FAMILY STUDY

Cardiovascular Disease in American Indians
(Phase V)

Operations Manual - Volume Five

SPECIAL STUDIES – CAROTID and POPLITEAL
ULTRASOUND 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 V)

Operations Manual

Volume Five

SPECIAL STUDIES - CAROTID and POPLITTEAL ULTRASOUND and ECHOCARDIOGRAPHY

July 01, 2006

For copies, please contact

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STRONG HEART STUDY
ULTRASOUND READING CENTER MANUAL OF OPERATIONS

Goals of the Study: The SHS Phase V exams will be conducted approximately 5-6 years after the original family exam and will permit evaluation of genetic factors that contribute to changes in CVD risk factors. This exam will include both carotid and cardiac ultrasound measures, so that the preliminary data on progression can be examined in more detail with a larger number of participants, and among participants as young 15 yrs. Because of the high rates of insulin resistance, obesity and diabetes among the young people in this population, the re-examination will permit detailed examination of the effects of these disorders on progression of preclinical CVD. Popliteal ultrasound has been added to provide better measures of peripheral arterial disease (PAD) because of the high rates of PAD observed during our cohort exams. Thus, we will be able to compare popliteal and carotid atherosclerosis and their risk factors with emphasis on smoking- and diabetes-related phenotypes. Carotid and popliteal arterial ultrasonography and echocardiography permit non-invasive assessments of arterial hypertrophy, detection and quantification of atherosclerosis, and estimation of hemodynamics. These methods will be used in Phase V 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 popliteal artery intimal-medial thickness (IMT) and discrete atherosclerotic plaques.
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 and plaque with LV structure, systolic and diastolic function, independent of risk factors and clinically-apparent cardiovascular (CV) disease.
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.
7. To examine changes in intermediate vascular phenotypes, to try to detect genes that are related to these changes, and to assess interactions of other risk factors (adiposity, insulin resistance, hyperglycemia) with these changes. In the first exam we found many young adults with diabetes or metabolic risk factors such as obesity and impaired fasting glucose. Identification of factors promoting atherosclerotic progression in this young at-risk age group is a high priority. Additionally, we will capitalize on members of the original cohort who also are in the Family Study (over 500) to examine long-term changes (for example, left ventricular hypertrophy and carotid intima medial thickness by standardized methods used since the 2nd SHS exam).
8. To examine subclinical atherosclerosis in peripheral arteries using popliteal ultrasound, assess its heritability and relations to risk factors (with focus on diabetes and smoking), and compare these to both carotid intima-media wall thickness (IMT) and ankle-brachial index (ABI).
9. To examine the relations between quantitative measures of systemic atherosclerosis,
cardiac hypertrophy, and cardiovascular dysfunction (e.g., LV mass, carotid plaque, carotid wall thickness) and CVD incidence and mortality? Are these potential predictors related to other established CVD risk factors, such as, diabetes?

SHS PHASE 5 CAROTID ARTERY ULTRASOUND STUDY

Prognostic Utility: A number of longitudinal studies involving population-based samples in different countries have examined the relation of baseline carotid IMT and/or discrete plaque to subsequent CVD event rates. These studies have varied in methodology: IMT and plaque are not always evaluated separately; risk is stratified based on thresholds, quintiles, standard deviations and/or increments of IMT; and multivariate analyses including standard CVD risk factors have not always been applied to examine the independent or additive utility of carotid US findings. Those studies which have analyzed IMT and plaque separately show the greatest risk of future myocardial infarction to be conferred by the presence of focal plaque rather than increased IMT, in keeping with preliminary analyses examining the prognostic utility of carotid ultrasound findings from Phase III of SHS (see below). Of note, discrete carotid plaques, an unambiguous sign of atherosclerosis, can be present even when the IMT of adjacent carotid segments is entirely normal. When traditional CVD risk factors are considered, the association of baseline carotid artery findings with outcome is usually attenuated but remains significant, particularly in women. Although carotid artery atherosclerosis is a manifestation of cerebrovascular disease, the majority of events predicted are due to coronary heart disease, underscoring the systemic nature of atherosclerosis. Autopsy studies have shown reasonable correlation between the severity (not presence) of carotid and coronary atherosclerosis. Similarly clinical studies have related carotid atherosclerosis detected by ultrasonography to obstructive coronary artery disease diagnosed by contrast angiography or clinically-manifest coronary disease. However, in view of the limited ability of coronary angiography to detect significant non-obstructive mural atherosclerosis, the association between the presence of coronary and carotid atherosclerosis is certainly even stronger than that suggested by the existing literature.

Measurement Technique: Ultrasound measurement of carotid wall IMT has been validated using gross and histopathologic reference standards and been found to be highly reproducible. The IMT can be measured in the common carotid artery (CCA), the bifurcation (bulb) and either of the branch vessels (usually the internal carotid artery [ICA]). Because of its tubular shape, perpendicular location relative to the transducer beam and virtual universal accessibility, measurement yield and reproducibility of the CCA IMT are higher than the ICA or bulb IMT. In the Atherosclerosis Risk in Communities (ARIC) study involving carotid US examinations in 13,824 individuals, IMT measurements were obtainable from the CCA in 91.4%, from the bifurcation in 77.3%, and from the ICA in 48.6% of participants. A report from The Rotterdam Study (n=1881 in the analysis) showed a similar trend in measurement yield: 96% in the CCA, 64% in the bifurcation, and 31% in the ICA.

One consideration in choosing the segment(s) to measure might be differences in the extent to which IMT of a given vessel correlates with prevalent CVD and/or outcome. In the Cardiovascular Health Study (CHS), the combination of CCA and ICA IMTs resulted in
minimally higher adjusted relative risks for subsequent myocardial infarction or stroke than did CCA or ICA IMT alone (1.36 vs. 1.27 and 1.30, respectively, for 1 SD increase)(29). In CHS ICA IMT had marginally higher adjusted relative risk for prediction of incident myocardial infarction (1.34 vs. 1.24), whereas CCA IMT was slightly better at predicting stroke (1.28 vs. 1.25). Similarly, the British Regional Heart Study noted that CCA IMT was a stronger correlate of prevalent stroke than was bifurcation IMT; the latter was not associated with prevalent coronary disease when the presence of plaque (measured separately from IMT) was considered. In the Insulin Resistance Atherosclerosis Study (IRAS), the presence of diabetes and fasting glucose were associated with CCA IMT but not ICA IMT. On balance there does not appear to be compelling evidence to suggest that combined measurements or measurement of a specific segment is clearly superior. The higher yield and superior reproducibility of measurement of the CCA IMT as well as its better suitability for semi-automated measurement favor its use, particularly in protocols that do not incorporate plaque (usually seen in the bifurcation or ICA) in the IMT measurement. In such circumstances, the predictive value of focal atheroma in these areas is preserved (by the categorical presence of plaque) without sacrificing a decrease in measurement yield or accuracy (by attempting to measure bifurcation or ICA IMT).

