Low-Density Lipoprotein Oxidation and Vitamins E and C in Sustained and White-Coat Hypertension

Sante D. Pierdomenico, Fabrizio Costantini, Anna Bucci, Domenico De Cesare, Franco Cuccurullo, Andrea Mezzetti

Abstract—Low-density lipoprotein oxidation and antioxidant vitamins E and C were investigated in white-coat hypertension in comparison with sustained hypertension and normotension. We selected 21 sustained hypertensive subjects, 21 white-coat hypertensive subjects, and 21 normotensive subjects matched for gender, age, and body mass index. White-coat hypertension was defined as clinical hypertension and daytime ambulatory blood pressure <139/90 (subjects were also reclassified using 134/90 and 135/85 mm Hg as cutoff points for daytime blood pressure). Blood samples were drawn for lipid profile determination, assessment of fluorescent products of lipid peroxidation in native LDL, evaluation of susceptibility to LDL oxidation in vitro (lag phase and propagation rate), and determination of LDL vitamin E and plasma vitamins E and C contents. Compared with sustained hypertensive subjects, white-coat hypertensives had significantly lower fluorescent products of lipid peroxidation (15.4 ± 3.4 versus 10.2 ± 3 units of relative fluorescence/mg LDL protein, \( P < 0.05 \)), longer lag phase (54 ± 10 versus 88 ± 10 minutes, \( P < 0.05 \)), lower propagation rate (8.2 ± 2.5 versus 5.95 ± 2.1 nmol diene/min per mg LDL cholesterol, \( P < 0.05 \)), higher LDL vitamin E content (8.3 ± 1.1 versus 10.1 ± 1.8 nmol/mg LDL cholesterol, \( P < 0.05 \)), and plasma vitamin C content (40 ± 13 versus 57 ± 9 μmol/L, \( P < 0.05 \)). No significant difference was observed between white-coat hypertensive and normotensive subjects. The results did not change after reclassification of subjects. Our data show that white-coat hypertensive subjects do not show an enhanced propensity to LDL oxidation or reduction in antioxidant vitamins. Given the role of LDL oxidation in the development of atherosclerosis and that of vitamin E and C in protecting against it, these findings suggest that white-coat hypertension per se carries a low atherogenic risk. (Hypertension. 1998;31:621-626.)

Key Words: hypertension, white-coat ■ oxidation ■ LDL ■ vitamin E ■ vitamin C

White-coat hypertension, ie, high clinical BP but “normal” ambulatory BP, is present in approximately 20% of mild to moderate hypertensive patients.1,2 The prognosis for white-coat hypertensive subjects is not yet completely clear.3–5 Some studies have shown that white-coat hypertension is a benign condition,3,5,6,10,12,13 whereas others have not.4,7–9,11 We have reported recently that subjects with white-coat hypertension do not show target organ damage and present a lipid profile similar to that in normotensive subjects,14 suggesting that they could be considered at low cardiovascular risk.

Several lines of evidence suggest that hypertension is associated with enhanced oxidative stress,15–23 although it is not yet clear whether this phenomenon occurs before or after the development of hypertension. An important consequence of increased oxidative stress is LDL oxidation. It has been suggested that beyond the known risk markers for cardiovascular disease, oxidation of LDL could play an important role in the development24 and progression25 of atherosclerosis. Moreover, it may contribute to the maintenance of hypertension.26–29 It has been reported that LDL is more oxidized in vivo and is more susceptible to oxidation in vitro in hypertensive patients than in normotensive subjects.30–32 In such a context, vitamins C33 and E34 represent the major antioxidants in the water- and lipid-soluble compartments, respectively, and they are devoted to protect against oxidative damage. To the best of our knowledge, no study apparently has evaluated whether sustained and white-coat hypertensives show differences in LDL oxidation and antioxidant vitamins.

The present study was designed to investigate LDL oxidation, evaluating fluorescent products of lipid peroxidation in native lipoproteins and susceptibility to oxidation in vitro, and antioxidant vitamins E and C in subjects with white-coat hypertension compared with subjects with normotension and those with sustained hypertension.

