FAMILY STUDY

Cardiovascular Disease in American Indians
(Phase IV)

Operations Manual - Volume Five

SPECIAL STUDIES – CAROTID ULTRASOUND,
TONOMETRY, AND ECHOCARDIOGRAPHY

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH
THE STRONG HEART STUDY

Cardiovascular Disease in American Indians (Phase IV)

Operations Manual

Volume Five

SPECIAL STUDIES - CAROTID ULTRASOUND, TONOMETRY, AND ECHOCARDIOGRAPHY

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STRONG HEART STUDY
ULTRASOUND READING CENTER MANUAL OF OPERATIONS

Goals of the Study: Carotid arterial ultrasonography and arterial pressure waveform analysis permit non-invasive assessment of arterial hypertrophy, detection and quantification of atherosclerosis, and estimation of arterial stiffness and hemodynamics. These methods will be used in Phase IV of the Strong Heart Study to accomplish the following specific aims:

1. To assess the heritability and genetic linkage of carotid artery intimal-medial thickness (IMT) and discrete atherosclerotic plaques.
2. To assess the heritability and genetic linkage of measures of arterial stiffness.
3. To determine the separate and joint effects of diabetes mellitus (DM), overweight and hypertension on arterial structure, function and atherosclerosis in individuals ≤45 years.
4. To examine the associations of arterial structure, plaque and stiffness with LV structure, systolic and diastolic function, independent of risk factors and clinically-apparent cardiovascular (CV) disease, in a population-based sample of individuals with DM.
5. To assess the prognostic significance of measures of arterial structure and function determined during the 3rd SHS exam.
6. To evaluate change of abnormalities of arterial structure and function in participants in the SHS pilot Family Study over a mean of nearly six years.

Research Design and Methods:

A. Carotid Artery Structure and Atherosclerosis:

Methods will be adapted from those used and refined at Cornell since 1989 and subsequently applied at other sites in multi-center studies (see Table 1). Imaging of both carotid arteries will be performed using Acuson 128 systems equipped with a 7 MHZ linear array arterial imaging transducer. B-mode scanning of the right and left extracranial carotid arteries will be done in multiple projections to optimize the detection of discrete atheromata, identified on two-dimensional images as the presence of a discrete plaque at least 50% greater than the surrounding wall within any segment of either carotid artery. Carotid plaque size is quantified by computer-assisted measurement of plaque thickness on two-dimensional frames. The maximum diameter of the plaque is measured along with percent encroachment of the lumen diameter. The severity of stenosis is quantified using standard Doppler techniques. Peak flow velocities in the 1.5 to 2.5 m/sec range are indicative of 50-74% lumen stenosis whereas velocity in excess of 2.5 m/sec is indicative of ≥75% stenosis. Two-dimensionally-guided M-mode tracings of both the right and left distal common carotid artery approximately 1 cm proximal to the bulb are obtained to measure carotid wall thicknesses and lumen diameter at end-diastole and peak-systole (maximum diameter).

At the Reading Center, suitable frames for measurement are acquired in real-time from the videotape using a frame-grabber interfaced with a high-resolution video monitor and stored on diskettes (Table 2). Following calibration for depth and time, the end-diastolic wall thickness
(combined intimal-medial thickness of the far wall) and end-diastolic and peak-systolic internal diameters (by continuous tracing of the lumen-intima interface of the near and far walls) are measured on several cycles using electronic calipers and averaged. Measurement of carotid wall thickness is never made at the level of a plaque (infrequent in the common carotid artery).

**B. Arterial Pressure Waveform and Compliance Estimates:**

Regional compliance characteristics of the carotid artery will be estimated using methods which incorporate carotid artery imaging and central arterial pressure waveforms back-calculated from the radial artery tonometry by means of validated transfer functions. The radial artery waveform is obtained using a high-fidelity solid-state strain-gauge transducer (Millar Instruments, Inc, Houston, TX) which functions as an applanation tonometer.

