1.5 RATIONALE FOR MEASUREMENTS

1.5.1 Blood Pressure

As blood pressure rises, so does risk of ischemic heart disease and its complications. The range of normal blood pressures is wide. Even within the "normal range", risk increases as the upper limits are approached. Usually, blood pressures are expressed as systolic pressure/diastolic pressure; values 140/90 mmHg or higher are considered to be hypertensive for adults. Middle-aged persons with a diastolic blood pressure of 90-104 mmHg (so called "mild" hypertension) have a risk of heart attack that is about 70 percent higher than that of persons with a diastolic pressure under 80 mmHg (normal value). Persons with a diastolic blood pressure exceeding 104 mmHg (moderately severe to severe hypertension) have a risk more than twice that of those with a normal value. Hypertension is an especially strong risk factor for stroke and, to a lesser extent, for peripheral vascular disease. Most of the knowledge of the consequences of high blood pressure arises from studies of sitting arm blood pressure.

1.5.2 Measurement of Body Fat

Population studies have always demonstrated a univariate association between obesity and CVD. However, in many early studies, the association between obesity and the incidence of CVD did not remain significant in multivariate analysis, and thus it was thought that obesity was a risk factor solely because of its influence on other risk factors such as blood pressure, plasma lipoproteins and diabetes. More recently, especially in longer term studies, significant independent associations between obesity and the incidence of CVD have been demonstrated.

Although early records are not conclusive, all evidence indicates that obesity among American Indians was rare until the last century. Their farming and hunting life styles which were associated with high degrees of physical activity and the lack of consistently abundant food sources, probably assured the maintenance of a lean population. However, with the advent of "Westernization" and the reservation system obesity has increased steadily among all Indian tribes and is now a major health problem. It is thus essential to evaluate the extent of obesity in the individuals in this study in order to ascertain its role in cardiovascular disease as well as its relationship to risk factors such as diabetes and hypertension.

In the past, assessment of obesity in population studies was invariably accomplished either by algorithms such as ratios of weight to height, or by measurements of skin folds using calipers. This was because assessment of body composition required either very expensive equipment or time consuming procedures, such as underwater weighing. Within the past few years, instrumentation has become available to allow estimates of body composition from measurements of tetrapolar impedance. This
measurement of bioelectrical impedance is quick and easy to perform and has been extensively validated against densitometry. These validations were first performed by Lukaski et. al. (53) and by Roche et. al. (54), in a wide variety of individuals. The conductivity increases in individuals with low percent body fat and the instrumentation calculates the percent body fat utilizing a computerized algorithm.

1.5.3 Anthropometric Measurements

It has been recently demonstrated that among obese individuals, the distribution of body fat is related to certain patterns of morbidity. Vague and co-workers (55) have observed that body fat distribution differs among obese individuals, and that obese subjects can be roughly divided into two groups depending on whether accumulation of body fat is subcutaneous and peripheral (referred to as gynecoid or female type obesity) or whether the fat accumulation is central and primarily in the omentum (referred to as central or android obesity). The latter distribution has been shown in a number of studies to be consistently associated with the triad of hypertension, insulin resistance, and cardiovascular disease. Most studies have shown that central obesity is a risk factor for coronary artery disease.

No systematic studies of body fat distribution have been made among the American Indians. However, visual observations suggest that central obesity is much more prevalent among this racial group.

The quantification of central vs. peripheral obesity is not well standardized. Original studies were done simply by photographs and visual evaluations. This was supplanted by body circumference measurements with investigators generally taking the ratio of the body circumference at the waist to the hip or the thigh as a measure of fat distribution. However, it is clear that the body fat of interest in central obesity is the non-subcutaneous, and therefore, whole body scanning devices are necessary for a precise evaluation of this depot. Nevertheless, it has been shown in a number of population studies that the comparative circumference measurements are an approximation of the body fat distribution and the only practical techniques usable in a field study.

1.5.4 Measurements of Peripheral Vascular Disease

The atherosclerotic process affects vessels in many parts of the body. While the most conspicuous morbidity and mortality arise from coronary atherosclerosis, large vessel peripheral arterial disease (PAD) often results in significant incapacitation of the lower extremities and has been also strongly associated with the incidence of coronary heart disease. Criqui and co-workers have shown that large vessel PAD is strongly and significantly predictive of all caused mortality in both sexes with a relative risk of 4 to 5, and this was independent of other cardiovascular risk factors in a multivariate analysis. Moreover, data from the Framingham study indicate that diabetes was associated with an
even greater magnitude of increase of peripheral vascular disease than was coronary heart disease.

A thorough evaluation of peripheral arterial occlusive disease usually entails both a history and a physical examination including measurements of pulses and segmental blood pressures and then more complex measures such as angiography or sonography. The latter two techniques are both expensive and difficult to apply in a field setting. On the other hand, both Criqui et al (56) and Beach et al (57) have used segmental blood pressures measured by a simple doppler instrument in studies involving hundreds of patients. In addition, the correlation between quantitative velocity measurements and segmental blood pressures with occlusion as measured directly by angiography has been established.

Because of time limitations and economic consideration for purchase of equipment, the following indices of peripheral vascular disease will be made in this study.

1. Rose Questionnaire for intermittent claudication.
2. Palpation of posterior tibial and dorsalis pedis pulses.
3. Auscultation for femoral bruits.
4. Measurement of the ratio between blood pressures taken at the antecubital fossa (brachial) and ankle (posterior tibial) using a doppler listening device (Imex Mascot Model).

1.5.5 Examination of the Lungs, Carotids and Neck Veins

The questionnaire currently used by Kriska, et al. (58) from the University of Pittsburgh, on the Gila River Reservation will be modified and adapted for this study. This consists of an interviewer administered questionnaire to assess general, leisure and occupational activities. It is designed primarily to evaluate the past year and past week activities and a summary measure of lifetime physical activity.

1. The lungs

Auscultation is done to detect signs of congestive heart failure (rales).

2. Carotids

Carotid bruits could be due to vascular disease or aortic valvular disease.

3. Neck veins

Dilatation in the upright position usually indicates congestive heart failure.
1.5.6 Electrocardiograms

All participants will have a resting electrocardiogram so that evidence for ischemic changes and left ventricular hypertrophy can be determined. The prevalence of such changes will reflect the prevalence in the population studied and can be compared to other population-based studies and among the three sites.

1.5.7. Overview of Laboratory Measurements

1. Lipoprotein Profile

**Lipoprotein Physiology:** Lipoproteins are basically spherical particles ranging widely in size and composed of two components: the lipids (or fats) in the core of the particle and the proteins on the surface of the particle. The two types of lipids which we are interested in measuring as part of the present research are triglyceride (TG) and cholesterol (CHOL). Depending on the relative amount of these two components and various associated proteins, different classes of lipoproteins can be defined (Table 1.1).

<table>
<thead>
<tr>
<th>Class</th>
<th>%Lipid</th>
<th>% Protein</th>
<th>Origin and Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>99</td>
<td>1</td>
<td>Intestine; transport of newly absorbed dietary fats; normally not detectable in plasma after a 12-hr fast; creamy layer on top of plasma tube after 12 hrs in the refrigerator.</td>
</tr>
<tr>
<td>VLDL, very low density lipoprotein</td>
<td>90</td>
<td>10</td>
<td>Liver; transport of newly synthesized triglycerides to peripheral tissue; approximately 80% of plasma TG is in this fraction</td>
</tr>
<tr>
<td>LDL, high density lipoproteins</td>
<td>75</td>
<td>25</td>
<td>Liver; derived from VLDL after the triglycerides have been metabolized; transport of cholesterol; approximately 75% of plasma cholesterol is in this fraction</td>
</tr>
<tr>
<td>HDL, high density lipoproteins</td>
<td>45</td>
<td>55</td>
<td>Liver and intestine transport of cholesterol from peripheral tissues back to the liver</td>
</tr>
</tbody>
</table>

*Strong Heart Study II 1/20/94 II- 29 Rationale for Measurements*
The evidence is overwhelming from both cross-sectional and prospective studies in a wide variety of populations that total and LDL cholesterol are significantly associated with the occurrence of atherosclerotic coronary vascular disease (ASCVD), and the HDL cholesterol has a negative or "protective" effect.

The relationship with total triglycerides or VLDL triglycerides has been more controversial. Several population studies have now demonstrated an independent positive association between elevated triglycerides and ASCVD. Triglycerides are also closely linked to obesity, hyperglycemia and low HDL, and are therefore important to measure because of their reflection of these disorders. Some of the ambiguity concerning the associations between triglycerides and coronary vascular disease stems from the possibility that all elevations in triglycerides may not be equal. That is, elevated VLDL with a high proportion of protein, or cholesterol rich VLDL such as that observed in many diabetics may be more atherogenic than large, triglyceride-rich VLDL.

