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

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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 embryos[3] 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 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

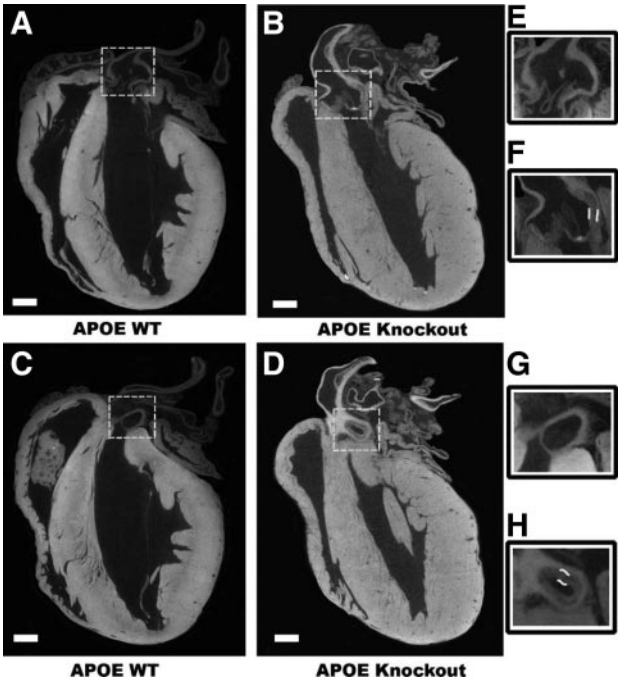

Figure 1. Coronal microCT-based virtual histology sections of wild-type (WT) mice (A and C) and ApoE null mice (B and D) (28-week-old females). Specimens were stained with a precommercial staining solution (Numira Biosciences, Salt Lake City, Utah) and scanned in a Scanco μCT40 instrument at 6 μm isometric 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.

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

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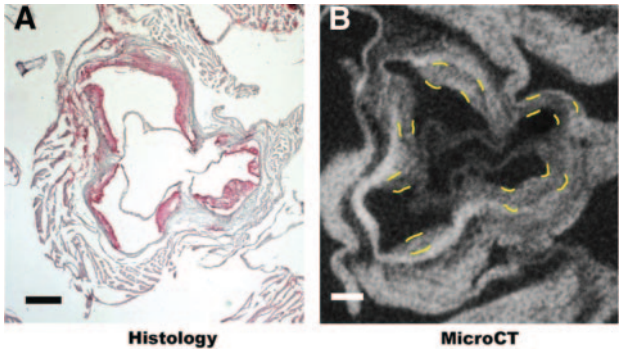

Figure 2. Oil Red O histological stain (red) (A) and microCT-based virtual histology scan (B) of the same atherosclerotic lesion from an ApoE null mouse. The yellow dotted lines highlight the area of atherosclerotic plaque in the aortic leaflet region. Bar=250 μm.

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 μm) microCT instrument scanning, these lesions are even more readily visualized (Figure III and Movies I through III in the online-only Data Supplement).

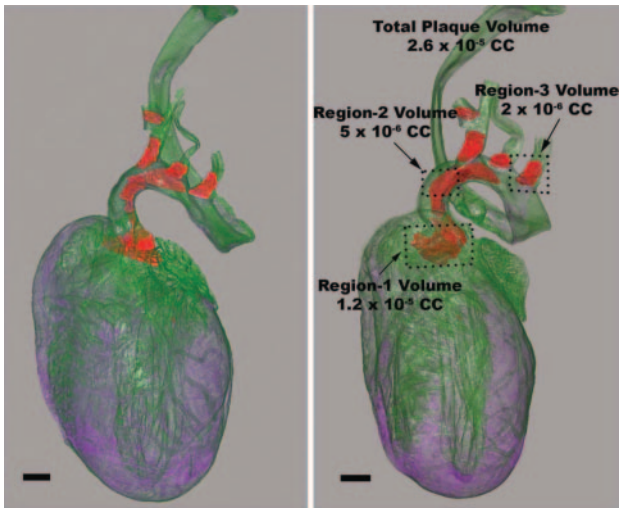

Figure 3. Left and right, Renderings of the same ApoE null mouse heart from different angles, demonstrating atherosclerotic plaques (red) that were segmented by image analysis and their volumes quantified. Total volume refers to the entire volume of the red region, which comprises several regions including 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.

