Adventitial vasa vasorum heterogeneity among different vascular beds

Offer Galili, MD,[a] Joerg Herrmann, MD,[a] Julie Woodrum, MS,[a] Katherine J. Sattler, MD,[a] Lilach O. Lerman, MD, PhD,[b] and **Amir Lerman, MD,[a]** *Rochester, Minn*

Introduction: **Different vascular beds show substantial variation in their susceptibilities for development of vascular disease like atherosclerosis, and thereby exhibit a variety of different clinical presentations. Yet, the underlying mechanism of this heterogeneity is not well defined. Recent evidence suggests a role for the vasa vasorum (VV) in vascular disease. We hypothesized that there is a differential distribution structure of adventitial VV in different vascular beds. Hence, the current study was designed to characterize and compare the structure of the adventitial VV in the coronary and the peripheral circulation.**

Methods: **Samples of vessels from different vascular beds were obtained from 6 female crossbred domestic pigs. The samples were scanned using micro–computed tomography, and the images reconstructed and analyzed to characterize VV architecture, including vessel wall area, VV count, VV density, intravessel spatial distribution, mean diameter of first- and second-order VVs and the ratio of second- to first-order VVs.**

Results: **There were significant differences in VV density among different vascular beds. Density was highest in coronary arteries (2.91 ± 0.26 vessels/mm^2, $P < .05$, vs renal, carotid, and femoral arteries), intermediate in renal arteries (1.45 ± 0.22 vessels/mm^2, $P < .05$, vs femoral artery) and carotid arteries (0.64 ± 0.08 vessels/mm^2, $P < .05$, vs femoral artery), and lowest in femoral arteries (0.23 ± 0.05 vessels/mm^2). A similar pattern for the ratio of second- to first-order VV was also observed. Random intravessel spatial distribution of VVs was seen in all vascular beds.**

Conclusion: **The current study demonstrates a differential structure of the adventitial VV in different vascular beds. This intra- and intervessel heterogeneity in VV anatomy is a phenotypic variability that might determine a differential local response to systemic risk factors and, thereby, variable propensity for vascular disease among different vascular beds. (J Vasc Surg 2004;40:529-35.)**

Clinical Relevance: **Atherosclerosis is a diffuse disease with differential expression among different vascular beds, inflicting a spectrum of vascular diseases. The majority of research efforts focus on the inner and medial vascular layers, which are in fact affected at the late stage of atherosclerosis. However, recent evidence suggests that the outer wall, including the adventitial layer and the vasa vasorum, plays a significant role in maintaining vessel integrity, contributing to the initiation and progression of atherosclerosis. The current article extends these previous observations. Using a novel imaging technology, micro–computed tomography, we demonstrate structural heterogeneity of the adventitial vasa vasorum among different vascular beds. This heterogeneity in VV anatomy is a phenotypic variability that might determine a differential local response to systematic risk factors and a concomitant variable propensity for vascular disease among different vascular beds. Furthermore, it suggests that anti-angiogenic treatment aimed at attenuating the VV neovascularization may have a potential preventive or reversal measure against the progression of atherosclerosis.**

Although considered a single system, different branches of the vascular tree show substantial variation in their susceptibility for development of vascular diseases such as atherosclerosis. Potential heterogeneity in the spatial density of the adventitial vasa vasorum among different vascular beds may be a mechanism underlying the subsequent differential expression of disease observed in different arterial branches.

Pathologies in the vascular tree exhibit a variety of different clinical presentations depending on the location of the disease process, the most severe consequences being myocardial infarction, gangrene, stroke, and ischemic nephropathy.[1] Furthermore, data recently published by the American Heart Association reflect that atherosclerosis affects different vascular beds to a different extent,[1]—a significant difference exists in the prevalence and severity of the atherosclerotic lesions in coronary, cerebrovascular, and peripheral circulation. These clinical findings are supported by autopsy-based studies demonstrating heterogeneity in vascular disease process, severity, and incidence among different arterial beds.[2-5] This heterogeneous distribution pattern is remarkable given the systemic exposure to cardiovascular risk factors such as hypercholesterolemia and hypertension, and attributes an important pathophysiologic role to local factors, including the vascular wall structure. However, the underlying mechanism of this heterogeneous response is not yet defined.

Previously, a passive role in the pathophysiology of vascular disease has been ascribed to the outer wall, which includes the adventitial layer and the vasa vasorum (VV). In contrast to this traditional view, recent evidence suggests

From the Divisions of Cardiovascular Diseases[a] and Nephrology and Hypertension,[b] Mayo Clinic College of Medicine.