Arterial stiffness (beta), the inverse of compliance, is estimated according to the formula: $\beta = \ln(P_s/P_d)/[(D_s-D_d)/D_d]$, where $P_s$ and $P_d$ are peak-systolic and end-diastolic pressures, respectively, $D_s$ and $D_d$ are peak-systolic and end-diastolic dimensions, respectively. Peterson's elastic modulus ($E_p$), an estimate of vascular stiffness which does not account for differences in distending pressure, is calculated according to the formula: $E_p = (|P_s-P_d|)/(D_s-D_d) \times D_d$. Young's modulus ($E$), which takes into account structural adaptive changes of vessel wall thickness, is calculated according to the formula: $E = (|P_s-P_d|)/(D_s-D_d) \times (D_d/h)$, where $h$ is carotid artery wall thickness (intima plus media). The augmentation index, a quantitative measure of the rapidity of wave reflection, will be measured from the arterial pressure waveform. All measurements are calculated on several cycles and averaged.

**C. Ultrasonographer Training and Quality Control:**

Arterial ultrasonography training, reading procedures and quality-control will be similar to those successfully employed in Phase III of the Strong Heart Study. Sonographers in the geographic locations will be identified and their credentials verified. One week of training will be provided at the Cornell Reading Center. Sonographers will observe the technique for the carotid ultrasound study as performed by a full-time research ultrasonographer. Dr. Roman will demonstrate use of the applanation tonometer. All sonographers will be observed and critiqued in their performance of arterial imaging and in use of the applanation tonometer. Sonographers will complete worksheets at the completion of each study which can subsequently be utilized for written or oral feedback.

Copies of videotapes will be made and kept at the field sites to facilitate feedback and prevent loss of tapes. Initial readings will be performed by the research sonographer and verified by the physician-investigator. The initial and verification readings of the ultrasound studies will be performed in a blinded manner and then merged with demographic descriptors for final quality-control check of extreme values. Measurements will be performed using established in-house custom measurement and database and statistical analysis programs, including computer support from the Clinical Research Center. Data will be electronically transmitted to the Coordinating Center. Clinical alerts, such as high-grade stenoses, will be immediately reviewed and results relayed by FAX immediately to the Field Center.
**TABLE 1: CAROTID ULTRASOUND PERFORMANCE PROTOCOL**

**Instrumentation:** Ultrasonographs will be calibrated against a phantom at installation and at regular intervals thereafter. The vascular probe will be set to default with processing curves and a persistence setting optimal for imaging of the carotid artery.

**Patient Preparation:** Imaging will be performed in a slightly darkened room with the subject in a supine position with slight hyperextension of the neck (a roll under the neck is optional). Electrodes will be placed for a modified three-lead electrocardiogram.

**Two-Dimensional Imaging and Doppler Study:** Two-dimensional (B-mode) long-axis imaging from multiple planes (posterior, lateral, anterolateral) will be done to maximize detection of discrete plaque. Following identification of the carotid bifurcation, the transducer should be moved caudally to examine the common carotid artery until its origin from the aortic arch (left) or innominate artery (right). Extensive imaging of the bifurcation should be performed given the high predilection for plaque in these regions. Both branch vessels should be scanned in a cephalad direction until their disappearance. If plaque is present, the transducer should be rotated to obtain a cross-sectional image identifying the maximum incursion of the plaque into the lumen. Pulsed Doppler analysis (with angle correction to ≤60°) should be performed to quantify the degree of stenosis.

**M-mode Study:** Following completion of the two-dimensional scanning protocol, the transducer should be positioned for optimal visualization of the distal common carotid artery perpendicular to the transducer beam (parallel to the linear probe). The M-mode cursor should be placed perpendicular to the long-axis of the distal common carotid artery to intersect the intima-lumen interfaces of both the near and far walls (in an area uninvolved by discrete plaque). Gain settings should be optimized to limit 'blossoming' of the brighter interfaces. Following conversion to a full-screen display, M-mode imaging of the distal common carotid artery should be recorded with particular attention to continuous imaging of the lumen-intima interface of both the near and far walls.

The complete protocol will be videotaped and the procedure will be repeated on the contralateral artery.
**TABLE 2: CAROTID IMAGE ANALYSIS PROTOCOL**

**Reading Center Equipment:** The Reading Center is equipped with a personal computer into which a frame-grabber has been inserted and connected to a high-resolution video monitor and professional videocassette recorder. Customized software allows acquisition in real time of two-dimensional or M-mode frames thus bypassing image degradation which might occur were analyses to be performed on stop-frame images.

