# Calcification and C-reactive protein in atherosclerosis : effects of calcium blocking and cholesterol lowering therapy

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# Chapter 7

# Anti-atherosclerotic effect of amlodipine, alone and in combination with atorvastatin, in apoE*3-Leiden/hCRP transgenic mice

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## Abstract

***Objective*** To investigate the pleiotropic effects of a calcium antagonist (amlodipine) on early atherosclerosis development in the presence and absence of an HMG-CoA-reductase inhibitor (atorvastatin) in apolipoprotein E*3-Leiden/human C-reactive protein (E3L/CRP) transgenic mice.

***Methods*** Male E3L/CRP transgenic mice were fed a cholesterol-containing diet either with or without amlodipine and/or atorvastatin. After 31 weeks, atherosclerosis in the aortic root area was quantified.

***Results*** Treatment with amlodipine did not significantly lower blood pressure, but resulted in a 43% reduction ($p < 0.03$) of lesion area as compared to the untreated group. Treatment with atorvastatin resulted in an 80% reduction of lesion area as compared to the untreated group ($p < 0.001$). Combined treatment with amlodipine and atorvastatin decreased the lesion area by 93%, significantly more than either treatment alone ($p < 0.008$). Plasma CRP levels were mildly elevated, on average 10 ± 6 mg/L, and did not differ between groups, neither on baseline nor during treatment.

***Conclusion*** Treatment with amlodipine, independently of blood pressure lowering, reduced atherosclerosis development in E3L/CRP mice. Atorvastatin had a strong anti-atherosclerotic effect, while co-treatment with amlodipine enhanced this effect significantly. Plasma CRP levels were not affected by any of the three treatments.

**Keywords:** atherosclerosis, calcium antagonist, statins, transgenic animal models

## Introduction

Atherosclerosis is a multifactorial disease, and the underlying cause of myocardial infarction and stroke. Factors contributing to the atherosclerotic process are hypertension, elevated low-density lipoprotein (LDL)-cholesterol levels, low high-density lipoprotein (HDL)-cholesterol levels, obesity, smoking, diabetes and genetic factors [1]. Drugs have been developed that effectively reduce these risk factors, such as calcium antagonists (CAs) which lower blood pressure, and HMG-CoA-reductase inhibitors (statins) which inhibit cholesterol synthesis and thereby lower plasma cholesterol levels.

The primary action of CAs is to reduce blood pressure by inhibiting $Ca^{2+}$ influx through voltage-gated transmembrane channels. However, CAs may influence a number of other key processes in atherosclerosis such as the oxidation of LDL and smooth muscle cell (SMC) migration and proliferation [2,3]. Several studies have demonstrated that the anti-atherosclerotic effect of CAs was limited to the first stages of atherosclerosis [4,5]. Pre-existing lesions were not influenced by CA therapy as far as angiographic progression or regression was concerned. More recent data have demonstrated that the CA amlodipine had no effect on the progression of atherosclerosis or cardiovascular events, but was associated with a reduction in cardiovascular morbidity [6]. These data failed to support the hypothesis that amlodipine alters the development or progression of minimal coronary artery lesions [6]. However, the recently published CAMELOT study has demonstrated that administration of amlodipine to patients with coronary artery disease (CAD) and normal blood pressure resulted in reduced cardiovascular events, and assessment of atherosclerosis progression with intravascular ultrasound showed that treatment with amlodipine slowed progression of atherosclerosis [7]. Therefore, the anti-atherosclerotic effects of CAs remain under debate.

Statins improve the survival rate of patients with hypercholesterolemia and CAD primarily by lowering circulating LDL cholesterol levels. Moreover, statins have a number of important pharmacological effects in addition to reducing the plasma LDL cholesterol level [8,9].

Combining CA and statin therapy may be more successful than either therapy alone as indicated by retrospective analysis of the data from the REGRESS study, where treatment of patients with both pravastatin and a CA was found to be more successful in retarding the progression of atherosclerosis than treatment with pravastatin alone [10]. Therapy with CAs alone had no effect on the progression of atherosclerosis in that study. This suggests that CAs only exert an anti-atherosclerotic effect in the presence of a cholesterol-lowering agent such as an HMG-CoA-reductase inhibitor.

The cardiovascular risk factor C-reactive protein (CRP) is a major acute phase reactant in man but not in mice. In humans, plasma CRP levels can be reduced by statin therapy [11-14].

Kleemann *et al* [15] have demonstrated that when CRP transgenic mice are treated for a short period with high doses of atorvastatin (0.1% $^{w}/_{w}$) constitutive expression of CRP as well as IL-1β-induced CRP expression can be lowered [15]. A lower dose of atorvastatin (0.01%) did not have such a short-term effect. In order to see if, in the long term, a lower dose of atorvastatin might have a similar effect, as well as to study a possible effect of a CA on the inflammatory marker CRP, we used in this study E3L mice into which the human CRP gene had been introduced.