This work was supported by the National Institutes of Health (R01 HL 63911, K-24 HL 69840-02 and R01 HL63282), the Miami Heart Research Institute, and the Mayo Foundation.

Competition of interest: none.

Reprint requests: Amir Lerman, MD, Division of Cardiovascular Diseases, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN, 55905 (e-mail: lerman.amir@mayo.edu).

0741-5214/$30.00

doi:10.1016/j.jvs.2004.06.032

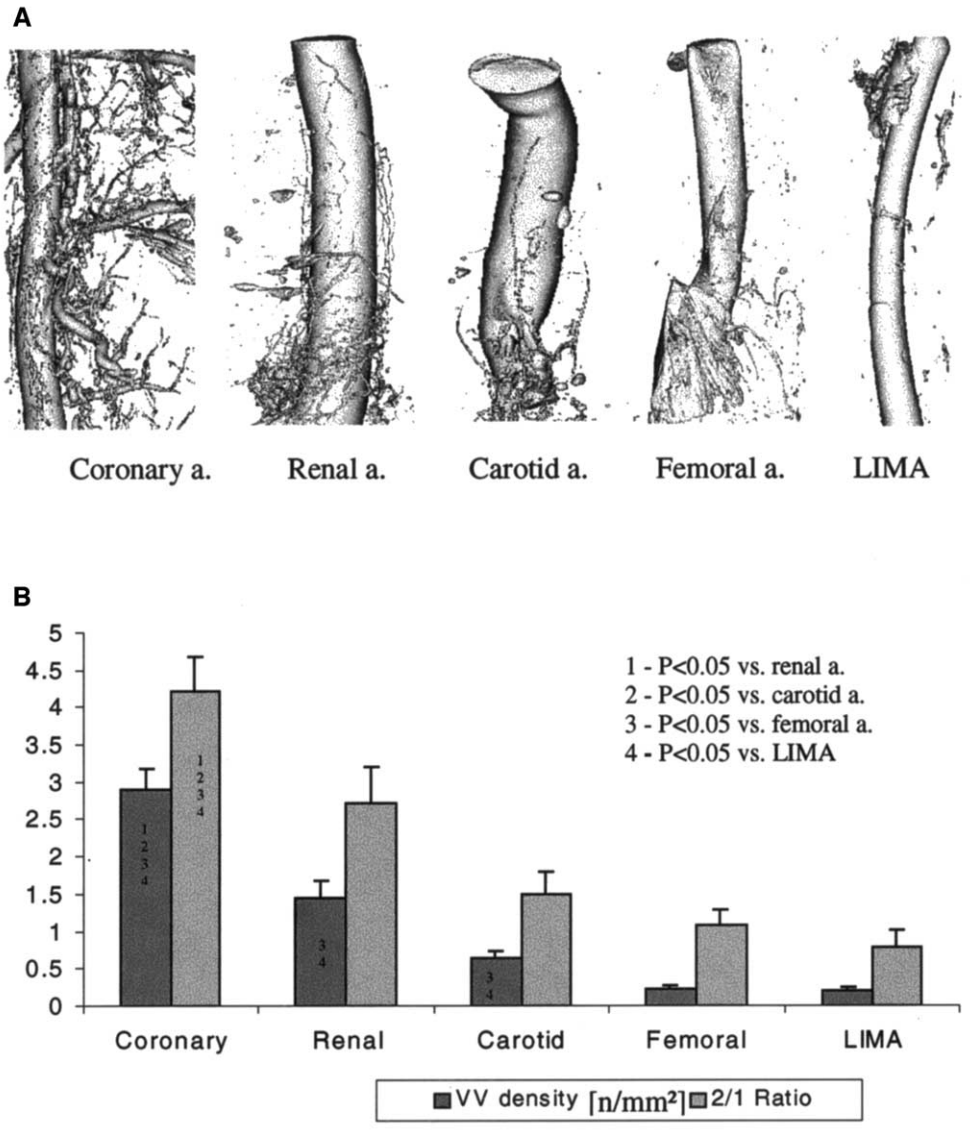

Fig 1. Representative micro–computed tomography images (**A**) and vasa vasorum density with second- to first-order ratio among the different vascular beds (**B**).

