Endothelial Function in Sustained and White Coat Hypertension

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**Background:** Endothelial dysfunction is a frequent finding in essential hypertension. The aim of this study was to assess endothelial function, by evaluating circulating nitric oxide metabolites, nitrate plus nitrite (NOx), and endothelium-dependent vasodilation (EDD), in white coat hypertension in comparison with sustained hypertension and normotension.

**Methods:** We selected 22 sustained hypertensive, 22 white coat hypertensive, and 22 normotensive subjects matched for age, gender, body mass index, and occupation. Women were also matched for menopausal status. Subjects with smoking habit, dyslipidemia, and diabetes mellitus were excluded from the study. White coat hypertension was defined as clinical hypertension and daytime ambulatory blood pressure (BP) <135/85 mm Hg. Groups received for 2 days a low-nitrate diet before obtaining blood samples for laboratory measurements. The NOx levels were measured by using the Griess reagent after enzymatic conversion of all nitrate to nitrite. Subjects also underwent brachial artery study by ultrasonography to evaluate EDD and endothelium-independent vasodilation.

**Results:** White coat hypertensive subjects had significantly higher levels of NOx than sustained hypertensive patients (30.8 ± 12 vs 22 ± 8.5 μmol/L, P < .05) and significantly higher EDD (7.8% ± 3.1% vs 4.6% ± 3.0%, P < .05). No significant difference was observed between white coat hypertensive and normotensive subjects regarding these parameters. Endothelium-independent vasodilation was not significantly different among sustained hypertensives, white coat hypertensives, and normotensives (18% ± 4.2% vs 18.3% ± 3.9% vs 18.6% ± 4.8%, respectively, P = not significant).

**Conclusions:** Our data suggest that middle-aged white coat hypertensive subjects without other cardiovascular risk factors do not show endothelial dysfunction in contrast with sustained hypertensive patients.

**Key Words:** Hypertension, white coat, nitric oxide, endothelium, ultrasound.

White coat hypertension (also known as isolated clinic hypertension), that is, high clinic blood pressure (BP) but “normal” ambulatory BP, has been reported to be present in approximately 20% of mild-to-moderate hypertensive patients. The risk profile for white coat hypertensive subjects is not yet completely clear. Some studies have shown that white coat hypertension represents a low-risk group whereas other investigators have not come to such a conclusion. In the same context, it is unknown whether white coat hypertension shares similarities in the pathophysiologic background with sustained hypertension. Indeed, the underlying mechanisms of white coat hypertension are not yet clear and it is not known whether it represents a distinct entity, a prehypertensive state or a low-risk form of essential hypertension. Some researchers have suggested that white coat hypertension is relatively specific to the clinical context and it is not a manifestation of generalized cardiovascular hyperreactivity.

It has been reported that nitric oxide (NO), synthesized by vascular endothelium, is implicated in the regulation of BP. Several lines of evidence suggest that NO availability may be reduced in patients with essential hypertension. Indeed, reduced levels of NO metabolites, nitrate plus nitrite (NOx), and impaired endothelium-dependent vasodilation (EDD) which is mainly mediated by NO have been reported in essential hypertension. The exact mechanism of this finding and whether it is a primary or a secondary phenomenon is not yet completely clear.
To the best of our knowledge, no study has evaluated endothelial function in white coat hypertension.

The present study was designed to evaluate circulating NOx levels and EDD in subjects with white coat hypertension compared with subjects with normotension and those with sustained hypertension.

Methods

Subjects

We selected 22 normotensive, 22 white coat hypertensive, and 22 sustained hypertensive subjects matched for age (within 5 years), gender, body mass index (within 10%), and occupation. Women were also matched for menopausal status. Exclusion criteria for entry in the study were antihypertensive (present or past) or other drug use, smoking habit, dyslipidemia, diabetes, impaired glucose tolerance, obesity, secondary hypertension, renal failure, cerebrovascular disease, ischemic heart disease, heart failure, peripheral vascular disease, gastrointestinal disease, systemic illness, recent history (within 1 month) of infection or trauma, and habit of regular aerobic exercise. Vegetarian persons were also excluded. Subjects were asked to avoid contact with smoking subjects. Women in perimenopausal status were studied in the same phase of the menstrual cycle. Subjects received for 2 days a normal-salt and 2 days a low-nitrate diet before obtaining blood samples for NOx evaluation. Study population came from the same geographic area (Chieti, Abruzzo, Italy). The study was in accordance with the Second Declaration of Helsinki and was approved by the Institutional Review Committee. Subjects gave informed consent.