In the present study we tested whether treatment with the CA amlodipine had anti-atherosclerotic effects, independent of blood pressure lowering, in E3L/CRP transgenic mice. Additionally, we investigated whether a potential anti-atherosclerotic effect of a CA is only seen after lipid lowering. To this end, mice were treated with amlodipine, atorvastatin or the combination of both, followed by quantification of atherosclerotic lesion area in the aortic root. Furthermore, to test whether atorvastatin has any anti-atherosclerotic effects beyond the lipid-lowering effect, a fifth group was added, the low-cholesterol group, having similar plasma cholesterol levels as the atorvastatin-treated mice.

## Methods

### *Mice*

ApoE*3-Leiden (E3L) transgenic mice are an established model for the study of hyperlipidemia and atherosclerosis [16] and have been shown to be responsive to lipid-lowering therapy [17-19]. Male $E3L^{+/-}/CRP^{+/-}$ mice were obtained by cross-breeding female E3L transgenic mice with heterozygous male CRP transgenic mice, originally described by Ciliberto *et al*. [20,21]. Identification of mice transgenic for E3L was performed by an ELISA for human ApoE [16]. The presence of the human CRP gene was assessed by PCR. Male E3L/CRP transgenic mice (mean age: 14 weeks at entry into the study) were used, because female CRP-transgenic mice do not constitutively express CRP [22].

### *Experimental design*

Before the start of the study, all animals were kept on a standard mouse chow (Hope Farms, The Netherlands). During a three-week run-in period, all animals received a semi-synthetic high-cholesterol diet [23] containing 1% ($^{w}/_{w}$) cholesterol and 0.05% ($^{w}/_{w}$) cholate. After the run-in period, total plasma cholesterol concentrations were measured, and the animals were divided into five groups of 15 to 16 mice, matched on the basis of age and cholesterol level. The high-cholesterol control (HC) group received a semi-synthetic diet [Table 1] to which 1% ($^{w}/_{w}$) cholesterol and 0.05% ($^{w}/_{w}$) cholate were added. To further increase the plasma cholesterol level, fructose (10%) was added to the drinking water. This

resulted in an additional increase in plasma cholesterol levels of approximately 3 mM. The amlodipine group received the same diet as the HC group with the addition of 0.003% ($^{w}/_{w}$) amlodipine (equivalent to 3.5 mg/kg body weight per day). This concentration of amlodipine was chosen so that it did not significantly lower blood pressure, since we wanted to study the effect of amlodipine independent of blood pressure lowering. The atorvastatin group received the same diet as the HC group with an addition of 0.0035% ($^{w}/_{w}$) atorvastatin (equivalent to 4 mg/kg body weight per day). This concentration was chosen to lower the plasma cholesterol levels by 30-40%. The combination group received the HC diet supplemented with both atorvastatin and amlodipine in the concentrations mentioned above. The diet of the low-cholesterol control (LC) group was titrated to reach the same plasma cholesterol level as in the atorvastatin group and contained 0.25% ($^{w}/_{w}$) cholesterol and 0.05% ($^{w}/_{w}$) cholate.

Animals had free access to water and food. Body weight and food intake were monitored every 4 weeks. The mice were sacrificed 31 weeks after randomisation.

The Institutional Animal Care and Use Committee of TNO had approved all animal experiments.

**Table 1.** Composition of semi-synthetic, cholesterol-raising diet.[23]

| **Diet T** | |
|---|---|
| consisting of: | 15% cacao butter<br>40.5% sucrose<br>10% corn starch<br>1% corn oil<br>20% casein<br>5.45% cellulose<br>5.1% mineral<br>1% choline chloride<br>0.2% methionine |
| Supplemented with: | 1% cholesterol<br>0.05% cholate |
| Drinking water | 10% fructose in water |

*Analysis of blood parameters*

Blood samples from each mouse were taken at baseline, at t = 4, 8, 12, 16, 20, 24, 28 weeks and at sacrifice. After a four-hour fasting period, blood samples were obtained by tail incision, collected in EDTA-coated tubes and centrifuged at 2000 x g for 10 min at 4°C to

obtain plasma. Total plasma cholesterol and triglyceride levels were measured enzymatically (kit No 236691 (Roche) and kit No 337-B (Sigma), resp.). Lipoprotein profiles were obtained by size fractionation, as previously described [19].

Plasma CRP concentrations were measured using a high-sensitivity in-house enzyme immunoassay using rabbit anti-human CRP IgG as capture and tagging antibody (DakoCytomation, Glostrup, Denmark). Human CRP Standard (Dade Behring) was used as a calibrator [24].

Plasma alanine aminotransferase (ALAT) activities were measured enzymatically (Reflotron kit No. 745 138, Roche).

Serum amyloid A (SAA) was determined by ELISA, as prescribed by the manufacturer (Biosource International, Nivelles, Belgium).

