

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kastelein JJP, Akdim F, Stroes ESG, et al. Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med* 2008;358:1431-43. DOI: [10.1056/NEJMoa0800742](https://doi.org/10.1056/NEJMoa0800742).

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SUPPLEMENTARY APPENDIX I:

Ultrasound Scans and Image Analyses Methods

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Ultrasound Imaging

In ENHANCE ultrasound examinations are performed in replicate at baseline and at planned (24 months) or unplanned end of study. Interim, single scans are performed at 6, 12, and 18 months. The carotid images of right and left common carotid, carotid bulb and internal carotid (Figure 1) as well as the left and right common femoral (Figure 2) arterial segments are obtained with the patient comfortably reclined on the examination couch. For the carotid scan the head tilted approximately 10 degrees to the contra lateral side of the scan. The transducer is pointed from the lateral position with the transducer axis parallel to an imaginary line from ear to ear. For the femoral scan the images are obtained with an anterior position of the transducer axis. The predefined and fixed angles of the head and leg position, the transducer angles and predefined anatomical landmarks, the carotid flow divider, the carotid bifurcation and the femoral bifurcation, contribute to the repeatability of subsequent measurements.

B-mode images to be used for the IMT analyses are obtained in a RES2 (a regional expansion selection of 2x2cm) mode; the maximum expansion of the image at time of scan as to maximize pixel resolution and, hence, measurement accuracy. All ultrasound images are obtained using an Acuson 128XP (Siemens AG, Erlangen, Germany) equipped with a 5-10 MHz linear array L7 transducer and Extended Frequency (EF) software. All machines automatically started up with standardized ENHANCE presets to ensure trial specific image characteristics. B (brightness) -mode ultrasound pre-sets include RES2 (Regional Expansion Selection view of a 2x2 cm area) and standardized persistence and processing settings (Figure 3, B-mode ultrasound image).

The protocol also includes M (motion) -mode investigations to investigate distal common carotid lumen (Figure 4) and arterial wall stiffness. M-mode ultrasound pre-sets include a duplex 1/3 RES2 B-mode, 2/3 M-mode view (Figure 5, M-mode ultrasound image) and fixed time scale settings. The M-mode results are not part of the main analyses of ENHANCE and will be reported elsewhere.

Ultrasound images are downloaded as digital still frames, TIFF-files, on a CD writer (Sony UPA-P100MD, Sony Corporation, Tokyo, Japan) in Tag Image File Format (TIFF). B-mode ultrasound images of the carotid and femoral arteries are acquired bilaterally according to a strictly adhered imaging protocol. In the carotid artery the segments are defined as the common carotid artery (the arterial far wall 1cm proximal to the carotid dilatation), carotid bulb (the arterial far wall between the dilatation and the flow divider) and the internal carotid artery (the arterial far wall 1cm distal of the flow divider). In the femoral artery the common femoral arterial far wall 1cm proximal to the femoral dilatation is visualized. The B-mode images provide the IMT measurements. Moreover, in the distal common carotid artery an M-mode image of the near and far walls for at least 2 heartbeats is obtained. The M-mode images provide the lumen measurements and arterial wall stiffness parameters.

In the image handling process, the images are stored on CD on site and sent to the Core Image Center (CIC, Perceptive Informatics, Waltham, MA, USA), at which point a quality assurance check is performed and all site, subject, and sonographer information is digitally masked from the images. The digital image files are then randomized and masked, and sent to the Core Echo Laboratory (CEL; Academic Medical Center, Amsterdam, The Netherlands), where IMT, lumen diameter, and carotid distensibility are calculated. During the sonographer

training and qualification period, images are sent by e-mail to the CEL Help Desk. The Help Desk is administratively separate from the readers of the CEL to ensure all image analysis is free of observer bias. All other CEL personnel, apart from those employees working for the CEL Help Desk, are blinded to site and sonographer identity throughout the entire trial.

Ultrasound Image Analyses

The Core Echo Laboratory uses trial specific designed image analysis software ('eTrack', Dr. Wim J. Stok, MSc, Mr. Will Hanselaar BSc, and Dr. Eric de Groot, MD PhD, Departments of Vascular Medicine and Physiology, Academic Medical Centre, Amsterdam, The Netherlands). Images for efficacy analyses were delivered per scan set (i.e., all images of all scans of a subject). Up to seven arterial wall segments (there are up to seven visits in this study) were prepared in a patient's subdirectory, such that the images of a given arterial wall segment could be easily browsed and used as reference images.

The masked TIFF image files are automatically defined as B- or M-mode images and automatically calibrated (based on the scaling notches of the 2x2cm RES box for the B-mode images and the time scaling and depth notches for the M-mode images). The reader was only allowed to proceed with image analysis if the following information was correctly present on all B- and M-mode images:

- Every image is identified by a unique number
- The settings of the Acuson ultrasound instrument must be according to protocol.

The quality of the image is evaluated according to subsequent criteria. Specifically, this included, but was not limited to: the correct arterial wall segment identified (ICA vs. ECA), presence of image noise, correct landmark identification, horizontal assessment of the wall,

consistency (symmetry of location) of the cursor placement along the wall, and transmit zone. Moreover, the quality of the interfaces and possible interference should be described (e.g. shadowing due to plaque, curved structure, etc.). Qualitative issues that may cause interference with the ability to do an IMT measurement are: shadowing due to near wall curvatures/plaques, tortuous vessel walls (in particularly in the CIA segments). The images are also evaluated according to the ability to do 1 cm measurements in the CCA and CIA and to do measurements between the dilatation and flow-divider cursors. Diagonal arterial walls are not deemed acceptable. The reader will focus on consistency to be able to identify the same interfaces (which can be difficult in the femoral arterial segments). Finally, a comparison to the reference images for similar vascular location within the segment on the image is performed, since the same location must be identified within all frames for accurate measurement.

