Cigarette Mainstream Smoke Exposure Increases Plaque Size in the Brachiocephalic Artery in Apolipoprotein E-Deficient Mice on Chow and Milk-Fat-Enriched Diets

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Introduction

Atherosclerosis is the pathologic condition for most of the coronary and arterial vascular diseases. Epidemiologic studies have demonstrated dose-response associations between smoking and the presence and progression of subclinical and clinical atherosclerosis.

The apolipoprotein E-deficient (ApoE-/-) mouse is an established model of atherosclerotic lesion development. In particular the brachiocephalic artery, which is the first branch from the aortic tree, is a site of predilection for the development of atherosclerotic plaques (1). Short-term experimental studies with ApoE-/- mice have revealed that cigarette smoke increases atherosclerosis (2,3,4) and intimal thickening after arterial cuffing (5).

Objective

Investigate the chronic influence of cigarette mainstream smoke (MS) on the development of atherosclerotic plaques, the amount of plaques in the aortic arch, and plaque size in the brachiocephalic artery in ApoE-/- mice.

Methods

Male ApoE-/- mice (20 per group) were whole-body-exposed for 12 months (6 h/d, 5 d/week) to diluted cigarette mainstream smoke at total particulate matter (TPM) concentrations of 100 µg/l and 200 µg/l or to filtered fresh air (sham). The mice were fed one of two different diets (ad libitum) differing in cholesterol/fat content: (i) chow (0.02%/4.5%), and (ii) milk fat (0.17%/21%).

Carboxyhemoglobin was measured as a biomarker of exposure in blood samples collected from the retrobulbar venous plexus of halothane-anesthetized mice.

To determine the amount of plaques that covered the intimal area of the aorta, the aortic arch was removed (**Figure 1A**), cut longitudinally, and opened out with acupuncture needles. One digital image was taken and the size of the aortic arch and the plaques were measured with Diskus software. The intimal area covered by plaques was calculated in percent of aorta size (**Figure 2**).

To determine the plaque size in the brachiocephalic artery, five consecutive cross sections of paraffin embedded tissue were prepared and stained with resorcin-fuchsin (**Figure 1B**). One digital image of each section was taken and analyzed stereologically with Diskus software as described elsewhere (6) (**Figure 3**).

Cholesterol in the aortic arch was determined by high performance liquid chromatography (HPLC).

For statistical analysis, one-way ANOVA and linear regression were performed. Data presented as means ± SE. Asterisks represent statistical significance (*: p <0.05; **: p <0.01; ***: p <0.001).

