A Volumetric Method for Quantifying Atherosclerosis in Mice by Using MicroCT: Comparison to En Face

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Abstract

Precise quantification of atherosclerotic plaque in preclinical models of atherosclerosis requires the volumetric assessment of the lesion(s) while maintaining in situ architecture. Here we use micro-computed tomography (microCT) to detect ex vivo aortic plaque established in three dyslipidemic mouse models of atherosclerosis. All three models lack the low-density lipoprotein receptor (Ldr−/−), each differing in plaque severity, allowing the evaluation of different plaque volumes using microCT technology. From clearly identified lesions in the thoracic aorta from each model, we were able to determine plaque volume (0.04–3.1 mm³), intimal surface area (0.5–30 mm²), and maximum plaque (intimal-medial) thickness (0.1–0.7 mm). Further, quantification of aortic volume allowed calculation of vessel occlusion by the plaque. To validate microCT for future preclinical studies, we compared microCT data to in situ surface area and volume, because they are in excellent correlation between microCT assessment and en face surface area (r² = 0.99, p < 0.0001 and r² = 0.95, p < 0.0001, respectively). MicroCT also identified internal characteristics of the lipid core and fibrous cap, which were confirmed pathologically as Stary type III-V lesions. These data validate the use of microCT technology to provide a more exact empirical measure of ex vivo plaque volume throughout the entire intact aorta in situ for the quantification of atherosclerosis in preclinical models.

Introduction

Ischemic heart disease resulting from coronary atherosclerosis is the leading cause of human mortality worldwide [1,2]. Atherosclerosis is characterized by deposition of cholesterol ester in the arteries that may progressively occlude the lumen and become prone to rupture and thrombosis. Therapies aimed at reducing the risk factors for developing atherosclerosis and/or directly reducing the size of the plaque are being actively pursued, creating the need for more robust methods to determine plaque alterations in preclinical models of atherosclerosis.

To date, different methods have been used to assess the extent of atherosclerosis in mice, or quantitatively compare lesion formation in various strains or treatment groups of mice. One method requires the sequential sectioning of the heart and aortic root [3] and subsequent histopathologic analysis to score and measure lesions in a 300 micron area at the level of the aortic sinus. Another method employs Sudan IV staining to determine the extent of atherosclerosis affecting the intimal surface throughout the entire aorta [4]. This method is commonly referred to as the en face technique and has been widely used as it yields morphometric data [5]. The technique consists of dissecting the aorta from the heart to the iliac bifurcation, opening it longitudinally to expose the luminal side, and staining it with Sudan IV to reveal lipid-laden plaques to measure lesional surface areas. These two methods provide qualitatively distinct estimates of lesion area in orthogonal axes. Another method uses high-resolution magnetic resonance imaging (MRI) to detect and quantify the plaque volume incorporating all dimensions assessed by the previous two methods in the intact aorta and to detect the lipid-rich necrotic core without requiring histopathology [6]. Despite the advancements in assessing aortic plaque, the need remains for the generation of very high-resolution three-dimensional plaque models, along with determining the degree of occlusion.

Currently, only the aortic root (Paigen) method is still routinely used for assessing the volume of an atherosclerotic lesion in preclinical models [3]. Though widely used, it is quite labor intensive and generates only a calculated estimate of aortic lesions. The estimate is often made on the basis of surface areas that are determined from only 10 sections (typically 10 µm) at fixed distances from the aortic sinus. The surface areas in turn are used to estimate the volume between the dropped sections. This approach provides only a limited volumetric assessment in the aortic root, and does not include the rest of the aorta or the adjoining arteries. We focused our work on validating the
microcomputed tomography (microCT) method by comparing it with the en face method. This was done because the en face method was previously validated by comparing it with the aortic root Paigen method [4,7] and because en face assesses the entire aorta and not just 10 transversal sections of the aortic roots.

