Effect of Low-Density Lipoprotein Cholesterol on Angiotensin II Sensitivity
A Randomized Trial With Fluvastatin

Nicole A.J. van der Linde, Eric J.G. Sijbrands, Frans Boomsma, Anton H. van den Meiracker

Abstract—Increased angiotensin II (Ang II) sensitivity predisposes to hypertension and plaque instability. Raised low-density lipoprotein cholesterol (LDL-c) may increase Ang II sensitivity, but evidence in humans for this effect of LDL-c is limited. In 28, healthy, nonsmoking subjects, aged 30±8 years, with familial hypercholesterolemia, we determined the difference in infusion rate of Ang II and norepinephrine required to increase systolic blood pressure by 20 mm Hg (Pd-20) after 4 weeks of placebo and fluvastatin 80 mg daily in a randomized, double-blind, placebo-controlled, crossover study. Before infusions were started, fasting blood samples were taken to measure lipids. After 4 weeks of placebo, the mean LDL-c concentration was 6.3±1.4 mmol/L. The average decrease of LDL-c was 1.7±0.7 mmol/L after 4 weeks of fluvastatin (P<0.001). The mean Pd-20 for Ang II increased by 1.28 ng/kg per minute (95% CI, 2.05 to 0.50; P=0.002) on fluvastatin, corresponding with a 26% decrease in Ang II sensitivity. Ang II sensitivity, however, remained increased compared with normocholesterolemic controls. The Pd-20 values for norepinephrine were unaffected by fluvastatin. 

The present study in healthy, young subjects with isolated hypercholesterolemia shows an increased sensitivity to Ang II that partly can be restored by LDL-c–lowering therapy. These findings indicate that LDL-c levels directly influence Ang II sensitivity. (Hypertension. 2006;47:1125-1130.)

Key Words: angiotensin ■ blood pressure ■ cholesterol ■ hypercholesterolemia ■ lipoproteins ■ statins

The role of low-density lipoprotein (LDL) cholesterol (LDL-c) in the pathogenesis of atherosclerosis has been firmly established.1 Angiotensin II (Ang II) has atherogenic properties as well. It activates the Ang II type 1 (AT1) receptor and stimulates reduced nicotinamide-adenine dinucleotide phosphate-oxidase in vascular cells, resulting in the generation of superoxide, leading to hypertrophy, hyperplasia, and oxidation of LDL.2–5 Moreover, an AT1-receptor antagonist inhibited LDL oxidation and streak formation in hypercholesterolemic monkeys.6 In vitro and animal data support the idea that raised LDL-c associates with increased AT1-receptor gene expression in vascular smooth muscle cells.7,8 Evidence for a direct effect of raised LDL-c on the sensitivity to Ang II in humans is limited. In previous studies we analyzed older subjects with additional cardiovascular risk factors, like hypertension and the metabolic syndrome, that could explain the increased sensitivity to Ang II as well.9–11 Proof of a direct effect of LDL-c on Ang II sensitivity could have preventive implications. We hypothesized that raised LDL-c increases sensitivity to Ang II, and, conversely, lowering LDL-c levels should decrease this sensitivity.

In the present randomized, double blind, placebo-controlled crossover study, the effect of fluvastatin on Ang II sensitivity was assessed in healthy, young subjects with familial hypercholesterolemia (FH). FH is a monogenetic disorder characterized by markedly raised LDL-c levels.

Methods

Subjects
We used predefined criteria to recruit 30 healthy, young, nonsmoking FH subjects without signs of cardiovascular disease. The diagnosis FH was based on LDL-c above age- and sex-specific 95th percentiles during a cholesterol-lowering diet with triglycerides and HDL-cholesterol (HDL-c) within the normal limits and a molecular diagnosis or the presence of tendon xanthomas or hypercholesterolemia in ≥1 first-degree relative.12 Exclusion criteria were secondary forms of hypercholesterolemia, hypertension, obesity, a history or signs of cardiovascular disease, smoking during the year before the trial, a history of alcohol or drug abuse, or noncompliance to treatment.

To obtain normal reference values, we previously studied 10 untreated, healthy, normocholesterolemic volunteers (6 males) matched as group for age (28±11 years), blood pressure (123±10/75±10 mm Hg), and body mass (23.4±2.0 kg/m²), who underwent an identical protocol as the subjects of the present study.13 Their fasting values of LDL-c, high-density lipoprotein (HDL) cholesterol (HDL-c), triglycerides and glucose were, respectively, 2.2±0.7 mmol/L, 1.4±0.6 mmol/L, 0.9±0.8 mmol/L, and 3.8±0.4 mmol/L. The study protocol was approved by the Review Board of the Erasmus Medical Center. Written informed consent was obtained from all of the subjects.