Endothelial activation was assessed by determination of the plasma levels of von Willebrand factor (vWF) by ELISA, using antisera from DakoCytomation, essentially as described by Ingerslev [25], and using pooled rat plasma for calibration.

### *Measurement of systolic blood pressure*

To evaluate the effect of amlodipine on blood pressure, systolic blood pressure was measured in all groups during the first and second week after randomisation by tail-cuff plethysmography. The Blood Pressure System for Rats and Mice (RTBP1001, Harvard Apparatus, Holliston, MA, USA) was used. Because the tails become more rigid as the mice age, the tail-cuff method did not allow blood pressure measurement at later time points. Systolic blood pressure of each mouse was measured in triplicate.

### *Assessment of atherosclerosis development*

31 weeks after the start of the study, mice were sacrificed under general fentanyl / fluanison / midazolam anaesthesia, the hearts were dissected, stored overnight in phosphate-buffered 3.8% formalin, embedded in paraffin, and sectioned. Sections of the aortic root (4 μm thick) were stained with haematoxylin-phloxin-saffron (HPS). Serial cross-sections were used for histological analysis. Per mouse, 4 sections at intervals of 50 μm were used for quantification and typing of atherosclerotic lesions. Each section was subdivided into three segments representing the three aortic valves. Total lesion area was determined using image analysis software (Leica Qwin), the same operator performing all analyses. Per mouse, the average lesion area per section was calculated. For the determination of the severity of atherosclerosis, the lesions were classified into five categories as described before [16,26]: type I) early fatty streak, type II) regular fatty streak, type III) mild plaque, type IV) moderate plaque, and type V) severe plaque.

*Statistics*

All data are presented as mean ± SD, or median (95% confidence interval). For statistical analysis SPSS 10.0 for Windows was used. Overall comparisons between groups were performed with the Kruskall-Wallis test. If only two groups were compared, Mann-Whitney rank sum tests were used. P values less than 0.05 were regarded as significant.

## Results

*Effects of amlodipine on cholesterol levels and blood pressure*

To test whether amlodipine had any lipid-lowering properties a pilot study was performed. At a concentration of 0.005% ($^{w}/_{w}$), amlodipine lowered plasma cholesterol levels and blood pressure in these mice. Because we aimed to have comparable cholesterol exposures in the amlodipine group and HC group, the amount of amlodipine in the diet was reduced to 0.003% ($^{w}/_{w}$). At this concentration, amlodipine no longer had a significant cholesterol-lowering, nor a blood pressure-lowering effect. This concentration was subsequently used in this study.

Mice that were treated with amlodipine had an average systolic blood pressure of 96 ± 3 mmHg compared to 99 ± 4 mmHg (n.s.) in mice that did not receive amlodipine.

*Effects of amlodipine, atorvastatin, and a combination of both on plasma cholesterol levels and lipoprotein composition*

No differences in body weight [table 2] or food intake (data not shown) were observed between groups.

**Table 2.** Plasma levels of lipids and inflammatory markers, and lesion area.

| | HC | Amlo | Atorva | Atorva + Amlo | LC |
|---|---|---|---|---|---|
| N | 12 | 13 | 15 | 16 | 13 |
| Triglycerides, mM (average) | 1.7 ± 0.8 | 1.3 ± 0.7* | 1.2 ± 0.6* | 1.0 ± 0.5* | 1.1 ± 0.5* |
| Cholesterol, mM (average) | 17.2 ± 6.2 | 15.6 ± 5.7 | 10.0 ± 2.5* | 9.5 ± 2.1* | 11.0 ± 3.2* |
| CRP, mg/L (average) | 10.2 ± 6.5 | 11.0 ± 6.2 | 10.6 ± 5.7 | 10.6 ± 4.9 | 10.2 ± 5.3 |
| Blood pressure (mm Hg), t = 10 | 98 ± 4 | 97 ± 3 | 99 ± 5 | 96 ± 3 | 98 ± 3 |
| **Endpoint measurements** | | | | | |
| Final body weight, g | 29.5 ± 2.3 | 28.6 ± 3.0 | 30.2 ± 3.3 | 29.7 ± 2.7 | 29.2 ± 5.1 |
| Plaque surface, $x10^3$ $\mu m^2$ | 27 ± 16 | 15 ± 19* | 5 ± 6 * | 2 ± 2* # | 8 ± 13* |
| SAA, mg/L | 114 ± 58 | 103 ± 57 | 61 ± 37 | 36 ± 19* • | 55 ± 53 |
| vWF % of pooled rat plasma | 189 ± 117 | 148 ± 79 | 101 ± 59 | 64 ± 56* | 56 ± 39* |

Data are presented as mean ± SD. * $p \leq 0.05$ compared to HC group, # $p < 0.05$ compared to amlo or atorva, • $p < 0.05$ compared to amlo (Mann-Whitney rank sum test).CRP = C-reactive protein, SAA = serum amyloid A, vWF = von Willebrand factor, HC = high-cholesterol control, amlo = amlodipine, atorva = atorvastatin