The IMT may be measured from the near (closest to the transducer) wall and/or the far wall. Although measurement reproducibility of the near and far walls has been reported to be comparable, measurement yield of the near wall is lower and may be less accurate than that of the far wall due to technical considerations. Current technology does not permit reliable separate measurement of the intima and media, hence the standard is combined intimal-medial thickness which has been anatomically validated for the far wall. Excess gain or ‘blossoming’ of the highly echogenic near-wall adventitia into the echo-lucent media or of the echogenic near-wall intima into the echo-lucent lumen will result in systematic under- or over-measurement, respectively, if IMT of the near wall is measured. In contrast, incursion of echoes from the far-wall intima into the media will not influence overall intimal-medial thickness measured from the far wall. In light of the foregoing considerations, we have chosen to measure IMT from the far wall of the CCA in SHS.

IMT has most commonly been measured from B-mode images. Alternatively, B-mode guided M-mode images of the distal CCA may be obtained. Although spatial resolution is comparable with the two techniques, temporal resolution is far superior with M-mode imaging thereby facilitating standardization of measurements at the time of minimum diameter, when the diastolic distending pressure is known, and estimation of pulsatility or vascular function. Because of the desire to assess measures of vascular function in Phases III and IV of SHS, we relied on M-mode measurement of CCA IMT. In Phase V of SHS we propose to make CCA IMT measurements from B-mode images using a semi-automated system that enhances reproducibility. We are employing this system in an intervention study of American Indians with diabetes (Stop Atherosclerosis in Native Diabetics Study [SANDS]) and have found high correlation between M-mode and B-mode measurements of carotid artery structure (r=0.89, p<0.001) with an intercept near zero and a slope approximating 1.0 in the first 230 subjects studied to date.

Internal diameter of the vessel lumen (usually the CCA) can be measured at a single point in time from B-mode images or throughout the cardiac cycle from M-mode tracings.
Measurement of lumen diameter as well as IMT permits calculation of vascular cross-sectional area, a surrogate measure of vascular mass comparable to left ventricular mass. Due to cyclic variations in IMT and lumen diameter, measurements should be performed using ECG gating and/or determination of minimal (end-diastolic) and maximal (peak-systolic) diameters.

Non-obstructive plaque, which may be defined as the presence of focal thickening at least 50% greater than that of the surrounding vessel wall, is usually readily identifiable, with the best appreciation of its encroachment into the lumen detected from the transverse plane. The most common location of plaque is within the carotid bifurcation when flow becomes less laminar, followed by the ICA; plaque is much less common in the CCA due to its usually laminar flow profile. Since Doppler velocity does not usually increase until significant (>50%) luminal obstruction develops, non-obstructive plaque cannot be reliably quantified using Doppler techniques. Because of its complex three-dimensional nature, the size of a single plaque or overall plaque burden is difficult to quantify; thus the categorical presence of plaque is more reproducible than measurement of its thickness. Plaque diameter, i.e. maximum incursion into the vessel lumen, may be measured but may not accurately reflect overall plaque burden. A semi-quantitative approach relies on the presence or absence of non-obstructive or obstructive plaque or the number of segments of the extracranial carotid arteries containing plaque.

**Carotid Artery Ultrasound Scanning Protocol**

**Instrumentation:**
Ultrasonographs will be calibrated against a phantom at installation and at regular intervals thereafter; sonographers should verify that this is performed by Acuson as part of routine maintenance. The 7.0 MHz vascular probe will be set to default with processing curves optimal for imaging of the carotid artery with no persistence. The usual depth is 30 to 40 mm.

**Patient Preparation:**
Imaging is 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) and lateral rotation, as necessary. Electrodes are placed for a modified three-lead electrocardiogram. The SHS study number should be entered before beginning the imaging study. In addition, the arterial system being imaged (left vs. right) should be entered on the screen.

**Two-Dimensional Imaging and Doppler Study:**
Two-dimensional (B-mode) long-axis imaging from multiple planes (posterior, lateral, anterolateral) should be done to maximize detection of discrete plaque. Following identification of the carotid bulb, the transducer should be moved caudally to examine the common carotid artery (CCA) until its origin from the aortic arch (left) or innominate artery (right). Both branch vessels should be scanned in a cephalad direction until their disappearance. Scanning should additionally be performed in the transverse plane. Identifying features of the internal carotid artery (ICA) on the imaging study include its larger size and motion away from the transducer as it proceeds intra-cranially, whereas the external carotid artery (ECA) is usually smaller and has extracranial branches. Pulsed Doppler analysis should also be performed to identify distinguishing characteristics of the ICA and ECA: the low resistance ICA is characterized by
spectral broadening and persistence of flow during diastole whereas the high resistance ECA has a rapid deceleration to the baseline with minimal diastolic flow.

Extensive imaging of the bulb and proximal bifurcation should be performed given the high predilection for plaque in these regions. A cross-sectional image identifying the maximum incursion of the plaque into the lumen should be obtained. Addition of color flow to the cross-sectional image may aid in distinguishing plaque from lumen and in wall detection. Pulsed Doppler analysis (with angle correction of 60 degrees) should be performed to quantify the degree of stenosis by obtaining the peak velocity at the level of the obstruction (1.5 to 2.5 m/sec = 50-74% obstruction, >2.5 m/sec ≥75% obstruction).

B-mode imaging of the distal CCA should be performed with the vessel positioned as perpendicular as possible to the transducer beam. When there is optimal definition of near and far wall IMTs of the distal 2 cm of the CCA, the freeze-frame button should be pressed, the image should be scrolled to an end-diastolic frame (largest diameter; approximately at the end of the QRS complex), and five seconds of the freeze-frame should be recorded.

