FRAMINGHAM HEART STUDY
GENERATION 3 EXAM 1
VASCULAR FUNCTION MANUAL

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Adapted from CHS, BMC and Conquer Protocols
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Appendices

Introduction to Brachial Artery Vascular Reactivity Test

The brachial artery vascular reactivity test is designed to look at the function of the endothelium (the blood vessel lining) using high-resolution ultrasound. We are studying vascular reactivity in the Framingham Study to more fully understand the determinants and prognosis of endothelial function in the community. It is hoped that the test results will help predict incident and recurrent CVD. For a more complete description of the specific aims please see appendix 7.

The examination takes approximately 20 minutes: a few minutes for participant preparation, 10-12 minutes of scanning time and a few additional minutes to enter information and complete forms at the end of the test.

Equipment

- Chattanooga Group, Inc. Triton Electric Hi-Lo Treatment Table
- Height adjustable sonographer chair
- Philips Medical Systems Agilent Sonos 5500 Ultrasound System, Model # M2424A
- Sonos 5500 Monitor, Model # DR5815
- Panasonic SVHS VCR Model # AG-MD835
- Laptop computer for Tonometry and Brachial acquisition from Gary Mitchell, M.D., Cardiovascular Engineering, Inc., Holliston, Massachusetts
- Gateway Computer. ATX Tower, Model # GP6-450 (manufactured 10/14/98)
- VX1100 19" Gateway Monitor
- Acquisition software provided by Gary Mitchell, M.D., Cardiovascular Engineering Inc., Holliston, Massachusetts.
- Analysis software from Medical Imaging Applications, Iowa City, Iowa, USA. Brachial Analyzer 3.2.3 sp2 and Brachial Converter – FD 3.2.2
- Hokanson E20 Automatic Rapid Cuff Inflator - with foot pedal
- Hokanson AG 101 Cuff Inflator Air Source
- Pediatric Blood Pressure Cuff - for bBrachial artery (goes with Hokanson equipment)
- For measuring Tonometry distances: Flexible measuring tape (Centimeter); Creative Health Products with cylinder at the end. (800-742-4478)
- Caliper for measuring femoral pulse distance; Cardiovascular Engineering, Inc. Holliston, Massachusetts

Supplies

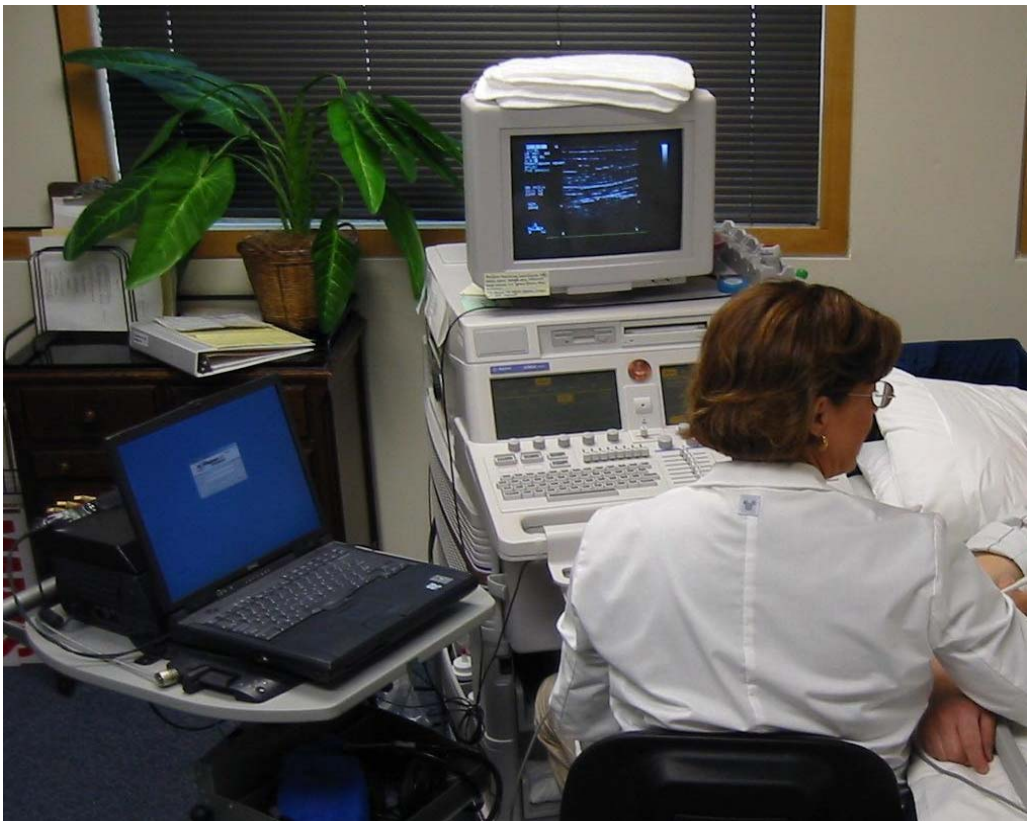
- Transducer gel
- ECG electrodes
- CD disks

Examination & Data Cleaning Documentation Materials

- Participant and Sonographer Worksheets
- Participant ID tape labels
- Log book
- Log-In sheets

Miscellaneous

- Standard pillow - for participant
- Hand and/or arm pillow support - for sonographer



Room setup for performing of brachial ultrasound test.

Performing the Brachial Ultrasound Test

A succinct **protocol flow sheet** is provided in appendix 4. Below we will expand on the elements of performing the brachial artery test in a standard fashion.

Initial Test Set Up

In the waiting room the participant reads a set of instructions about the **Non-Invasive Cardiovascular Testing Station tests** (see appendix 1) and signs an informed consent form before arriving at the Brachial station. If not, have the participant read the instructions and sign the consent form before proceeding.

The sonographer asks the participant the questions on the **Vascular Testing Participant Worksheet** and fills out the worksheet. (see appendix 2). The reverse side of this form, the **Brachial Scan Sonographer Worksheet**, is then used by sonographer to CD #, temperature in the testing room, etc. (see appendix 2 for exact items), and to enter information regarding the interpretation of the Brachial Scan.

Acquisition. Enter the participant ID#, Exam #, and Sonographer ID# in the Mitchell acquisition computer. Click on Brachial Reactivity.

Labeling storage media, log in sheet & ultrasound system. The sonographer should also enter the participant ID # and name, exam date, sonographer ID#, comments, CD #, and miscellaneous information regarding interpretation and data management, on the **Generation 3 Exam1 Log Book Sheet for Tonometry, Brachial and Echo Tests in the Log Book** (see appendix 3). Enter the participant ID# and name on the Sonos 5500 Ultrasound System. Put participant ID# and name label on the image CD disk. Save remaining participant labels for data CD disks.

Verbally preparing the participant

The sonographer then reviews the test with the participant to make sure he/she understands the procedure and to gain his/her cooperation (see appendix 1). Make sure to include specific details not mentioned in the participant description as follows (see also appendix 4 - **FHS Brachial Vascular Reactivity Protocol Flowsheet**):

- *Blood pressure will be taken on the left arm once before the scan with an automated blood pressure cuff using the NIHem system.*
- *First we will take pictures of the right brachial artery, the blood vessel near your elbow.*
- *Then the right arm BP cuff will be inflated above your blood pressure.*
- *You may feel pins and needles or tingling of the right hand during inflation, as if the hand has "gone to sleep".*
- *The cuff is then deflated for a 2-minute deflation scan.*
- *You will be asked to keep your right arm still and not to move your hand or fingers until I announce that the scan is completed.*
- *I will announce each minute of the 8-minute scan, how many more minutes until deflation, i.e. "4 more minutes, 3 more minutes, " etc.*

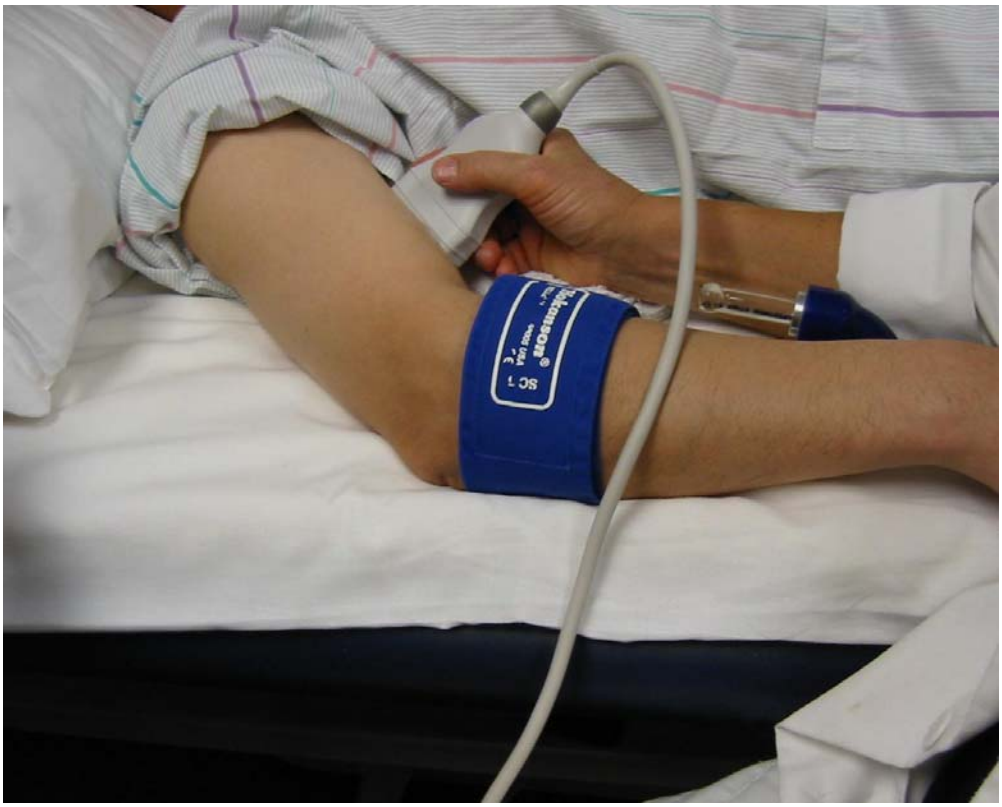
It is extremely important to let the participant know that the most critical images during the test are obtained immediately after the cuff is deflated to watch how the artery reacts after the cuff is released. It should be emphasized that the participant not move his/her right arm and hand until sonographer announces that she is finished taking pictures.

Participant set up

(See appendix 10, illustration 1, for station layout.) The test is performed with the participant lying down with the head resting on one or two pillows. A blood pressure cuff (always pediatric size) is placed on the right forearm, 1-2 cm below the bend in the elbow. Attach this cuff to the automatic cuff inflator. The elbow should be slightly bent, palm facing down and fingers relaxed. A small pillow support for the sonographer's hand may be placed next to the brachial artery, where he/she will hold the transducer.

Four ECG leads are attached to the patient's chest to allow visualization of the participant's heart rhythm during the brachial artery scan. Two ECG leads are placed on participant's chest, left side and right side, and two below the participant's collarbones (clavicles), left side and right side. The amplitude should be sufficient to trigger the Rwave capture of frames for the computer. To adjust the amplitude, turn the (Sensi) knob located under ECG on the Sonos 5500.

Before the sonographer starts the brachial scan, she has measured the particpant's blood pressure in the left brachial artery. The systolic blood pressure is recorded on the Brachial Scan Sonographer Worksheet. When the right arm blood pressure cuff is inflated, it will be inflated to a pressure of 200 mm Hg or 50 mm Hg higher than the systolic pressure – whichever is higher - or to a maximum of 250 mm Hg. The pressure is set on the Hokanson Automatic Rapid Cuff Inflator.



Cuff location on lower arm and transducer placement on brachial artery.

Ergonomic Concerns

A critical issue in designing and implementing the brachial ultrasound protocol is the ergonomic stress on the sonographer, particularly in research sites performing more than a few studies a day. We have instituted the following measures to minimize ergonomic stress on the sonographer:

- Try to lay out the equipment in the room to minimize the amount of reaching the sonographer has to do (see appendix 10, illustration 1).
- Adjust chair and table height to minimize reaching and elevation of shoulders.
- Arrange pillows, if desired, to support sonographer's right forearm and elbow (the scanning arm).
- The automatic BP cuff inflator is essential, so the sonographer can focus on the scanning and keeping the transducer still.
- Only do 1-2 studies in a row. It is very important to get up, stretch and walk around for a few minutes between participants.
- Neck, shoulder and back exercises throughout the day are essential. Roll shoulders, stretch upper trapezius, stretch rhomboids, stretch levator scapulae, and stretch the scalleni (neck muscles) to relieve tension.

The Preliminary Scan

Confirm that the Mitchell computer is ready for **Brachial Reactivity acquisition**. (For Brachial Acquisition Tools, see separate Manual.)

Confirm that the **Brachial Program** has been selected on the Sonos 5500. Press **Bmode** on the right control panel to obtain 2-D images of the brachial artery. The sonographer puts ultrasound gel on the transducer and then places the transducer, orientaton point or point of reference pointing toward participant's right shoulder, on the participant's skin surface at an area 2 – 9 cm (ideal is 3.5-5.5 cm) proximal to the elbow crease, to perform the preliminary scan of the right brachial artery. To verify that vessel is an artery and not a vein, she turns on the color and/or the PW Doppler and verifies pulsatile flow.

To expand image on the screen, press **Zoom** on the right-hand control panel**. DGC** levels are adjusted until the image is optimal. Adjust **Focus** on the right control panel.

As the sonographer starts the preliminary scan, she tries to obtain optimal quality images of the four principal boundaries in the artery wall: the media/adventitia boundaries (also referred to as the M-line) in the near border, and the media/adventitia boundaries on the far border. The brachial artery diameter measurements will be made between the M-line on the near border and the M-line on the far border. The sonographer then finds the long axis of the artery. Aspects of an ideal image include:

- The artery lies horizontal on the screen.
- The artery is vertically centered on the screen.
- The straightest segment of the artery is visualized.
- A clear media adventia hypoechoic line – the 'M-line' is well visualized on both the near and far walls over > 50% of the vessel length.
- The lumen is sonolucent – i.e. without 'schmutz' & shadowing in the center of the lumen.
- A readily identifiable landmark is well seen.

The sonographer can adjust the transducer in four "basic" ways to improve the image: angling the transducer, rotating the transducer, "rocking" (toe/heel) the transducer, and applying more or less pressure with the transducer. The participant's arm may also be repositioned if necessary to obtain higher quality images. Since these images need to be maintained for exactly 8 minutes during the test, the position should be comfortable for the sonographer as well. The pillow supports for the sonographer come in handy for this purpose.

It is essential that optimal quality images are acquired with the M-line visible and the artery steady. Do not move up or down the artery or change the angle, once the test has begun! The Brachial Analysis Software works best when images are steady and show the M-line clearly!

The sonographer then presses PW Doppler on the right control panel and places the Doppler cursor in the middle of the artery. Adjust Cursor angle with the knob below the right control panel and line up the Doppler signal so it is parallel with the brachial artery.

It is important to remind the participant that talking may interfere with the scan, and that it is critical not to move during the scan, especially the right arm and hand.
The sonographer states to the participant: *"I am now going to take pictures of your brachial artery. Are you comfortable?"* Then the sonographer states: *"I'm placing cold gel on your arm."* The sonographer now reminds the participant that he/she *may terminate the test if at any time the test becomes too uncomfortable.*

When the sonographer is satisfied with the angle and the location of the images of the artery, two markers are positioned on the image screen to facilitate scanning and making measurements. On left control panel, press **Annotate**, and then press **Baseline**.

Using the trackball, the sonographer then moves the cursor to the middle of the vessel at the center of the segment of the artery, called the "**Area of Interest"** or **AOI.** The area with optimal quality of the near border and the far border and positioned as close to the center of the screen as possible is chosen as the AOI. Try to place the AOI in the portion of the artery where you are most likely to want to measure the artery. Press the key with the* symbol on the left control panel to mark the AOI.

Next, place the cursor on an identifiable "**landmark"** anywhere on the image. Use one of the various directional arrows, → ← ↓ ↑ on the left control panel, to point the arrow to the landmark chosen. Criteria for choosing a landmark include: a) an unusual or distinctive area; b) preferably an area relatively near the center of the screen; c) good landmarks include a small branch off the artery or bright echoes ("ridges") or hollow black spaces. If the artery is scanned consistently during the test without the sonographer moving, the degree marker and the arrow marker will be in the same location at the beginning of the baseline scan until the end of the deflation scan.

The duplex images on the Mitchell computer screen during the examination also help the sonographer to consistently scan the same segment of the artery during the entire test. After the baseline scan, the baseline images stay on the screen to guide the sonographer. Anytime during the deflation scan, she can look at the baseline image on the screen and double check that she is scanning the artery at the same segment and with the same angle during the deflation scan. The baseline images will be there for comparison to all subsequent images.

During the preliminary scan, the sonographer also makes a mental note of the transducer location and angle relative to the participant's upper arm and tries to keep the same location and angle throughout the scan.

The Baseline Scan

The beginning of the baseline scan is indicated by the appearance of the word **Baseline** at the top of the image screen. Sonographer verifies that the participant is comfortable. She also verifies that she is getting high quality images before starting to acquire and record images.

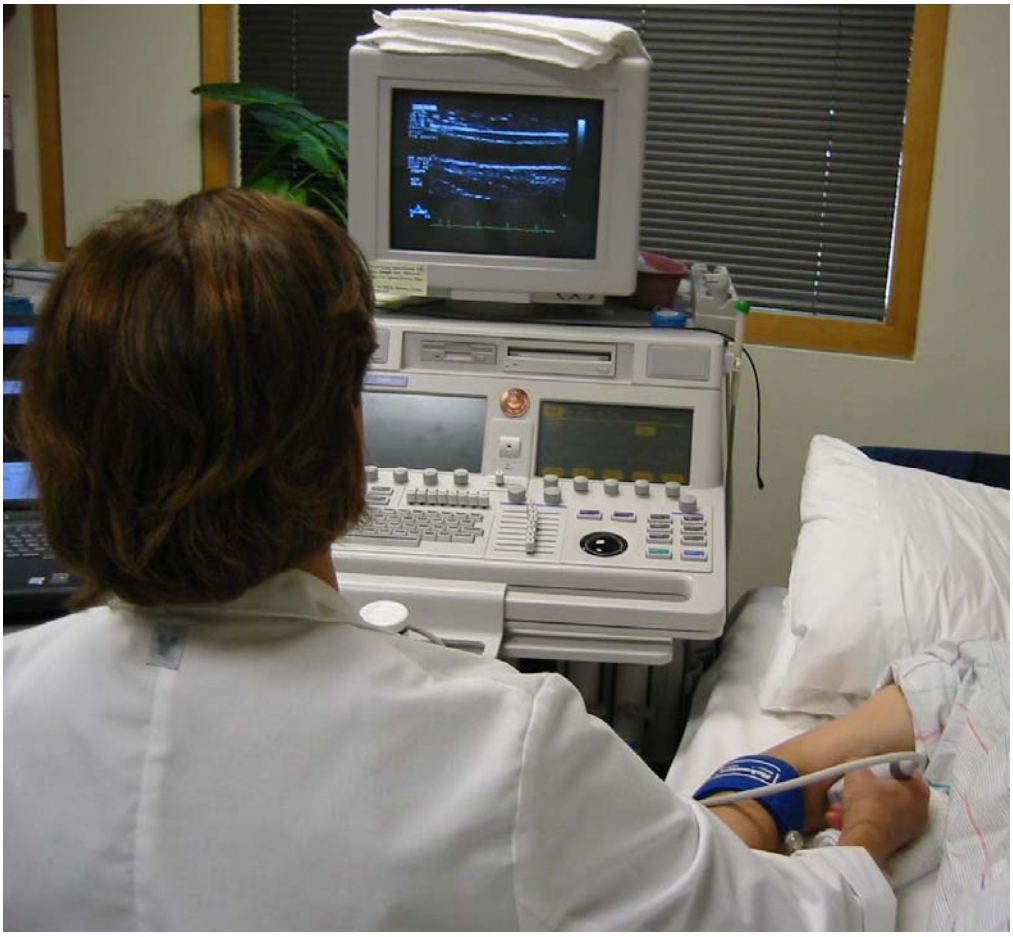
From now on, all the images are acquired on the Mitchell computer only. The image seen on the Sonos 5500 is not recorded on video.

Register the patient on the Mitchell computer by opening the NIHem program.
Then press Register. Register the patient by entering the Participant's initials, the Exam number and the sonographer number. Then press OK.

Press Brachial Reactivity. Now the program is ready to acquire brachial images.

For the **Baseline scan, c**lick Baseline on the Computer to start acquiring BMode images.
After 15 beats of Bmode images, press **PW Doppler** on the Sonos 5500**.** Record 10 beats of PW Doppler signals on the computer, then hit Save Images on the computer.

The baseline images will stay on the screen on the computer, in a duplex format, to guide and help the sonographer during the deflation scan. During the baseline sequence, measurements of the brachial artery diameter from a minimum of 10 cardiac cycles will be made for comparison with the brachial artery diameter of the corresponding segment during the two-minute deflation period.



Sonographer is ready to start the baseline scan.

The Inflation Scan

A few seconds before 1:00, the sonographer warns the participant that "*the cuff will inflate suddenly"* and that the participant will feel a lot of pressure on their arm. The sonographer reminds the participant not to move his/her arm. **At 1:00** exactly, the sonographer inflates the cuff by stepping on the foot pedal connected to the Automatic Cuff Inflator. Immediately thereafter, she presses **Stop** on the VCR on the Toshiba and hits **Save Images** on the computer. It is important to capture 3-5 triggers of Doppler signals @ 6K PRF on the computer to note the **Trigger of Inflation.** She then returns to B-single and continues to obtain 2-D images of the brachial artery during the 5-minute occlusion period.

The occlusion cuff is inflated to 50 mm Hg above the participant's baseline systolic pressure or at least 200 mm Hg for a maximum of 250 mm Hg. The occlusion systolic measurement is recorded on the work sheet.

The cuff remains inflated for 5 minutes and the sonographer stays focused on obtaining optimal quality images. It is crucial to keep arm and transducer steady, and also to keep AOI and landmark in the same position throughout this period. In some participants, the artery will move as the cuff is inflated. Since the two markers placed on the image are frozen on the screen, they will also appear to have moved slightly relative to the artery.

The two markers need not be repositioned.
Often, the artery will return to its original position at the end of the exam, which will be confirmed by the markers reappearing in its original position.

Great care must be taken to image the same arterial segment during the occlusion period as during the baseline period, to help sonographer continue to image the same arterial segment during the deflation period to the greatest extent possible.

During this 5-minute occlusion period, the sonographer announces to the participant: *"4 more minutes until deflation,... 3 more minutes,… 2 more minutes, etc."*

At 15 seconds before deflation, the alarm on the manual timer will sound. The sonographer then presses **Start** on the VCR on the Toshiba, and starts acquiring PW Doppler images on 6K PRF with duplex imaging. She also hits **Deflation** on the Annotation menu.

At 10 seconds before deflation, she clicks on **DEFLATION** on the Computer, to start acquiring the deflation sequence of images on the computer, while announcing to the participant that the cuff will be deflated in 10 seconds. The sonographer also reminds the participant that he may feel pins and needles or a warm or cold feeling in the hand.

The Deflation Scan

The sonographer deflates the cuff at exactly :00 by stepping on the foot pedal of the automatic cuff inflator. It is important to acquire images with PW Doppler on the computer to capture **the trigger # of deflation.** Immediately thereafter, she switches the PW Doppler from 6K PRF to 12K PRF and captures 3-5 beats on 12K PRF as well as hits **Save Doppler** on computer. She then immediately switches to B-single and records optimal quality 2-D images of the brachial artery for the remaining 2 minutes on the computer as well as on the VCR on Toshiba.

The two minutes immediately following cuff deflation is the most critical time of the entire exam. This is when the flow-mediated dilation occurs. Every effort should be made to avoid movement of sonographer transducer or the participant's arm. The sonographer should therefore announce to the participant: *"It is critical that you not move your arm for the next two minutes."*

The artery should be horizontally displayed on the screen. Adjust transducer by doing toe or heel, if artery is displayed diagonally. Every attempt should be made to have artery vertically centered with the anterior and posterior intima parallel to each other. The artery will be in the focal zone, and the lumen should be free of echoes as much as possible. Fine-tune the image by rotating and/or angling transducer to optimize near wall and far wall. Ideally the artery will "look like a railroad track" with intima, M-line, and adventia fully visible!

At end of scan, the sonographer announces *"End of scan – you may move your arm, hand and fingers!"* Click on **Save Images** on the Computer. Press **STOP** on the Toshiba VCR.

Test Wrap Up

A final blood pressure determination is made in the left brachial artery by pressing the Start button on the Dinamap. Write down blood pressure, pulse and mean arterial pressure on the **Endothelial Function Sonographer Worksheet.** Determine the probe placement distance on the participant's arm. Recheck the probe angle. Record information on the work sheet. Also record the start and ending times of the video recording. Write any additional comments on the **Log-In Sheet.** Remove the electrodes, the BP cuff on the left arm, and the cuff on the right arm.

Before participant leaves the room, have him fill out the Discomfort Survey at the bottom of the Endothelial Function Participant Worksheet (see appendix 2).

He/she should also be told the following: *"You should be aware that about 0.5% of participants develop painless red spots on the arm after the test, which resolve on their own within a few days. This is harmless, but if it occurs, you should call the sonographer, so that we can track the frequency and the time to resolution".*

Thank the subject for participating.

Appendix Item 1

The Vascular Function Tests – Handout for Participant

The Framingham Study's Noninvasive Cardiovascular Testing Station

In the cardiovascular testing station you will receive four tests that noninvasively examine your heart and blood vessels' structure and function. None of the tests involve radiation. You will receive the following tests:

1. Blood pressure

- The sonographers will carefully measure your blood pressure while listening with headphones.

2. Arterial tonometry

- The sonographer will hold a flat pressure-sensing device (the tonometer) against the superficial pulses in your arm, leg and neck for approximately a minute at each of these four sites. This approach allows us to assess blood vessel stiffness. **Details of the test are provided on the reverse side.**
- At the very end of all 4 tests, the sonographers will measure the distances between the 4 sites where the recordings were taken.

3. Echocardiogram

- The sonographer will hold an ultrasound transducer at several points over your left chest. The echocardiogram uses sound waves to take a picture of your heart. The test measures the heart's size and function.

4. Brachial ultrasound

- The sonographer will hold an ultrasound transducer over your left arm artery (brachial) and measure the size and the flow in the artery at baseline. Then the sonographer will inflate a blood pressure cuff over your lower arm for 5 minutes. After the cuff is released the sonographer will take a picture of the size and blood flow in the artery for two minutes after the cuff is released. The test measures the ability of the brachial artery to get bigger (dilate) when exposed to increased blood flow; this ability is a measure of the health of the blood vessel lining. The test may cause temporary numbness and tingling. Rarely subjects develop painless red spots, which disappear in a few days. **Details of the test are provided on the reverse side.**

- **If you have a very abnormal echocardiogram test the results will be sent to your physician.** Since the test is performed in a research context, and read without any knowledge of your symptoms or history, the results would need to be interpreted by your doctor in the context of your clinical history.

- **The Brachial ultrasound and arterial tonometry are solely used for research purposes. They are not used in clinical practice or to guide medical decisions. For this reason we will not be sending the results to your physician.**

If at any point during the testing you are uncomfortable and would like to terminate the tests, please tell the technicians.

Thank you for your support of the research at The Framingham Study.

If you have further questions about the noninvasive tests please contact Dr. Emelia Benjamin by leaving a message at 508-935-3445 or 617-638-8968 or Dr. Ramachandran S. Vasan at 508-935-3450.

The Framingham Study's Noninvasive Cardiovascular Testing Station

The Arterial Pressure Waveform Test (tonometry)

How is the test performed?

- Measurements are made by gently pressing the tip of flat pressure sensing device (the tonometer) against the superficial pulses in the arm, leg and neck for approximately a minute at each of four sites. This device records the pressure waveform that is associated with each pulse or heartbeat.
- Next, the distance from the base of the neck to each of the pulse sites is measured.
- You will be asked to lie quietly during this phase of the testing. There should be no discomfort. This test has been performed safely in thousands of patients.
- At a later date, using a computerized analysis, we will examine the shape of the pressure waveforms and calculate the speed at which pressure waves travel through the large arteries.

Why are we doing this test?

- The arterial pressure waveform test is a noninvasive method to evaluate the stiffness of the large arteries.
- This test will allow us to evaluate the relationship between cardiac risk factors, arterial stiffening and the development of cardiovascular disease.

The Brachial Artery Vascular Reactivity Ultrasound Test

For this test you will be asked to do the following:

- Have an ultrasound picture taken of the artery located in your upper arm.
- Have a blood pressure cuff inflated on your lower right arm for 5 minutes.
  When the cuff is inflated your arm may feel like it is going to sleep or numb.
- After the cuff is released we will take pictures of your artery for 2 more minutes.
  When the cuff is released your arm may feel pins & needles, warm or cold.
- At a later date we will make computer measurements of the amount that your artery expands after the cuff was released. The changes are very small, so we cannot tell you the results while we are doing the study.
- ***To get the best information it is very important that you not move when we are taking the ultrasound pictures.***
- This noninvasive test has been performed in thousands of research participants safely.
- Approximately 0.5% of participants develop painless red spots on the arm after the test, which resolve on their own within a few days. This is harmless, but if it occurs please call the sonographer (508-935-3445 or 508-935-3406) so we can track the frequency & the time to resolution.

Why are we doing this test?

- This test is designed to look at the function of the blood vessel lining.
- We are doing the test to understand if the results relate to risk factors for heart disease and to understand if the results will help predict the development of heart disease and stroke.