**Review of Videotape:** The videotape of each study will be reviewed in its entirety at the Cornell Reading Center. Whenever a plaque is detected, that frame showing maximum diameter of the plaque (either longitudinal or cross-sectional) will be acquired in real time using the frame grabber and stored on a diskette. Suitable frames including M-mode imaging of the both distal common carotid arteries demonstrating continuous tracing of the lumen-intima interfaces of the near and far walls will be acquired in real-time and stored on diskette. The frame number of each image acquired in this context will be recorded on a worksheet.

**Measurement Techniques:** Measurements will be recorded on a worksheet. Plaque will be graded as present/absent, unilateral/bilateral (including location) and quantified by maximum diameter and percent lumen stenosis (using calibrated electronic calipers). Maximum velocity at the level of a plaque causing significant stenosis will be recorded. Following calibration for depth and time, measurement of the intimal-medial thickness of the far wall will be made on as many cycles as are available on the acquired frame and averaged. Measurements will be obtained at end-diastole (minimum diameter) with the aid of the electrocardiogram. Minimum and maximum diameters will be measured by continuous tracing of the lumen-intima interface of the near and far walls on sequential cycles and averaged.

**Data Summary and Transmission:** Measurements on the worksheet will be verified by an investigator for faithfulness to the analyzed image and for outlier values before being transferred by diskette for incorporation in the main computer database. Variables to be transmitted will be: plaque (absent/present [unilateral/bilateral]); plaque location(s); plaque diameter(s); percent lumen stenosis; maximal Doppler velocity; right and left end-diastolic intimal-medial thicknesses; mean end-diastolic intimal-medial thickness; right and left minimum and maximum internal diameters; mean minimum and maximum diameters.
TABLE 3: PROCEDURE FOR ARTERIAL APPLANATION TONOMETRY

1. Subject will remain in the supine position following completion of the carotid ultrasound study.

2. Necessary information will be typed into the SphygmoCor system, including subject age, gender, and brachial blood pressure. The brachial systolic and diastolic pressures to be entered here will be calculated by averaging the last 2 of 3 sequential brachial pressure readings made at the end of the echocardiogram procedure (see pp. V-10 to V-11).

3. The applanation tonometer will be positioned over the left radial artery and manipulated to obtain an arterial pressure waveform of an appropriate contour with the highest pulse pressure. Following stabilization of the recording, the space bar on the laptop computer will be pressed and a series of waveforms will be acquired for processing.

4. The data acquired will be stored on diskette and sent to the Cornell Reading Center. Following verification of fidelity of the pressure waveform, the following data will be transferred by diskette for incorporation in the main computer database: central aortic systolic and diastolic blood pressures, augmentation index and estimates of carotid artery compliance (beta, Peterson's elastic modulus, Young's modulus).
Echocardiography

It is the Reading Center principal investigator's experience over many years that the percentage of echocardiograms that are suitable for accurate measurement is enhanced if the examining sonographer makes preliminary measurements on each study and is then provided feedback as to how to improve the suitability of the study for quantitation and the selection of interfaces to measure. To obtain reproducible M-mode measurements of LV structures, dominant lines representing the necessary interfaces should be recorded, and recognized during interpretation, that exhibit continuous motion in the correct pattern for the structure for at least 0.10 second but ideally through the entire cardiac cycle (6, 26, 38).

Echocardiography Performance at Field Centers

Principles: The most important primary echocardiographic measurements and derived variables to assess the heart in an epidemiologic context can be obtained from a relatively simple echo examination (28, 39). Correct orientation of the ultrasound beam and imaging planes to LV structure and blood flow is essential.

The LV resembles an ellipse of rotation that is nearly circular in short-axis views, with a long-axis about twice its minor axis. To measure the LV minor axis accurately it is necessary to orient the echocardiographic plane from the parasternal (or less commonly the subcostal) window to pass perpendicularly through the interventricular septum and posterolateral LV wall at the junction of papillary muscle tips and mitral chordae (Figure 2A). Rotation of the 2-D sector 90° to the short axis projection allows one to measure the true, maximum LV diameter (Figure 2B). If, as is common in older subjects, the best parasternal window is in a low interspace, LV minor-axis dimensions and wall thicknesses should not be measured in the usual fashion, although it may be possible to measure correctly the aortic root and left atrium (Figure 2C). Instead, a higher interspace should be used, which may image only a narrow sector that includes the LV minor axis (Figure 2D). If this is not possible, linear measurements of LV minor axis and wall thicknesses should be made at the correct level and orientation by the leading-edge method from 2-D long-axis views that maximize LV cavity size.