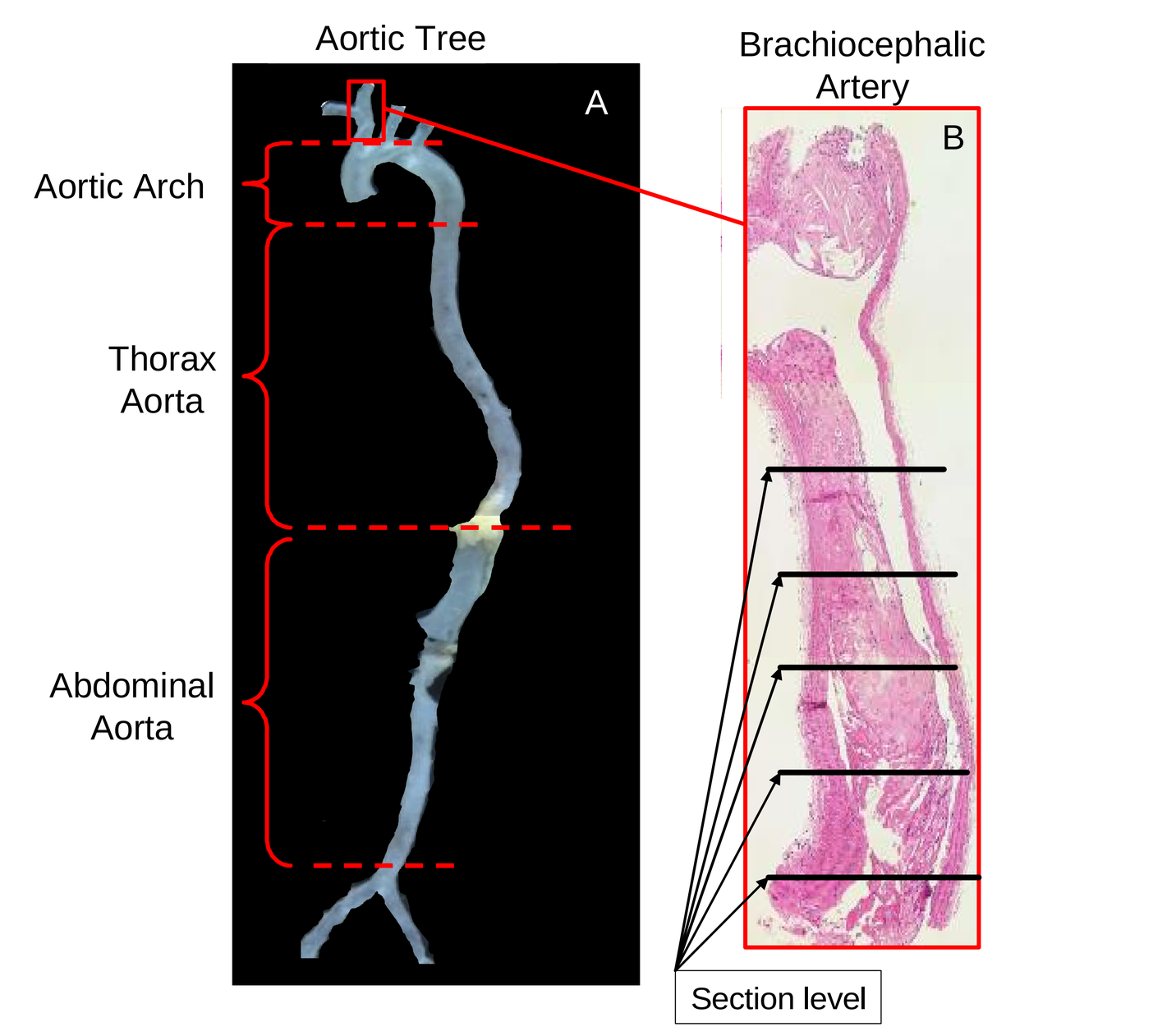

Figure 1: Image of the Aortic Tree of an ApoE-/- Mouse
A) whole tree with sections labeled B) hematoxylin + eosin-stained longitudinal section of the brachiocephalic artery.

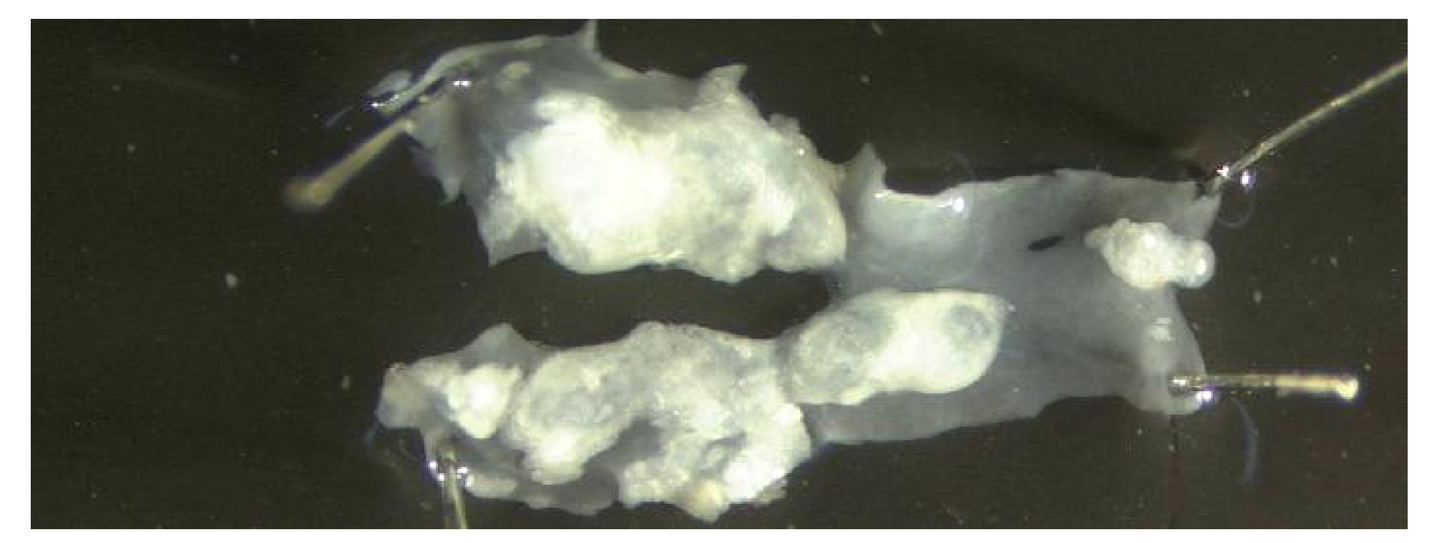

Figure 2: Opened Aortic Arch with Atherosclerotic Plaques (6 months)