Appendix Item 2

FHS Vascular Testing Participant Worksheet

&

FHS Brachial Scan Sonographer Worksheet

Vascular Testing

|7|0|2|1|4| FORM NUMBER OMB NO=0925-0216

Exam 1
Framingham Study Vascular Function Participant Worksheet

	Keyer 1: _____	Keyer 2: _____
0 1 9 If yes, ☞ discontinue brachial	**Do you have active Raynaud's disease, as manifested by daily attacks of Raynaud's currently blue fingers or ischemic finger ulcers?** (0=No, 1=Yes, 9=Unknown)	
0 1 2 3 8 9 If 1(right), ☞ discontinue brachial If 2(left), ☞ BP on right	**Women Only: Have you had a radical mastectomy on right side?** A radical mastectomy is the removal of the breast, associated lymph nodes, and underlying musculature. Does NOT include lumpectomy or simple mastectomy. (0=No, 1=Yes, right, 2=Yes, left, 3=Yes, both, 8=Male, 9=Unknown)	
0 1 9 if yes fill ☞	**Have you had any caffeinated coffee, caffeinated tea, or other caffeinated drinks in the last 6 hours?** (0=No, 1=Yes, 9=Unknown) |__|__| How many cups? (99=Unkown)	
0 1 9	**Have you eaten anything else this morning?** (0=No, 1=Yes, 9=Unknown)	
0 1 9	**Have you had a fat free cereal bar in clinic?** (0=No, 1=Yes, 9=Unknown)	
0 1 9 if yes fill ☞	**Have you smoked cigarettes in the last 6 hours?** (0=No, 1=Yes, 9=Unkn) |__|__|:|__|__| If yes, how many hours and minutes since your last cigarette? (99:99=Unknown)	

Tonometry

|__|__|/|__|__|/|__|__|	**Date of tonometry scan?** Mo/Day/Yr
|__|__|__|	**Tonometry Sonographer ID**
0 1	**Was tonometry done?** 0 = No, test was not attempted or done 1 = Yes, test was done, even if all 4 pulses could not be acquired and recorded

Updated 5/21/03

Brachial Scan

|7|0|2|1|5| FORM NUMBER OMB NO=0925-0216

|__|__|/|__|__|/|__|__|	**Date of brachial scan?** (mo/day/yr)
|__|__|__|-|__|__|__|	**Brachial Video CD number**
|__|__|__|	**Brachial Sonographer ID**
|__|__|.|__|	**Room temperature** (Celsius)
|__|__|__|	**Mean systolic baseline blood pressure**
|__|__|__|	**Cuff inflation pressure** (Baseline SBP + 50 or 250)
0 1 2	**Was brachial protocol completed?** (Determined at time of scan or at time of interpreting) 0=No: protocol was not completed i.e. none of the 3 parts completed of Baseline, Doppler, Deflation 1=Yes: protocol was done and completed i.e. all 3 parts completed of Baseline, Doppler, Deflation 2=Yes, Partial: protocol was partially completed i.e. 1 part of 3 completed, 2 of 3 completed of Baseline, Doppler, Deflation
If no (0) or partial (2) ☞	Brachial scan deviations: **circle ALL that apply** **1**: Subject refusal **2**: Subject discomfort **3**: Time constraint **4**: Equipment problem (if not #5 or #6), specify _____ **5**: Foot pedal problem/cuff sequence problem **6**: Doppler problem **7**: Other, specify _____
|__|__|__|	**Interpreter ID** (mo/day/yr)
|__|__|/|__|__|/|__|__|	**Interpretation date**
0 1 2 9	**Baseline measurable?** (0=No, 1=Yes, 2=Suboptimal, 9=Unknown)
0 1 9	**Do you see occlusion?** (0=No, 1=Yes, 9=Unknown)
0 1 9	**Do you see normal release?** (0=No, 1=Yes, 9=Unknown)
0 1 2 9	**Deflation measurable?** (0=No, 1=Yes, 2=Suboptimal, 9=Unknown)
0 1 2 9	**OK to calculate FMD?** (0=No, 1=Yes, 2=Suboptimal, 9=Unknown)
0 1 9	**Significant rhythm disturbance** (0=No, 1=Yes, 9=Unknown)
|__|__|__|-|__|__|__|	**Measurement Video CD#**
|__|__|__|-|__|__|__|	**Brachial data floppy #**

Not for Data Entry.

Distances:

_____Carotid(mm) _____Brachial(mm) _____Radial(mm) _____Femoral(mm)

(Added 10/02/02, version# not changed)

Appendix Item 3

FHS Generation 3 Exam 1 Log Book Sheet For Tonometry, Brachial and Echo Tests

GENERATION 3 EXAM 1 LOG BOOK SHEET FOR TONOMETRY, BRACHIAL AND ECHO TESTS

|7|0|2|1|7| FORM NUMBER OMB NO=0925-0216

Date of Clinic Visit ____ - ____ - _____ **Room #** **108** **110**
Mo Day Yr

TONOMETRY

Test done? ☐ **yes** (test was done, even if all 4 pulses could not be acquired and recorded) ☐ **no** (test was not attempted or done)

If no, why: Circle all that apply
1. Subject refusal
2. Subject discomfort
3. Time constraint
4. Equipment problem, specify _____
7. Other, specify _____

_____ **Sonographer ID#**

|__|__|__|-|__|__|__| **Video CD#**

____/____/____ **TONOMETRY test date** if different from Clinic Date above.

ECHO

Test done? ☐ **yes** (test was done, even if recorded on video only) ☐ **yes, partial** (i.e. only apical OR only parasternal images were acquired) ☐ **no** (test was not attempted or done)

If no or partial, why: Circle all that apply
1. Subject refusal
2. Subject discomfort
3. Time constraint
4. Equipment problem, specify _____
7. Other, specify _____

_____ **Sonographer ID#**

|__|__|__|-|__|__|__| **SVHS#**

____/____/____ **ECHO test date** if different from Clinic Date above.

MD overread required: ☐ **yes** ☐ **no**

BRACHIAL

Test done? ☐ **yes** (test was done, even if problems with Baseline, Doppler, and/or Deflation) ☐ **no** (test was not attempted or done)

If no, why: Circle all that apply
1. Subject refusal
2. Subject discomfort
3. Time constraint
4. Equipment problem, specify _____
5. test contraindication
7. Other, specify _____

_____ **Sonographer ID#**

|__|__|__|-|__|__|__| **Video CD#**

____/____/____ **BRACHIAL test date** if different from Clinic Date above.

Updated 5/21/03

Appendix Item 4

FHS Vascular Station Flowsheet

With Succinct Description of

Vascular Reactivity & Tonometry Tests

FHS Vascular Protocol Flowsheet

Phase	Time, mins Total	Phase	Worksheet	Agilent Sonos 5500	Cardiovascular Engineering Computer	Verbal	Cuff, Timer, Light, Table
Set up	0-4	4	• Ask participant to read description sheet before the test • Fill out logbook sheet • Fill out participant worksheet • Place BP cuff on LA	• Machine on, Preset Cardiac Exam, select Probe Center S-3 Transducer • Enter name, ID • Enter sonographer's ID • Apply 4 ECG leads (R &L collarbone, L & R ribcage) • Verify ECG is appropriate amplitude	• Machine on, [Register] • Enter pt initials, ID type & ID • **Double check the subject ID against the subject's FHS ID** • Study date (default) • Study type (enter 3-_____) • Sonographer #	• **Verify read test description** • Ask eligibility questions • Ask subject to lie supine & rest • Ask other work-sheet questions	• If wearing shorts, have subject remove shorts in dressing room • If dressing gown tight, remove sleeves • Adjust automatic exam table to correct height
Blood Pressure	4-6	2	Enter systolic BP on Work sheet		• Click Waveforms on toolbar • Click BP 1 on bottom toolbar	• "I am inflating the cuff."	• Make sure cuff is not touching pt. chest.
Acquire Tonometry	6-14	8			• Click BRA on bottom toolbar • Find BA & keep L finger on pulse • Place tonometer over artery, adjust pressure, maximize amplitude • Save waveform, left click • Repeat for radial, femoral & carotid [Rad] [Fem] [Car] • Close	• 'Now I'm checking your pulses with a blunt instrument' Before Femoral • 'I'm going to check the pulse at the top of your leg'	• Put on gloves
Perform echocardiogram per protocol. [25-30 minutes]							
Return to brachial protocol							

FHS Vascular Protocol Flowsheet

Brachial Setup	40-45	5		*If subject eligible* • Find artery long axis, find straightest segment of the artery • Annotate baseline • Color Doppler verifies artery • Zoom on artery • **Find best image** • Adjust FOCUS closest to near wall • **Annotate AOI & landmark** • Set up PW Doppler	*If subject eligible* • Click [Brachial Reactivity]	*If eligible* • 'I'm placing cold gel on your arm' • Describe test • **'To enhance test, during scan it's critical that you not move'** • **If at any time the test becomes too uncomfortable ask terminate test**	*If subject eligible* • Apply cuff R forearm 3 cm distal to antecubital fossa • Turn off room light
Baseline	45-47	2		• **Verify that you are comfortable** • Record BMode 15 beats • Switch to PW Doppler • Verify PW position • Record flow for 10 beats	• Verify good image quality before acquiring • Hit [Baseline] to acquire 15 triggers B mode • 10 beats of Doppler flow	• **Verify 'Are you comfortable?'** • 'I'm taking pictures of your artery'	•
Inflation	47-52	5		• **Return to BMode,** keep AOI & landmark steady for 5 minutes • **Keep arm & transducer in same position**	• 3-5 triggers after inflation hit [Save images] • No images are acquired during occlusion • Hit [Deflation] 5 sec before deflating the cuff	• 'Now I'm going to inflate the cuff on your arm for 5 mins' • Announce minutes • 'Your arm will feel like it is going to sleep'	• **Pressfootpedal &inflate** cuff to 200 mmHg, or 50 mm above pt's SBP • Computer timer rings 00 secs pre-deflation
Deflation	52-54	2		• **Keep transducer in same position** • **Annotate deflation** • Switch to PW Doppler • Deflate cuff @-:00 seconds • Record flow for 10-15 beats • Turn off Doppler: go to BMode • Record brachial images till 2 minutes post.	• 10-15 triggers Doppler • Record images for 2 minutes • Computer saves images automatically	• 'I'm about to deflate the cuff, you will feel pins & needles warm/cold' • **'It's critical that you not move your arm for 2 minutes**	• **Press footpedal & deflate cuff @:00**
Tonometry Wrap up	54-56	2	• Measure distances to pulse sites • Record on worksheet			• 'Now I'm going to measure the distances to your pulses'	Take off gloves
Finish	56-60	4		• Wipe gel off subject	• Hit [Distances] • Enter distances & hit [OK]	• **Review rare 'red spots'** • **Thank subject for participation**	• Turn on room light
Change gloves & wash hands after each subject **At end of clinic save to 2 CDs - Verify image CD ok on brachial**							

Appendix Item 5

FHS List of Rules for

Measuring Brachial Artery Ultrasound Images

FRAMINGHAM HEART STUDY
List of Rules for Measuring Brachial Artery Ultrasound Images
Milan Sonka's Program

Conversion

- Review the images and decide which will be the first and last image. Enter the frame numbers in appropriate boxes. Do the same for both **baseline & deflation** images.
- Annotate baseline versus deflation sequence.
- Include **"Inflation trigger"** and **"Deflation trigger"** in the sequence you save.
- **"Inflation trigger"** and **"Deflation trigger"** frame is the frame where Doppler signal first demonstrates reduced flow and augmented flow.

Training

- Reader should sit straight in front of the screen, not at an angle!
- Open baseline images as well as deflation images simultaneously and place them next to each other, baseline images to the left on the screen, deflation images to the right on the screen.
- **Baseline study and deflation study should be measured on the same day.** (Only exception is during data cleaning, if only baseline study or deflation study needs to be remeasured.)
- Review the images first, before you decide which image you want to train on.
- Try to choose **one of the 5 best frames for training** - for **baseline** images.
- Choose **one of the 5 best frames at 1:00 for training post deflation** - for **deflation** images.
- When all frames are equal, train on the first of the "good" frames.
- If results are not accurate when training on the first few frames, go several frames forward (10-50) and train on frame that will give the most accurate results.
- Try to have "training box" include AOI (area of interest), if possible.
- However, if reader decides that another segment is more accurate, train on this segment.
- Try to leave the "training box" the default size, if possible.
- **DO NOT reject the frame you have trained on.**
- Train on an image where "confidence level" is 70% or higher.
- When reviewing or playing, change the speed of the frames playing to 1 FPS or 5 FPS. (Default is 10 fps.)
- Retrain if you have less than 70% of frames measured correctly.
- Always adjust the measuring lines to measure the "M-line", the line between the adventitia and the intima (see figures 1 and 2).
- If measuring line jumps to the intima, adjust "near border" or "far border". Only when the M-line is **not** measurable, measure the intima, but ONLY when you can consistently measure the intima throughout the baseline and the deflation studies.
- "Launch" the measuring program once, with measurements. Then review once and look for accuracy and confidence level.
- If results will improve, make "training box" smaller, particularly if the edge detection is jumping because of a bifurcation or "gap" in the intima.
- **Train on the same segment for baseline & deflation images. Extremely important!**

- The baseline diameter and the beginning of the deflation diameter should measure within 0.20 mm. Flow-mediated dilation is calculated from baseline images and peak dilation of deflation images.
- **Flow-mediated dilation is calculated from baseline images and peak dilation of deflation images.**

Measuring

- "Launch" the measuring program when satisfied with training. **DO NOT STOP** during the launch, unless you plan on retraining. Adjust speed to 1FPS or 5 FPS. Watch measuring lines carefully.
- Then look at the summary and the outliers. Reject all Doppler frames.
- Go back to frame 1 and play the program frame by frame. Reject outliers or remeasure , if frames are measurable. (Use Control X on the keyboard to reject. Control Z will undo rejection.)
- If more than 70% of frames seem to be measured accurately, reject frames with "wrong" lines.
- If less than 70% of frames seem to be measured accurately, retrain (= too many outliers!).
- **Measure at least 10 frames for baseline sequence.** It is OK to reject "bad" frames and not edit them, if you already have 10 measured frames.
- If you have lots of frames with wrong measurements, reject them. Then manually (black person icon) edit every third frame, if they are measurable.
- When done measuring, look at report (see figure 3). Make sure 70% of frames are measured, either by program or manually.

Rejecting

- When reviewing or playing, keep mouse on STOP button and write down frame numbers of the frames you want to reject. i.e. frames with Doppler images.
- Reject all frames where Doppler is turned on.
- Before rejecting a frame, decide whether the "lines" are measuring accurately or not.
- Do not reject "outliers" where the measuring lines are accurate.
- Do not reject a frame because the diameter is different from other frames. Always go back and look at the accuracy of the measuring lines.
- Do not reject the frames with confidence level less than 70% if the measuring lines look accurate. (Let Marty Larson make this decision later.)
- The diameter of the artery on the first few frames of deflation should be similar to the baseline diameter, within 0.1 mm.
- If more than 5 continuous frames are rejected, measure manually (black person icon)every third frame.
- **DO NOT reject more than 30% of frames.**

Manual measurements

- Manual measurements are made by clicking **the black person icon.**

- It is preferable to go with the computer generated lines. However, if it is more efficient to measure manually 10-15 frames than to retrain multiple times, do so.
- For intermittently rejected isolated frames, there is no need to measure manually.
- When multiple frames in a row have been measured by the computer in an unsatisfactory way, manually measure every third frame.

Training box – ROI

- Training box should preferably be used in its default size (horizontally around 7-8 millimeters).
- However, if it is obvious while training that there is a segment where the computer lines "jump" off the appropriate borders, it is reasonable to narrow the ROI.
- The default ROI is 155 pixels long and therefore measures 115 diameters. It is reasonable to narrow the ROI to as little as 55 pixels (1/2 of default ROI).

Saving

- **Save study, with measuring lines** on the frames, to D:drive - Shuxia's folder and Birgitta's folder.
- **Save data in a separate data file,** on D:drive, then on two floppies on A:drive; one to give to data people and one as a back-up for our own file.

Measuring difficult studies

- When measuring difficult studies, measure manually. Click on the black person icon, and manually draw the measuring lines on the near border and the far border.
- **Only measure images that are measurable.** If baseline images are measurable, but deflation images are **NOT** measurable (participant moved, sonographer moved, artery moved, etc.), measure the baseline sequence and **DO NOT** measure the deflation sequence. **Baseline diameters alone may be prognostic.** (Make a note to data people that deflation sequence is missing.)
- Repeat measuring manually every other two frames throughout the study where diameter is measurable.
- If possible, try to measure as many frames as possible around 60 seconds post deflation (5 frames before 1:00 post deflation, and 5 frames after 1:00 post deflation).

Optimal Images

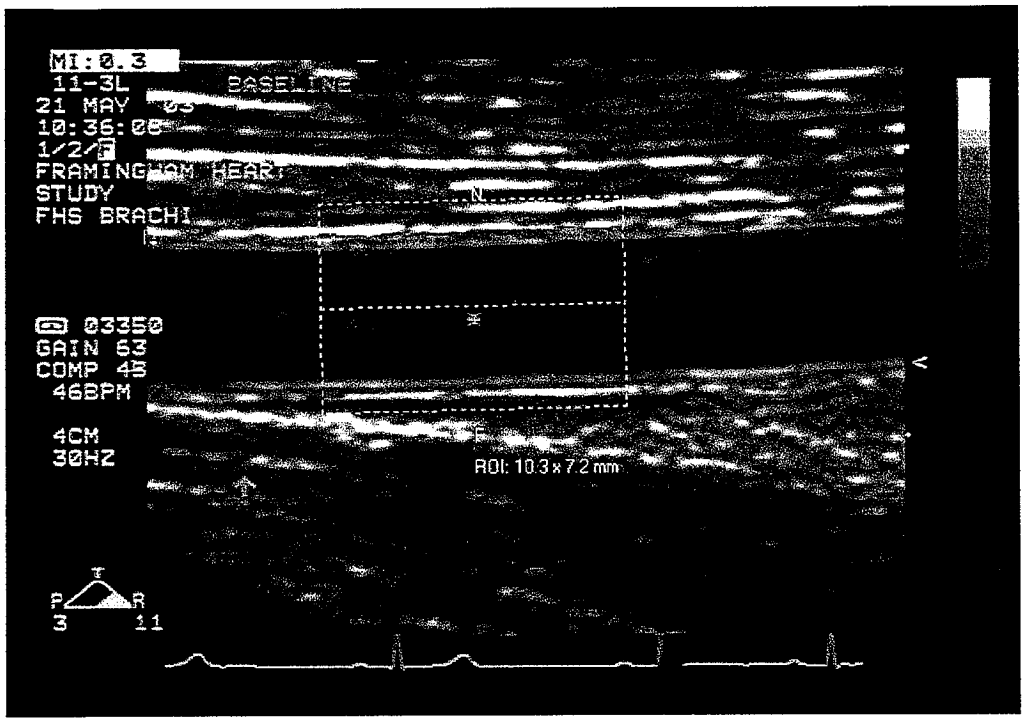


Fig 1. – Baseline sequence. Measuring lines (purple lines) are in the correct place on the M-line between the intima and the adventitia.

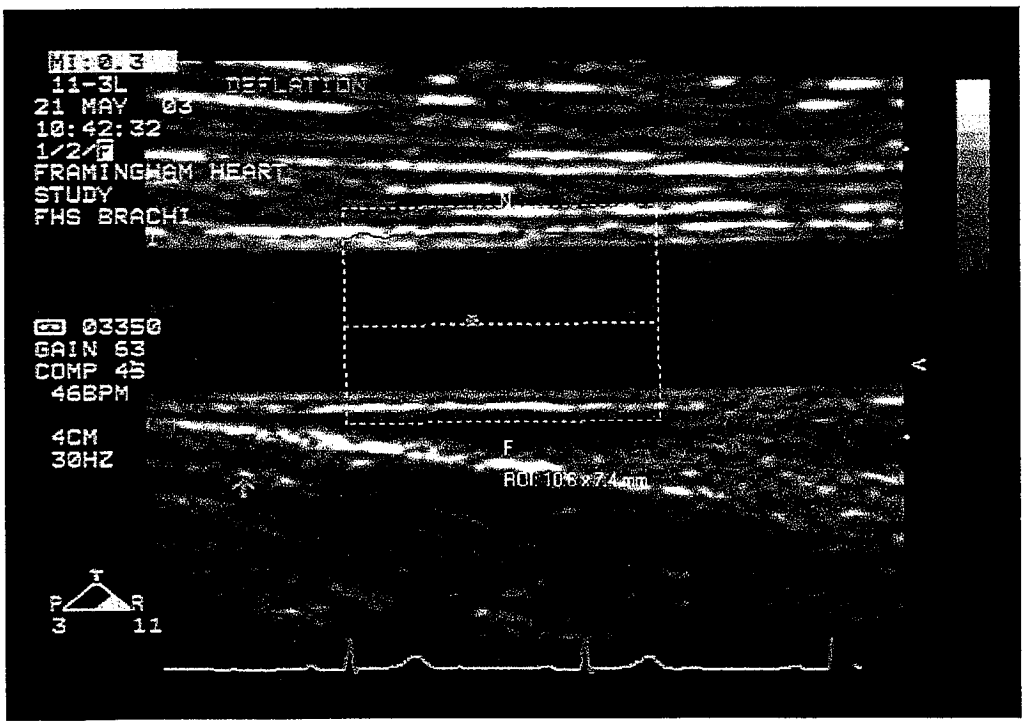


Fig 2. – Deflation sequence. Measuring lines (purple lines) are in the correct place on the M-line between the intima and the adventitia.

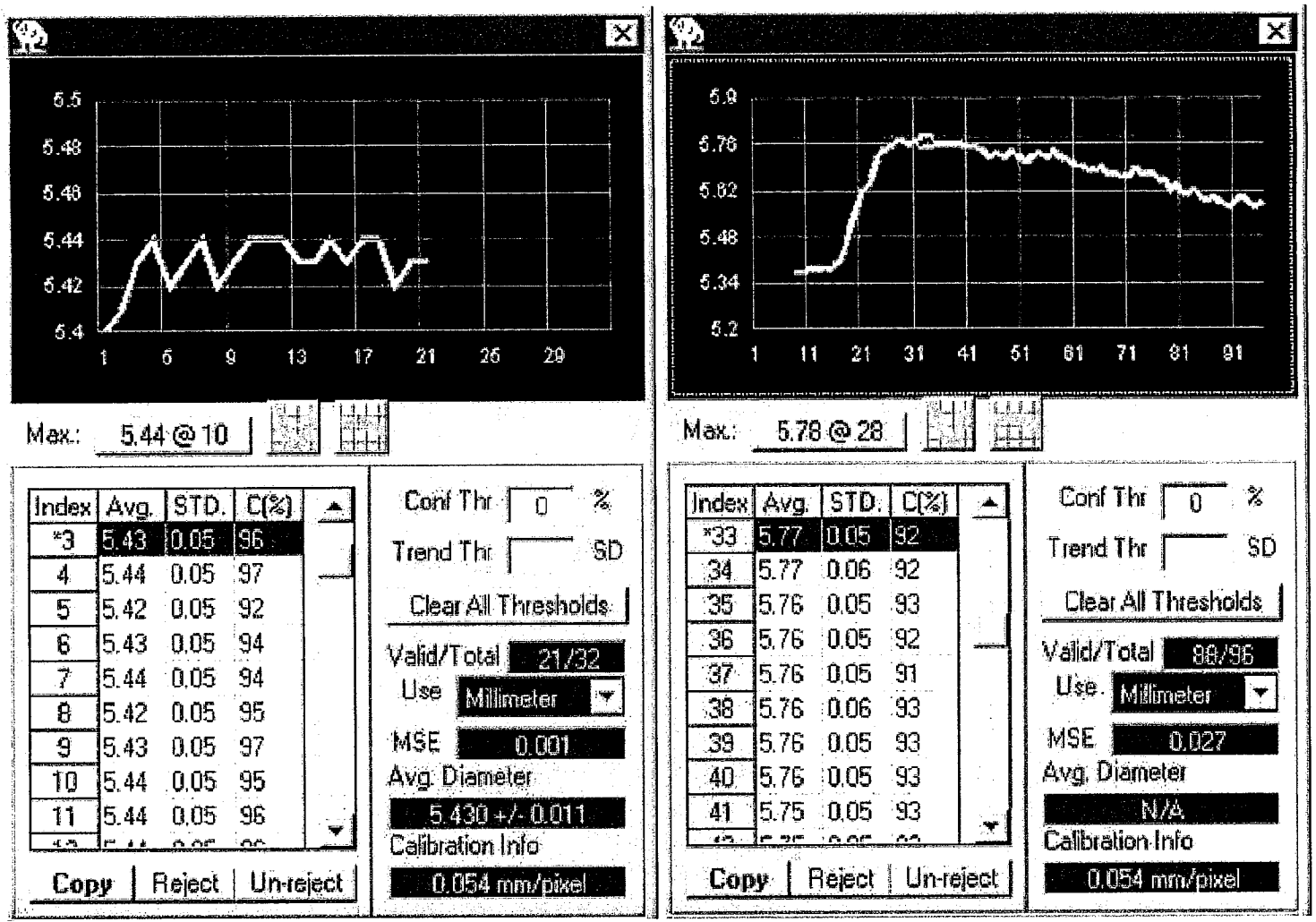


Fig 3. – Report of baseline and deflation measurements. Flow-mediated dilation is calculated from baseline images and peak dilation of deflation images.

Suboptimal Images

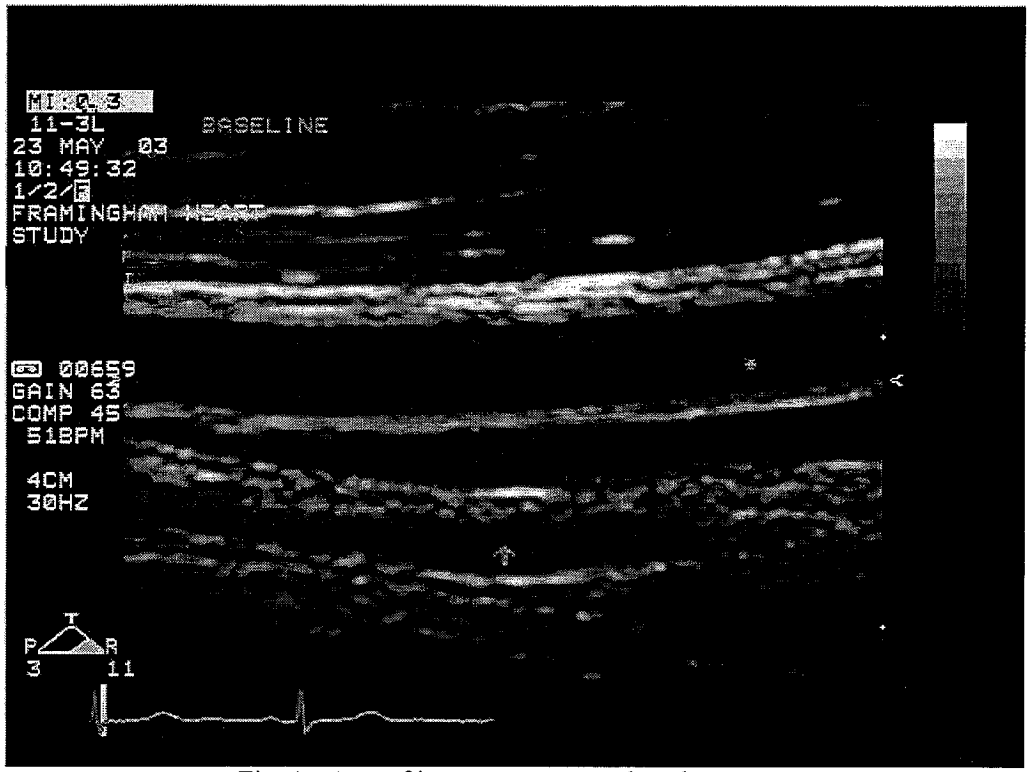


Fig. 4 – Area of interest not centered on the screen.

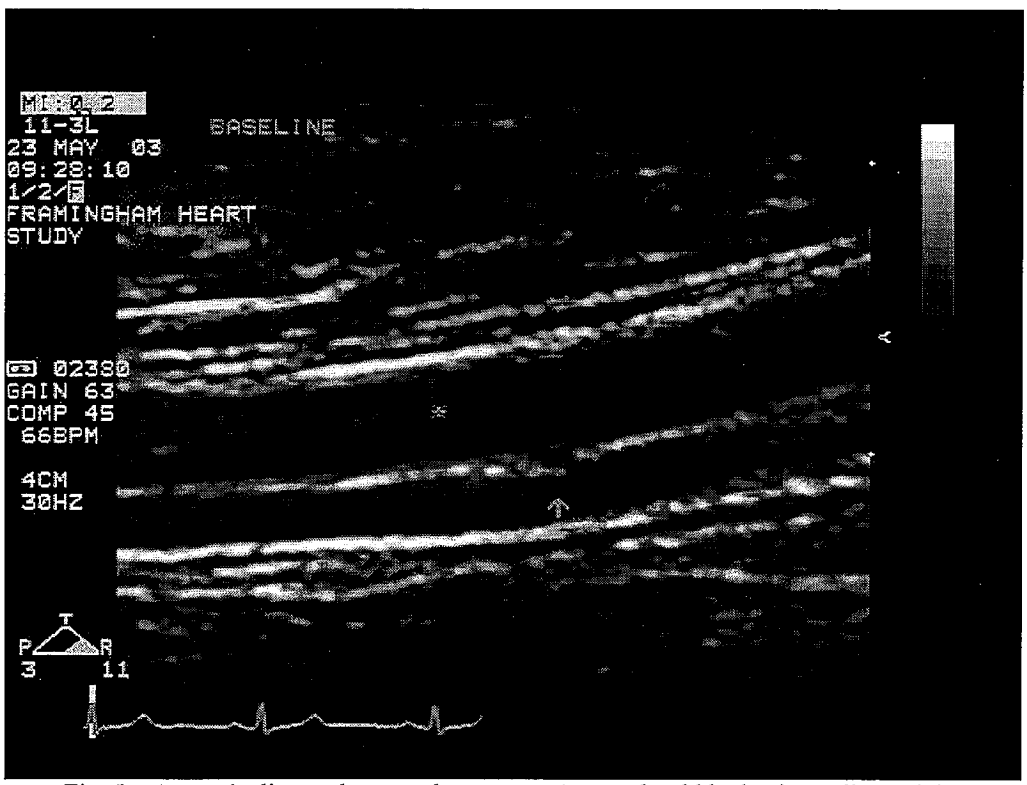


Fig. 5 – Artery is diagonal across the screen. Artery should be horizontally straight.