The complete protocol is videotaped and the procedure is repeated on the contralateral artery.

Clinical Alerts and Referral Criteria

The presence of significant obstruction (>50%) constitutes a clinical alert. Such studies will be identified at the Reading Center and processed within 48 hours of receipt. Results of such studies will be reported by telephone to the Field Center. The presence of ≥75% obstruction should result in immediate referral whereas obstruction of 50-74% should result in routine referral. The detection of non-obstructive plaque (<50%) should provoke assessment of risk factors for atherosclerosis and discussion between the physician and the SHS subject regarding their reduction at the next routine visit.
SONOGRAPHER WORKSHEET

SHS V STUDY
Weill Medical College of Cornell University
New York Presbyterian Hospital
525 East 68th Street
New York, New York 10021
212-746-4654

Participant ID#: ______________________
Location: __________________________
Date of exam: ________________________
Sonographer: _______________________

<table>
<thead>
<tr>
<th>Plaque Location</th>
<th>Plaque +/-</th>
<th>Peak Velocity (m/sec) (if obstruction is present)</th>
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<tbody>
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<td>R ECA, far</td>
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Sonographer signature  Date
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<th>Date</th>
<th>Sonographer</th>
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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 B-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.

Measurement Techniques: Plaque will be graded as present or absent. A plaque score (0 to 8) will be generated based on the number of extracranial segments containing focal plaque. Maximum velocity at the level of a plaque causing significant stenosis will be recorded. End-diastolic (minimum dimension) lumen diameter and intimal-medial thickness of the far wall of the distal common carotid artery will be measured from digitized images using semi-automated edge-detection software. The software averages 100 separate measurements taken over a 1cm segment of the distal common carotid artery.

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), plaque severity (non-obstructive, significant stenosis, severe stenosis), plaque score (0-8), right and left end-diastolic diameters, and intimal-medial thicknesses.

SHS PHASE 5 POPLITEAL ARTERY ULTRASOUND STUDY

Popliteal Artery Structure and Atherosclerosis. To provide direct measures of peripheral arterial disease, a highly prevalent condition in the SHS population, the geometry and presence of atherosclerotic plaque in the popliteal arteries (PA) will be assessed by ultrasound. B-mode scanning of the right and left PAs will be performed using Acuson Sequoia systems with a 5-7 MHz linear array arterial imaging transducer in multiple projections to optimize detection of discrete atheromata, identified on 2-D images as the presence of discrete plaque >50% thicker than the surrounding wall within any segment of either PA. In the SHS cohort, discrete atherosclerosis identified in this way in the carotid circulation predicted subsequent myocardial infarction, (relative risk=6.3), definite coronary heart disease events (relative risk=2.3) and stroke (relative risk=2.7). Maximum plaque diameter is quantified by computer-assisted measurement of plaque thickness on 2-D frames as described for carotid evaluation. Color Doppler is used to identify abnormalities such as aliasing and areas of turbulence indicative of obstructive disease. The severity of stenosis is further quantified using standard Doppler techniques. Stenosis severity criteria are determined by spectral broadening, flow reversal, and
peak systolic velocity ratios identified on pulse Doppler analysis. Vessels are classified as normal or having 0-19% stenosis, 20-49% stenosis, 50-99% stenosis or occlusion (Table 1). Focal areas of doubling of the measured peak systolic velocity have been shown to correspond to hemodynamically significant lesions of greater than 50% narrowing of arterial lumen diameter.

<table>
<thead>
<tr>
<th>Stenosis Category</th>
<th>Velocity</th>
<th>Waveform</th>
<th>Spectral Broadening</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-19%</td>
<td>Normal</td>
<td>Triphasic</td>
<td>--</td>
</tr>
<tr>
<td>20-49%</td>
<td>&lt;double the proximal segment plaque present</td>
<td>Triphasic</td>
<td>+</td>
</tr>
<tr>
<td>50-99%</td>
<td>&gt;double the proximal segment, and &gt;200 cm/sec beyond stenosis</td>
<td>Loss of reverse component</td>
<td>+</td>
</tr>
<tr>
<td>Occlusion</td>
<td>No flow</td>
<td>Monophasic pre-occlusive thump</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 1. Categorization of Popliteal Artery Stenosis Severity

Two-dimensionally-guided M-mode tracings of both the right and left popliteal artery are obtained to measure popliteal 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 (Imaging Technology, Inc., Woburn, MA) interfaced with a high-resolution (480 x 640 pixel field) video monitor and stored on diskettes. Following calibration for depth and time, the end-diastolic wall thickness (combined intimal-medial thickness of the far wall) and end-diastolic and peak-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 wall thickness is never made at the level of a plaque. In addition, B-mode measurements of the popliteal artery (end-diastolic near and far wall IMTs and diameter) will be made using the Artery Measurement System (AMS II, v.1.111).

Popliteal Artery Ultrasound Scanning Protocol

Instrumentation:

Ultrasoundographs will be calibrated against a phantom at installation and at regular intervals thereafter; sonographers should verify that this is performed by Acuson as part of routine maintenance. Popliteal artery imaging will be performed with Acuson Sequioa systems with 5-7 MHz linear array arterial imaging transducers. The 7.0 MHz vascular probe will be set to default with processing curves and a persistence setting optimal for imaging the popliteal artery.

Patient Preparation:

Imaging is performed in a slightly darkened room with the subject in a prone position with the knee slightly flexed, which will allow for a more direct, easier approach to popliteal artery imaging. Alternatively, if the prone position is difficult for the patient, the patient may remain in the supine position with the leg bent slightly and positioned out to the side away from
the patient, or the patient may be placed in a lateral decubitus position with the knee slightly flexed. Electrodes are placed for a modified three-lead electrocardiogram. The SHS study number should be entered before beginning the imaging study. In addition, the arterial system being imaged (left vs. right) should be entered on the screen.