If a Beta Estimate is performed, measurements are made of total plasma cholesterol and triglyceride. HDL is measured after precipitation of LDL and VLDL. LDL is calculated by the Friedewald formula:

\[
\text{LDL chol} = \text{Total Chol} - \text{HDL Chol} - (\text{Total TG}/5)
\]

This estimate is based on the assumptions that VLDL cholesterol is a minor portion of the total cholesterol, that the majority of the total triglyceride is in VLDL, and that the composition of the VLDL is "normal", that is, VLDL cholesterol is approximately one fifth that of triglyceride.

This method has two advantages:

1. Can be performed on plasma that was frozen.
2. Requires much less technician time.

The disadvantages are:

1. It is inaccurate in individuals with high triglycerides (> 400).
2. It is inaccurate in individuals with altered VLDL composition.
3. It will not allow the isolation and examination of VLDL composition and relations to ASCVD.

If Beta quantitation is performed, total cholesterol and triglyceride are measured, and HDL cholesterol is measured after precipitation, as in the beta estimate. In addition, VLDL is isolated by ultracentrifugation, and the ratio of
cholesterol to triglyceride is measured in VLDL. From this we can directly calculate:

\[
\text{LDL-Chol} = \text{Bottom-chol} - \text{HDL-CHOL} \\
\text{VLDL-Chol} = \text{Total chol} - \text{Bottom chol} \\
\text{VLDL-Triglyceride} = \text{VLDL-Chol} \times \left[\frac{\text{VLDL-TG}}{\text{VLDL-Chol}}\right]
\]

The advantages are:

1. LDL cholesterol is measured directly, not estimated.
2. A measure of VLDL composition is obtained.
3. VLDL and bottom fractions are available for further apoprotein measurements or for storage.

The disadvantages are:

1. The ultracentrifugation is laborious, time consuming and costly.

The Beta quantitation procedure is selected because of the need for accuracy in the measurement of LDL and because it yields a VLDL fraction of particular interest in a population with high prevalence of diabetes. People with diabetes frequently have abnormal composition of VLDL.

2. Glucose Tolerance Test (Glucose and Insulin)

Although it may be argued that 75 gm glucose load is not a measure of glucose disposal that is analogous to carbohydrate ingested during daily meals, it is the standard measure of glucose tolerance which can be compared to other studies, and forms the basis for all the currently used criteria for diagnosis of diabetes. Because of the high prevalence of diabetes in all three centers, and because of the multiple previous studies reporting associations between diabetes and CVD, a glucose tolerance test is essential for the current study. The most simple to perform is one where blood samples are drawn by venipuncture at fasting, and then two hours after ingestion of the glucose. All other fasting blood samples may be obtained at the time of the fasting sample, thus limiting the venipunctures to two.

Glucose concentrations will be measured in both fasting and two hour samples. Blood for this is obtained in tubes containing fluoride to prevent consumption of glucose by WBCs. Previous studies in Phoenix have shown that tubes of blood containing fluoride can be held on ice for up four hours before isolating the plasma, and glucose values are stable. Glucose is measured on the Hitachi analyzer using a glucose oxidase technique.
Insulin concentration in blood has been reported in several recent studies to be an independent risk factor for the development of CVD. Although the mechanism of this association has not been established, there are several intriguing possibilities involving its link with insulin resistance, hypertension, hypertriglyceridemia, and thrombosis. The first three factors have been linked in several population studies in individuals with central obesity. However, some studies suggest that these factors are not universally associated. It will thus be of interest to measure fasting insulin concentrations in individuals at the three centers, to evaluate its relationship to vascular disease and also to blood pressure, triglycerides, waist/hip ratio and fibrinogen.

Insulin will be measured using an overnight radioimmunoassay developed as a modification of the method of Morgan and Lazarow (50). It utilizes a double antibody method; both antibodies and labeled insulin can be obtained efficiently from commercial sources. Although no absolute reference plasma pools are available for insulin, we have constructed our own control pools. The assay has proven to be stable over time with a coefficient of variation of 8-10%. One source of error in insulin measurements occurs in some individuals who have been previously treated with insulin, and thus have circulating insulin antibodies. Samples from insulin treated diabetics will be flagged at the time of drawing, so that their data can be separately evaluated.

3. **Glycated Hemoglobin**

The relationship between glycemia and the occurrence of CVD is an important one. Although it is well established that diabetes is associated with an increase in CVD, it is not clear whether there is a significant correlation between plasma glucose and either prevalence or incidence of CVD, and in fact several studies have failed to show a relationship between macrovascular disease and glucose tolerance, especially in diabetics.

One explanation that has been cited for this is that tests such as GTT do not reflect long term glycemia, and also have high intra-subject variability. An alternative for the integrated assessment of glucose levels over time is the measurement of glycated proteins, since the nonenzymatic glycation of proteins is a constant process which increases directly with increasing concentrations of glucose. Thus, the measure of extent of glycation of a protein with a relatively long half-life is an assessment of the ambient levels of glucose during the life of that protein.

The most commonly employed is the measure of Hemoglobin Alc. It can provide an assessment of glucose status which reflects approximately a two month period. Although there is an excellent correlation between HbAlc and glucose during GTT over the entire range of glucose intolerance, the correlation in the
nondiabetic to IGT range is not as strong. A recent measure of HbA1c in Framingham has shown a very strong positive correlation between it and CVD over the entire range.

HbA1c is much more laborious to measure than is glucose. Although several electrophoretic and chromatographic techniques have been employed in the past, currently an HPLC assay appears to be the method of choice. HbA1c will be measured by HPLC in the laboratory.

4. **Fibrinogen**

Disorders of the coagulation system could play a major role in ASCVD. There has been special interest in the role that abnormalities in the clotting system might play in the increased risk for atherosclerosis observed in diabetics. Abnormalities in several factors have been reported to be associated with atherosclerosis. One of the most commonly and easily studied is fibrinogen, and it has been shown to be an independent risk factor for CVD in both nondiabetics and diabetics in the Framingham study.

Fibrinogen has been most commonly measured using a chronometric technique. For this thrombin is added to plasma to induce clotting, and the clot is quantitated on a fibrometer or automated coagulometer. Since the lab at the MRI does not possess this equipment, measurements will be made by Dr. Russell Tracy at the University of Vermont.

5. **DNA**

Because CVD is a clinically heterogeneous disorder and involves a complex interaction between genetic and environmental factors, it will probably be explained by a complex polygenic transmission. Recent development in recombinant DNA technology, including using restriction enzymes to identify polymorphisms, are now frequently being used in study of genetic disorders and may be very helpful in sorting out the genetics of complex diseases such as atherosclerosis. Methods are now available for detecting altered nucleotide sequence in the human genome, which may be used as genetic markers of CVD or risk factors. Certain alterations in DNA sequence may be demonstrated by cleaving genomic DNA with restriction enzymes, hybridizing with cloned DNA probes and by detecting changes in the length of gene fragments by autoradiography. These techniques have allowed the chromosomal mapping of the genes for diseases such as muscular dystrophy and Huntington's chorea.

Although we do not yet have evidence in Indians that CVD shows familial aggregation, there is certainly ample indication in other populations that CVD and
several of its risk factors are familial and thus would lend themselves to genetic studies. Although genes for cardiovascular disease have been localized in animals, attention in human studies has been focused on identifying alleles that may be associated or linked with other diseases. Since diabetes, hypertension and altered lipoprotein concentrations are strong risk factors for the development of CVD, attention has been focused on the possibility that abnormalities in apoprotein or insulin gene loci might be associated with susceptibility to CVD. Mandrup-Poulsen et al. (60) have suggested that a polymorphic region of DNA close to the human insulin gene is a genetic marker for atherosclerosis. Karathanasis et al. (61) have shown that the genes for ApoA-I and apoC-III are physically linked, and that polymorphism of the apoA-I gene inherited as a trait linked to premature atherosclerosis in one affected family. Ordovas et al. (62) have also shown that the apolipoprotein A-I gene polymorphism was associated with CAD in a study of 88 patients, and was also found in 8 out of 12 kindreds with familial hypopalphalipoproteinemia. Finally, the possible association between NIDDM and arteriosclerosis is further suggested by a recent report of an association between a apoA-I gene polymorphism and susceptibility to NIDDM.

Because of the distinct possibility that the next several years will lead to greatly increased availability of genetic markers and likely specific gene loci with documented association with CVD, it is of interest to study these in the Indian groups to be examined in the current survey. The present study will undoubtedly include many related people and gives the opportunity to identify families for linkage studies. For this reason it is proposed in the present protocol to isolate and store DNA from lymphocytes of blood sample. This can be easily accomplished in an efficient and economical way and would, therefore, serve as a store for future genetic studies.