If the 2-D short-axis is correctly oriented, M-mode LV recordings may provide clearer delineation of wall interfaces, and in that case will be used for LV measurements.

A major advantage of 2-D echocardiography is its ability to visualize the LV long-axis and wall segments near the apex. To accomplish this, one must obtain the true (longest) long-axis dimension and visualize the LV walls in approximately orthogonal apical 4- and 2-chamber views. The LV long-axis is commonly foreshortened in the 4-chamber view (Figure 4A), as seen when the transducer is rotated to the 2-chamber view and the LV apex is out of the field of view (Figure 4B). The transducer should then be moved inferolaterally until the LV apex is as nearly centered at the top of the image "fan" in both views as possible (Figures 4C and 4D).

The accuracy of Doppler recordings depends on the ultrasound beam being parallel to the axis of blood flow. Variants of the apical 2- and 4-chamber views should be used to sample LV inflow across the mitral anulus or valve orifice; the apical long-axis should be used to measure systolic flow across the aortic anulus to calculate stroke volume and cardiac output; and sample volume placement between the...
inflow and outflow tract to measure isovolumic relaxation time.

**Protocol for Echocardiogram Performance**

Standardized methods will be employed to obtain high-quality recordings. Echocardiograms will be performed in an area that provides room for the examining table, echocardiograph, etc., and has dimmable lighting to prevent glare on the echocardiograph screen that would interfere with study performance. Participants will change their top for a light gown to permit discrete exposure of the chest wall overlying the parasternal and apical acoustic windows. Disposable ECG lead attachments (set of 3) will be attached to the skin to monitor a single ECG lead for timing purposes. The participant will then lie down and assume a partial left decubitus position (with pillows or a foam-rubber wedge to support the back) with the head of the examining table modestly elevated. The participant’s last name, initials, SHS study number and the date and site of recording will be entered so they will be recorded on videotape. Echocardiographic recordings will then be made using procedures outlined in Table 1. Careful performance of this protocol will require 40 minutes of participant's time including the period required to get in and out of a gown and to step from the SHS examining area to and from the adjacent echocardiography area.

**TABLE 1**

**ECHOCARDIOGRAPHIC TECHNIQUES FOR LEFT VENTRICULAR MEASUREMENTS**

<table>
<thead>
<tr>
<th>Instrument Calibration:</th>
<th>Calibrate against phantom at installation and at regular intervals thereafter.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echocardiographic Performance:</strong></td>
<td>Standardize and record decubitus position. Use mattress cut-out for apical imaging. Record images in held expiration.</td>
</tr>
<tr>
<td><strong>Location of Imaging Planes:</strong></td>
<td>From short-axis view with correct angulation of short-axis plane defined in long-axis view or in long axis with maximization of left ventricular cavity diameter.</td>
</tr>
<tr>
<td>2-Dimensionally guided M-mode:</td>
<td>Define correct orientation of short-axis and apical views by use of 90 degree orthogonal planes.</td>
</tr>
<tr>
<td>2-Dimensional Echo:</td>
<td></td>
</tr>
<tr>
<td><strong>Recognition of Measurable Images:</strong></td>
<td>Dominant lines with correct motion representing interfaces for at least 0.10 seconds (5mm at standard recording speed).</td>
</tr>
<tr>
<td>M-mode:</td>
<td>Visualization of complete interface in motion with continued visualization in stop-frame mode. Do not use “persistence” on the echocardiograph.</td>
</tr>
<tr>
<td>2-Dimensional Echo:</td>
<td>Use three or more cardiac cycles.</td>
</tr>
<tr>
<td><strong>Enhancement of Reproducibility:</strong></td>
<td>Record imaging window location and patient position. For research use readings by two or three investigators.</td>
</tr>
</tbody>
</table>

Modified from Devereux et al (reference 28).

Specific recordings will be made as outlined in Table 2 and the following text: Parasternal...
Long-Axis 2-D recordings will be obtained first, with the interspace and degree of left decubitus positioning chosen to allow correctly oriented linear measurements of interventricular septal (IVS), LV internal diameter and posterior wall (PW) dimensions.

**TABLE 2**

**ECHO/DOPPLER SCANNING AND RECORDING SEQUENCE**

I. **Parasternal Long-Axis Primary View**
   A. Two-dimensional echocardiography during quiet respiration: Maximize left ventricular and aortic diameter and record 10 beats on tape.