that the adventitial layer may play a significant role in maintaining vessel integrity, and may contribute to the initiation and progression of arthritis and atherosclerosis.[6,7] Indeed, experimental studies demonstrated that manipulation of the adventitia and more specifically of the VV (removal of the adventitia or obstruction or hypoperfusion of the VV) could lead to the atherosclerotic changes of the intima.[8-12] Studies have also demonstrated that lesion formation is associated with proliferation of VVs at the site of the lesion formation.[13-15] Attenuation and regression of the atherosclerotic process with medical intervention (HMG-CoA) has been associated with a decrease in VV density.[16] These studies underscore the role of the adventitial VV in the pathophysiology of vascular diseases. However, these studies were performed on the coronary vasculature and none of them addressed the heterogeneous distribution pattern among different vascular beds as it relates to adventitial and VV structure. As stated earlier, potential heterogeneity in the spatial density of the adventitial VV among different vascular beds may be one of the mechanisms underlying the subsequent differential expression of disease observed in these beds.

The current study was designed to test the hypothesis that there is a differential structure of adventitial VV in different vascular beds. Specifically, the spatial density, distribution, diameter of first- and second-order VVs, and the ratio of second- and first-order VVs in the coronary, renal, carotid, and femoral circulation were examined.

Micro–computed tomography analysis data

	Coronary artery	*Renal artery*	*Carotid artery*	*Femoral artery*	*Left internal mammary artery*
Vessel wall area (mm^2 mean/section)	$2.1 \pm 0.1^{*\dagger\ddagger\S}$	$7.3 \pm 0.7^{\ddagger}$	$12.3 \pm 1.6^{\S}$	$12.6 \pm 1.2^{\S}$	7.2 ± 0.3
VV count (n mean/section)	$6.5 \pm 0.6^{\ddagger\S}$	$7.6 \pm 1^{\ddagger\S}$	$5.7 \pm 0.9^{\ddagger\S}$	1.9 ± 0.3	1.6 ± 0.3
VV density (n/mm^2 mean/section)	$2.9 \pm 0.3^{*\dagger\ddagger\S}$	$1.5 \pm 0.2^{\ddagger\S}$	$0.6 \pm 0.1^{\ddagger\S}$	0.2 ± 0.05	0.2 ± 0.04
Ratio 2nd/1st order VV (mean/section)	$4.2 \pm 0.5^{*\dagger\ddagger\S}$	$2.7 \pm 0.5^{\S}$	1.5 ± 0.3	1.1 ± 0.2	0.8 ± 0.2
Diameter 1st order VV (μm)	$84.2 \pm 2.4^{*}$	$111.4 \pm 5.4^{\dagger\ddagger\S}$	84.5 ± 5.1	94.9 ± 7.4	71.2 ± 10
Diameter 2nd order VV (μm)	$58.1 \pm 2.2^{*\ddagger}$	$72.3 \pm 3.6^{\S}$	$70.3 \pm 4.1^{\S}$	$74.9 \pm 6^{\S}$	44.1 ± 5

VV, Vasa vasorum.
*$P < 0.05$ vs renal artery.
†$P < 0.05$ vs carotid artery.
‡$P < 0.05$ vs femoral artery.
§$P < 0.05$ vs left internal mammary artery.

METHODS

Animals. This study was reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee. Experiments were conducted on 6 normal female crossbred domestic pigs of similar ages (50-70 kg, 5-6 months old) (Larson Products, Sargeant, Minn). The swine is an appealing model for studying human disease because its cardiovascular anatomy and physiology are comparable to humans', and lipid profile and development of vascular disease in pigs closely resembles human disease.[17] The animals were euthanized with an overdose of sodium pentobarbital (10 mL Sleepaway; Fort Dodge Laboratories, Fort Dodge, Iowa) and the coronary (left anterior descending [LAD]-medial segments), carotid, renal, and femoral arteries (both sides, medial segments) were harvested immediately after euthanasia. Due to its low incidence of atherosclerosis,[18,19] the left internal mammary artery (LIMA) was examined as well.

Micro–computed tomography analysis. Segments of the arteries were prepared and scanned by micro–computed tomography (micro-CT) as described previously.[20-22] Briefly, a glass cannula was advanced into the most proximal part of each artery, followed by injection of 500 mL heparinized saline (0.9% sodium chloride with 5,000 IU of heparin) at an infusion rate of 10 mL/min to clear the circulation from remaining blood and microthrombi. Next a low-viscosity (20-centipoise) lead chromate–doped silicon polymer (MicrofilTM, MV-122; Canton Biomedical Products, Boulder, Colo) was injected into the regional circulation at a rate of 0.6 mL/min (pressure 70 mm Hg) until the polymer emerged from the veins. After overnight refrigeration at 4°C to allow polymerization of the compound, the arteries were carefully dissected, with the adventitia preserved intact, and placed in a 36.5% formaldehyde solution. Subsequently, specimens were placed in glycerin solution at increasing concentrations (30%, 50%, 75%, and 100% changed at 24-hour intervals) for dehydration of the segments and were thereafter rinsed with acetone, air-dried for 24 hours, and embedded in a paraffin mold for 3-dimensional (3D) Micro-CT imaging.