IMT measurements are done after comparison to the reference images of the same arterial wall segment. The region of interest (ROI) should be consistent with the other frames used in the synchronous reading as much as possible. The identification of the landmarks is checked (readers may overrule if appropriate and length criteria apply based on the reader identified landmark identification). Measurements are done using cursors (which look like upside-down pyramids). The leading edges of the lumen-intima (LI) and media-adventitia (MA) interfaces are identified, using an interactive cursor. The leading edges of the lumen-intima and media-adventitial interfaces of the arterial far wall are determined from the B-mode images. This distance that represents the intima-media complex of the carotid wall and is hence defined as intima-media thickness, or 'IMT'. The eTrack program calculates the MEAN IMT (the average of the distance between the interfaces, the MEAN IMT and the maximum thickness, the MAX IMT, of a given arterial wall segment)(Figure 6). The results are downloaded as a.

ASCII-text data and b. an associated control image. The control image shows the ROI, the identification of the interfaces and correctness of image interpolation (Figure 7). In the ENHANCE study, near wall measurements were not obtained, since this measurement includes two gain-dependent trailing edges of interfaces of which only one interface, the intima-lumen interface, has a well defined leading edge. For those reasons, near walls contribute only to statistical 'noise' and are not to be used in comparative trials.

From the M-mode images of at least 2 heart beats the MEAN distal common carotid lumen is calculated (Figure 8). The lumen is defined as the distance from the leading edge of the near-wall intima-lumen interface to the leading edge of the far wall lumen-intima interface. The additional M-mode parameters are calculated automatically based on blood pressure information (according to RR, prior and after each M-mode observation) and the derivatives of the net excursions of the arterial walls. As for the B-mode, eTrack provides control images with the M-mode measurements (Figure 9).

The quantitative and qualitative assessments of the B- and M-mode images are downloaded in separate, read-only, B-mode and M-mode databases; each measurement, in ASCII-text format, is accompanied with its adjoining JPEG control image (as in Figures 7 and 9), so issues of both qualitative and quantitative nature can be addressed directly with the concerning sonographer and/or reader.

All image analyses have been performed by certified readers.

Rejected images and those of poor quality were identified for further evaluation by consistency checks, over-reads and the medical director of the Core Echo Lab. Intima-media wall thickness was analyzed on the B-mode images.

REFERENCE

1. John J. P. Kastelein, Philip T. Sager, Eric de Groot, Enrico Veltri, MD. Comparison of ezetimibe plus simvastatin versus simvastatin monotherapy on atherosclerosis progression in familial hypercholesterolemia: Design and rationale of the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial. *Am Heart J* 2005;149:234- 9)

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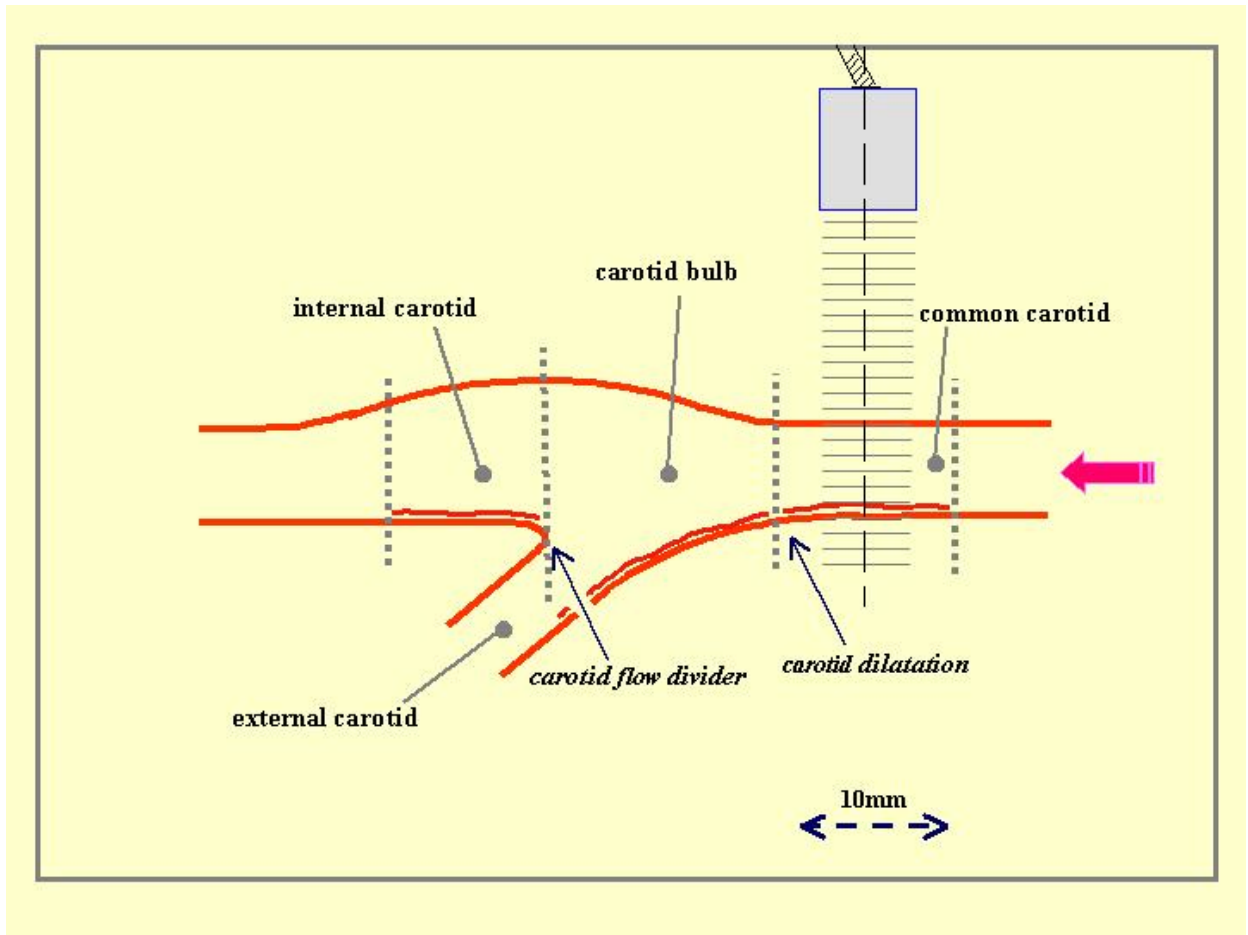


Figure 1.