Two-Dimensional Imaging and Doppler Study:

The ultrasound examination is begun by 2-D (B-mode) imaging in the transverse plane to establish the vascular anatomy. The transducer is placed posteriorly at the site of the popliteal fossa at the level of the knee joint. The PA is located posterior and slightly lateral to the popliteal vein. At the level of the knee joint, the PA sends off small geniculate branches. The PA is followed 6 to 8 cm superiorly into the thigh as it moves more medially within the adductor canal (above knee segment). The PA is then followed from the fossa inferiorly, approximately 6 to 8 cm from the knee joint to the superior aspect of the calf (below knee segment). Longitudinal imaging is then done along the course of the PA beginning proximally, in order to directly evaluate the vessel wall anatomy and the vessel lumen for obstructive disease. The segment being imaged (popliteal fossa, above knee segment and below knee segment) should be entered on the screen.

If plaque is present, the cine function should be activated to allow frame-by-frame scrolling to obtain the maximum plaque diameter. The transducer should then be rotated to obtain a cross-sectional image identifying the maximum incursion of the plaque into the lumen. Color Doppler is used to identify abnormalities, such as aliasing and turbulence, indicative of obstructive disease. Pulsed Doppler analysis, at an insonation angle of $\leq 60^\circ$, is performed to quantify the degree of stenosis. Maximum velocity distal to a plaque causing significant stenosis is recorded. A point that is free of plaque, 2 to 4 cm proximal to sites of abnormal velocity is sampled to obtain a velocity ratio. The presence of collateral branches is noted whenever possible. Plaque location, peak systolic velocities and velocity ratios should be entered on the Sonographer Worksheet (see worksheet below and Table 1 above).

Evaluation for aneurysm of the popliteal artery (dilatation $>1.5$ times the diameter of the adjacent normal vessel) will require measurement of transverse and sagittal diameters of the above knee and below knee segments. The localization of lesions will be recorded in centimeters below and above the cranial edge of the patella, which will serve as a reference point in all patients.

Popliteal artery IMT will be evaluated at the site of the popliteal fossa, using the patella as a landmark. B-mode imaging of the popliteal artery should be performed with the vessel positioned as perpendicular as possible to the to the transducer beam. The 2-D imaging plane is oriented perpendicular to the intima-lumen interfaces of the near and far PA walls (in an area free of discrete plaque). Gain settings are optimized to limit “blossoming” of brighter interfaces. When there is optimal definition of near and far wall IMTs of the PA, the freeze-frame button should be pressed, the image should be scrolled to an end-diastolic frame (largest diameter; approximately at the end of the QRS complex), and five seconds of the freeze-frame should be recorded.

The complete protocol is videotaped, and the procedure is repeated on the contralateral
artery.

Clinical Alerts and Referral Criteria

The presence of a >50% obstruction or an occlusion of the popliteal artery or the presence of a popliteal artery aneurysm ≥ 2.0 cm in diameter constitute a clinical alert in the presence of the following signs/symptoms:

- Foot REST pain
- Limb weakness / paralysis
- Limb coolness
- Foot/toe discoloration
- Foot/toe ulcers
- An ABI < 0.4

The incidental finding of a deep venous thrombosis (DVT) of the popliteal vein constitutes a clinical alert. Such studies will be identified at the Reading Center and processed within 48 hours of receipt. Results of such studies will be reported by telephone to the Field Center. Such patients should be referred for immediate evaluation.

The presence of >50% obstruction or occlusion of the popliteal artery without signs or symptoms of critical limb ischemia as described above should result in routine referral (within one month). The detection of non-obstructive plaque (<50%) should provoke assessment of risk factors for atherosclerosis and discussion between the physician and the SHS subject regarding their reduction at the next routine visit.
SONOGRAPHER WORKSHEET

SHS V STUDY
Weill Medical College of Cornell University
New York Presbyterian Hospital
525 East 68th Street
New York, New York 10021
212-746-4654

Participant ID#: ______________________
Location: __________________________
Date of exam: ______________________
Sonographer: ______________________

<table>
<thead>
<tr>
<th>Plaque Location</th>
<th>Plaque +/-</th>
<th>Peak Velocity (m/sec) (if obstruction is present)</th>
<th>Peak Velocity (m/sec) proximal to obstruction</th>
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<td>L Below knee segment, far</td>
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<td>_______________________________________________</td>
<td></td>
</tr>
<tr>
<td>R Popliteal fossa, near</td>
<td>_____</td>
<td>_______________________________________________</td>
<td></td>
</tr>
<tr>
<td>R Popliteal fossa, far</td>
<td>_____</td>
<td>_______________________________________________</td>
<td></td>
</tr>
<tr>
<td>R Above knee segment, near</td>
<td>_____</td>
<td>_______________________________________________</td>
<td></td>
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<tr>
<td>R Above knee segment, far</td>
<td>_____</td>
<td>_______________________________________________</td>
<td></td>
</tr>
<tr>
<td>R Below knee segment, near</td>
<td>_____</td>
<td>_______________________________________________</td>
<td></td>
</tr>
<tr>
<td>R Below knee segment, far</td>
<td>_____</td>
<td>_______________________________________________</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Popliteal aneurysm +/-</th>
<th>Diameter (cm)</th>
<th>Location of popliteal aneurysm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sonographer signature: ______________________
Date: ______________________

Strong Heart Study V  07/01/2006
V-12  Carotid & Popliteal US & Echo
Ultrasound machine settings for imaging of the popliteal artery:

Set the **frequency** of the probe to the highest that it will allow (**6 MHz**).

Adjust the following (tabs are located at upper right hand corner of control panel; the adjustments made are indicated on the screen on the upper right hand corner, usually below the tape counter):

- **Space time** = set to S2
- **Edge** = set to +2
- **Delta** = set to 4
- **Persistence** = use 1
- **Color filter** = set to lowest filter (1)
- **Color scale** (seen on upper left hand corner when color Doppler is turned on) = set to 0.20 m/s

Once these settings are adjusted on the machine, these settings can be stored as a **popliteal artery preset**, which you can then use for imaging of the popliteal artery (instead of the carotid preset):

- Click Data setup on the keyboard
- Pick presets
- On the lower left, type in “popliteal”
- Then hit the STORE button
Popliteal Artery Ultrasound Scanning Protocol

B-mode/transverse

Mid segment (popliteal fossa)
- Above knee segment (6-8 cm above top edge of patella)
- Below knee segment (6-8 cm below bottom edge of patella; popliteal artery dips down with the patella seen to left of the artery)

Record each segment for 5-7 seconds, freeze & record each segment as well
* Measure dilated segments (trailing to leading edge)
* Image maximum plaque incursion, freeze & record