Study Protocol
After 4 weeks washout of statin treatment, computerized randomization was performed to assign the treatment order of 80 mg daily

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Samples for the determination of plasma norepinephrine were collected in heparinized tubes on ice containing glutathione. All of the samples were immediately centrifuged at 4°C, and plasma was stored at -80°C until assayed. Norepinephrine was measured by high-performance liquid chromatography with fluorometric detection. PRA was measured by the formation of angiotensin I during incubation of plasma for 1 hour. VWF was measured in duplicate by an ELISA with the use of rabbit polyclonal anti-VWF antibodies (Dako, Glostrup, Denmark).

Computing of Hemodynamics
Finger blood pressure, heart rate, and ECG were monitored and recorded continuously. After stable baseline values had been obtained for 30 minutes, intravenous infusion of Ang II (Clinalfa) was started at rates of 2, 4, and 8 ng/kg per minute, with each infusion step lasting 10 minutes. At the end of each infusion step, blood was collected for the determination of plasma norepinephrine and von Willebrand factor (VWF) antigen, and plasma renin activity (PRA) were taken.

Determination of Lipids, Ang II, Norepinephrine, VWF Antigen, and PRA
Serum total cholesterol, HDL-c, LDL-c, and serum triglycerides were determined with automated methods (Hitachi 911, Boehringer Mannheim). For the measurement of plasma Ang II, blood was collected in tubes on ice containing an inhibitor mixture (2.4 mg of EDTA, 0.02 mg of remikiren, and 0.02 mg of lisinopril). Determination was done by radioimmunoassay after SepPak extraction. Samples for the determination of plasma norepinephrine were collected in heparinized tubes on ice containing glutathione. All of the samples were immediately centrifuged at 4°C, and plasma was stored at -80°C until assayed. Norepinephrine was measured by high-performance liquid chromatography with fluorometric detection. PRA was measured by the formation of angiotensin I during incubation of plasma for 1 hour. VWF was measured in duplicate by an ELISA with the use of rabbit polyclonal anti-VWF antibodies (Dako, Glostrup, Denmark).

Statistical Analysis
To enable the comparison of responses to vasoactive substances of each individual, the infusion rate of Ang II and norepinephrine to increase systolic blood pressure by 20 mm Hg (Pd-20) was calculated with linear regression analysis of the dose–response curves. Before performing the present study, we estimated that, in 26 subjects, a difference of 2 (±3) ng/kg per minute in Ang II-20 values in subjects between placebo and active therapy could be detected with a power of 90%. We recruited 30 subjects to compensate for loss to follow-up. Data are expressed as mean±SD or mean and 95% CI. Lipid values after 4 weeks of placebo or fluvastatin and baseline values of Ang II, norepinephrine, PRA, and VWF antigen before infusions were compared with a paired t test. The Pd-20 values in subjects at 4 weeks were compared with a paired t test. The effects of fluvastatin on Ang II and norepinephrine infusion in subjects were studied by a 2-way ANOVA. A P<0.05 was considered to indicate a significant difference.

Results
Of 30 FH subjects, 28 (16 men) fully completed the studies. Their mean (±SD) age was 30±8 years, their body mass index was 24.6±3.8 kg/m², and their fasting glucose concentration was 4.1±0.4 mmol/L. Two subjects discontinued the study because of career-related obligations. The calculated compliance to study medication was 98%.

Baseline values of lipids and baseline hemodynamics after a 30-minute supine resting period are shown in Table 1. As expected, serum total cholesterol and LDL-c were increased. Compared with placebo, fluvastatin decreased serum total cholesterol (mean paired difference, 1.82 mmol/L; 95% CI, 1.54 to 2.09; P<0.0001) and LDL-c (1.72 mmol/L; 95% CI, 1.45 to 1.98; P<0.0001) by, respectively, 24% and 27%. The baseline serum triglyceride and HDL-c levels were well within the normal range. HDL-c did not change with fluvastatin, whereas serum triglyceride decreased slightly. The baseline values of systolic and diastolic blood pressure during placebo and fluvastatin were similar.

The Ang II–induced increase in systolic blood pressure and diastolic blood pressure during placebo was reduced after 4 weeks of treatment with fluvastatin (P<0.001; Table 2 and Figure 2). Heart rate did not change during Ang II infusion, neither during placebo nor during fluvastatin administration.
The Pd-20 for Ang II increased by 1.28 ng/kg per minute (95% CI, 0.50 to 2.05; \(P < 0.002\)) corresponding with 26% (95% CI, 10 to 42%) after 4-weeks administration of fluvastatin (Figure 3).