Carotid arterial segments. The common carotid (defined as the segment 1cm proximal to the dilatation), carotid bulb (between the dilatation and the flow divider) and the internal carotid (1cm distal from the flow divider) arterial segments. Carotids are scanned bilaterally.

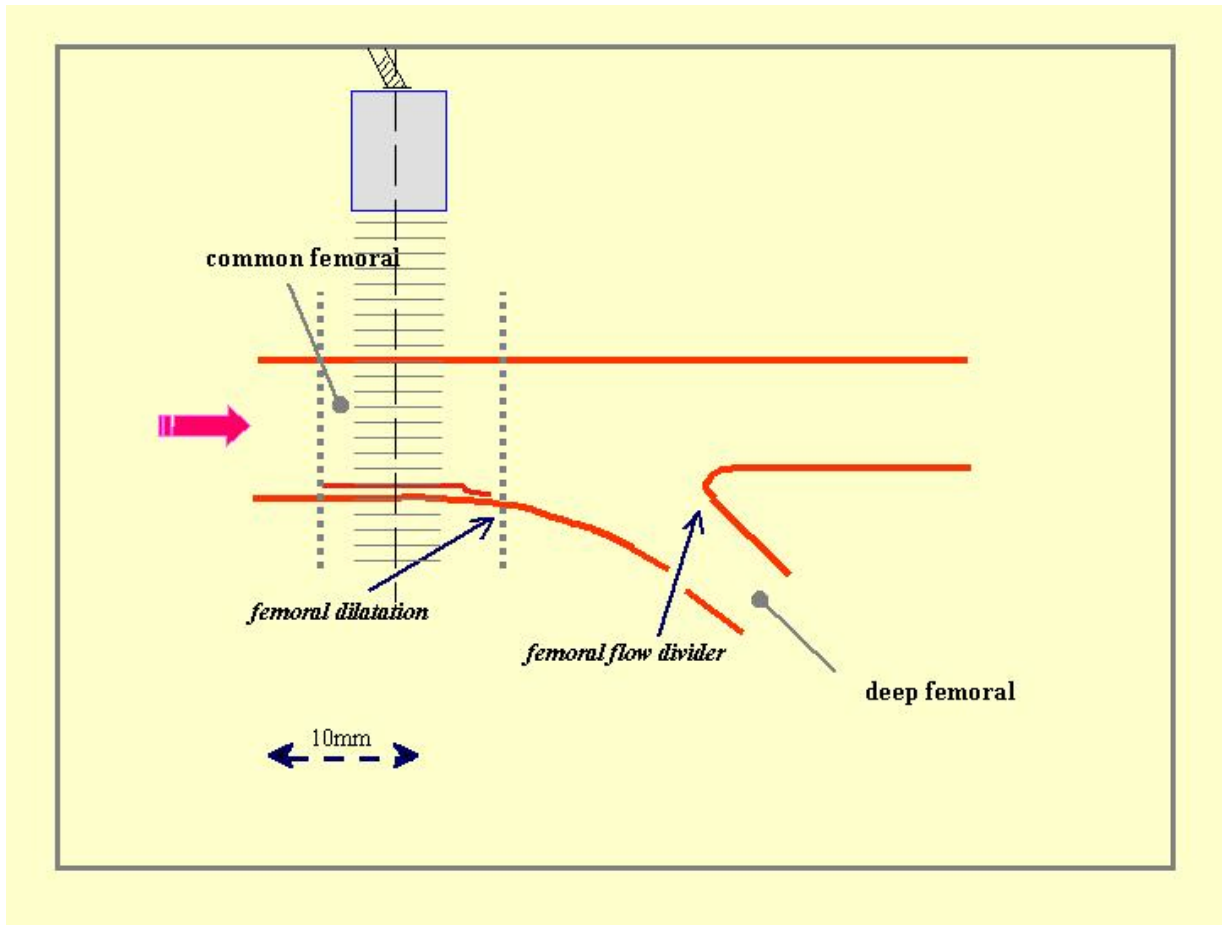


Figure 2.

Femoral arterial segment. The common femoral (defined as the segment 1cm proximal to the dilatation) arterial segment. Femorals are scanned bilaterally.

