

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Microscopic Computed Tomography Based Virtual Histology for Visualization and Morphometry of Atherosclerosis in Diabetic Apolipoprotein E Mutant Mice
Hernan G. Martinez, Suresh I. Prajapati, Carlos A. Estrada, Fabio Jimenez, Marlon P. Quinones, Isabel Wu, Ali Bahadur, Allen Sanderson, Christopher R. Johnson, Minsub Shim, Charles Keller and Seema S. Ahuja

Circulation 2009;120;821-822

DOI: 10.1161/CIRCULATIONAHA.108.829531

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2009 American Heart Association. All rights reserved. Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/cgi/content/full/120/9/821>

Data Supplement (unedited) at:

<http://circ.ahajournals.org/cgi/content/full/120/9/821/DC1>

Subscriptions: Information about subscribing to *Circulation* is online at
<http://circ.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/reprints>

Microscopic Computed Tomography–Based Virtual Histology for Visualization and Morphometry of Atherosclerosis in Diabetic Apolipoprotein E Mutant Mice

Hernan G. Martinez, MD*; Suresh I. Prajapati, MSc*; Carlos A. Estrada, MD; Fabio Jimenez, BSc; Marlon P. Quinones, MD; Isabel Wu, BSc; Ali Bahadur, MSc; Allen Sanderson, PhD; Christopher R. Johnson, PhD; Minsub Shim, PhD; Charles Keller, MD†; Seema S. Ahuja, MD†

Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the arteries and is a leading cause of heart disease and stroke in developed and developing countries.¹ Animal models have become increasingly important tools for addressing key mechanistic and therapeutic questions that cannot be answered from human studies of atherosclerosis. However, the small-scale vascular structures in genetically engineered mice require labor-intensive histomorphometric techniques to quantify lesions.

Recently, a new technique has emerged to image *ex vivo* blocks of soft tissue by staining tissue with metal solutions, then scanning with a microscopic computed tomography (microCT) instrument (Figure I in the online-only Data Supplement).² This technique was originally applied to the study of the developing heart in embryos³ and fetuses (Figure II in the online-only Data Supplement) but can also be applied to the *en bloc* imaging of the heart, great vessels, and lesions thereof. By this method, tissues are left intact, but one can employ image analysis to create “virtual” histological sections that allow the identification of an individual lesion in a 2-dimensional image. Furthermore, the 3-dimensional nature of microCT scans permits the volumetric assessment of multifocal atherosclerotic lesions.

Shown in Figure 1 are excised hearts from an apolipoprotein E knockout mouse (ApoE null), a well-established murine model for atherosclerosis, as well as a control mouse. Both mice are on a diabetic background strain, KK-Ay, maintained on a standard diet. ApoE null mice are spontaneously hypercholesterolemic and develop fatty streaks as well as advanced lesions at aortic sites where atherosclerosis is also typically seen in humans.⁴ In these coronal virtual histological sections, the ventricular myocardium and atria can be appreciated (Figure 1A through 1D), as well as

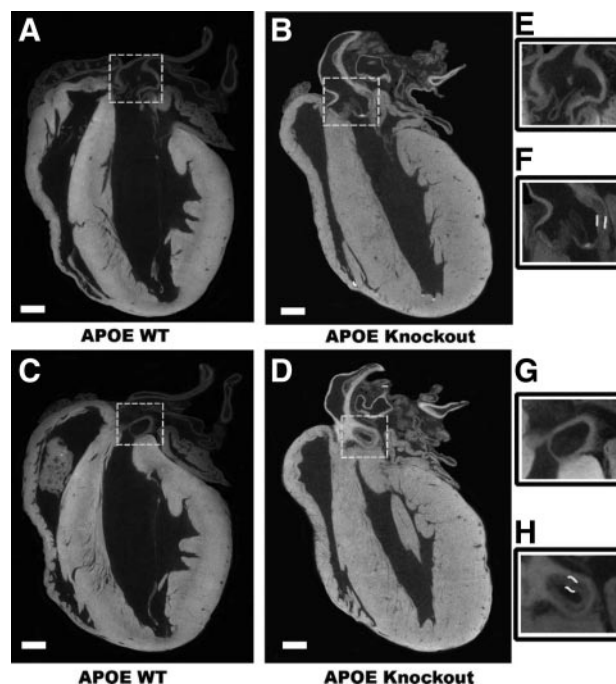


Figure 1. Coronal microCT-based virtual histology sections of wild-type (WT) mice (A and C) and ApoE null mice (B and D) (28-week-old females). Specimens were stained with a precommercial staining solution (Numira Biosciences, Salt Lake City, Utah) and scanned in a Scanco μ CT40 instrument at 6 μ m isotropic resolution. Insets E, F, G, and H correspond to yellow boxed regions in A, B, C, and D, respectively. Insets of the aortic leaflet (F) and ascending aorta (H) demonstrate atherosclerotic plaques (bracketed by yellow lines) in the ApoE null mice. Bar=1 mm.