Office BP Measurements

Clinic systolic and diastolic BP recordings were performed according to the standard technique with the subject in the supine position. Measurements were performed in triplicate, and the average value was used as the BP for the visit. Clinic hypertension was defined as systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg at each of three visits. Clinic normotension was defined as BP <140/90 mm Hg.

Ambulatory BP Monitoring

Ambulatory BP monitoring was performed with a portable noninvasive recorder (SpaceLabs 90207, Redmond, WA) on a day of typical activity. Technical aspects have been previously reported.10 Ambulatory BP readings were obtained at 15-min intervals from 6 AM to midnight, and at 30-min intervals from midnight to 6 AM. The following ambulatory BP parameters were evaluated: average daytime (awake period), nighttime (asleep period), and 24-h systolic and diastolic BP. Awake and asleep periods were calculated from diary times. Recordings were automatically edited.10 Subjects included in the study had recordings of good technical quality. White coat hypertension was defined as clinic hypertension and daytime BP <135/85 mm Hg.31

Laboratory Procedures

Blood samples were drawn after a fasting period of 12 h. Glucose, creatinine, total cholesterol, triglycerides, and low-density lipoprotein (HDL) cholesterol were determined by standard methods. Low-density lipoprotein (LDL) cholesterol was calculated with Friedewald’s formula. Blood samples for NOx determination were centrifuged immediately at 3000 g for 15 min and the supernatant was collected and frozen at −20°C until assayed. Serum NOx levels were measured with the use of the Griess reagent as previously described.32 Briefly, 5 μL of reconstituted nitrate reductase and 10 μL of 2 mmol/L NADH (Oxis International, Inc., Portland, OR) were added to the samples and incubated at room temperature for 20 min to convert all nitrate to nitrite. Samples were deproteinized, and then 100 μL of Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) was added. After color development at room temperature, absorbance values were measured at a wavelength of 540 nm. Each sample was assayed in duplicate. Nitrite in the serum was estimated by a standard curve obtained from enzymatic conversion of potassium nitrate to nitrite. Results are reported as NOx (nitrate plus nitrite) and expressed as micromoles per liter. The reproducibility of measurements was studied by performing a repeat determination 1 week later in a randomly drawn sample of 15 subjects in the same conditions. The coefficient of variation for NOx levels was 3.4%.

Vascular Study

The EDD and endothelium-independent arterial vasodilation were assessed as previously described.33 Brachial artery diameter and flow were measured from B-mode ultrasound images and pulsed wave Doppler, respectively, by using a Hewlett Packard model 77030A (Andover, MA) ultrasound imaging system with a 7.5-MHz transducer. The brachial artery was scanned in longitudinal section 2 to 10 cm above the elbow and when a satisfactory transducer position was found the skin was marked and the arm remained in the same position throughout the study. Scans were taken at rest, during reactive hyperemia (EDD), again at rest, and after sublingual glyceryl trinitrate (endothelium-independent vasodilation). After the basal scan, a pneumatic cuff placed around the forearm was inflated to a pressure of 250 mm Hg for 4.5 min and then rapidly deflated to induce increased flow. A second scan was performed continuously for 30 sec before and 90 sec after deflation of the cuff including a recording of flow velocity for the first 15 sec after the cuff was released. Thereafter, 10 min was allowed for vessel recovery after which a further resting scan was taken. Sublingual glyceryl trinitrate spray (400 μg) was then administered and 4 min later the last scan was performed. Images were re-
corded on super VHS videotape for later analysis. The arterial diameter was measured at a fixed distance from an anatomic marker using ultrasonic callipers. Measurements were taken from the anterior to the posterior “m” line (interface between the media and adventitia). The mean diameter was calculated from four cardiac cycles incident with the R-wave on the electrocardiogram. For the reactive hyperemia scan, diameter measurements were taken 50 to 60 sec after cuff deflation. Diameter changes were derived as percentage change relative to the mean of baseline scans (100%). Examinations were performed by a single investigator who was unaware of subjects’ characteristics. The reproducibility of measurements was studied by performing a repeat scan 1 week later in a randomly drawn sample of 20 subjects. The coefficient of variation for EDD was 3.6%.