Suboptimal Images

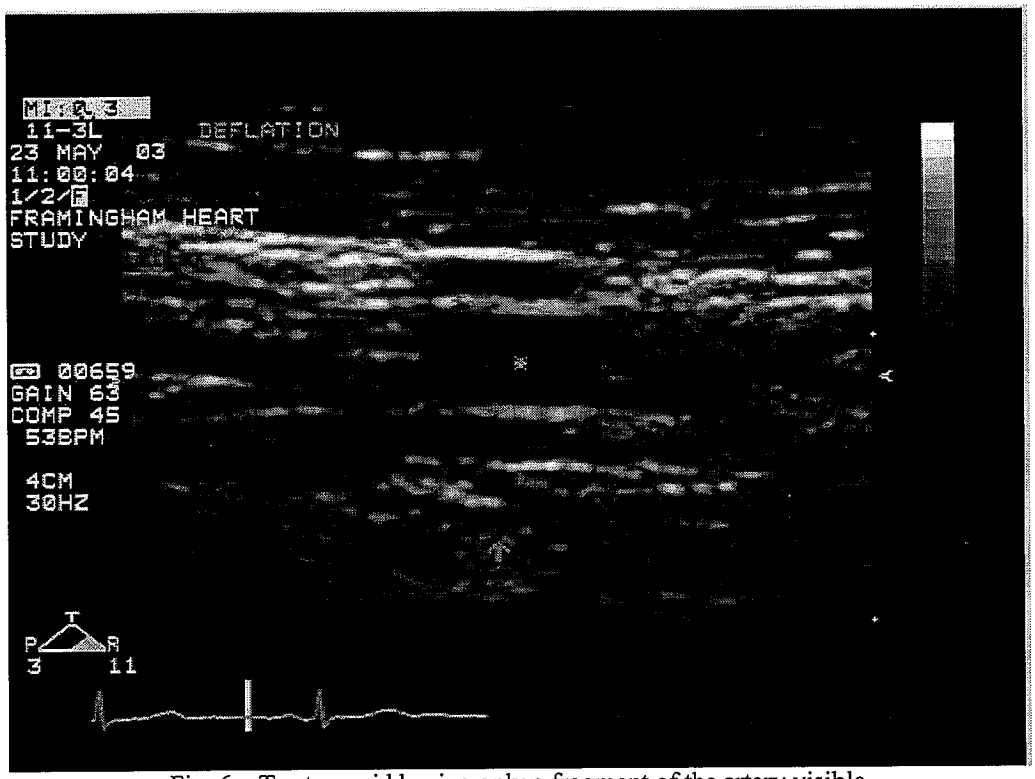


Fig. 6 – Try to avoid having only a fragment of the artery visible.

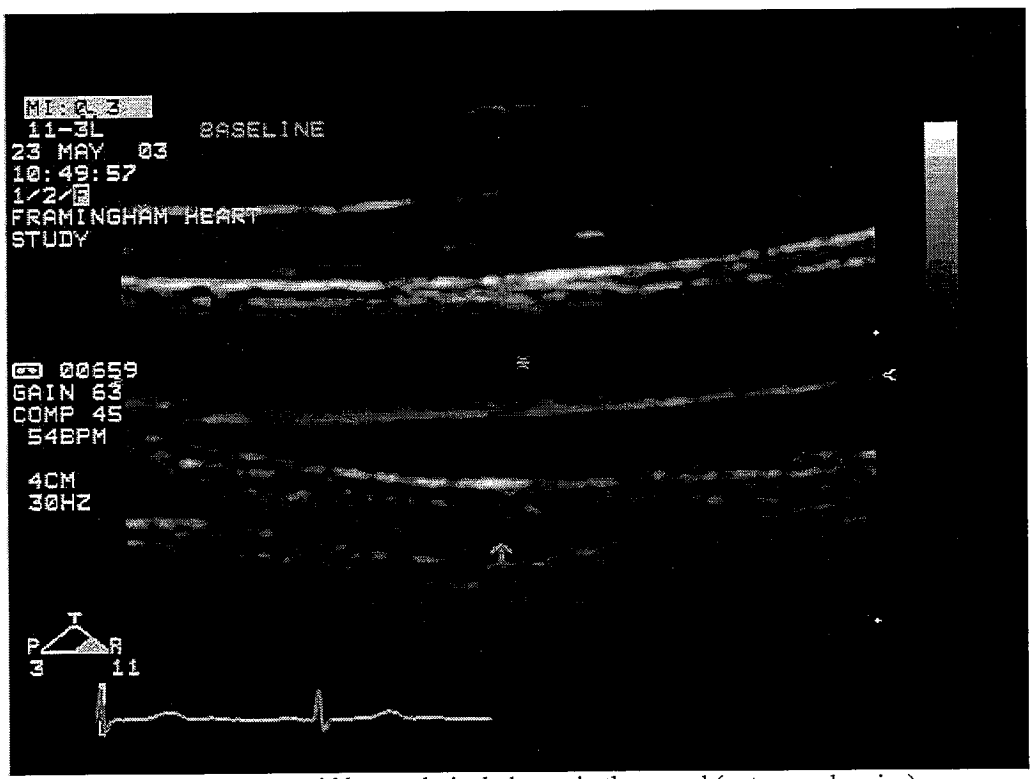


Fig. 7 – Try to avoid hypoechoic darkness in the vessel (not enough gains).

Suboptimal Images

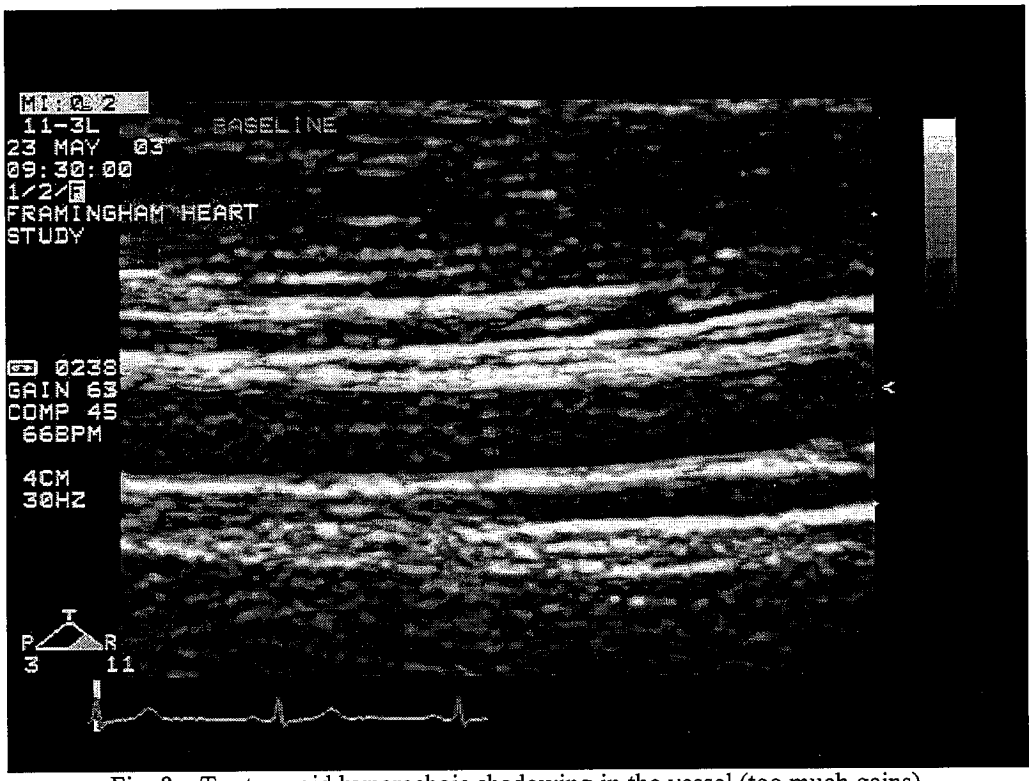


Fig. 8 – Try to avoid hyperechoic shadowing in the vessel (too much gains).

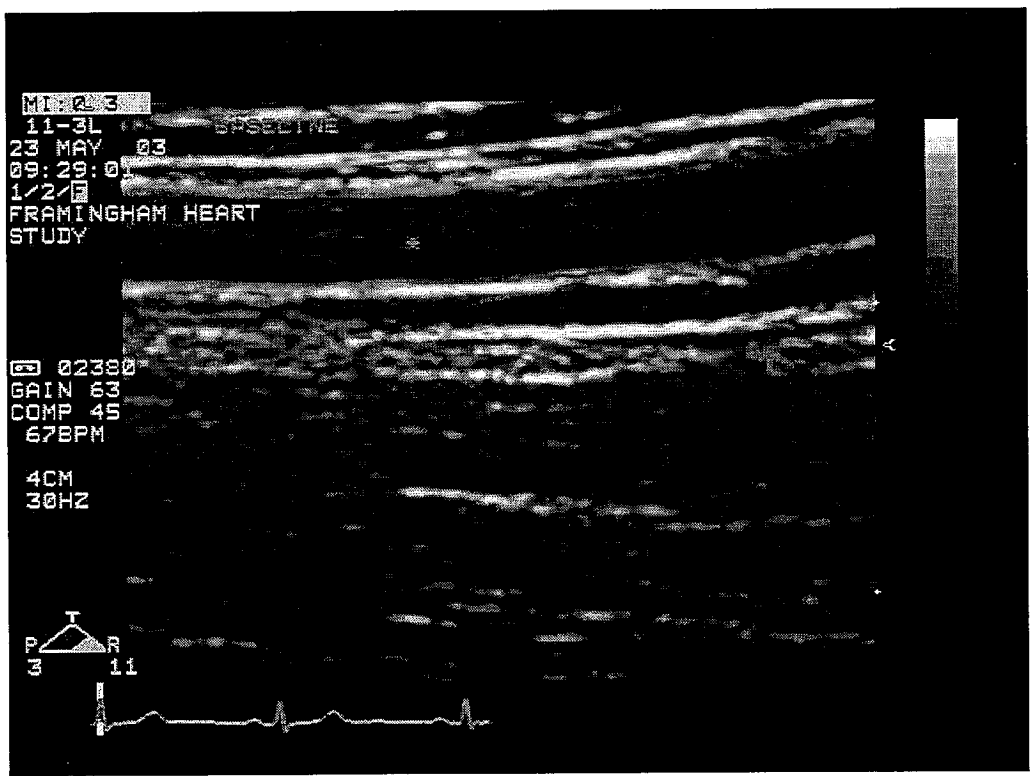


Fig. 9 – Try to avoid imaging the vessel too superficially. Chosen segment is also slightly curved.

Suboptimal Images

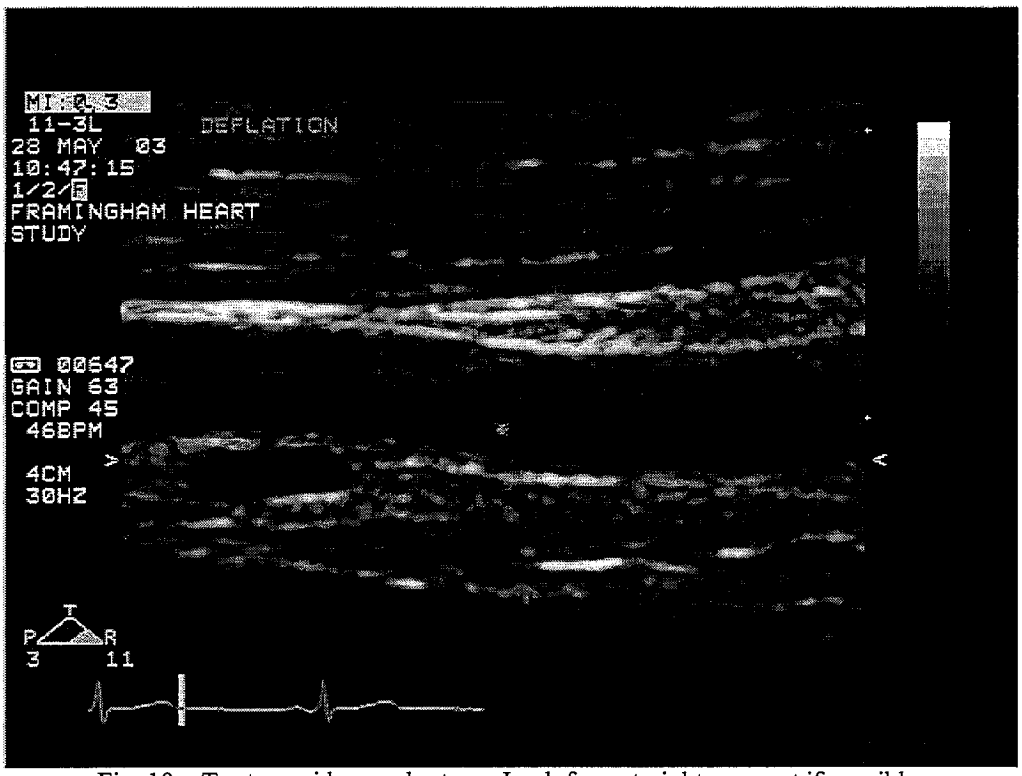


Fig. 10 – Try to avoid curved artery. Look for a straight segment if possible.

Appendix Item 6

FHS Data Cleaning Procedures

Gen 3 Brachial Cleaning:

This is documentation on how to clean the brachial data. Each brachial record is made up of two components: a quantitative part and a qualitative part.

- The **quantitative** part consists of brachial diameter measurements made offline by the sonographers using automated edge detection software. The measurement data includes information about the diameter and time because the brachial diameter is measured at baseline and for two minutes post-deflation.
- The **qualitative** part includes subject data recorded by the sonographers at the time of the test on paper forms entitled the "*Endothelial Function Participant and Sonographer worksheets.*" The qualitative data is entered into the Ingres database by two keyers. The qualitative information includes subject eligibility criteria, subject blood pressure, etc.

Joining together these two parts forms a complete record. It is challenging to clean this data because each record has multiple lines of data, and this makes it very difficult to manage the datasets.

Step 1- Clean the Brachial Worksheets:

This is an explanation of how to clean the brachial worksheets. The cleaning programs are located in /home/brachial/gen3/worksheets. These programs allow you to get a single keying of the worksheets so they can be hooked up to the measurement data.

First, run prelim1.sas and see how many single/double/multiple keyings there are and decide if you want to key all sheets up to this point or just take the id's that are double keyed. Then run prelim2.sas and prelim3.sas. Prelim2.sas outputs keyings into two different data sets and prelim3.sas makes sure the keyers are not equal.

IMPORTANT: At this point, make sure to run the before.sas program. This program takes a snapshot of the tables before any changes are made to them.

Run the compare.sas program. You should be comparing prelim3a.ssd01 to prelim3b.ssd01- this is comparing the two keyings. Print this out and look up what the appropriate answers should be. Also, look for any sheets the keyers may have flagged and also make the necessary changes- I tend to write those down on an additional sheet if they do not pop out on the compare.

Once the compare and flags are all set, then you can begin making the changes in the table. Make sure to make corrections to BOTH keyings. Once this is done, run the after.sas program. This outputs the after.ssd01 dataset. Now use beftoaft.sas to compare before.ssd01 to after.ssd01 and make sure that all your changes made it into the table.

Prelim4.sas takes in the after.ssd01 data set and just outputs a single keying. Then prelim5.sas outputs prelim5.ssd01 and changes -1's, 9's, etc. to .'s.

Prelim6.sas compares the worksheet data in prelim5.ssd01 to the log book data. Any changes that need to be made on the worksheet are done here in if/then statements. (This was only done in round 1 – all of these checks are also in the cleaning program Brach4.sas, so are done here for round 2 and after).

Step 2- Process the Data:

1. Take all floppy disks from technicians and use WS_FTP to transfer them from floppy to UNIX. Put all Birgitta's studies into /home/brachial/gen3/data/curdatbl, all of Shuxia's studies into /home/brachial/gen3/data/curdatsf, and all of Eva's studies into /home/brachial/michelle/gen3/curdateo. If a study is a remeasured study (on a floppy disk of numbered 500 or higher) then it should go into one of the following directories: /home/brachial/gen3/data/remdatbl (Birgitta), /home/brachial/gen3/data/remdatsf (Shuxia), and /home/brachial/gen3/data/remdateo (Eva).
2. Use the bprocess.sas (for Birgitta's data), sprocess.sas (for Shuxia's data), and eprocess.sas (for Eva's data) to create sas datasets from the text files stored in /home/brachial/gen3/data/curdatbl, /home/brachial/gen3/data/curdatsf, and /home/brachial/gen3/data/curdateo respectively. If the study is a remeasured study, use the following programs instead to process the text files into sas datasets: remeasbl.sas (Birgitta), remeassf.sas (Shuxia), and remeaseo.sas (Eva). IMPORTANT: In these programs, you must change the dataset name that you want to get outputted. For example, if Birgitta has remeasured studies that are being processed on 1/22/2001, change the name to b012201r in the program anywhere there is a dataset getting outputted. Note that there is a variable called "pdate" created in these programs. This variable is the process date and should get changed to the date that the date gets processed.
3. Print out the .lst files for each tech. At this point the id's need to get checked against the logbook. Place a check mark next to each id once it is found on the printout and use a yellow highlighter to place a check mark in the logbook for that id. This is a very important step. If any data are missing then ask tech to put it on a new floppy and transfer it into the curdat__ folder. Once you have all the data, then run __process.sas again to output a file containing the missing data. Then move all data from the curdat__ folder to the olddat__ folder and move files in remdat__ to remold__.
4. The programs listed above in (2) put the sas datasets into the directory /home/brachial/gen3/data/output. The programs output the datasets named as bmonthly.ssd01 (Birgitta), smonthly.ssd01 (Shuxia), and emonthly.ssd01 (Eva). You must go into this directory each time you process new data and rename these sas datasets. The naming convention is as follows: use the first letter of each techs name plus the date you process the data. For example, if you processed data on 1/22/2001 and it was Birgitta's data, you would rename bmonthly.ssd01 to b012201.ssd01. If it is a remeasured study you do not need to rename it in the directory because it was all ready outputted with the correct name (it should have been changed in the program).
5. Once each techs dataset is in place then the cleaning process can begin.

Step 3- Cleaning:

1. First create a new directory under /home/brachial/michelle/finaldat. The naming convention that is used is br_____ . Insert the date that cleaning is started on; for example, if you start cleaning on 1/22/2001 then make a new directory called br012201.

2. Copy all the .sas program files from the directory with the most current date.

SAS Program	Description	Data File Output
Brach1.sas	This program sets all the sas datasets that were created from the text files together. Each time a new cleaning round is started, add in the new names of the datasets. It also checks to make sure there are no duplicates and prints out a list of the new data that was added on to the old data. Printout the .lst file and check it against the sheets that were used to check against the logbook. Also check to make sure the code1 variable is correct- change any "10's" that have re-reads to "9's", etc.	f1d_1.ssd01
Brach2.sas	Make any changes to code1 that are necessary. Create round variable and change to the correct round of cleaning.	f1d_2.ssd01
Brach3.sas	This program takes any id that has interpretation date not equal between the baseline and deflation reading and changes it to be equal by updating it to the later of the two dates. The worksheet data gets hooked up to the data at this point. Note that only the unclean id's get the worksheet hooked on to them at this point because the clean data should not be touched. This program also tells me if anyone is missing worksheet.	f1d_3.ssd01
Brach4.sas	This program includes the first round of checks to be done on the data. There are 33 checks in total and they are mostly looking for discrepancies between the worksheet and data. This program provides a list of all the id's with the problem number- any changes that need to be made must be made on this list.	probs_1.ssd01
Brach5.sas	This program is almost the same as the one above but this program provides a description of the problem, one page per id. Place the printout of the problem and the brachial worksheet into a maroon folder and give to the techs. If it is a simple problem it may just be corrected by the data cleaner.	---
Brach6.sas	Type in any changes/corrections that need to be made (if-then statements). Be sure to change floppy # for rereads.	f1d_4.ssd01
Brach7a.sas	Tells me who is missing baseline data or deflation data – these id's need to be deleted before check 2 in brach7.sas	
Brach7.sas	This program includes the second round of checks on the data.	probs_2.ssd01

	There are 8 checks in total, most dealing with the study time and millisecond variables.	
Brach8.sas	This program is very similar to brach7.sas, but produces a description of the problem, one page per id. Place the printout of the problem and the brachial worksheet into a folder and give to the techs. Make any corrections or changes to be made on the printout from brach7.sas	
Brach9.sas	Use this program to printout any of the id's that have problems with their study times. The printout of the whole record should be included in the folder with the description of the problem and the brachial worksheet.	
Brach10.sas	Type in any changes/corrections that need to be made that deal with the study time (if-then statements)	fld_5.ssd01
Brach11.sas	This program includes the third round of checks to be done on the data. There are 5 checks in total and they mostly check for problems with the brachial diameter. You will need to know the which ids only have a baseline or deflation reading and these id's need to be deleted before check 7 (those ids from brach7a.sas)	probs_3.ssd01
Brach12.sas	This program is very similar to brach11.sas, but produces a description of the problem, one page per id. Place the printout of the problem and the brachial worksheet into a maroon folder and give to the techs. Make any corrections of changes to be made on the printout from brach11.sas.	probnum2.ssd01
Brach13.sas	Use this program to printout any of the ids that have problems with their diameters. The printout of the whole record should be included in the folder with the description of the problem and the brachial worksheet.	
Brach14.sas	Type any changes/corrections that need to be made to specific diameters, for example, if just one frame needs to get deleted.	fld_6.ssd01
Brach15.sas	Make any changes to code2 that are necessary. Do not change those that have been remeasured – leave them as code2 = 0 so they will get picked up again in next cleaning	fld_7.ssd01
Brach16.sas	This program just double-checks that the techs made changes to the brachial floppy number on the worksheet if they remeasured a study	fld_8.ssd01
Brach17.sas	This program checks to be sure all codes are correct. Also checks to be sure those with only a baseline or only a deflation have BR15 = 0.	fld_9.ssd01

Description of Coding Variables in Brachial Dataset:

Variable Name	*Variable Value*	*Comment*
Code1 (Status variable)	Code1 = 9	This is an original reading and a re-read coded as '11' should exist for any id coded as a '9'.
	Code1 = 10	This is the original reading but no other readings exist for id's coded as a '10'.
	Code1 = 11	This is a re-read; there should be a matching id in the dataset that has a code1 = '9'.
	Code1 = 50	Reproducibility Reading that should be in final dataset.
	Code1 = 51	Extra Reproducibility reading or extra reading.
Code2 (Cleaning Variable)	Code2 = 0	This means that the id has not been through cleaning yet.
	Code2 = 1	This means an id is cleaned.
	Code2 = 2	This means an id has gone through the cleaning process but will pop up again with cleaning issues.
Reprod (Reproducibility Round)	Reprod = 0	Not a reproducibility study.
	Reprod = 1	Reproducibility Round 1.
	Reprod = 2	Reproducibility Round 2.
	Reprod = 3	Reproducibility Round 3.
	Reprod = 4	Reproducibility Round 4.
	Etc…	
Round (Round of Cleaning)	Round = 1	Round 1 of cleaning.
	Round = 2	Round 2 of cleaning.
	Round = 3	Round 3 of cleaning.
	Etc…	

List of checks that are done in Brach4.sas:

Check Number	Check
Check 1	Checking idtype is a 3
Check 2	Checking that the data interpretation date is the same as the worksheet interpretation date
Check 3	Checking Sonographer ID in data is the same as the worksheet sonographer id
Check 4	Checking Interpreter ID in data is the same as the worksheet interpreter id
Check 5	Making sure that the study date is not later than the interpretation date
Check 6	If a participant has Raynaud's disease, should not continue with exam. Making sure that this question is not coded as 'Yes' and the rest of worksheet is filled out
Check 7	If a participant has had a radical mastectomy, should not continue with exam. Making sure this question is not coded as 'Yes' and the rest of worksheet is filled out
Check 8	Checking sex from Roster with a sex variable created from the worksheet
Check 9	Making sure that Cuff inflation pressure is not less than initial SBP
Check 10	Checking that if person said they had caffeinated beverages that subquestion is filled in ('if yes, how many cups?') or if they didn't have any caffeinated beverages that the subquestion is coded as missing
Check 11	Checking if person said they smoked in last 6 hours that the subquestion is filled in ('if yes, how many hours and minutes since your last cigarette') or if they had not smoked in the past 6 hours that the subquestion is coded as missing
Check 12	Checking range of initial SBP (80-200 mm Hg)
Check 13	Checking range of Cuff inflation pressure (200-240 mm Hg)
Check 14	Checking for any missing values
Check 15	Checking range of room temperature (15-28 degrees Celsius)
Check 16	Making sure that the variable reprod in the data which stands for whether or not a study is a reproducibility is coded correctly
Check 17	Making sure that if a study has a code1=9 or 10 that the variable reading = 1
Check 18	Making sure code1=11 that the variable reading=2
Check 19	Making sure that the code1 variable is coded correctly
Check 20	Making sure that the round variable is coded correctly
Check 21	Making sure that the interpreters are either 30, 49, or 88
Check 22	Making sure that on the brachial floppy disk number from worksheet that the first portion of the variable is one of the technicians id numbers
Check 23	Making sure that the variables baseline measurable, deflation measurable, and ok to calculate FMD are filled out correctly.
Check 24	Checking to see if scan was complete then no reason was checked on worksheet or if scan was not complete then a reason was checked.
Check 25	Checking that log book test done variable is the same on the worksheet.
Check 26	Checking that log date matches worksheet scan date.
Check 27	Checking that log date matches the date in the data.
Check 28	Checking that first part of the floppy number on worksheet matches the interpreter id.
Check 29	Checking to see if the video number on the log matches that on the worksheet.
Check 30	Checking to see if the sonographer id on the log matches what is on the worksheet
Check 31	Making sure the study date on the worksheet is the same as the contact date in the gen3 status table
Check 32	Check that the first part of the floppy # on the worksheet matches the data interpreter ID.
Check 33	Making sure if code1=11 that the techs updated the worksheet interpretation date and brachial floppy number

List of checks that are done in Brach7.sas:

Check Number	Check
Check 1	Checking for any studies that have msec > 200000
Check 2	Checking that the difference between the first and last stime is about 8 minutes
Check 3	Checking for any negative msec
Check 4	Checking that msec are continuously increasing
Check 5	Checking that the first frame of deflation is less than 1000 msec
Check 6	Checking that Baseline has at least 5 measured frames
Check 7	Checking that Deflation has at least 15 measured frames
Check 8	Checking that the last Deflation is at least 90000 msec

List of checks that are done in Brach11.sas:

Check Number	Check
Check 1	Checking the range of the brachial diameter (bdia): range goes from 2.2-6.5 mm
Check 2	Checking that the difference between the mean of the baseline brachial diameters and the mean of the first five deflation diameters is not greater than 0.2 mm
Check 3	Checking that any adjacent frames are not greater than 0.2 mm different from each other
Check 4	Checking that the difference between the mean of the baseline diameters is not greater than 0.4 mm different than any one of the baseline diameters
+Check 7	Checking that the FMD raw 60 second is between –2% and 16%
*Check 8	Checking that the FMD regressed 60 second is between –2% and 16%
*Check 9	Checking that the FMD regressed maximum is between –2% and 16%

+NOTE: The range was determined by doing boxplots on diameters and taking the upper/lower 1% as cutoff for cleaning.
*NOTE: We did not do Check 8 or Check 9 as of right now

Cleaning Rounds That Have Been Completed:

Round Number	Continue with SAS program…	Complete	Latest Clean Data File:
Round 1		✓	/home/brachial/gen3/finaldat/br102102/f1d_9.ssd01
Round 2		✓	/home/brachial/gen3/finaldat/br042903/f1d_9.ssd01
Round 3			
Round 4			
Round 5			

Appendix Item 7

FHS Original Specific Aims of RO1 Grant Submission

A. SPECIFIC AIMS

Standard risk factors and atherosclerotic lesion severity do not fully define the risk for cardiovascular disease (CVD). Increasingly, researchers have come to understand that vascular dysfunction contributes at both *early and advanced* stages to the pathogenesis of coronary heart disease and stroke. **We propose to noninvasively characterize vascular function by examining two related vascular phenotypes, endothelial dysfunction, with brachial artery flow-mediated dilation, and vascular stiffness, with arterial tonometry, in 3850 adult offspring of the Framingham Heart Study Offspring and OMNI cohorts,** known as the **Third Generation** (Gen 3) cohort. Invasive and noninvasive studies suggest that impairments of both vascular phenotypes are associated with CVD risk factors and the presence of CVD. Correlates of conduit vessel stiffness, such as pulse pressure, have emerged as powerful predictors of CVD risk. Additionally, preliminary patient studies have suggested that endothelial dysfunction, with the attendant loss of the vasodilator, anti-thrombotic, and anti-inflammatory properties, is predictive of increased CVD events. However, prior studies have not definitively determined the clinical correlates of vascular dysfunction, because they have been limited to small highly selected patient samples. Most prior studies have examined vascular function in older adults. Additionally, no prior study in the community has established whether noninvasively assessed indices of vascular function predict the development of hypertension (HTN) and CVD. Critically, while HTN and the nitric oxide (NO) pathway have genetic determinants, no study has established the heritability and genetic basis of vascular stiffness and endothelial function.