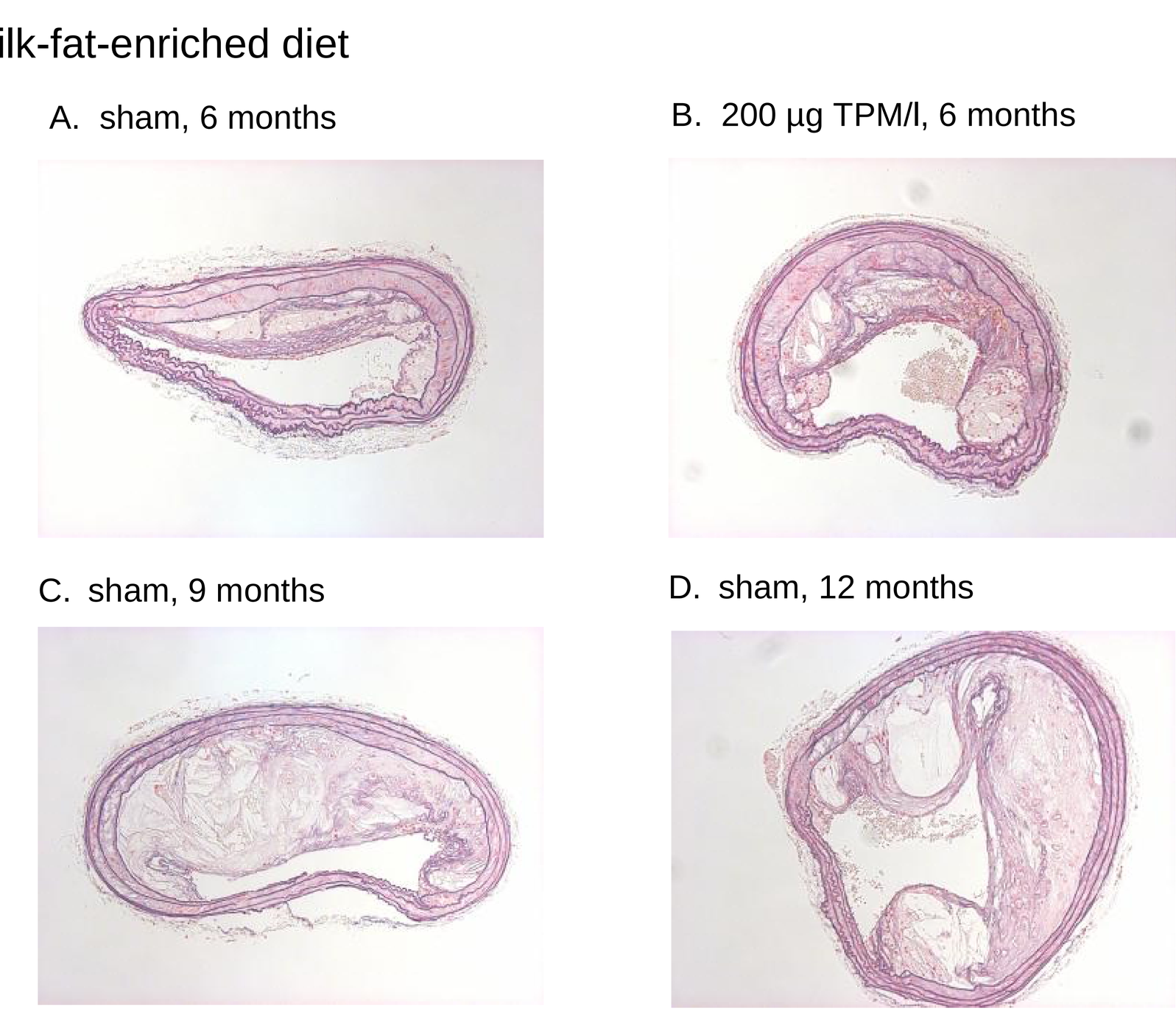

Figure 3: Representative Images of Resorcin-Fuchsin-Stained Cross Sections of the Brachiocephalic Artery Showing Atherosclerotic Plaques

Results

Carboxyhemoglobin

Carboxyhemoglobin levels were 10% ± 0.3 in the 100 µg TPM/l groups and 20% ± 0.6 in the 200 µg TPM/l groups.

