Increased Arterial Intima-Media Thickness and In Vivo LDL Oxidation in Young Men With Borderline Hypertension

Jyri O. Toikka, Hanna Laine, Markku Ahotupa, Arto Haapanen, Jorma S.A. Viikari, Jaakko J. Hartiala, Olli T Raitakari

Abstract—We used borderline hypertension as a model for prehypertension to examine the early influences of elevated blood pressure on subclinical atherosclerosis, lipoprotein oxidation, and cardiac adaptation. Healthy men (age 37±4 years) were classified prospectively into 2 groups on the basis of having either borderline hypertension (systolic 130 to 140 mm Hg or diastolic 85 to 89 mm Hg, n=16) or normal (<130/85 mm Hg, n=22) blood pressure values during the previous 2 years. The groups were matched for age, body size, and serum cholesterol levels. High-resolution ultrasound was used to measure intima-media thickness (IMT) of the carotid and brachial arteries, cardiac dimensions, and brachial artery endothelial function. Baseline low-density lipoprotein (LDL)-diene conjugation was measured as an estimate of in vivo LDL oxidation (ox-LDL). Compared with normotensive controls, men with borderline hypertension had higher IMT of the carotid artery (0.58±0.06 versus 0.75±0.07 mm, P<0.001) and IMT of the brachial artery (0.45±0.05 versus 0.37±0.07 mm, P<0.001), and increased levels of ox-LDL (29±9 versus 47±17 mol/L, P<0.001), but similar endothelial function. Left ventricular mass was similar in both groups, but there were significant differences in left ventricular geometry. In multivariate analyses, the predictors of carotid IMT were 24-hour systolic blood pressure (P<0.001) and ox-LDL (P=0.10). The current study demonstrates evidence of increased subclinical atherosclerosis and ox-LDL in borderline hypertension. These results are consistent with the idea that enhanced ox-LDL may be one of the pathophysiological events related to development of atherosclerosis in men with borderline elevated blood pressure. (Hypertension. 2000;36:929-933.)

Key Words: hypertension, borderline oxidized LDL intima-media thickness

Several mechanisms have been suggested to describe how elevated blood pressure might confer the increased risk of coronary artery disease and stroke. These include structural and functional changes in the circulatory system, increased oxidative stress, and accelerated atherogenesis. Because hypertension is often associated with other conditions, such as obesity and dyslipidemia, which can also modify vascular structure and function, studies in patients with established hypertension are not ideal in assessing the natural history of vascular and metabolic changes directly associated with blood-pressure elevation. Mildly elevated blood pressure or borderline hypertension (BHT) is a strong risk factor for established hypertension, and this condition offers a useful model for studying prehypertension in humans. The development of noninvasive ultrasound techniques has opened up the field for in vivo studies of arterial structure and function in asymptomatic young subjects. By using BHT as model for prehypertension and studying only healthy young subjects, it is possible to examine the early cardiovascular and metabolic manifestations related to elevated blood pressure levels without the confounding effects mediated by other risk factors commonly clustering with advanced stages of hypertension.

Therefore, to study the early cardiovascular and metabolic changes in BHT, we compared 2 matched groups of asymptomatic young men that were prospectively selected according to previously measured blood pressure values over 2 to 3 years. Our results suggest that the early pathophysiological events related to blood pressure elevation include increased peripheral artery wall thickness and LDL oxidation (ox-LDL), and that these changes seem to occur before significant adaptations are seen in cardiac structure.

Subjects
Healthy men were enrolled from the STRIP-project, which is an ongoing study aimed to assess the effects of dietary intervention on risk factors in young children. Young (age <45 years), nonsmoking, and nondiabetic men (fathers of the children in the STRIP-project)

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were included in the current study. Subjects were divided into 2 groups on the basis of earlier blood pressure measurements taken on 3 occasions ≈1 year apart. The group with BHT included men with blood pressure values defined as high normal (systolic blood pressure 130 to 140 mm Hg or diastolic blood pressure 85 to 89 mm Hg in all previous measurements). The control group included men with normal blood pressure (<130/85 mm Hg) in each measurement. The study protocol was performed on 16 men with BHT and 22 controls matched for age, body mass index, and serum cholesterol concentration. All men gave their written informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocol had been approved by the Ethics Committee of Turku University and Turku University Central Hospital.