Average plasma cholesterol levels from 8 blood draws (at t = 3, 8, 12, 16, 20, 24, 28 weeks, and at sacrifice) are presented in table 2. Total cholesterol exposure (plasma cholesterol times weeks of exposure) per group is shown in figure 1A. There was no significant difference in cholesterol exposure between the HC group and the amlodipine group. As compared to the HC group, atorvastatin lowered total cholesterol exposure by 42% ($p<0.005$). There was no significant difference in cholesterol exposure between the group treated with the combination of atorvastatin/amlodipine and the group treated with atorvastatin alone [Fig. 1A]. Titration of cholesterol levels in the LC group resulted in comparable cholesterol exposures in the atorvastatin and LC groups [Fig. 1A]. Triglyceride levels of the HC group were significantly higher than in all other groups [table 2].

Lipoprotein profiles showed that atorvastatin mainly reduced VLDL, IDL and LDL cholesterol [Fig. 1B]. Amlodipine did not affect the lipoprotein profile.

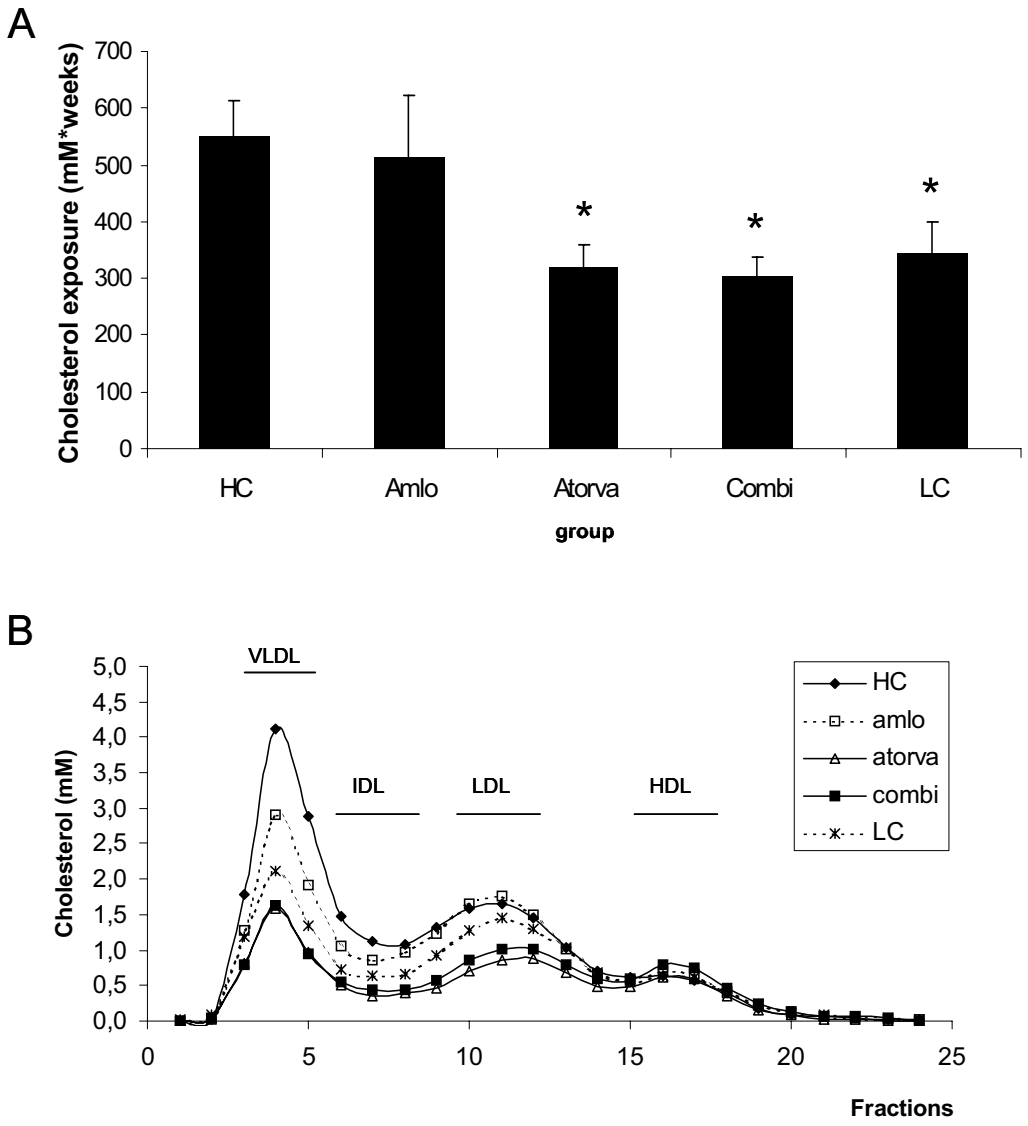



**Figure 1.** The effect of treatment with atorvastatin, amlodipine and the combination of both on plasma lipids. **A.** Cholesterol exposure (plasma cholesterol times weeks of exposure; mM*weeks). ∗ $p < 0.05$ when compared to HC. **B.** Cholesterol profiles of plasma lipoproteins (t = 20 weeks).