II. **Left Ventricular Imaging**:
   A. M-mode cursor perpendicular through left ventricle just below the level of the mitral leaflet tips: Record 10 beats of 2-D update image with M-mode recording, then record at least 10 beats of full-screen M-mode during quiet respiration and attempt at least 5 beats at held-expiration.
   B. Turn 90° into parasternal short-axis view.
   C. Two-dimensional echocardiography at or just above level of papillary muscle tips during quiet respiration: Record 15 beats on tape.
   D. M-mode cursor through the meridian of the left ventricle at level of papillary muscles: Record 10 beats of 2-D update image with M-mode recording and 10 beats of full-screen M-mode recording during quiet respiration on tape. Then attempt at least 5 beats at held-expiration.
   E. Sweep two-dimensional short-axis views superiorly to the mitral valve and aortic valve level. Record 10 beats on tape.

III. **M-mode sweep** from LV through mitral valve to left atrium/aortic view recorded on videotape.

IV. **Aortic Left Atrial Imaging**:
   A. Two-dimensional echocardiography in long-axis views during quiet respiration at level of aorta and left atrium with maximization of aortic diameter at the sinuses of Valsalva: 10 beats.
   B. M-mode cursor perpendicular through aorta and left atrium with maximization of aortic diameter by "tilting" medially and laterally of the 2-D imaging plane: Record 10 beats of 2-D update image with M-mode recording, then record 10 beats of full-screen M-mode during quiet respiration.
C. Color Doppler will be turned on to record 10 beats of a view encompassing the left ventricular outflow tract and left atrium.

V. Apical Four-Chamber View

A. Two-dimensional echocardiography in quiet respiration. Record at least 10 beats with maximum chamber dimensions and good LV endocardial definition on tape.

B. Pulsed Doppler transmitral flow recording with sample volume at the mitral anulus leaflet tips during diastole: Using a 2.5 MHz transducer, record 10 beats of 2-D update image with Doppler recording, then record 10 beats of full-screen Doppler during quiet respiration. Repeat the same process moving the sample volume towards the tips of the MV leaflets.

C. Direct cursor across the base of the anterior mitral leaflet and use pulsed or, if necessary, continuous wave Doppler to record simultaneous LV inflow and outflow patterns for measurement of isovolumic relaxation time.

D. Move sample volume along left side of interventricular septum to right superior pulmonary vein; record 10 beats of full-screen Doppler of pulmonary vein flow.

E. Doppler color flow mapping during quiet respiration: Using the 2.5 MHz transducer, turn on color to look for mitral regurgitation: Record 15 beats on tape while sweeping from the 4- to the 5-chamber view.

F. Turn approximately 90° into apical two-chamber view.

VI. Apical Two-Chamber and Apical Long-Axis Views

A. Two-dimensional echocardiography in the true apical two-chamber view during quiet respiration or held expiration: Record 15 beats with maximum LV chamber dimensions and good LV endocardial definition on tape.

B. Two-dimensional echocardiography in the apical long-axis view during quiet respiration or held expiration: Record 10 beats taking care to include the left ventricle, left atrium, aorta and right ventricle in the image.

C. Pulse Doppler recording in the apical long-axis view: Record 10 beats of 2-D update image with pulsed Doppler recording at the plane of the aortic valve anulus (hinging points of the aortic cusps), then record 10 beats of full-screen Doppler during quiet respiration.

Left Ventricular Imaging

While recording on VHS tape the imaging plane will be tilted medially and laterally to maximize the LV cavity area in the long-axis view. Gain and dynamic range settings, imaging frequency in mHz,
location of the transition zone and used of harmonic imaging should be adjusted to optimize interface
definition. Recordings will then be made of at least 10 cycles of optimally oriented 2-D long-axis views
followed by 5 beats in held expiration. The M-mode cursor line will than be optimally oriented in the 2-
D long view just basal to the level of the papillary muscle tips; at least 10 cycles of LV M-mode
recordings with 2-D update and a second 10 cycles of full-screen M-mode will be made on videotape. If
feasible, 5 cycles of full-screen M-mode will be recorded in held expiration. If another imaging window
is subsequently recognized to be superior, the primary 2-D view and full-screen M-mode recordings
during quiet respiration will be repeated. An attempt will be made to include a period of held expiration
in LV recordings unless this interferes with LV visualization. The 2-D imaging plane will be rotated
from the chest wall position that permitted optimal long-axis M-mode cursor orientation, approximately
90° to visualize the LV short-axis view, at or just towards the LV base from the visible landmark of the
papillary muscle tips. Recordings will then be made as indicated in Table 2.