While traditional histopathology analysis remains the gold-standard technique for the assessment of ex vivo tissues, these methods are labor intensive, time consuming, and often rely on subjective, qualitative measures. For that reason, there is increasing use of medical imaging technologies, primarily micro-MRI and CT, as alternatives or supplements to histopathology. When coupled with appropriate contrast agents, these methods can highlight tissue with high resolution and three-dimensional visualization. These methods are non-destructive, enabling scanning of the entire fixed tissue and allowing for subsequent histopathology. Finally, a key advantage of the imaging methodologies is the 3D visualization and analysis options wherein image segmentation and analysis algorithms quantify visualized pathologies into statistically measurable parameters. Because of the high contrast for bone, microCT has traditionally been used for the analysis of bone to examine changes in density and structure in response to treatments [8,9,10,11]. However, recent method and contrast development have allowed improved imaging of soft tissue [12,13,14] and vessels [15,16,17], facilitating the assessment of disease states.

Along these lines, the feasibility of microCT has been used to assess atherosclerosis in 8 coronary arteries from human autopsy specimens [18] with excellent correlation to histological findings. In this report we demonstrate the use of microCT in 3 mouse models with different levels of atherosclerotic lesions all associated with deficiency of the Ldlr. Recently, the same technology was previously validated by comparing it with the aortic root method. This was done because the en face method was previously validated by comparing it with the aortic root Paigen method [4,7] and because en face assesses the entire aorta and not just 10 transversal sections of the aortic roots.

In this report we demonstrate the use of microCT in 3 mouse models with different levels of atherosclerotic lesions all associated with deficiency of the Ldlr. Recently, the same technology was previously validated by comparing it with the aortic root method. This was done because the en face method was previously validated by comparing it with the aortic root Paigen method [4,7] and because en face assesses the entire aorta and not just 10 transversal sections of the aortic roots.

Materials and Methods

Ethics Statement

All animal studies were approved by the Amgen Inc. IACUC under protocol number 2006-00010.

Animals

Four male Ldlr-3KO mice (Ldlr<sup>−/−</sup>Apop<sup>100/100</sup>Lep<sup>ob/ob</sup>) and 4 male Ldlr-2KO mice (Ldlr<sup>−/−</sup>Apop<sup>100/100</sup>Lep<sup>ob/+</sup>) were bred at Charles River Laboratories (San Diego, CA). The generation of the mice has been discussed previously [20]. Mice were fed standard chow (8640; Harlan Teklad; Indianapolis, IN) for the duration of the study. Five Ldlr<sup>−/−</sup> (Ldlr-1KO mice) were obtained from Jackson Laboratories (Bar Harbor, ME) for microCT imaging and quantitation. Specimens were used for imaging due to the size constraints of scanning necessary for sufficient resolution; instead carcasses were trimmed and the excised heart and aorta specimens intact with spine, ribs and kidneys were shipped in fixative to Numira Biosciences (Salt Lake City, UT) for microCT imaging and quantitation. Specimens were allowed to fix by gentle agitation in 10% neutral-buffered formalin for an additional 4–5 days at room temperature.

After complete fixation the samples were immersed in a 5% Phosphotungstic Acid solution for 48 (±2) hours. The samples were rinsed with 1x PBS both before and after reagent exposure. The described specimen preparation, staining, and scanning process is a patent-pending method of Numira Biosciences Inc (www.numirabio.com). Numira performs this method as a service to its clients. The contrast reagent was only used during the soaking of the carcass and not injected during perfusion of the mouse. Full immersion of the sample was necessary for uptake of the agent into the aorta and plaque to allow them to be distinguished from each other.

MicroCT imaging and data processing

MicroCT Imaging: A high-resolution, volumetric microCT scanner (µCT40; ScanCo Medical, Zurich, CH) was used to scan the tissue with the following parameters: 10 µm isometric voxel resolution, 200 ms per view, 2000 views in a 360 degree rotation, 55 kVP for tube voltage and averaging the five frames into one. The scanners utilized in this study are calibrated weekly as recommended by the manufacturer. The calibration block is supplied by the manufacturer and contains a metal rod of known volume. The instrument scans the block and calculates the volume of the rod and the calculated volume must be within 2.0% of the actual volume. Initially only 1 cm of the descending aorta was scanned (for Ldlr-2KO and -3KO mice), in later experiments (Ldlr-1KO AD mice) the aorta were scanned to the iliac bifurcation. The microCT data was reconstructed and files were converted to DICOM format for further processing.

