in vivo thromboxane (TX) A2 biosynthesis.9–11 8-Isoprostanes, resulting in persistent platelet activation in RVD, are closely related to TXA2/PGH2 receptors.12 These activities are mediated by the interaction with receptors that are distinct from but closely related to TXA2/PGH2 receptors.13,14 Thus, we tested the hypothesis that angiotensin II–dependent oxidant stress is responsible for enhanced formation of bioactive F2-isoprostanes, resulting in persistent platelet activation in RVD hypertension.

### Methods

#### Subjects

We studied 25 patients with RVD, 25 subjects with essential hypertension, and 25 healthy subjects (Table 1). Patients were recruited among those admitted to the departments of Internal Medicine of our Institutions. Diagnosis of hypertension associated with RVD was made on the basis of angiographic evidence of renal artery stenosis in patients suspected of having renovascular hypertension on clinical grounds and positive renal scintigraphy and/or echo-Doppler scan of the renal arteries.15,16 Angiographic studies revealed the presence of significant stenosis (exceeding 65% of the lumen diameter) of a renal artery that was due to fibromuscular dysplasia in 4 subjects (aged 17 to 28 years; 3 women) and atherosclerotic lesions in the other 21 patients (aged 52 to 77 years). Four patients had bilateral stenosis. All patients were on antihypertensive treatment at the time of study. Angiotensin-converting enzyme (ACE) inhibitors and antagonists of the angiotensin receptor were replaced with other antihypertensive drugs at least 15 days before sampling to avoid interference with angiotensin II release or activity. Five patients receiving low-dose aspirin continued their antiplatelet treatment. Five patients were cigarette smokers (10 to 30 cigarettes per day) and 2 had type 2 diabetes mellitus (one treated).

All RVD patients underwent a revascularization procedure of the ischemic kidney. Percutaneous transluminal angioplasty of the stenotic renal artery (PTRA) was performed in 24 patients. Stents were also implanted in patients with atherosclerotic lesions, and both renal arteries were dilated in patients with bilateral stenosis. Because of the presence of an aneurysm of the abdominal aorta, a prosthetic graft was applied surgically to the remaining patient.

Essential hypertension was diagnosed on the basis of negative results of investigation for secondary hypertension that included renal scintigraphy and/or echo-Doppler scan of the renal arteries. To match the RVD patients for potentially confounding factors, 2 of the 25 patients with essential hypertension had type 2 diabetes mellitus and 5 were cigarette smokers (10 to 30 cigarettes per day). Essential hypertensive patients were being treated with calcium antagonists, α-receptor blockers, or diuretics (n = 15) or were untreated (n = 10) at the time of study.

Atherosclerotic lesions of carotid arteries (>30% reduction in lumen diameter) were detected in 15 RVD patients and 13 patients with essential hypertension.

Twenty-five healthy subjects were also recruited among members of the medical and nursing staff and their relatives in Verona and were matched with hypertensive patients for sex and age.

#### Study Protocol

A cross-sectional study was performed to compare the urinary excretion rates of 8-iso-PGF2α, and 11-dehydro-TXB2 in hypertensive patients with RVD and control subjects. The contribution of renal artery stenosis to oxidative stress and platelet activation was further investigated by comparing the urinary excretion of 8-iso-PGF2α, and 11-dehydro-TXB2 before and after 6 months after PTRA.

In the cross-sectional study, all subjects were studied using the same protocol, which consisted of clinical evaluation, blood pressure measurement, and blood and urine sampling. Both RVD patients and those with essential hypertension were hospitalized during the study and received a standard diet containing ~150 mmol of sodium per day. They also underwent an echo-Doppler evaluation of the carotid arteries.

The urinary excretion of 8-iso-PGF2α, and 11-dehydro-TXB2 was evaluated from overnight urine collections (from 8 PM to 8 AM). Urine sampling was repeated twice, 2 days apart. The timing and total volume were recorded, and two 50-mL aliquots were stored at