An M-mode "sweep" will then be made from the LV through the mitral valve to the aorta/left
atrium level. At the aorta/left atrium level, 2-D long-axis recording will be resumed, the imaging plane
will be manipulated to maximize aortic anular and root diameter, the cursor beam will be oriented
through the sinuses of Valsalva at their maximum diameter and M-mode recordings will be made as
described in Table 2. Color Doppler will be turned on to record 10 beats of a view encompassing the
LV outflow tract and left atrium.

At the completion of these recordings the transducer will be shifted to the apical window,
identified by palpating the location of the LV impulse on the chest wall and then moving the transducer
inferolaterally until the LV apex is visualized in both 2- and 4-chamber views. Repositioning of
subjects may be needed to obtain a good apical acoustic window. When this is accomplished, the 2- and
4-chamber views that maximize LV cavity size will be recorded (at least 10 cycles of each); in the 4-
chamber view pulsed Doppler recordings of blood flow velocity at the mitral anulus and mitral leaflet
tips will be recorded (Figure 5A)(10 cycles) Color Doppler will then be turned on for another 15 cycles
as the imaging plane is swept from the 4- to 5-chamber view, following which pulsed or continuous
wave recordings of colored outflow and inflow signals will be made to measure the isovolumic
relaxation time. After completion of these recordings, the transducer will be rotated to the apical long-
axis view (which visualizes the aortic valve and root as opposed to the 2-chamber view which excludes
them in favor of the anterior LV wall) and pulsed Doppler recordings of blood flow at the aortic annular
plane (10 cycles) will be performed (Figure 5B).

**Brachial Pressure Measurement**

At this point the subject will be returned to a supine position without turning up the lights or any
other change, and the brachial blood pressure will be measured using the appropriate-size cuff and a
mercury sphygmomanometer. The first and fifth Korotkoff sounds (appearance and disappearance of
sound) will be used as systolic and diastolic pressures, based on the average of the last two of three
sequential determinations.

With the participant remaining in the supine position, the bend of the elbow should be at heart
level and the legs should be uncrossed. The participant should be able to relax the neck and shoulder
muscles as much as possible. Note: because the participant has been at rest on the examining table,
there is no need for the 5-min waiting period used as part of BP measurement during the clinic exam.
The brachial artery is palpated (just medial to and above the ante-cubital fossa), and this location is marked for stethoscope placement. The appropriate size cuff is then wrapped around the participant’s arm with the center of the bladder over the artery. Use the arm that is closest to the sonographer. Connect the cuff to a standard mercury manometer and establish the pulse obliteration pressure by slowly inflating the cuff while palpating the radial artery until the pulse is no longer felt. Then, deflate the cuff and record the obliteration pressure. Conversation should be limited but the procedure may be briefly explained to the participant at any time.

For each of the stethoscope BP measurements, the cuff is inflated to +30 mmHg above the obliteration pressure, the pressure is held constant for 5 sec, and then the cuff is slowly deflated (2 mm/sec) while reading pressures for Korotkoff sounds. Record the 1st and 5th phases, reading the pressure in mmHg to the nearest even number. If the mercury column falls in between two scale marks (mmHg) at the time the first or fifth Korotkoff sound is heard, the higher number should be recorded. The first sound heard in a series of two sounds is recorded as the systolic blood pressure (phase 1), and the first silence in a series of two silences is recorded as the diastolic blood pressure (phase 5), not the last sound heard. If the sounds do not cease completely, the fourth Korotkoff sound will be used. The sonographer records all 3 of these auscultated BP readings. Using a calculator, average the second and third readings and mention the results to the participant, clearly stating the systolic and diastolic pressures.

If the Korotkoff sounds are heard at the outset of cuff deflation, the peak inflation level used was too low. The cuff should be immediately deflated by releasing the thumbscrew and disconnecting the cuff tube. Make another blood pressure measurement, starting at a peak inflation level, which is 10 mmHg above the previous level.