Ultrasound Imaging
All measurements were performed with Acuson 128XP/10 (Acuson Inc) ultrasonography, with the use of 7 MHz scanning frequency linear-array transducer in peripheral artery imaging (carotid and brachial arteries) and 2.5/3.5 MHz scanning frequency phased-array transducer in echocardiographic imaging.

Intima-Media Thickness
Both sides of the common carotid artery diameter were scanned, and the images of the distal 10 mm of the common carotid artery (far wall) were recorded. Ultrasound scans were recorded on videotape for later, off-line analysis. Several end-diastolic frames were selected and analyzed with a custom-made software program to determine mean carotid IMT. The interobserver and intraobserver coefficient of variation of carotid IMT measurements were 5.2 ± 4.1% and 4.0 ± 3.2%, respectively. Brachial artery far-wall IMT was measured in end-diastole in at least 3 different locations and in 3 different cardiac cycles. The mean of these 9 measurements was used as the carotid IMT.

Echocardiography
The men were examined in a left lateral decubitus position. Left ventricular dimensions were obtained from M-mode tracings. Left ventricular mass (LVM) was calculated according to the Penn convention. The left ventricular mass index was calculated dividing LVM by body surface area. Septal to posterior wall ratio and relative wall thickness (2 × posterior wall thickness/ septal thickness) were calculated as measures of left ventricular geometry. Left ventricular (LV) systolic function was assessed by calculating the ejection fraction as [(LV diastolic volume - LV systolic volume)/LV diastolic volume]. LV diastolic function was assessed by calculating the ratio of the early and late mitral inflow peak velocities (E/A-ratio) measured from the mitral flow velocity tracings.

Endothelial Function
Brachial artery scans were obtained at rest, during reactive hyperemia (with increased flow causing flow-mediated, endothelium-dependent dilation), and after sublingual nitrate (causing endothelium-independent vasodilation). The brachial artery was scanned in longitudinal section ≈5 to 15 cm above the elbow. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes and then released. A second scan was taken continuously for 30 seconds before and 90 seconds after cuff deflation. Thereafter, 10 to 15 minutes was allowed for vessel recovery, after which sublingual nitroglycerin (isosorbide dinitrate spray 2.5 mg) was administered, and the last scan was acquired 3 to 4 minutes later. For the reactive hyperemia scan, diameter measurements were taken ≈60 seconds after cuff deflation. The vessel diameter in scans after reactive hyperemia and nitroglycerin administration was expressed as the percentage relative to the average diameter of the artery in the resting scan (100%). The interobserver variation for measurements of flow-mediated dilatation in our laboratory was 0.63 ± 0.45% (range 0.14% to 1.63%; CV, 8.6 ± 6.4%) and the intraobserver variation of 2 consecutive (6 months apart) measurements was 0.48 ± 0.43% (range 0.07% to 1.34%; CV, 6.2 ± 4.4%).

Ambulatory Blood Pressure Measurements
Ambulatory blood pressure was monitored with an auscultatory method (Novacor Diastys). The device was installed in the left arm in the morning, and blood pressure was verified against a mercury sphygmomanometer both when installing and taking off the automated device. The ambulatory blood pressure was recorded at 20-minute intervals during daytime and at 60-minute intervals during night-time.

Ox-LDL, Serum Lipoproteins, and Insulin
Venous blood was collected after an overnight fast. Serum total cholesterol, HDL cholesterol and triglyceride concentrations were measured with the use of standard enzymatic methods (Boehringer Mannheim GmbH) with a fully automated analyzer (Hitachi 740, Hitachi Ltd). HDL cholesterol concentration was measured after polyethylene glycol precipitation. LDL cholesterol concentration was calculated using Friedewald’s equation. Serum insulin concentration was measured by radioimmunoassay kit (Pharmacia).