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Subjects (n = 25)</th>
<th>Essential Hypertensives (n = 25)</th>
<th>RVD Hypertensives (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>11 (44)</td>
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</tr>
<tr>
<td>Age, y</td>
<td>57 (22–69)</td>
<td>64 (30–74)</td>
<td>65 (17–77)</td>
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<tr>
<td>Cigarette smoking, n (%)</td>
<td>0 (0)</td>
<td>5 (20)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.4 (17.5–30.1)</td>
<td>26.6 (18.4–33.3)*</td>
<td>24.8 (18.4–34.4)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120 (105–140)</td>
<td>150 (130–185)‡</td>
<td>165 (110–220)§</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80 (70–90)</td>
<td>95 (65–110)‡</td>
<td>90 (65–120)§</td>
</tr>
<tr>
<td>PRA, ng/mL per hour</td>
<td>. . .</td>
<td>0.70 (0.25–4.18)</td>
<td>1.45 (0.25–7.60)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.38 (4.16–8.54)</td>
<td>5.44 (4.21–6.60)</td>
<td>4.99 (3.88–9.20)</td>
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<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.15 (4.13–7.70)</td>
<td>5.11 (3.43–7.49)</td>
<td>5.45 (3.59–7.36)</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.55 (1.19–2.27)</td>
<td>1.34 (0.98–2.09)†</td>
<td>1.16 (0.67–2.00)§</td>
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<td>Creatinine clearance, mL/min</td>
<td>93 (62–141)</td>
<td>102 (50–147)</td>
<td>60 (19–133)§‡</td>
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<td>Homocysteine, μmol/L</td>
<td>9.9 (7.1–20.7)</td>
<td>14.1 (8.7–38.0)‡</td>
<td>15.3 (8.4–40.1)§</td>
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<td>Vitamin A, μmol/L</td>
<td>3.17 (0.31–6.66)</td>
<td>2.93 (0.97–4.59)</td>
<td>3.64 (0.87–7.53)</td>
</tr>
<tr>
<td>Vitamin C, μmol/L</td>
<td>39.7 (2.5–88.0)</td>
<td>25.5 (2.8–70.9)</td>
<td>29.0 (4.0–81.1)</td>
</tr>
<tr>
<td>Vitamin E, μmol/L</td>
<td>32.5 (16.2–67.3)</td>
<td>26.2 (14.1–39.4)†</td>
<td>23.4 (7.8–38.9)§</td>
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Data are expressed as median (range) or number (percent).

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Data are expressed as median (range) or number (percent).
Blood samples were taken to determine plasma glucose, total and HDL cholesterol, triglycerides, and creatinine. Plasma concentrations of homocysteine and the antioxidant vitamins A, C, and E were also determined to assess the oxidant-antioxidant balance. Blood was collected from a peripheral vein to measure plasma renin activity (PRA) in patients with RVD and essential hypertension. Before blood sampling, patients were kept in the supine position for at least 1 hour.

Blood pressure was recorded (the mean of 3 readings) between 10 AM and noon using a mercury column sphygmomanometer with the subjects in the supine position for 30 minutes. Body mass index was also calculated.

The effects of revascularization of the ischemic kidney on lipid peroxidation were evaluated in hypertensive patients with RVD. Blood samples were collected by catheterization from renal veins and the inferior vena cava distal to the ostium of the renal veins (for technical reasons, blood sampling was not done in 4 patients). The renal vein renin ratio (ie, the ratio of PRA in blood draining the stenotic kidney to PRA in venous blood from the contralateral kidney) was used as an index of the activation of the renin-angiotensin system and renal hyperperfusion in patients with unilateral stenosis. Plasma concentration of angiotensin II was also measured in blood from the renal veins and vena cava.

Urine samples were obtained from RVD hypertensives 6 months after revascularization (7 patients were lost to follow-up). Two urine collections and blood sampling were performed 2 days apart according to the previously described procedure (ACE inhibitors and antagonists of the angiotensin receptor were replaced with other antihypertensive drugs at least 15 days in advance). Patency of the treated vessels was evaluated and confirmed by echo-Doppler investigation of the renal arteries 6 months after PTRA.

The study protocol was approved by the ethics committees of the medical centers involved, and informed consent was obtained from all subjects.

Biochemical Analyses
PRA was measured using a radioimmunoassay to quantify the amount of angiotensin-I generated during 1 to 3 hours of incubation of plasma at 37°C and pH 5.7. The sensitivity of the assay was 0.25 ng/mL per hour, and its interassay variability was <11%. Angiotensin II was measured by radioimmunoassay after extraction of the peptide from plasma, as previously described. Immunoactive 8-iso-PGF2α and 11-dehydro-TXB2 were extracted from 20-mL urine aliquots and analyzed by previously validated radioimmunoassay techniques. Total homocysteine concentration in plasma was determined by high-performance liquid chromatography after derivatization using fluorescence detection. Plasma vitamins A, C, and E were also measured by high-performance liquid chromatography using a fluorescence detection system. The additional biochemical parameters were determined using a Technicon DAX 96 automated analyzer (Technicon Instruments).
Effects of Revascularization of the Ischemic Kidneys

The revascularization procedure had limited effects on blood pressure and renal function (Table 2), and all but 5 patients (3 with fibromuscular dysplasia of renal arteries) required long-term antihypertensive therapy after the procedure, although the number of administered drugs was reduced.