Methods

Subjects
We selected 21 normotensive, 21 white-coat hypertensive (see below for definition), and 21 sustained hypertensive subjects matched for gender, age, and body mass index. Exclusion criteria for entry in the study were smoking habits, diabetes mellitus, hypercholesterolemia (>5.7 mmol/L), hypertriglyceridemia (>2.9 mmol/L), antihypertensive and lipid-lowering drug use (present or past), antioxidant sub-
Selected Abbreviations and Acronyms
BP = blood pressure
FPL = fluorescent product(s) of lipid peroxidation
HDL = high-density lipoprotein
LDL = low-density lipoprotein
URF = units of relative fluorescence

LDL Oxidation and White-Coat Hypertension

stances use, known secondary hypertension, chronic renal failure, cerebrovascular disease, ischemic heart disease, congestive heart failure, and gastrointestinal and liver disease. Subjects came from the same geographical area (Chieti, Abruzzo, Italy) and had a similar dietary pattern. The diet composition was assessed by a well trained diettian who collected diet histories. The study was in accordance with the Second Declaration of Helsinki and was approved by the institutional review committee. All participating subjects gave informed consent.

Office BP Measurements
Clinical systolic and diastolic BP recordings were performed on the same arm, with the subject in the supine position after 10 minutes of rest, according to the standard technique. Phase V was used to determine diastolic BP. Measurements were performed in triplicate, and the average value was used as the BP for the visit. Clinical hypertension was defined as systolic BP >140 mm Hg and diastolic BP >90 mm Hg in three visits.

Ambulatory BP Monitoring
Ambulatory BP monitoring was performed with a portable noninvasive recorder (SpaceLabs 90207) on a day of typical activity. Technical aspects have been previously reported.47 The following ambulatory BP parameters were evaluated: average daytime systolic and diastolic BP (awake period), average nighttime systolic and diastolic BP (asleep period), and average 24-hour systolic and diastolic BP. Awake and asleep periods were calculated from diary times. Recordings were automatically edited.48 All subjects included in the study had recordings of good technical quality. White-coat hypertension was defined as clinical hypertension and daytime BP >139/90 (the daytime upper limit of a previously reported normotensive population).14 Subjects were also reclassified according to different cutoff points (134/90 and 135/85 mm Hg for daytime BP).59

Laboratory Procedures

Biochemical Analyses
Blood samples for total serum cholesterol, HDL cholesterol, triglycerides, and glucose were drawn after a fasting period of 12 hours. Total cholesterol, triglycerides, and glucose were determined by standard methods. HDL cholesterol was measured by the immunoturbidimetric technique. LDL cholesterol was calculated with Friedewald's formula.

LDL Isolation
Venous blood was taken from subjects and placed in tubes containing EDTA (2.7 mmol/L), and plasma was immediately separated by centrifugation. The LDL fraction was isolated from freshly drawn plasma by single-vertical-spin ultracentrifugation (Centricon TVF 6513, Kontron Instruments) using a discontinuous NaCl/KBr density gradient.57 To protect LDL against oxidative modification during isolation, EDTA (2.7 mmol/L) was added to density solutions. LDL was recovered from the mid-to-upper part of the gradient and dialyzed for 22 hours in the dark against three changes of PBS containing EDTA (10 μmol/L), pH 7.4, at 4°C. LDL cholesterol was measured by a commercially available enzymatic reagent (CHOD-PAP MPR1, Boehringer Mannheim), and LDL protein was determined by the method of Lowry et al.46

LDL Oxidation
Oxidation of LDL (fresh preparations at a concentration of 0.2 mg LDL cholesterol/mL) was triggered by the addition of 5 μmol/L CuSO4 in PBS, pH 7.4, at 37°C and continuously monitored spectrophotometrically at 234 nm to evaluate the formation of conjugated dienes.68 Determinations were carried out in a computer-assisted diode array spectrophotometer (Hewlett-Packard 8452-A) equipped with 7-position automatic sample changer. As previously reported,37 the oxidation curve is characterized by the lag phase, the propagation phase, and the decomposition phase. The lag phase and the propagation rate were calculated as previously reported.37 Two LDL preparations of the same sample were oxidized in two consecutive oxidation runs on the same day. The values reported for lag phase and propagation rate are means of the values thus obtained. The coefficients of variation for lag phase and propagation rate were 3.2% and 3.8%, respectively.