### *Atherosclerosis development*

The atherosclerotic lesion area and the severity of the lesions were assessed in cross sections of the aortic root. For each mouse, 4 sections over a 150 µm interval, representing the area in which the aortic valves are clearly visible, were analysed by computer image analysis. Representative photomicrographs of atherosclerotic lesions found in the different groups are shown in figure 2. Atherosclerotic lesions were mostly mild lesions (type I-III) that did not cover the entire vessel wall between the valves.

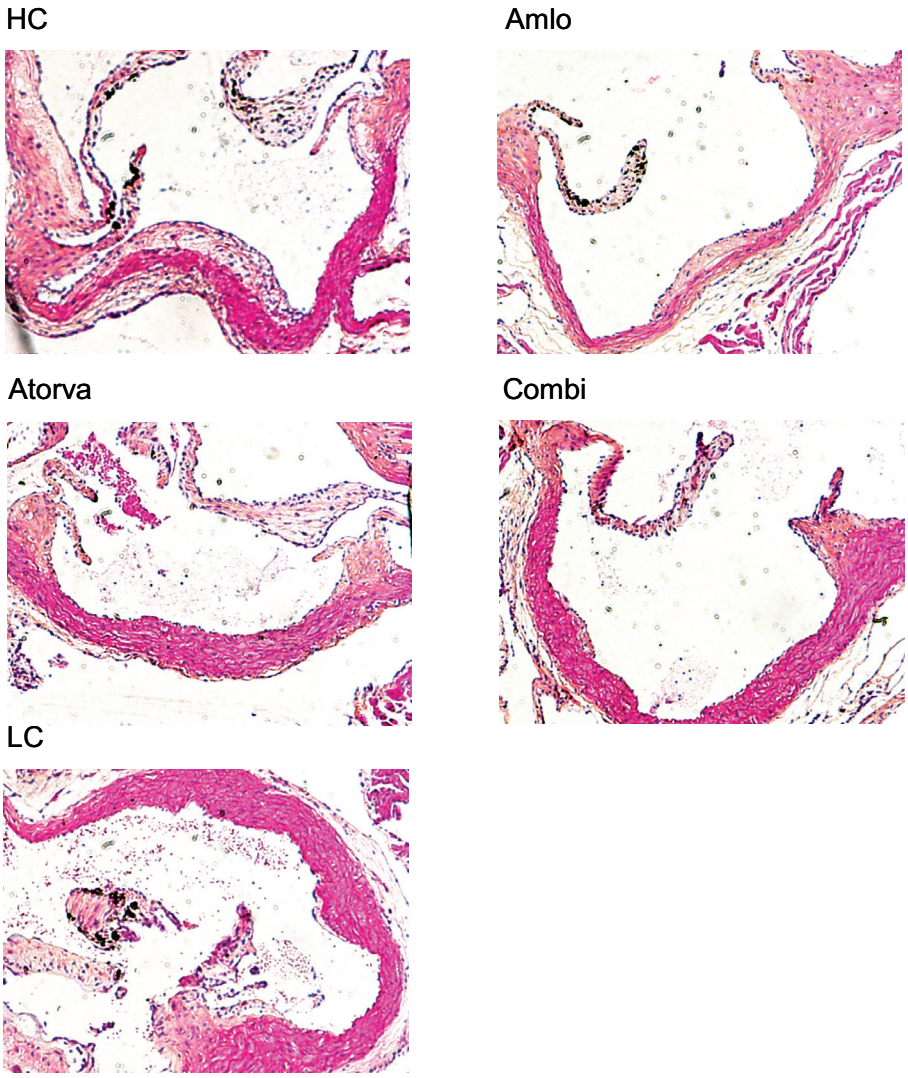



**Figure 2.** Representative photomicrographs of atherosclerotic lesions found in cross sections of the aortic root area of the five treatment groups (haematoxylin-phloxine-saffron staining).

The individual lesion area per section for each group is shown in figure 3. For the HC group the average lesion area per section was 27 ± 16 $\mu m^2$*1000 [table 2]. Treatment with amlodipine resulted in a 43% reduction to 15 ± 19 $\mu m^2$*1000 ($p < 0.03$). Treatment with atorvastatin resulted in an 80% reduction of lesion area (5 ± 6 $\mu m^2$*1000, $p < 0.001$). Combined treatment with atorvastatin and amlodipine decreased the lesion area by 93% to 2 ± 2 $\mu m^2$*1000, which was significantly more than either treatment alone ($p < 0.008$). The lesion area in the atorvastatin group was 30% lower than in the LC group (5 ± 6 $\mu m^2$*1000 vs. 8 ± 13 $\mu m^2$*1000), but this difference did not reach statistical significance.