A trend toward reduction in the urinary excretion of 8-iso-PGF$_{2\alpha}$, and 11-dehydro-TXB$_2$, was observed after angioplasty (8-iso-PGF$_{2\alpha}$: median, $-66.6$ pg/mg creatinine; range, $-889.3$ to $226.5$ pg/mg creatinine; 11-dehydro-TXB$_2$: median, $-284.0$ pg/mg creatinine; range, $-4628$ to $518.0$ pg/mg creatinine). We observed that the variability in individual changes in urinary 8-iso-PGF$_{2\alpha}$ excretion could be largely accounted for by the variable baseline levels of lipid peroxidation (Figure 4). Moreover, the reduction in urinary 8-iso-PGF$_{2\alpha}$ after PTRA was detected in the patients who had baseline values $>300$ pg/mg creatinine (median, $-190.5$ pg/mg creatinine; range, $-889.3$ to $-29.0$ pg/mg creatinine; n=8; P=0.008); no significant change was observed in subjects with lower baseline F$_2$-isoprostane excretion (median, $-24.7$ pg/mg creatinine; range, $-87.5$ to $226.5$ pg/mg creatinine; n=10; P=0.37). Changes in 8-iso-PGF$_{2\alpha}$ excretion were also related to baseline values of renal vein renin ratio (Figure 5A), renal vein angiotensin II ratio (Figure 5B), and peripheral PRA ($r_s=-0.61$; n=18; P=0.007). Stepwise multivariate regression analysis identified baseline 8-iso-PGF$_{2\alpha}$ as the only variable independently related to changes in urinary 8-iso-PGF$_{2\alpha}$ after revascularization (coefficient $\beta=-0.81$; P=0.002).

**Discussion**

Measurement of the urinary excretion of 8-iso-PGF$_{2\alpha}$ has been characterized as a reliable method of investigating lipid peroxidation in vivo and has been shown to reflect a
status of enhanced oxidative stress, regardless of the underlying pathophysiological triggers. A number of clinical conditions in which increased oxidative stress was postulated on the basis of experimental data or ex vivo tests have been reported to be associated with increased urinary excretion of 8-iso-PGF$_{2\alpha}$. In the present study, we demonstrated that this noninvasive index of lipid peroxidation is markedly increased in hypertensive patients with RVD compared with patients with essential hypertension with comparable levels of blood pressure and healthy normotensive subjects. A number of coexisting conditions may independently affect the oxidant/antioxidant balance and the level of lipid peroxidation. In our group of RVD hypertensives, the urinary excretion of 8-iso-PGF$_{2\alpha}$ does not seem to be related to the presence of conventional cardiovascular risk factors that may independently be associated with increased oxidative stress: none of them had hypercholesterolemia; moreover, low HDL cholesterol, diabetes mellitus, cigarette smoking, and overt atherosclerosis were equally represented in essential and essential hypertensives with baseline 8-iso-PGF$_{2\alpha}$, angiotensin II ratio, and renal vein renin ratio. This functional test is a reliable noninvasive index of lipid peroxidation and whether the source of urinary F$_{2}$-isoprostanes is located within the ischemic kidney or in the systemic circulation, although the observed relations between urinary 8-iso-PGF$_{2\alpha}$ and both renin ratio and angiotensin II ratio in renal veins suggest that lipid peroxidation may occur primarily in the hypoperfused kidney.

We also investigated the relationship between lipid peroxidation and platelet activation in RVD. Davi et al have described a consistent correlation between urinary excretion rates of 8-iso-PGF$_{2\alpha}$ and 11-dehydro-TXB$_{2}$ in patients with hypercholesterolemia, diabetes mellitus, and homozygous homocystinuria. In addition, further supportive evidence was obtained for a biochemical link between lipid peroxidation and platelet activation in vivo by showing that vitamin E dose-dependently reduced both 8-iso-PGF$_{2\alpha}$ and 11-dehydro-TXB$_{2}$; excretion in these diverse clinical settings. Consistent with these observations, we found that the rate of TXA$_{2}$ biosynthesis was markedly enhanced in RVD patients and closely correlated with the rate of F$_{2}$-isoprostane formation.

It is tempting to speculate that increased generation of bioactive F$_{2}$-isoprostanes and other isoeicosanoids may be responsible, at least in part, for increased platelet activation, the development of hypertension, and ischemic nephropathy in this setting. 8-iso-PGF$_{2\alpha}$ promotes platelet aggregation and induces platelet adhesion. Moreover, 8-iso-PGF$_{2\alpha}$ is a powerful constrictor of the renal vasculature and increases blood pressure. Besides being suggestive of a direct involvement in these functional responses, the increased rate of 8-iso-PGF$_{2\alpha}$ may reflect the presence of more abundant biologically active species formed by similar mechanisms. The hypothesis of a pathophysiological role of increased isoprostane generation is also supported by the observation that TXA$_{2}$/PGH$_{2}$ receptor blockers, but not cyclooxygenase inhibitors, reduced blood pressure in experimental renovascular hypertension.

In conclusion, we have identified RVD with hypertension as a clinical condition characterized by increased lipid peroxidation and platelet activation, presumably as a consequence of renin-angiotensin system activation. The use of ACE inhibitors or angiotensin receptor antagonists may represent a suitable strategy to probe the pathophysiological role of angiotensin II–dependent lipid peroxidation in this setting.

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