Lipid Peroxidation in Native LDL
Lipid peroxidation in native LDL was evaluated via the assessment of FPL, which essentially reflects the interaction of polyunsaturated fatty acids peroxidation products with amino groups of phospholipids and apolipoprotein B.39,40–45 They are more sensitive and specific than thiobarbituric acid reactive substances and tend to remain localized at the site of oxidant burden.44 The samples were irradiated with ultraviolet light to remove the fluorescence contribution of such compounds as retinol just before fluorescence measurements. Fluorescence was estimated spectrophotometrically at 360-nm excitation and 430-nm emission using a Kontron SFM 25 spectrophotometer calibrated with quinine sulfate. The 430-nm fluorescence in freshly prepared LDL is most likely indicative for remnants of in vivo lipid peroxidation.37 Results were expressed as units of relative fluorescence (URF) per milligram of LDL protein. FPL were evaluated twice from the same sample on the same day. The data reported are means of the values thus obtained. The coefficient of variation for FPL was 3.1%.

Vitamin E and Vitamin C Determination
LDL and plasma vitamin E were measured with high-performance liquid chromatography using a Kontron System 450 and expressed in nanomoles per milligram LDL cholesterol and in micromoles per liter, respectively. Procedures were as previously reported.46 Vitamin E and internal standard were detected by a ultraviolet-visible spectrophotometer (Kontron Detector 430) at different wavelengths (290 and 280 nm, respectively). Plasma vitamin C was determined by spectrophotometry18 and is expressed in micromoles per liter.

Statistical Analysis
Data are expressed as mean±SD. Groups were compared with one-way ANOVA followed by Scheffe’s test (or modified t test using the Bonferroni method to adjust the probability values) for multiple comparisons or with the Kruskal-Wallis test followed by the Mann-Whitney U test for multiple comparisons where appropriate.66 ANCOVA was also used when needed. All analyses were made with the SYSTAT program implemented on an Apple Macintosh SE/30. Statistical significance was defined as P<.05.

Results
Characteristics and BP values of study population are reported in Table 1. Age, gender distribution, and body mass index did not differ among the groups. Clinical BP was significantly higher in sustained hypertensives and in white-coat hypertensive subjects than in normotensives; it was also slightly but significantly higher in sustained hypertensives than in white-coat hypertensives. Ambulatory BP was significantly higher in sustained hypertensives than in white-coat hypertensives and normotensives but similar between white-coat hypertensives and normotensives.

Laboratory findings are reported in Table 2. Glucose, total cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol were not different among the groups as a result of the selection process. FPL content in native LDL was significantly
TABLE 1. Characteristics and BP Values of the Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sustained Hypertensives</th>
<th>White-Coat Hypertensives</th>
<th>Normotensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/10</td>
<td>11/10</td>
<td>11/10</td>
</tr>
<tr>
<td>Age, y</td>
<td>46±6</td>
<td>47±7</td>
<td>47±6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7±3.1</td>
<td>26.4±2.9</td>
<td>26.4±4</td>
</tr>
<tr>
<td>Clinical SBP, mm Hg</td>
<td>156±10*</td>
<td>148±4*</td>
<td>129±5</td>
</tr>
<tr>
<td>Clinical DBP, mm Hg</td>
<td>97±3*</td>
<td>79±4</td>
<td></td>
</tr>
<tr>
<td>Daytime SBP, mm Hg</td>
<td>152±11§</td>
<td>128±4</td>
<td>126±4</td>
</tr>
<tr>
<td>Daytime DBP, mm Hg</td>
<td>99±3§</td>
<td>81±3</td>
<td>79±3</td>
</tr>
<tr>
<td>Nighttime SBP, mm Hg</td>
<td>133±12§</td>
<td>110±7</td>
<td>109±6</td>
</tr>
<tr>
<td>Nighttime DBP, mm Hg</td>
<td>85±5§</td>
<td>70±3</td>
<td>69±3</td>
</tr>
<tr>
<td>24-hour SBP, mm Hg</td>
<td>148±11§</td>
<td>123±5</td>
<td>122±4</td>
</tr>
<tr>
<td>24-hour DBP, mm Hg</td>
<td>96±4§</td>
<td>77±4</td>
<td>76±4</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. *P<.0003 vs normotensives; †P<.01 vs white-coat hypertensives; §P<.03 vs white-coat hypertensives; ‡P<.0003 vs white-coat hypertensives and normotensives.