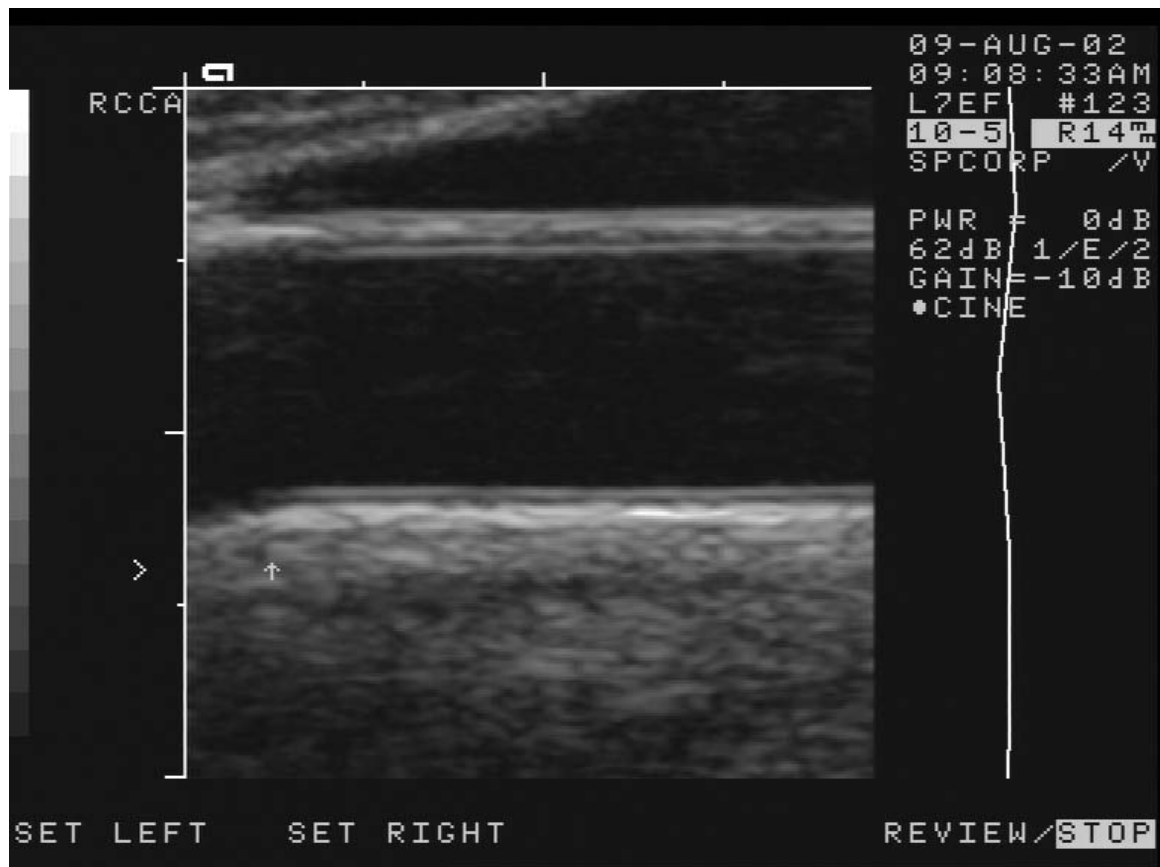


Figure 3.

B-mode ultrasound still image of the common carotid arterial wall segment and its standard settings as used in the imaging trial proptocol. The arrow in the far wall indicates the carotid flow divider. The image is saved as a TIFF-file.

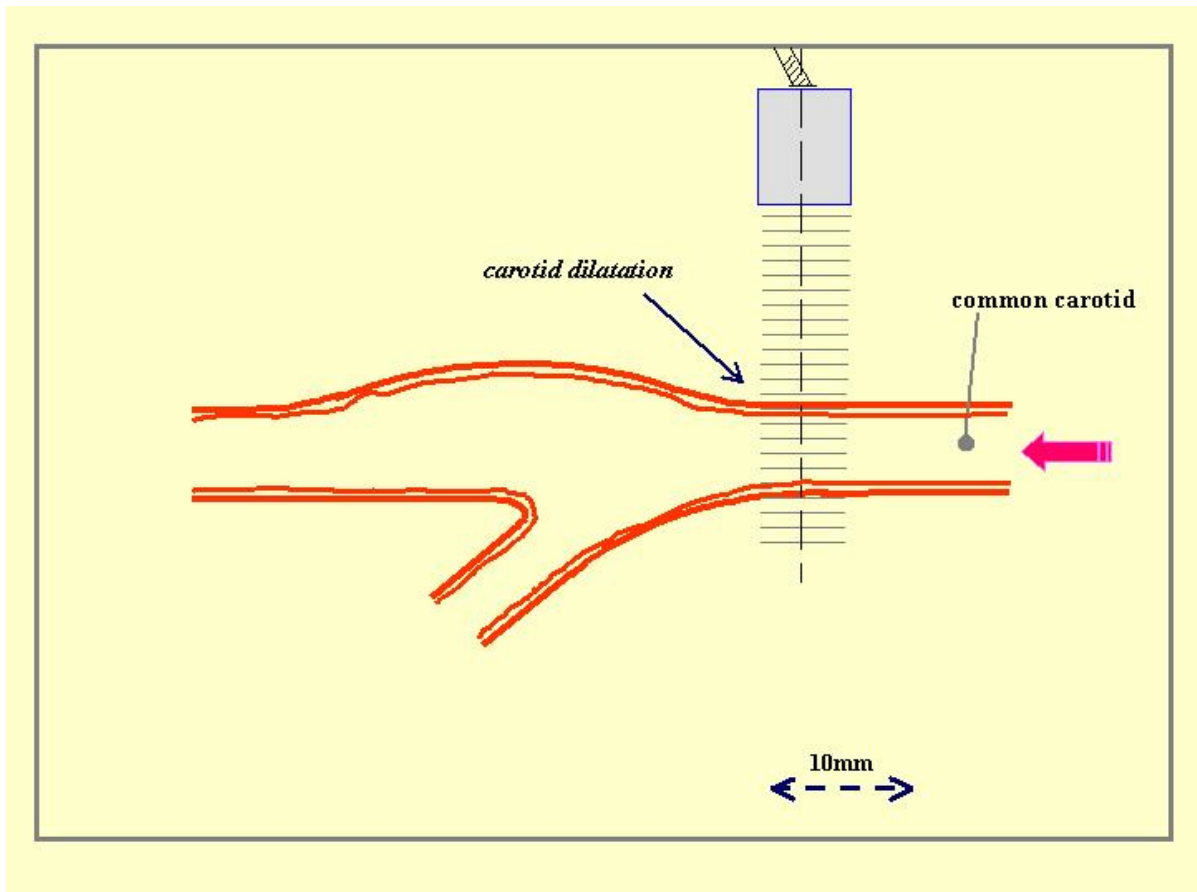


Figure 4.

M-mode images are created in the distal common carotid artery.

