Accelerated Atherosclerosis and Calcification in Vein Grafts
A Study in APOE*3 Leiden Transgenic Mice

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Abstract—Vein grafts fail due to development of intimal hyperplasia and accelerated atherosclerosis. Many murine genetic models in which genes are overexpressed, deleted, or mutated have been introduced recently. Therefore, mouse models are very well suited to dissect the relative contribution of different genes in the development of accelerated atherosclerosis. In the present study, we evaluated whether accelerated atherosclerosis in human vein grafts could be mimicked in hypercholesterolemic APOE*3 Leiden transgenic mice. Venous bypass grafting was performed in the carotid artery in APOE*3 Leiden mice fed either a standard chow diet or a high cholesterol–rich diet for 4 weeks. At several time points (0 hour to 28 days), mice were euthanized and the morphology of the vein grafts was analyzed. In normocholesterolemic mice, vein graft thickening up to 10-fold original thickness, predominantly consisting of α-smooth muscle cell actin–positive cells, was observed after 28 days. In hypercholesterolemic mice, accelerated atherosclerosis with accumulation of lipid-loaded foam cells was observed within 7 days after surgery. This accelerated atherosclerosis progressed in time and resulted in significant increase in vein graft thickening up to 50 times original thickness with foam cell–rich lesions and calcification within 28 days after surgery. The atherosclerotic lesions observed in these murine grafts show high morphological resemblance with the atherosclerotic lesions observed in human vein grafts. This accelerated, diet-dependent induction of atherosclerotic-like lesions in murine vein grafts provides a valuable tool in evaluating the mechanisms of accelerated atherosclerosis and therapeutic interventions of vein graft disease. **(*Circ Res*. 2002;91:577-584.)**

Key Words: vein graft ■ mice ■ accelerated atherosclerosis

Vein bypass grafting remains the most common method of vascular reconstruction to treat obstructive arterial lesions.[1] However, aorta-coronary and peripheral vein grafts are known to have a high failure rate of 10% to 40% after 1 year and 50% to 60% after 10 years.[2] These vein grafts undergo early intimal thickening and accelerated atherosclerosis,[3–6] both of which may contribute to eventual graft failure.

In patients with angiographic evidence of occlusive disease after vein grafting, atherosclerotic lesions within the graft have been demonstrated as early as 6 to 12 months after surgery.[4,5,7,8] These histological studies revealed that the structural changes in venous bypass grafts are due to the development of a rapidly progressive and structurally distinct form of atherosclerosis. This specific, rapidly progressing form of atherosclerosis observed in venous bypass grafts is generally known as accelerated atherosclerosis[9,10] and is morphologically different from native atherosclerosis. Vein graft atherosclerotic lesions are more diffuse, concentric, and friable with a poorly developed or absent fibrous cap, whereas native vessel atheroma are proximal, focal eccentric, and nonfriable with a well-developed fibrous cap.[11,12] Also, accelerated atherosclerotic lesions contain more foam cells with varying degrees of lipid accumulation and macrophage/mononuclear and inflammatory cell infiltration than native atherosclerotic lesions.[13]

Studying the effect of hypercholesterolemia on intimal hyperplasia after a vascular intervention in an atherosclerotic animal model is indispensable to clarify the underlying mechanisms of accelerated atherosclerosis in patients after venous bypass grafting. Several animal models manifesting lesions resembling human vein graft intimal hyperplasia have been developed.[6,14–16] These studies all provide a morphological description of intimal hyperplasia in vein grafts; however, the underlying mechanism of accelerated atherosclerosis could not completely be clarified. For studying accelerated atherosclerosis in vein grafts, the choice of mice allows for the advantages of being able to preform advanced genetic studies such as transgenesis and gene targeting. Transgenic technologies have provided numerous inbred lines with well-defined genetic maps enabling advanced genetic studies of the mechanism of vein graft disease and accelerated atherosclerosis in particular.

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APOE*3 Leiden transgenic mice[17] express the human APOE*3 Leiden gene. These mice have an impaired clearance of chylomicron and very-low-density lipoprotein (VLDL) remnant lipoproteins. As a consequence, these mice develop diet-dependent hyperlipidemia and are highly susceptible to diet-induced native atherosclerosis. This is in contrast to ApoE-deficient mice, in which atherosclerosis is not diet dependent. Furthermore, when fed a cholesterol-rich diet (HFC 0.5%), APOE*3 Leiden mice have been shown to develop typical atherosclerotic lesions similar to APOE$^{-/-}$ mice. However, the lipoprotein profile of the APOE*3 Leiden mice differs from the profile of the APOE$^{-/-}$ mice and resembles more the human situation.[1,2,5] Also, APOE*3 Leiden mice express murine APOE in peripheral cells such as macrophages, whereas in APOE$^{-/-}$ mice, this does not occur. This animal model, in which the atherosclerotic lesions have many features in common with human atherosclerotic lesions,[18,19] is currently considered as one of the animal models of native atherosclerosis closest to that occurring in humans. Taken together, we think that using the APOE*3 Leiden mice is to be preferred above working with wild-type versus APOE$^{-/-}$ mice for studying accelerated vein graft atherosclerosis.