Baseline values of blood pressure and heart rate before infusion of norepinephrine were similar to the values before infusion of Ang II (Table 1). The norepinephrine-induced dose-dependent increase in blood pressure was not affected by administration of fluvastatin (Table 2 and Figure 2). Incremental infusion rates of norepinephrine decreased heart rate (\(P < 0.05\)) versus baseline. These infusion-induced changes of heart rate were similar during placebo and fluvastatin treatment (Figure 2).

The Pd-20 for norepinephrine during placebo did not differ from the Pd-20 during fluvastatin administration.

The plasma concentration of Ang II, norepinephrine, and PRA at baseline and infusion-induced elevations of Ang II and norepinephrine were similar in the placebo and fluvastatin group (Table 3). The effects on blood pressure of Ang II and norepinephrine were independent of the order of placebo and fluvastatin. VWF antigen values were not affected by fluvastatin; values were 1.07 ± 0.31 U/mL during placebo and 1.05 ± 0.38 U/mL during fluvastatin (\(P = 0.57\)).

**Discussion**

We have shown that subjects with FH have a clearly increased Ang II sensitivity that partially recovers during cholesterol-lowering treatment. Young healthy FH subjects without overt additional cardiovascular risk had ~2-fold increased sensitivity to Ang II compared with normocholesterolemic controls. Treatment with fluvastatin recovered the Ang II sensitivity, but, compared with the normocholesterolemic controls, Ang II sensitivity remained increased. Most probably this was because of the ~2-fold higher LDL-c in the fluvastatin-treated FH subjects. Taken together, our findings strongly support the hypothesis that raised LDL-c increases the sensitivity to Ang II.

**Ang II and Norepinephrine Sensitivity**

Our findings are in line with an observational study in which the systolic blood pressure response to Ang II was ~2-fold greater in 14 hypercholesterolemic than in 13 normocholesterolemic subjects. In that study, sensitivity to norepinephrine in the hypercholesterolemic and normocholesterolemic subjects was similar. Eight of the hypercholesterolemic subjects were treated with statins, reducing LDL-c by 32% and...
decreasing the sensitivity to Ang II by 30%. A challenge with norepinephrine was not performed. The body mass index and the waist circumference were not reported, but relatively high mean triglyceride levels suggest that a proportion of these subjects had the metabolic syndrome.

The only previous placebo-controlled study was performed in 14 subjects with a mean age of 56 years, increased body mass index, hypercholesterolemia, and hypertension: pravastatin reduced LDL-c by 31% and decreased the sensitivity to Ang II and norepinephrine by 30% and 160%, respectively.10 In contrast, an in vitro study showed equal norepinephrine sensitivity in human peripheral small arteries derived from untreated and treated hypercholesterolemic and from normocholesterolemic subjects, whereas the norepinephrine-induced aortic ring constriction of hypercholesterolemic rabbits was profoundly lower than the constriction of normocholesterolemic animals.8,19 Our findings on Ang II sensitivity accord with the literature, but the human data on norepinephrine sensitivity are conflicting and disagree with the findings in animals. This suggests a complex interaction between LDL-c and norepinephrine, likely involving additional factors.

Remarkably, infusion of Ang II was not associated with a decrease in heart rate, whereas infusion of norepinephrine was. The absence of decrease in heart rate during Ang II infusion has been noticed in previous studies.13,20 It is caused by a centrally mediated inhibitory effect of Ang II on the response to baroreflex activation and not to an Ang II–mediated increase in sympathetic tone.21,22 If this inhibitory effect on baroreflex activation had not occurred, the blood pressure dose–response curve to Ang II with the infusion rates applied would have been lower and likely comparable to that of norepinephrine.

Some studies have shown that statins have a blood pressure–lowering effect in poorly controlled hypertensive patients who are either treated or not.23–25 In contrast, studies including normotensive individuals or well-controlled hypertensive patients were unable to demonstrate a statin-induced blood pressure–lowering effect.26,27 In agreement with these latter studies, no blood pressure–lowering effect of fluvastatin was observed in our normotensive FH subjects. The reason for the absence of a blood pressure–lowering effect despite the decrease in Ang II sensitivity is unknown. In any case, it was not a consequence of reciprocally increased renal renin release, because PRA and plasma Ang II concentrations during placebo and fluvastatin were the same.