Body Weight Development

- Body weight was up to 10% lower in the normal chow smoke-exposed groups.

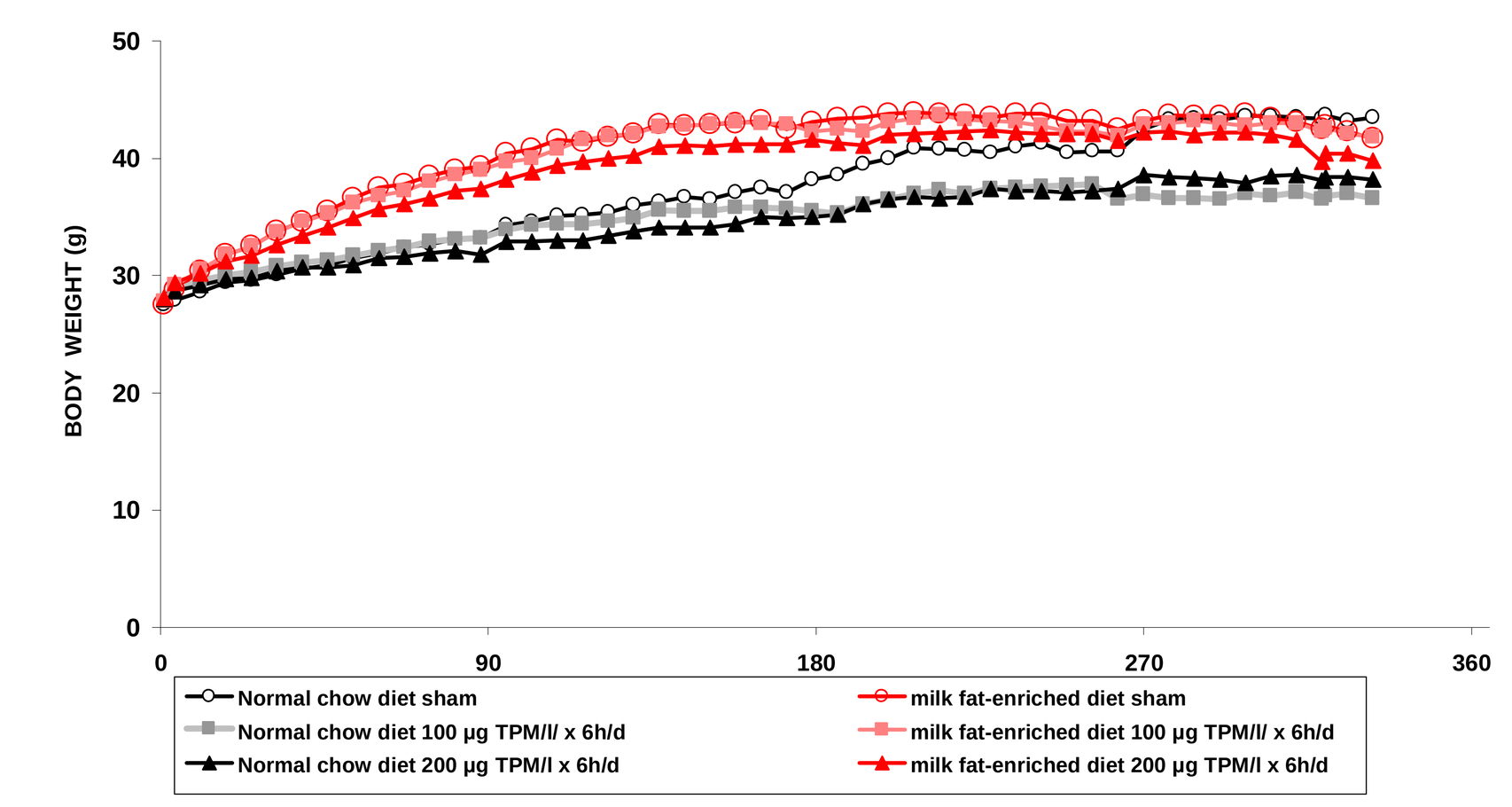

Figure 4: Body Weight Development of ApoE-/- Mice

Cholesterol Content

- Aortic cholesterol content was significantly higher in milk-fat-enriched diet groups compared to chow diet groups (p <0.001).
- Smoke exposure significantly increased the amount of cholesterol in the milk-fat-enriched diet group (200 µg TPM/l vs sham) over the 12-month time course (p <0.05).