TABLE 2. Laboratory Findings of the Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sustained Hypertensives</th>
<th>White-Coat Hypertensives</th>
<th>Normotensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical Analyses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.7±0.27</td>
<td>4.66±0.22</td>
<td>4.6±0.38</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.09±0.80</td>
<td>4.88±0.46</td>
<td>4.78±0.57</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.19±0.13</td>
<td>1.24±0.18</td>
<td>1.27±0.15</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.45±0.73</td>
<td>1.32±0.5</td>
<td>1.24±0.24</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.23±0.57</td>
<td>3.05±0.41</td>
<td>2.95±0.42</td>
</tr>
<tr>
<td>LDL Oxidation Measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPL, URF/mg LDL protein</td>
<td>15.4±3.4*</td>
<td>10.2±3.0</td>
<td>10.1±1.9</td>
</tr>
<tr>
<td>Lag phase, min</td>
<td>54±10*</td>
<td>88±10</td>
<td>89±9</td>
</tr>
<tr>
<td>PR, nmol diene/min per mg LDL-C</td>
<td>8.2±2.5*</td>
<td>5.96±2.1</td>
<td>4.8±1.3</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL vitamin E, mmol/mg LDL-C</td>
<td>8.3±1.1*</td>
<td>10.1±1.8</td>
<td>10.5±1.5</td>
</tr>
<tr>
<td>Plasma vitamin E, μmol/L</td>
<td>25.5±3.7</td>
<td>27.5±4.5</td>
<td>28±3.6</td>
</tr>
<tr>
<td>Plasma vitamin C, μmol/L</td>
<td>40±13*</td>
<td>57±9</td>
<td>58±13</td>
</tr>
</tbody>
</table>

LDL-C indicates LDL cholesterol; PR, propagation rate. *P<.05 vs white-coat hypertensives and normotensives.

When subjects were reclassified using 134/90 and 135/85 mm Hg as the cutoff points for daytime BP, 2 and 6 white-coat hypertensives, respectively, were reclassified as sustained hypertensive subjects. This redistribution (Table 3) did not change the results previously observed. All differences observed between sustained and white-coat hypertensive subjects, both in the original groups and in the reclassified groups, remained largely significant after adjustment for clinical BP.

Discussion

In the present study, we have evaluated LDL oxidation and antioxidant vitamins E and C in sustained hypertensive, white-coat hypertensive, and normotensive subjects. Our data show that sustained hypertensives have higher susceptibility to LDL oxidation and lower LDL vitamin E and plasma vitamin C contents than white-coat hypertensives and normotensives, whereas we did not find differences between white-coat hypertensives and normotensives.

FPL in native LDL could be indicative of subtle oxidation that had already occurred in vivo.34,42,43 Radical mediated oxidant damage of LDL particles seems to occur in or near the arterial wall34; however, it has been suggested that modified lipoproteins could escape into the bloodstream to account for the presence of lipid peroxidation products in plasma.17 Indeed, oxidatively modified LDL has been detected in human plasma.49 FPL in native LDL were found to be significantly higher in sustained hypertensives, which suggests that persistent hypertension enhances LDL infiltration into the arterial wall, thus increasing the availability of LDL for oxidation.
Lag phase is a measure of LDL resistance to oxidation in vitro. It especially depends on LDL antioxidant content and amounts of preformed lipid hydroperoxides. The major antioxidant in LDL is vitamin E. The higher content of lipid peroxides, which is suggested by the higher content of FPL, and the lower content of vitamin E in LDL of sustained hypertensives could account for the significantly lower duration of the lag phase in comparison with white-coat hypertensives and normotensives. Contrasting effects of LDL fatty acid composition pattern on lag phase have been reported. Some authors have documented that an increased n-6 polyunsaturated fatty acid content in LDL decreases the lag phase; however, the n-3 polyunsaturated fatty acid ratio reduces the lag phase, whereas others have not. It has also been reported that an increased LDL content in n-3 polyunsaturated fatty acids decreases the lag phase; however, the n-3 polyunsaturated fatty acid content in our typical diet is almost negligible. In the present study, we did not evaluate LDL fatty acid composition pattern; thus, we cannot exclude that n-6 polyunsaturated fatty acid content could have influenced to some extent the lag phase duration, if such an influence exists at all.

Peroxidation rate is indicative of the autocatalytic chain reaction of lipid peroxidation after depletion of antioxidant content. It seems to be mainly influenced by polyunsaturated/monounsaturated fatty acid ratio, being higher in the presence of an increased content of n-6 polyunsaturated fatty acids. Peroxidation rate was significantly higher in sustained hypertensive subjects than in white-coat hypertensives and normotensives. It can be speculated that despite having similar nutritional habits, sustained hypertensives tend to have an increased content of n-6 polyunsaturated fatty acids in LDL, as previously reported.