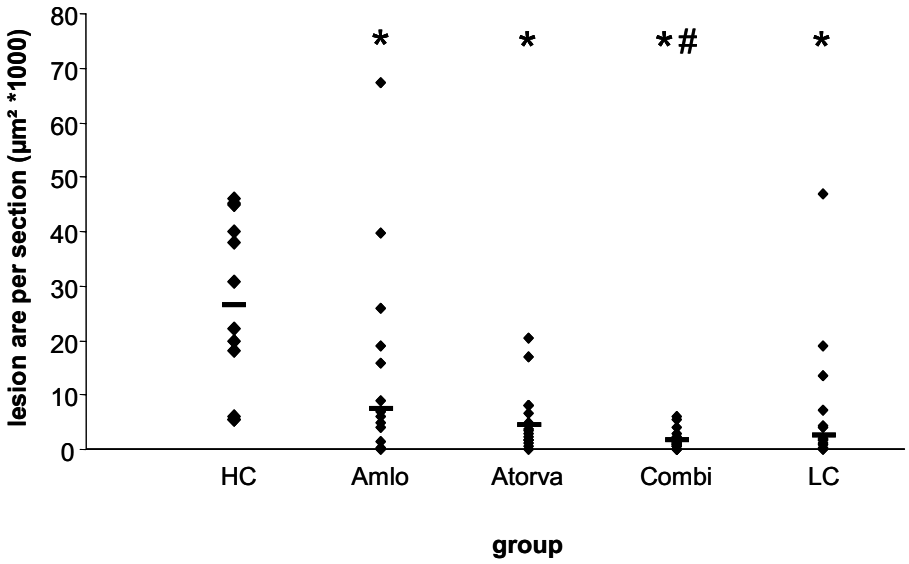



**Figure 3.** Atherosclerotic lesion development. Shown are individual values (♦) for average atherosclerotic lesion area per section per mouse; (—) indicates the median value for each study group. * $p \leq 0.05$ when compared to HC, # $p < 0.05$ when compared to atorvastatin, and amlodipine groups. HC = high-cholesterol control

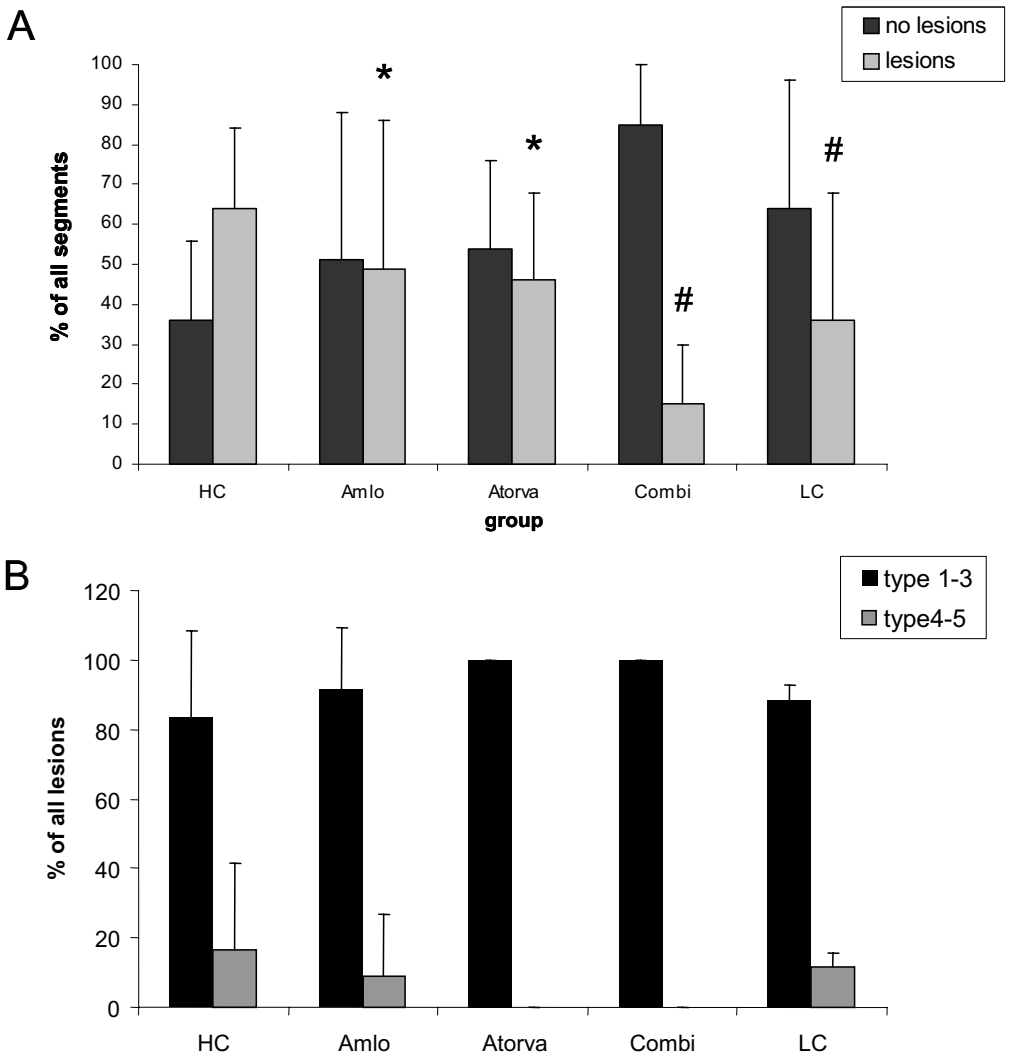
The severity of atherosclerosis was also expressed as the percentages of all segments that contained lesions [Fig. 4A]. This percentage was significantly reduced in all treatment groups ($\chi^2$ test). Furthermore, in the HC and atorvastatin groups all mice had lesions. In the amlodipine, the combination and the LC groups, mice were found that had no lesions at all (1 out of 13, 3 out of 16, and 1 out of 13 mice, respectively).