Zou et al[20] described a new method to perform venous bypass grafting in mice. In this model, arterialization of the venous graft predominantly caused by proliferation of vascular smooth muscle cells in normocholesterolemic C57bl/6 mice was described. This smooth muscle cell (SMC) proliferation was significantly reduced in ICAM knockout mice, demonstrating the role of ICAM-1 in vein graft thickening.[21] Also, pretreatment of the vein grafts with the growth factor receptor antagonist Suramin resulted in a significant reduction of neointima hyperplasia, indicating that the PDGF-receptor is involved in vein graft thickening.[22] Recently, Dietrich et al[23] reported the development of vein graft atheroma in ApoE-deficient mice.

The aim of the present study is to assess the early morphology of murine vein grafts implanted into a hypercholesterolemic environment in more detail by examining thickening, graft composition, and cell accumulation after venous bypass grafting in APOE*3 Leiden mice with special attention for vein graft calcification. Our observations indicate that the cholesterol-fed APOE*3 Leiden mouse provides a suitable model to evaluate the mechanisms and therapeutic interventions of accelerated atherosclerosis in vein grafts.

Materials and Methods

Mice

All experiments were approved by the committee on animal welfare of the Netherlands Organization for Applied Scientific Research (TNO). Specific pathogen-free transgenic APOE*3 Leiden mice were crossbred for 18 generations with C57BL/6 mice. Male animals, aged 8 to 10 weeks, were allocated randomly to one of the 2 experimental diets on the basis of age and litter.

Diets

During the experimental period, animals were fed either a chow-diet or a cholesterol-enriched high-fat diet containing 0.5% cholate to improve intestinal cholesterol uptake and suppress bile acid synthesis, both leading to increased plasma cholesterol levels (high fat and cholate [HFC] enriched diet 0.5%: casein 20%, choline chloride 1%, methionine 0.2%, cocoa butter 15%, cholate 0.5%, cholesterol 1%, sucrose 40.5%, cornstarch 10%, corn oil 1%, cellulose 5.1%, and mineral mixture 5.1%) 4 weeks before surgery and continued after surgery. All mice received water and food ad libitum during the entire experiment.

Vein Graft Procedure

After 4 weeks of chow or HFC 0.5%, mice were anesthetized with Hypnorm (Bayer, 25 mg/kg) and Dormicum (Roche, 25 mg/kg) IP Atropine sulfate (1.7 mg/kg body weight) was administered to maintain the respiratory tract in good condition. The procedure used for vein grafts was similar to that described by Zou et al.[20] In brief, the right common carotid artery was dissected free from its surrounding from the bifurcation at the distal end toward the proximal end. The artery was cut in the middle and a cuff placed at the end on both sides. Next, both ends of the artery were everted over the cuffs and ligated with an 8.0 silk ligature. The vena cava was harvested and grafted between the 2 ends of the carotid artery by sleeving the ends of the vein over the artery cuff and ligating them together with an 8.0 silk suture.

Lipids and Lipoprotein Analysis

Blood samples were taken under general anesthesia from the tail vein at the time of surgery and euthanasia. Total plasma cholesterol (Boehringer Mannheim GmbH, kit 236691) and triglyceride (Sigma Diagnostics, kit 337-B) concentrations were measured enzymatically.

Histological Assessment of Vein Graft Lesions

At euthanasia, mice were anesthetized with Hypnorm/Dormicum. The thorax was opened and mild pressure-perfusion (100 mm Hg) with 3.7% formaldehyde in 0.9% NaCl (wt/vol) for 10 minutes was performed by cardiac puncture. After perfusion, the vein graft was harvested, fixed overnight in 3.7% formaldehyde in phosphate buffered saline, and paraffin-embedded.

To assess the effect of hypercholesterolemia on the composition of intimal thickening in time, 3 mice from each group were euthanized at hour 0, and 1, 7, 14, and 28 days after placement of the graft. Serial cross sections (5-μm thick) were used throughout the entire length of the graft for histological analysis. Incorporation of 5-bromo-2-deoxyuridine (BrdU) into DNA as a marker of DNA synthesis was studied in 3 mice from each group (time point 14 and 28 days) by IP BrdU injection 72, 48, and 24 hour before euthanasia. All samples were routinely stained with hematoxylin-phloxine-saffron (HPS). Weigert's elastin staining was used to visualize elastic laminae, von Kossa staining for calcium deposits, and thionin staining for cartilage.[24] Lipid deposition was visualized with Oil red O (Burr) staining. Smooth muscle cells were visualized by immu-