Image Processing: Seg3D, a segmentation software package from the Scientific Computing and Imaging Institute (SCI, University of Utah, Salt Lake City, UT) was used to semi-automatically label plaques within the aortic arch. No filtering was used during the instrument reconstruction process. The aorta was first labeled based on prior knowledge of its cylindrical shape and the grayscale values of its wall. To differentiate the different areas, plaque, lumen and aorta, the basic grayscale criteria differences were used, as each entity has a unique value that can be distinguished from the others. The software was then used to label the appropriate areas.

The software applications Teem (http://teem.sourceforge.net/) and SCIRun (SCI) were then used to calculate the plaque volumes and surface areas, and to create the intimal-medial thickness-map movies. Presence of plaques was verified by an external radiology consultant (MicroRad, LLC; Salt Lake City, UT).

Volume Measurements: As part of the segmentation process, Seg3D reported the number of voxels associated with the aorta and plaque. The voxel count for each region of interest was converted into cubic millimeters by multiplying by the volume per voxel element.
Total Surface Area Measurements: To create smooth surface representations of the regions of interest, the boundary faces of the segmented regions were smoothed using Taubin’s algorithm [21]. The surface area for each region was then calculated by summing the areas of all of the triangles associated with each boundary.

Attached Plaque Surface Area: We define the attached portion of the plaque as the region of the surface that abuts the aorta surface (in contrast to that portion of the surface that faces the interior of the aorta). The “Attached Plaque Surface Area” measurement was also used when computing the “Percent Coverage” (percentage of aorta wall that is covered by plaque), as seen in the “5-Panel Percent Occlusion Map” movies. Since the attached plaque surface is relatively smooth and represents just one side of the three-dimensional plaque surface, it was anticipated that the Intimal (attached) Plaque Surface Area would correspond well with the plaque surface area measured with the en face method.

*En face* analysis

After Numira Biosciences completed the CT analysis, aortas were soaked for one week in 4% paraformaldehyde/7.5% sucrose fixative to remove the CT contrast agent as well as to make the aorta more flexible for dissection. Aortas were excised from the carcass, separated from the heart, flat-mounted on wax boards, and stained with Sudan IV using previously published methods [20]. After staining, aortas were trimmed to yield the aortic arch and a 1 cm section of the descending aorta. The segments were individually positioned on a glass plate and microCT images as a guide to assist in trimming, the Sudan IV stained aortic whole mount confirmation with the use of the conventional *en face* staining technique for the Intimal-Medial Thickness Map Generation: For each point on the plaque surface, we computed the minimum distance to the opposite side of the plaque (i.e., vessel-side to lumen-side and vice versa). The plaque was pseudo-colored to indicate the plaque thickness at each point by using the color map shown on the left corner of the thickness map images.

Histological analysis

After *en face* imaging, the Sudan IV stained aortic whole mount samples from mice were returned to fixative. Later, by using both the *en face* and microCT images as a guide to assist in trimming, the aorta lengths were transected at specific points, yielding segments several millimeters in length, and were processed in paraffin using standard histological methods. Once infiltrated, the specimens were embedded perpendicularly in blocks with the trim edge down, providing selected cross-sectional profiles across the aorta. Sequential sections were stained with H&E, Verhoeff-Van Gieson, and picrosirius red. Lesions were graded according to Stary type [22,23].

MicroCT reproducibility assessment

The reproducibility of microCT measurements was assessed by four independent operators who were instructed to analyze the microCT images for an aorta and to derive lesion surface areas and volumes. We calculated the coefficient of variation (CV) to assess reproducibility.

Statistical analysis

Data analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc.). Linear regression and Spearman’s Rank Correlation coefficient calculations were performed to compare the lesional data generated by the microCT and *en face* methodologies. Data in Table 1 comparing Ldlr-2KO and -3KO mice were analyzed using a Student’s T-Test; data comparing all three strains were analysed by using a One-Way ANOVA and a Tukey post hoc test.