6. Lipoprotein(a)

Lp(a) is a heterogenous lipoprotein class which consists of an LDL particle to which a large glycoprotein moiety - apo(a) - is attached via a disulfide bridge. It was first identified by Berg66 as a genetic variation of the immunologic response in rabbits to human LDL. In 1972, interest in Lp(a) was heightened by 3 independent reports67, 68, 69 suggesting a link between the presence of a pre-beta migrating lipoprotein fraction66 identified shortly thereafter as Lp(a) and patients with coronary artery disease. Subsequent retrospective66,67 as well as prospective66 studies have confirmed this association. Elevated levels of Lp(a) have also been associated with the prevalence of cerebrovascular disease67 and coronary artery bypass restenosis. Lp(a) shares structural homology with plasminogen activator inhibitor and it may thus interfere with fibrinolysis.75 The impact of co-existent hyperglycemia or diabetes on Lp(a) concentrations and function is controversial.76
7. Genetic Admixture

The relative proportions of genes that parental populations contribute to a hybrid population can be estimated from allele frequency distributions when the parental populations have been surveyed for the genetic markers used in the analysis. The power of a particular genetic polymorphism to estimate admixture proportions depends upon the degree of variation at the genetic locus and the relative differences in allele frequencies between the parental groups. For instance, in Native Americans, when estimating the proportion of Caucasian admixture, the Gm allotype system is very powerful because it has five major haplotypes and one of these, Gm3:5,13,14, has a very high frequency in European Americans, 0.650, but is absent in non-admixed Native Americans. However, modern mathematical techniques do not rely on one allele or haplotype alone. Rather, they incorporate all of the alleles and haplotypes over multiple loci. The Gm and Km systems, and the traditional red blood cell loci, ABO, MNs, Rhesus, Duffy, and Kidd, provide the starting point for the method of pooled admixture estimates that is within the budget and logistical constraints of the study. Together they represent 24 alleles and haplotypes that are well characterized in European, African, and Native Americans.

Pooled admixture analyses using these multiallelic techniques have led to a better understanding of genetic admixture in human populations and its effect on genetic epidemiology. Williams, et al.,77 have shown that pooled admixture estimates in the Pima and Tohono O'odom Native Americans of the Gila River Indian Community are highly correlated with estimates derived from stated admixture. This suggests that the subjects are generally aware of their amount of non-Indian alleles. Knowler, et al.,78 have shown in this same population that the genetic admixture can be a confounding variable in disease-allele association studies, and that it is important to have a control on the amount of non-Indian alleles in a Native American community that is the subject of an epidemiological study. The pooled admixture analyses can also be jointed with demographic data to present a complete description of the relative proportions of Native American and non-Indian alleles and to describe the populations from which the genetic admixture was derived.79

Methods for individual admixture estimates, the proportion of admixture in an individual rather than a population, have been worked out by Chakraborty and co-workers80, 81 and applied to studies of Mexican Americans in Texas. These techniques, and modifications that are being prepared by Robert C. Williams and Jeffrey Long, also use allele and haplotype frequencies that have been well characterized in the parental populations. However, because the reliability of the individual proportion improves as more polymorphic loci are added, in the future, consideration will be given to adding DNA systems, particularly the highly polymorphic VNTR markers and the short, tandem repeat loci.
Both pooled and individual estimates of genetic admixture will be calculated for the Strong Heart sample. First the Gm and Km systems will be employed to determine the amount of genetic admixture in the entire sample of Native Americans, and within subsets of this sample, by tribe. The magnitude of this admixture will then be analyzed with respect to geographic location to determine whether there is a cline for genetic admixture in Native Americans. When the red blood loci are finished, they will be added to the Gm and Km systems to refine the above estimates and to calculate the individual admixture values and weights for each subject in the sample. These pooled and individual admixture values can then be compared with the prevalence of cardiovascular disease in the Strong Heart sample to determine whether non-Indian alleles are a risk factor.

1.6 PROCEDURE FOR GLUCOSE TOLERANCE TEST (GTT)

For all subjects, a fasting glucose value will first be obtained by using One-Touch (see Section 6.1.1 for procedure). Query subjects as to whether they are a known diabetic. If they are, ask if they take insulin or oral agents.

Note that all diabetic participants taking insulin will be exempted from the glucose tolerance test (GTT). Those diabetics who take oral agents and who have two random glucose values ≥ 250 mg/dl. or any participant with a fasting glucose ≥ 225 mg/dl. by One-Touch will also be EXEMPTED from the GTT. For individuals on renal dialysis or who have had a kidney transplant, blood will be drawn at the time of the examination, if possible.

1. Have the bottle of glucose (Glutol 75g.) and blood drawing equipment ready. Although the Glutol has proved to be very dependable and consistent when its concentration per ml has been measured in numerous samples, the volume supplied per bottle is not consistent. Thus it is necessary to measure both the Glutol and the water into which it will be diluted for each patient. The easiest way to accomplish this is to have a plastic graduated container such as that used for urine collections. The person administering the glucose tolerance test can thus pour 135 ml of Glutol into the measuring container, pour that into the cup supplied to the patient and then measure out 135 ml of water using the same measuring container. The cup containing the diluted Glutol is then ready for the patient. This measuring container can be used for all patients visiting on that day, but it should be discarded at the end of the day and a fresh measuring container used for the next clinic.

2. Ask subject if he/she has been fasting for 12 hours and whether he/she has refrained from smoking and beverages other than water, and record the response on the GTT check list given in Appendix 6.
3. Draw fasting blood samples as described in Procedure for Blood Drawing. Record the time of blood collection.

4. Describe the purpose of the GTT to the subject.

5. Ask the subject to drink the glucose solution quickly, within 3 minutes - Record the time the process started on form.

6. Instruct the subject that he/she should not eat, drink or smoke anything until the second blood sample is obtained two hours later.

7. Instruct the subject to take the specimen container to the bathroom for the urine sample - Record the time of urine collection.

8. Place urine sample in the refrigerator.

9. Obtain second blood sample at exactly 2 hours post load - Effort should be made so that the second blood sample is obtained at exactly 2 hrs ± 3 min. Record the time of collection.

1.7 PHYSICAL EXAMINATION

During the examination, participants wear a gown, or loose fitting clothes that do not impair accurate body measurements and the examination. It is helpful to have them wear large scrub pants to enable the pant legs to be rolled up for the ECG examinations. Shoes and socks are removed for the supine examination and weight and height measurements. The form to be used is given in Appendix 5.

1.7.1 Anthropometry

Anthropometry is performed before the clinic snack with the participant’s bladder empty. The subject may wear a scrub suit or clothing into the station. Measurements may be taken over the scrubsuit or light clothing only. Make sure that the pockets are empty and the belt is removed. Height and weight measurements are not to be taken with the participant wearing shoes.

Measurements if possible are taken by a team of two persons (one acting as observer, the other as recorder). If two are available, the first observer takes the measurements, calling out the value of the measurement.
The first observer keeps the measuring instrument in place until the recorder repeats the number. The recorder also checks the examinee's position during the procedure. If a single observer performs the measurements, each should be recorded immediately after they are taken. Values taken are rounded to the nearest unit indicated for each measure. Fractions less than 0.5 will be omitted and fractions greater than or equal to 0.5 will be rounded up to the next higher unit.

1. Height and Weight

a) Standing Body Height

The participant stands erect on the floor or the horizontal platform with his/her back against the vertical mounted ruler, heels together and against the vertical ruler, looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane which includes the lower margin of the bony orbit and the bony socket containing the eye the most forward point in the supratragal notch just above the anterior cartilaginous projections of the external ear) (Figure 1). The right angle is brought down snugly but not tightly on the top of the head. A foot stool is used if the examiner is shorter than the participant so that the examiner's view is level with the point of measurement on the head of the participant. The participant's height is recorded to the nearest centimeter using the rounding method described above. The participant is instructed to stand as straight as possible but with feet flat on the floor. (A check is made to be sure the floor is level, the wall is at a 90 degree angle to the floor, the wall is straight and the metal ruler is mounted perpendicular to the floor). A chart converting centimeters to inches is on the wall or available for use in informing the participant of his/her height in inches.

b) Body Weight

Before a participant is weighed, the scale is balanced so that the indicator is at zero when no weight is on the scale. The scale must be level and on a firm surface (not a carpet). The participant is instructed to stand in the middle of the platform of the balance scale (Detecto, model 683-p) with head erect and eyes looking straight ahead. Record the results to the nearest kilograms using the rounding method described above. To maintain accuracy, the scale is zeroed daily and must be calibrated with a known weight (50 lbs.) every month or whenever the scale is moved. To calibrate the scale, check that the 50 lb. weight weighs 50 lbs. after zeroing the scale. Furthermore, the operator should make sure that an adult must weigh 50 lbs. more when standing on the scale holding the weight.
Figure 1 (a). General Description: The **scapulae**, or shoulder blades, are large, triangular, flat bones situated in the dorsal part of the thorax between the levels of the second and seventh ribs. A sharp ridge, the spine, runs diagonally across the posterior surface of the flattened, triangular body. The end of the spine projects as a flattened, expanded process called the **acromion**. This process articulates with the clavicle.