Statistical Analysis

Data are expressed as mean ± SD. Groups were compared with one-way ANOVA followed by Scheffé’s test for multiple comparisons or with the Kruskal-Wallis test followed by the Mann-Whitney U test for multiple comparisons, where appropriate. Spearman correlation was used to evaluate the association between clinic and ambulatory BP and markers of endothelial function. Multiple regression analysis was also used. Analyses were made with the use of SYSTAT 10 package (SPSS Inc., Chicago, IL). Statistical significance was defined as \( P < .05 \).

Results

The main characteristics of study population are reported in Table 1. Age, gender distribution, and body mass index did not differ among the groups by selection. Occupation was not different among the groups. In the sustained hypertensive, white coat hypertensive, and normotensive groups, 14 subjects were employees (office staff and civil servants with sedentary jobs), 4 subjects were teachers, and 4 subjects were housewives, respectively.

Clinic BP was significantly higher in sustained hypertensives and white coat hypertensives than in normotensives; it was also higher in sustained hypertensives than in white coat hypertensives. Ambulatory BP was significantly higher in sustained hypertensives than in white coat hypertensives and normotensives, and similar between white coat hypertensives and normotensives.

Glucose \( (4.7 ± 0.38 \text{ mmol/L}) \), creatinine \( (73.3 ± 13 \text{ mg/dL}) \), total cholesterol \( (4.52 ± 0.41 \text{ mmol/L}) \), HDL cholesterol \( (1.21 ± 0.15 \text{ mmol/L}) \), triglycerides \( (1.34 ± 0.31 \text{ mmol/L}) \), and LDL cholesterol \( (2.62 ± 0.26 \text{ mmol/L}) \) were not different among sustained hypertensives, white coat hypertensives, and normotensives, respectively, as a result of the selection process.

Serum NOx levels in the study population are reported in Fig. 1. The NOx levels were significantly different among sustained hypertensives, white coat hypertensives, and normotensives; there was no significant difference between white coat hypertensives and normotensives.

Brachial artery diameter at baseline was not significantly different among sustained hypertensives, white coat hypertensives, and normotensives \( (3.85 ± 0.47 \text{ mmol/L}) \), respectively, \( P = \text{not significant} \). The EDD of study groups is reported in Fig. 1. It was significantly lower in sustained hypertensives than in white coat hypertensives and normotensives; no significant difference was observed between white coat hypertensives and normotensives. Endothelium-independent vasodilation was not significantly different among sustained hypertensives, white coat hypertensives, and normotensives \( (18% ± 4.2% \text{ mmol/L}) \), respectively, \( P = \text{not significant} \). In the study group as a whole, there was no association

Table 1. Characteristics and blood pressure of 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>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Age (y)</td>
<td>45 ± 7</td>
<td>45 ± 8</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>25.4 ± 2.1</td>
<td>25.3 ± 2.0</td>
<td>25.1 ± 2.0</td>
</tr>
<tr>
<td>Clinic systolic BP (mm Hg)</td>
<td>153 ± 8( \dagger )</td>
<td>148 ± 3( * )</td>
<td>128 ± 6</td>
</tr>
<tr>
<td>Clinic diastolic BP (mm Hg)</td>
<td>99 ± 4( \dagger )</td>
<td>96 ± 2.5( * )</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>Daytime systolic BP (mm Hg)</td>
<td>145 ± 7( \dagger )</td>
<td>128 ± 4.5</td>
<td>127 ± 5</td>
</tr>
<tr>
<td>Daytime diastolic BP (mm Hg)</td>
<td>95 ± 4( \dagger )</td>
<td>78 ± 4</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Nighttime systolic BP (mm Hg)</td>
<td>130 ± 10( \dagger )</td>
<td>112 ± 6</td>
<td>112 ± 6</td>
</tr>
<tr>
<td>Nighttime diastolic BP (mm Hg)</td>
<td>82 ± 7( * )</td>
<td>68 ± 7</td>
<td>67 ± 6</td>
</tr>
<tr>
<td>24-h systolic BP (mm Hg)</td>
<td>140 ± 7( * )</td>
<td>123 ± 5</td>
<td>122 ± 6</td>
</tr>
<tr>
<td>24-h diastolic BP (mm Hg)</td>
<td>91 ± 5( * )</td>
<td>79 ± 4</td>
<td>74 ± 5</td>
</tr>
</tbody>
</table>