As mentioned, vitamin E is the major antioxidant of LDL, whereas the other antioxidants play a minor role. Vitamin E is a lipophilic chain-breaking antioxidant that scavenges lipid peroxyl radicals both in lipoproteins and in cellular membranes. We have found that vitamin E in LDL was lower in sustained hypertensives than in white-coat hypertensives and normotensives and similar between white-coat hypertensives and normotensives. Concentration of vitamin E in LDL is the result of exogenous intake, its transfer from LDL to cellular membranes or other lipoproteins, and its metabolic redox reactions in LDL. It can be hypothesized that oxidation of vitamin E via intermediate formation of chromanoxyl radical would reduce its content in LDL of sustained hypertensives; this phenomenon could occur in or near the arterial wall and account for a reduction of vitamin E in LDL until it can be regenerated by other antioxidant systems. On the other hand, a loss of vitamin E from LDL due to physicochemical properties or accelerated transfer to cell membranes could help explain our findings. Indeed, some authors have reported a lower erythrocyte cell membrane concentration of vitamin E in hypertensive patients, which could suggest an increased turnover of this antioxidant.

Vitamin C is a water-soluble chain-breaking antioxidant that reacts with oxygen free radicals and represents the outstanding antioxidant in plasma. In addition, it can regenerate vitamin E from the radical form. A number of epidemiological studies have shown a negative correlation between BP and vitamin C. Several mechanisms by which vitamin C might influence BP have been proposed, including a free radical-scavenging property preventing prostacyclin synthetase inhibition. Moreover, it has also been suggested that BP may influence vitamin C metabolism. We have found lower levels of vitamin C in sustained hypertensives than in white-coat hypertensives and normotensives and similar values between white-coat hypertensives and normotensives. It can be speculated that the lower vitamin C level found in sustained hypertensives may be the result of a greater antioxidant consumption, either for direct reactions or for regeneration of vitamin E, in response to an increased oxidant load associated with sustained hypertension. In any case, vitamin C reacting with oxygen free radicals could stop a vicious circle that contributes to the maintenance of hypertension, thus exerting an antihypertensive effect.

This study has some limitations. We used a cutoff point of 139/90 mm Hg for daytime BP to distinguish sustained and white-coat hypertensive subjects. However, in the selected white-coat hypertensive group, the highest daytime BP values were 136/87 mm Hg and the majority of subjects had BP values ≤135/85 mm Hg. Thus, we do not know whether the same results would have been achieved in white-coat hypertensives with daytime BP in the ranges of 137 to 139 mm Hg and 88 to 90 mm Hg or in white-coat hypertensive subjects clustered in the ranges of 136 to 139 mm Hg and 86 to 90 mm Hg of daytime BP. Moreover, because of the selection process, we excluded subjects with smoking habit, diabetes, and dyslipidemia. The susceptibility to LDL oxidation in white-coat hypertensives with such factors in comparison to sustained hypertensives and normotensives with the same additional risk factors has to be determined. We and others have reported that white-coat hypertensives do not seem to share metabolic abnormalities with sustained hypertensives; on the contrary, Julius et al have reported an increased prevalence of metabolic alterations in white-coat hypertensives. In such a context, at present, our results cannot be extrapolated to all white-coat hypertensive subjects.

As previously reported, it is not yet clear whether enhanced oxidative stress occurs before or after the development of hypertension. In the former hypothesis, our data suggest that sustained hypertension and white-coat hypertension are two distinct conditions. In the latter hypothesis, our results suggest that persistent hypertension, and not transitory hypertension, is associated with enhanced LDL oxidation and increased atherogenic risk. The previously reported findings that BP variability and white-coat effect are probably devoid of prognostic significance add further support to this hypothesis and to our data.

In conclusion, our data suggest that white-coat hypertension, in contrast with sustained hypertension, is not associated with enhanced LDL oxidation and reduced antioxidant vitamins. Given the role of LDL oxidation in the development of atherosclerosis and that of vitamin E and C in protecting against it, our findings suggest that white-coat hypertension per se carries a low atherogenic risk, adding further insight into its clinical significance.

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References


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