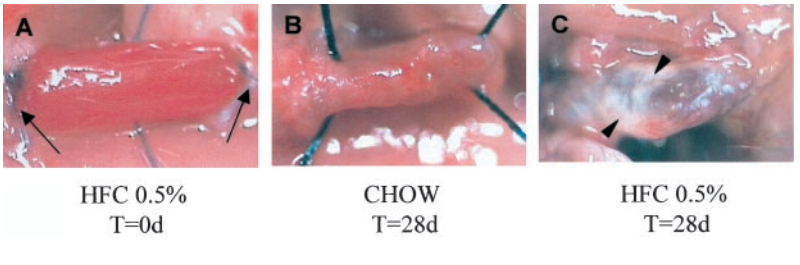

Figure 1. Macroscopic examples of vein grafts in transgenic APOE*3 Leiden mice. A, Vein graft directly after engraftment. Arrows indicate anastomosis. B, Typical example of vein graft 28 days after graft placement in a mouse on a normocholesterolemic diet (chow). C, Typical example of vein graft 28 days after graft placement in a mouse on a hypercholesterolemic diet (HFC 0.5%). Note extensive atherosclerotic plaque formation indicated by arrowheads.

nohistochemistry with α-smooth muscle cell actin (αSM-actin) antibodies (Dako), monocytes/macrophages by Mac-3 (Pharmingen) staining, and endothelial cells by antibodies against PECAM-1 (Pharmingen) and von Willebrand Factor (vWF) (Sigma). Bone related proteins were detected by immunohistochemistry with antibodies against osteocalcin and osteonectin (Anawa Trading SA).[25]

Quantification of Vein Graft Lesions

To quantify the effect of hypercholesterolemia on intimal thickening in murine vein grafts, mice on either a chow diet or HFC 0.5% diet were euthanized 28 days after surgery. Six equally spaced cross-sections throughout the center of the graft were used in all mice to quantify intimal lesions. Using image analysis software (Qwin, Leica), total vessel wall cross sectional area, luminal area, and outer vessel wall circumferential area was measured between the lumen and the adventitia.

Statistics

All data are presented as mean±SEM. Overall comparisons between groups were performed with the Kruskal-Wallis test. If a significant difference was found, groups were compared with their control using Mann-Whitney rank sum tests. Values of $P<0.05$ were regarded significant.

Results

Plasma Lipid Levels

Body weights were monitored at the day of surgery and at euthanasia. No significant changes in body weights were registered in any of the animals. Compared with the standard mouse diet (chow), the HFC 0.5% diet increased plasma cholesterol concentrations significantly (2.1±0.19 versus 26.7±7.0 mmol/L; $P<0.05$), while serum-triglyceride concentrations were decreased as described earlier.[26] The distribution of lipids among the different lipoprotein classes in APOE*3 Leiden mice on a HFC 0.5% diet are highly comparable to the human situation. A shift in lipoprotein profile from the HDL-sized fraction toward the VLDL/LDL-sized fractions, especially when fed HFC 0.5% diet, was observed as reported previously (data not shown).[17,18]

Time Course of Development of Vein Graft Lesions

To study the effect of hypercholesterolemia on the development of intimal hyperplasia in vein grafts in time, 3 mice on the chow diet and 3 mice on HFC 0.5% diet were killed at hour 0, and 1, 7, 14, and 28 days after surgery, respectively. Macroscopic examples of vein grafts directly and 28 days after surgery are depicted in Figure 1. Abundant plaque formation is observed in vein grafts of mice on the hypercholesterolemic diet, whereas in normocholesterolemic mice, no plaque formation was observed. Representative histological sections of a vein graft directly after surgery (t=0) are shown in Figure 2. Both in the animals on a chow diet and on the HFC 0.5% diet, 1 day after surgery the endothelial cell lining of the vein grafts showed evidence of injury. Based on both HPS and vWF staining, a disturbance of the endothelial layer is observed after 1 day, suggesting injury of the endothelium. Also, 1 day after surgery, numerous platelets and polymorphonuclear granulocytes (PMNs) were observed adherent to the endothelial surface in both groups. In both groups, a decrease of αSM-actin–positive cells was observed 7 days after surgery. This phenomenon is in concordance with