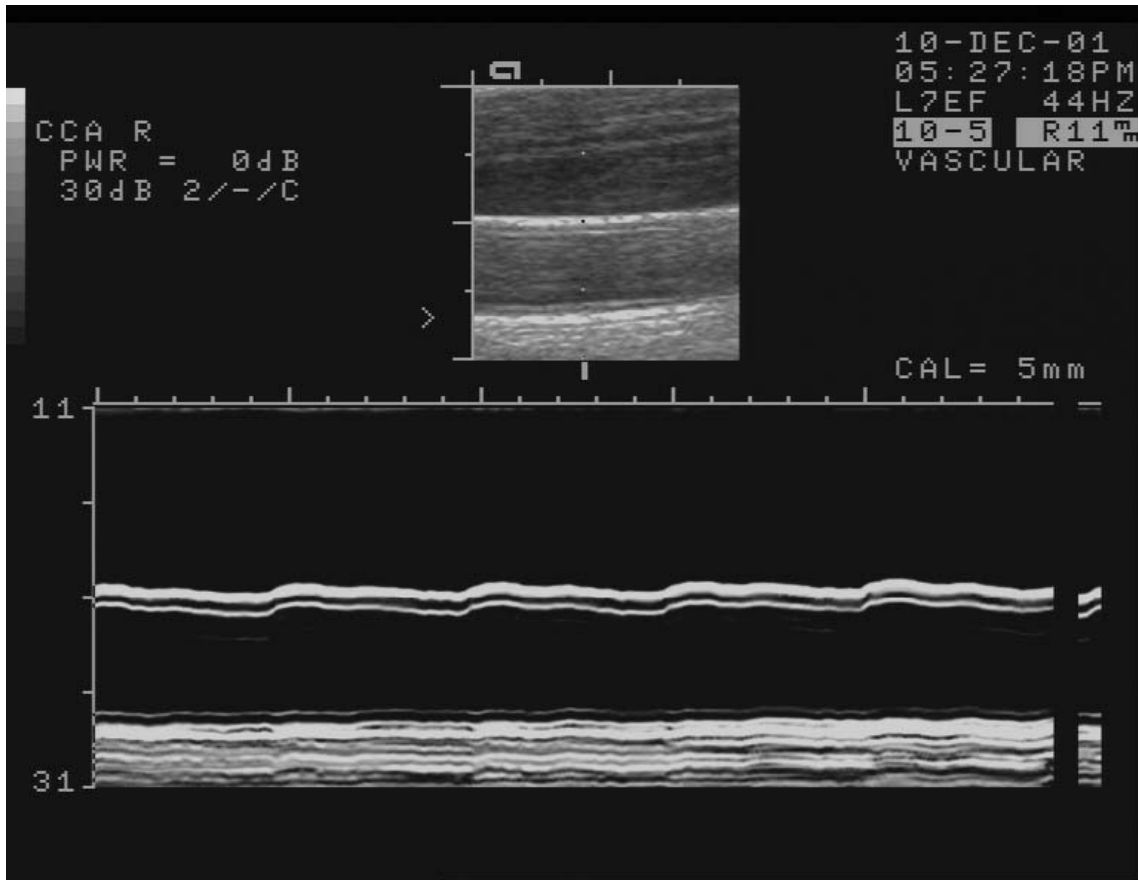


Figure 5.

The near and far walls are visualized in the 1/3 B-mode; the movement in time is depicted in the 2/3 M-mode image. The 1/3-2/3 image is saved as a TIFF-file of the same format as the B-mode image.