Once all measurements (carotid ultrasound, echocardiography, and tonometry) have been completed, the ECG leads will then be disconnected and the subject allowed to dress and to go to the SHS clinic or to leave. The technician will then complete the logging information on the unblinded echo performance worksheet (Appendix 2) and on the "blinded" label (without identifiers that would reveal age, gender, blood pressure or body size) for videotape box (Appendix 3) that includes, the subject's initials, SHS participant number, date of performance and sonographer, and prepare the performance area for the next subject. Total technician time for echo performance (30 minutes), initial logging and area preparation will be 40 minutes per subject.

After completion of the day’s studies, Field Center technicians will continue the procedure begun during the training period of making preliminary measurements on each study of LV dimensions from 2-D guided M-mode recordings, recording the qualitative normality or abnormality of LV systolic function from 2-D recordings, and noting any clinical abnormalities. The worksheets with preliminary readings (Appendix I) will then be assembled with videotapes for shipment to the Reading Center, preparations (videocassettes, ECG electrodes, gel, etc.) for the next day completed, and the technicians will complete the day by reviewing teaching comments returned from the Reading Center.

Central Coordination and Echocardiogram Reading at The New York Hospital-Cornell Medical Center
The Reading Center is responsible for design of the echocardiogram protocol, training and continuous feedback for quality control of echocardiogram performance by Field Center technicians, central reading of echocardiograms with careful procedures to assure accuracy and reproducibility of data, and on-going analyses (in appropriate conjunction with the Coordinating Center at the University of Oklahoma Health Sciences Center) to assure quality control. The Center will take advantage of procedures and skills developed in this laboratory in performing over 15,000 research echocardiograms in clinical patient groups, defined population samples, and large numbers of family units to study echocardiographic methodology, the heart in hypertension, heritable cardiovascular diseases, valvular heart diseases and a variety of other conditions.

**Evaluation of LV Structure**

LV measurements will be made by the ASE recommendations, in which measurements are made from leading edge to leading edge, time end-diastolic measurements at the QRS onset. The 2-D parasternal long-axis recordings will be played backward and forward to find the cycle or up to three cycles that maximize(s) the LV cavity area. In this view, septal and PW thicknesses and LV internal dimension will be measured by the leading-edge ASE convention at the level of the papillary muscle tips along an axis perpendicular to the LV walls (40). If the M-mode beam is correctly oriented and demonstrates clearer interfaces than 2-D images, these recordings will be used for linear dimension measurements. This procedure has been used for the past 9 years in the Cornell laboratory, and increases the proportion of subjects with measurable LVs by about 10%. LV mass values by this technique with the ASE correction (9) have proven nearly identical to those from good quality M-mode recordings in the same research subjects in the Cornell laboratory, indicating their interchangeability.

With the parasternal long-axis 2-D view on the monitor, the cycle illustrating the largest LV outflow tract and aortic root diameter will be visualized to measure the aortic annular diameter at the QRS onset between the hinging points of the two visualized aortic cusps, a measurement needed to calculate stroke volume (28). On this same 2-D image the aortic root diameter will be measured by the leading-edge technique at the level of the sinuses of Valsalva as described by Roman et al (41-42), and the videotape will be advanced to end-systole (end of the T wave of the ECG) for measurement of left atrial diameter by the trailing edge to leading edge technique. The choice of the trailing edge of the posterior aortic wall, rather than the leading edge, is based on the fact that a space containing loose connective tissue exists between the aortic and left atrial walls that would otherwise be included in the left atrial diameter measurement.

The videotape will then be advanced to the apical 4- and 2-chamber views and played (several times if necessary) to allow completion by the reader of semi-quantitative scoring of wall motion (from normal to mildly, moderately or severely hypokinetic to akinetic) (40). In addition, a summary impression of global LV systolic function (normal/abnormal/severely depressed) will be made. The videotape will be advanced to the 4-chamber view recording Doppler flow across the mitral anulus. Early and late diastolic flow will be traced by the leading edge (black-white interface) method to measure peak E and A velocities and the E and A time-velocity integrals on the three cycles illustrating the highest velocity. The videotape will then be advanced to the combined recordings of anterior mitral LV inflow and outflow along the midportion of the anterior mitral leaflet to measure the isovolumic relaxation time. The videotape will then be advanced to the apical long-axis view illustrating transaortic
flow and the aortic flow time-velocity interval measured on three cycles by the leading-edge black-white method as described by Dubin et al (30). The recordings of color Doppler flow will be used in conjunction with imaging information to record the presence, etiology and estimated severity of valvular regurgitation or stenosis by established methods (41, 43-44).