This yielded an image with a 3D matrix of 42-μm cubic voxels with 16 bits of gray scale (Fig 1). Data analysis was performed using Analyze software (Biomedical Imaging Resource, Rochester, Minn). On average, 6 to 12 cross sections at 1-mm intervals, in areas between branch points, were chosen as regions of interest for analysis. The area of VV analysis was determined as previously described by Edelman et al,[23]—in short, the radius of the vessel, including the lumen and the wall, is doubled to determine the VV area borders. In this way the VV area is normalized to the vessel lumen area and designated vessel wall area.[16,22,24] VVs were manually traced and measured in this area on each cross section, yielding the following parameters: vessel wall area, VV count, density (ie, number VV/mm^2 vessel wall area), intravessel spatial distribution, and mean diameter and ratio of second- and first-order VVs. First-order VVs are defined as those originating from the vessel lumen and running longitudinally, and second-order VVs as those originating from first-order VVs and running circumferentially.[25]

Statistical analysis. Continuous data are expressed as mean ± standard error. Multiple group comparison was based on Kruskal-Wallis one-way analysis of variance on ranks and Spearman Rank Order Correlation. Statistical significance was accepted for a value of $P < .05$.

RESULTS

Histology cross sections from all sites showed that the vessels were free of atherosclerotic lesions.

Vessel wall area, VV count, and VV density. Vessel wall area was highest for the femoral artery, decreasing significantly in the renal and coronary arteries (left anterior descending–medial segment). The VV count was significantly low in the femoral artery versus the other vascular beds (Table). Consequently, as displayed in Fig 1, VV density was highest in the coronary arteries, intermediate for renal and carotid arteries, and lowest in the femoral arteries.

Ratio of second- to first-order VVs. The ratio of second- to first-order VVs was highest in the coronary arteries, followed by the renal, carotid and femoral arteries, respectively (Fig 1). A significant positive correlation was found between VV density and the ratio of second- to

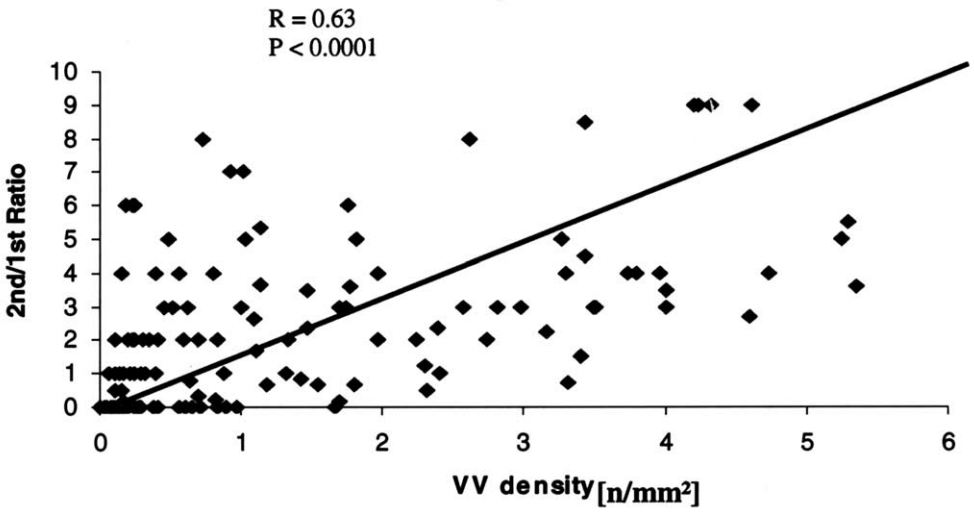

Fig 2. Correlation between second- to first-order ratio and vasa vasorum density in different vascular beds. The figure shows all the vessels compiled. When tested separately all the vascular bed showed a positive correlation except for the carotid artery.

first-order VVs in all vascular beds except for the carotid artery (Fig 2).