---

Figure 1 (b). the **Frankfort Plane**: The horizontal plane which includes the lower margin of the bony orbit, the bony socket containing the eye and the most forward point in the supratragal notch, the notch just above the small prominence of skin covered cartilage projecting over the meatus of the external ear.
2. **Supine Waist (Abdominal) Girth**

An anthropometric tape is applied at the level of the umbilicus (naval) with the patient supine (Figure 2) and the participant is instructed to "breathe quietly". The measurement is made and recorded to the nearest centimeter using the rounding method described above.

3. **Erect Hip Girth**

Instruct the participant to stand erect yet relaxed with weight distributed equally over both feet. The hip girth is measured at the level of maximal protrusion of the gluteal muscles (hips) (Figure 3). Keep the anthropometric tape horizontal at this level and record the measurement to the nearest centimeter using the above rounding method. Only one measurement is made. The greatest source of error for this measurement is due to not having the tape horizontal. Technician(s) should check the position of the tape to assure its correct position from both the front and back.

4. **Upper Arm Circumference**

The participant sits on a table or stool so that the right arm hangs freely with the right hand resting on the right knee. The observer applies the tape measure horizontally at the midpoint between the acromion and olecranon (Figure 3). Record the measurement to the nearest centimeter using the rounding method described above. This measurement is used to select the proper size blood pressure cuff.

A Novel Products tension tape is used to measure both abdominal and hip girth and the upper arm circumference.

1.7.2 **Training and Certification for Anthropometry**

Each technician must undergo training and certification by individual experienced anthropometrist. The training program for taking body size measurements consists of the following components.

1. Training is conducted centrally by an expert in anthropometry.

2. Each field center trains one or two individuals before the baseline examination. One individual from each center is designated the center's anthropometry supervisor.

3. If additional personnel are needed by a center to perform anthropometry, training is provided by the center's anthropometry supervisor.
4. Training includes:

a. Introduction - rationale for body size measurements, overview of technique, expected limits of reproducibility, and pitfalls related to anthropometry.

b. Demonstration of technique - an expert demonstrates the proper technique of each measurement on a volunteer subject. This includes a description of proper and improper techniques, as well as recording of data.

c. Practice - technicians divide into groups of three, and two techs perform measurements on the third in a round-robin fashion. This is done under the observation of a trained anthropometrist. Differences in technique and clarification of problem areas are discussed.

d. Testing - several subjects are assessed independently and blindly by each technician. Each technician's measurements are compared with the expert's measurements and the results discussed in class. The four subjects examined have four distinctly different body types: lean, obese, athletic, and aged.

e. Certification - technicians must measure one or more test subjects and be within certain standards of error:

1) The arm, waist and hip measurements must agree within + 1 cm on each subject.
2) Weight must agree within + 1 kg. Height within 1 cm.

If these are met, the subject receives certification for field work. Trainees who have problems are identified, and they are allowed to practice and try again to be certified.

1.7.3 Sitting Blood Pressure

1. Introduction

In the Strong Heart Study, sitting blood pressure is measured in a resting state, using 3 measurements with a Baum mercury sphygmomanometer. Within any one individual, variation in blood pressure is substantial, even within a few minutes and particularly under conditions perceived as stressful. Use of three replicate readings tends to reduce this short-term variation.

2. Standardized Clinic Procedure

Correct measurement of blood pressure is of the utmost importance to the success of this study. It is essential that the procedure described below for measuring blood pressure be followed exactly. Precision is essential for valid comparisons of blood pressure between groups of people and in individuals on different occasions.
Supine waist girth at level of umbilicus

Figure 2. Location of Waist Girth Measurement
Figure 3. Location of Upper Arm, Hip, and Calf Circumference
3. Description of the Equipment

a) Stethoscope

A standard stethoscope with a bell is used. Korotkoff sounds are best heard with the bell because of their low pitch. Stethoscope tubing should be about 10-12 inches from the bell piece to "Y" branching. This length provides optimal acoustical properties and allows the observer to read the sphygmomanometer at eye level and in a comfortable position. Earpieces should fit comfortably and snugly in the ears. Four points should be observed in using the stethoscope.

i) The ear piece should be directed downwards and forwards into the external ear canal.
ii) The ear pieces should be tight enough to exclude outside sound but not so tight that they cause discomfort.
iii) The valve between the bell and the diaphragm should be turned in the correct direction.
iv) The bell of the stethoscope should be placed lightly on the skin overlying the brachial artery - immediately below the cuff and medial to the cubital fossa above the medial epicondyle of the radius and posterior to the biceps muscle. Light pressure accentuates the low-pitched sound and avoids compression murmurs. When pressing too heavily with the bell on the artery a murmur can be heard which may prolong the apparent duration of phase 4 and give inaccurate readings.

b) Sphygmomanometers

Standardized Baum mercury instruments are used for all clinic visits. The mercury manometer consists of a screw cap, a face with numbers, a lined glass column, a reservoir containing mercury, rubber tubing, and a metal case. The rubber tubing from the mercury manometer connects to the rubber tubing from the inflatable rubber bladder of the cuff. As the inflatable rubber bladder is filled with air, the air pressure in the bladder travels through the connecting rubber tubing. The pressure pushes the mercury out of the reservoir and into the lined glass column. The number for each line is read when the rounded top of the mercury, the meniscus, is level with it. If the meniscus is exactly between the lines, the reading is made from the line immediately above, i.e., rounded up the nearest even number.

c) Cuffs and Bulbs
Proper size of the cuff is essential for accurate blood pressure measurement. Study Centers have three standardized Baum cuffs available - adult, large adult, and thigh cuff.

The range markings on commercial cuffs overlap from size to size and do not offer a precise guideline. In the Strong Heart Study arm size is measured and the cuff size is selected as follow:

Table 1.2 Determination of cuff size based on arm circumference (Mid humeral)

<table>
<thead>
<tr>
<th>Cuff Size</th>
<th>Arm Circumference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>24 to 32 cm</td>
</tr>
<tr>
<td>Large Adult</td>
<td>33 to 41 cm</td>
</tr>
<tr>
<td>Thigh</td>
<td>&gt;41 cm</td>
</tr>
</tbody>
</table>

4. Blood Pressure Measurement Instructions

Some of the many extraneous factors influencing blood pressure are controlled by standardizing the measurement technique and the environment in which the measurement is made. Uncontrolled factors, such as time of day, arm circumference, recent use of caffeine, and identity of the observer are recorded, so that they can be taken into account during analysis.

The Strong Heart Study participants are asked to avoid caffeine (tea, coffee, chocolate, and soft drinks), eating, heavy physical activity, smoking and alcohol intake for twelve hours and to refrain from smoking for at least one-half hour prior to the clinic visit. Current drug intake, including medications affecting blood pressure, and non-prescription drugs is recorded on the day of the examination. A detailed history of alcohol intake history and recent physical activity at work and leisure are also recorded.

5. Staff Preparation for Participant Visit

In relating to the Strong Heart participants, remember that participation in the study is voluntary. Participants are given full explanation and instructions about the preparation for the blood pressure examination and an opportunity for questions. The setting in which blood pressure measurements are made is standardized.
6. Measurement Procedures

The sitting arm blood pressure is measured three times at each clinic visit. It takes approximately 10 minutes to make three blood pressure measurements including the initial five minute rest. The blood pressure measurements are made early in the clinic visit sequence immediately following the reception and informed consent, and more than 15 minutes after phlebotomy.