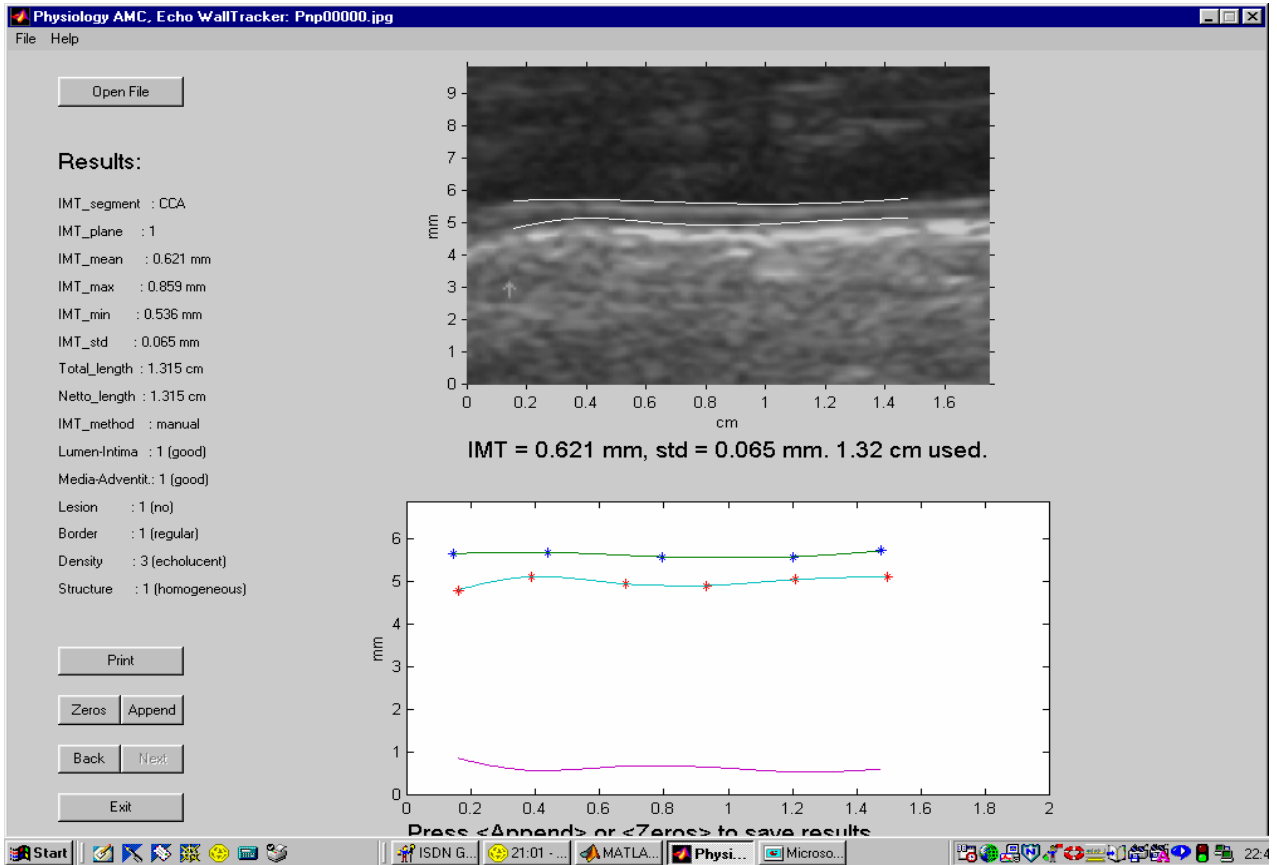


Figure 6.

Image analysis of a the region of interest of a B-mode image, using the eTrack program. The image analyst (or ‘reader’) indicates the lumen-intima and media-adventitia interfaces with cross-hairs using an interactive cursor. The cross-hairs are splined.

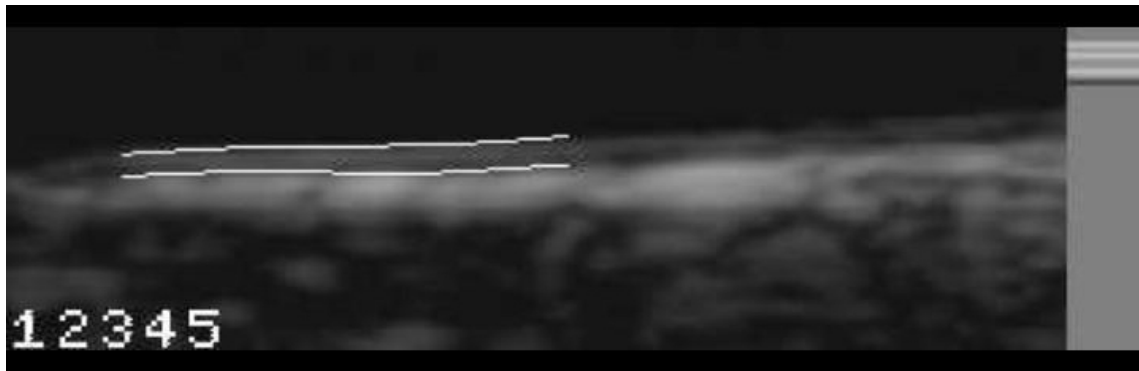


Figure 7.

Control image as used for B-mode image analysis. The control image represents the ROI of the image. The splined (manual) tracings delineate the lumen-intima and the media adventitia interfaces. The light gray bar on the right side of the control image indicates the videolines that could (homogeneous gray) and could not be (striping) evaluated by the image interpolation function of the image analysis program. In this example, the striping is in the lumen and hence outside the region of interest of and of no influence on the IMT measurement (Courtesy Dr J.M. Karemaker and Dr W.J. Stok, Department of Physiology, Academic Medical Centre, Amsterdam, The Netherlands).

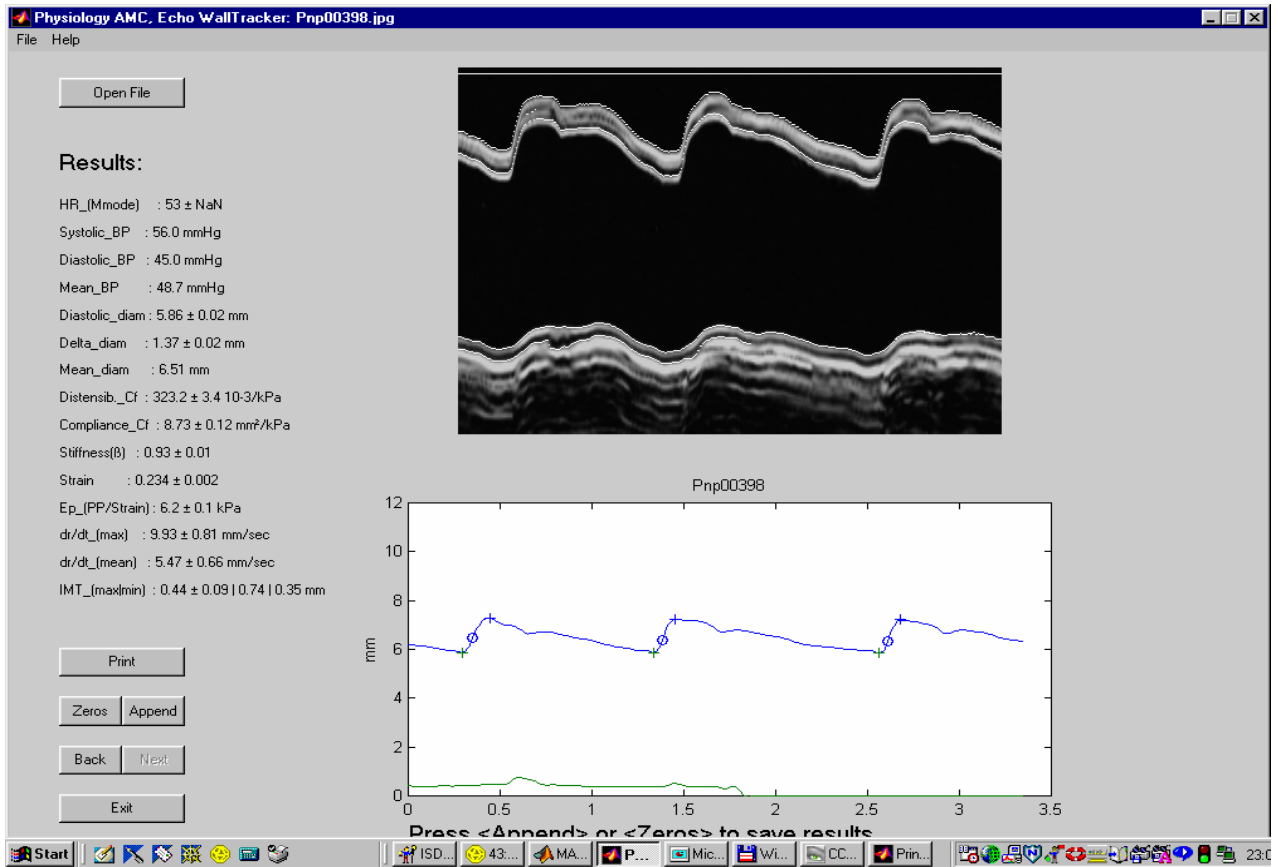


Figure 8.