Vasa vasorum diameter. First-order VV diameter was highest in the renal artery, with a significant difference between it and the other vascular beds that were similar to each other. Second-order VV diameter was lowest in the coronary artery, with a significant increase in size in the carotid, renal, and femoral arteries (Table).

Intravessel spatial distribution of VV. Analyzing the spatial distribution of the VVs within each vessel[26] showed intravessel heterogeneity, but no evident pattern was detected in any of the vascular beds.

Left internal mammary artery. The LIMA was examined due to its low incidence of atherosclerosis. As displayed in the Table, VV area, count, and density differed significantly between the LIMA and the coronary, renal, and carotid arteries. Compared with the LIMA, the second- to first-order VV ratio was significantly higher in the coronary and renal arteries and second-order VV diameter was significantly higher in all vessels.

DISCUSSION

The current study demonstrates heterogeneity in the distribution of VVs among different vascular beds. Notably, the variability in this anatomic feature can support the theory of phenotypic differences among different vascular beds and may relate to the differential expression patterns of atherosclerotic cardiovascular disease (ASCVD). The tendency of atherosclerosis to localize in arterial bifurcation led to the recognition that hemodynamic features, such as low shear stress forces, turbulence, and oscillatory flows, which are present in bifurcation areas, play a major role in the localization of the atherosclerotic plaque. Nevertheless, different vascular sites are sensitive to various extents with regard to systemic factors such as hypercholesterolemia,[27,28] genetic background,[29] increased oxidative stress,[30] gender,[31] and immune status,[29] resulting in the heterogeneous distribution of disease. VanderLaan et al[32] suggested differential gene expression profiles of the vascular cells in different vascular beds in response to the local flow patterns. Although speculative, such differences in the gene expression can determine a different local response to systemic risk factors resulting in differences in size, composition and progression of the atherosclerotic lesion. In addition, anatomic heterogeneity can contribute to variations in vascular vulnerability.

Previous studies demonstrated proliferation of adventitial VV in the atherosclerotic vessels.[8-12] Micro-CT is a novel technology, which by tomographic reconstruction of the adventitia enabled us to outline and quantify the 3D anatomy of VV.[20-22] We have previously demonstrated that early atherosclerosis is characterized by neovascularization of the porcine coronary arteries, suggesting a significant role of the adventitial vasa vasorum prior to vasofunctional alterations and plaque formation.[16,24]

In the current study we applied the micro-CT technology to the porcine peripheral circulation, showing heterogeneity of VV density among different vascular beds. The VV is regarded as the vascular bed of the vessel wall. Hence, this observation of anatomical variability might be the result of vessel wall exposure to different oxygen tension.[33,34] Increase in wall thickness causes a decrease in the oxygen content of the blood within the vessel wall as the diffusion distance from the lumen increases. Hence, a difference in wall thickness between different vascular beds necessitates different extents of VV network in order to deliver nutrients and oxygen to the vascular wall. The finding of a positive direct correlation between VV spatial

density and the second- to first-order VV ratio indicates that this intervessel variability is achieved by variation in the second order of the VVs. In a previous work we observed a similar correlation in vessels exposed to hypercholesterolemia, indicating that proliferation of second order VVs is related to the early atherosclerotic process.[35] On the other hand, in addition to VV density, the regulation of the perfusion of the vessel wall is dependent also on the endothelium, which is a highly vulnerable target organ in ASCVD. Exposure to risk factors causes endothelial dysfunction at an early stage. Since VV has the complete structure of a blood vessel, endothelial dysfunction is present at its level as well as at the level of the host vessel.[36-40] Under baseline conditions (eg, the absence of risk factor) like those in the current study, the host vessel is in a steady state, receiving ample nutrient and oxygen supply. However, as demonstrated in our previous studies,[16,24,35,41] once this equilibrium is disturbed and vessel wall hypoxia occurs, vessel growth, eg, VV neovascularization, is triggered by signaling through hypoxia inducible transcription factors and downstream angiogenic factors such as vascular endothelial growth factor.

Having a significantly (more than 70 times) higher endothelial surface area, the presence of endothelial dysfunction at the VV level might have more of an impact on vascular wall perfusion than the host vessel and might render it more susceptible to atherogenesis. This is supported by a study in ApoE-deficient mice, showing that anti-angiogenic therapy not only reduced plaque neovascularization but eventually plaque growth.[42] Thus, when vascular beds are exposed to risk factors, one may anticipate variability in vasoreactivity between different vascular beds due to the difference in VV spatial density; eg, vessels with low VV density at their steady state will be less affected. Furthermore, the extensive VV network can function as a conduit for entry of macrophages and inflammatory factors that may potentially promote the progression of angiogenesis and plaque formation. Indeed, inhibition of angiogenesis has been shown to reduce macrophages in the plaque and around the VV.[43] The relationship between the adventitial VV and the development of atherosclerosis is further supported by our observation that the LIMA, a vessel with low incidence of atherosclerosis,[16,17] shows a significantly lower VV density.