Once the participant is given instructions and explanations, and the equipment has been checked, blood pressure measurement begins. The following steps must be followed precisely.

a) If the participant indicates that there is a medical or postsurgical reason for not having the blood pressure measured on the right arm (or if the right arm is missing), reverse chairs and proceed with the left arm. Indicate on the data collection form that the left arm is used. If in doubt, or if the participant prefers not to have a blood pressure taken on either arm, consult with the supervisor.

b) Seat the participant with the right arm on table. The bend at the elbow (ante-cubital fossa) should be at heart level. Legs should be uncrossed and head support comfortable. The participant should be able to relax the neck and shoulder muscles as much as possible.

c) Connect the cuff to a standard manometer and establish the pulse obliteration pressure by slowly inflating while palpating the radial artery until pulse is no longer felt. Deflate and record the pulse obliteration pressure.

d) Palpate the brachial artery (just medial to and above the ante-cubital fossa), and mark this location for stethoscope placement. Choose the correct cuff size and wrap the cuff on the arm with the center of the bladder over the artery. If the participant seems particularly apprehensive, delay wrapping the cuff until after the five minute wait.

e) Record the time. Allow a five minute wait before taking the blood pressure. Conversation should be limited. However, a brief explanation of the procedure can be repeated at this time if necessary.

f) Measurement 1: Connect the cuff to the manometer. Inflate rapidly to the pulse obliteration level + 30 mm. Holding the pressure constant with the bulb, wait 5 seconds. Place the bell of the stethoscope on the brachial artery
and slowly deflate the cuff (2 mm per second) while listening. Record the 1st and 5th phases, reading the pressure in mmHg to the nearest even number. The first sound heard in a series of at least two sounds is recorded for systolic blood pressure (phase 1). The first silence in a series of at least two silences is recorded for diastolic blood pressure (phase 5), not the last sound heard. If the sounds do not cease completely, the fourth Korotkoff sound will be used.

g) Measurements 2 and 3: Have the participant raise measurement arm for five seconds. After waiting another 25 seconds with the participant's arm on the table, repeat the measurement in step f above and disconnect cuff.

Average blood pressure readings are calculated for the second and third readings. Because of the importance of the blood pressure averages, to inform the participant and for the purposes of referral, all arithmetic is done with a calculator.

If for any reason the observer is unable to complete, or has forgotten to complete any portion of the examination (and the participant is gone), draw two horizontal lines through the space(s) on the form. This is the correct way to indicate missed information. If an entire reading is missed and the participant is still sitting at the blood pressure work station, completely deflate the cuff and start over with a replacement reading.

7. Reporting the Blood Pressure Results to the Participant

Using a calculator, average the second and third readings and mention the results to the participant. State clearly the systolic and diastolic pressure.

8. Procedure for changing the peak inflation level

Occasionally the Korotkoff sounds may be heard as soon as one places the stethoscope over the brachial pulse. If this happens, the peak inflation level used was too low. The observer immediately deflates the cuff by releasing the thumbscrew and disconnecting the cuff tube. Then have the participant hold the cuff-wrapped arm vertically for five seconds. Proceed with blood pressure measurement, starting at a new peak inflation level, 10 mmHg above the previous level.

9. Sitting Blood Pressure Training and Certification

At each field center a minimum of two clinic staff persons are trained for measuring sitting blood pressure. They need not be health professionals, but they
must be trained and certified in the blood pressure measurement technique. Observers should also have experience in relating to people.

The first training session begins with a description and demonstration of the correct blood pressure measurement procedure. Trainees watch the American Heart Association blood pressure instruction video tape. Checklist is used for certifying all persons taking BPs (Appendix 7 (a) & (b)). Simultaneous BPs will be recorded using a Y stethoscope as described in Appendix 9.

It is the responsibility of each field center to conduct these procedures and report to the Coordinating Center when the procedures are completed.

Y Tube stethoscope observations are made in conjunction with the blood pressure training video during initial training and for quarterly quality control. The trainer has the observer-trainee go through the entire blood pressure measurement procedure using a quality control checklist. The observer and trainer listen with the Y Tube and record the values on separate sheets. Two measurements on one subject are obtained. Measurements by the trainer and the trainee should agree within 4 mmHg on any one reading (systolic or diastolic) and averages should agree within 3 mmHg.

10. Quality Control

To ensure the accuracy of the blood pressure measurements throughout the study, quality control measures are developed centrally and applied at all field centers. These measures include:

a) recruitment of the most qualified personnel
b) standardized training and certification
c) retraining as necessary
d) observation of data collection by supervisors, using the checklist given in Appendix 9. One checklist is used for each technician and mailed to the Coordinating Center each month
e) frequent staff meetings to provide feedback
f) editing of data, both manual and by computer
g) a quality assurance program administered by the Coordinating Center
h) simultaneous Y Tube observation of each technician by the blood pressure supervisor
i) equipment and maintenance program

11. Technician Training and Quality Control
Blood pressure technicians are trained centrally prior to participant recruitment. New technicians hired after the start of the study are trained locally by the Study Coordinator or a designated "Blood Pressure Supervisor".

The Coordinating Center directs a blood pressure quality assurance program to review six-monthly data. This includes quality analysis and review of blood pressure data every 3 months, comparing means for each technician with the values for all technicians, by center. These statistics are adjusted for weight, age and sex of the participants by the use of Z-scores. Arbitrary levels of Z-scores, (which can be modified according to performance) are used to detect possible systemic deviations in blood pressure measurement by individual technicians. Digit preference is also monitored for each technician. The Form for Recording Simultaneous Blood Pressure Observations in Appendix 9 will be used.

12. Equipment Maintenance

Each study center is responsible for the proper operation and maintenance of its equipment. Maintenance responsibility is assumed by the nurse clinician, and all staff are instructed to report any real or suspected equipment problems to that person promptly.

All checks, inspections, cleanings and problems indicated are documented and recorded by date in a permanent log. Problems and solutions are also recorded. The local nurse clinician sends a copy of this log monthly to the Coordinating Center. A copy of this log is given in Appendix 10.

The standard sphygmomanometer is inspected once a month. These inspections include a check of:

i) the zero level  
ii) mercury leakage  
iii) manometer column for dirt or mercury oxide deposit  
iv) condition of all tubing and fittings

The equipment is cleaned if inspection indicates it is needed, or at least once a year. Specific maintenance instructions for the standard sphygmo-manometer are provided in Appendix 11.

1.7.4 Ankle Systolic Blood Pressure

1. Move the Participant to the Supine Position

Assist the participant in moving to the supine position on the examination table.
2. Applying the Blood Pressure Cuff

The appropriate ankle blood pressure cuff is applied on the right calf. The same size cuff should be used on the ankle as the one used on the arm. In special instances, different cuff size may be used.

At this point, a blood pressure cuff is applied above the ankle of the right leg, as shown in Figure 4. Place the cuff flat on the table (the surface marked "side to the patient" face up) with the appropriate ankle centered on the cuff. At this time disregard the "over the artery" marker. The lower edge of the cuff (from which the hoses extend) should be approximately 2-2 1/2 inches above the medial malleolus. Following the contour of the lower leg, wrap the end of the cuff with the velcro "fabric" over the ankle, as shown in Figure 5. Note that depending on the degree of tapering in this area, the cuff corner will be offset from parallel toward the knee. Holding the cuff from sliding, wrap the other end over the ankle (step III in Figure 5), again following the contour of the ankle, and secure the velcro. Check to be sure that the corners of the cuff extending above the upper edge of the cuff are about equal: if one end extends more than the other, loosen the velcro and adjust the wrap. Next, locate the "over the artery" marker of the cuff, and rotate the cuff so that this line is directly over the posterior tibial artery. The cuff may be rotated more easily by sliding it toward the malleolus, and after alignment, the cuff can be made snug by pulling it up toward the calf. The cuff should conform closely to the shape of the ankle, with the lower edge 2-2 1/2 inches above the malleolus.

The posterior tibial artery is usually palpated as it courses posteriorly to the medial malleolus. Even if the posterior tibial pulse is not palpable, the posterior tibial artery is used as the location for the marker line on the cuff for the "over the artery position". Any kinks in the tubing are removed, and any "tugging" of the tubing on the participant's leg is relieved.

3. Procedure for Measuring Ankle Blood Pressure

a) Palpate posterior tibial pulse and mark these locations. Apply ultrasound gel to the posterior tibial area over the pulse or in the area shown on Figure 4.

b) Listen for the pulse using the Imex Mascot Doppler. If no pulse is audible or palpable, then try to use the dorsalis pedal pulse for the determination of blood pressure. If no pulse is audible, record zero for ankle blood pressure after the absence of pulse is verified by a second observer.
c) Inflated cuff to a pressure reading 20 mm higher than the "Peak Pressure" used for the sitting arm pressure (i.e., obliteration plus 50 mmHg) and utilize identical deflation techniques while listening with the Doppler.

d) Take a second blood pressure, and record both blood pressure in the Physical Examination Form. This procedure also applies to the doppler arm blood pressure.

e) Record the first sound as described above.

The blood pressure cuff is applied over the brachial artery according to the instructions found in the Sitting Blood Pressure section of this manual. By consulting the participant's Data Form, the observer verifies that the same arm and the same cuff size are used as for the sitting blood pressure readings.

If the participant had his/her sitting blood pressure taken on the right arm earlier in the clinic examination, the cuff is applied on the right arm at this time. The observer then uses the Doppler to record the brachial pressure. The pressure recorded in the right arm is used to calculate the brachial/ankle systolic pressure ratio for both lower extremities.

