Lipid Peroxidation Is Not Increased in Patients With Untreated Mild-to-Moderate Hypertension

Jean-Luc Cracowski, Jean-Philippe Baguet, Olivier Ormezzano, Janine Bessard, Françoise Stanke-Labesque, Germain Bessard, Jean-Michel Mallion

Abstract—In contrast with the huge amount of experimental data available, only few and somewhat unconvincing clinical studies support the hypothesis that oxidative stress is involved in the early stages of essential hypertension in humans. Isoprostanes are chemically stable lipid peroxidation products of arachidonic acid, the quantification of which provides a novel approach to the assessment of oxidative stress in vivo. The main objective of this study was to quantify the urinary levels of 15-F₂₅-IsoP in the early stages of essential hypertension, using gas chromatography/mass spectrometry, by comparing 30 patients with never-treated mild-to-moderate hypertension with 30 gender- and age-paired healthy controls. Urinary 15-F₂₅-IsoP levels were not significantly different in hypertensive patients (69 ± 36 pmol/mmol creatinine) compared with controls (75 ± 34 pmol/mmol creatinine, 95% confidence intervals on differences: −23 to 13). No significant correlation was found between basal urinary 15-F₂₅-IsoP levels and age, low-density lipoprotein cholesterol, glucose, clinical pulse pressure, carotid intima-media thickness, left ventricular mass index, or aortic pulse wave velocity. In conclusion, this study shows that lipid peroxidation is not increased in never-treated mild-to-moderate hypertension. This suggests that oxidative stress is not implicated in the pathogenesis of human essential hypertension, at least in the early stages. (Hypertension. 2003;41:286-288.)

Key Words: hypertension, essential ■ prostaglandins ■ lipid peroxidation ■ oxidative stress ■ blood pressure monitoring, ambulatory

Hypertension is a common cardiovascular disorder, the pathogenesis of which remains unclear. Many animal experiments or in vitro studies in humans support the hypothesis that increased oxidative stress may be one of the initial triggers of vascular remodeling and elevated blood pressure.¹–³ In contrast to the huge amount of experimental data available for different species and strains, only few and relatively unconvincing clinical studies support such a hypothesis in the early stages of essential hypertension in humans because of the lack of a reliable clinical biomarker of lipid peroxidation in vivo.⁴

Isoprostanes are chemically stable lipid peroxidation products of arachidonic acid, and their quantification provides a novel approach to the assessment of oxidative stress in vivo.⁵ Among the isoprostane isomers, 15-F₂₅-IsoP is a stable, sensitive, and specific product of lipid peroxidation that is currently quantified in urine and plasma as a biomarker of lipid peroxidation.⁶

The main objective of this study was to quantify the urinary levels of 15-F₂₅-IsoP as a biomarker of lipid peroxidation in the early stages of essential hypertension by comparing patients with never-treated mild-to-moderate hypertension with gender- and age-paired healthy controls. The secondary objectives were to test whether 15-F₂₅-IsoP levels correlate with clinical blood pressure, left ventricular mass index, carotid intima media thickness, and aortic pulse wave velocity.

Methods

Patients

Thirty white patients referred to the hypertension department at Grenoble University Hospital were included in the study between January 2000 and October 2001. The inclusion criterion was untreated (never-treated) mild-to-moderate essential hypertension, clinically diagnosed according to the World Health Organization criteria (systolic blood pressure = 140–159 mm Hg and/or diastolic blood pressure = 90–99 mm Hg on 3 consecutive occasions). Exclusion criteria included secondary hypertension, previously treated essential hypertension, coronary heart disease, and the following potential confounding factors associated with increased F₂-isoprostane production: current cigarette smoking,⁷ hypercholesterolemia,⁸ and diabetes.⁹ Secondary hypertension was excluded by means of clinical and biochemical assessment. Coronary heart disease was excluded on the basis of clinical history, ECG, and echocardiography. The mean duration of hypertension was defined as the time between the diagnosis and inclusion in the study.

One healthy volunteer was selected for each patient, matched in terms of age (± 10 years), gender, and date of inclusion in the study (± 2 months). These healthy volunteers were selected from the general population, excluding in-hospital subjects. The same exclusion criteria applied to the control group.