BP = blood pressure.

* \( P < .05 \) v normotensives; \( \dagger P < .05 \) v white coat hypertensives.
between NOx and clinic systolic \((r = -0.19; P = .13)\) and diastolic \((r = -0.19; P = .13)\) BP, and between EDD and clinic systolic \((r = -0.20; P = .1)\) and diastolic \((r = -0.21; P = .9)\) BP. On the contrary, there was a significant association between NOx and 24-h systolic \((r = -0.44; P = .0004)\) and diastolic \((r = -0.42; P = .0008)\) BP, and between EDD and 24-h systolic \((r = -0.48; P = .0001)\) and diastolic \((r = -0.45; P = .0003)\) BP.

The relationships among clinic and 24-h systolic BP and markers of endothelial function are reported in Fig. 2.

In this selected population, multiple regression analyses showed that 24-h systolic (or diastolic) BP was an independent correlate of NOx \((\beta = -0.522 \text{ and } P = .0004)\) and EDD \((\beta = -0.629 \text{ and } P = .0001)\), whereas clinic systolic (or diastolic) BP, LDL cholesterol, HDL cholesterol, triglycerides, glucose, and age were not.

### Discussion

White coat hypertension has been widely studied in the past years but it remains a matter of controversy. Various questions regarding its mechanisms and its prognostic relevance are still waiting for answers. In the present study, we have evaluated circulating NOx levels and EDD in sustained hypertensive, white coat hypertensive, and normotensive subjects. We have found lower circulating levels of NOx and lower EDD in sustained hypertensive patients than in white coat hypertensive subjects, whereas no significant difference was found between white coat and normotensive subjects.

Circulating NOx can be used as an index of endogenous formation of NO, provided that oral intake of nitrate and nitrite is restricted for at least 48 h and other potential confounders are taken into account. Some studies evaluated circulating levels of NOx in patients with essential hypertension but inconsistent results were reported. In any case, using various indexes of NO formation, previous studies concluded that NO production may be reduced in essential hypertension. In our study, patients with clinical hypertension were divided into those with sustained and those with white coat hypertension by ambulatory BP monitoring. Thus, the present findings are not comparable with those observed in previous studies in which the clinical hypertensive population was studied as a whole. Our data suggest that NO synthesis may be reduced in sustained hypertensive subjects in comparison with white coat and normotensive subjects. The exact reason for decreased synthesis of NO in essential hypertension is at present unclear. Various hypotheses have been suggested, including the presence of endogenous inhibitors of NO synthase (eg, asymmetrical dimethyl-L-arginine), altered transport of L-arginine through the endothelial membrane, alteration in the intracellular signal transduction pathways, decreased activity of NO synthase, and decreased release of NO. Some of these alterations could be present in sustained hypertension but not in white coat hypertension. For example, it has been reported that oxidized LDL can inhibit the uptake of L-arginine and the synthesis or release of NO by endothelial cells. We have recently reported that sustained hypertensives show enhanced oxidative stress and LDL oxidation and reduced levels of antioxidant vitamins in comparison with white coat hypertensives. This aspect could contribute to explain differences in NOx levels between sustained and white coat hypertensive subjects. It cannot be totally excluded, however, that to some extent lower levels of NOx in sustained hypertension may result from increased degradation of NO by oxygen free radicals. At present it is unclear to what extent metabolites deriving from the interaction between NO and oxygen free radicals and those deriving from the interaction between reactive NO species and biologic molecules could contribute to circulating NOx levels.