If it is impossible to obliterate the sounds after increasing the pressure to above 250 mmHg, no systolic measurement should be made in that ankle.

f) Repeat the procedure for the left leg and record the pressure as soon as the cuff is in the proper position.

The observer now removes all conduction jelly. Socks and a robe or other garments are now replaced and the participant is escorted to the next work station.

1.7.5 Electrocardiogram

1. Basic description

a) A Marquette Mac-PC (or Mac-12) based system will be used.

b) All ECGs will be transmitted centrally to Fitzsimons Army Medical Center in Denver electronically by modem.

c) All ECGs will be read in a standard manner at the ECG Reading Center by Board Certified or Board Eligible Staff Cardiologists and transmitted or mailed back to the site of origin for clinical correlation or other action, if required. In any case, all ECGs will be overread and promptly returned.
d) All ECGs will be forwarded to the University of Minnesota ECG center for application of Minnesota codes.

e) The Strong Heart Study will itself maintain a permanent copy of all cardiograms in its possession to assure "perpetual" availability of the study data for study members.

f) A standard level of competence must be demanded of our personnel performing ECGs at each site. A "competency exam" would be conducted of all persons recording ECGs at individual sites by a physician (or other designated person) who would judge the ability of the person being examined to adhere to standard protocol.

2. Minimal Equipment Requirements

a) A new Mac-PC with modem will be used at each clinic. Mac-12 machines may be used if they are available.

b) Fitzsimons Army Medical Center will provide free use of their mainframe CAPOC (Computer Associated Practice of Cardiology software) system (except for study hook-up costs and paper costs) for the duration of the study. This system can be accessed 24hrs/day by modem and stores all study cardiograms together or by center. Also, floppy disc downloading can be accomplished to a Mac-12 compatible format.

Transmission instructions on Mac-PC, Standardized ECG, instructions and Minnesota Codes are given in Appendices 33, 34, and 35, respectively.

Procedures will differ at each center concerning how ECG readings are supplied to local physicians and IHS health records. A copy of the ECG obtained at the time of performance, if marked "unconfirmed" can be included in the patients chart that day. A clinical reading will be performed at Fitzsimons and returned by reverse transmission procedure WITHIN one week. A hard copy of this clinical reading will also be sent to the Coordinating Center for storage.

All ECGs will be sent to the University of Minnesota to be coded. The Minnesota codes will then be added to the ECG data set by the Coordinating Center for data analyses.
Figure 4. Placement of the blood pressure cuff on the ankle. Step 1 - Positioning the lower leg on the cuff.
Step 2. Wrap fabric end of the cuff following contour of ankle

Step 3. Wrap and secure cuff

"ears" about equal

Figure 5. Placement of the blood cuff on the ankle. Step 2 and Step 3: Wrapping and securing the cuff
1.7.6 Impedance Measure

The measurement of body fat is accomplished using the Impedance Meter, Model # B1A101, made by RJL Equipment Company. This involves a small low frequency current which travels across the body through the extracellular fluids. The measurement of bioelectrical impedance is related to the volume of the conductor and when expressed as impedance or conductance, it is proportional to fat free mass.

1. Procedure

a) Before beginning explain why you are making the measurement to the subject and check to see that the subject has not exercised vigorously for the past 12-hours and has not consumed alcohol in the past 24-hours. Make sure that the subject is not dehydrated. Record past vigorous exercise or alcohol consumption on the data form.

b) Before beginning the test, be sure that the subject cable is securely attached to the RJL spectrum, have the subject remove the right shoe and sock and lie down with the right side nearest to the analyzer;

c) If the examination table is metallic, it must have a foam pad - all of the body must be on the pad.

d) For best results:

i) Use electrodes only once.

ii) Legs should be far enough apart so that thighs do not touch each other. A towel may be used to prevent the legs and thighs being touching.

iii) Hands and arms should be far enough apart so that the arms and hands do not touch the torso. A towel can be used to prevent the arms from touching the body.

iv) No body parts should be in contact with any external metal (jewelry and pins in bones will not effect the results).

v) Subject's skin should be clean, dry and warm to the touch. If the skin is oily, clean it with an alcohol swab before attaching the electrodes.
Prior to the attachment, cut the electrodes in half bisecting the foil tab. The cut edge of the electrode placed on the ankle and wrist should face toward the shoulder and thigh respectively. The cut edge of the other two may face in either direction.

e) Electrode Placement:

i) Attach the black wires to the foot with the red clip connected to electrode at ankle (F1). Attach the red wires to the hand with the red clip connected to electrode at the wrist (H1).

ii) Put H1 on an imaginary line from the protruding bone of the wrist to bisect the ulnar head; make sure that the cut edge of the electrode is toward the shoulder.

iii) Put H2 just above the knuckles of the right hand or on any finger; there should be at least 5 cm difference between H1 and H2.

iv) Put F1 on an imaginary line between the protruding ankle bones to bisect the medial malleolus; make sure that the cut edge of the electrode is toward the thigh.

v) Put F2 just above the toes of the right foot or on the great toe (there should be 5 cm difference between F1 and F2)

Once the electrodes have been properly attached to the subject, the values for resistance and reactance will appear on the screen. Record these on the results sheet. Make sure that the toggle switch is set on x1.

2. Instructions for Impedance Meter

Battery Charging

Unit has rechargeable batteries that must be charged before use. They are charged by plugging instrument in with power switch in off position. Manufacturer suggests charging for 8 hours prior to use. Instrument should not be plugged in longer than 8 hours; damage to batteries may occur.

For our use they suggest the following: Plug unit in first thing in the morning before clinic and at least 15 minutes before the first test. Leave unit plugged in for the duration of each clinic, but have the power on only when testing a patient. At the end of clinic, the meter should indicate high charge (green area).

Checking Instrument
Before testing the first patient, be sure that the cables are not crimped or damaged. Check battery charge using the following procedure. Disconnect power cord. Place the Resistance/Reactance switch in the resistance position. Place the switch labeled x1/x10 in the x1 position. Attach the 2 clips from one patient cable to one side of 500 ohm resistor provided.

Attach the two clips from the other cable to the other side of the resistor. Turn power on. Resistance displayed should be between 490 and 510 ohms. If resistance is in this range, proceed with patient testing.

If resistance is not within this range, the batteries may not be fully charged, or another problem may be present. If charge appears to be low, charge batteries for 8 hours, then retest. If unit is fully charged and resistance is still not acceptable, see manual, page 9, for trouble shooting.

Note: Patient cables are made of silver. Take care not to bend or abuse cables. They should be left plugged into instrument to minimize handling, except when relocating instrument.

3. Quality Control for Impedance Measure

Training for the measurement of body fat using the bioelectric impedance meter was accomplished by a representative from the RJL Equipment Company who attended the training followed the following steps:

a. Instructions concerning the use and verification of the machine.

b. Demonstration by instructor of the procedure.

c. Practice by the individual operators.

d. Certification of operators if instructor and operator achieve an impedance measure where resistance and reactance were each within 3%.

For ongoing quality control in each center, one individual will be designated as supervisor of the impedance measures. This individual will assure that each of the other operators of the instruments is recertified quarterly by having him/her perform an impedance measure on the same individual as the instructor. These should agree within 3%.

In addition, the instructor is responsible for the monitoring of the impedance meter. This includes checking the battery charge daily before the instrument is used, following the
instructions in the manual. Further, the instructor should observe individual operators performing impedance measures at least quarterly to verify consistent and proper technique.

1.7.7 Examination of the Lungs, Bruits and Pulse

1. Sitting Examination

   Neck - Presence or absence of venous column in 90° upright position is recorded.

2. Sitting or Supine Examination

   Neck, Carotid Bruits - The participant is asked to stop breathing momentarily. With the stethoscope bell, the examiner listens first above the clavicle for the common carotid artery and second, at the angle of the jaw for carotid bifurcation. In each position, the stethoscope is placed for three cardiac cycles, alternating sides of the neck.

3. Auscultation

   Auscultation can be performed while patient is sitting quietly waiting (five minutes) for blood pressure measurements.

a) Lungs

   Lungs - Rhonchi, Rales- The participant is in the sitting position. It may be best for men to remove the scrub top or shirt entirely and for women to lift it. The stethoscope diagram (which should be warmed in the palm of the hand) is used. The participant is instructed to take deep breaths through the mouth. After the first five or six breaths and as needed thereafter, the participant is asked about symptoms of light-headedness. Auscultate posteriorly beginning at the apices with at least one full breath in each location. Three locations on each side are examined: apex, mid-lung field (approximately at the 6th intercostal space) and the base, which may need to be determined by percussion. Rhonchi are described as coarse breathing noises.

