SUPPLEMENTARY APPENDIX B

Ultrasonographic assessment of common carotid artery intima-media thickness, and the measurement of coronary artery calcium using The Multi-Detector Computerized Tomography
A. Ultrasonographic Assessment of Common Carotid Artery Intima-Media Thickness

Acquisition of ultrasound images and measurement of carotid artery intima-media thickness (CIMT) were conducted using standardized procedures and technology specifically developed for longitudinal measurements of atherosclerosis (1-10) (Patents 2005, 2006, 2011). In brief, high resolution B-mode ultrasound carotid artery images were acquired using a linear array 7.5 MHz transducer. Single lead electrocardiogram (ECG) and ultrasound images were simultaneously recorded. The jugular vein and carotid artery were imaged transversely and longitudinally with the former vessel stacked above the latter. All images contained internal anatomical landmarks for reproducing probe angulation. The baseline image for each individual was used as a guide for follow-up examinations. For each individual, depth of field, gain, ultrasound input power, dynamic range, monitor intensity setting and all other instrumentation settings used at baseline examination were maintained for all follow-up examinations. This establishes standardization for instrument setup encompassing the full dynamic range of the ultrasound echo across all examinations within the same participant. These standardized procedures result in reproducible imaging and processing of the same portion of the arterial wall at each examination necessary for accurately tracking atherosclerosis change (1-8). Arterial dimensions including the far wall CIMT was measured at sub-pixel resolution using automated computerized edge detection software (7,8) (Patents 2005, 2006, 2011). CIMT was determined as the average of 70 to 100 measurements between the intima-lumen and media-adventitia interfaces along a 1 cm length just proximal to the carotid artery bulb at the same point of the cardiac
cycle. This method standardizes the location and the distance over which CIMT is measured, ensuring comparability within and across participants (7,8). This CIMT method is correlated with the change in coronary artery disease assessed by quantitative coronary angiography (9) and is predictive of clinical coronary events (10). The coefficient of variation of repeated CIMT measurements is typically <3% and often approaches 1% (3,5,11).

**Equipment Certification.** The ultrasound machines used in this study were calibrated by using a standardized phantom. If the calibration of an ultrasound imager was inaccurate, this problem was rectified by a service technician and the accuracy of calibration was retested before the imager was certified.

**Ultrasonographer Training.** Ultrasonographers were trained by the staff of the USC Atherosclerosis Research Unit (ARU) Core Imaging and Reading Center (CIRC). Carotid anatomy was reviewed and excellent to poor quality IMT images were reviewed. The pitfalls leading to poor images were demonstrated. The ultrasonographers were certified if they followed the standardization protocol and were able to obtain carotid artery images that yielded IMT measurements with a variability of 4% or less with repeated imaging.

**Quality Control.** Initially, images were sent to CIRC for evaluation and if corrective procedures were needed, the information was sent back to the ultrasonographer within 48 hours. Once established, ongoing image quality and standardization procedures were monitored at CIRC. The images were checked for the input power, echo detector gain, and dynamic range values to ensure that identical conditions were used for each follow-up examination. The ultrasound image was assessed for quality, including
brightness and clarity of the lumen-intima and media-adventitia boundaries as well
inclusion of structures such as the bulb and stacking of the jugular vein. All follow-up
images were compared with the baseline image to ensure reproducibility of probe
angulation and proper image acquisition. If deterioration of image quality occurred, the
ultrasonographer was retrained in the image acquisition procedures. To monitor
operation of the system, a standardized phantom was regularly scanned. The images
obtained from the phantom were measured with our IMT assessment software; any
change in system performance was investigated.

Variability of CIMT acquisition was monitored. Variability was determined from the
repeat baseline ultrasound scans obtained at screening and at month 0 approx. 1 to 2
weeks apart. The subjects had a repeat ultrasound examination 1 to 2 weeks following
the end of study scan. The repeat ultrasound at baseline and as subjects completed the
trial were a part of our quality control procedures.

References


healthy postmenopausal women: a randomized controlled trial. Stroke 2011;42:3168-75.


B. The Multi-Detector Computerized Tomography.

The MDCT scan was performed using General Electric Lightspeed Model VCT 64 channel scanner in high-resolution volume mode, using 100-ms exposure time. EKG-triggering synchronized images in the same point in diastole, corresponding to 40% of R-R interval. Proximal coronary arteries were visualized and at least 30 consecutive images were obtained at 3-mm intervals. Coronary calcium was defined as a plaque of at least 3 contiguous pixels (area=1.02 mm²) with a density >130 Hounsfield units.

Lesion score was calculated by multiplying lesion area by a density factor derived from Hounsfield units. Total CAC score was determined by summing the lesion scores from the left main, left anterior descending, circumflex, and right coronary arteries using the Agatston method. A cardiac CT trained physician blinded to interventions interpreted all scans at CT reading center at the Los Angeles Biomedical Research Institute, Torrance, CA under Dr. Budoff’s supervision. The CT reading center staff supervised the initial training and quality control of the CT image analysts and the reading process.

At the CT reading center, custom software was used to measure calcified plaque for these studies; the custom software was based on software used during the year 10 examination in 1994–1995 of the CARDIA study. Key aspects of the software and reading process include the blinding of CT image analysts to any clinical information about study participants and to the calculated results (eg, Agatston score). The software checks the technical parameters used to obtain the scan and notifies the image analyst if values are outside those specified in the protocol. On the CT images, the software locates the calibration phantom standards and measures the CT attenuation of each calibration phantom by computing the mean CT number for pixels contained within each.
of the four regions of interest on the image. These data are used to calibrate the image
to a standardized level across all study sites.

The image analysts identified the anatomic course of the coronary arteries on the CT
images by assigning waypoints along the length of the major arteries. The waypoints
were used by the software, along with image data, to define a line corresponding to the
trajectory of the coronary artery across the surface of the heart. The image analysts
review the coronary artery trajectories determined by the program and adjust the
computer-generated trajectory if it deviates from the observed course of the coronary
artery. The coronary artery trajectories allow quantification and location of calcified
plaque within the coronary arteries and were saved to facilitate future analysis. By using
the coronary artery trajectories, the software automatically identifies candidate calcified
plaques on the basis of predefined minimum criteria for CT attenuation (130 HU),
minimum calcified plaque size, and distance from the coronary artery trajectory. The
image analyst systematically reviewed each candidate calcified plaque and either
accepted or rejected its inclusion as calcified coronary artery plaque. The software
computes several measures of calcified coronary artery plaque, and these measures
include the Agatston score (by using the standard 130-HU threshold, modified to adjust
for section thickness), calcified plaque volume, and interpolated calcified plaque volume
along the section direction. After image calibration, the measures of calcified plaque
were recalculated to provide phantom-adjusted Agatston scores. The image analyst
then completes a quality control assessment of quality in several categories: motion
artifact, misregistration artifact, noise artifact, phantom placement, and coverage of the
heart.
References