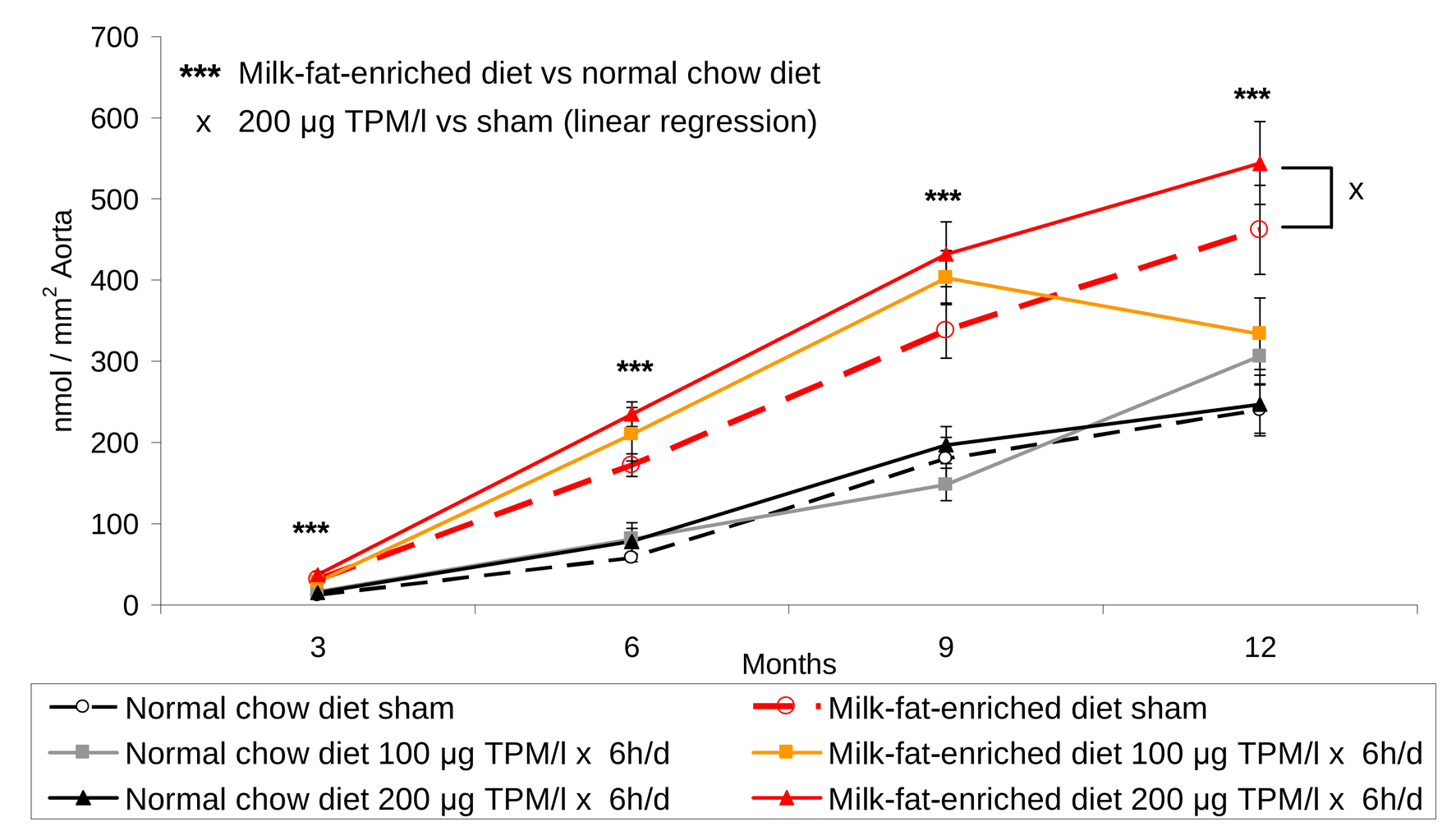

Figure 5: Amount of Cholesterol in the Aortic Arch of ApoE-/- Mice

Absolute Plaque Size in the Brachiocephalic Artery

Smoke exposure significantly increased plaque size

- in the normal chow diet group (200 µg TPM/l vs sham) over the 12-month time course (p <0.05);
- in the milk-fat-enriched diet group (200 µg TPM/l vs sham) over the 12-month time course (p <0.05) and at 6 months (p <0.05) (compare **Figures 3A** and **3B**).