Furthermore, the contribution of VVs to vascular disease may also be mediated by their physical fragility, although the notion of VV fragility is not tested in the current study. Intraplaque hemorrhage is common in advanced atherosclerotic lesions and its occurrence is considered an important event in the manifestation of atherosclerotic disease, causing acute processes such as myocardial infarction as well as cerebrovascular and peripheral acute ischemia.[44-46] Previous studies have demonstrated the association between plaque neovascularity and quantity of intraplaque hemorrhage.[47-49] In the current study, the variability in VV density was accounted for by the second-order VVs. These microvessels are smaller in diameter than the first-order VVs, less mature, and might be more susceptible to hemorrhage, especially in the presence of hypertension. Thus, this observation can support the association between neovascularization and intraplaque hemorrhage.

Gössl et al[26] recently showed patchy distribution of VV territories in the vessel wall of the LAD artery. Using the same methodology, our findings show similar random distribution of VVs within the vessel walls of the carotid, renal and femoral as well as the coronary arteries. This absence of a detectable pattern in different vascular beds is in accord with the non-uniform distribution of the atherosclerotic plaque on the intimal surface of the vessels[3,4] and further supports the possible pathophysiologic role of VV density in plaque formation.

Study limitations. The current study describes the structural micro-anatomy of the normal vessel wall in 5 different vascular beds. Although the increase in VV density in the atherosclerotic process is well documented, it is possible that the VVs observed in the normal vessels are not related to the newly formed VVs observed in vessels under atherogenic stimuli. Furthermore, the current study is descriptive and involves neither molecular pathophysiologic mechanism of the vascular disease nor physical properties of the VV (eg, VV fragility). Nevertheless, the significant anatomic differences between the different vascular beds are in line with the growing evidence in the literature supporting the role of VV in vascular disease.

In conclusion, different vascular beds exhibit intra- and intervessel heterogeneity in VV anatomy, a phenotypic variability that might determine their differential local response to systemic risk factors and propensity for development of atherosclerosis. The coronary vasculature, which is most affected by the atherosclerotic process[1,2,4] was found to have the highest VV density, thereby supporting a possible pathophysiologic role of VV in atherosclerosis. Inflammatory arterial diseases such as Takayasu's disease, Bechet disease, and temporal arteritis show progression of the inflammatory process from the adventitia, (eg, vasa vasoritis) toward the inner layers of the vessel wall (vasculitis).[50] Whether higher VV density exposes the host vessel to an increase inflammatory process is unknown; however, the inflammatory process is well recognized in the atherosclerosis pathophysiology. Hence, further investigation exploring the variability in vasoreactivity and expression of growth factors and inflammatory markers, with and without exposure to risk factors, is warranted in order to elucidate the underlying mechanism and potential of ASCVD heterogeneity.

Dr Amir Lerman is an Established Investigator of the American Heart Association.

REFERENCES

1. American Heart Association. Heart disease and stroke statistics—2003 update. Dallas (Tex): Am Heart Assoc; 2003. p. 5-21.
2. Pasterkamp G, Schoneveld AH, Hillen B, Banga JD, Haudenschild CC, Borst C. Is plaque formation in the common carotid artery representative for plaque formation and luminal stenosis in other atherosclerotic peripheral arteries? A post mortem study. Atherosclerosis 1998;137: 205-10.