Image analysis of a the region of interest of an M-mode image, using the eTrack program. At least 2 heartbeats are required for a proper tracing of the lumen and arterial wall stiffness parameters.

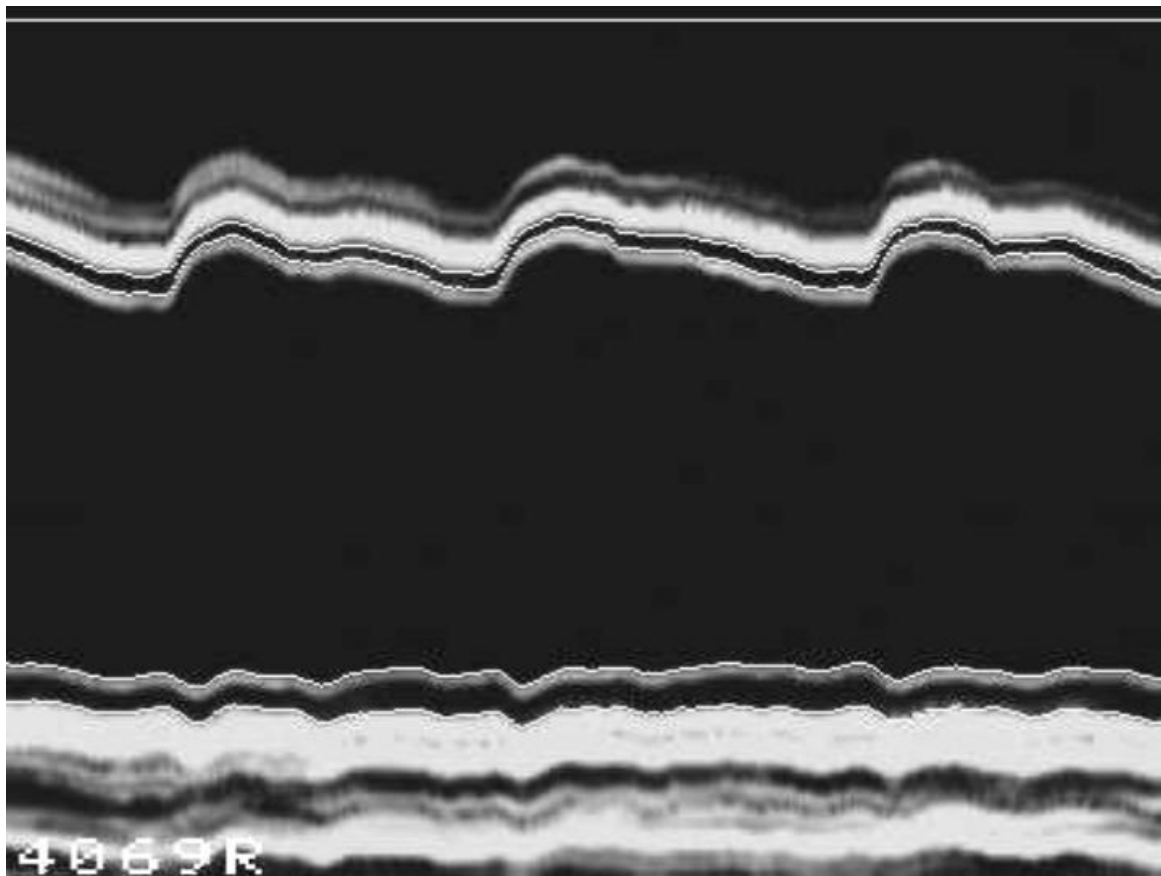


Figure 9.

Control image as used for M-mode image analysis. The (automatic) contour tracings delineate the peri-adventitial – adventitia interface and the intima-lumen interfaces of the near wall, and the lumen-intima and the media-advential interfaces of the far wall. The lumen is measured between the leading edges of the interfaces of the intima-lumen of the near and the lumen-intima of the far wall interfaces (Courtesy Dr J.M. Karemaker and Dr W.J. Stok, Department of Physiology, Academic Medical Centre, Amsterdam, The Netherlands).

SUPPLEMENTARY APPENDIX II:

a. Most common Treatment Related Treatment Emergent Adverse Events

	Simvastatin (n=363)	Simvastatin plus Ezetimibe (n=357)
Subject reporting any adverse event (%)		
Blood and lymphatic system disorders	107 (29) 1(<1)	122 (34) 0
Cardiac disorders	1(<1)	1(<1)
Ear and labyrinth disorders	1(<1)	0
Endocrine disorders	0	1(<1)
Eye disorders	3(1)	1(<1)
Gastrointestinal disorders	32 (9)	36 (10)
General disorders and administration site conditions	13 (4)	17 (5)
Hepatobiliary disorders	0	1(<1)
Infections and infestations	1(<1)	0
Injury, poisoning, and procedural complications	1(<1)	1(<1)
Investigations	17(5)	27(8)
Neoplasm benign, malignant and unspecified	1(<1)	0
Reproductive system and breast disorder	1(<1)	1(<1)
Respiratory, thoracic and mediastinal disorders	3(1)	6(2)
Skin and subcutaneous tissue disorders	9(2)	6(2)
Musculoskeletal and connective tissue disorders (%)	45 (12)	56 (16)
Nervous system disorders (%)	20 (6)	19 (5)
Psychiatric disorders (%)	4 (1)	8 (2)
Renal and urinary disorders (%)	5 (1)	3 (1)
Vascular disorders (%)	2 (1)	1 (1)