Results

Detection of atherosclerotic plaque in Ldlr-2KO and -3KO mice

Ldlr-2KO and -3KO mice were used to evaluate the feasibility of identifying plaque in the aortas using microCT since they exhibit different amounts of plaque. As expected, the Ldlr-3KO mice were obese, extremely hypercholesterolemic (including elevated HDL-cholesterol) and hypertriglyceridemic compared with Ldlr-2KO mice (Table S1, [20]). The Ldlr-2KO mice were also hypercholesterolemic compared with typical levels in wildtype mice (Table S1, [24]). Two-dimensional microCT images were generated and used to visualize the aorta and resident plaque from the Ldlr-2KO and -3KO mice. Figure 1 shows a representative 10 μm microCT coronal section of the aorta and arteries and clearly defines artery wall, lumen and plaque. The aorta and lesions were segmented from the microCT dataset and a 3D rendering of the microCT data was generated. A rotational loop sequence of these frames was then generated and composited into a movie (Movie S1). Single images from the movies were captured to compare the aortas from the two mouse strains side-by-side (Figure 2). Since linear measurements were generated throughout the aortas and lesions, plaque thickness was modeled. It is important to note that microCT detection of the plaque area was confirmed with the use of the conventional *en face* staining technique in the same aortas. Figure 2 (lower panels, *en face*) confirms the gross 2D shape of the lesions found in each aorta.

Each aorta from Ldlr-2KO and -3KO mice was measured using microCT analysis (Table 1) and *en face* methods (Figure S1). We also calculated plaque area normalized to total aorta area, for comparison between the mouse strains. As expected, smaller lesion sizes were found in the Ldlr-2KO mice than in Ldlr-3KO mice. In two Ldlr-2KO mice no lesions were detected by microCT, whereas small areas of Sudan IV-positive staining were evident from *en face* analyses (Figure S1, aortas 6928 and 6930). Interestingly, a severe aneurysm was observed in the aorta from an Ldlr-3KO mouse (aorta 6944; Figures S1 and S2). The vessel volume was approximately double that of the other Ldlr-3KO mice; however, the plaque volume was not similarly increased.

We found that both readouts of lesion surface and volume were highly reproducible; plaque surface area – SD = 0.59, CV = 5.25%, plaque volume – SD = 0.014, CV = 2.98%.

We evaluated whether the lesion measurements between Ldlr-2KO and -3KO aortas generated with microCT showed similar fold differences with *en face* (Figure S3) and found a 14.6-fold difference in total plaque surface area between Ldlr-2KO and Ldlr-3KO using *en face* and a 17.3-fold difference using microCT (Figure S3A), demonstrating comparable fold differences between the two techniques. We also calculated an 11.7-fold difference in plaque volume between the Ldlr-2KO and Ldlr-3KO aortas (Figure S3B).

Detection of atherosclerotic plaque in a standard preclinical model of atherogenesis

To evaluate microCT for its utility in preclinical studies, we quantitatively analyzed atherosclerotic plaques in 5 Ldlr single knockout mice fed an atherogenic diet (Ldlr-1KO-AD) (a common model of atherogenesis [25,26,27,28]). In these mice plasma cholesterol levels were extremely elevated (3137 +/- 63 mg/dL).
Table 1. Aortic and lesion dimensional characteristics of Ldlr-1KO mice fed atherogenic diet (AD) and Ldlr-2KO and -3KO mice as determined by microCT and en face.