In addition, lesions were typed as being type I-III lesions (fatty streaks and mild plaques) or as type IV-V lesions (moderate and severe plaques). In the HC, amlodipine and LC groups, 10-20% of the lesions consisted of severe (type IV-V) lesions. In the atorvastatin and combination groups no severe lesions were found [Fig. 4B].

### *The effect of amlodipine and atorvastatin on levels of C-reactive protein, serum amyloid A and von Willebrand factor*

At the endpoint of the study plasma CRP and SAA levels were measured as markers of general inflammation. Von Willebrand factor (vWF) in plasma was measured as a marker of endothelial activation.

**Figure 4.** Atherosclerotic lesion severity. **A.** Severity of atherosclerosis development depicted as the percentage of segments containing lesions.* $p \leq 0.05$ when compared to the HC group, # $p < 0.0001$ when compared to HC group. **B.** Severity of the atherosclerotic lesion as determined by the percentage of lesions classified as type I-III lesions (fatty dots and streaks and mild plaques) and type IV-V (moderate and severe plaques). Data are presented as mean ± SD. HC = high-cholesterol control




Plasma CRP levels were mildly elevated, on average 10 ± 6 mg/L, and did not differ between groups (table 2).

SAA levels were significantly lower only in the atorvastatin + amlodipine-treated group, when compared to the HC group. vWF levels were lower in the atorvastatin + amlodipine-treated and LC groups as compared to the HC group (table 2). There was no significant effect of either atorvastatin treatment alone or amlodipine treatment alone on SAA and vWF levels.

## Discussion

In this study, the anti-atherosclerotic effects of amlodipine, alone and in combination with atorvastatin, on early atherosclerosis development in the aortic root of male hypercholesterolemic E3L/CRP transgenic mice were quantified. Although amlodipine treatment did not significantly lower cholesterol levels or blood pressure, it reduced lesion area by 43%, indicating that amlodipine has an atheroprotective effect even in the absence of blood pressure lowering. The types of lesions found in the amlodipine-treated mice were mostly of type I-III, and 10-20% of type IV-V. In contrast to the HC and atorvastatin groups, in the amlodipine group as well as in the combination and LC groups some mice even had no lesions at all.

The HMG-CoA reductase inhibitor atorvastatin has potent anti-atherosclerotic effects in female E3L mice [18]. The present study shows identical results in male E3L/CRP transgenic mice. In male E3L/CRP mice, atorvastatin (4 mg/kg body weight per day) decreased plasma cholesterol levels by 42%, which was associated with a reduction in lesion area by 80% as compared to the HC group.

Delsing *et al*. [18] treated female E3L mice with atorvastatin (10 mg/kg body weight), amlodipine (2 mg/kg body weight) and a combination of both, and found no effect of amlodipine treatment on atherosclerosis. This contrasts with our findings in male E3L/CRP mice, which could be due to differences in the drug concentrations used, or due to the fact that the lesions Delsing *et al*. studied were advanced lesions, whereas we studied early lesions. Any benefit to be gained with amlodipine could be restricted to effects on the early stages of atherosclerosis only. This suggestion is supported by previous angiographic trials, that have shown that CAs can significantly reduce new lesion formation among patients with documented disease [4,5], while existing lesions were not affected.

Novel actions of amlodipine have been described which suggest that some of its atheroprotective effect may be due to its unique physical and pharmacokinetic properties. Amlodipine is highly lipophilic which enables this drug to partition into the cell membrane. In vascular smooth muscle cells (VSMCs) derived from the atherosclerotic rabbit aorta amlodipine restores cholesterol-induced membrane bilayer abnormalities [27,28]. Other proposed mechanisms through which CAs may affect atherosclerosis development include the inhibition of proliferation and migration of VSMCs [5,29-31], and the inhibition of lipoprotein oxidation [32,33]. In addition, CAs modify the binding of monocytes to the endothelium and the synthesis of matrix components [34].