Several studies have demonstrated impaired EDD.
which is mainly mediated by NO,\textsuperscript{22,24} in patients with hypertension. This alteration has been shown by using different methods and in various vascular beds.\textsuperscript{22,23} The EDD may be considered as an index of vascular NO bioactivity. Impaired EDD may be due to decreased endothelial production of NO or to decreased bioavailability of NO in the vessel wall because of its inactivation by increased formation of oxygen free radicals. Concerning NO production, it has been previously discussed. Some hypotheses have been suggested for increased breakdown of NO by oxygen free radicals in essential hypertension, a condition with increased oxidative stress. Notably, potential sources of endothelial-derived superoxide anion include xanthine oxidase, cyclooxygenase, nicotinamide adenine dinucleotide-phosphate-H oxidase, and leakage from mitochondrial respiratory chain.\textsuperscript{36} It has been suggested that cyclooxygenase-derived superoxide anions could be responsible for NO degradation and endothelial dysfunction in essential hypertension.\textsuperscript{26} Another mechanism by which increased oxidative stress may reduce NO availability is LDL oxidation. In addition oxidized LDL may directly inactivate or attenuate NO biologic activity.\textsuperscript{36} As aforementioned, we have previously reported enhanced oxidative stress and LDL oxidation in sustained hypertension in comparison with white coat hypertension.\textsuperscript{13} These findings could contribute to explain differences in EDD between sustained and white coat hypertensive subjects.

In summary, our data suggest lower NO production or increased NO degradation in sustained hypertension in comparison with white coat hypertension leading to differences in endothelial function. At present it is unclear whether endothelial dysfunction is a primary or a secondary phenomenon in essential hypertension. Some investigators suggest that it may be a primary phenomenon because it can be detected in offsprings of essential hypertensive patients, shows discordant correlation with BP, and does not seem to be normalized by all antihypertensive drugs in spite of BP lowering.\textsuperscript{27} Other researchers suggest that endothelial dysfunction may be a secondary phenomenon related to increased BP per se, which may stimulate (or may be associated with) the production of oxygen free radicals that inactivate NO.\textsuperscript{28,29} Concerning the latter hypothesis, it has also been reported that transient hypertension may impair directly EDD.\textsuperscript{29} In that context, however, transient hypertension lasted 1 h.\textsuperscript{29} Such a situation is not comparable with white coat hypertension,
and it is unclear whether this phenomenon is temporary or permanent. Moreover, it has been recently reported that acute BP lowering does not improve EDD in the brachial artery.30

In the hypothesis that endothelial dysfunction is a primary phenomenon, our data suggest that sustained hypertension and white coat hypertension show some differences in pathophysiologic background. In the hypothesis that it is a secondary phenomenon, our results suggest that persistent hypertension throughout the 24-h period, and not isolated increase in BP during the clinic visit, may induce endothelial dysfunction.

Beyond its role in the pathogenesis or maintenance of hypertension, endothelial dysfunction predisposes to thrombosis, leukocyte adhesion, and proliferation of smooth muscle cells in the arterial wall.19 Notably, all these events are implicated in the development of atherosclerosis.19 Moreover, it has been recently reported that endothelial dysfunction is a marker of future cardiovascular events in patients with essential hypertension.38 Thus, our results could also contribute to explain the lower reported cardiovascular morbidity and mortality in white coat hypertensive subjects in comparison with sustained hypertensive patients.8,14,15

The present study has some limitations. First, because we excluded subjects with a smoking habit, dyslipidemia, and diabetes mellitus, our results cannot be extrapolated to all white coat hypertensive subjects. Second, because we studied only white subjects, our conclusions may not be extended to other racial groups.

In conclusion, our data suggest that middle-aged white coat hypertensive subjects without other cardiovascular risk factors do not show endothelial dysfunction in contrast with sustained hypertensive patients.

References