| Aorta No. | Ldlr-1KO-AD Mean +/- SEM | Ldlr-2KO Mean +/- SEM | Ldlr-3KO Mean +/- SEM |
|-----------|-------------------------|----------------------|----------------------|
|           | 4 | 5 | 7 | 17 | 18 | 6941 | 6928 | 6930 | 6962 | 6927 | 6943 | 6944 | 6945 |
| MicroCT   |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Total Aorta Volume (mm$^3$) | 11.7 | 10.2 | 11.1 | 10.6 | 11.2 | 11.1±0.3 | 7.2 | 6.2 | 7.42 | 8.2 | 7.3±0.4 | 8.5 | 8.3 | 23.6 | 12.3 | 13.2±3.6 * |
| Total Plaque Volume (mm$^3$) | 2.5 | 3.1 | 3 | 2.5 | 2.3 | 2.7±0.2 | 0.1 | 0 | 0 | 0.1 | 0.1±0.0 | 0.5 | 0.4 | 0.7 | 0.7 | 0.6±0.1 ** |
| Plaque volume (mm$^3$)/Aorta volume (mm$^3$)$^a$ | 0.21 | 0.30 | 0.27 | 0.24 | 0.21 | 0.25±0.02 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01±0.00 | 0.06 | 0.05 | 0.03 | 0.06 | 0.05±0.01 *** |
| Total Aorta Surface Area (mm$^2$) | 63.1 | 50.9 | 53.5 | 52.2 | 52.4 | 54.4±2.2 | 33.2 | 28.1 | 29.7 | 36.8 | 32±1.9 | 34.3 | 35.3 | 70.7 | 45.4 | 46.4±8.5 ** |
| Total Attached Plaque Surface Area (mm$^2$)$^a$ | 22.2 | 21.7 | 30 | 19.2 | 19.6 | 22.5±2.0 | 0.5 | 0 | 0 | 1 | 0.4±0.2 | 4.2 | 5.3 | 7.9 | 8.3 | 6.4±1.0 ** |
| Plaque area (mm$^2$)/Aorta area (mm$^2$)$^a$ | 0.35 | 0.43 | 0.36 | 0.37 | 0.37 | 0.42±0.04 | 0.02 | 0 | 0 | 0 | 0.03 | 0.01±0.01 | 0.12 | 0.15 | 0.11 | 0.18 | 0.14±0.01 *** |
| Maximum Plaque Thickness (mm)$^a$ | 0.4 | 0.7 | 0.3 | 0.5 | 0.4 | 0.5±0.1 | 0.1 | 0 | 0 | 0 | 0.1 | 0.1±0.00 | 0.5 | 0.2 | 0.2 | 0.2 | 0.3±0.1 * |
| Average % Occlusion$^a$ | 24.6 | 34.1 | 35.7 | 26.1 | 23.4 | 28.8±2.5 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| En Face |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Total Plaque Surface Area (mm$^2$) | 14.9 | 13.5 | 16.8 | 12.4 | 11.6 | 13.8±0.9 | 0.4 | 0.1 | 0.2 | 0.7 | 0.4±0.1 | 2.6 | 3.7 | 8.4 | 5.5 | 5.1±1.3 * |

* Aortas scanned from aortic valve to iliac bifurcation.
** Aortas scanned from aortic valve to 1 cm down descending aorta.
* Aorta with aneurysm.
* Only data from Ldlr-2KO and -3KO were analysed using a T-Test, as they were generated similarly (1 cm of descending aorta).
* Data from all three strains (Ldlr-1KO-AD, 2KO and -3KO) were analyzed using ANOVA, as these readouts of plaque were standardized to aorta dimension.
* P < 0.05;
** P < 0.01 vs. Ldlr-2KO aortas using a T-Test.
*** P < 0.0001 vs. Ldlr-1KO AD aortas.
* P < 0.05 vs. Ldlr-2KO aortas using ANOVA and Tukey post hoc test.
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are in excellent agreement with the typical (r² = 0.95, en face predictor of plaque quantity, and to confirm its correlation with
images on left). and the lesion and is demarcated in pink after segmentation (arrows; difference in gray scale values of the CT data between the aortic wall and can be distinguished from the aortic wall by the arteries are clearly seen. Aortic lesion is evident within the luminal space of the aorta and can be distinguished from the aortic wall by the difference in gray scale values of the CT data between the aortic wall and the lesion and is demarcated in pink after segmentation (arrows; images on left).