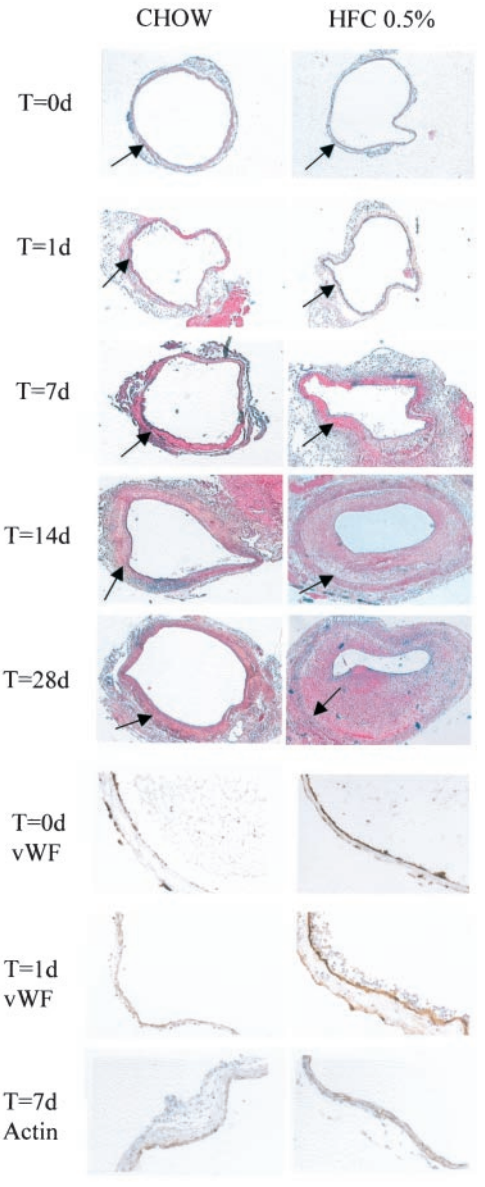

Figure 2. Cross sections of murine vein grafts on several time points (0, 1, 7, 10, 14, and 28 days) after placement of graft in mice on a chow diet and HFC 0.5% diet. In mice of both diet groups, endothelial injury and adherence of PMNs was observed 1 day after engraftment. SMC loss was observed within 7 days after graft placement. In mice on the HFC 0.5% diet, foam cell accumulation was observed after 7 days, whereas in mice on the chow diet, no foam cells were observed. After 14 days, vessel wall thickening was increased in mice on the HFC 0.5% diet compared with mice on the chow diet. This increase in vessel wall thickening was even more pronounced 28 days after surgery. Arrows indicate outer vessel wall border. Magnification, 10× to 25×.

previous reports of SMC loss in human saphenous vein bypass grafts in the first week postoperatively.[13] Also, already after 7 days, first foam cell accumulation was observed in vein grafts of mice on the HFC 0.5% diet. In late vein grafts (14 and 28 days), intimal thickening progressed in both groups. However, the vein grafts in the mice on a chow diet displayed intimal thickening up to 10 times the original thickness 28 days after surgery, whereas vein grafts of mice