TABLE 3. Concentrations of Plasma Ang II and Norepinephrine at Baseline and During Infusion of Ang II or Norepinephrine and Plasma Renin Activity at Baseline

<table>
<thead>
<tr>
<th>Baseline and Infusion</th>
<th>Placebo</th>
<th>Fluvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.4±1.5</td>
<td>4.0±1.8</td>
</tr>
<tr>
<td>2 ng/kg per minute</td>
<td>7.5±4.8*</td>
<td>7.9±4.3*</td>
</tr>
<tr>
<td>4 ng/kg per minute</td>
<td>31.1±15.1†</td>
<td>29.8±15.0†</td>
</tr>
<tr>
<td>8 ng/kg per minute</td>
<td>57.1±21.8†</td>
<td>57.3±18.1†</td>
</tr>
<tr>
<td>Norepinephrine (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>146±57</td>
<td>148±51</td>
</tr>
<tr>
<td>30 ng/kg per minute</td>
<td>392±227*</td>
<td>387±175*</td>
</tr>
<tr>
<td>60 ng/kg per minute</td>
<td>810±407†</td>
<td>760±305†</td>
</tr>
<tr>
<td>120 ng/kg per minute</td>
<td>1880±1019†</td>
<td>1806±772†</td>
</tr>
<tr>
<td>PRA, ng Ang I/mL per h</td>
<td>4.5±2.5</td>
<td>4.6±2.8</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P<0.01 vs baseline; †P<0.001 vs baseline.

LDL Cholesterol

Our FH patients had increased LDL-c without other cardiovascular risk factors, like hypertension and insulin resistance. This enabled analysis of the specific interaction between LDL-c and Ang II. Raised LDL-c increases the expression of AT1 receptors in vascular smooth muscle cells explaining the increased sensitivity to Ang II.7,8 LDL lowering by statins may reduce the density of AT1 receptors and the sensitivity to Ang II. Alternatively, some statins, including fluvastatin, may directly inhibit AT1-receptor gene expression by reducing its promoter activity.28 The observation that Ang II sensitivity was lower in the normocholesterolemic control subjects with substantially lower LDL-c levels than the FH subjects treated...
with fluvastatin is supportive for a direct effect of LDL-c rather than the use of fluvastatin as an underlying mechanism modulating AT1 receptor density.

We have found only partial recovery of Ang II sensitivity. Larger reductions of LDL-c might have normalized Ang II sensitivity. It could also be argued that our follow-up period of 4 weeks was too short. However, prolonging the treatment period probably would not have decreased Ang II sensitivity any further, because the cholesterol-lowering effect of fluvastatin is maximal within 4 weeks.29 Endothelial dysfunction at young age is a characteristic of FH.30 Recently, we induced endothelial dysfunction by a systemic subpressor dose of Nω-nitro-L-arginine methyl ester, as well as by a systemic NO-clamp in normocholesterolemic subjects and found an enhanced response to Ang II but not to norepinephrine similar to the present findings in hypercholesterolemic subjects.13 As a marker of endothelial function, VWF antigen was measured in the present study. No decrease in the concentration of VWF antigen occurred with fluvastatin, likely excluding the possibility that the decrease in Ang II sensitivity was caused by endothelial function improvement induced by LDL-c reduction.

In conclusion, this double-blind, placebo-controlled study in a homogeneous group of young subjects with FH demonstrates that LDL-c increases sensitivity to Ang II. This interaction between LDL-c and Ang II sensitivity appears to be specific, because it was not observed for norepinephrine. Furthermore, this interaction is unlikely mediated by a statin-induced improvement of endothelial function but by a LDL-c-induced increase in AT1-receptor density in vascular smooth muscle cells.

Perspectives

It has been well established that hypercholesterolemic patients often have hypertension and that the presence of hypercholesterolemia and hypertension is associated with an excessive cardiovascular risk.31 Upregulation of AT1 receptors in vascular smooth muscle cells may provide a link for the association of hypercholesterolemia and hypertension. In addition, oxidative stress caused by AT1-receptor stimulation may speed up LDL-c-induced atherosclerosis and plaque instability and, hence, the clinical manifestations of cardiovascular disease.12–14 Future research is required to analyze whether more aggressive LDL-c lowering in hypercholesterolemic subjects is associated with normalization of Ang II sensitivity. If, in spite of aggressive cholesterol-lowering treatment, the recovery of Ang II sensitivity remains incomplete, add-on treatment with agents that interfere with the renin–angiotensin system is a logical second step.

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