B-mode/sagittal

Mid
- Above knee
- Below knee

Record each segment for 5-7 seconds, freeze & record
* Image maximum plaque incursion, freeze & record

Color/sagittal

Mid
- Above knee
- Below knee

Record each segment for 5-7 seconds, no measurements

PW Doppler

Above knee
- Mid
- Below knee

Obtain at least 1 spectral Doppler waveform, measure PSV, EDV (insonation angle of ≤60°)
* If plaque present or turbulence by color Doppler, walk sample volume ON VIDEO through the area of stenosis to obtain peak velocity; freeze, measure PSV, EDV; Obtain a spectral Doppler waveform at a point that is free of plaque, 2 to 4 cm proximal to sites of abnormal velocity, measure PSV, EDV

IMT

Mid segment; B-mode

Record 3 sweeps at 50 speed; freeze, measure 3 beats of the far wall @ end of QRS (leading edge of intima to leading edge of adventitia)

Important points:

- Obtain flow velocity profiles for all lesions
- Align cursor to vessel wall
  - Angle ≤ 60 degrees
- Electronic beam-steering
- Transducer heel-toe maneuvers

- During PW Doppler, always show good color image
  - Freeze Doppler spectrum with the right kidney key (color image will be in real-time update mode)
  - Freeze color image with the wheel
  - Use manual cine to scroll back to good color image
  - THIS IS KEY for interpreting physician

- Indicator orientation: toward subject’s RIGHT or HEAD

- Labels:
  - AK = above knee popliteal
  - BK = below knee popliteal
  - PF = mid popliteal (popliteal fossa)
  - Post = posterior approach
  - Med = medial approach

- Above knee popliteal artery will include 6 cm proximal to the top of patella

- Medial approaches can be used as alternative window

- Phased-array transducer helpful for Doppler of popliteal artery bifurcation & distal SFA

- Criteria for aneurysm = dilatation >1.5 times the diameter of the adjacent normal vessel

- Aneurysm & lesion locations noted in distance (cm) relative to cranial edge of patella

- Plaque characteristics may be observed:
  - Surface
  - Morphology
  - Density

### Criteria for Popliteal Artery Stenosis Severity

<table>
<thead>
<tr>
<th>Stenosis Category</th>
<th>Velocity</th>
<th>Waveform</th>
<th>Spectral Broadening</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-19%</td>
<td>Normal</td>
<td>Triphasic</td>
<td>--</td>
</tr>
<tr>
<td>20-49%</td>
<td>&lt;double the proximal segment plaque present</td>
<td>Triphasic Monophasic</td>
<td>+</td>
</tr>
<tr>
<td>50-99%</td>
<td>&gt;double the proximal segment, and &gt;200 cm/sec beyond stenosis</td>
<td>Loss of reverse component</td>
<td>+</td>
</tr>
<tr>
<td>Occlusion</td>
<td>No flow</td>
<td>Monophasic pre-occlusive thump</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Echocardiographic evaluation of LV geometry, LV systolic and diastolic function and valvular heart disease is an increasingly useful tool in epidemiologic research as the yield of technically satisfactory echocardiograms in middle-aged to elderly adults has risen from 70% in the initial Framingham experience to 96-98% in the 4th SHS examination and other multi-center studies coordinated by the Cornell Reading Center. A central contribution of echocardiographic studies, mostly performed in white populations, is the demonstration that high LV mass predicts CV morbidity and mortality more strongly than conventional risk factors other than advancing age. In the SHS cohort we have demonstrated that LV geometry on Phase II echocardiograms strongly predicts CV mortality and CV events, independent of LV ejection fraction, standard risk factors or measures of renal dysfunction. In one of the first analyses of the prognostic implications of abnormal LV diastolic filling in a population-based sample, we showed that an elevated ratio of early diastolic/atrial phase LV filling (E/A ratio>1.5) predicted CV death more strongly (p=.0003) than age (p<.01), DM (p=.001), or systolic BP (p=.04) and that reduced E/A ratio (<0.8) also predicted CV death. High prevalences of echocardiographic abnormalities in SHS may contribute to high CV death rate in American Indians.

Despite progress made by the SHS and other studies in elucidating stimuli to increases in LV mass, only 50% of the variability of LV mass can be predicted by clinical (age, gender, height, BMI and DM) and hemodynamic (BP, stroke volume and myocardial contractility) variables. An important addition to this knowledge has been demonstration in the SHS as well as the HyperGEN and Framingham studies of significant phenotypic heritability of LV mass independently of recognized stimuli to LV growth. In a parallel NHLBI-funded epidemiologic study, the Hypertension Genetic Epidemiology Network (HyperGEN), echocardiographic measurements of LV geometry made at the Cornell Reading Center showed substantial phenotypic heritability (h²) in hypertensive sib-pairs: there was significant inheritance of both LV mass and relative wall thickness in both African-Americans and in whites, with stronger inheritance of LV mass in African-American than white participants (h²=.70 vs. 26) and of LV relative wall thickness in whites than African-Americans (h²=.45 vs. 18, both comparisons, P<.01). These findings, combined with the results from relative-pair analyses in SHS, document substantial heritability of LV mass, independent of age, gender, body habitus and BP, indicating that heritability is sufficient to have adequate power in the proposed research design to detect linkage with genes contributing to regulation of LV mass, geometry and function. Evidence has also been obtained that arterial structure and function influences LV geometry independent of other known factors and that arterial size shows even stronger heritability in the SHS population than LV mass. The performance of echocardiograms on 3,637 members of 95 large multi-generational families in SHS Phase IV at the same time as arterial imaging and pressure waveform recordings provides a unique opportunity to clarify pathophysiologic inter-relations between the heart and arterial tree, to establish the role of heredity in determining CV structure and function, and to find genes responsible for this heritability.

An additional area in which knowledge is evolving rapidly is the way in which abnormalities of LV structure and function participate in causal pathways leading to morbidity and mortal CV events. A line of evidence developed in studies at Cornell has shown associations between, on the one hand, arterial wall thickness, discrete plaque, measures of arterial stiffness...
and the augmentation index (a measure of the increase that occurs in the central [aortic and carotid] systolic arterial pressure due to early return of reflected pressure waves from the peripheral arteries) and, on the other, LV wall thickness, mass and midwall systolic function. However, these studies have been limited by their cross-sectional nature and lack of full characterization of the levels over time of potentially important covariates (e.g., lipid and lipoprotein profile, body composition beyond calculation of BMI, insulin levels, etc). The proposed study will present an ideal opportunity to examine the temporal evolution of these arterial-cardiac parallelisms and to relate progression of cardiac and arterial abnormalities to DM, obesity and other risk factors that are becoming increasingly prevalent in most populations.