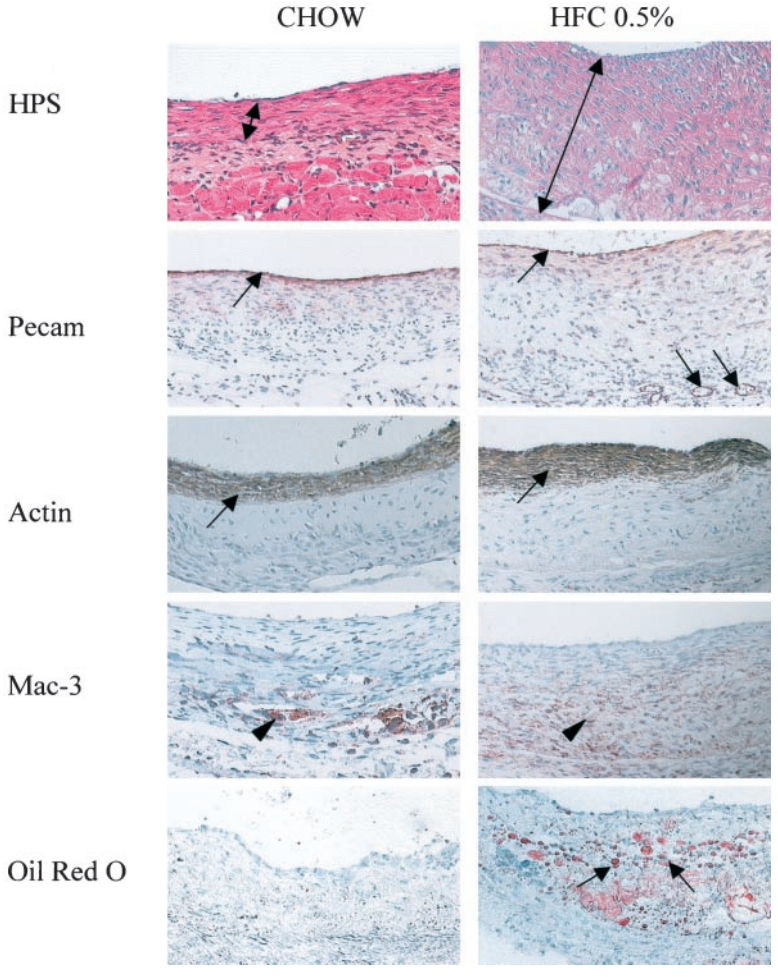

Figure 3. Cross sections of murine vein grafts 28 days after surgery in mice on a chow diet and HFC 0.5% diet; Hematoxylin-phloxine-saffron staining. In mice on the HFC 0.5% diet, a significant increase in intimal hyperplasia is observed, double-headed arrows indicate total vessel wall thickness; PECAM staining for endothelial cells. In both diet groups, endothelial cells are present at the luminal side of the vessel wall.; αSM-actin staining for smooth muscle cells. Intimal hyperplasia in mice on a chow diet predominantly consists of α-smooth muscle cell–positive cells. In mice fed a HFC 0.5% diet, smooth muscle cells occur mainly on the luminal side of the vessel wall; MAC-3 staining for macrophages. In mice on the chow diet, few Mac-3–positive cells are observed in the vessel wall. In granulation tissue surrounding the graft, Mac-3–positive cell are observed (arrows). In mice on the HFC 0.5% diet, abundant lipid-loaded Mac-3–positive cells are present in the graft; Oil red O lipid staining. Abundant lipid deposition in foam cells in vessel wall of grafts in mice on a HFC 0.5% diet, opposed to no Oil red O–positive cell is grafts of mice on the chow diet. Arrows indicate positive cells. Magnification, 25×.

on the HFC 0.5% diet thickening up to 50 times original thickness was observed (Figure 3).

Cellular Composition of Vein Graft Lesions

To characterize the effect of hypercholesterolemia on the cellular composition of late vein grafts, APOE*3 Leiden mice were randomized into 2 groups. One group (n=8) was fed a chow diet, and one group (n=8) fed a HFC 0.5% diet, 4 weeks before the placement of the graft in order to obtain stable plasma cholesterol-levels. Twenty-eight days after surgery, light microscopy of transverse sections through the vein grafts revealed that in mice on a chow diet, a thickening of the vessel wall occurred (Figure 3) as described above, whereas proximal and distal segments of carotid artery possessed normal histology (data not shown). This intimal thickening was approximately 10 cell-layers thick and consisted predominantly of αSM-actin–positive cells, although monocytes/macrophages were also detected. An almost intact endothelial layer was observed in the grafts 28 days after surgery. No foam cells were detected in any of the sections taken from the vein grafts of normocholesterolemic animals at 28 days.

In animals fed the HFC 0.5% diet for 4 weeks, a profound increase in vessel wall thickening compared with normocholesterolemic animals was observed. In the vein grafts of these hypercholesterolemic animals, numerous macrophages and foam cells were present in the subendothelial space and interspersed between the smooth muscle cells. Also, abundant foam cell deposition was observed on the adventitial side of the vein graft. In addition, Oil red O staining revealed excessive lipid accumulation in the vein grafts of mice on the HFC 0.5% diet. This lipid deposition was localized both on the luminal side and on the adventitial side of the vein graft.

In addition, in hypercholesterolemic mice, cartilage formation, calcified cartilage, and amorphous calcification was frequently observed. Less frequently, ectopic bone formation was found in areas of amorphous calcification, whereas in normocholesterolemic mice, less cartilage, calcification, or bone formation could be visualized (Figure 4).

Proliferation was studied by examining incorporation of BrdU into DNA at 14 and 28 days after surgery in mice on a chow diet and on the HFC 0.5% diet. A profound incorporation of BrdU was observed 14 and 28 days after surgery in the vein grafts in both groups (Figures 5A and 5B). No significant difference in increase of BrdU-positive SMCs was observed in the both groups of animals at 14 days, whereas an increase was observed in the normocholesterolemic mice 28 days after surgery when compared with the hypercholesterolemic mice (Figure 5C).

Quantification of Vein Graft Lesions

To evaluate the correlation between serum cholesterol levels and the development of atherosclerotic lesions in the vein