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Similarly, the number of aortic plaques from the Ldlr-1KO-AD was higher than those from Ldlr-2KO and -3KO mice (Table 1 and Figure 3). In these analyses, 3-panel movies incorporating many images and readouts were created (See Methods and Movie S2). Figure 3A–C shows a single frame from a 5-panel movie. Extensive atherosclerotic lesions in the Ldlr-1KO-AD throughout the aortic tree and descending aorta are clear, along with other dimensions of atherosclerotic lesions in all three Ldlr-deficient models, providing data that can be processed to generate movies to view the relevant features from any orientation. In the first example, we produced a rotating movie around a ventral/dorsal axis (Movie S1), allowing viewing of the aorta and plaque from all perspectives. In the second example we produced a multipanel movie that travels through the aorta, displaying relevant dimensional statistics with reference to the original microCT images (Movie S2). Importantly, we determined the precise amount of lesional volume and calculated luminal occlusion by the lesions based on volumetric data. The attached surface area and volume of the lesions determined by microCT were highly reproducible between operators (CV = 5.3% and 3% respectively) and correlated highly (p<0.0001) to en face surface areas. In addition to quantifying the dimensions of the plaque, we found that microCT can also yield information about the interior of the lesion, notably the necrotic area confirmed by histologic analysis, without resorting to those methods that may that may also alter the morphology and therefore affect volumetric measurements.

The use of microCT to detect lesions in mice offers numerous advantages over traditional techniques. 1) From the perspective of the researcher, hands-on time is possibly reduced; the aortas need not be precisely removed from the animal and only minimal organ trimming is necessary. Although staining with a contrast agent takes 2 days, the aortas once positioned within the CT scanner, can be scanned for overnight data collection, and data processing and image/3D rendering is mostly performed in silico. The current method also reduces artifact or operator error as the collection of data is performed in an unbiased manner by software that has been provided guidance to determine the area of plaques by trained personnel. 2) Accurate volumetric data can be collected which reflect the precise volume of the lesion and aorta, unlike the aortic root in which estimations of plaque volume are used between dropped sections. Furthermore, microCT generates volumetric data of the aorta that is mostly still within its normal environment, potentially retaining all of its in vivo characteristics (with the exception of blood volume/displacement). In contrast,
the aorta may be physically distorted by handling, shrunken by fixation/embedding with en face and aortic root sectioning.

The aneurysm detected in one of our Ldlr-3KO mice is a good example of the benefits of in situ 3D (Figure S2) imaging over the flattened en face preparation, where the architecture of this lesion was significantly distorted (aorta 6944; Figure S1). The in situ microCT scanning also allows the viewing and analysis of surrounding tissues and organs, for example the carotid arteries or perivascular adipose. 3) Anatomical planes can be chosen after processing, as opposed to the careful selection of one anatomical plane before traditional histologic analysis, or cutting of the tissue before en face analysis. One can choose the desired orientation of a

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**Figure 2. Three-dimensional renderings of atherosclerotic plaque.** The microCT images are rendered to generate a 3D representation of the aortic arch and attached plaque. A rotating movie (Movie S1) allows the viewing of the plaque from any aspect. Coronal and oblique aspects obtained from the movies are shown for both Ldlr-2KO (left images) and -3KO mice (right images). The distance of the surface of the plaque from the aorta luminal wall is indicated using a color spectrum scale (0–340 μm) shown on the left-hand side of each image. Both coronal and oblique sections illustrate differences in the amount of atherosclerosis detected by microCT between the two strains. The same aortas were excised from the carcass and assessed by en face methods (see Materials and Methods) to allow comparison of the aortic arch plaque by using the two techniques. doi:10.1371/journal.pone.0018800.g002
particular histological projection by simple digital re-orientation of a data set. 4) The nondestructive in situ methodology leaves the aorta intact for future analyses, including histology to confirm potential necrotic areas and immunohistochemistry. Further, the precise area of the aorta can be selected for follow up based on reference to the microCT data. 5) Lastly internal composition of the lesions, specifically necrotic areas, can be identified. Presumably microCT will also be of use in those models of advanced atherosclerosis that exhibit lesions harboring areas of calcification.

Despite the obvious quantitative value of microCT imaging, there are significant hurdles to wide-spread use, including the cost and technical expertise required. Further, there are perhaps some limitations on the ability of microCT to detect the smallest of lesions. In two of the Ldr-2KO mice we analyzed tiny lipid-positive areas that were detected and measured (both less than 0.2 mm²) by the en face method (Figure S1, Table 1) but that were not detected in these same samples by microCT. Further studies will be required to optimize the detection of lesions within this range.