Conjugated dienes of LDL as markers of ox-LDL were measured by determining the level of LDL diene conjugation. In brief, serum LDL cholesterol was isolated by precipitation with buffered heparin. The amount of peroxidized lipids in samples was determined by degree of conjugated diene double bonds. Lipids were extracted from the samples and analyzed spectrophotometrically at 234 nm.

Statistical Methods
Results are expressed as mean ± standard deviation. Distribution of serum triglyceride and insulin values were skewed and therefore included as their logarithms in the analyses. Comparisons between the study groups were conducted by use of the Student’s t test and ANCOVA (to control for the differences in triglyceride and insulin values between the groups). Univariate associations were studied by calculating Pearson’s correlation coefficients; multivariate modeling was performed with stepwise multivariate linear regression analysis with P < 0.15 as model entry criteria.

Results
The characteristics of subjects are shown in Table 1. Men with BHT had similar LDL-cholesterol and HDL-cholesterol levels compared with controls but significantly higher triglycerides and insulin values.

The findings of the echocardiographic examination are shown in Table 2. LVM was similar between the study groups (P = 0.55), but men with BHT tended to have increased

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 22)</th>
<th>Borderline Hypertension (n = 16)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>36±4</td>
<td>37±4</td>
<td>...</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.4±2.3</td>
<td>26.1±2.5</td>
<td>...</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>123±9</td>
<td>140±13</td>
<td>...</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73±5</td>
<td>83±11</td>
<td>...</td>
</tr>
<tr>
<td>Systolic 24-h blood pressure, mm Hg</td>
<td>118±8</td>
<td>135±10</td>
<td>...</td>
</tr>
<tr>
<td>Diastolic 24-h blood pressure, mm Hg</td>
<td>70±8</td>
<td>81±9</td>
<td>...</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1±0.8</td>
<td>5.6±1.0</td>
<td>0.12</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.2±0.28</td>
<td>1.1±0.17</td>
<td>0.23</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.4±0.8</td>
<td>3.5±0.9</td>
<td>0.53</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.13±0.52</td>
<td>1.95±0.98</td>
<td>0.002</td>
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<tr>
<td>Serum insulin, μU/L</td>
<td>8.0±3.3</td>
<td>10.3±4.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
relative wall thickness ($P=0.07$), decreased septal to posterior wall ratio ($P=0.04$), and decreased E/A-ratio ($P=0.09$).

### IMT and Ox-LDL

Luminal diameters of carotid (5.8±0.5 versus 5.7±0.4 mm, $P=0.46$) and brachial (4.6±0.4 versus 4.6±0.6 mm, $P=0.93$) arteries were similar in the controls and in men with BHT, respectively. Carotid IMT was 0.58±0.06 mm in the control group and 0.75±0.07 mm in the BHT group ($P<0.001$; Figure 1). Brachial IMT was 0.45±0.05 mm in controls and 0.57±0.07 mm in men with BHT ($P<0.001$; Figure 1). There was a significant correlation between carotid IMT and brachial IMT ($r=0.60$, $P<0.001$). Ox-LDL was 29±9 mmol/L in the control group and 47±17 mmol/L in the BHT group ($P<0.001$; Figure 2). The differences in carotid and brachial IMTs and in ox-LDL between the study groups remained highly significant (all $P<0.05$) after adjustment for triglyceride and insulin levels.

Carotid artery IMT correlated with 24-hour systolic blood pressure ($r=0.59$, $P<0.001$) (Figure 3), 24-hour diastolic blood pressure ($r=0.50$, $P<0.001$), ox-LDL ($r=0.49$, $P=0.002$) (Figure 3), systolic blood pressure ($r=0.46$, $P=0.004$), triglycerides ($r=0.59$, $P<0.001$) and insulin ($r=0.39$, $P=0.01$).