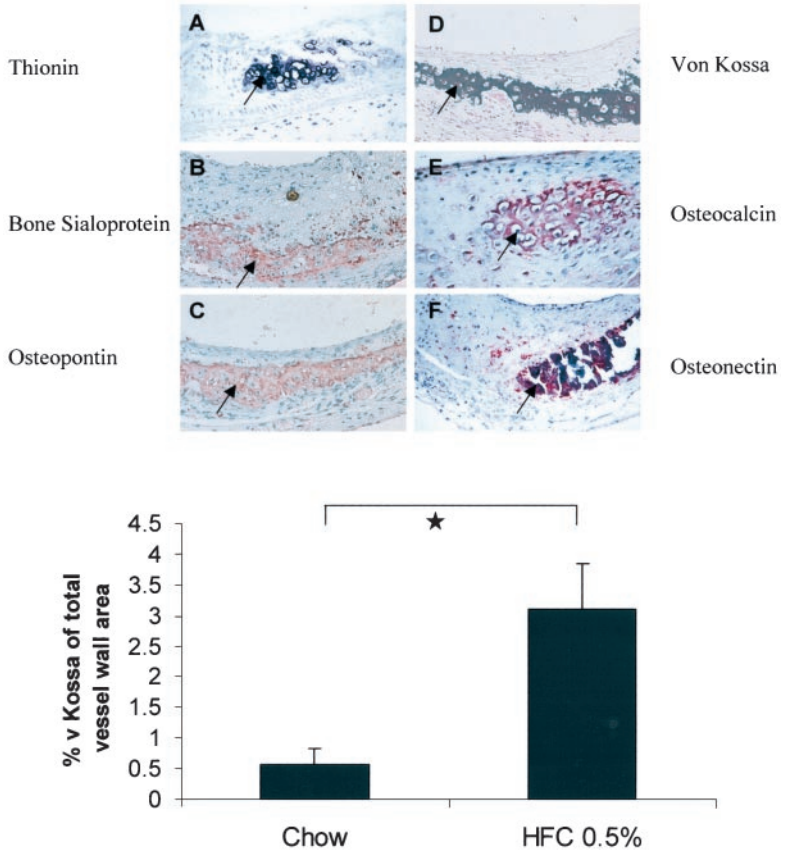

Figure 4. Cartilage formation and calcification in cross sections of murine vein grafts 28 days after surgery in hypercholesterolemic mice on HFC 0.5% diet. Cartilage formation demonstrated by thionin staining (A) could frequently be demonstrated. In the same area, expression of the bone-related proteins, bone sialoprotein (B) and osteopontin (C), could be visualized. Areas of cartilage formation start to calcify as demonstrated with the von Kossa staining (D). Osteocalcin expression was found in areas of calcified cartilage (E). Furthermore, areas of amorphous calcification and, less frequently, ectopic bone formation (not shown) could be visualized. F, Osteonectin-positive areas and cells within and around amorphous calcification. Arrows indicate positive cells or areas of staining. Magnification 25× to 64×. Quantification of calcification displayed by percentage of Von Kossa positive area of total vein graft area is given in G. In hypercholesterolemic mice, a significant increase in total calcified areas in vein grafts can be observed compared with vein grafts of normocholesterolemic mice (n=6). *$P<0.05$.

grafts, 2 groups of 8 APOE*3 Leiden mice received either chow diet or HFC 0.5% diet 4 weeks before bypass surgery. At time of surgery, these groups of mice had a mean serum cholesterol level of 2.3 and 28.6 mmol/L, respectively. Quantification of total vessel wall thickness of 2 groups of APOE*3 Leiden mice revealed a significant 4.5-fold increase in mean total wall thickness in mice fed HFC 0.5% diet ($P<0.001$) compared with mice on a chow diet (Figure 6). Although there was a reduction in luminal area in mice on the HFC 0.5% diet, this was statistically not significant. A significant difference (45.5% increase; $P<0.05$) in vein graft circumference was observed between mice on the 2 different diets. This indicates a positive remodeling to preserve luminal diameter in hypercholesterolemic conditions. Accelerated atherosclerosis was further quantified in both diet groups by quantifying the total MAC-3 positive vein graft area as percentage of total vein graft area (Figure 7). In hypercholesterolemic mice, total MAC-3 positive area was significantly increased compared with normocholesterolemic mice (89%; $P<0.05$). Furthermore, the extent of vein graft calcification was calculated by measuring total Von Kossa positive area as percentage of total vein graft area. Significant increase of total calcified area was observed in vein grafts in hypercholesterolemic mice compared with vein grafts of normocholesterolemic mice (81.6%; $P<0.05$) (Figure 4).

Discussion

The present study describes the effect of a hypercholesterolemic environment on the formation of accelerated atherosclerosis in early vein grafts in mice. The accelerated atherosclerotic lesions observed in murine vein grafts in this study show high morphological resemblance with the atherosclerotic lesions observed in human vein grafts.

Because of the specific nature of accelerated atherosclerosis in human vein grafts, we have to verify that the morphological characteristics of accelerated atherosclerosis in murine vein grafts is comparable with pathological features observed in human vein graft disease. Therefore, the effects of hypercholesterolemia on the early morphological features of accelerated atherosclerosis in early vein bypass grafts are evaluated in APOE*3 Leiden mice, the mice with a human-like lipoprotein profile.

Histological features observed in human vein grafts were recognized in the vein grafts of hypercholesterolemic mice in this study, as discussed in the next paragraphs.

The endothelial cell injury in murine vein grafts is comparable with endothelial damage occurring in the first week after vein grafting in humans. Surgical and ischemic trauma together with altered shear stress is presumed to be accountable for this endothelial damage. Consequently, due to this, aggregation of PMNs on the luminal surface occurred. This has been described previously in animal and human studies.[27,28]

Smooth muscle cell loss was observed in murine grafts in the first week after surgery. This is in accordance with smooth muscle cell loss described in specimens of early vein graft in patients by Kockx et al[13] and in various experimental studies.[27,29]