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Study Design
This was a single-blinded, parallel-group, one-center study. Subjects were assessed as outpatients for eligibility and information. All subjects gave written informed consent, and the study was approved by the institutional review board of the hospital.

Both patients and control subjects underwent the following investigations over a period of 1 day: noninvasive 24-hour ambulatory blood pressure monitoring, echocardiography, carotid ultrasonography, carotid-femoral pulse wave velocity, total-, high-density lipoprotein-, and low-density lipoprotein–plasma cholesterol, and glucose quantification. These investigations were performed with a methodology previously described in detail.10,11

Urinary 15-F_{2t}-IsoP Measurements
Urine samples (20 mL) were collected between 8 and 10 AM in polyethylene tubes, immediately refrigerated and transferred to the laboratory, aliquoted, and stored at −20°C. Urinary levels of 15-F_{2t}-IsoP were measured using gas chromatography/mass spectrometry as previously described.12 The results were corrected to urinary levels of creatinine. Final results were expressed as picomole per millimole of creatinine. Observers were blinded to the source of samples for technical analysis.

Statistical Analysis
Sample size calculations were based on the main objective of detecting a difference of at least 25 pmol/mmol between patients and healthy controls, with α=0.05 and a power (1−β)=0.8. In our records, the mean 15-F_{2t}-IsoP levels in urine samples in healthy subjects is 79 pmol/mmol creatinine, with a standard deviation of 33. Using such data, 29 subjects are required in each group. Therefore, we decided to include 30 patients and 30 controls. Statistical analyses were performed using SPSS software (SPSS Inc). When normality of the distribution was verified, data were expressed as mean±SD. Comparisons were performed using the Student t test or one-way ANOVA. Where appropriate, a univariate ANOVA model was used to take into account age, cholesterol, and glucose as covariables. Relationships between continuous variables were evaluated by the Pearson correlation coefficient. Values of P<0.05 were considered significant.

Results
Subject Characteristics
The demographic, clinical, and baseline cardiovascular data of the patients and controls are listed in the Table.

Main Objective
Urinary 15-F_{2t}-IsoP levels were not significantly different in hypertensive patients (69±36 pmol/mmol creatinine) compared with controls (75±34 pmol/mmol creatinine, 95% confidence intervals on differences: −23 to 13).

Secondary Objectives
No significant correlation was found between basal urinary 15-F_{2t}-IsoP levels and age, low-density lipoprotein cholesterol, glucose, clinical pulse pressure, carotid intima-media thickness, left ventricular mass index, and aortic pulse wave velocity.

Discussion
This study shows that mild-to-moderate hypertension is not associated with increased lipid peroxidation, as reflected by the similar isoprostane levels observed in patients and controls.

F_{2t}-isoprostanes are free-radical–dependent metabolites of arachidonic acid, currently used as clinical biomarkers of lipid peroxidation.5 Among the different stereoisomers, 15-F_{2t}-IsoP is a stable, sensitive, and specific marker of lipid peroxidation, easily quantifiable in urine, whose the physiological variations are well documented.6 Among the cardiovascular risk factors, cigarette smoking, homozygous familial hypercholesterolemia, type II diabetes, and homocystinemia are associated with increased urinary levels of 15-F_{2t}-IsoP, demonstrating enhanced lipid peroxidation in such conditions. For other risk factors, such as polygenic hypercholesterolemia and type I diabetes, the results are more controversial, especially as 15-F_{2t}-IsoP levels are normal at the early stages of diseases such as type I diabetes with normal microalbuminuria and children’s type IIa hypercholesterolemia.6 Essential hypertension is a common condition often associated with other cardiovascular risk factors. To date, no study has clearly tested the hypothesis that lipid peroxidation is involved in the pathogenesis of hypertension. To provide a clear-cut answer, the present study was carefully conducted to avoid bias. To avoid the interference of drug therapy, only patients who had never been treated for hypertension were included. Precautions were taken to avoid a potential confounding bias in isoprostane measurement.6 Each patient was matched with an age- and gender-paired control. Patients with previously diagnosed diabetes or hypercholesterolemia and cigarette smokers were excluded. The diagnosis of high blood pressure was clinically performed. Although only mild-to-moderate hypertensive subjects were included, ambulatory blood pressure monitoring and aortic pulse wave velocity