SUPPLEMENTARY APPENDIX II:**b. Commonly Reported Treatment Emergent Adverse Events**

	Simvastatin	Simvastatin plus Ezetimibe
	(n=363)	(n=357)
Subject reporting any adverse event (%)		
Influenza	338 (93) 79(22)	338 (95) 99(28)
Nasopharyngitis	50(14)	57(16)
Myalgia	32(9)	43(12)
Headache	41(11)	35(10)
Arthralgia	41(11)	34(10)
Back pain	33(9)	38(11)
Sinusitis	22(6)	29(8)
Fatigue	27(7)	22(6)
Cough	17(5)	26(7)
Upper respiratory infection	19(5)	23(6)
Dyspepsia	21(6)	20(6)
Nausea	22(6)	18(5)
Pain in Extremity		23(6)
Diarrhea	16(4) 14(4)	
Insomnia	17(5)	20(6) 19(5)
Chest pain	18(5)	13(4)
Gastroenteritis	18(5)	99(3)

SUPPLEMENTARY APPENDIX II**c. Adverse event leading to withdrawal of consent.**

	Simvastatin	Simvastatin plus Ezetimibe
	(n=363)	(n=357)
Subject reporting any adverse event (%)		
Cardiac disorders	34(9) 4(1)	29(8) 2(1)
Gastrointestinal disorders	4(1)	5(1)
General disorders and administration site conditions	3(1)	4(1)
Infections and infestations	1(<1)	0
Investigations	6(2)	11(3)
Neoplasm benign, malignant and unspecified	2(1)	0
Skin and subcutaneous tissue disorders	6(2)	1(<1)
Musculoskeletal and connective tissue disorders (%)	6(2)	13(4)
Nervous system disorders (%)	2(1)	3(1)
Psychiatric disorders (%)	2(1)	0
Metabolism and nutrition disorders	1(<1)	0
Pregnancy, puerperium and perinatal conditions	1(<1)	0

SUPPLEMENTARY APPENDIX III

Additional secondary endpoints

1. Change from baseline in the mean IMT, separately for the three carotid artery segments (common carotid, carotid bulb, and the internal carotid artery) and the femoral artery
2. The percent change from baseline in lipid parameters (LDL-c, HDL-c, total cholesterol, apoB, and triglycerides)
3. The percent change from baseline in lipid indices: total cholesterol, calculated LDL-c, HDL-c, triglycerides, apolipoprotein B, apolipoprotein AI, and CRP.

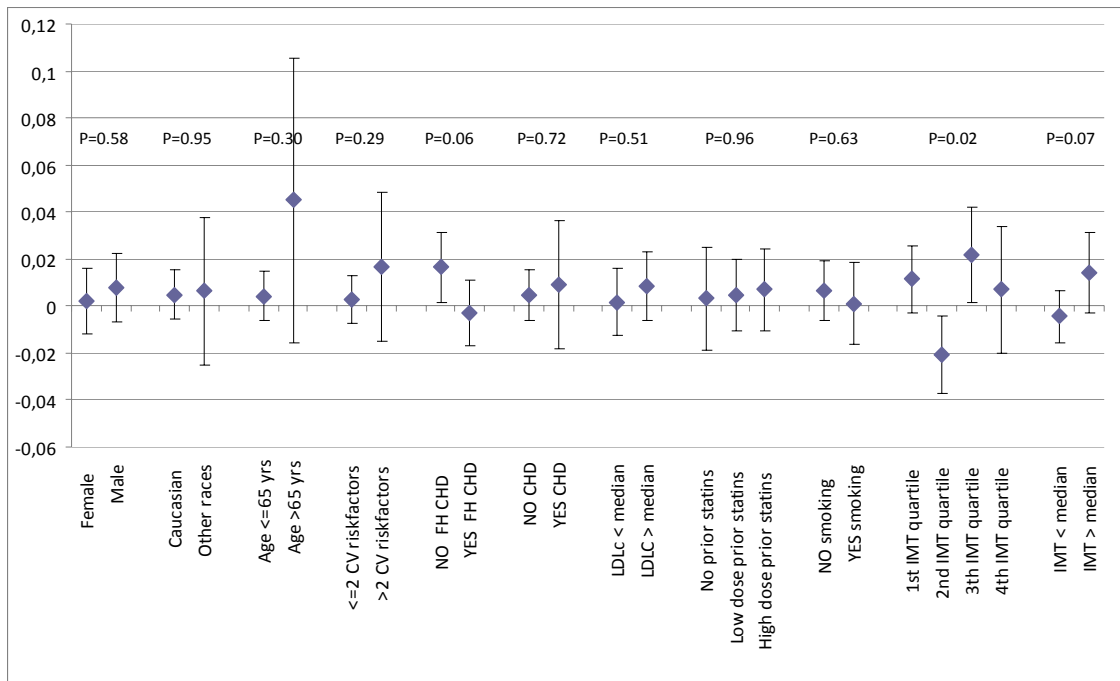
SUPPLEMENTARY APPENDIX IV:

Prespecified exploratory analyses:

1. Age (≥ 65 years or < 65 years)
2. Gender
3. Race (Caucasian or non-Caucasian)
4. LDL cholesterol (greater or less than the median)
5. Cigarette smoking
6. History of coronary heart disease
7. Pre-statin dose (high, low, or no statin)
8. Baseline cIMT (greater or less than the median).

SUPPLEMENTARY APPENDIX V:

Treatment difference in change in cIMT in prespecified subgroups



Treatment difference in change in cIMT in prespecified subgroups

Bars represent (95% confidence interval)