Currently, we are studying vascular function in about 3600 upper middle-aged and elderly participants of the Framingham Offspring Cohort and minority OMNI Cohort. By pooling those results with the results from Gen 3, we will have over 7000 vascular examinations in an extensively studied *multi-generational* community-based cohort. Thus, we will have the opportunity to characterize the environmental determinants, the heritability, and the prognostic implications of abnormal vascular function throughout the years of adulthood. Our study will potentially pave the way for identifying disease-promoting genes. **We hypothesize that vascular dysfunction will be determined by both environmental and genetic factors. We further hypothesize that abnormal endothelial function and vascular stiffness phenotypes will be associated with each other, will predispose to the development of HTN and CVD, and will be early harbingers of atherosclerosis and cardiovascular remodeling.** This proposal has the following specific aims:

Aim 1. **To determine the cross-sectional relations between risk factors and vascular function in young and middle-aged adults in the community.** Taking advantage of the risk factor information that will be available in the Gen 3 Framingham Study, multivariable models will be used to examine the relations between vascular function and standard and novel CVD risk factors, including metabolic, inflammatory and hemostatic factors.

Aim 2. **To study the heritability of vascular function.** The rich family structure of the Framingham Offspring and Gen 3 cohorts will allow an investigation of the heritability of vascular function. Analysis of intermediate vascular phenotypes will enhance our ability to define genetic and environmental factors that predispose to HTN and CVD. If, as anticipated, subjects have cell lines available, further genetic analyses will be facilitated.

Aim 3. **To relate endothelial function and vascular stiffness to each other and to other markers of subclinical CVD in early and middle adulthood**. Multivariable models will be used to assess the relations between endothelial function and conduit vessel stiffness. Additionally, we will evaluate the association of vascular function to markers of subclinical CVD, including left ventricular mass by echocardiography.

Aim 4. **To assess the relation of vascular function to longitudinal changes in blood pressure.** Vascular function and blood pressure are associated cross-sectionally, but it remains uncertain whether vascular dysfunction predisposes to HTN or vice versa. **The prospective design of the Framingham Heart Study will allow us to observe the relations between vascular function, and longitudinal blood pressure changes in young and middle- aged adults at the crucial early stages of atherogenesis and vascular remodeling.**

The Framingham Heart Study and the investigators are uniquely suited to carry out this proposal. Dr. Benjamin has over 12 years of experience with rigorous, large-scale studies involving ultrasound. She has already established a highly efficient, integrated vascular function testing station at Framingham and has extensive experience with the proposed endpoints. The co-investigators, Dr. Vita, Dr. Larson, Dr. Mitchell, Dr. Levy, Dr. Vasan are experts in the fields of endothelial function, statistics, vascular tonometry, HTN and echocardiography, respectively. The Gen 3 cohort provides a large, single site, population-based sample of young and middle-aged subjects with a wealth of contemporary risk factor data and the availability of longitudinal follow up beyond the length of the proposed grant. **The Gen 3 data will greatly enhance the data already collected in their parents by virtue of increasing our power to do cross sectional and heritability analyses, and by virtue of examining intermediate phenotypes in a younger cohort prior to the development of HTN, clinical CVD and CVD medication usage.** This unique combination of investigator expertise and a well-characterized sample will provide the largest family-based data set of vascular phenotypes in the world, and will increase our understanding of the pathophysiology of HTN and CVD.

Appendix Item 8

Non-Invasive Hemodynamics Workstation
Blood pressure, Tonometry, Brachial Reactivity and Echo Doppler
Acquisition Notes

NIHem

Noninvasive Hemodynamics Workstation
Data Acquisition Notes

Note: This workstation is not approved for routine clinical use. It is intended to be used for research purposes only under the supervision of an experienced physician.

Warning: The NIHem Workstation utilizes multiple high resolution and multimedia timers, including those supplied with the Windows NT® operating system. Unauthorized software or hardware may interfere with the proper function of these timers. Therefore:

- **Do NOT install unauthorized hardware or software onto the NIHem Workstation.**
- **Do NOT modify the Windows NT® configuration in any way without prior approval from Cardiovascular Engineering, Inc.**
- **Do NOT activate or load screen savers onto the computer.**

Rev. 2.0 (DRAFT)
October 22, 2004

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1.0. Overview: Setup

1.1. Have the patient change into a hospital gown (open in front). Undershorts are OK, but please have them remove a bra or T-shirt.

1.2. Inform the patient that he/she should lie down and make him/herself comfortable. Inform them that you are going to place electrodes on their back, ECG electrodes on their chest and a BP cuff on their left arm.

1.3. Apply 4 ECG leads to chest. Place arm leads under left and right collarbones and leg leads on lower left and right ribcage.

1.4. Apply the cuff to the patient's left arm. The cuff should be snug but not tight, with the microphone wire and hose exiting the cuff along the medial aspect of the arm, with the microphone wire overlying the patient's brachial artery. If you consider a cross-section through the patient's left upper arm viewed from below, the brachial artery will be at about 2 o'clock (just medial to the biceps tendon), which is where the microphone cable should be placed. This will place the pressure hose at about 5 o'clock, which is along side the medial edge of the triceps muscle. This requires that the hose and cable assembly pass between the patient's arm and chest.

1.5. If gown is tight on arm, remove left arm from sleeve of hospital gown before applying BP cuff. Allow the patient to rest supine for at least 5 minutes before starting blood pressure acquisitions. This is a good time to register the patient, who will acclimate to the surroundings and to the presence of the operator.

1.6. Attach tonometer to the Patient Junction Box.

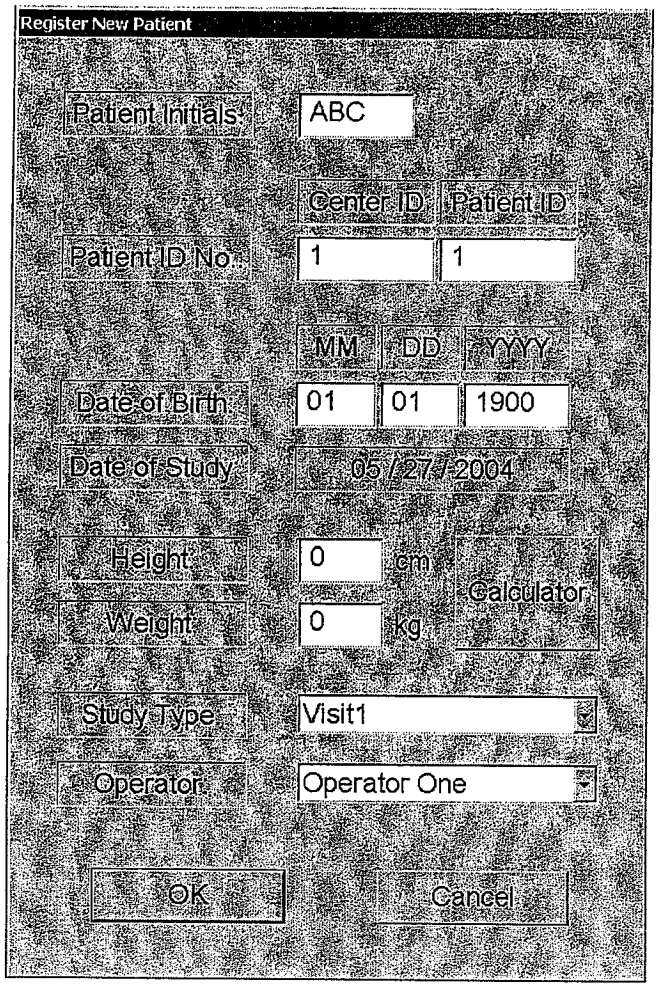
1.7. Turn the machine on, and open NIHem.exe. Click [Register].

1.8. Enter Patient Initials (see Figure 1), Center ID, Patient ID, Date of Birth, and Height and Weight if applicable. The Study Date defaults to the computer's current date.

1.9. Select Study Type and Operator.

1.10. Click [OK].

Figure 1. Patient Registration Dialog Box.



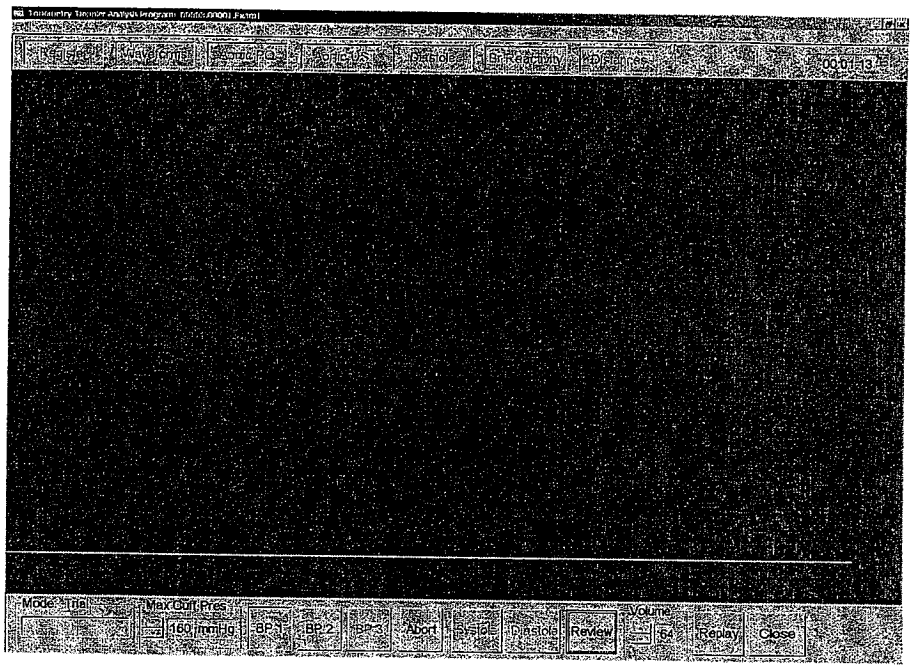
2.0. Blood Pressure Acquisition

2.1. Patient preparation

2.1.1. The patient should be supine and otherwise prepared for the NIHem evaluation. The PPG probes and ECG leads should be attached prior to placing the blood pressure cuff on the patient.

2.1.2. The patient's arm should rest comfortably at their side on the bed during acquisitions. The arm should not be against the chest wall as this will produce respiratory artifacts in the pressure and microphone waveforms.

Figure 2. NIHEM Opening Blood Pressure Acquisition Toolbar.



2.2. Recording the Blood Pressure

2.2.1. After registering the patient, select [Waveforms] from the main menu toolbar at the top of the screen.

2.2.2. Select [BP] from the Waveforms toolbar at the bottom of the screen.

2.2.3. Put on the headphones. If you have an analog system, turn the headphone volume down (fully counterclockwise). If you have a digital system, it will automatically mute the microphone channel.

2.2.4. Set the maximum cuff pressure to approximately 40 mmHg higher than the expected systolic pressure.

2.2.5. Click [BP1] (see Figure 2, above) and wait for the cuff to inflate (cuff will inflate and deflate automatically).

2.2.6. If you have an analog system, adjust the volume knob to the nominal listening level, which should sound comparable to a stethoscope. If you have a digital system, you may adjust the volume using the on-screen control. Avoid excessively high volume levels.

2.2.7. Click [Systole] when the first beat with a Korotkoff sound is heard. If the first Korotkoff sound is too soon (less than 10 seconds) after the motor stops inflating, the maximum cuff pressure is too low. Hit [Abort], increase maximum cuff pressure, wait a minute, and redo that blood pressure.

2.2.8. Click [Diastole] when the first beat with no Korotkoff sound is heard.

- If either landmark is clicked prematurely, it is fine to click again and update the landmark to a later event. However, it is not possible to update landmarks to an earlier event unless the BP acquisition is repeated or played back. Therefore, the user should maintain a low threshold for clicking.

2.2.9. When the cuff reaches 40 mmHg, the deflate valve will close for 20 seconds so that pressure waveforms can be acquired. It is important that the patient remain still during this period. Therefore, the user should remain focused on the computer monitor during this "hold period" so that the patient does not think that the blood pressure acquisition is complete. When a blood pressure acquisition is complete, the data is saved automatically (see Figure 3 for an example of an optimal recording).

2.2.10. If an error is made during a given blood pressure acquisition, click the [Abort] button and redo that blood pressure prior to proceeding to the next blood pressure. This will minimize the need for redoing multiple blood pressures later. Examples of errors that require aborting and restarting include:

- Korotkoff sounds start as soon as cuff inflation stops, indicating that the maximum cuff pressure is too low.
- The patient moves or the cuff is against the chest creating substantial artifacts on the pressure waveforms (see Figures 4 and 5 for additional examples).

It is not necessary to redo or abort blood pressure acquisition if the blood pressure landmarks are not marked correctly. These can be edited later, as described in Section 2.3.

Figure 3. Optimal BP Acquisition.

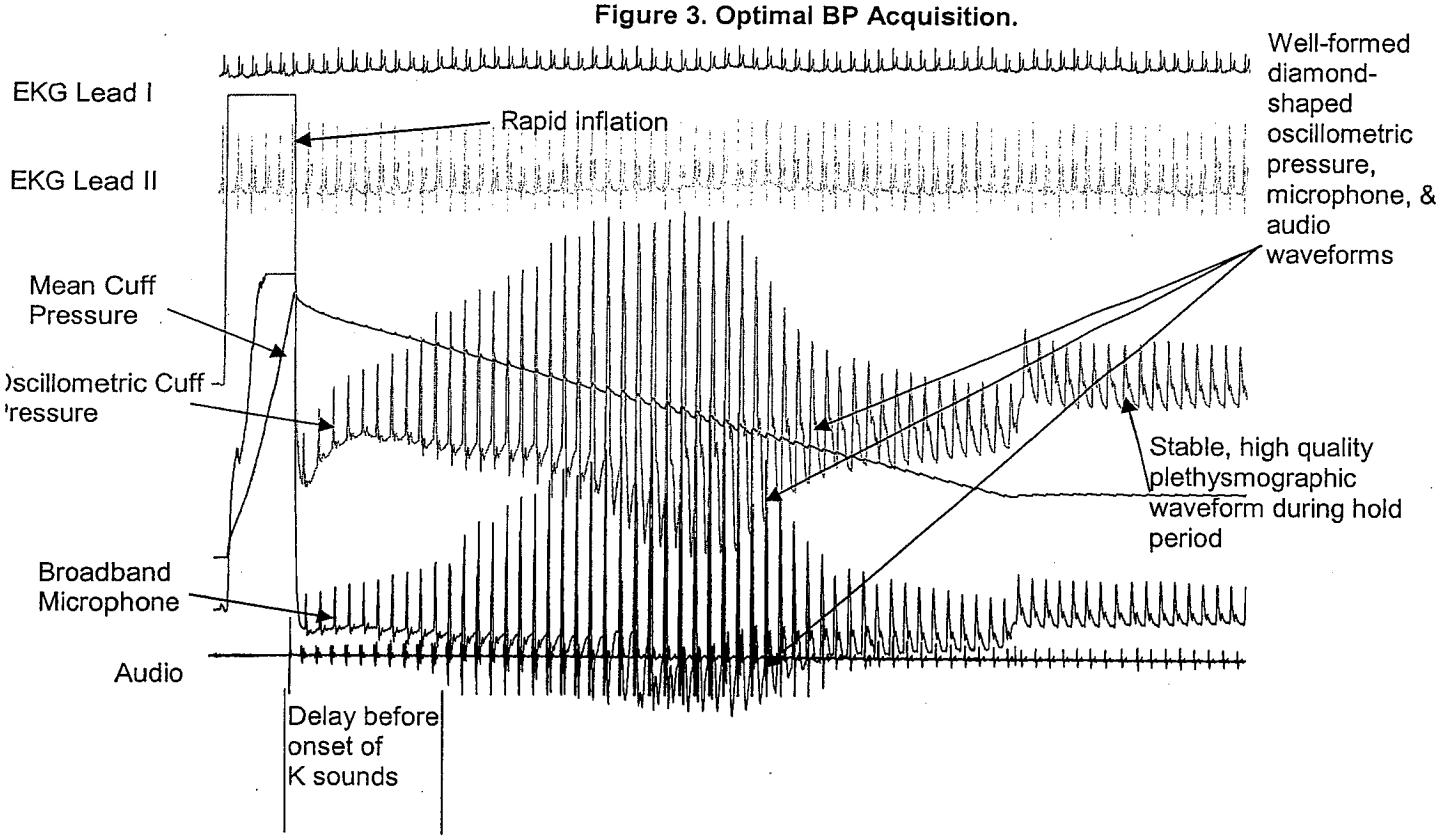


Figure 4. BP Acquisition with Cuff too Loose and Poor Cuff Positioning.

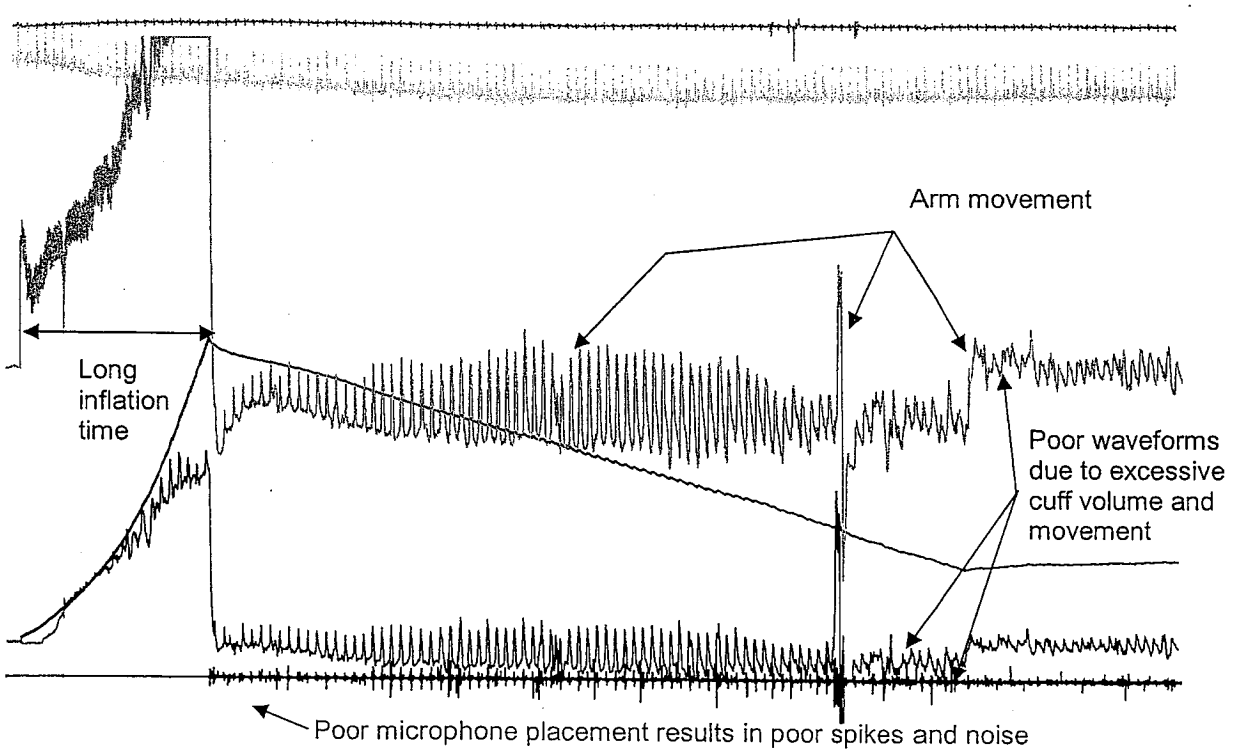
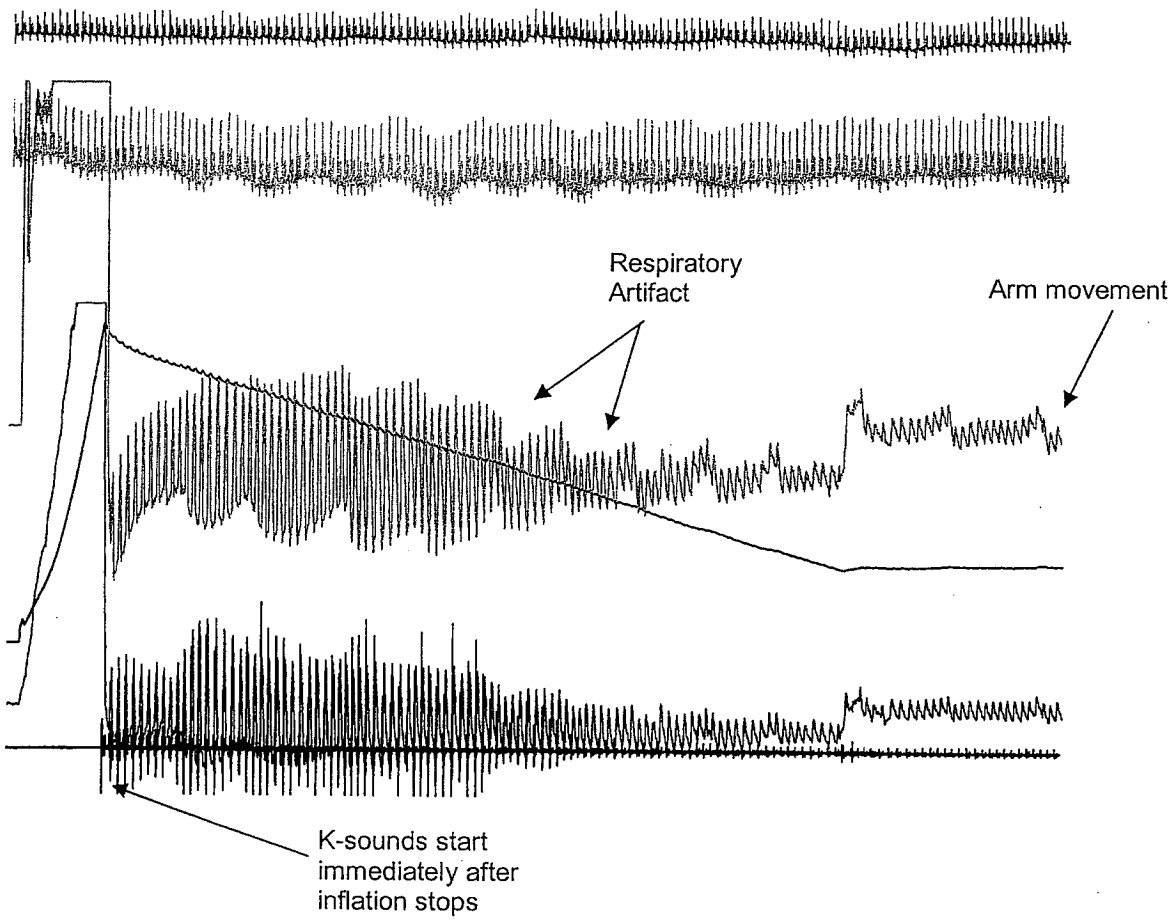


Figure 5. BP Acquisition with Maximum Cuff Pressure too Low



2.3. *Reviewing and Repeating Blood Pressures*

2.3.1. After performing the blood pressure, click the [Review] button to load the "Blood Pressure Summary" dialog box. This dialog box tabulates the individual blood pressure value.

2.3.2. If it is necessary to correct the systolic or diastolic marks, click [Replay] ***before*** proceeding to the next set of measurements. This will playback the acquired BP audio and also draw a vertical position cursor as playback progresses. Press the [Systolic] or [Diastolic] button as appropriate when the cursor falls on the correct beats. It is also possible to skip the cursor to the desired playback position by clicking on the screen at the desired position. *It is not possible to correct the BP marks once you leave the present screen.*

2.3.3. Overwrite any of the BP measurements with a new acquisition if needed by simply clicking on the corresponding [BP*x*] button and clicking OK when prompted.

2.3.4. Remove BP cuff and headphones and store after BP acquisition is complete.

3.0. Tonometry Acquisition

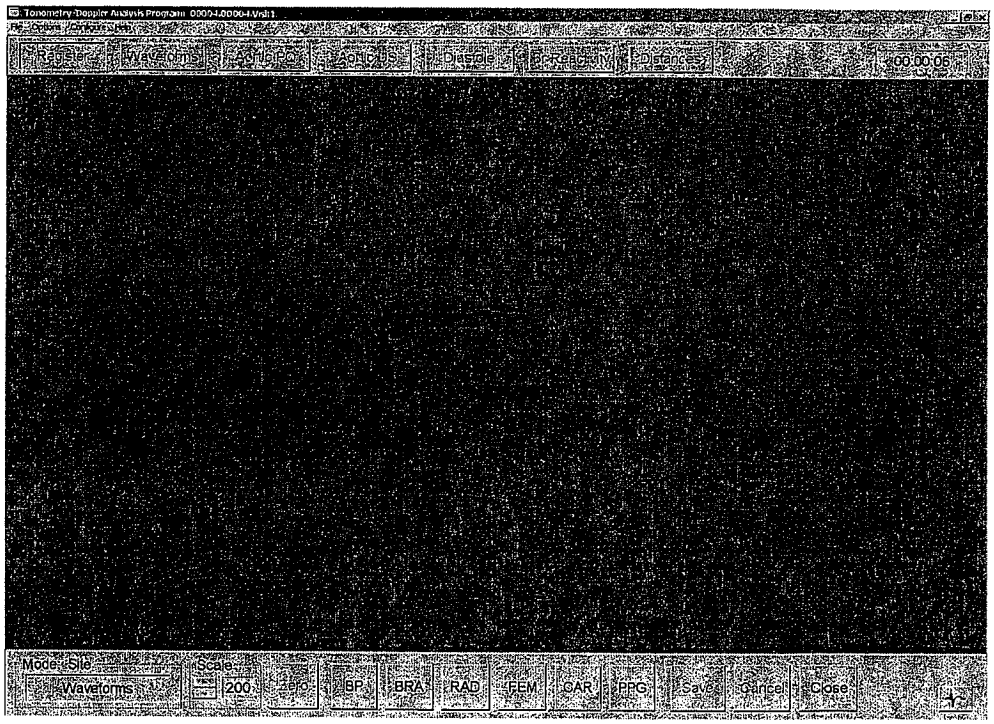
3.1. Overview

3.1.1. With gloves on, confirm that all pulses (especially the femoral artery) are accessible prior to initiating the pulse acquisition sequence. Establish the course of the artery to find the point of maximal pulsation at each site.

3.1.2. Ask the patient not to talk unless necessary during the pulse acquisition. Proceed through the sequence of pulses as quickly as possible without feeling rushed. Do not comment to the patient on the quality of the recordings during the acquisition.

3.1.3. Click [Bra] (Figure 6) and ensure that you have a stable ECG recording with a tall, upright R-wave on at least one of the two ECG leads.

Figure 6. NIHem Tonometry and PPG Acquisition Toolbar.



3.2. Use of the NIHem Low Profile Tonometer

3.2.1. Tonometry uses dry contact. Generally, the index finger is used to hold down the tonometer. It is better to rest the entire finger flat on the patient's skin rather than having only the fingertip in contact with the tonometer. Resting the fingers of the hand flat against the patient stabilizes hand position and minimizes tremor and baseline drift.

3.2.2. Place the white sensor of the low profile tonometer at the midpoint of the long axis of the vessel at the point of maximal pulsation and apply approximately as much pressure as was required to optimally palpate the pulse. For brachial arteries, this will elevate the blue pressure tracing to about ¼ to ½ way up the screen on a 200 scale, depending on arm size, artery depth, etc. Radial arteries are superficial with bone immediately behind the artery; thus light pressure (¼ screen) should be used to start. Femoral arteries are

deeper and require more pressure (generally ½ screen or more). Carotids generally require light pressure (often ¼ screen or less) if the optimal location is chosen, as detailed below.

3.2.3. Use the tonometer and the following steps to find the midline of the vessel:

- Position the tonometer at the presumed (palpated) midline of the vessel and apply moderate pressure as appropriate for the site.
- Then use skin traction to purposefully displace the sensor medial to the long axis of the vessel.
- Next, slowly and systematically move laterally, using skin traction (i.e., do not attempt to actually slide the tonometer across the skin), until a pulsation is seen. Continue to move laterally until the pulsation becomes maximal and then declines. In this manner, the center of the vessel and optimal point for tonometry is clearly established since the tonometer has moved across the entire spectrum from too medial to optimal to too lateral.
- Move back medially until at the optimal central point (maximal amplitude for the current amount of hold-down pressure and sharpest waveform features).
- With experience, you will notice that it is possible to feel the pulsations of the vessel through the tonometer. This tactile feedback will help with optimal positioning of the sensor.
- Note that it is important to maintain a constant amount of hold-down pressure during this maneuver and to move slowly. If hold-down pressure is varied during the sweep, the baseline will wander, making it difficult to see pulsations as the tonometer passes over the center of the vessel. If the sweep is performed too rapidly, it is possible to move across the vessel in a single diastole and never see a pulsation. Recall that the entire sweep from too medial to too lateral is only a few millimeters.

3.2.4. Optimize the hold-down pressure. This is accomplished by gently and gradually applying increased amounts of pressure. Assuming that minimal pressure is applied initially, as more pressure is applied the pressure waveform amplitude (i.e., the pulse height from waveform foot to peak) will become larger and the features (especially the dicrotic notch) will become clearer. The waveform tracing will also move higher on the screen in proportion to the amount of pressure applied—this is not what we are referring to as "amplitude." As even more pressure is applied, the waveform amplitude will become smaller, the dicrotic notch will fall, and diastole will become flat, approximating a square wave, because the vessel is actually collapsing in diastole. This indicates that the optimal pressure point has been passed. Reduce hold-down pressure until the maximal waveform amplitude is obtained, hold this position for 20 seconds and save.