One particularly promising approach to assessing myocardial systolic and diastolic function directly is use of tissue Doppler imaging (TDI) to measure the velocities of LV shortening during systole and lengthening during different phases of diastole. Spectral pulsed TDI can measure myocardial systolic shortening and diastolic lengthening velocities, which provide more direct measures of heart muscle systolic and diastolic function than previously available. Measurement of the systolic myocardial velocities at various sites of the LV provides information on global systolic function as well as regional myocardial contractile dysfunction. Of note, systolic myocardial velocity has been shown to be subnormal in hypertensive patients, paralleling the reduction of calculated midwall shortening seen in hypertensive patients with normal systolic function by conventional parameters. TDI is particularly useful in identifying abnormal LV diastolic relaxation. Because it is less affected by changes in preload, mitral anular velocity assessed by TDI is able to identify pseudonormal LV filling (abnormal LV relaxation with high filling pressures) and thereby is more sensitive for identifying impaired LV relaxation than the mitral inflow velocity flow pattern measured by conventional pulsed Doppler. Of note, the latter less sensitive method has already been shown to predict prognosis in Strong Heart Study participants. We have shown that diabetes is independently associated with abnormal LV relaxation. Thus, accurate and comprehensive assessment of diastolic function is particularly important in the Strong Heart Study.

A new method that we have developed is based on the early demonstration by Sarnoff and colleagues that myocardial oxygen consumption per heart beat per gram of myocardium is closely determined by the tension-time index (the integral of LV wall stress throughout systole) and evidence that myocardial oxygen consumption closely tracks with echocardiographic measurements of LV wall stress. We use standard echo-Doppler measures of LV mass, end-systolic stress, ejection time and heart rate to derive an estimate of myocardial work energy expenditure (MEE) in calories per minute. Compared to findings in a reference group of apparently normal SHS cohort members, MEE was appreciably higher in participants in the 2nd SHS exam with DM or hypertension, nearly 300% higher in individuals with LV ejection fractions <40% and over 150% higher in those who suffered subsequent CV death.

**Measurement Technique:** Standardized examinations will include 2-D guided M-mode echocardiograms and selected 2-D and Doppler recordings. Recordings to measure tissue Doppler parameters of LV systolic and diastolic function (peak systolic contraction velocity and the peak velocity of early diastolic and atrial phase myocardial lengthening of the LV myocardium at the interventricular septal- and lateral LV wall-mitral anular junctions (E’ and A’ velocities) will be added to those obtained in SHS Phase IV, using presets for this purpose that
were already added to the Siemens Sequoia echocardiographs during the SHS Phase IV examination. Studies will be sent to the Reading Center for blinded interpretation by experienced technician and physician readers. Study performance and interpretation will focus on measures of LV mass and geometry, global and regional systolic function, and diastolic filling to maximize the yield of reliable data to answer the specific study questions.

Because of the geographical dispersion of the Field Centers, multiple steps are used in the SHS to maximize quality control of echocardiogram performance. These include preliminary measurement of LV dimensions and other variables by examining technicians, which increases awareness of aspects of image orientation and definition needed for a measurable study. A copy of videotaped 2-D and M-mode views on study subjects is made by the Field Centers for the sonographer to review with final measurements, comments and suggestions from the Reading Center about how to enhance technical quality, for continuing education of performing sonographers. Site visits to Field Centers by Reading Center staff will be made as necessary. The Reading Center uses procedures adapted from those developed and refined in the Cornell laboratory over 20 years and used in SHS Phases II and IV. Steps to assure data quality include blinded performance of measurements, checking of initial measurements against the visual appearance of the echocardiograms, verification of technician-reader measurements by experienced investigators, and repeat verification of measurements that fall outside expected ranges for a normal to mildly diseased population or reveal unexpected relations among variables. Computer support and assistance with data management and statistical analyses is provided by the Computer Center of the General Clinical Research Center. Echocardiogram performance and measurement protocol is described in more detail in the Appendix.

PROCEDURE FOR ECHOCARDIOGRAM PERFORMANCE:

For performance of the echocardiograms, examining tables with cut-outs designed to facilitate performance of standardized, quantifiable echocardiograms are used. At the Reading Center quantitative and qualitative assessment of echocardiograms is performed using Digisonics Review Centers.

Initial Training/Start-Up: Once selected, technicians undergo phased training before the performance period. Formal training consists of a course in New York that combines didactic teaching of selected general aspects of echocardiography and the specific study protocol, and hands-on training in performing echocardiograms by the study protocol. The training course at the Reading Center can be combined with that for carotid ultrasound for sonographers who are already familiar with procedures for quantitative evaluation of the heart, with discretion to adjust the amount of training depending on the individual trainee’s skill level.

Sonographer Measurements: It is the Reading Center’s experience 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 given feedback as to how to improve the suitability of the study for quantitation and the selection of interfaces to measure.
Echocardiography Performance at Field Centers: 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 beam 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 under 2-D guidance. Rotation of the 2-D sector 90 degrees to the short axis projection allows one to measure the true, maximum LV diameter. If, as is common in older subjects, the best parasternal window is in a low interspace, a higher interspace should be used, which may image only a narrow sector that includes the LV minor axis. 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.

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, as seen when the transducer is rotated to the 2-chamber view and the LV apex is out of the field of view. 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. The accuracy of Doppler recordings depends on the ultrasound beam being parallel to the axis of blood flow. The apical 2- and 4-chamber views are used to sample LV inflow across the mitral anulus and valve orifice while the apical long-axis view, or the 5-chamber view, are best to measure systolic flow across the aortic anulus to calculate stroke volume and cardiac output. Color Doppler recordings in parasternal long-axis and apical 4-chamber views are used to identify significant valvular regurgitation.