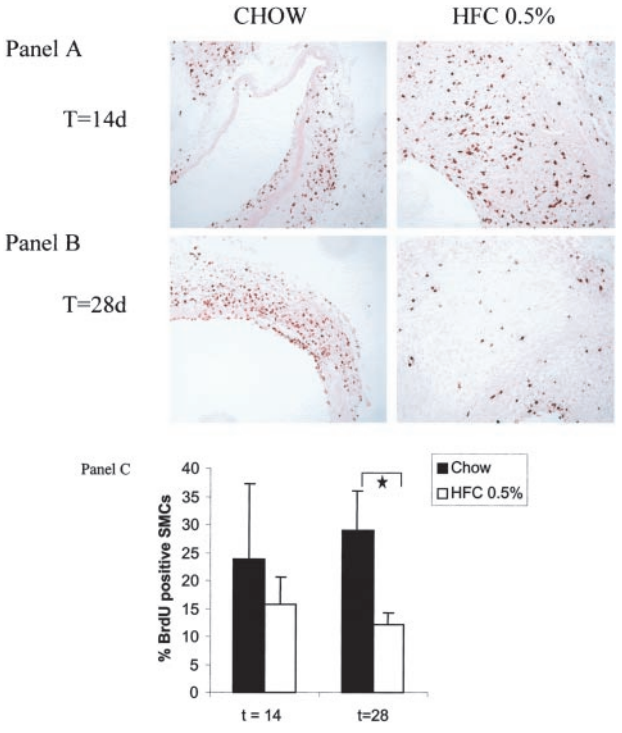

Figure 5. DNA synthesis in cross sections of murine vein grafts 14 and 28 days after surgery in mice on a chow diet and HFC 0.5% diet. Staining for BrdU: A, Extensive BrdU-positive cells 14 days after graft placement in both diet groups; B, Decrease in BrdU-positive cells 28 days after surgery in hypercholesterolemic mice compared with 14-day time point. Magnification, 25×. C, Quantification of BrdU-positive SMCs as percentage of total SMCs in the vein graft in both diet groups at 14 and 28 days after vein grafting. No significant difference in percentage BrdU-positive SMCs was observed in vein grafts 14 days after surgery, whereas at 28 days in hypercholesterolemic mice a decrease in percentage of BrdU-positive SMCs can be observed as compared with the chow diet group. *$P<0.05$.

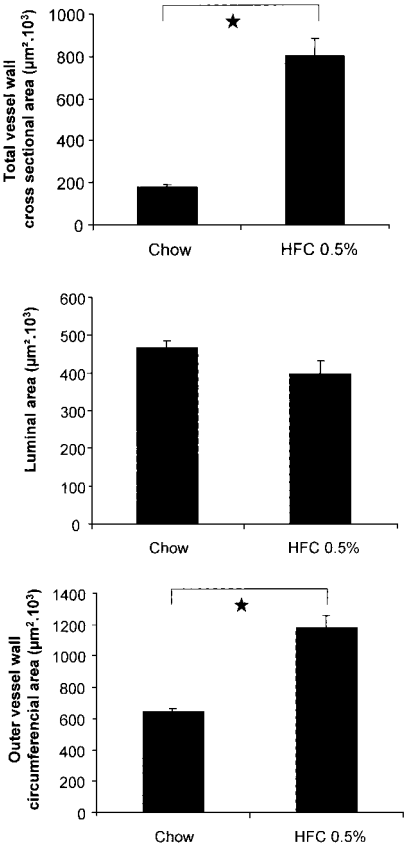

Figure 6. Quantification of total vessel wall area, luminal area, and vein graft circumferential area of APOE*3 Leiden mice fed HFC 0.5% diet (n=8) and chow diet (n=8) 28 days after engraftment. Total areas were quantified by image analysis using 10 serial sections per vein graft segment and expressed in μm^2 (mean±SEM). Arrows indicate positive cells. *$P<0.001$.

In mice on the hypercholesterolemic diet, lipid-loaded macrophages were demonstrated in the subendothelial space as early as 7 days after vein bypass grafting. Also, massive accumulation of foam cells in the vessel wall was detected in the hypercholesterolemic mice. The abundant accumulation of these foam cells within the first week illustrates the extremely fast initiation of the atherosclerotic process in these murine vein grafts. This foam cell deposition is a typical feature of accelerated atherosclerosis in late human vein graft disease.[13,30,31]

Cartilage and amorphous calcification of the vessel wall were frequently seen in mice on the hypercholesterolemic diet. Less frequently, ectopic bone formation was found in areas of amorphous calcification. Calcification of atherosclerotic lesions is a typical feature of advanced stages of atherosclerosis in native arteries[32] but is also observed in late human vein grafts.[33] The occurrence of vascular calcification in this study is especially of interest, because to our knowledge, this observation has not been described in an in vivo experimental animal model before.