3.2.5. Characteristics of an optimal waveform include:

- Steadily falling diastolic tracing
- Clean, smooth transition into the upstroke at the "foot" of the waveform
- Monophasic early systolic upstroke
- Recognizable reflected wave or secondary pressure peak either in systole or diastole
- Crisp dicrotic notch
- Stable baseline
- These features apply to all waveforms, although the shape of the waveform and timing of the reflected wave will vary from site to site (see Figure 7). Specifically, in the femoral

artery, the dicrotic notch will appear as an abrupt change in slope rather than a true notch and the reflected wave will merge with the primary wave producing a single peak.

3.2.6. Signs of poor positioning or too much pressure include:

- Flat diastolic phase, producing a "square wave" tracing
- Scooped diastole that reaches an early minimum and is rising prior to the foot of the waveform
- Sudden negative dip just prior to the upstroke, producing a "square root sign" at the foot of the waveform
- Inverted pressure waveform, suggesting that the tonometer is off to one side of the vessel

3.2.7. Beware of excessive pressure, especially in slender patients with superficial arteries and in all patients at the radial artery. The artery will collapse or will slide out from under the tonometer if too much pressure is used. A common error is to use excessive pressure in an attempt to rectify improper centering. Make certain that you locate the optimal centering before attempting to optimize pressure (see Figure 8 for examples).

3.2.8. Mark the site of each waveform acquisition with a lip liner as soon as a suitable pulse waveform has been recorded. The impression of the tonometer should be visible on the skin and the dot should be placed where the center of the sensing button was located.

Figure 7. Optimal Tonometry Acquisitions.

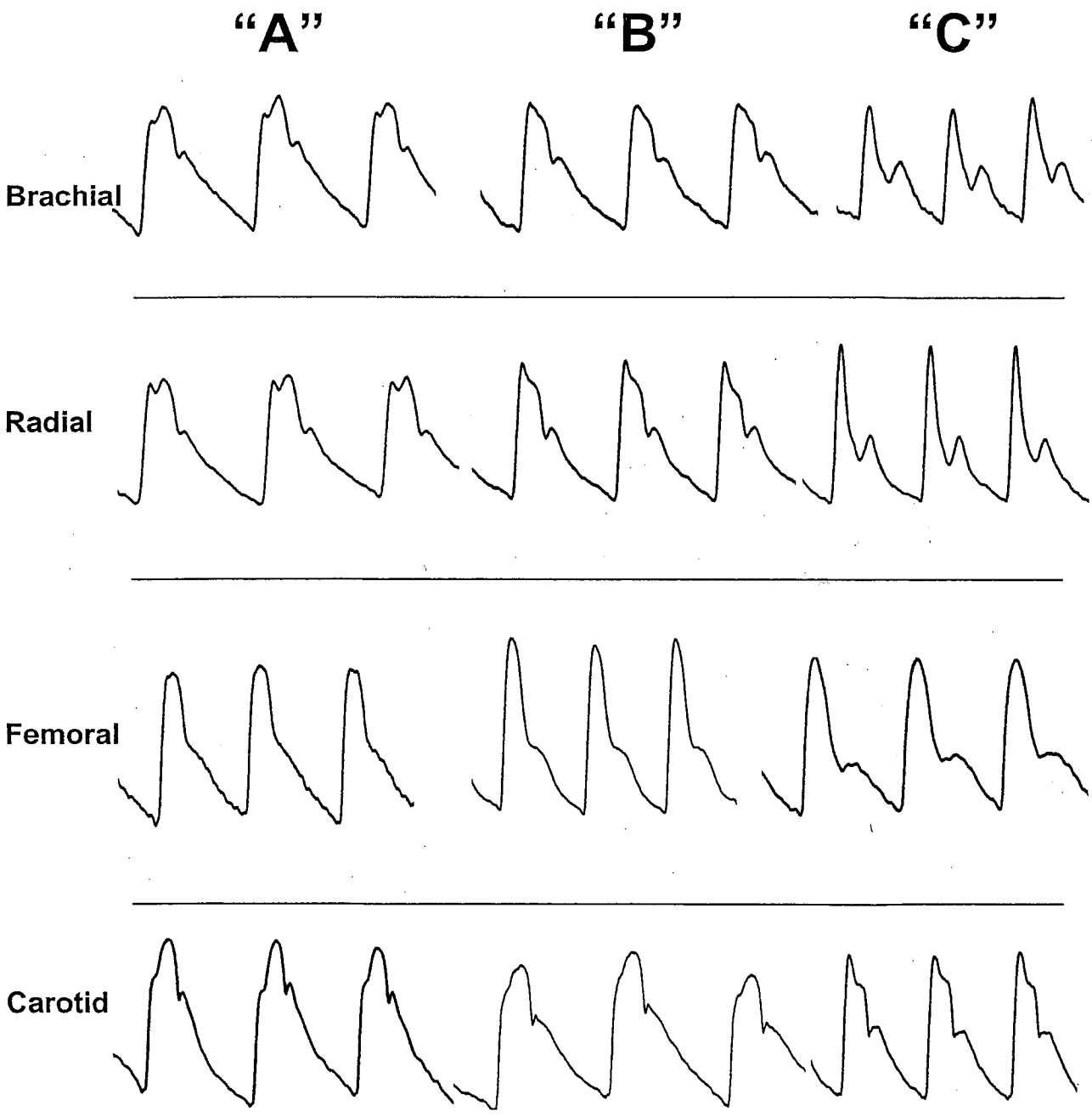
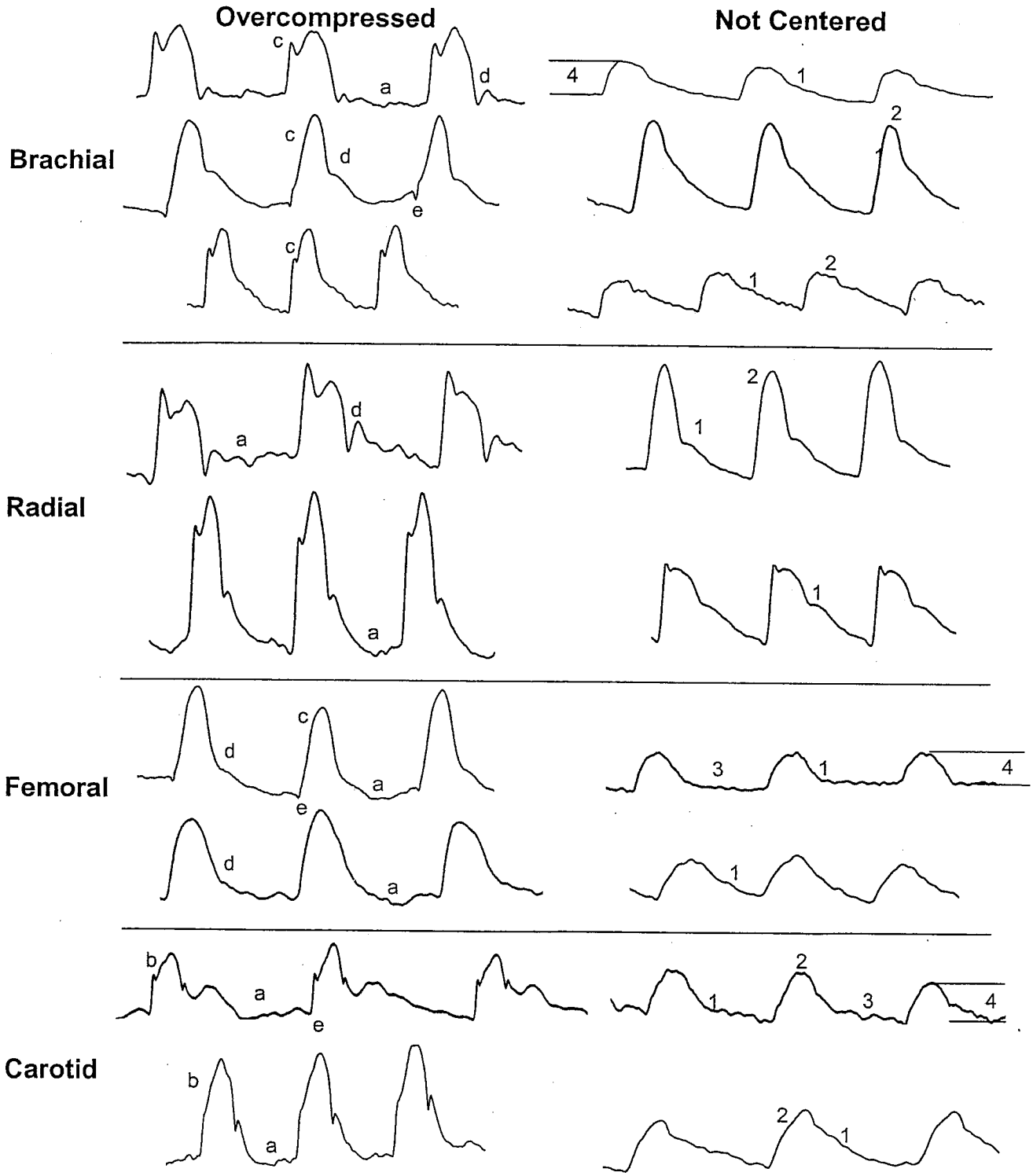


Figure 8. Common Tonometry Acquisition Problems.



Legend:

a = Flat or scooped diastole	d = Depressed dicrotic notch	2 = Poorly defined reflected wave
b = Turbulence	e = Square root sign	3 = Flat diastole
c = Distorted upstroke	1 = Poorly defined dicrotic notch	4 = Low amplitude

3.3. Site Specific Notes: Brachial

3.3.1. Start with the patient's arm fully extended on the bed at their side with the hand in a neutral position (palm toward their thigh) or palm up.

3.3.2. Place the index and middle fingers of your right hand along the antecubital crease with your index finger lateral to the biceps tendon and your middle finger medial to the tendon. The pulse should be palpable under one of the two fingers, indicating whether to approach the artery from the medial or lateral side of the tendon. In most cases, medial to the tendon and just above the antecubital crease is the optimal location. Avoid palpating the artery through the tendon, as this will produce a damped tracing.

3.3.3. The initial location to look for the pulse should be at or just above the antecubital crease, realizing that the maximal pulsation may be 1-2 cm above or (less often) below this reference point, depending on the relationship between the artery and biceps tendon. If the pulse is weak or not apparent, try turning the patient's hand palm down. If you still cannot find the artery, palpate along the medial edge of the biceps muscle to find the artery as it emerges between the biceps and the triceps.

3.3.4. Once the pulse is located, use the index, middle and ring fingers of the left hand to identify the course of the artery along the long axis of the arm.

3.3.5. When all three fingers are on the artery, use small amounts of medial/lateral traction to roll the artery under your fingers to determine its size, course and mobility.

3.3.6. Move your index finger aside and place the tonometer at the point of maximal pulsation. Use only enough pressure to elevate the blue pressure tracing to about ¼ the height of the screen (200 scale).

3.3.7. Optimize location and hold-down pressure as detailed above.

3.3.8. Once an optimal waveform is obtained, stabilize your position, hold for 10 seconds and save.

3.4. Site Specific Notes: Radial

3.4.1. The radial artery is superficial and reasonably constrained just proximal to the first of the wrist creases, at approximately the location of the styloid process of the radius.

3.4.2. The radial artery is generally very superficial and is surrounded by bone. Therefore, use only enough pressure to elevate the blue pressure tracing to about ¼ the height of the screen (200 scale).

3.4.3. If the artery is recessed below the level of the flexor tendons of the wrist, it may be necessary to place the tonometer body and cable in-line with (rather than perpendicular to) the long axis of the vessel in order to avoid a bridging effect of surrounding tendons and bone.

3.4.4. Optimize location and hold-down pressure as detailed above.

3.4.5. Once an optimal waveform is obtained, stabilize your position, hold for 10 seconds and save.

3.5. Site Specific Notes: Femoral

3.5.1. The femoral artery is the most deeply located of the vessels. Therefore, finding the optimal pulse location prior to attempting tonometry is important.

3.5.2. In general, "high and inside" is a good starting place. Thus, start at or above the inguinal crease, and stay well medial to the insertion of the quadriceps tendons.

3.5.3. Locate the pulse with the right hand by placing all four fingers along the inguinal ligament (essentially perpendicular to the long axis of the vessel). One of the fingertips should fall on the artery.

3.5.4. Place the index, middle and ring fingers of the left hand along the long axis of the artery, realizing that the proximal vessel is angling toward the midline. Once the fingers are aligned with the artery, move the hand along the vessel as needed to position the index finger at the point of maximal pulsation. Then move the index finger aside and, using your right hand, place the tonometer at the point of maximal pulsation while maintaining patient contact with the middle and ring fingers of the left hand. This will help establish localization of the vessel as the tonometer is properly positioned.

3.5.5. The femoral artery will require more pressure than the brachial or radial. Apply enough pressure with the tonometer to elevate the blue pressure tracing to mid-screen on 200 scale. Find the vessel midline as detailed above in the section on use of the tonometer. Once the waveform is visible, if you have kept the fingers of your left hand on the pulse, gradually reduce the pressure being applied by the fingertips of the left hand, if possible. Occasionally, especially with obese patients, it will be necessary to maintain some pressure with the left hand in order to compress or displace overlying adipose tissue. However, do not press hard enough with the left hand to compress the artery.

3.5.6. If it is difficult to apply and maintain sufficient hold-down pressure with the index finger of your right hand alone, use your left hand to apply additional pressure to your right index finger.

3.5.7. Waveform morphology will be somewhat less well defined with the femoral artery. The dicrotic notch will be evident more as an abrupt change in slope rather than an actual notch in most patients. Furthermore, the reflected wave will generally merge with the forward wave to form a single peak. However, pulse amplitude should be comparable to that of the brachial and radial.

3.5.8. Once an optimal waveform is obtained, stabilize your position, hold for 10 seconds and save.

3.6. Site Specific Notes: Carotid

3.6.1. Position the patient's head so that the chin is slightly up and the patient is looking slightly away from you. Exaggeration of either of these adjustments will make tonometry more difficult as it will place excessive tension on the sternocleidomastoid (SCM) muscle and skin.

3.6.2. The optimal location for carotid tonometry is just lateral to the larynx, in the angle between the SCM muscle and the larynx, i.e., cranial and medial to the SCM. It is possible to compress the carotid against the pre-vertebral muscles in this region. Do not palpate the carotid from the side of the neck through the SCM. This will require excessive hold-down pressure and will produce a damped tracing.

3.6.3. Locate the carotid pulse and determine the mobility of the vessel. Then use the very tip of your fully extended right index finger (as though pointing at the pulse) to imitate a tonometer and determine the optimum approach, i.e., one which allows you to palpate a suitable pulse without causing the vessel to move. The initial approach should be approximately 45 degrees lateral to the midline and perpendicular to the long axis of the vessel. Once the vessel is located, decreasing the angle with respect to the midline may help to stabilize the vessel against the pre-vertebral muscles. If your finger is properly

located in the angle between the SCM and larynx, you will find that very light pressure will be needed to palpate the pulse even in patients with fairly large necks.

3.6.4. Attempt tonometry using the optimum approach that was established using your finger. Note that once the vessel is located, a more stable tracing is often obtained by adjusting the hold-down angle of the tonometer to be more parallel with a plane through the midline, i.e., in an anterior-posterior direction.

3.6.5. It is important to stabilize your arm and hand during carotid tonometry. Your arm should lie across the patient's deltopectoral groove or chest; thus, you must be seated just across from the patient's shoulder. This position is facilitated by setting up the room with the head of the bed pulled away from the wall by 18-24" so that when the NIHem cart is pushed against the wall it is out of the way. The arm can also rest on the patient's chest, although respiratory artifact may be a problem. Additionally, the fingers of the hand holding the tonometer should be flat against the skin of the neck if possible.

3.6.6. Tell the patient that it is OK to swallow as needed. If the patient swallows during a suitable recording period, it will create an artifact that will require a few seconds of additional recording; however, the patient will be more relaxed.

3.6.7. In the average patient, very little pressure will be required to obtain a waveform. Therefore, use only enough pressure to elevate the blue pressure tracing to about ¼ the height of the screen (200 scale). In heavier patients, it may be necessary to apply more pressure, though rarely to beyond the mid-screen level.

3.6.8. Once an optimal waveform is obtained, stabilize your position, hold for 20 seconds and save.

Figure 9a. Performing a sweep across the long axis of the brachial artery.

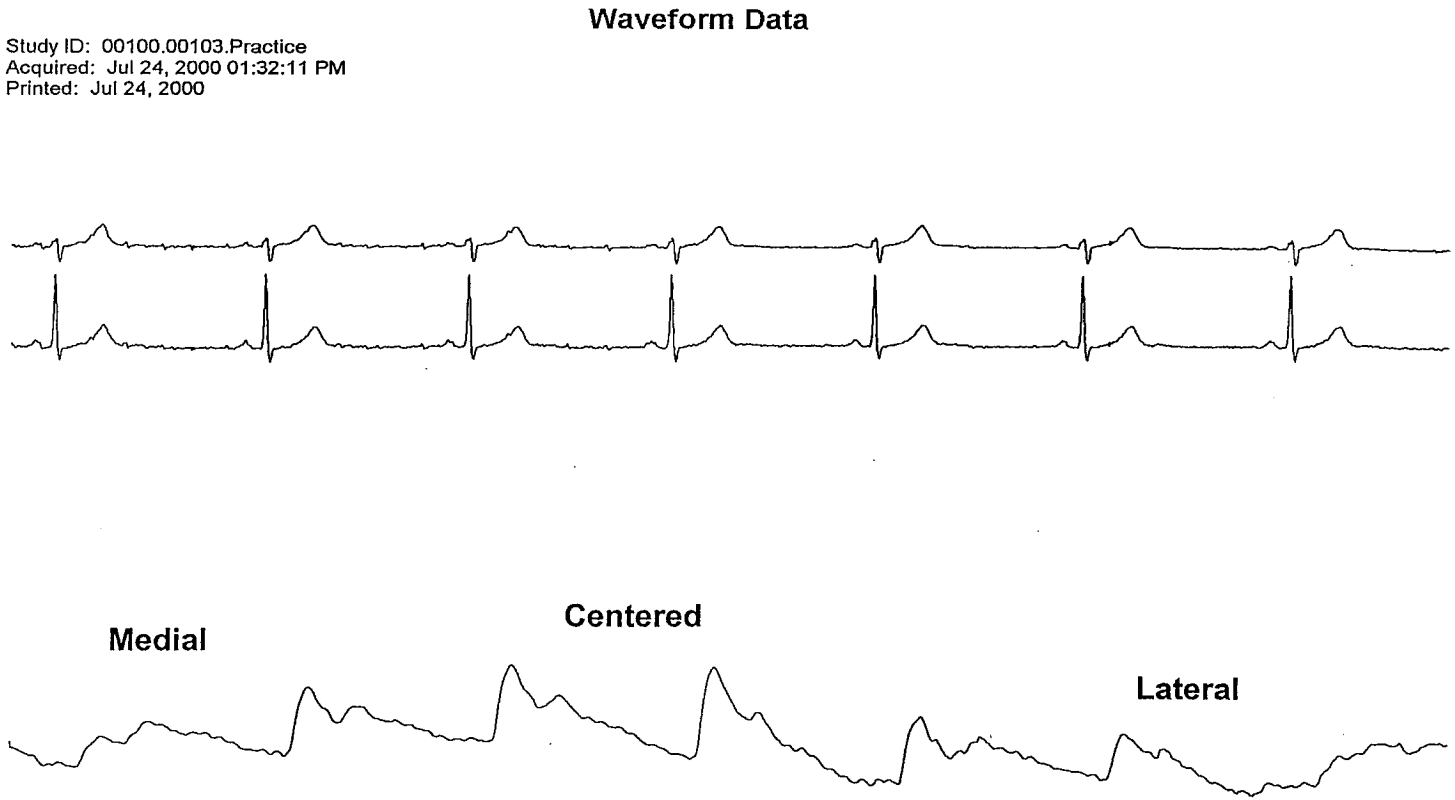


Figure 9b. Performing a sweep across the long axis of the radial artery.

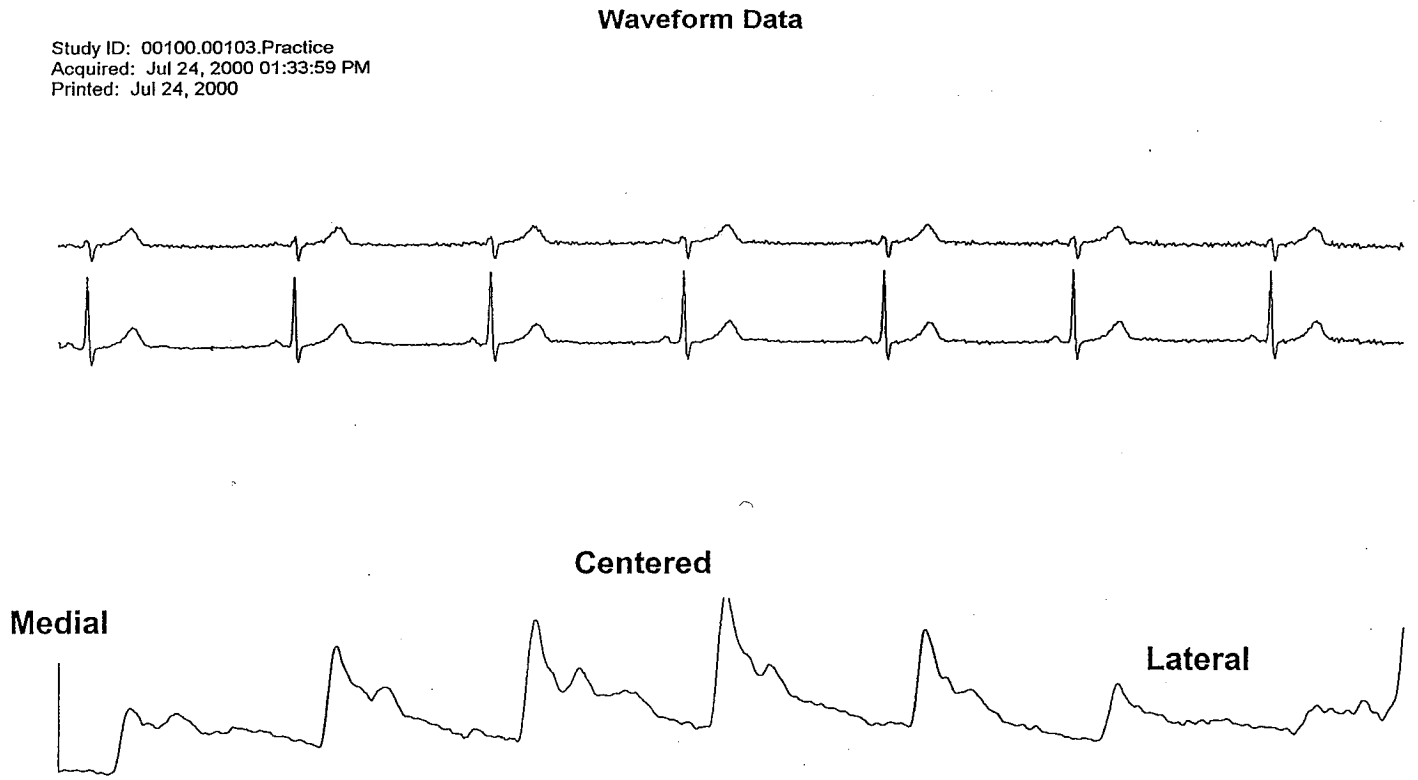


Figure 9c. Performing a sweep across the long axis of the femoral artery.

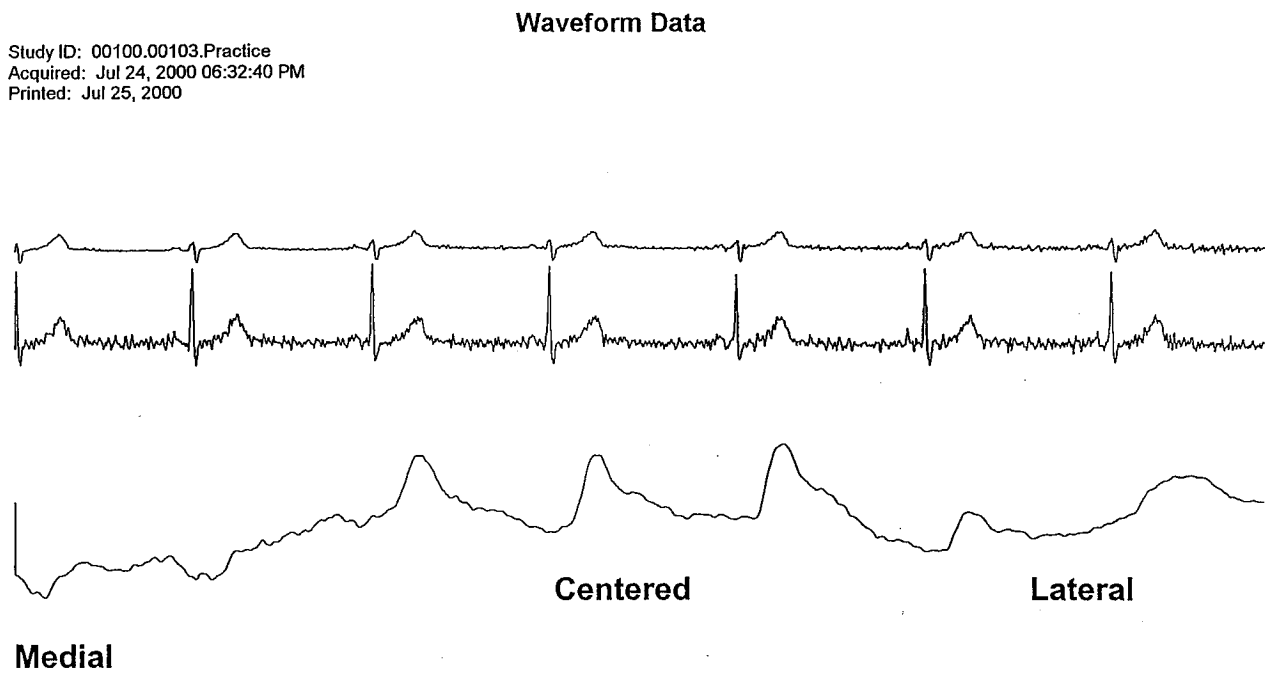
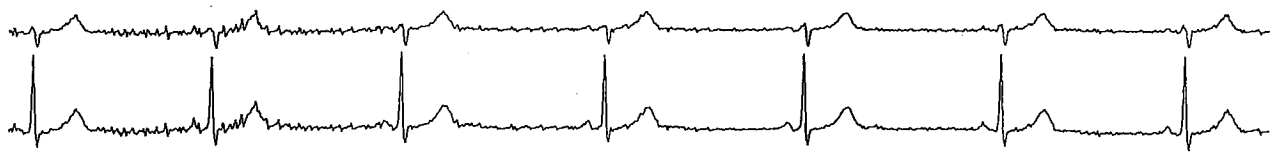


Figure 9d. Performing a sweep across the long axis of the carotid artery.

Waveform Data

Study ID: 00100.00103.Practice
Acquired: Jul 24, 2000 01:39:47 PM
Printed: Jul 24, 2000



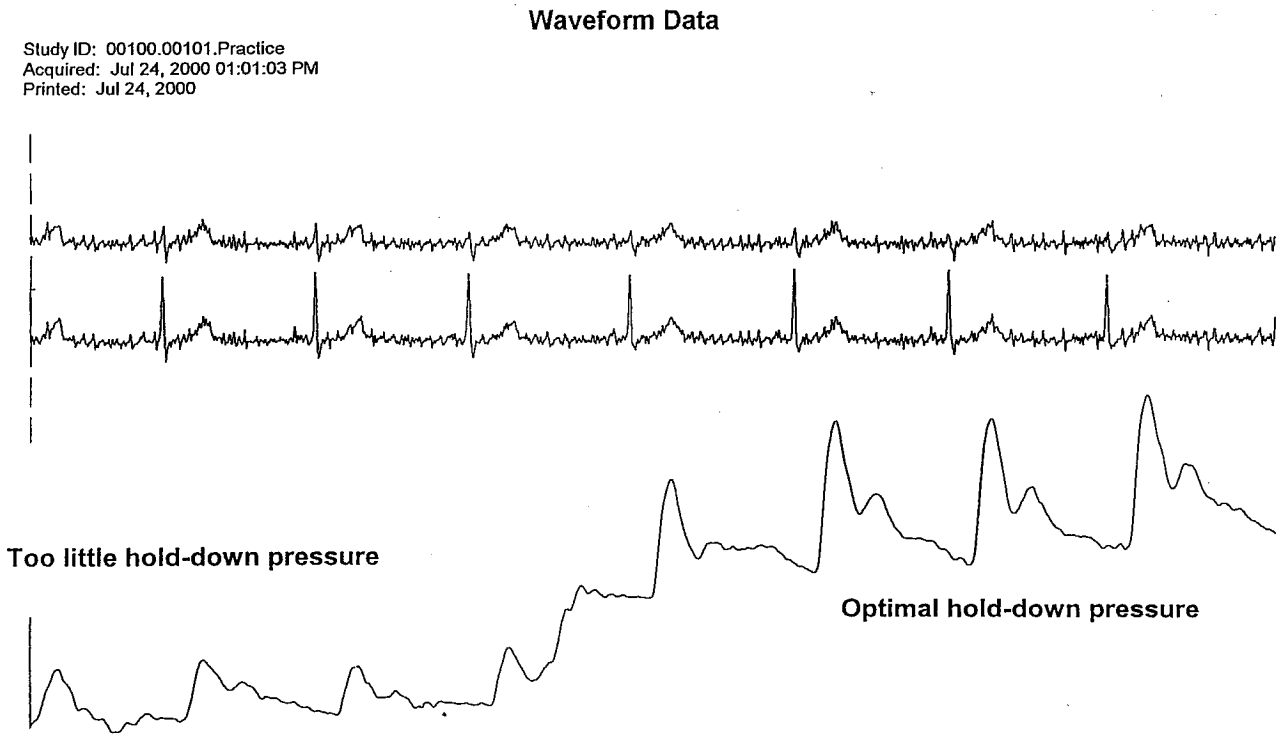
Note the loss of features and merging of the dicrotic notch with a flattened diastolic baseline as the tonometer moves off center, giving a low amplitude, square-wave appearance to the pressure waveform

Centered

Medial **Lateral**

Waveforms: Carotid Scale: 200 Sweep: 10 Origin: 0 Thresh: 100

Figure 9e. Changing brachial hold-down pressure from too little to optimal.