   Rales are fine moist noises. Basilar rales are reported as those within two stethoscope diameters of the base of the lung. "Lower lung" means from above the base to mid-lung, at the 6th space posteriorly.

4. Supine Examination

a) Femoral Bruits
The femoral artery should be auscultated by stethoscope using the diaphragm at the inguinal crease bilaterally for the evidence of bruits. (This is a large artery readily palpable in all but the most obese individuals).

b) Ankle Edema

The socks or other foot covering are removed. The participant is examined in the supine position. Gentle but firm pressure is applied along the mid-tibia, anteriorly down to the ankle in each leg. Pitting or indentation remaining after pressure is removed constitutes definite edema. The examiner identifies the mid-point between the prominence of the medical malleolus and the inferior border of the patella. Pitting at or above that mid-point is recorded as "marked" edema. Pitting only below that point is recorded as "mild" edema. The degree of edema is based on the extent.

c) Posterior Tibial Pulse

The examiner palpates inferior to the medial malleolus of each foot. The presence or absence of arterial pulsation is recorded. If in doubt, the examiner compares with the radial pulsation.

d) Dorsalis Pedis Pulse

The superior aspect of each foot is palpated for the presence or absence of this pulse.

e) Peripheral neuropathy using monofilaments.

1.7.8 Physical Findings to be Confirmed by a Physician to Assure for Presence of CHF

The participants should be referred for evaluation by a physician if these findings were noted so they can be confirmed and evaluated for the presence of congestive heart failure.

1. Lung Examination

Rales in at least three of four lower lung field in the sitting position.

2. Other findings

Bilateral ankle edema, orthopnea or paroxysmal nocturnal dyspnea.
The standard IHS referral form should be used to refer patients with newly observed physical findings described above to an internist or cardiologist so that the diagnosis can be confirmed and the prevalence of congestive heart failure can be determined. In such cases, use the Physician Referral Form for Diagnosis of CHF in Appendix 14.

1.7.9 Referral Guidelines

It is the intention of the Strong Heart Study that individuals who participate in the physical examination will be provided both with education and encouragement concerning a healthy lifestyle aimed at preventing cardiovascular disease and, also that the participants receive assistance in securing medical care for any significant medical conditions uncovered during the course of the study exam.

1. Referral procedure:

a) All participants reporting for the medical exam will receive appropriate educational materials concerning a heart health lifestyle. In addition, the examining personnel, when possible, will endeavor to educate the participants during the exam concerning the importance of risk factor reduction and modifications that the individual might make to improve his/her risk for cardiovascular disease.

b) After all laboratory results are completed from the physical examination, a follow-up letter will be mailed to each participant thanking him or her for participation and supplying him/her with basic medical information obtained during the exam. (See example of letter and suggested interpretation in Appendix 15).

c) After all results from the medical examination are complete, a form will be generated by the Coordinating Center which will be available to the Indian Health Service for insertion into the patients medical record. This will contain results of the electrocardiogram, measurements of body fat, glucose tolerance test, and blood measurements, which might be of benefit for their future medical care.

d) In order to insure that the patient receives appropriate referral and treatment for significant medical conditions uncovered during the course of the study, consistent referral levels have been established as described below which will be applied at each center.

2. Referral Levels and Medical Data Review

The Strong Heart Study refers participants using established guidelines for referral. Uniform criteria for referral of participants are implemented at all centers. Emergency, immediate, urgent, and routine referrals are made. Methods for referring participants who
have no physician are established with the participant. All referrals are documented on a separate log and copies of the referrals are kept in the Strong Heart Study folders. The following are the levels of referral established for the Medical Data Review.

a) Emergency Referral: The patient is immediately escorted to a physician or an emergency squad is summoned. In such situations study personnel will provide emergency care to the best of their ability and training as appropriate to the emergencies that arise.

b) Immediate Referral: The participant is urged to see his/her physician within one day.

The physician assistant/nurse clinician notifies the participant's physician or nearest IHS facility and the Strong Heart Study physician, if applicable. The participant is provided with an IHS referral form to take to his/her physician.

c) Urgent Referral: The participant is urged to see his/her physician within one week.

The physician assistant/nurse clinician confirms the decision with the Strong Heart physician, if applicable, of the referral. An IHS referral form is filled out and an appointment is made with the assistance of the clinic staff and/or CHRs.

d) Routine Referral: The participant is asked to see his/her physician within one month, or at first convenient appointment.

The physician assistant/nurse clinician advises a visit to the participant's physician. Appointments for the patients are made by the CHRs or clinic staff.

e) No Referral: The study results are summarized for participant and held for routine results letters.

3. Referral and Review Guidelines for Independent Patient Follow-up

Guidelines for referral at medical data review are provided in the table below. The reviewer determines the acuteness of the findings, as well as whether or not the condition is being followed by a physician.

If the participant is aware of and being followed medically for a condition, judgement is exercised about whether to refer. The standard IHS referral form is used to provide appropriate clinical information to the health care professional who will evaluate the patient. A copy of this referral will be retained with the research forms to document the referral that was made.
## Referral at the time of examination

<table>
<thead>
<tr>
<th>Emergency Referral</th>
<th>Statement to Participant</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP $\geq$ 260 mmHg&lt;br&gt;DBP $\geq$ 130 mmHg</td>
<td>Your BP is very high&lt;br&gt;Your BP is very high</td>
</tr>
<tr>
<td>Pulmonary edema or any finding or symptom suggestive of a life-threatening illness, including evidence of acute MI.&lt;br&gt;Use Referral form (Appendix 14)</td>
<td>Describe rationale for referral to participant</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immediate Referral</th>
<th>Statement to Participant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting One Touch glucose $&gt; 400$</td>
<td>Your blood sugar is very high</td>
</tr>
<tr>
<td>SBP 240-259 mmHg&lt;br&gt;DBP 115-129 mmHg</td>
<td>Your BP is very high&lt;br&gt;Your BP is very high</td>
</tr>
<tr>
<td>Diabetic foot ulcer</td>
<td>Your foot must be seen by a physician</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>Your chest pains may be important</td>
</tr>
<tr>
<td>Neurologic symptoms in past week</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Other severe symptoms or findings</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Echocardiogram finding: Pericardiac tamponade Intercardiac mass</td>
<td>You may have serious problem in your heart</td>
</tr>
<tr>
<td>Urgent Referral</td>
<td>(&quot;Consult M.D. within a week&quot;)</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>SBP 200-239 mmHg</td>
<td>Your BP is high</td>
</tr>
<tr>
<td>DBP 105-114 mmHg</td>
<td>Your BP is high</td>
</tr>
<tr>
<td>Angina, stable but untreated/not being followed</td>
<td>Your chest pains may be important</td>
</tr>
<tr>
<td>Neurologic symptoms, untreated, one week to six months ago</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Suspected congestive heart failure (Use Referral Form in Appendix 14)</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Other acute, but less severe symptoms</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Inappropriate medication usage may be dangerous</td>
<td>Taking medication incorrectly</td>
</tr>
<tr>
<td>Non-diabetic with a fasting One Touch glucose of $\geq$ 200</td>
<td>Your blood sugar is high</td>
</tr>
<tr>
<td>Pulmonary function test findings:</td>
<td>You may have serious problem in your lungs</td>
</tr>
<tr>
<td>Undiagnosed severe pulmonary disease</td>
<td>You may have serious problem in your lungs</td>
</tr>
<tr>
<td>Clinic cough, fever, weight loss, and other symptoms suggestive of active TB or valley fever</td>
<td>You may have serious problem in your lungs</td>
</tr>
<tr>
<td>Echocardiogram: Previously undiagnosed potentially show stenosis</td>
<td>You may have serious problem in your heart</td>
</tr>
<tr>
<td>Gallbladder suspicious for cancer</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Condition</td>
<td>Recommendation</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>SBP 140-199 mmHg</td>
<td>Your BP is elevated into borderline range. Recommend that participant confirm blood pressure reading within 2 months</td>
</tr>
<tr>
<td>DBP 90-104 mmHg</td>
<td>Your BP is elevated into borderline range. Recommend that participant confirm blood pressure reading within 2 months</td>
</tr>
<tr>
<td>Old MI (Rose Questionnaire), previously unrecognized</td>
<td>Your chest pain may be important</td>
</tr>
<tr>
<td>Neurologic problem (stroke, TIA findings) &gt; 6 months ago, unrecognized</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Claudication, previously unrecognized</td>
<td>Your leg pain may be important</td>
</tr>
<tr>
<td>Both pedal pulse are missing in one extremity and not previously referred or the ratio of doppler pressure of ankle/arm &lt; 0.8</td>
<td>You may have a problem in your arm or foot. You should check with your doctor</td>
</tr>
<tr>
<td>Carotid Bruit: previously undiagnosed</td>
<td>You have a heart murmur and carotid murmur and should be checked by your doctor</td>
</tr>
<tr>
<td>Undiagnosed peripheral neuropathy</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Undiagnosed moderate pulmonary disease</td>
<td>Your symptoms may be important</td>
</tr>
<tr>
<td>Mild valvular disease</td>
<td>You may need antibiotics when you have dental work</td>
</tr>
<tr>
<td>Symptomatic gallstone</td>
<td>Results from reading center to be reviewed by physician</td>
</tr>
<tr>
<td>Condition</td>
<td>Possible Action</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Referral after results are available</td>
<td>Consult M.D. within 1 month (If critical values: FBG&gt;400, TG&gt;1000, creatinine&gt;2, cholesterol &gt;300, laboratory will call field center)</td>
</tr>
<tr>
<td>Fasting blood glucose $\geq 140$ or 2 hr-glucose $\geq 200$, and non-diabetic</td>
<td>You may have diabetes</td>
</tr>
<tr>
<td>Fasting blood glucose $\geq 200$ and diabetic</td>
<td>Your diabetes is not under control</td>
</tr>
<tr>
<td>2-hr blood glucose 140-199</td>
<td>Your blood sugar is high. You may develop diabetes</td>
</tr>
<tr>
<td>Cholesterol $&gt; 200$</td>
<td>Your blood cholesterol must be rechecked</td>
</tr>
<tr>
<td>Triglycerides $&gt; 1000$</td>
<td>Your triglycerides is very high</td>
</tr>
<tr>
<td>Triglycerides $&gt; 250$</td>
<td>Your triglycerides must be rechecked</td>
</tr>
<tr>
<td>Plasma Creatinine $&gt; 2$ and no previous history of kidney problems</td>
<td>Your kidneys are not functioning well</td>
</tr>
<tr>
<td>Urine Albumin $&gt; 1000$ mg/day or Plasma creatinine 1.5-2.0</td>
<td>Your urine test shows you should be checked</td>
</tr>
<tr>
<td>Undiagnosed valvular disease</td>
<td>Results from reading center to be reviewed by physician</td>
</tr>
<tr>
<td>Positive PPD and no previous therapy or preventive therapy</td>
<td>Refer participant to preventive therapy</td>
</tr>
</tbody>
</table>
ECG RE Ferral: ECG Findings Requiring Review by M.D. before Participant leaves the clinic