In the present study, the significant increase in intimal hyperplasia is accompanied by a significant increase in circumference in hypercholesterolemic animals compared with normocholesterolemic animals. This indicates an expansive remodeling of the grafted vein, which can be considered as compensatory enlargement to preserve lumen diameter and, thereby, flow. This compensatory enlargement was documented previously in patients after venous bypass grafting.[34]

The formation of accelerated atherosclerosis in vein grafts is poorly understood. However, the endothelium, as a mediator of vessel wall homeostasis, is believed to play an important role in this process. Hyperlipidemia increases the duration of endothelial cell recovery, thereby delaying reestablishment of control of barrier function and cell growth.[28] Endothelial injury followed by adherence and penetration of macrophages to the endothelium is correlated to hyperlipidemia.[9] Also, production of superoxide anions by macrophages in hyperlipidemic conditions is documented previously.[9,35] In this study, vein grafts in the mice on the hypercholesterolemic diet displayed a significant increase in total intimal hyperplasia compared with normocholesterolemic animals. This increase of the degree of intimal hyperplasia in the presence of hypercholesterolemia has been described in human studies.[36] This increase of intimal hyperplasia under hypercholesterolemic conditions is attributed primarily to the abundant accumulation of lipid-loaded mac-

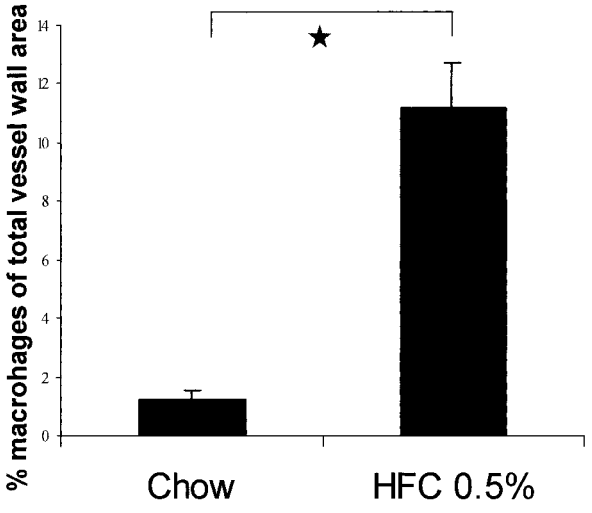

Figure 7. Quantification of accelerated atherosclerosis in vein grafts in normocholesterolemic and hypercholesterolemic total mice by calculation of total MAC-3–positive area as percentage of total vein graft area. A significant increase in percentage of total MAC-3 positive area in vein grafts of hypercholesterolemic mice compared with vein grafts of normocholesterolemic mice 28 days after engraftment was observed (n=6). *$P<0.05$.

rophages, because the foam cell accumulation in hypercholesterolemic animals is not accompanied by an increase in smooth muscle cell index over normocholesterolemic mice.[29] Furthermore, Kockx et al[13] observed a close spatial relationship between foam cell accumulation, pronounced smooth muscle cell loss, and cell death in segments of occluded human vein grafts.

Recently, Dietrich et al[23] described the rapid development of vein graft atheroma in APOE knockout mice. Several similarities between that study and our study can be observed. In both studies, venous bypass grafting was performed in transgenic hypercholesterolemic mice resulting in atherosclerotic lesions within the graft with morphological features of human vein graft disease. However, Dietrich et al mainly focuses on the development in vein graft atheroma between 4 and 16 weeks after surgery, whereas our focus is on the early development of accelerated atherosclerosis within 4 weeks after operation. Furthermore, the presently observed calcified cartilage formation, expression of bone related protein, and ectopic bone formation in murine vein grafts within 4 weeks after surgery are aspects of late human vein graft disease and have never been described, to our knowledge, so early after surgery in an experimental animal model. These early pathological changes, which in patients take several years to develop, may be of value in studying atherosclerotic vein graft calcification in patients. Another difference with the study of Dietrich et al is the choice of murine strain ($APOE^{-/-}$ versus APOE*3 Leiden). When fed a cholesterol-rich diet (HFC 0.5%) APOE*3 Leiden mice have been shown to develop typical atherosclerotic lesions similar to $APOE^{-/-}$ mice. However, the lipoprotein profile of the APOE*3 Leiden mice differs from the profile of the $APOE^{-/-}$ mice and resembles more the human situation.[17,26] Also, APOE*3 Leiden mice express murine APOE in peripheral cells such as macrophages whereas in $APOE^{-/-}$ mice this does not occur. Taken this together, we think that using the APOE*3 Leiden mice on different diets is to be preferred above working with wild-type versus $APOE^{-/-}$ mice.

In conclusion, hyperlipidemia in APOE*3 Leiden mice resulted in a significant increase in accelerated atherosclerosis in vein grafts with profound vein graft thickening and excessive foam cell accumulation within 4 weeks after surgery. Foam cell accumulation was even observed within 7 days after vein bypass grafting, which illustrates the extreme fast initiation of this accelerated atherosclerosis. The atherosclerotic lesions observed in these murine grafts, show high morphological resemblance with the atherosclerotic lesions observed in human vein grafts. This accelerated, diet-dependent induction of atherosclerotic-like lesions in murine vein grafts is of value in studying the mechanisms of accelerated atherosclerosis and provide valuable information for therapeutic intervention in vascular diseases.

Acknowledgments

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