The pulse is located using enough pressure to elevate the waveform to about mid-screen. Once a waveform is located, additional hold-down pressure is applied, resulting in higher amplitude and sharper waveform features (dicrotic notch and reflected wave).

Figure 9f. Changing radial hold-down pressure from too little to optimal.

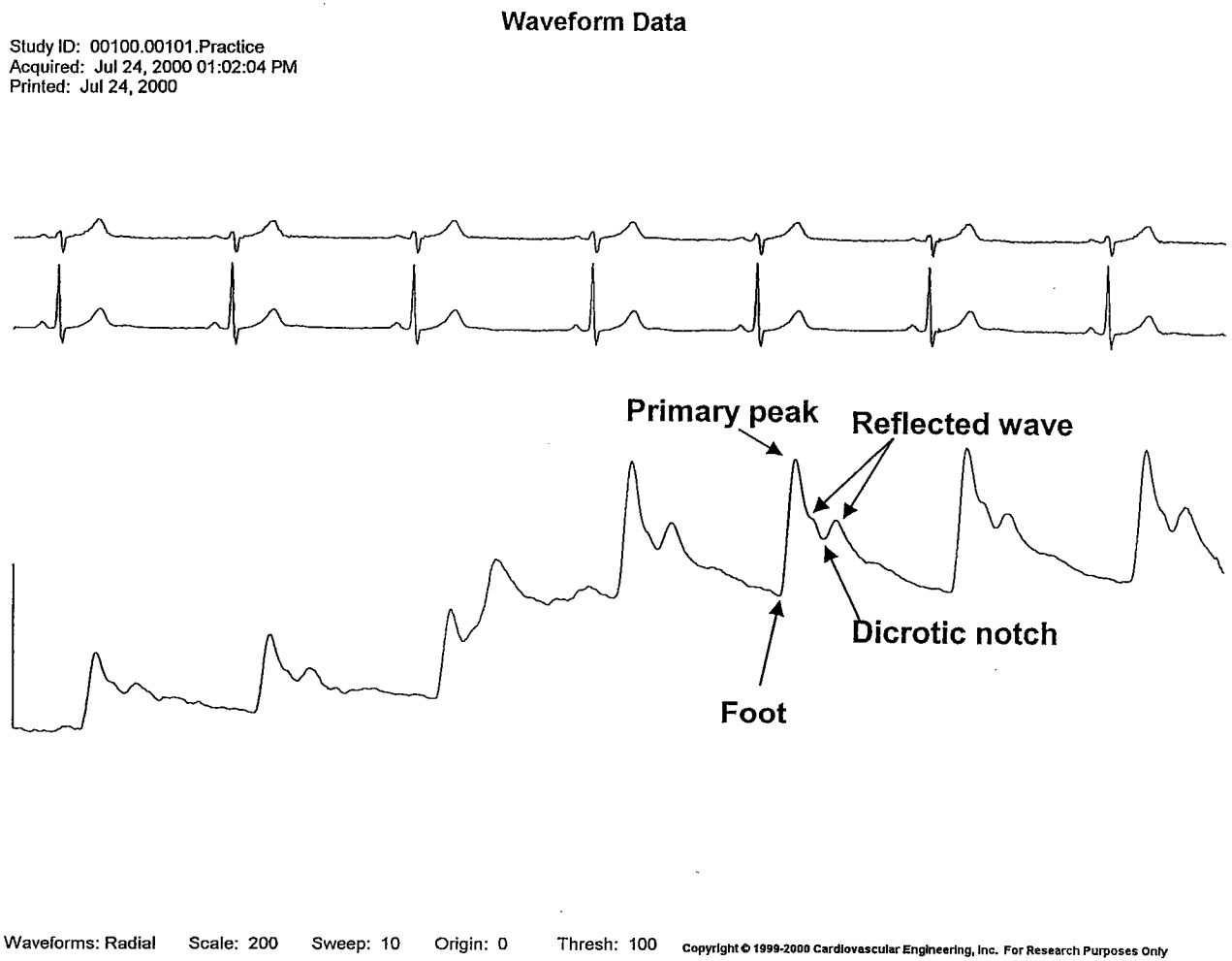


Figure 9g. Changing femoral hold-down pressure from too little to optimal.

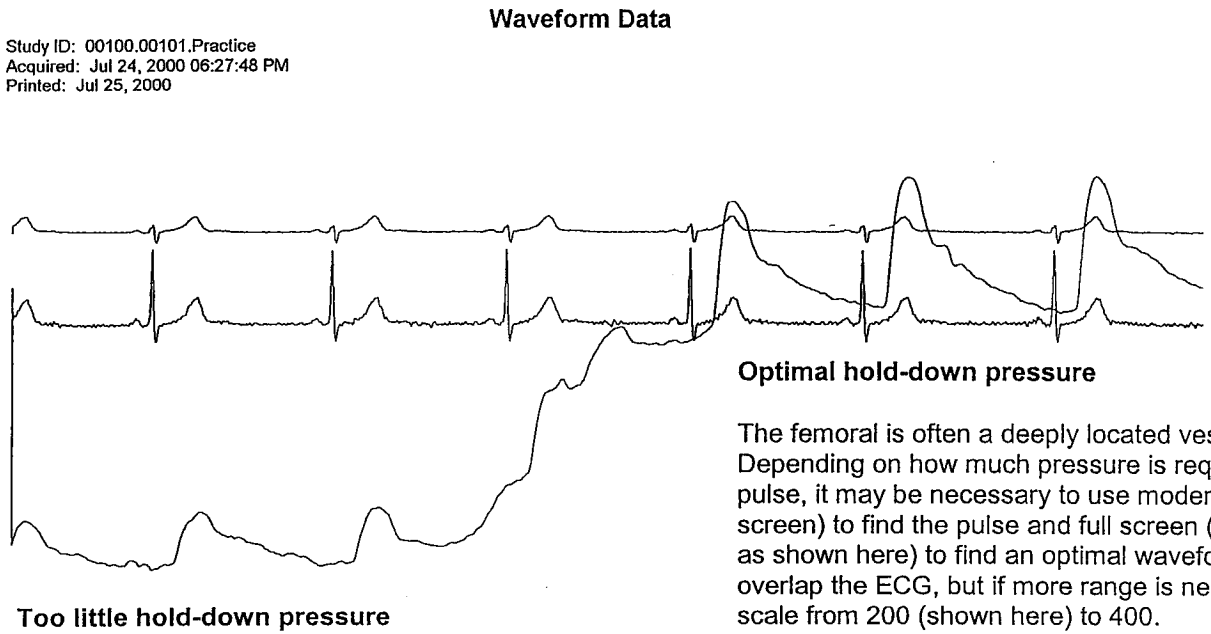


Figure 9h. Changing carotid hold-down pressure from too little to optimal.

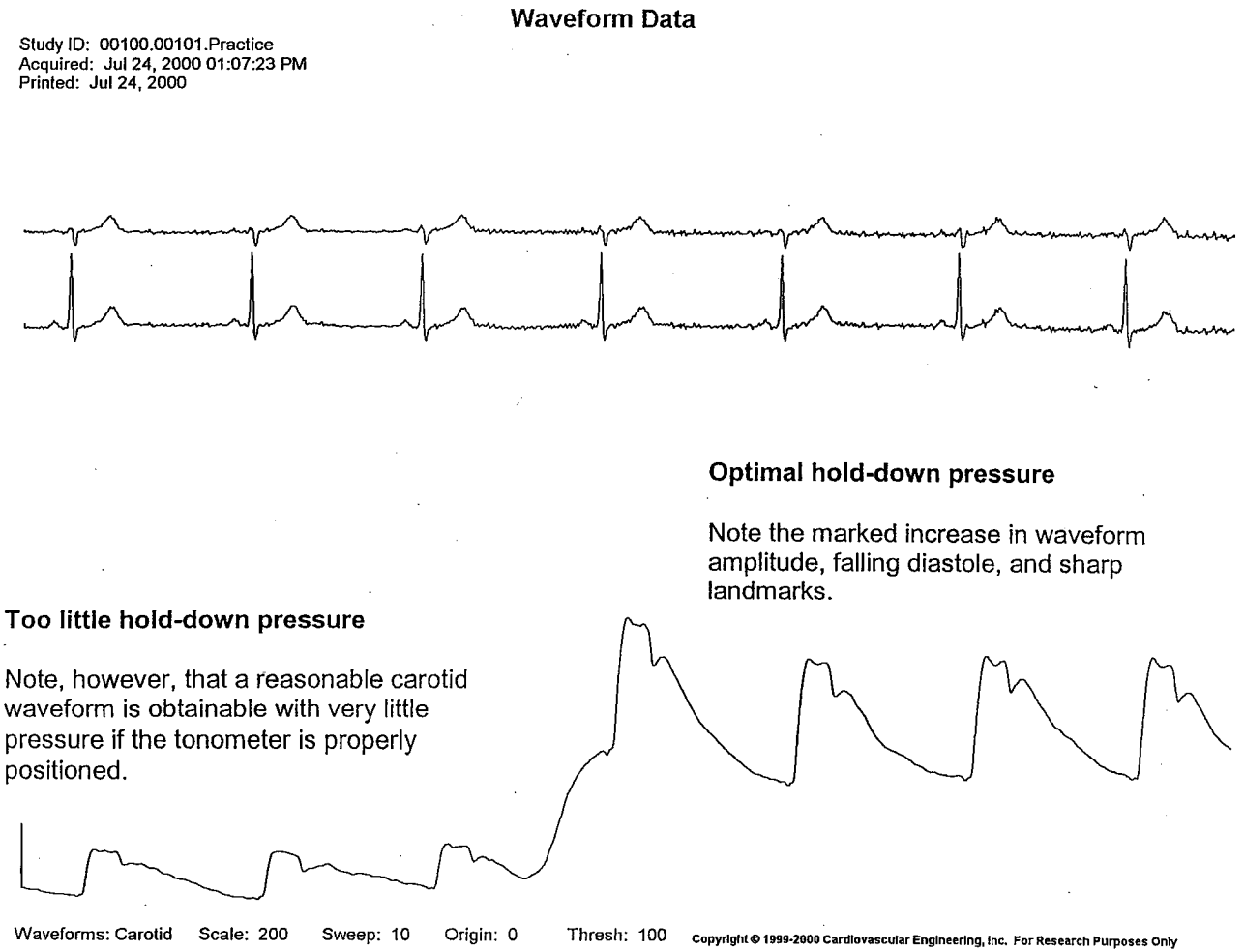
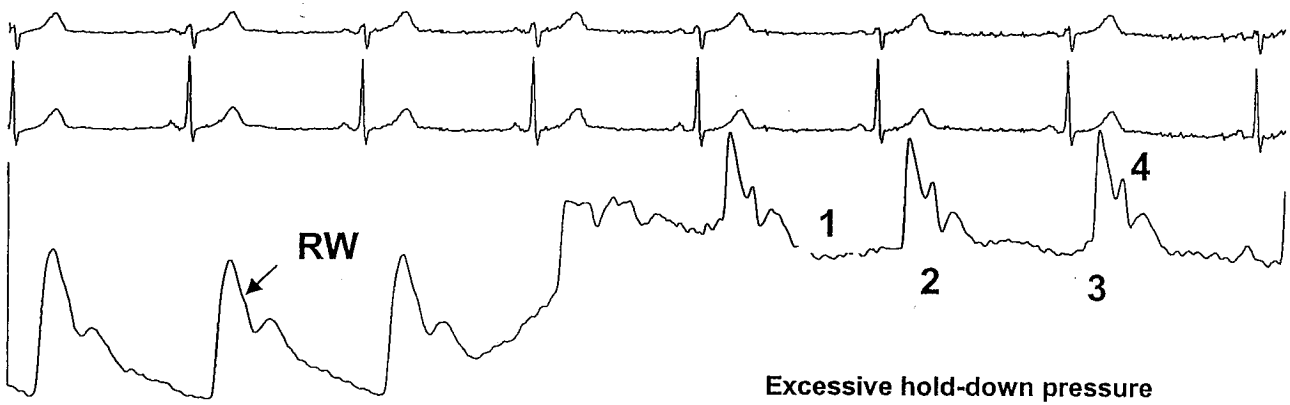


Figure 9i. Changing brachial hold-down pressure from optimal to excessive.

Waveform Data

Study ID: 00100.00102.Practice
Acquired: Jul 24, 2000 01:10:48 PM
Printed: Jul 24, 2000



Optimal hold-down pressure

Optimal waveform has falling diastole, sharp foot, monophasic upstroke, reflected wave (RW), which begins in systole with remainder in diastole and obvious dicrotic notch.

Excessive hold-down pressure

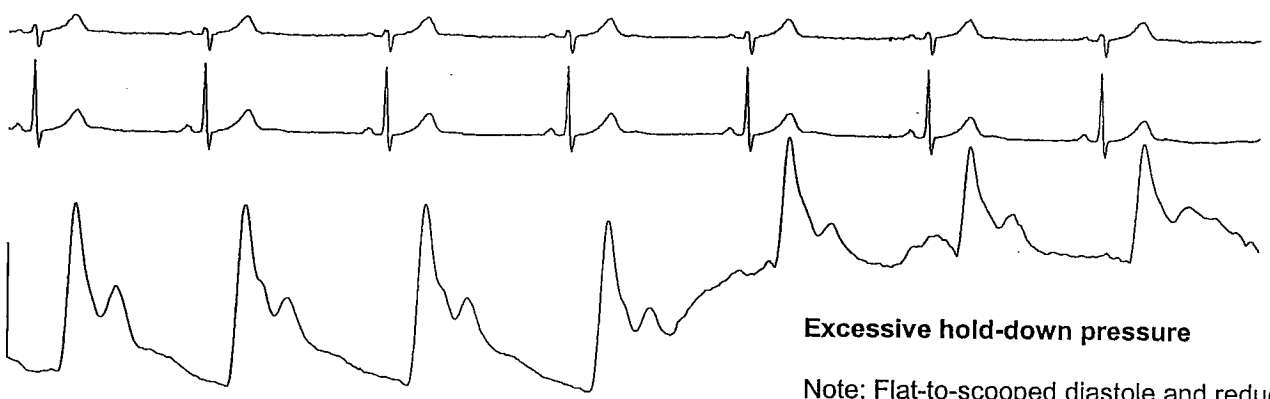
When excessive pressure is applied diastole flattens (1), waveform amplitude is reduced (2), waveform foot is slurred (3) and reflected wave and dicrotic notch become excessively peaky (4).

Waveforms: Brachial Scale: 200 Sweep: 10 Origin: 0 Thresh: 100

Figure 9j. Changing radial hold-down pressure from optimal to excessive.

Waveform Data

Study ID: 00100.00102.Practice
Acquired: Jul 24, 2000 01:13:55 PM
Printed: Jul 24, 2000



Optimal hold-down pressure

Excessive hold-down pressure

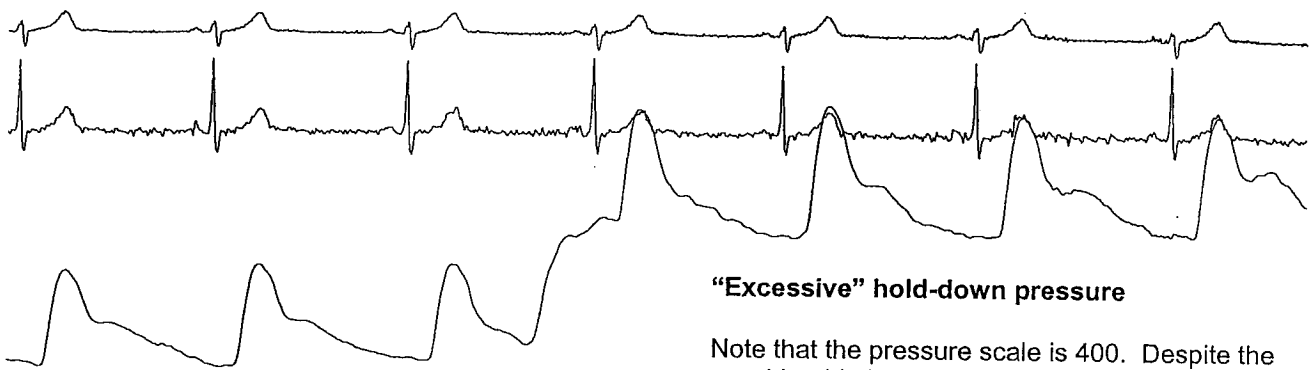
Note: Flat-to-scooped diastole and reduced waveform amplitude when excessive hold-down pressure is applied.

Waveforms: Radial Scale: 200 Sweep: 10 Origin: 0 Thresh: 100

Figure 9k. Changing femoral hold-down pressure from optimal to "excessive."

Waveform Data

Study ID: 00100.00102.Practice
Acquired: Jul 24, 2000 06:13:28 PM
Printed: Jul 25, 2000



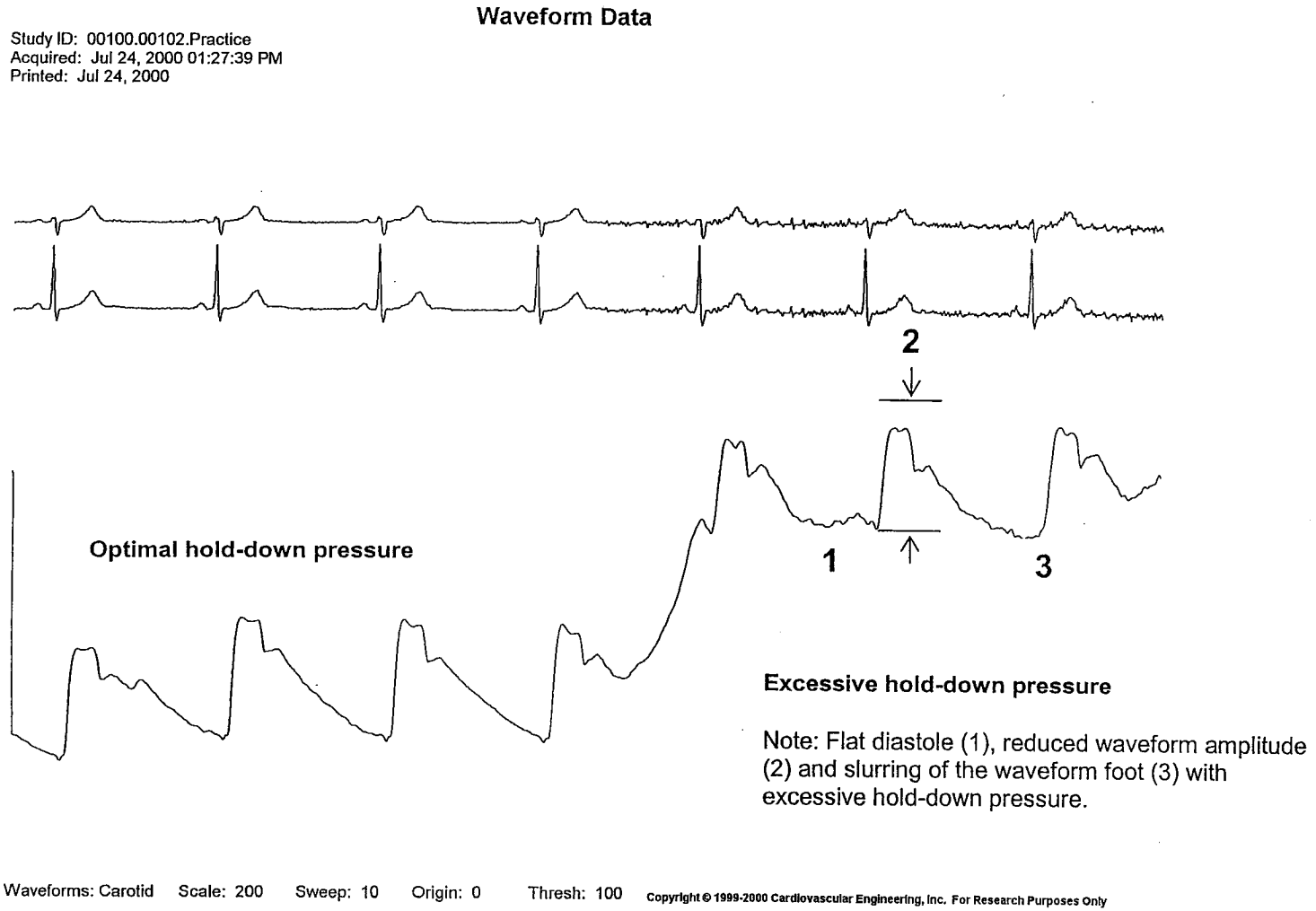
Optimal hold-down pressure

"Excessive" hold-down pressure

Note that the pressure scale is 400. Despite the considerable hold-down pressure, the waveform still looks reasonable, with the possible exception of mild scooping of diastole. Waveform amplitude continues to increase due to improved coupling with increased pressure in this deeply located vessel.

Waveforms: Femoral Scale: 400 Sweep: 10 Origin: 0 Thresh: 75

Figure 9I. Changing carotid hold-down pressure from optimal to excessive.

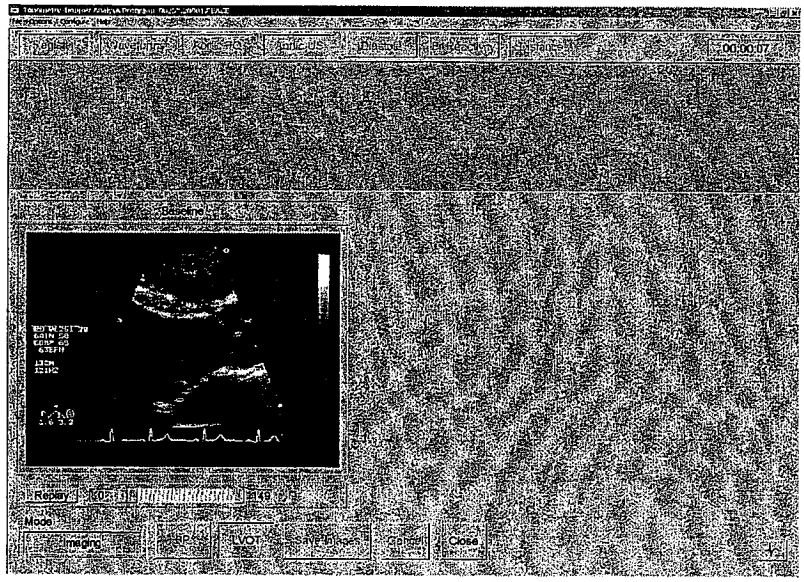


4.0. LVOT Diameter Imaging Acquisition

4.1. Place patient in the left lateral decubitus position. Use pillows as props to ensure patient comfort. Confirm that the ECG tracing is intact after moving the patient.

4.2. Click [Aortic US] from main menu bar.

Figure 10. NIHem LVOT Imaging Acquisition Screen.



4.3. Images are obtained using a parasternal long axis view with the aortic valve plane at the horizontal center of the image (Figure 10).

4.4. Depth setting should be minimized if your machine does not have zoom capability. Otherwise, please minimize the depth setting first (11-13 cm) and then use 'zoom' with a 4-6 cm region of interest in order to maximize the size of the valve (Figure 11).

4.5. Aortic leaflets should be visible throughout the cardiac cycle so that the hinge points of the leaflets can be established.

4.6. Measurements are taken in systole, so it is important to ensure that the LVOT does not rock out of plane during systole.

4.7. Watch for three or four consecutive analyzable cardiac cycles and click [Save Images].

4.8. Click [Replay] and ensure that there are at least two good images that clearly demonstrate the full diameter of the LVOT during systole.

Figure 11. Optimal Aortic Root and LVOT Imaging Acquisition.

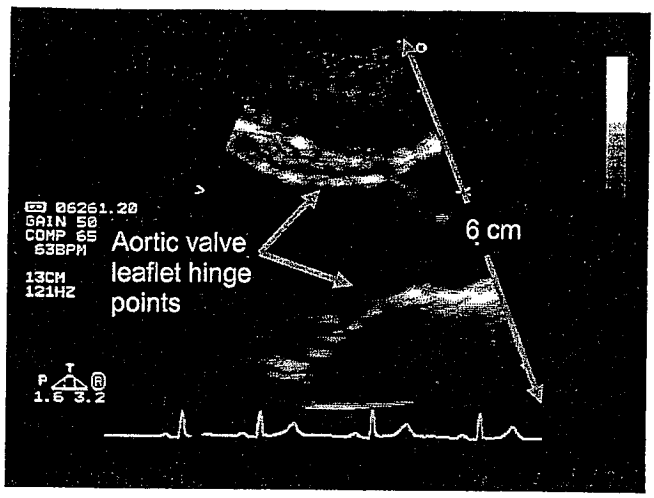


Figure 12. Aortic Root and LVOT Imaging Acquisition that is not Zoomed.

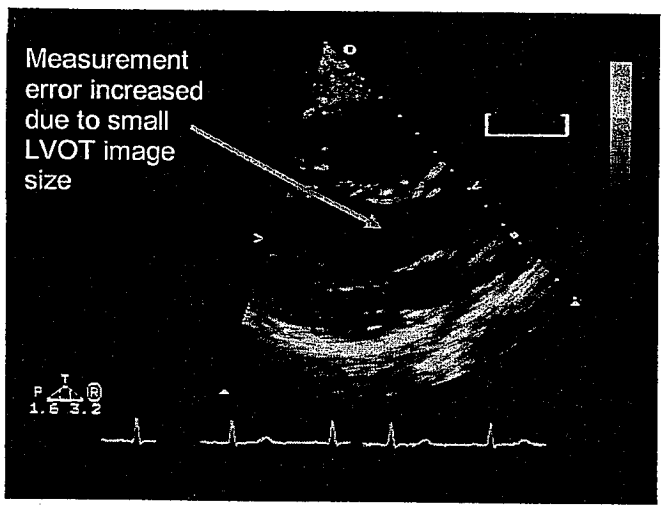
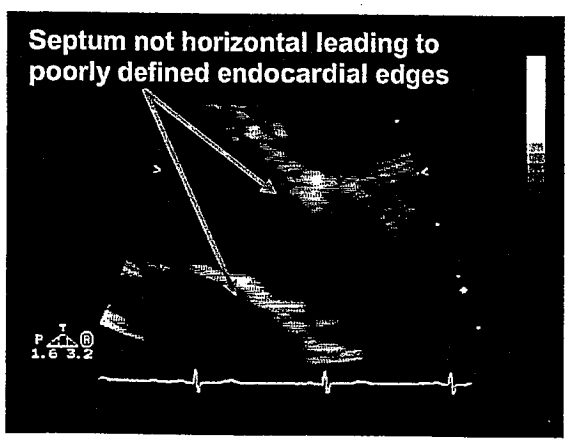


Figure 13. Aortic Root and LVOT Imaging Acquisition with Acoustic Window too Low.

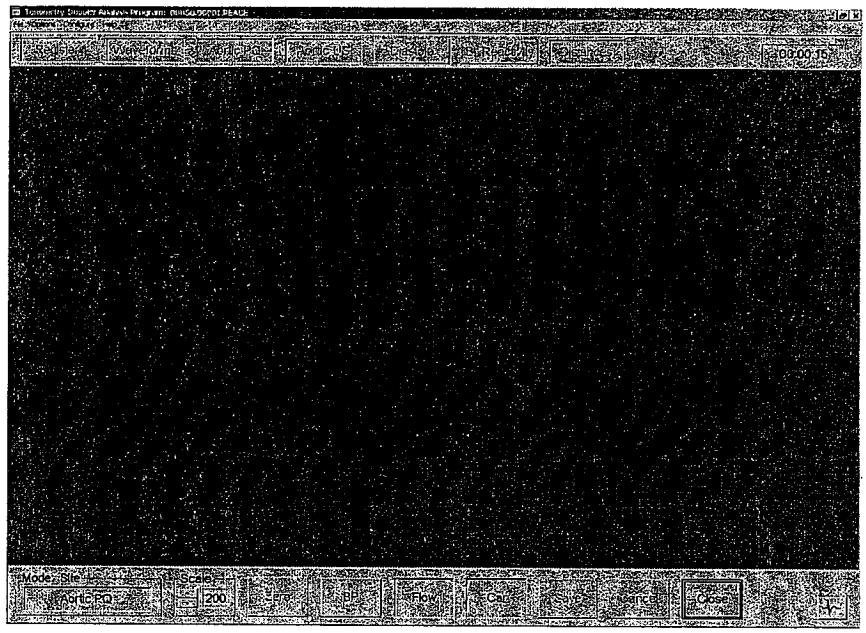


5.0. *Left Ventricular Outflow Tract (LVOT) Doppler Acquisition*

5.1. Click [Aortic PQ] from main menu bar.

5.2. Click [Flow] from the Aortic PQ toolbar (Figure 14).

Figure 14. NIHem LVOT Doppler Acquisition Toolbar.