Would like to review with M.D.,
Call should be made to Reading Center by field staff at (303) 361-8133

* Acute pattern abnormalities (MI, ischemia)

* Rhythm disturbances
  2nd or 3rd degree block, ventricular tachycardia,
  any type of ectopic beat > 6/minute, couplets bigeminy, R on T,
  multifocal premature ventricular contractions,
  atrial fib/flutter with ventricular rate < 60/min or > 110/min,
  sinus bradycardia < 40/min, sinus tachycardia > 110/min, PR interval ≥ 0.26 sec.

* Any other ECG findings, alone or in conjunction with symptoms, causing concern

Other ECG Findings to be reviewed the same day; if possible

QT Prolongation (confirm medications)

ECGs where Routine Referral is usually appropriate

New left bundle branch block
New right bundle branch block
Wolff Parkinson White
Left Ventricular Hypertrophy

Examples of Usually Benign ECGs (always obtain old comparison ECG when available)

Left Axis Deviation/Left Anterior Hemi (Fascicular) Block
Atrial Abnormalities, Intraventricular Conduction Delay
Unusual P Wave Axis, Wandering Atrial Pacemaker
S₁ S₂ S₃ Pattern, Old Right Bundle Branch Block
Incomplete Right Bundle Branch Block
ST Elevation compared with Early Repolarization
First Degree AV Block

Copies of each ECG obtained as part of the Strong Heart Study will be forwarded to either
the local clinical director or other identified local clinical personnel.
QUALITY CONTROL

1.8  

1) Anthropometry and blood pressure

Duplicate measures of arm blood pressure (systolic and diastolic), ankle blood pressure, and anthropometry (height, weight, waist/hip ratio, and electrical impedance measurements) should be performed by a second observer on an approximate 10% randomly selected sample of participants. These data must be sent to the Coordinating Center for monthly analysis. Results of the analysis will be provided to the field centers and the Steering Committee on a monthly basis. Criteria for unacceptable differences are as follows:

1) Systolic Blood Pressure: 15 mmHg
2) Diastolic Blood Pressure: 15 mmHg
3) Height: 1 cm
4) Weight: 0.5 Kg
5) Resistance: 15 units

Duplicate data for blood pressure, height, and weight will be compiled by the Coordinating Center and reported to the clinics and Steering Committee quarterly; in addition, distributions of measurements and digit preference for each staff member will be compiled and repeated quarterly.

Anthropometric measurements and blood pressure by standard sphygmomanometer and by Doppler should be observed and evaluated quarterly by the clinic supervisor. This person will also assure that each of the other operators of the impedance meter is recertified quarterly by having him/her perform an impedance measure on the same participant as the supervisor. In addition, a simultaneous Y-tube observation of each observer by the blood pressure supervisor should be made. All results will be analyzed by the Coordinating Center on a quarterly basis. Duplicate blood pressures taken by Doppler will be performed quarterly by the supervisor.

To maintain accuracy, the scale should be zeroed daily and should be calibrated with a known weight (50 lbs.) every month or whenever the scale is moved. The impedance meter should be calibrated daily, follow manufacturer's instructions. This includes checking the battery charge daily before the instrument is used. The standard sphygmomanometer should be inspected once a month. These inspections include a check of (i) the zero level, (ii) mercury leakage, (iii) manometer column for dirt or mercury oxide deposit, and (iv) condition of all tubing and fittings. Record equipment monitoring or a checklist. The Coordinating Center will compile the data and document staff performance.
2) Laboratory tests

Duplicate blood and urine specimens should be collected on approximately 10% of the participants and sent to the Core Laboratory in a blind fashion. Results obtained for each test will be analyzed monthly by the Coordinating Center for accuracy and consistency. The percent of pairs with differences within 5%, 10%, and 20% will be computed. Correlation coefficients and coefficients of variation will be calculated and technical errors estimated. Poor correlation or unreasonably high technical error will be reported to the Laboratory and the Steering Committee.

3) Personal interview

Personal interviews must be observed monthly by the study coordinator. Problems and errors should be identified using a checklist and corrected immediately.

4) Pulmonary function tests and echocardiography

Refer to PFT section and Echo section, respectively.

5) Data quality at the Coordinating Center

As in Phase I, every data form received from the field centers should be checked for completeness by staff at the Coordinating Center. If there are any apparently incorrect entries or missing data, the staff will contact the respective field center for clarification. The entire form will be returned to the field center if the problem cannot be solved by telephone. After all the questionable items are clarified, the form will be entered and verified by two different persons.

The data entry program provides a second quality control check. Range checks and logical checks should be built into the data entry program. The program will refuse to accept such data until the errors are corrected. Computer printouts of data received will be sent to each field center. Summary statistics such as mean, median, range, maximum and minimum for continuous variables and frequency distributions for categorical variables will be calculated monthly for each center and data not meeting consistency checks will be flagged.

6) Quality control for surveillance data

In the mortality and morbidity surveys, decisions on cardiovascular disease (CVD) deaths and CVD events of interest will be made by the three physicians on the Mortality and Morbidity Review Committee. Duplicate records of every death and 10% of all morbid events will be sent to a second member. Each physician independently determines the classification of a cause of death or CVD event, and the Coordinating Center then compares
the results from both physicians. If the primary reviewer feels a decision on the cause of
death or that regarding a possible CVD event is particularly debatable, all information will
be sent to another reviewer so that a joint decision can be reached. The committee will meet
annually to discuss equivocal cases.

The Mortality Committee will also evaluate the quality of chart reviews and advise
clinic staff if changes are needed.

7) Quality control site visits

Quality control site visits will be scheduled every six months. The site visit team
which consist of the Program Manager from NHLBI and representatives from every center
will visit each center, observe every component of the study, identify inconsistencies,
discrepancies, and other problems, and provide advice for improvement.

8) Certification of technicians

Each center will recruit the most qualified personnel. Clinical staff will be centrally
trained and certified before the examination begins and newly hired personnel are trained at
each clinic. Recertification occurs every six months to ensure accurate and consistent
performance.

9) Confidentiality and safety of data

All personnel with access to data collected for the study are required to sign a
confidentiality pledge. Completed data forms are placed in locked file cabinets at every
center and only authorized staff members have access to the data.
REFERENCES


5. DHHS. Indian Health Service. Indian Health Service Chart Rook Series. June 1984.


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