**Calculation of Derived Variables**

After the technician reader has completed accepting or correcting the initial primary measurements of cardiac dimensions, flow patterns and grading of the motion abnormalities, the data will be transferred to the Clinical Research Center computer, where mean values for these measurements will be utilized to calculate derived variables. A second step will merge blood pressure and body size measures for further calculations before range checks and additional physician-investigator verification of primary data.

Linear ASE convention 2-D or M-mode measurements at end-diastole by ASE measurements are used to calculate LV mass by the anatomically validated formula:

Left Ventricular Mass = 0.8(1.04 [(IVS + LVID + PWT)\(^3\) - LVID\(^3\)]) + 0.6g

Estimates of LV mass by this method were closely related to actual LV weight at necropsy (r=0.90, p<0.001) in 52 adults (9) showed excellent reproducibility (RHO=0.93, p<0.001) in a series of 183 hypertensive patients (45) and predicted prognosis in patients with CHF (17).

Overall LV mass is the best measure of myocardial cell size, since the number of cardiac myocytes remains relatively constant after infancy, and is the most sensitive echocardiographic index of LV hypertrophy (24, 27). However, additional useful information is provided by the LV wall thickness/radius ratio, or "relative wall thickness" (RWT). This increases in proportion to chronic elevation of LV systolic pressure due to adaptive LV hypertrophy (46) and adds to LV mass for prediction of complications of hypertension (4). RWT is calculated from M-mode measurements as 2PWT/LVID (47); increased LV mass is classified as concentric hypertrophy if RWT is >0.41 and eccentric hypertrophy when RWT is normal (48). If LV relative wall thickness is increased but LV mass is normal, the subject is considered to have "concentric LV remodeling", an LV geometric pattern first described from the Cornell Laboratory (4, 48).

**Evaluation of Ventricular Performance and Load**

Systolic function of a symmetrically contracting LV, such as occurs with uncomplicated hypertension, diabetes or alcoholism, can be assessed by measurement of the fractional shortening of LVID between end-diastole (d) and end-systole(s):

Fractional Shortening (%) = \([(LVIDd-LVIDs)/LVIDd]\) x 100

If LV wall motion is uniform, fractional shortening is closely correlated with global LV ejection...
Linear dimensions can be used in the Teichholz formula (50) to calculate EF, as has been validated by comparison with angiographic reference studies (51-52). Increased rates of cardiovascular death have been shown to be associated with mildly reduced (40-54%) EF by this method (OR=3.5) and severely reduced (<40%) EFs (OR=6.8, both p<0.01) in Strong Heart Study participants.

Because ejection-phase indices of LV performance are highly dependent on afterload, measurement of myocardial afterload is helpful in determining whether or not observed ventricular function reflects normal myocardial contractility. The most direct measure of myocardial afterload is midwall circumferential end-systolic stress (ESS), which can be measured using end-systolic LV measurements by the ASE convention and cuff blood pressure, measured with the subject on the examining table at the end of the echocardiogram, in a catheterization-validated formula (53-54). A close inverse relation exists between LV shortening and ESS in both normal and hypertensive subjects (55-56), which becomes most linear when ESS is plotted on a logarithmic scale (ESS$_{10}$). Expression of observed midwall shortening as a percent of that predicted for end-systolic stress provides an afterload-independent measure of LV contractile performance. Afterload-corrected midwall shortening is subnormal in patients with congestive cardiomyopathy (57) and in a subset of patients with uncomplicated essential hypertension.

**2-D Evaluation of LV Performance** will rely primarily on evaluation of LV function by the semi-quantitative scoring system developed by Mayo Clinic investigators (16). This system utilizes parasternal short-axis views at mitral valve and midventricular level, apical 2 and 4-chamber views, and parasternal and apical long-axis views to visualize 6 wall segments in each short-axis plane and 4 segments in the LV apical region. Careful adherence to the described protocol permits scoring of wall motion in all segments in more than 80% of subjects studied under difficult circumstances (portably in a CCU setting) and in well over 90% of subjects in the Strong Heart Study and HyperGEN echocardiographic surveys.