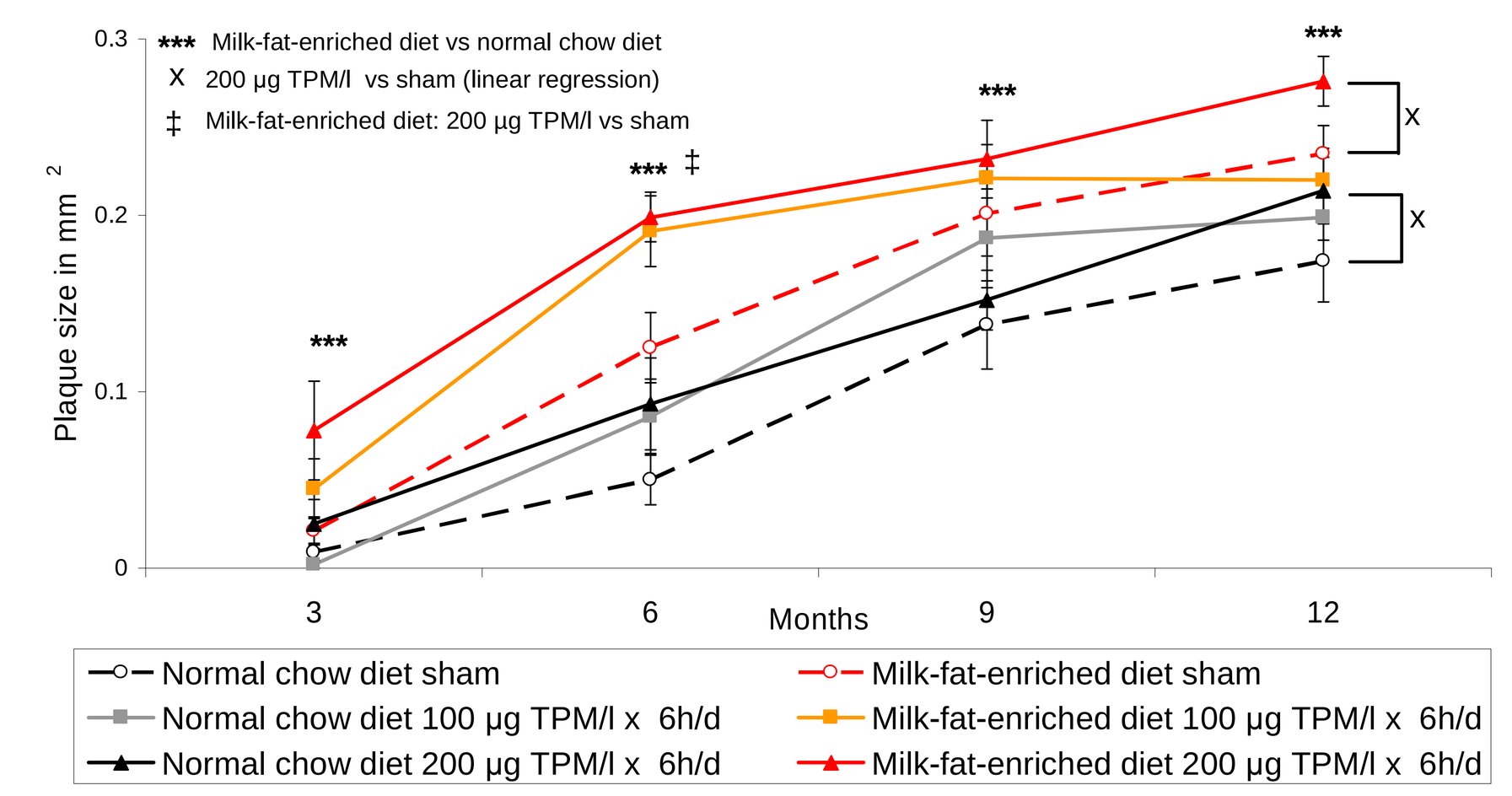

Figure 6: Absolute Size of Plaques in the Brachiocephalic Artery

Relative Plaque Size in the Brachiocephalic Artery

Smoke exposure significantly increased the fraction area of plaque size

- in the normal chow diet group (200 µg TPM/l vs sham) over the 12-month time course (p <0.05);
- in the milk-fat-enriched diet group (200 µg TPM/l vs sham) over the 12-month time course (p <0.05) and at 6 months (p <0.05).