5.3. Obtain pulsed Doppler from the left ventricular outflow tract just proximal to the aortic valve in an apical five-chamber view (see Figure 15). Use the following echo settings (adjust as necessary):

- Filter: 300Hz – 400Hz
- Gain: 65% (Avoid high PRF)
- Scale: 120cm/sec (marked 120cm/sec but actually visible is 180cm/sec
- Sweep: 50

5.4. Find maximal peak flows, which should correspond to the proper placement of the sample volume in the LVOT.

5.5. Keep the zero baseline at the center of the spectral display so that the scale is symmetrical, e.g. –80 to +80, -120 to +120, etc. If aliasing is seen, change the scale in order to eliminate the aliasing. Do not shift the zero baseline, as this only obscures the aliasing (but does not eliminate it from the audio signal that is recorded by the computer).

5.6. Use minimal wall filter settings in order to avoid a loss of information on either side of the zero baseline. Excessive filtering creates a wide gap, which makes it difficult to assess the onset and end of flow.

5.7. Problems can arise from positioning the sample volume too low in the LVOT or overlying tissue. Improper placement of a sample volume can result in tissue artifact and low amplitude Doppler signal, as shown in Figures 16 and 17. Placement of the sample volume too low in the LVOT will record excessive mitral inflow signal and will underestimate true peak LVOT flow, as shown in Figure 18. Flows should be laminar in these patients with no aortic valve disease. Flow envelopes should be bright (on screen) and clearly audible, indicating a good signal-to-noise ratio.

Figure 15. Optimal LVOT Doppler Acquisition.

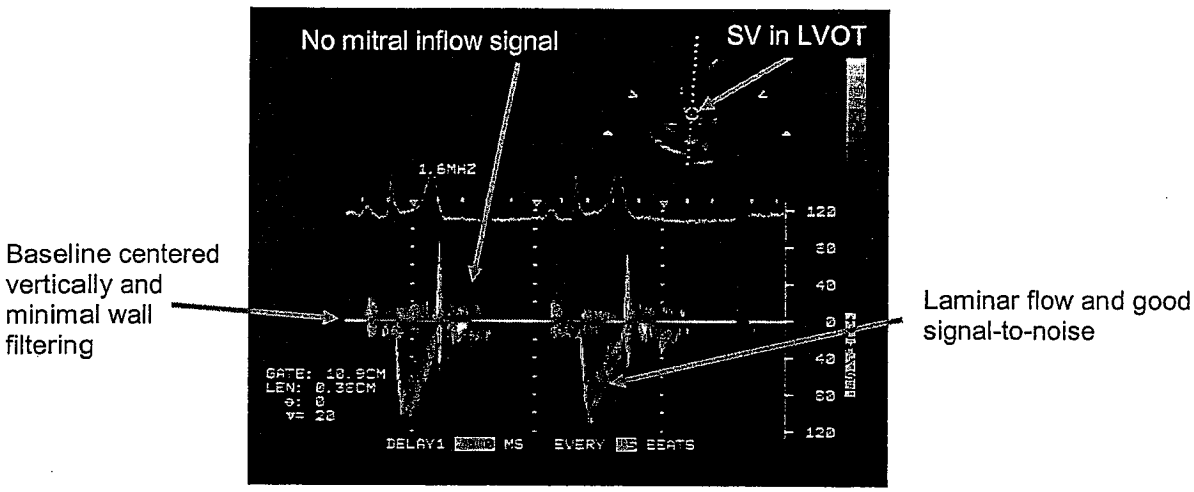


Figure 16. LVOT Doppler Acquisition with Septal Artifact.

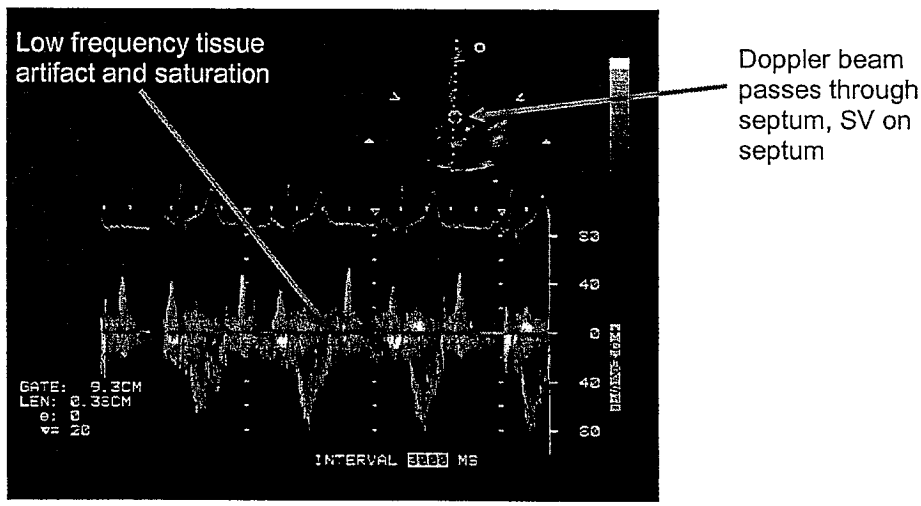


Figure 17. LVOT Doppler Acquisition with Poor Signal.

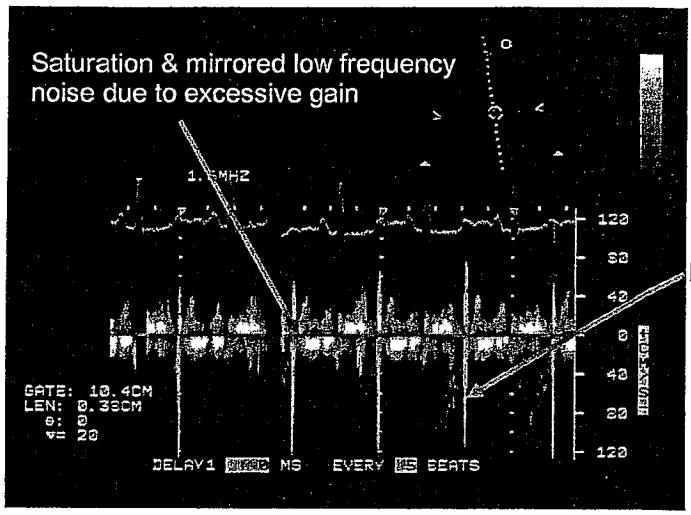
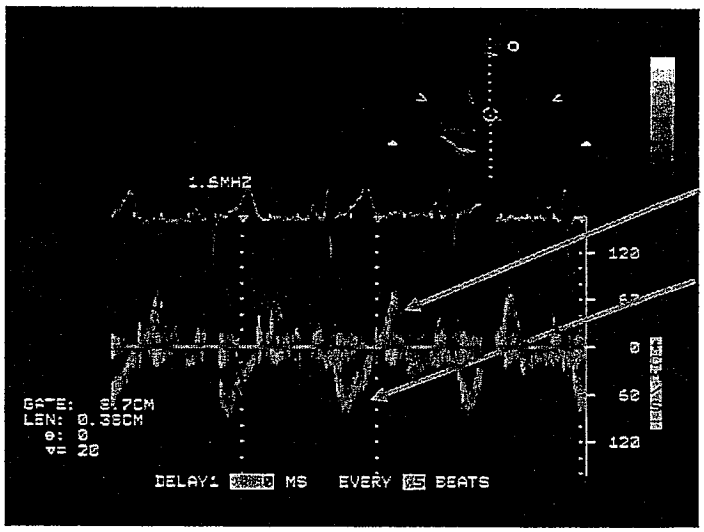


Figure 18. LVOT Doppler Acquisition with Sample Volume too Low.



5.8. Once 20 seconds of continuous optimal Doppler signal has been acquired, click [Save].

5.9. Click [Car]. Optimize the Carotid site tonometry and record 20 seconds of good waveforms. See tonometry acquisition section above and attached documents for more information on obtaining optimal tonometry.

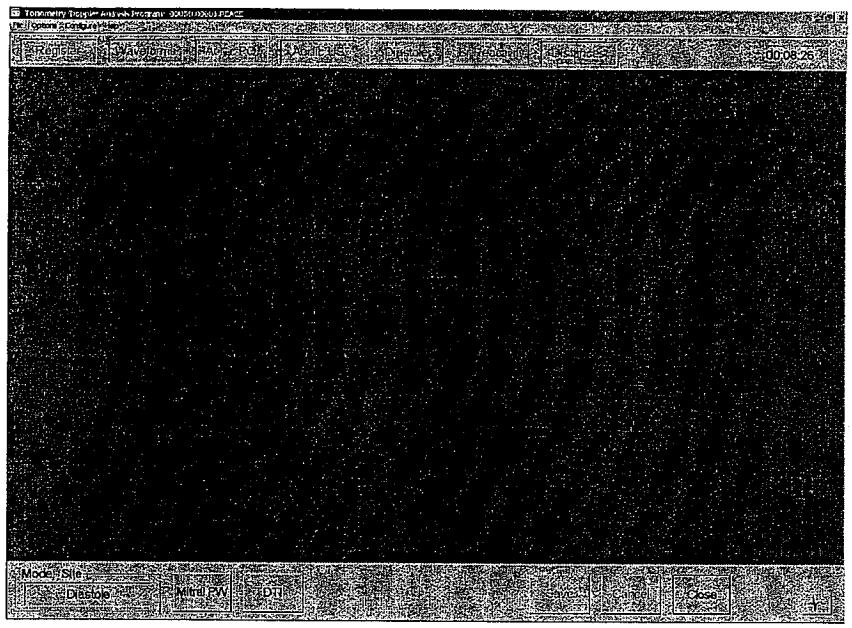
5.10. Close [Aortic PQ] toolbar.

6.0. *Mitral Inflow/Diastolic Function Acquisition*

6.1. Keep patient in left lateral decubitus position. Do not manipulate the patient's breathing pattern during the diastolic function acquisition as this may adversely influence the quality of the data acquired. There may be loss of signal during certain phases of the respiratory cycle, but this is preferable to breath-holding.

6.2. Click [Diastole] and then [Mitral PW] (Figure 19).

Figure 19. NIHem Diastolic Function Acquisition Toolbar.



6.3. Use Pulsed Doppler mode from an apical 4-chamber view of the heart. The sample volume should be placed at the level of the mitral leaflet tips during diastole. See Figure 20 for optimal recording and Figures 21-23 for recordings with common errors.

6.4. The following echo settings (with adjustments as necessary) should be used:

- Filter: 300Hz – 400Hz
- Gain: 60%
- Scale: 80 cm/sec
- Sweep: 50

Figure 20. Optimal Mitral Inflow Acquisition.

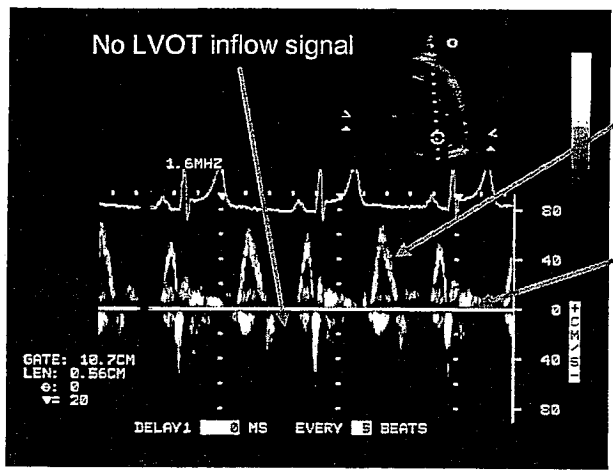


Figure 21. Mitral Inflow Acquisition with Sample Volume not Centered.

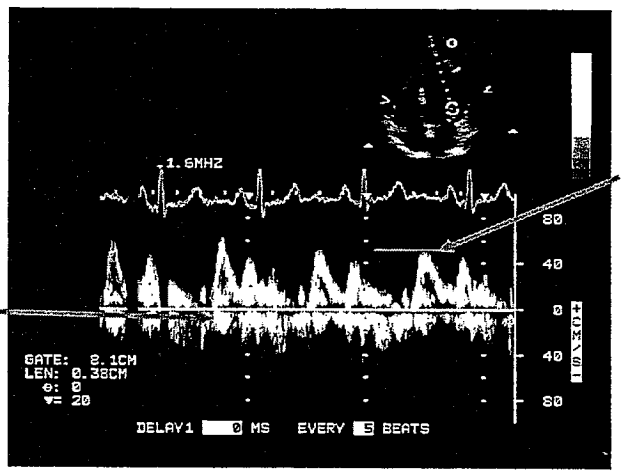


Figure 22. Mitral Inflow Acquisition with Sample Volume too Low.

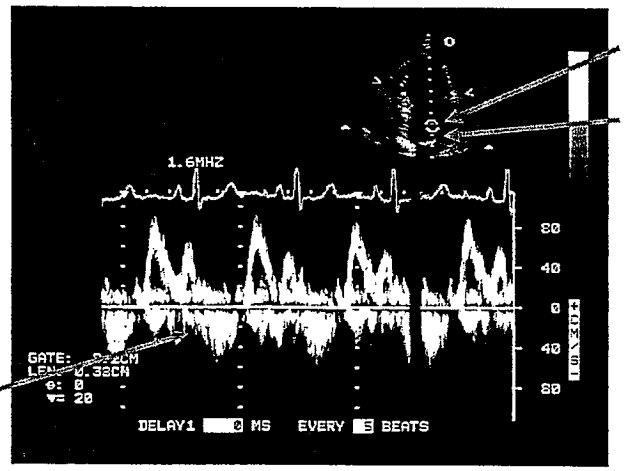
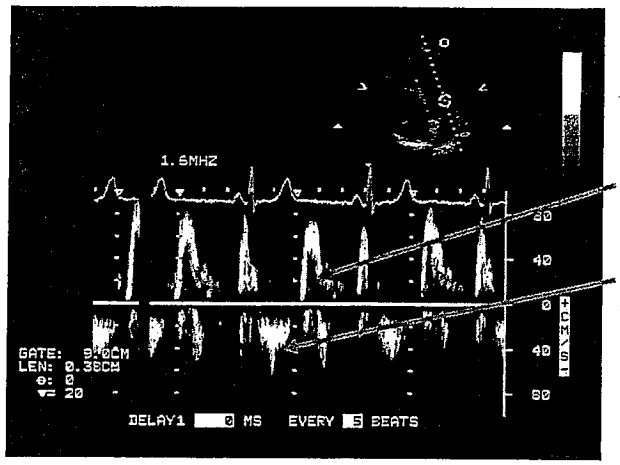


Figure 23. Mitral Inflow Acquisition with Excessive Filtering.



6.5. Once 20 seconds of continuous optimal Doppler signal has been acquired, click [Save].

6.6. Click [Close].

7.0. *Doppler Tissue Velocity Acquisition*

7.1. Keep patient in left lateral decubitus position. Do not manipulate the patient's breathing pattern during the diastolic function acquisition as this may adversely influence the quality of the data acquired. There may be loss of signal during certain phases of the respiratory cycle, but this is preferable to breath-holding.

7.2. Use Tissue Doppler mode from an apical 4-chamber view of the heart. The sample volume should be placed at the center of the lateral mitral annulus. See Figure 24 for optimal recording and Figures 25-26 for recordings with common errors.

7.3. Use the "Tissue Doppler" preset on the echo machine. The following settings are contained within this preset, and can be entered manually if necessary:

- Filter: 50Hz
- Gain: 55%
- Scale: 20 cm/sec
- Sweep: 50

7.4. Click [Diastole] and then [DTI] to begin acquiring data (see Figure 19).

Figure 24. Optimal Doppler Tissue Velocity Acquisition.

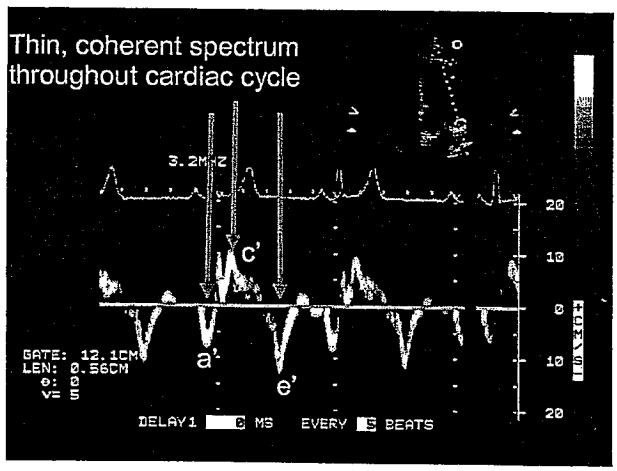
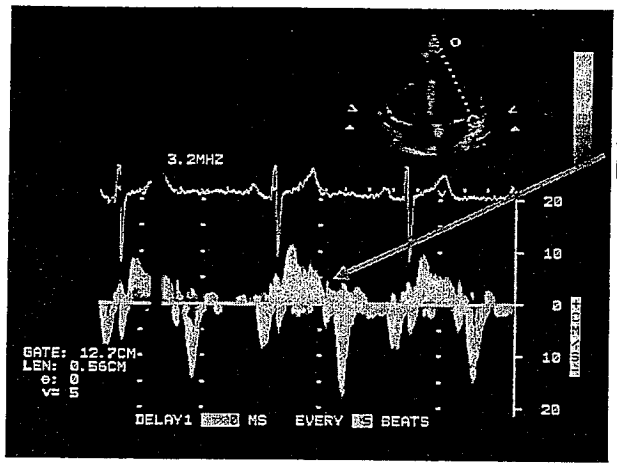
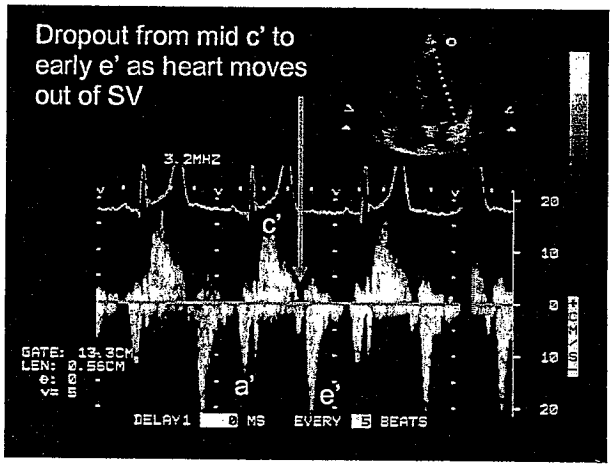


Figure 25. Doppler Tissue Velocity Acquisition with Excessive Gain.



Saturation and regions of broadband spectral content

Figure 26. Doppler Tissue Velocity Acquisition with Sample Volume behind Endocardium.



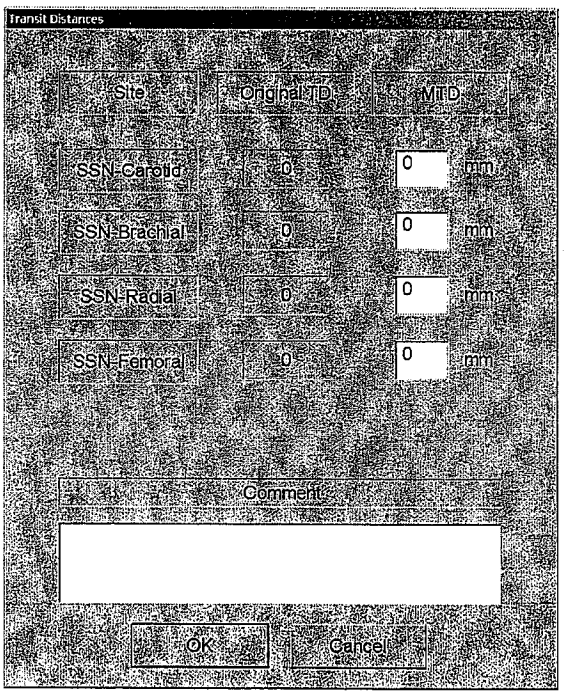
7.5. Once 20 seconds of continuous optimal tissue Doppler signal has been acquired, click [Save].

7.6. Click [Close].

8.0. Transit Distances Acquisition

- All measurements are made from the base of the supra-sternal notch (SSN) to the various pulse acquisition sites.
- The patient should remain supine exactly as they were positioned during the tonometry acquisition sequence.
- Use the tape measure supplied with the NIHem system to measure the SSN-Carotid, SSN-Brachial, and SSN-Radial transit distances. This fiberglass tape measure will not stretch. It also has an extension at the zero end, which is used as a handle. This allows the operator to place their thumbnail exactly on the zero mark and to then keep the zero mark firmly seated in the SSN without the need to look at both ends of the tape measure.
- Use the supplied oversized calipers for the SSN-Femoral transit distance measurement. This will avoid overestimation of this distance in obese patients.
- Click [Distances] to access the screen where the values are inputted (Figure 27).

Figure 27. Transit Distances Entry Dialog Box.



8.1. Measure the SSN-Carotid transit distance.

8.1.1. Place your right thumbnail on the zero mark, and then place the zero mark in the "V" at the midline at the base of the SSN.

8.1.2. Position the patient's head just as it was during the tonometry acquisition.

8.1.3. Extend the tape measure up to the dot at the carotid site and pull it moderately tight.

8.1.4. Record the distance in mm to the nearest 1 mm.

8.2. Measure the SSN-Brachial and SSN-Radial transit distances.

8.2.1. Abduct the patient's arm to 90° to form a straight line from supra-sternal notch to brachial site to radial site.

8.2.2. The arm must be kept in the plane of the body to avoid under- or over-estimating either distance.

8.2.3. Place your right thumbnail on the zero mark, and then place the zero mark in the "V" at the base of the SSN.

8.2.4. Pull the tape measure across to the dot at the brachial acquisition site. The tape measure should just touch the surface of the body as it touches the brachial site, indicating that the arm is in plane with the chest wall. Specifically, there should be no bridging from the SSN to the Brachial site as will occur if the arm is anterior to the plane of the chest. Note the distance in mm to the nearest 1 mm.

8.2.5. Use your left hand to anchor the tape measure at the brachial location. Release the zero point with your right hand, transfer your right hand to the brachial location and anchor the tape measure at the brachial site so that the brachial distance is still aligned with the brachial dot. Then use your left hand to further extend the tape measure to the radial site. This approach provides a surface measurement of the full distance from SSN to radial site and avoids a bridging effect that is possible if the tape measure is pulled directly between SSN and radial.

8.3. Measure the SSN-Femoral transit distance.

8.3.1. This distance is measured with the provided oversized calipers. Open caliper arms until visibly wider than distance to be measured.

8.3.2. Align the inner (i.e. nearest to other caliper arm) surface of the free end of the fixed arm of caliper in the "V" at the base of the SSN (be aware of the close proximity to patient's chin!). The calipers should extend down the body and NOT towards the head of the patient (Figure 28).

8.3.3. Caliper scale should align approximately with the mid-sagittal body plane.

8.3.4. Grasp the free end of the caliper with your right hand, just to the right of the block. Use your right thumb to gently push the block to the appropriate distance. Holding the caliper with the right hand creates the leverage to stably and accurately move the block.

8.3.5. Align the inner edge (i.e. nearest to other caliper arm) of the free end of the moveable arm of the caliper with the dot at the femoral site by gently pushing the free arm with right hand thumb.

8.3.6. Record the distance from the left (inner) side of the sliding block on the calipers in mm to the nearest 1 mm.

Figure 28. Caliper placement for SSN-Femoral transit distance.

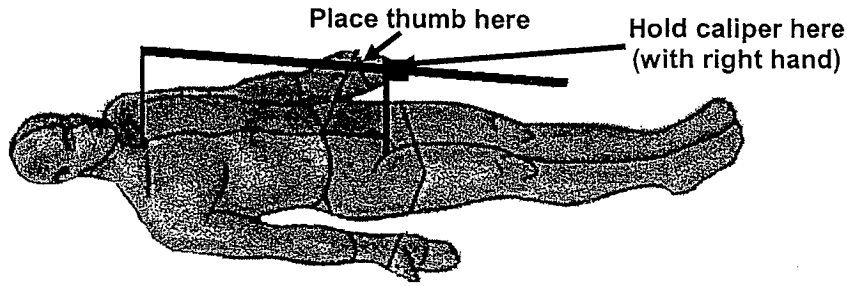


Table 1. Typical transit distances (mm).

Site	Mean	SD	Minimum	Maximum
SSN-Carotid	87	10	60	110
SSN-Brachial	415	30	345	500
SSN-Radial	650	40	550	765
SSN-Femoral	520	40	400	600

9.0. Wrap Up

- Remove ECG probes from patient. Thank patient. He/she may leave to change.
- Close NIHem program. Record data onto CD.

9.1. Recording a CD-R

9.1.1. Insert a blank Data CD-R into the drive.

9.1.2. Double Click on the "Record a CD" icon which is located on the top right of the desktop. This will start EasyCD.

9.1.3. In the left upper window (Explorer), double click the "NIHem" folder.

9.1.4. Double click the "Data" sub-folder.

9.1.5. This gives a list of all studies (folders) on the hard drive. In the left hand window, highlight the folder corresponding to the patient that was studied. Folder naming convention is XXXXX.YYYYY.ZZZZZ, where X is the zero-padded center ID, Y is the zero-padded patient ID and Z is the study type, e.g., "00999.00012.Baseline" is a Baseline study on patient 12 at center 999.

9.1.6. Click the Add (+) button on the toolbar.

9.1.7. Click the Create CD (red dot) button on the toolbar.

9.1.8. Click "OK" on the CD Creation Setup dialog box.

9.1.9. When the CD recording has finished, the CD will be ejected and you will be informed whether the recording was successful. Click [OK]. If any errors are reported, discard the CD and start again.

9.1.10. Insert an Archive CD into the drive and wait 10 seconds for the drive to lock the disk.

9.1.11. On the main menu under "CD" select "Import Session." In the Import Session dialog box, highlight the last available session in the list and then click the "Import" button. This will load the existing recordings into the data window along with the new study that is to be added.

9.1.12. Click the Create CD (red dot) button on the toolbar. The "CD Creation Setup" dialog box will popup. Click "OK."

9.1.13. When the CD recording has finished, the CD will be ejected and you will be informed whether the recording was successful. If successful, click "OK." If any errors are reported, do NOT discard the Archive CD. Create a new Archive CD using a fresh disk and call Cardiovascular Engineering, Inc., for further instructions on how to recover the old Archive CD.

9.1.14. Close Easy CD Creator. Click "No" on the "Save CD Layout...?" dialog box.

9.2. Labeling a Data CD-R

9.2.1. Use a felt tip permanent marker and write directly onto the top surface of the CD-R as it was packaged in the jewel case.

9.2.2. Write the patient's initials on line one. Write the folder name on line two. This can be found by referring to the CD project in Easy CD Creator or by using Windows NT Explorer to look in the C:\NIHem\Data directory. The folder name is constructed as noted in Section 1.5 above.

9.2.3. Write the date of the study on line 3.

9.2.4. As an example, see Figure 28, or the following example. For patient initials GFM, center 999, patient 12, Baseline visit, done on June 7, 2000, the front of the CD-R would be labeled as follows:

GFM

00999.00012.Baseline

June 7, 2000

9.3. Labeling an Archive CD-R

9.3.1. It is sufficient to write only the Patient ID and Visit Number of each backed up study on the surface of the disk, since there is not enough room for all of the above data when multiple studies are on the disk

9.3.2. Record Patient ID and Study Date on the jewel case insert (Figure 29).

9.4. Shipping a Data CD-R to Cardiovascular Engineering, Inc.

9.4.1. Complete a domestic or international airbill as appropriate for your site location.

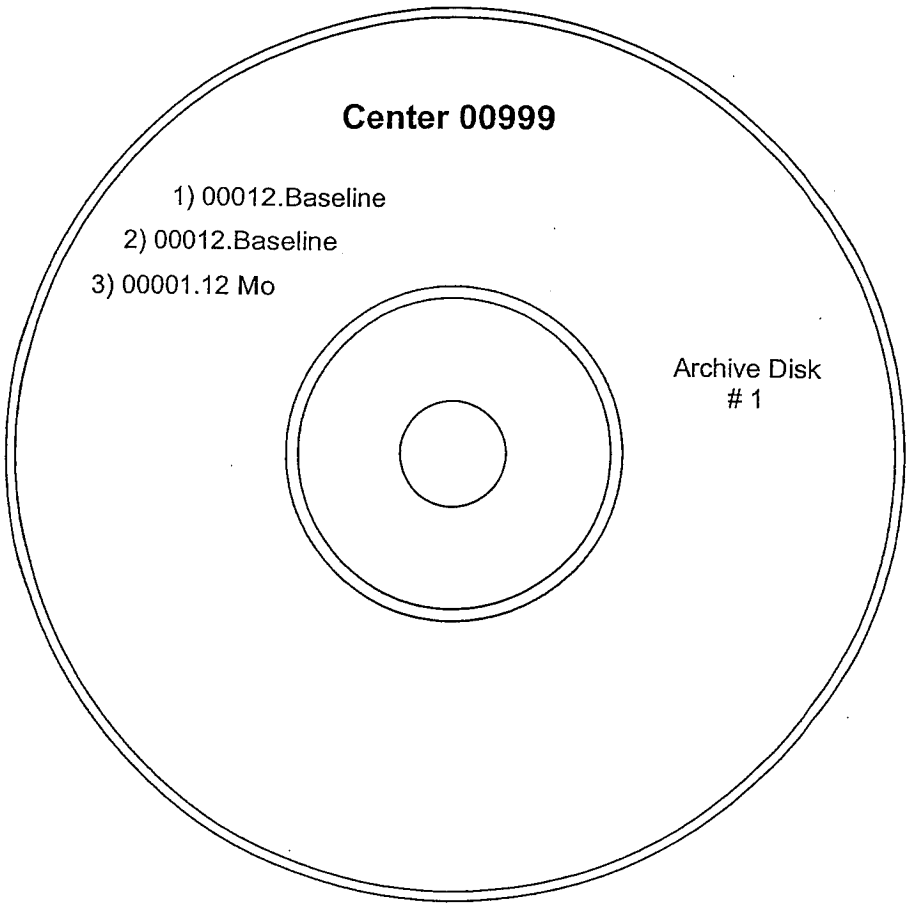
9.4.2. Place the Data CD-R inside a FedEx Letter envelope and attach the airbill.