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**Table 2. Echocardiographic Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Borderline Hypertension</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass, g</td>
<td>176±28</td>
<td>182±35</td>
<td>0.55</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>86±12</td>
<td>89±16</td>
<td>0.51</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>55±3</td>
<td>54±3</td>
<td>0.40</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>35±3</td>
<td>33±4</td>
<td>0.05</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>7.9±0.7</td>
<td>8.0±1.1</td>
<td>0.67</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>7.5±0.7</td>
<td>8.1±1.2</td>
<td>0.09</td>
</tr>
<tr>
<td>IVSd/LVPWd-ratio</td>
<td>1.05±0.08</td>
<td>1.00±0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.28±0.03</td>
<td>0.30±0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>E, cm/s</td>
<td>68±10</td>
<td>72±8</td>
<td>0.17</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>45±10</td>
<td>54±10</td>
<td>0.01</td>
</tr>
<tr>
<td>E/A-ratio</td>
<td>1.54±0.28</td>
<td>1.38±0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>65±5</td>
<td>69±7</td>
<td>0.08</td>
</tr>
</tbody>
</table>

LVMI indicates left ventricular mass-index; LVDd, left ventricular diameter in diastole; LVDs, left ventricular diameter in systole; IVSd, septum in diastole; LVPWd, left ventricle posterior wall in diastole; E/A-ratio, early (E) and late (A) mitral flow peak velocities.

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**Figure 1.** Carotid (top) and brachial (bottom) IMT in normotensive and BHT men (both $P<0.001$). Means and 95% confidence intervals are shown.

**Figure 2.** Ox-LDL in subjects with BHT and normotensive controls ($P<0.001$). Means and 95% confidence intervals are shown.

**Figure 3.** Relationship between carotid IMT and 24-hour systolic blood pressure ($r=0.59$, $P<0.001$, top) and between carotid IMT and ox-LDL ($r=0.48$, $P=0.002$, bottom).
and tended to correlate with pulse pressure \( (r=0.27, P=0.11) \). Brachial artery IMT correlated with 24-hour systolic blood pressure \( (r=0.51, P=0.001) \), 24-hour diastolic blood pressure \( (r=0.44, P=0.006) \), ox-LDL \( (r=0.33, P=0.042) \), systolic blood pressure \( (r=0.44, P=0.006) \), and diastolic blood pressure \( (r=0.43, P=0.007) \).

In stepwise multivariate linear regression analyses, the predictors of carotid IMT were 24-hour systolic blood pressure \( (P<0.001) \) and ox-LDL \( (P=0.10) \), which were both directly associated with carotid IMT. These 2 variables explained 40% of the variance in carotid IMT. The only predictor of brachial IMT was 24-hour systolic blood pressure \( (P=0.001) \), which explained 26% of the variance of brachial IMT.

**Endothelial Function**

Flow-mediated dilatation did not differ between the groups \( (3.82\pm3.73\% \text{ versus } 3.73\pm4.97\%, \ P=0.95) \). Nitrate-mediated dilatation was similar in both groups \( (14.5\pm7.0\% \text{ versus } 15.1\pm6.9\%, \ P=0.81) \). Flow-mediated dilatation correlated significantly with total cholesterol concentration \( (r=0.42, P=0.009) \), LDL cholesterol concentration \( (r=0.49, P=0.002) \), and brachial artery baseline diameter \( (r=0.48, P=0.002) \), but not with 24-hour systolic \( (r=0.13, P=0.42) \) or diastolic \( (r=0.13, P=0.43) \) blood pressure.

**Discussion**

The present study demonstrates that increased peripheral intima-media thickening and enhanced ox-LDL are related to blood pressure elevation in young asymptomatic men. Both potential markers of increased atherosclerosis have previously been shown to be present in older subjects with established hypertension, and the present data suggest that these changes occur already in mild hypertension.

