Supplemental appendix

Note: These files are very large and will be available in the public domain at the NIH/NHGRI public ftp website: <u>ftp://ftp.nhgri.nih.gov/pub/outgoing/ArteryImages</u>

Movie of internal elastic membrane. This low resolution video is made from the image shown in full detail in supplement 2. It can be viewed in various formats, including Quicktime and Microsoft Mediaplayer or Realplayer. The movie is a composite of screenshots of the high-resolution image and starts and ends with the entire cross section of the artery. The image zooms in to the internal elastic lamina of the main lumen of the femoral artery, showing variations in the lamina, duplications, breaks and changes in elasticity. The lamina bifurcates just beyond a point where it is compressed into a squiggle and forms a very large intra lamina bone-like calcification that is 1000 times as large as the width of the lamina. (The video zooms out to show this feature.) At the other side of the feature the video zooms in to show the continuation of the elastic lamina around the lumen. There is an additional high magnification of the elastic lamina where the histology appears to be disorganized at a very fine level. The video completes the tour of the lamina where it began and zooms out to reveal the entire artery. Boxes were added to the image to help with scale during the zooming process and are not meant to show a specific feature.

1. Link to site to download the ultra high-resolution H&E image of the entire artery. This is an EPS image, a PSD image, or a pdf image of the entire cross section of the femoral artery resected during the bypass surgery. The image consists of approximately 500 Megapixels and was constructed from a mosaic of 624 individual 40X micrographs of the H&E stain of the paraffin-impregnated formalin fixed tissue. This image is taken from a microtome slice just below a bifurcation in the artery. The bifurcation lumen is in the upper quadrant, and is not the major lumen. There is a single large region of calcification and many smaller regions that involve the entire circumference of the internal elastic lamina that can be traced completely around the perimeter of the main lumen. The approximate resolution is ± 2 um over a 10,000 um diameter. There is one cutting artifact that runs vertically and just off center to the image. The micrograph white balance was unintentionally readjusted in the (horizontal) lower 1/3 of this image during acquisition. No post photomicrographic rebalancing or other retouching was performed. To align all high resolution images properly, an initial 10X image was assembled automatically from 36 mosaic pictures, then 230 - 20X images were overlayed and aligned to the 10X mosaic image. Finally, the 624 40X images were overlayed and aligned to the 20X images.

(Composites of other slices were stained and composite 20X images automatically aligned with Zeiss algorithms in the main paper figure 4).

2. Rotating non-contrast CT of the lower extremities demonstrating severe calcification and arteriomegaly, especially of the distal femoral and popliteal arteries.