atherosclerotic lesions of the aortic root (Figure 1B) and ascending aorta (Figure 1D). After microCT-based virtual histology scans, the ApoE null aortic root was sectioned by traditional histology for staining of atherosclerotic lesions

From the Departments of Medicine (H.G.M., C.A.E., F.J., M.P.Q., S.S.A.), Pediatrics (C.K.), and Cellular and Structural Biology (C.K.) and Greehey Children's Cancer Research Institute (S.I.P., I.W., A.B., C.K.), University of Texas Health Science Center, San Antonio; Scientific Computing and Imaging Institute, University of Utah, Salt Lake City (A.S., C.R.J.); National Institute of Environmental Health Science, Research Triangle Park, NC (M.S.); and South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio (S.S.A.).

The online-only Data Supplement is available with this article at <http://circ.ahajournals.org/cgi/content/full/120/9/821/DC1>.

*Drs Martinez and Prajapati contributed equally to this work and †Drs Keller and Ahuja contributed equally to this work.

Correspondence to Charles Keller, MD, or Seema S. Ahuja, MD, Division of Nephrology, Department of Medicine, University of Texas Health Science Center, 7703 Floyd Curl Dr, MC7870, San Antonio, TX 78229-3900. E-mail ahuja@uthscsa.edu

(*Circulation*. 2009;120:821-822.)

© 2009 American Heart Association, Inc.

Circulation is available at <http://circ.ahajournals.org>

DOI: 10.1161/CIRCULATIONAHA.108.829531

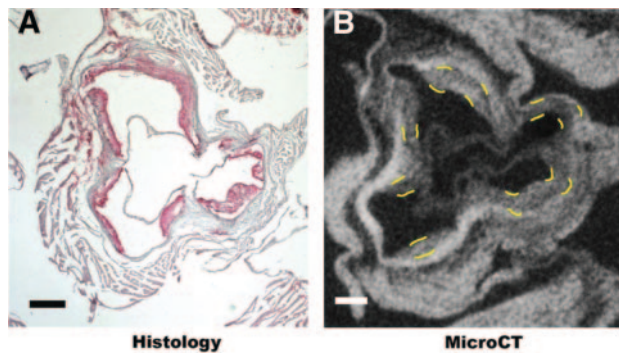


Figure 2. Oil Red O histological stain (red) (A) and microCT-based virtual histology scan (B) of the same atherosclerotic lesion from an ApoE null mouse. The yellow dotted lines highlight the area of atherosclerotic plaque in the aortic leaflet region. Bar=250 μ m.

with Oil Red O, which stains the fatty plaque red (Figure 2A), thereby confirming the atherosclerotic lesion seen by virtual histology. With higher-resolution (1.8 μ m) microCT instrument scanning, these lesions are even more readily visualized (Figure III and Movies I through III in the online-only Data Supplement).

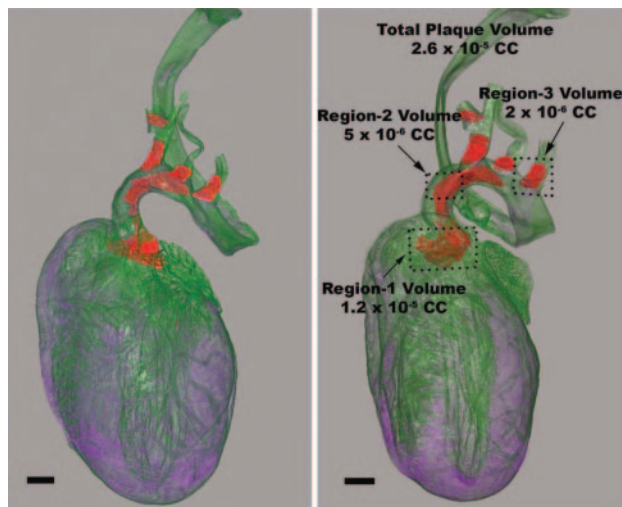


Figure 3. Left and right, Renderings of the same ApoE null mouse heart from different angles, demonstrating atherosclerotic plaques (red) that were segmented by image analysis and their volumes quantified. Total volume refers to the entire volume of the red region, which comprises several regions including 1, 2, and 3 (shown by dotted boxes). Purple indicates myocardium; green, atria and vessels. Bar=1 mm.

An advantage of microCT-based virtual histology is that the data are entirely digital and not subject to loss of individual sections in handling or improper plane of sectioning. Furthermore, freely available software tools exist for the visualization of natural edge boundary features of 3-dimensional tissues (Figure 3, left) as well as volume quantification of atherosclerotic lesions at multiple foci to microliter accuracy (Figure 3, right) (Seg3D, <http://www.sci.utah.edu/cibc/software>).