3. Weber G, Bianciardi G, Bussani R, Gentilini R, Giarelli L, Novelli MT, et al. Atherosclerosis and aging. A morphometric study on arterial lesions of elderly and very elderly necropsy subjects. Arch Pathol Lab Med 1988;112:1066-70.
4. Vink A, Schoneveld AH, Poppen M, de Kleijn DP, Borst C, Pasterkamp G. Morphometric and immunohistochemical characterization of the intimal layer throughout the arterial system of elderly humans. J Anat 2002;200:97-103.
5. Libby P. The pathogenesis of atherosclerosis. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine. 15th ed. New York: Mc Graw Hill Companies, Inc; 2001. p. 1377-82.
6. Wilcox JN, Scott NA. Potential role of the adventitia in arteritis and atherosclerosis. Int J Cardiol 1996;54(suppl): S21-35.
7. Barker SGE, Beesley JE, Baskerville PA, Martin JF. The influence of the adventitia on the presence of smooth muscle cells and macrophages in the arterial intima. Eur J Vasc Endovasc Surg 1995;9:222-7.
8. Booth RFG, Martin JF, Honey AC, Hassall DG, Beesley JF, Moncada S. Rapid development of atherosclerotic lesions in the rabbit carotid artery induced by perivascular manipulation. Atherosclerosis 1989;76:257-68.
9. Barker SGE, Tilling LC, Miller GC, Beesley JE, Fleetwood ??, Stavri GT, et al. The adventitia and atherogenesis: removal initiates intimal proliferation in the rabbit which regresses on generation of a 'neoadventitia'. Atherosclerosis 1994;105:131-44.
10. Nakata Y, Shionoya S. Vascular lesions due to obstruction of the vasa vasorum. Nature 1966;212:1258-9.
11. Martin JF, Booth RFG, Moncada S. Arterial wall hypoxia following hypo-perfusion through the vasa vasorum is an initial lesion in atherosclerosis. Eur J Clin Invest 1990;20:588-92.
12. Barker SGE, Talbert A, Cottam S, Baskerville PA, Martin JF. Arterial intimal hyperplasia after occlusion of the adventitial vasa vasorum in the pig. Arteroscler Thromb 1993;13:70-7.
13. Barger AC, Beeukwes R III, Lainey LL, Silverman KJ. Hypothesis: vasa vasorum and neovascularization of human coronary arteries. N Engl J Med 1984;310:175-7.
14. Zhang Y, Cliff WJ, Schoefl GI, Higgins G. Immunohistochemical study of intimal microvessels in coronary atherosclerosis. Am J Pathol 1993; 143:164-72.
15. Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Hum Pathol 1995;26:450-6.
16. Wilson SH, Herrmann J, Lerman LO Holmes DR Jr, Napoli C, Ritman EL, et al. Simvastatin preserves the structure of coronary adventitial vasa vasorum in experimental hypercholesterolemia independent of lipid lowering. Circulation 2002;105:415-8.
17. Bloor CM, White FC, Roth DM. The pig as a model of myocardial ischemia and gradual coronary artery occlusion. In: Swindle MM, Moody DC, Phillips LD, editors. Swine as models in biomedical research. Ames: Iowa State University Press; 1992. p. 163-75.
18. Julke M, von Segesser L, Schneider J, Turina M, Heitz PU. Degree of arteriosclerosis of the internal mammary artery and of the coronary arteries in 45-to-75-year-old men. An autopsy study. Schweiz Med Wochenschr 1989;119:1219-23.
19. Lytle BW, Loop FD, Cosgrove DM, Ratliff NB, Easley K, Taylor PC. Long-term (5 to 12 years) serial studies of internal mammary artery and saphenous vein coronary bypass grafts. J Thorac Cardiovasc Surg 1985; 89:248-58.
20. Wan SY, Ritman EL, Higgins WE. Multi-generational analysis and visualization of the vascular tree in 3D micro-CT images. Comput Biol Med 2002;32:55-71.
21. Jorgensen SM, Demirkaya O, Ritman EL. Three-dimensional imaging of vasculature and paranchyma in intact rodent organs with X-ray micro-CT. Am J Physiol 1998;275:H1103-14.
22. Kwon HM, Sangiorgi G, Ritman EL, McKenna C, Holmes DR Jr, Schwartz RS, et al. Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia. J Clin Invest 1998;101:1551-6.
23. Edelman ER, Nugent MA, Smith LT, Karnovsky MJ. Basic fibroblast growth factor enhances the coupling of intimal hyperplasia and proliferation of vasa vasorum in injured rat arteries. J Clin Invest 1992;89:465-73.
24. Rodriguez-Porcel M, Lerman A, Ritman EL, Wilson SH, Best PJM, Lerman LO. Altered myocardial microvascular 3D architecture in experimental hypercholesterolemia. Circulation 2000;102:2028-30.