Oxidation of LDL is an important early event in the pathogenesis of atherosclerosis. Ox-LDL has been implicated in the increased formation of fatty streaks in the arterial intima, which represent the earliest form of atherosclerotic lesion. Previous studies have suggested that hypertension may be related to increased ox-LDL by showing either increased in vitro oxidizability of LDL or elevated titers of autoantibodies against ox-LDL in subjects with essential hypertension. Our study extends these observations and provides the first direct evidence of enhanced ox-LDL in men with BHT. In contrast to our findings, Wu et al recently described decreased titers of autoantibodies against ox-LDL in subjects with BHT. This apparent discrepancy might be explained by the possibility that in certain circumstances, decreased titers of autoantibodies against oxidatively modified LDL may not reflect decreased lipoprotein oxidation, but instead indicate either altered immunoresponse to ox-LDL or increased consumption of autoantibodies due to binding to early atherosclerotic lesions.

Some insights on the mechanisms by which blood pressure elevation might increase ox-LDL have been gained by experimental studies suggesting the importance of pressure changes on the arterial wall in the development of atherosclerosis and lipoprotein oxidation. Meyer et al induced luminal pressures on rabbit aorta in vitro and observed that this stretching increased the uptake of LDL into the arterial wall. More recently, Inoue et al observed that mechanical stretching of cultured smooth muscle cells enhances ox-LDL and superoxide production in the exposed cells. Thus, the combination of increased influx of LDL into the arterial wall and increased oxidative stress may offer a mechanistic explanation of how elevated blood pressure enhances the development of atherosclerosis and ox-LDL.

The results of the present study show that in prehypertension, structural changes appear first in the peripheral arteries before significant adaptation is seen in cardiac size. Although we did not find any increase in the LVM in men with BHT, our data nevertheless revealed subtle changes in indices of cardiac function and geometry, such as increased ejection fraction and relative wall thickness, and altered diastolic filling patterns. If the number of men had been greater, more of these indices might have been significantly different between the study groups. These early adaptive cardiac changes may precede the development of overt left ventricular hypertrophy. The change in LV geometry in BHT may also partly explain increased ox-LDL, since myocardial stretch may be associated with up-regulation of endothelial ox-LDL receptor.

Men with BHT had similar brachial artery endothelial function compared with controls. Hypertension appears to have little effect on flow-mediated conduit artery capacity, although most studies, but not all, have suggested impaired endothelium-dependent vasodilation in the resistance vessels of subjects with elevated blood pressure. These observations may suggest difference in endothelial susceptibility between conduit and resistance vessels to the effects of hypertension. In our study, endothelial function was, nevertheless, closely related with serum LDL cholesterol, demonstrating the importance of standard lipid risk factors in determining endothelial function in young men.

The ultrasound method for IMT does not allow for differentiation between intimal thickening due to atherosclerotic process or medial hypertrophy (smooth muscle growth) due to pressure effects. Therefore, it is not clear whether the increase in IMT in men with BHT represents the former or the latter, although previous observations suggest that IMT may be more closely related to intimal atherosclerotic process. Increased IMT correlates significantly with traditional vascular risk factors, which predicts the likelihood of cardiovascular events in population groups, and is related to the severity and extent of coronary artery disease.

We measured ox-LDL with the use of an assay that measures LDL diene conjugation based on spectrophotometric analysis of LDL lipids. Therefore, it is possible that lipophilic substances of plasma that are transported within the LDL fraction and absorb light at 234 nm may potentially interfere with this assay. We have recently shown in healthy volunteers, however, a close correlation between LDL baseline diene conjugation and the in vivo ox-LDL measured as the titer of serum autoantibodies against oxidized LDL particles.

These data provide evidence of increased in vivo ox-LDL and subclinical atherosclerosis in men with BHT. Our results are consistent with the idea that enhanced ox-LDL may be...
one of the pathophysiological events related to development of atherosclerosis in hypertension.

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References

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