Figure 3. Plaque Detection by microCT in Ldlr-1KO atherogenic diet-fed mice; correlation of en face with microCT. A single frame of a 5-panel movie (Movie S2) of an Ldlr-1KO fed an atherogenic diet. (A) Aortic arch and descending aorta (gray) showing location of the plaque (yellow). Cross section of aorta is show by the black line to indicate the section of interest in subsequent panels. (B) Both upper and lower graphs represent sections along the aorta (proximal to distal) along the x-axis and plaque dimensions along the y-axis. Upper graph shows average aorta radius (red), maximum plaque thickness (blue) and average plaque thickness (yellow) at each section is shown continuously along the aorta. Lower graphs show percent plaque coverage (green) and percent occlusion of the plaque within the aorta. The vertical line in both graphs indicates position in relation to section in panel A. (C) MicroCT image of the section indicated in panels A and B shows a cross section of the aorta with accumulation of plaque. The plaque which is detected by the imaging has been demarcated in pink (right image). (D, E) Correlation of lesional readouts using microCT and en face. En face plaque surface area data derived from aortas of Ldlr-1KO-fed atherogenic diet (AD) (green squares), -2KO (red circles), and -3KO (blue triangles) mice were compared with plaque surface area (D) or plaque volume (E) derived by microCT methodology. Linear regression and correlation coefficients were calculated for each data set comparison.

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Lesional surface areas are reported in Table 1. Although the entire descending aorta was analyzed and reported, here we show only the aortic arch.

Table S1 Body weight and plasma lipid characteristic of Ldlr-2KO and -3KO mice (N = 4): Ldlr-3KO mice were obese, extremely hypercholesterolemic (including elevated HDL-cholesterol) and hypertriglyceridemic compared with Ldlr-2KO mice. The Ldlr-2KO mice were hypercholesterolemic compared with typical levels in wildtype mice.

Supporting Information

Figure S1 En face assessment of atherosclerotic plaque. Aortas from Ldlr-2KO (top images) and -3KO mice (bottom images) used for microCT analysis were dissected from the carcass and assessed by en face methods and Sudan IV staining to allow comparison of the aortic arch plaque using the 2 techniques. Lesional surface areas are reported in Table 1. Although the entire descending aorta was analyzed and reported, here we show only the aortic arch.

Table S1 Body weight and plasma lipid characteristic of Ldlr-2KO and -3KO mice (N = 4): Ldlr-3KO mice were obese, extremely hypercholesterolemic (including elevated HDL-cholesterol) and hypertriglyceridemic compared with Ldlr-2KO mice. The Ldlr-2KO mice were hypercholesterolemic compared with typical levels in wildtype mice.

Movie S1 Intimal-medial plaque thickness movie of Ldlr-3KO aorta 6945 (Table 1): This movie ties together the three-dimensional aortic arch with the segmentation and quantitative metrics. The movie is split into five panels; each panel is described below: 3D Rendering Frame (left): we display the 3D aortic arch, with a perpendicular slice. The arch is rendered as two surfaces: the vessel is drawn in a transparent gray; the plaque is shown in dark yellow. At the bottom of this frame, we report the slice number and slice percent occlusion as the movie progresses (and indicated by the black ring), as well as total specimen measurements for the average occlusion, total plaque volume, and total attached surface area. Linear Distance Measurements (top-right): for each slice along the aorta, we graph the average aorta radius, maximum plaque thickness, and the average plaque thickness. As the animation proceeds, a black time-bar indicates the current slice position. Percent Measurements (middle-right): for each slice along the curved centerline, we graph the percent coverage (percent of the vessel wall that has plaque attached) and percent occlusion (percent of the vessel cross-section that is occluded with plaque). As the animation proceeds, a black time-bar indicates the current slice position. Planar Slice (bottom-middle): slice of microCT volume that cuts through and is centered on the aorta, and which is oriented perpendicular to the centerline. Planar Slice with Segmentation Overlay (bottom-right): planar slice (same as above) with plaque segmentation overlaid in red. Total Percent Occlusion: The total percent occlusion for each specimen is taken as the average of the percent occlusion values calculated at each centerline-reformatted slice. We note that this formulation is different than the value that results from the ratio of the total plaque volume to the total vessel volume (which we do not report).

(MOV)
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