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### Table: Characteristics of Controls and Never-Treated Mild-to-Moderate Hypertensive Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n=30)</th>
<th>Patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52±9</td>
<td>53±12</td>
</tr>
<tr>
<td>Female gender</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23±3</td>
<td>25±2</td>
</tr>
<tr>
<td>Disease duration, mo</td>
<td>41±57</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, g/L</td>
<td>2.17±0.32</td>
<td>2.24±0.33</td>
</tr>
<tr>
<td>LDL cholesterol, g/L</td>
<td>1.30±0.29</td>
<td>1.40±0.37</td>
</tr>
<tr>
<td>HDL cholesterol, g/L</td>
<td>0.67±0.13</td>
<td>0.60±0.21</td>
</tr>
<tr>
<td>Triglyceride, g/L</td>
<td>0.92±0.71</td>
<td>1.21±0.46</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.62±0.54</td>
<td>5.02±0.90</td>
</tr>
<tr>
<td>SBP clinic, mm Hg</td>
<td>125±9</td>
<td>152±10*</td>
</tr>
<tr>
<td>DBP clinic, mm Hg</td>
<td>81±5</td>
<td>95±9*</td>
</tr>
<tr>
<td>Pulse pressure clinic, mm Hg</td>
<td>44±6</td>
<td>58±15*</td>
</tr>
<tr>
<td>Ambulatory blood pressure monitoring:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour SBP, mm Hg</td>
<td>119±7</td>
<td>139±12*</td>
</tr>
<tr>
<td>24-hour DBP, mm Hg</td>
<td>75±5</td>
<td>88±10*</td>
</tr>
<tr>
<td>24-hour pulse pressure, mm Hg</td>
<td>44±5</td>
<td>52±10*</td>
</tr>
<tr>
<td>Left ventricular mass, g</td>
<td>143±40</td>
<td>164±51</td>
</tr>
<tr>
<td>Left ventricular mass index, g/m²</td>
<td>81±18</td>
<td>91±27</td>
</tr>
<tr>
<td>Intima-media thickness, μm</td>
<td>669±127</td>
<td>708±168</td>
</tr>
<tr>
<td>Pulse wave velocity, m/s</td>
<td>9.1±1.5</td>
<td>10.4±2.2*</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean±SD. Qualitative variables are expressed as percentages.

*P<0.05 vs controls.
were higher in patients compared with controls, whereas there was a trend toward an increased left ventricular mass index and carotid intima media thickness.

No difference in urinary 15-F_{2t}-IsoP levels was observed in patients compared with controls. Isoprostanes are the most sensitive markers of lipid peroxidation in vivo. Increased 15-F_{2t}-IsoP levels in urine are detected following coronary angioplasty, but are also detected following transient myocardial ischemia such as a diagnosis coronary angiography. Increased 15-F_{2t}-IsoP levels in urine were also described at the early stages of other vascular diseases such as systemic sclerosis. The present study did not lack power to detect a difference between patients and controls because the number of subjects (30 in each group) was calculated to provide a power of 80%. Furthermore, analysis of the mean and of 95% confidence intervals on differences clearly shows that no trend toward a difference between controls and patients is suggested. Although this study does not rule out the possibility that lipid peroxidation is increased in later stages of essential hypertension, it shows that it is not enhanced in the early stages of the disease already associated with vascular and cardiac morphological changes. This strongly suggests that oxidative stress is not involved in the pathogenesis of essential hypertension.

**Perspectives**

This study shows that lipid peroxidation is not increased in mild-to-moderate hypertension in never-treated patients. This suggests that oxidative stress is not implicated in the pathogenesis of human essential hypertension, at least in the early stages. Further clinical studies including more severe patients are required to determine whether lipid peroxidation may be a consequence of hypertension and may correlate to other biomarkers of cardiovascular diseases.

**Acknowledgments**

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**References**

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