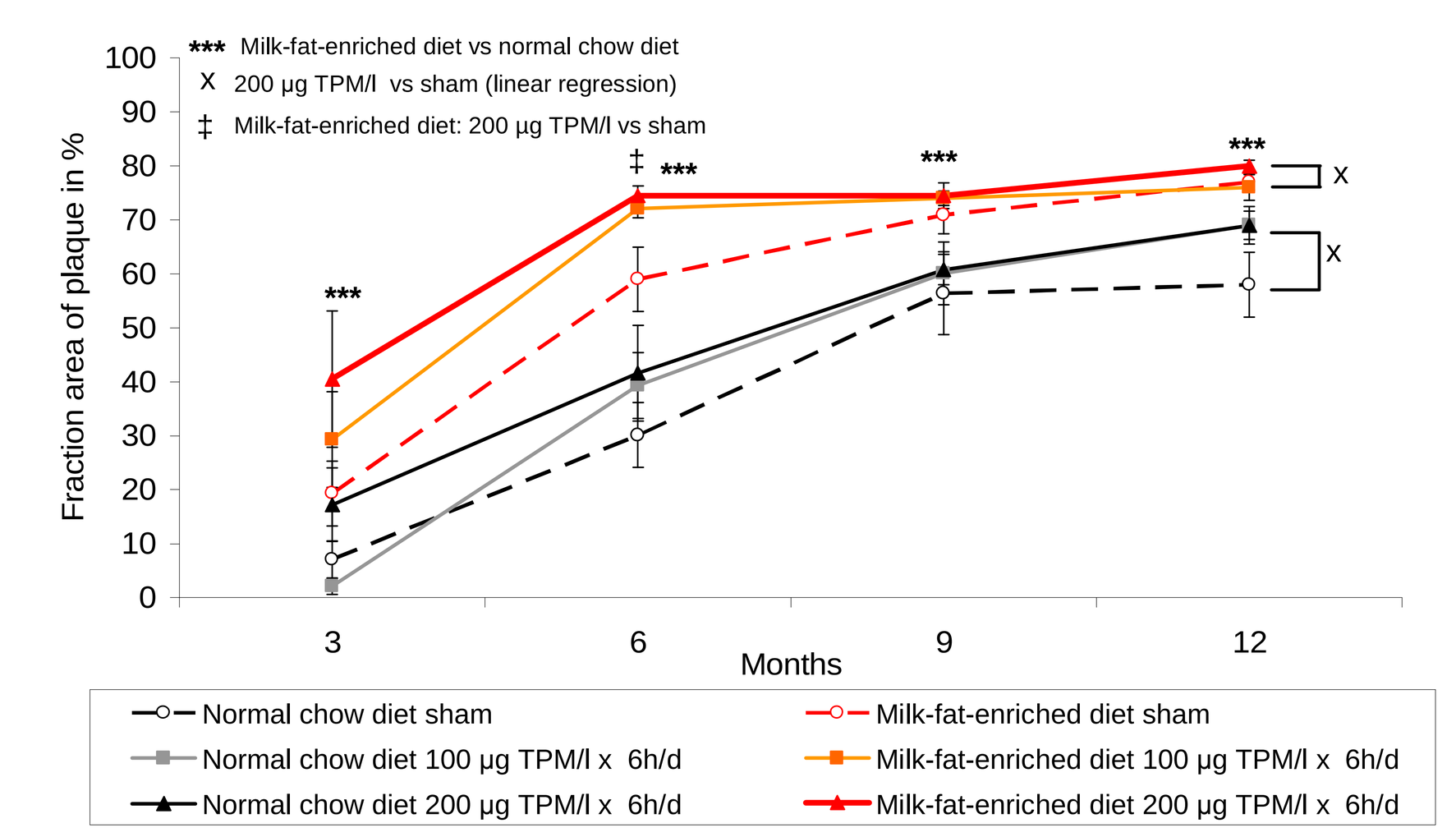

Figure 7: Fraction Area of Plaque Size as a Percentage of the Brachiocephalic Artery
Values level out after 6 months due to an increased vessel size (compare **Figures 3C** and **3D**).