Protocol for Echocardiogram Performance: Echocardiograms are performed in the same van or fixed facility as the carotid ultrasounds, described previously. Subjects change their top for a light gown to permit discrete exposure of the chest overlying parasternal and apical acoustic windows. One ECG lead is continuously monitored for timing purposes. The subject assumes 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 subject’s SHS study number and the date and site of recording are entered on the videotape. Careful performance of this protocol will require 30 minutes of subjects' time.

Specific recordings to be made: Parasternal long-Axis 2-D recordings will be obtained first, with the interspace and degree of left decubitus positioning chosen to allow an M-mode cursor line to traverse the interventricular septum (IVS) and LV posterior wall (PW) perpendicularly.

A. 2-D echocardiography during quiet respiration: Maximize LV and aortic diameter and record ≥10 beats on tape.

B. M-mode cursor perpendicular through LV at the LV minor axis; Record 10 beats of 2-D update image with M-mode recording, then record at least 10 beats of M-mode at 50 mm/sec during quiet respiration and attempt at least 5 beats at held expiration; the imaging plane is tilted medially and laterally to maximize the LV cavity area in the long-axis view.
C. Turn 90° into parasternal short-axis view to visualize the LV short-axis at papillary muscle tips.
D. 2-D echocardiography at the papillary muscle tips during quiet respiration: 15 beats on tape.
E. M-mode cursor through the LV meridian at papillary muscle tips: 10 beats of full-screen 2-D and 10 beats of M-mode with 2-D update during quiet respiration. Then attempt 5 beats at held expiration.
F. M-Mode Sweep from LV through mitral valve to left atrium/aortic view recorded on videotape.
G. Aortic/Left Atrial Imaging: 2-D echocardiography in long-axis views during quiet respiration aorta/left atrial level with maximization of aortic diameter at the sinuses of Valsalva: 10 beats. Color Doppler to search for aortic and mitral regurgitation in long-axis view.
H. M-mode cursor perpendicular through aorta and left atrium with maximization of aortic diameter by "tilting" medially and laterally of 2-D imaging plane: 10 beats of 2-D update image with M-mode recording.
I. At completion of these recordings the transducer will be shifted to the apical window, identified by palpating the LV impulse on the chest wall and then moving the transducer inferolaterally until the LV apex is visualized in 2- and 4-chamber views, with repositioning of the participant if needed. Pulsed Doppler transmitral flow recording with sample volume at the middle of the mitral anular plane during diastole: Using a 2.5 mega Hz transducer, record 10 beats of 2-D update image with Doppler recording, then record at least 10 beats of full-screen videotaped Doppler at 100 mm/sec during held expiration if possible. Move sample volume to leaflet tips (highest E wave velocity) and repeat recording. Locate a pulsed Doppler sample volume or continuous wave Doppler beam orientation that depicts simultaneously clear envelopes of systolic ejection of blood and diastolic transmitral flow to visualize the isovolumic relaxation period, increase sweep speed to 100 mm/sec and record ≥ 10 beats of full-screen Doppler during held expiration. Preset TDI controls are present on the Sequoia ultrasonographic systems that allow the system to filter out the high-velocity signals of blood flow within the cardiac chambers and display only the low-velocity and high amplitude signals representing wall motion velocities. Tissue Doppler acquisition is performed with placement of sample volumes in the basal interventricular septum and lateral LV wall, which takes no longer than 2 minutes to complete. 4- and 5-chamber view color Doppler recordings will then be made to search for aortic and mitral regurgitation.
J. Turn approximately 90° into apical 2-chamber view. 2-D echocardiography in the true apical 2-chamber view during quiet respiration or held expiration: Record 15 beats with maximum LV chamber dimension and good endocardial definition.
K. The transducer is 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 10 cycles of pulsed Doppler blood flow at the aortic annulus (hinging points of aortic cusps) is recorded during quiet respiration or held expiration.

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 and popliteal ultrasound and echocardiography) 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 echo performance worksheet, add the subject's SHS number, and date of performance to a label attached to the videocassette, and prepare the performance area for the next subject. Field Center technicians will continue the procedure begun during the training period of making preliminary measurements on each study of LV dimensions, recording the qualitative normality or abnormality of LV systolic function from 2-D recordings, and noting any clinical abnormalities. The worksheets with preliminary readings 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 previous studies returned from the Reading Center with teaching comments.
ECHOCARDIOGRAM MEASUREMENT AT READING CENTER:

Central Echocardiogram Reading: Measurement and qualitative interpretation of echocardiograms will be performed primarily by technician-readers with extensive over-reading and supervision by physician-investigators. Upon receipt of studies from Field Centers, they will be logged into a hard-copy book and into a computer that already contains participant SHS number, and other demographic information needed to assure unambiguous subject identification. Videotapes will be assigned to technician and physician readers who will enter SHS number to assure a match into the Revue Center.

Videocassettes are placed in the VCR and advanced to the start of each study (identified by code number). Parasternal long- and short-axis 2-D views will be reviewed to ascertain correct M-mode beam angulation, and scored for semiquantitative wall motion of visualized wall segments. If the M-mode beam is correctly angulated, the technician-reader will choose the visually-best LV cycles (up to 6) and identify the QRS onset for each cycle on the simultaneous ECG to time end-diastolic measurements of interventricular septal and posterior wall thickness and LV internal dimension by the ASE convention (at QRS onset) and at the nadir of posterior septal motion for ASE end-systolic measurements. If the M-mode beam is not correctly oriented, 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 technique at the level of the papillary muscle tips along an axis perpendicular to the LV walls. This procedure contributed to the substantial increase in the proportion of subjects with measurable LVs to about 90% in the second SHS examination and to >95% in the subsequent HyperGEN echocardiography study, compared to about 70% in the Framingham and Cardiovascular Health Studies. LV mass values by this technique with the ASE correction 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 (trailing to leading edge), a measurement needed to calculate stroke volume.