25. Zamir M, Silver MD. Vasculature in the walls of human coronary arteries. Arch Pathol Lab Med 1985;109:659-62.
26. Gössl M, Rosol M, Malyar NM, Fitzpatrick LA, Beighley PE, Zamir M, et al. Functional anatomy and hemodynamic characteristics of vasa vasorum in the walls of porcine coronary arteries. Anat Rec 2003;272A: 526-37.
27. Goerge J, Afek A, Shaish A, Levkovitz H, Bloom N, Cyrus T, et al. 12/15-Lipooxigenase gene disruption attenuates atherogenesis in LDL receptor–deficient mice. Circulation 2001;104:1646-50.
28. Cyrus T, Witztum JL, Rader DJ, Tangirala R, Fazio S, Linton ME, et al. Disruption of the 12/15-lipooxigenase gene diminishes atherosclerosis in apo-E deficient mice. J Clin Invest 1999;103:1597-1604.
29. Reardon CA, Blachowicz L, Lukens J, Nissenbaum M, Gets GS. Genetic background selectivity influence innominate artery atherosclerosis: immune system deficiency as a probe. Arterioscler Thromb Vasc Biol 2001;21:585-593.
30. Witting PK, Pettersson K, Letters J, Stocker R. Site-specific antiatherogenic effect of probucol in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2000;20:26-33.
31. Whitman SC, Ravisankar P, Daugherty A. INFγ deficiency exerts gender-specific effect on atherogenesis in apoE-′- mice. J Interferon Cytokine Res 2002;22:661-670.
32. VanderLaan PA, Reardon CA, Getz GS. Site specificity of atherosclerosis, site-selective responses to atherosclerotic modulators. Atheroscler Thromb Vasc Biol 2004;24:12-22.
33. Hueper WC. Arteriosclerosis: the anoxemia theory. Arch Pathol 1944; 38:173.
34. Gainer JL. Hypoxia and atherosclerosis: re-evaluation of an old hypothesis. Atherosclerosis 1987;68:263-6.
35. Herrmann J, Lerman LO, Rodrigez-Porcel M, Holmes DR Jr, Richardson DM, Ritman EL, et al. Coronary vasa vasorum neovascularization precedes epicardial endothelial dysfunction in experimental hypercholesterolemia. Cardiovasc Res 2001;51:762-6.
36. Kullo IJ, Mozes G, Schwartz RS, Gloviczki P, Tsutsui M, Katusic ZS, et al. Enhanced endothelium-dependent relaxations after gene transfer of recombinant endothelial nitric oxide synthase to rabbit carotid arteries. Hypertension 1997;30:314-20.
37. Tsutsui M, Onoue H, Iida Y, Smith L, O'Brien T, Katusic ZS. Adventitia-dependent relaxations of canine basilar arteries transduced with recombinant eNOS gene. Am J Physiol 1999;276:H1846-52.
38. Heistad DD, Marcus ML, Martin JB. Effects of neural stimuli on blood flow through vasa vasorum in dogs. Circ Res 1979;45:615-20.
39. Scotland R, Vallance P, Ahluwalia A. Endothelin alters the reactivity of vasa vasorum: mechanisms and implications for conduit vessel physiology and pathophysiology. Br J Pharmacol 1999;128:1229-34.
40. Scotland RS, Vallance PJT, Ahluwalia A. Endogenous factors involved in regulation of tone of arterial vasa vasorum: implication for conduit vessel physiology. Cardiovasc Res 2000;46:403-11.
41. Herrmann J, Best PJ, Ritman EL, Holmes DR Jr, Lerman LO, Lerman A. Chronic endothelin receptor antagonism prevents coronary vasa vasorum neovascularization in experimental hypercholesterolemia. J Am Coll Cardiol 2002;39:1555-61.
42. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 1999;99:1726-32.
43. Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvin E, et al. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advaned atherosclerosis. Natl Acad Sci 2003;100:4736-41.
44. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med. 2003;349:2316-25.

45. von Maravic C, Kessler C, von Maravic M, Hohlbach G, Kompf D. Clinical relevance of intraplaque hemorrhage in the internal carotid artery. Eur J Surg 1991;157:185-8.
46. Persson AV. Intraplaque hemorrhage. Surg Clin North Am 1986;66: 415-20.
47. Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. Br J Surg 2001;88:945-50.
48. McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg 1999;30:261-8.
49. Fryer JA, Myers PC, Appleberg M. Carotid intraplaque hemorrhage: the significance of neovascularity. J Vasc Surg 1987;6:341-9.
50. Numano F. Vasa vasoritis, vasculitis and atherosclerosis. Int J Cardiol 2000 Aug;75 Suppl 1:S1-8.

Submitted Apr 1, 2004; accepted Jun 26, 2004.