Thus, evaluation of mechanisms underlying atherosclerosis and interventions can be accelerated by this time-saving, higher-accuracy research imaging technique. In addition, because microCT-based virtual histology is inherently digital, data analysis for virtual histology is poised to take advantage of an emerging new generation of powerful software tools such as those mentioned above, which will further speed vascular disease research.

Acknowledgments

We thank Itzik Goldberger and Tiffany Fong for the scans graciously provided by Xradia Inc.

Sources of Funding

This research was supported by the Veterans Administration (Merit Review) and National Institutes of Health grant R01 AR 052755 to Dr Ahuja. microCT scans were performed at Numira Biosciences (Salt Lake City, Utah) through a reciprocal scanning agreement. This work was made possible in part by software from the National Institutes of Health/National Center for Research Resources Center for Integrative Biomedical Computing (5P41RR012553).

Disclosures

Dr Keller is cofounder of Numira Biosciences (<http://www.numirabio.com>), which licenses microCT-based virtual histology from the University of Texas Health Science Center at San Antonio for commercial applications. The other authors report no conflicts.

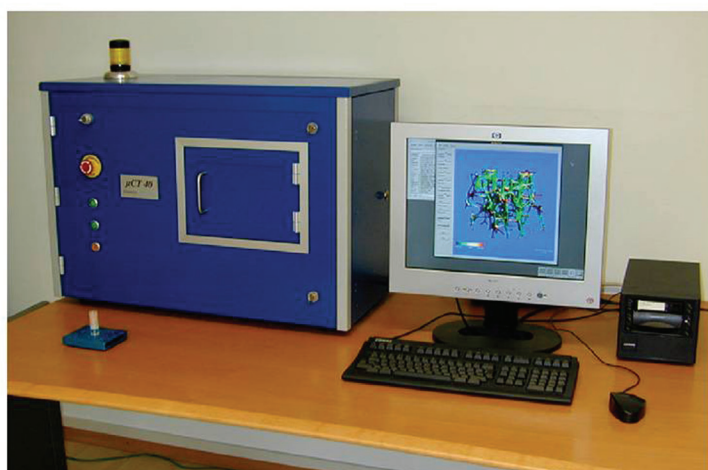
References

- Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420:868–874.
- Langheinrich AC, Bohle RM, Greschus S, Hackstein N, Walker G, von Gerlach S, Rau WS, Holschermann H. Atherosclerotic lesions at micro CT: feasibility for analysis of coronary artery wall in autopsy specimens. *Radiology*. 2004;231:675–681.
- Johnson JT, Hansen MS, Wu I, Healy LJ, Johnson CR, Jones GM, Capecchi MR, Keller C. Virtual histology of transgenic mouse embryos for high-throughput phenotyping. *PLoS Genet*. 2006;2:e61.
- Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science*. 1992;258:468–471.

SUPPLEMENTAL MATERIAL

Supplementary Figure S1

A



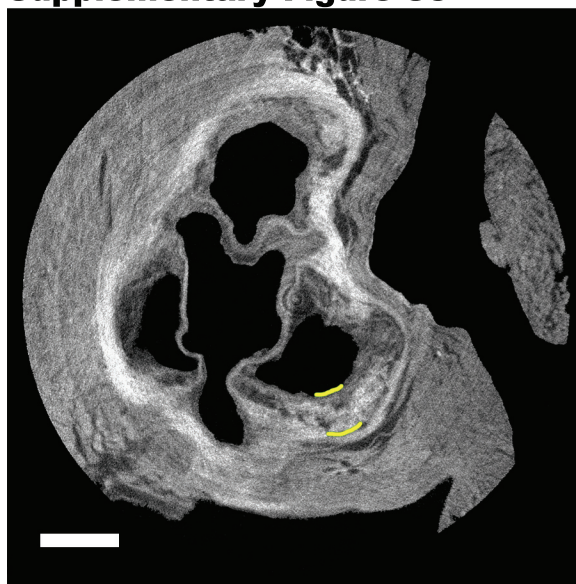
B



Supplementary Figure S2



Supplementary Figure S3



Video File Legend

Sagittal

Sagittal plane movie of microCT-based Virtual Histology of the great vessels of an ApoE $-/-$ mouse heart at 1.8 μ m isometric resolution. (Windows Media Player)

Coronal

Coronal plane movie of microCT-based Virtual Histology of the great vessels of an ApoE $-/-$ mouse heart at 1.8 μ m isometric resolution. (Windows Media Player)

Axial.

Axial plane movie of microCT-based Virtual Histology of the great vessels of an ApoE $-/-$ mouse heart at 1.8 μ m isometric resolution. (Windows Media Player)