The videotape will then be advanced to the apical 4- and 2-chamber views to allow detection of segmental areas of akinesia or dyskinesia that would suggest the presence of myocardial infarction and undermine the validity of linear measurements for evaluation of LV geometry and systolic function. LV systolic function in 14 segments will be made on a scale ranging from normal to dyskinetic. The videotape will be advanced to the 4-chamber view recording Doppler flow across the mitral anulus and at the level of the leaflet tips (highest E-wave velocity). 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 integral on the three cycles illustrating the highest velocity. 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. A pulsed sample volume location or continuous wave Doppler beam orientation that
encompasses both LV inflow and outflow signals will be identified to measure the isovolumic relaxation time. For each set of measurements the depth (or Doppler velocity) and time calibrations will be repeated. As an immediate quality-control step, the measurements obtained by the CardioRevue Center will be compared to the videotaped images with the use of calipers. The preliminary reports prepared by the Field Center technicians will be reviewed and comments made confirming the quality of recordings and measurements or indicating needed corrections and how to accomplish them that will be returned to the Field Centers. After the technician-reader has checked the computer print-out of primary and derived measurements for appropriate correspondence to the primary recordings, blinded studies will be arranged in batches for over-reading of all studies and verification of all measurements by a physician investigator.

After measurements are investigator-verified and transmitted to the computer center, they will be merged electronically with demographic data to facilitate checking of echocardiographic measurements against ranges of expected values for body size and gender. A random sample of studies will be selected for duplicate readings and ones with measurements or inter-reader differences that fall outside a priori limits for further verification as well as other quality control procedures, as well as a sample of "in range" studies will be reviewed by the physician-readers. Procedures for computerized data tracking and management will utilize standard data bases and ASCII files with limited custom programming done in house in coordination with the Coordinating Center. M-mode or analogous linear 2-D measurements at end-diastole by the American Society of Echocardiography recommendations are used to calculate LV mass by the anatomically validated formula:

\[
\text{LV Mass} = 0.8 \times (1.04 [(\text{IVS} + \text{LVID} + \text{PWT})^3 - \text{LVID}^3]) + 0.6 \text{ g}
\]

Estimates of LV mass by this method were closely related to actual LV weight at necropsy \((r=0.90, p<0.001)\) in a study of 52 adults.

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. 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 and adds to LV mass for prediction of complications of hypertension. RWT is calculated from M-mode measurements as \(2\text{PWT}/\text{LVID}\); increased LV mass is classified as concentric hypertrophy if RWT is \(\geq 0.43\) and eccentric hypertrophy when RWT is normal. If LV RWT is increased but LV mass is normal, the subject is considered to have "concentric LV remodeling", an LV geometric pattern newly described from the Cornell Laboratory.

Normalization of LV mass for body size will be done using body height to the appropriate power of its allometric (or growth) relation with LV mass (height\(^{2.7}\)), which the Cornell laboratory has shown to optimize both the sensitivity of echocardiography for detection of LV hypertrophy in hypertensive patients and the capacity to predict CV complications, and alternatively using FFM calculated from bioelectric impedance as well the traditional normalization for body surface area.

**Systolic Function and Mechanics:** 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):

\[
\text{Fractional Shortening (\%)} = \frac{(\text{LVID}_{d} - \text{LVID}_{s})}{\text{LVID}_{d}} \times 100
\]

If LV wall motion is uniform, fractional shortening is closely correlated with global LV ejection fraction, and is a simple substitute for it. LV ejection fraction will be assessed by a 2-D method that we have previously shown to be even more strongly predictive of cardiovascular and all-cause death in SHS participants than LV fractional shortening. Because of the recognition that endocardial shortening may be normal while myocardial function is depressed in LVs with thick walls (as occur commonly in hypertension), LV midwall shortening will also be calculated using a method that the Cornell laboratory has shown to enhance both the detection of abnormal LV systolic function in hypertensive patients and the capacity to predict an adverse prognosis in the SHS population.

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 end-systolic stress (ESS), which can be calculated from end-systolic LV measurements and cuff BP, determined with the subject on the examining table at the end of the echocardiogram. A catheterization-validated formula will be used to calculate LV circumferential end-systolic stress. Inverse relations exist between endocardial and midwall LV fractional shortening and ESS in both normal and hypertensive subjects which becomes most linear when ESS is plotted on a logarithmic scale. The fractional shortening expected for a given level of ESS may be predicted from findings in normal subjects studied in our laboratory. Calculation of the observed/predicted fractional shortening ratio provides stress-corrected fractional shortening as an afterload-adjusted measure of LV chamber performance. Stress-corrected midwall fractional shortening has already been shown to be an extremely strong predictor of CV death in very short-term follow-up of the SHS cohort. Peak systolic shortening velocity of the basal interventricular septum and lateral LV wall will be assessed by tissue Doppler imaging.

**Evaluation of LV Pump Performance and Systemic Hemodynamics:** The systolic pump performance of the heart will be primarily assessed by Doppler measurement of stroke volume and cardiac output, with secondary measurements of potential interest provided by the measurement package of peak ejection rate and mean acceleration of trans-aortic blood flow. When it is appropriate in analyses to take body size into account, these measures of pump performance will be normalized for body surface area, body height to its appropriate allometric power and fat-free body mass to either eliminate from consideration or take into account the influence of overweight on cardiac pump performance. Total peripheral resistance and the ratio of pulse pressure/stroke volume will be calculated as indices of the function of the peripheral arterial tree.

**Evaluation of LV Diastolic Filling:** The "E" and "A" velocities and integrals of transmitral blood flow will be used as measures of LV filling a) during ventricular relaxation and shortly thereafter and b) in response to atrial contraction. The isovolumic relaxation time, calculated as the interval between the end of systolic forward flow in the LV outflow derived and the onset of transmitral flow from the Doppler recordings described above will be used as an index of the time constant of early diastolic LV relaxation. The atrial contribution to filling
calculated from the ratio of the “A” wave integral to the total integral of diastolic flow across the mitral anulus will be used a measure of the dependence of LV filling on atrial contraction, and atrial systolic force will be calculated as described by Chinali et al as a measure of the active atrial contribution to LV filling. Diastolic LV myocardial function will be assessed by tissue Doppler measurement of E’ and A’ velocities of the basal interventricular septum and lateral LV wall, with E’ velocities <8cm/sec taken as evidence of impaired early diastolic relaxation and an E/E’ ratio >15 as evidence of elevated LV filling pressure.

**Ultrasonographer Training and Quality Control:**

Sonographer training, reading and quality-control procedures will be similar to those successfully employed in SHS Phases II through IV with addition of training in PA imaging. One week of intensive training will be provided at the Cornell Reading Center. Sonographers will observe the technique for echocardiography, carotid and popliteal ultrasound study as performed by a highly-experienced research sonographer. Then, sonographers will be observed and critiqued in their performance of arterial imaging. 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.