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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.

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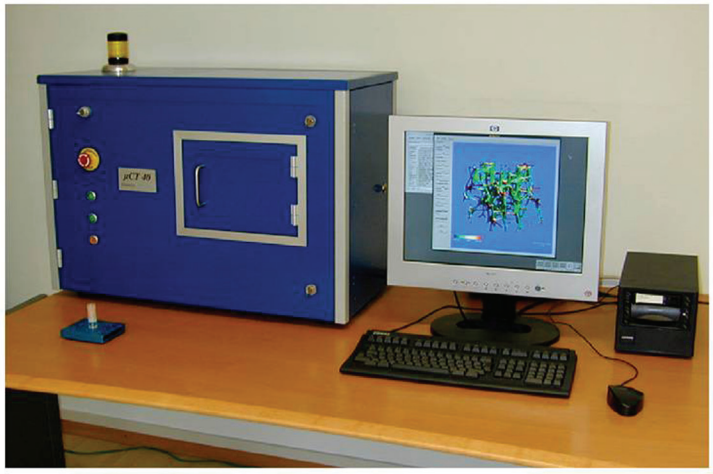
SUPPLEMENTAL MATERIAL

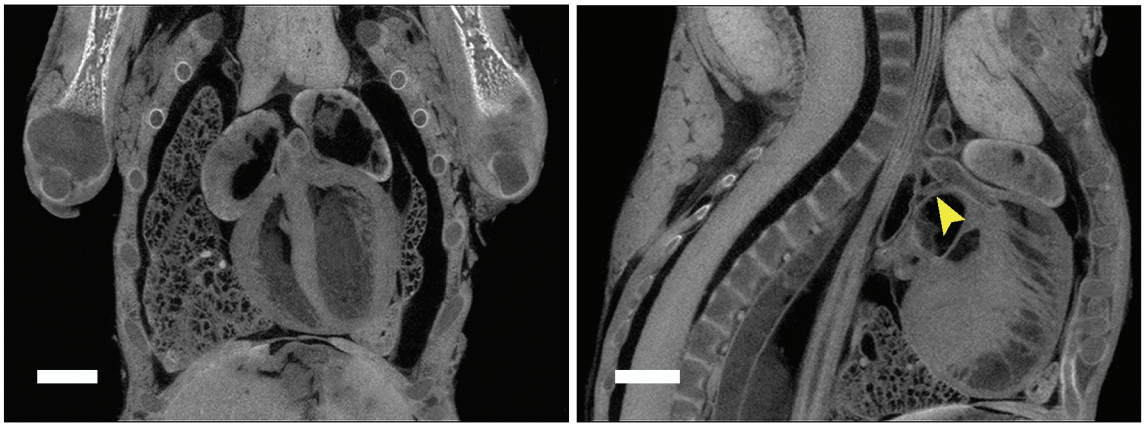
Supplementary Figure S1

A

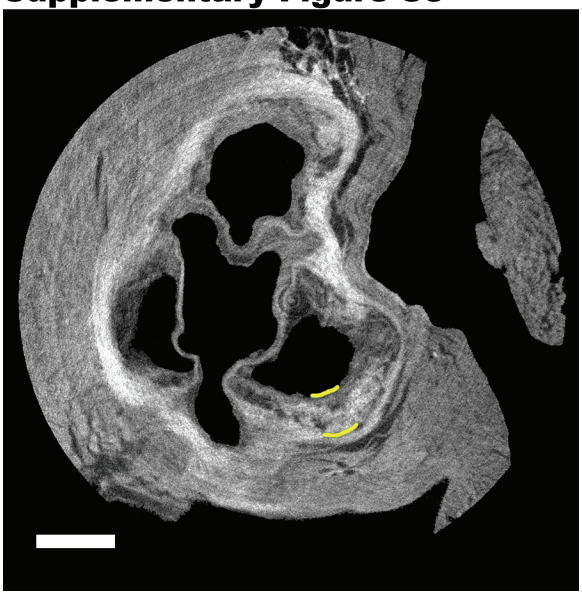


B

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 um 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 um 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 um isometric resolution. (Windows Media Player)