The present study was designed to detect possible additive effects of atorvastatin and amlodipine treatment. Combined treatment with atorvastatin and amlodipine reduced lesion area more extensively than either treatment alone, leading to a reduction of lesion area by

93%. Also, in the combination group the largest number of mice without lesions was found (19%, 3 out of 16). This additive effect of co-treatment on early atherosclerosis might be mediated through the inhibition of LDL oxidation since combining amlodipine and lovastatin treatment decreases the susceptibility of LDL to oxidation more effectively than amlodipine alone [35].

The recently presented Encore 2 study (presented at the European Society of Cardiology in Munich, Sept 2004, by T. Lüscher), a randomised study comparing plaque progression as measured by intravascular ultrasound in patients treated with 1) statins or 2) a combination of statins with the CA nifedipine, also showed a tendency to a reduced atherosclerosis progression in the patients treated with the combination of statin and nifedipine. Even though these results did not reach statistical significance (probably due to a lack of power), the results pointed essentially in the same direction as the retrospective REGRESS study analysis by Jukema *et al*, showing a reduced progression of atherosclerosis in patients on a combination of statin and CA therapy, compared to statins alone [10].

Our study included, as did the Encore 2 and the REGRESS study, a group treated with both a statin and a CA. In our mice, this combination therapy proved more effective than either compound alone, as in the two clinical studies cited. This may suggest that, in these respects, similar processes are involved in both human atherogenesis and in the murine model used.

*In vitro* and *in vivo* studies have documented cholesterol-lowering independent effects of statins that may be beneficial in atherosclerosis. These include the inhibition of leukocyte adhesion and migration [36], and decreased production of cytokines. Stabilisation of the atherosclerotic plaque, reduction of oxidative stress [37] and inhibition of vascular inflammation have also been attributed to the use of statins [38]. It remains unclear, however, whether direct anti-inflammatory effects contribute to the beneficial role of statins, since cholesterol lowering *per se* is also anti-inflammatory [8]. In this study, we compared mice on a high-cholesterol diet supplemented with atorvastatin with mice on a low-cholesterol diet that resulted in a cholesterol exposure similar to that of the atorvastatin-treated mice. In this way we aimed to assess whether atorvastatin has any effects independent of lipid lowering. Lesion area was reduced by 30% in the atorvastatin group as compared to the LC group. However, this effect was not statistically significant, possibly because the extent of atherosclerosis was very low and the development of atherosclerosis was in the early phase in these groups. Therefore, we could not demonstrate an anti-atherosclerotic effect of atorvastatin in E3L/CRP mice that was independent of cholesterol lowering.

Besides lipid metabolism and high blood pressure, inflammation is an important component of atherosclerosis [39], and plasma levels of inflammatory markers can be used as predictors of cardiovascular risk [40,41]. Therefore, we measured serum amyloid A (SAA) at the

endpoint of the study and C-reactive protein (CRP) levels at several time points during the study. Additionally, we measured Von Willebrand factor (vWF) as a measure of endothelial activation. Although CRP levels gradually declined during the study period, there was no difference in CRP levels between groups. So, none of the drugs had any effect on CRP levels in E3L/CRP mice.

In humans statins reduce CRP levels [11-14]. Kleemann *et al.* have demonstrated that when CRP transgenic mice are treated with a high dose of atorvastatin (0.1% $^{w}/_{w}$) constitutive expression of CRP as well as IL-1β-induced CRP expression can be lowered [15]. We did not observe an effect of 0.0035% $^{w}/_{w}$ atorvastatin on CRP levels in E3l/CRP mice. This lower dose, which was not effective in the short-term experiments described by Kleemann *et al.* [15] and was used here not to lower cholesterol too much, is apparently also ineffective during long-term treatment. Therefore, the absence of an effect of atorvastatin on CRP expression in the present study is most likely a dose-related effect.

Amlodipine alone had no effect on plasma vWF and SAA levels, indicating that the compound has no anti-inflammatory properties. Cholesterol lowering, either by atorvastatin or low-cholesterol feeding, lowered SAA levels in these mice; this effect did not reach significance. Using amlodipine in combination with atorvastatin resulted in a significant decrease of SAA levels when compared to the HC group. The effect of the different diets on vWF was similar, with the exception that lipid lowering did not reduce vWF levels equally in the atorvastatin and LC groups.

## Conclusion

This study in ApoE*3-Leiden/hCRP transgenic mice has demonstrated the atheroprotective potential of amlodipine and atorvastatin on plaque development in the aortic root of male E3L/CRP transgenic mice. The atheroprotective effect of amlodipine was independent of blood pressure. Atorvastatin also had a strong anti-atherosclerotic effect. Co-treatment with amlodipine significantly enhanced this effect. Plasma CRP levels were not affected by any treatment.

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