Plaque Size in the Aorta

- Aortic plaque content was significantly higher in milk-fat-enriched diet groups compared to chow diet groups (p <0.01).

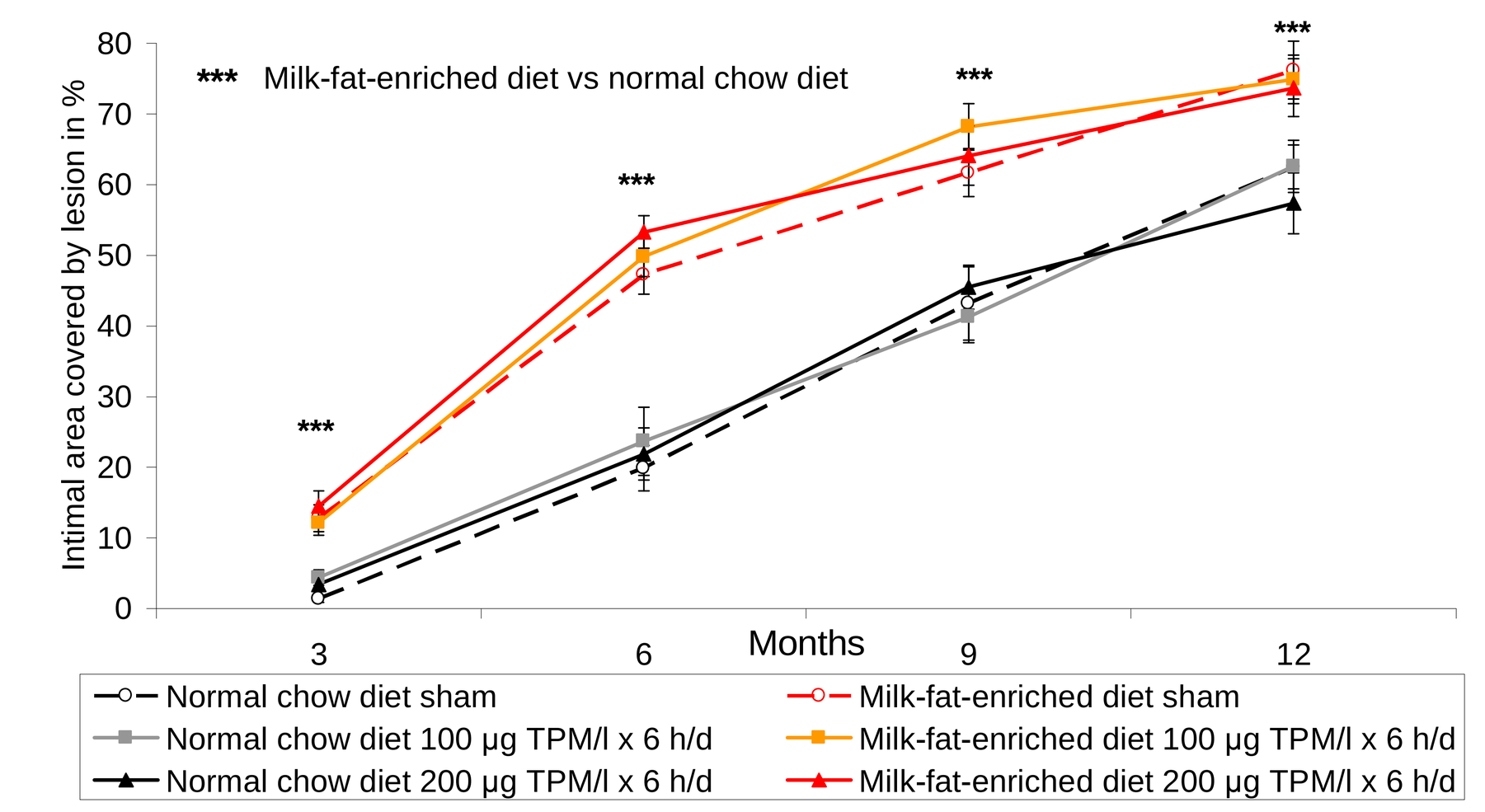

Figure 8: Amount of Plaques in the Aortic Arch of ApoE-/- Mice

Conclusion

The data presented here suggest that atherosclerosis as measured in the brachiocephalic artery is significantly increased by exposure to 200 µg TPM/l cigarette mainstream smoke in ApoE-/- mice on chow and milk-fat-enriched diets.

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