9.4.3. It is EXETREMELY important that you complete the Data Transmittal Notification Fax form and fax the form to Cardiovascular Engineering, Inc. on the date of the study, so that we will be aware that a disk has been sent. Keep the original form in the patient's study binder for future reference.

Figure 28. Sample CV137-090 Data CD.

- Note the patient initials (GFM) followed by the CenterID.PatientID.Visit number (i.e. the folder name as seen in Easy CD creator) followed by the date of the study.

Figure 29. Sample CV137-090 Archive CD.



- Note that the Center ID is written once at the top of the disk. Each study is identified by the Patient ID and the visit number. For example patient number 00012, Baseline study is written "00012.Baseline", etc.

15.4.2 After CD recording is complete, begin procedure for next patient (if applicable) or shutdown computer from the NT [Start] menu. Turn power off and unplug main power cord after shutdown is complete.

15.4.3 Disconnect tonometer and store.

Appendix Item 9

Overview of Training & Quality Assurance[a]

FHS Quality Control Protocol

[a] Note: Developed by Drs. Corretti and Benjamin for International Brachial Reactivity Task Force

Training and Quality Assurance
Framingham Heart Study

Table. Training and Quality Improvement Protocol		
Elements	**Scanning**	**Measurement**

Manuals	• Written description of procedure for participants
	• Succinct protocol flow sheet @ station
	• Longer protocol documentation manual to train sonographers
	  • Acquisition protocol outlines
	  • Equipment presets
	  • Study sequence
	  • Sonographer script to convey to subjects
	  • Specification of optimal 2-D images
	  • Near & far artery wall interface with continuous & distinct intima-media layers over > 50% of vessel length, sonolucent lumen
	  • Specification of inadequate 2-D images
	  • Near & far artery wall interface with discontinuity, lack of ability to recognize intima-media interface, oblique artery view
		• Explicit written documentation to enhance consistency
		• Manual & automated measurements
		  • Frame selection techniques
		  • Segment selection
		  • Criteria for unmeasurable studies
		  • Criteria to reevaluate measurements
		• Automatic analysis packages
		  • Technique for selecting image & segment for 'training'
		  • Criteria for rejecting frames
		  • Criteria delineating when to make manual measurements

Worksheets	• Record subject factors
	  • If ineligible, why
	  • Refused, mastectomy, Raynaud's
	  • Potential modifiers of FMD
	  • Time of day, last cigarette, caffeine, medications, food, menses
	  • Systolic blood pressure & cuff inflation pressure
	• Record scan factors
	  • Equipment issues, any deviations from protocol
		• Log book to track status of studies
		  • Dates performed, saved, measured
		  • SVHS and/or disk numbers
		• Worksheet to record technical quality of study

Training	• Scientific rationale and physiology of flow-mediated dilation	

	• Basic knowledge of ultrasound equipment, 2-D & Doppler analysis
	• Demonstration, review of technical tips & pitfalls, hands-on experience
	• Ergonomic issues
	  • Essential to train in techniques to minimize stress related injuries
	• Qualification criteria
	  • Training period with close supervision & feedback on protocol adherence & image quality
	  • Periodic review of scan performance
	  • Minimum number of studies
	  • At least 20 supervised scans prior to scanning independently
	  • At least 50 per year to maintain competency
		• Demonstration, technical tips & pitfalls of segment selection, hands-on experience
		• Develop & adhere to written protocol
		• Qualification criteria
		  • Training period with close supervision & feedback
		  • Formal observer-specific reproducibility assessment prior to independent reading
		  • Minimum number of studies
		  • All observers from a given study measure 20 studies together prior to reading independently
		  • At least 50 per year to maintain competency

<table>
<tr><td rowspan="2">Descriptive Statistics</td><td>• Assess for systematic differences by sonographer & site</td><td>• Assess for systematic differences by observer & site</td></tr>
<tr><td colspan="2">• Mean baseline and peak deflation diameters, and FMD. Doppler data if reported.
• Per time period & over time to assess for secular drifts in measurements</td></tr>
<tr><td>Data Cleaning</td><td colspan="2">• Missing worksheet or measurement data
• Criteria to reevaluate study
• Range checks
• FMD out of range (-2 to 12%)
• Diameter out of range (2.5 to 5.5 mm)
• Any image > 0.2 mm different than adjacent images in acquisition sequence
• Consistency checks
• For continuous analysis data are baseline & immediately post-deflation diameters within 0.1 mm?
• Do subject visit date and time and scan date and time match?</td></tr>
<tr><td>Lab Meetings</td><td colspan="2">• Periodic lab meetings
• Review work flow
• Review compliance with scan & measurement protocols
• Measure random and difficult studies together
• Review results of data cleaning and reproducibility analyses</td></tr>
<tr><td rowspan="3">Reproducibility</td><td>• Image variability
• In single site study each sonographer scan the same participants under similar conditions to assess systematic differences in scanning technique</td><td>• Multi-site studies should have core reading laboratory
• Intra- & interobserver variability: Interpreters measure a minimum of 20 scans twice to assess for systematic differences within and between observers
• Temporal variability Interpreters measure a calibration set over the duration of a longitudinal study to assess for secular drifts in measurement technique</td></tr>
<tr><td colspan="2">Potential sources of variability
• Subject
• Day to day, time of day, exercise, smoking, food, caffeine, medications, menstrual cycle
• Sonographer
• Protocol
• Cuff: duration of occlusion, occlusion above vs. below elbow, mm Hg of occlusion, automatic vs. manual cuff inflation
• Transducer held manually vs. with stereotactic device
• Observer
• Intraobserver, interobserver, temporal measurement differences
• Equipment
• Ultrasound
• Transducer frequency, proprietary differences in ultrasound equipment, settings
• Digital analysis
• Image storage on SVHS vs. digital media, ECG gating & point in cardiac cycle, manual vs. automated measurements, different analysis algorithms, upgrades</td></tr>
<tr><td colspan="2">Statistics
• Correlations, mean and absolute differences, components of variability [systematic vs. random differences]
• Assess on baseline and peak deflation diameter and FMD. Doppler, if assessed.</td></tr>
</table>

Education	• For those beginning to adopt technique, currently, education is most efficiently gained by visiting experienced labs • The field would benefit from the availability of more formal course opportunities
Certification	• While noninvasive measurement of endothelial function is a research tool, certification will remain study-specific • Prior to becoming a clinical tool, formal certification requirements, courses, & ongoing continuing medical education will be necessary

Appendix Item 10

Illustrations

Tonometry Pulse Aquisition Sites

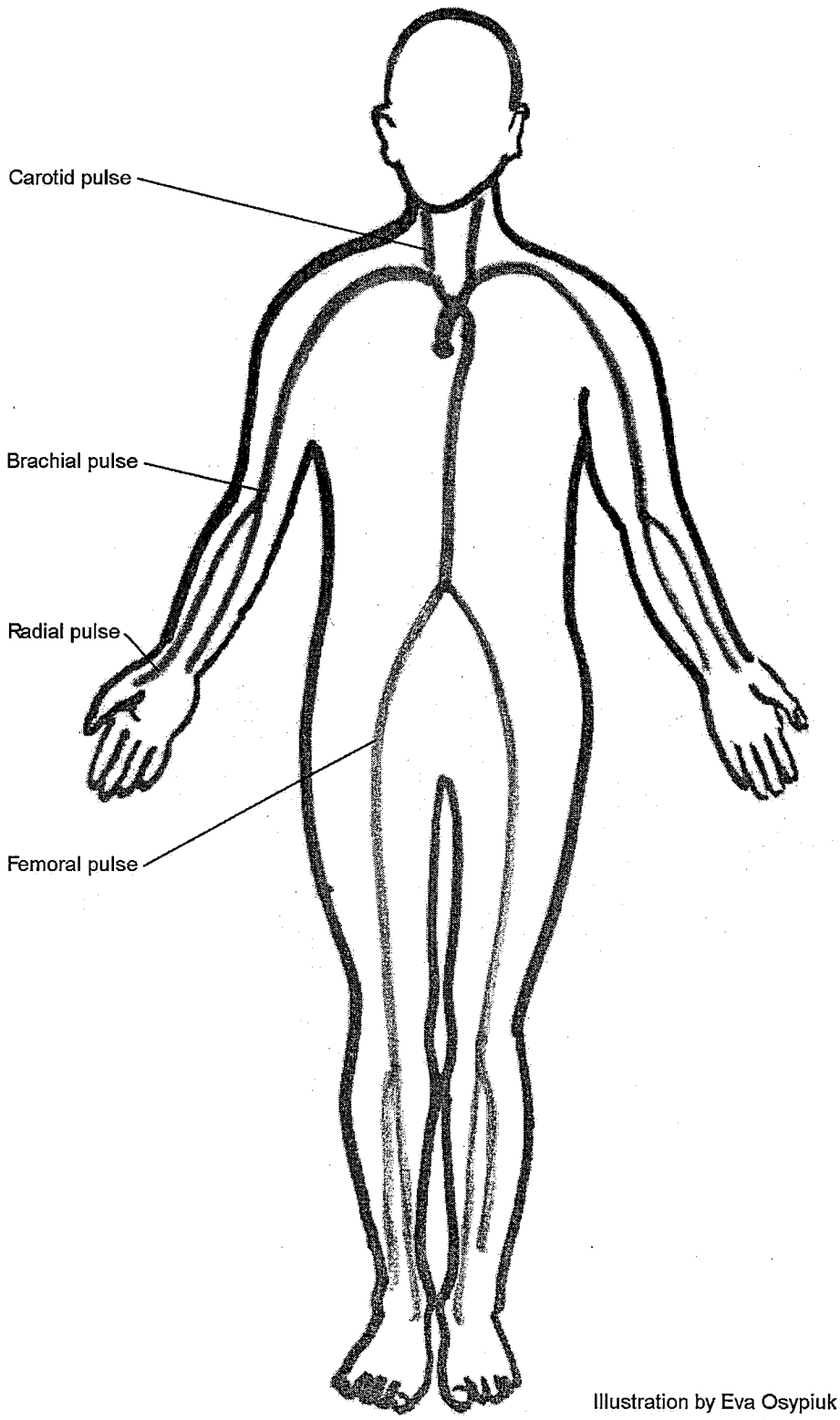
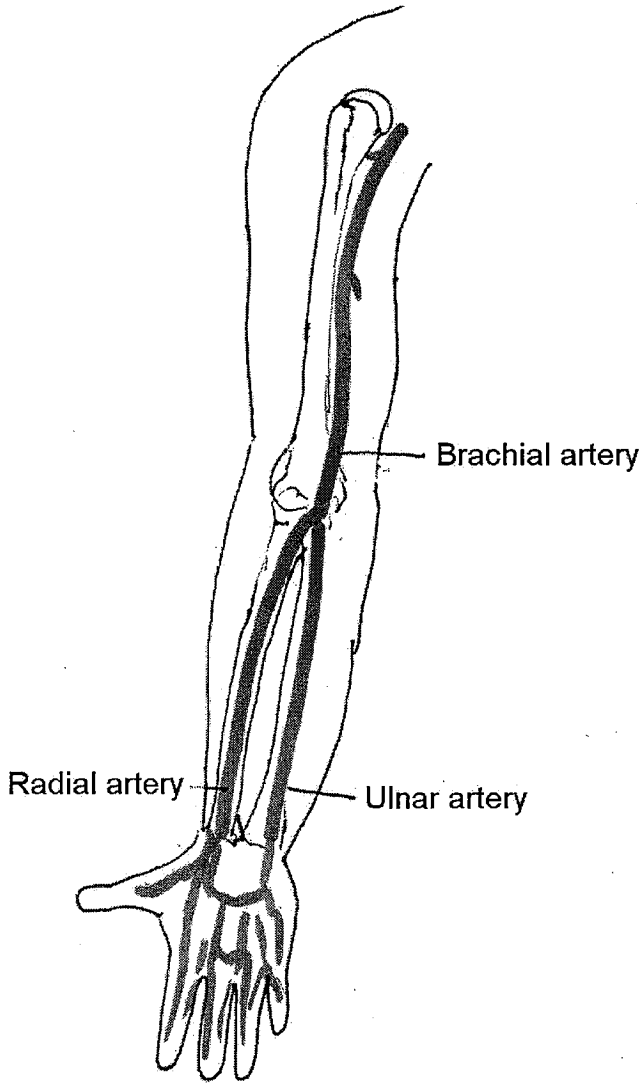


Illustration by Eva Osypiuk

Brachial Artery and Radial Artery

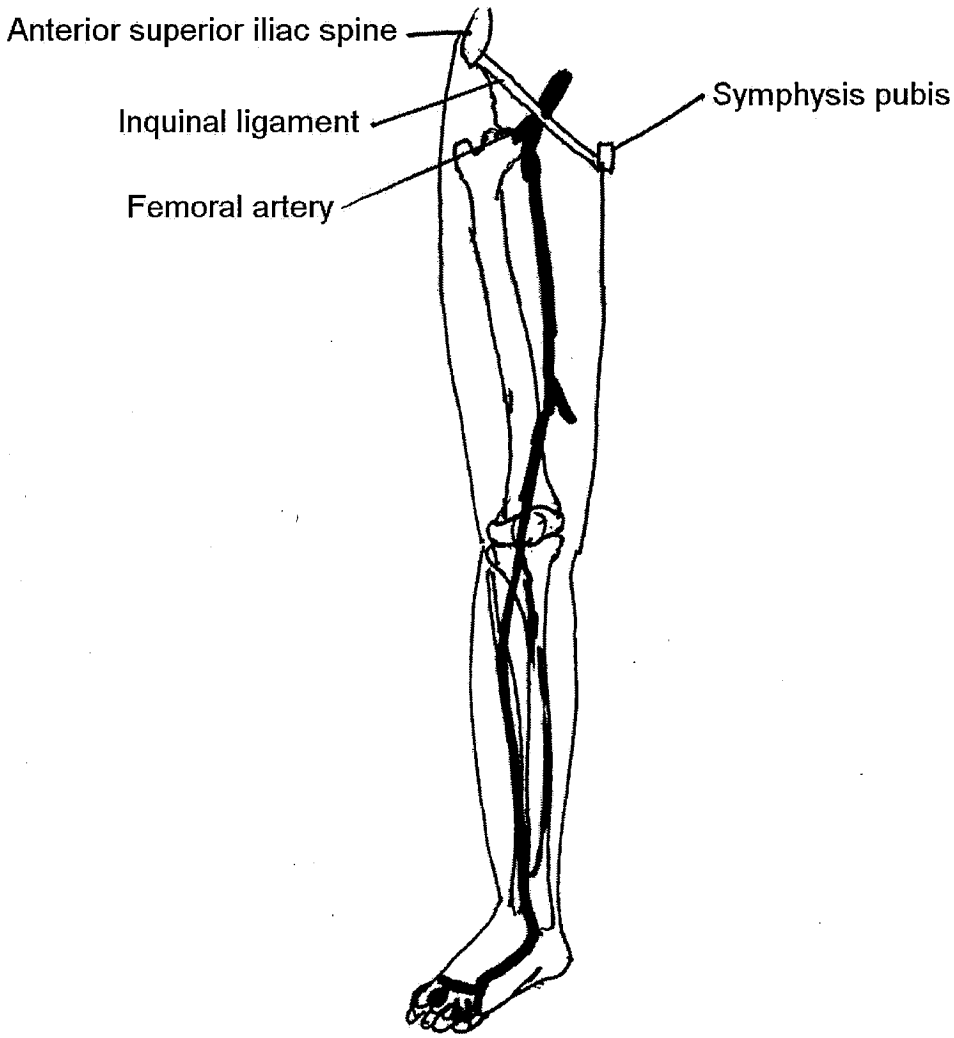


Brachial artery can be felt in and above the bend of the elbow, just medial to the biceps tendon and muscle.

Radial artery can be felt on the flexor surface of the wrist laterally.

Illustration by Eva Osypiuk

Femoral Artery



Femoral artery is palpable below the inquinal ligament, midway between the anterior superior iliac spine and the symphysys pubis.

Illustration by Eva Osypiuk

Carotid Artery

Optimal location for carotid tonometry is just lateral to the larynx, in the angle between the SCM muscle and the larynx.

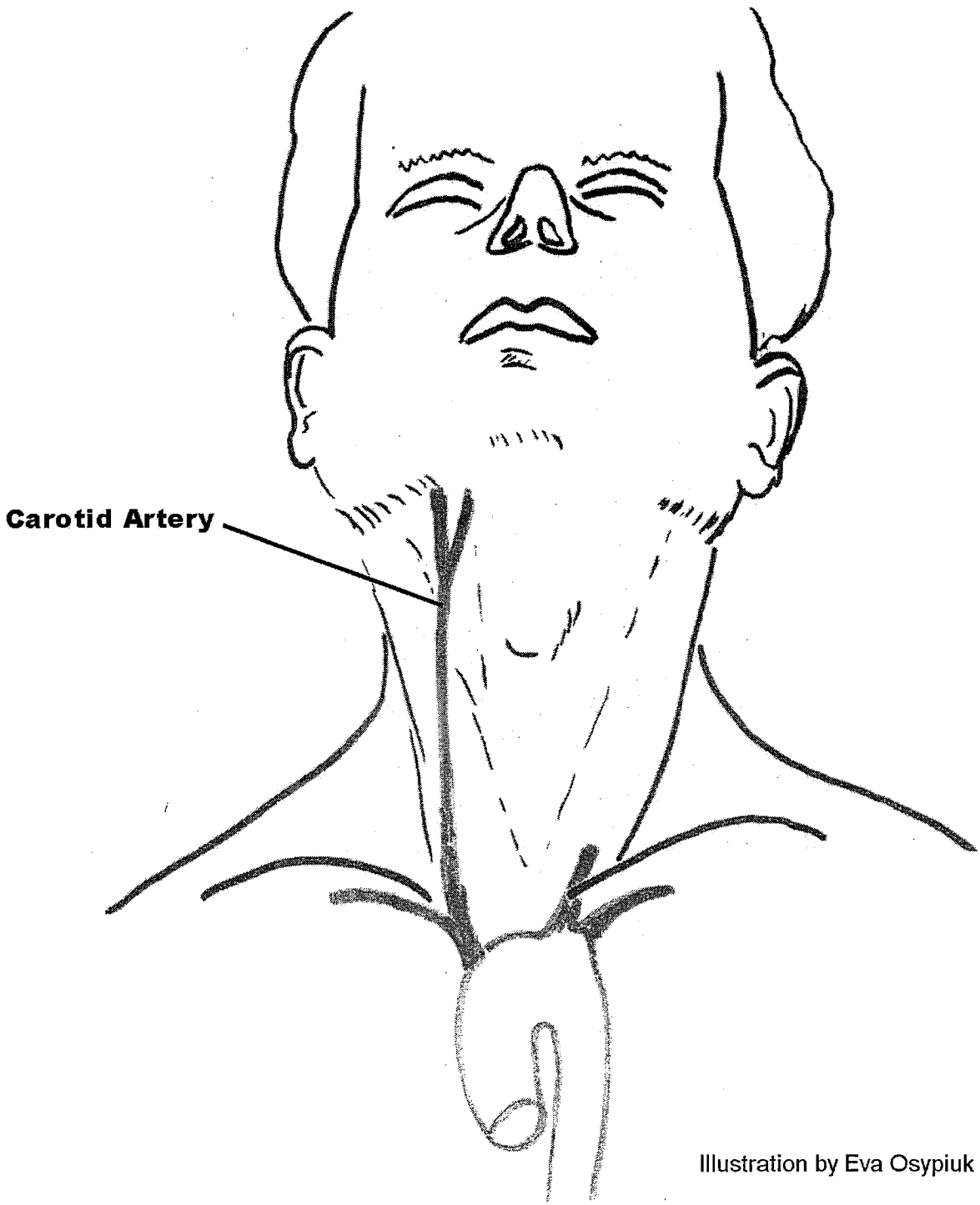


Illustration by Eva Osypiuk

Appendix Item 11

NIHem Data Acquisition Instructional Poster

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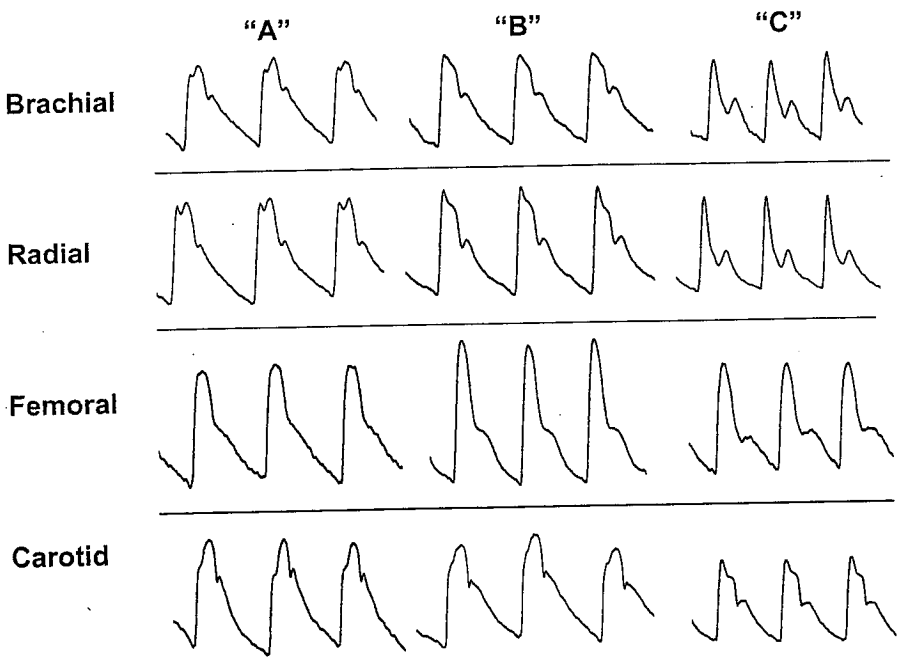
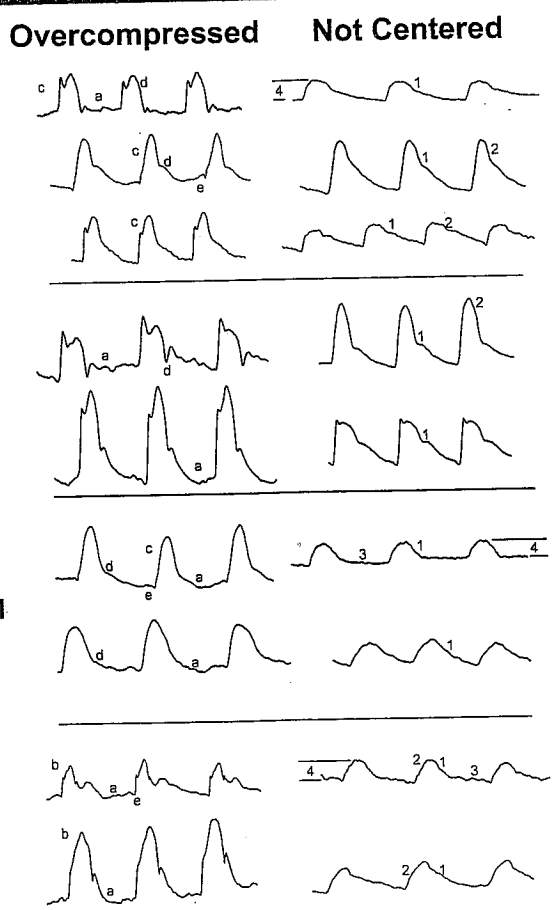
NIHem Data Acquisiti

Tonometry

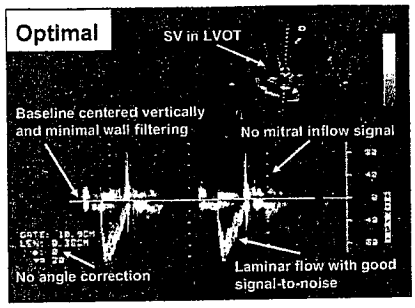


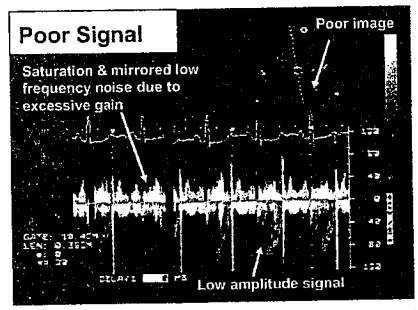


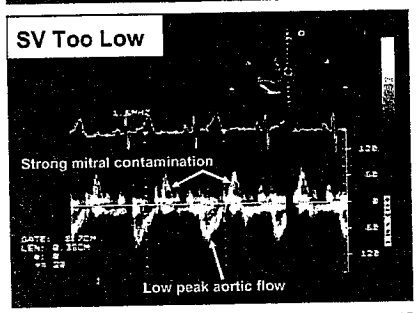
Overcompressed:
a = Flat or scooped diastole
b = Turbulence
c = Distorted upstroke
d = Depressed dicrotic notch
e = Square root sign

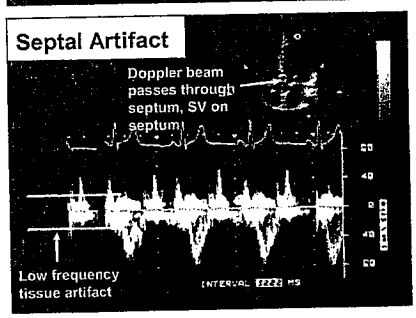
Not Centered:
1 = Poorly defined dicrotic notch
2 = Poorly defined reflected wave
3 = Flat diastole
4 = Low amplitude

LVOT Flow

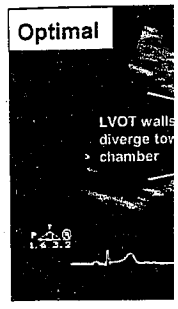


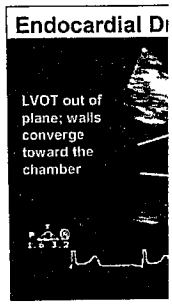




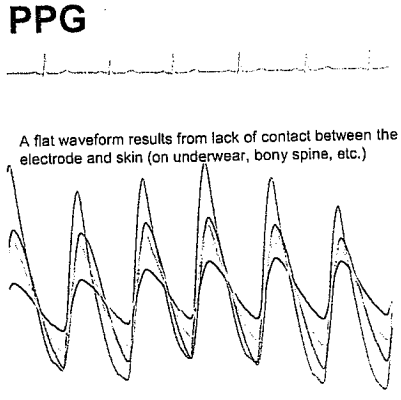


LVOT Dia



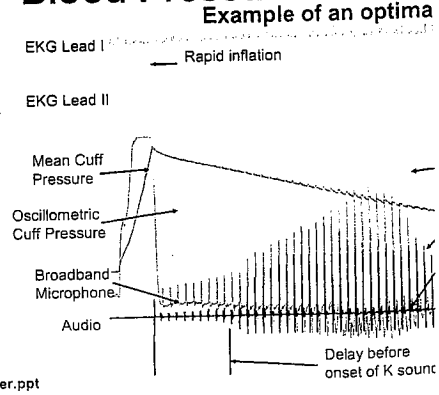


PPG



Blood Pressure

Example of an optima



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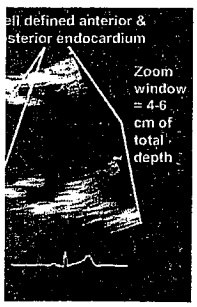
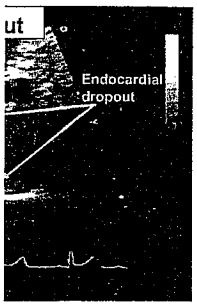
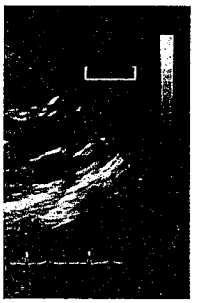
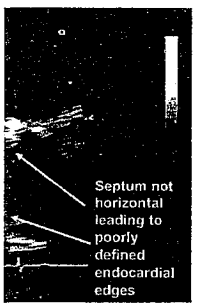
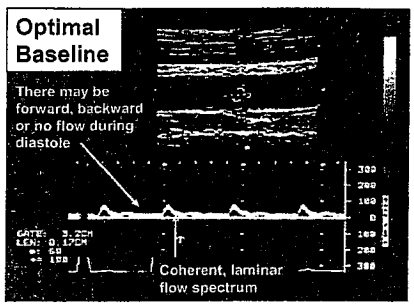


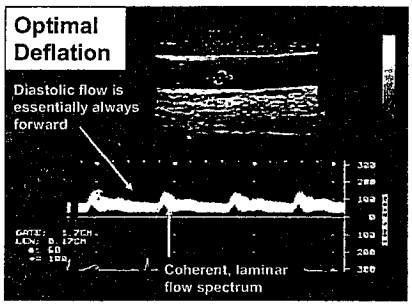


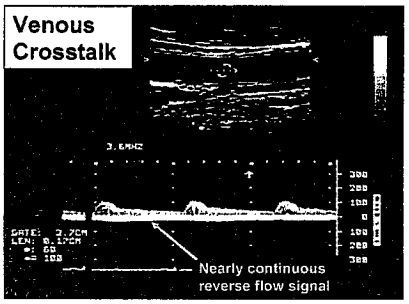


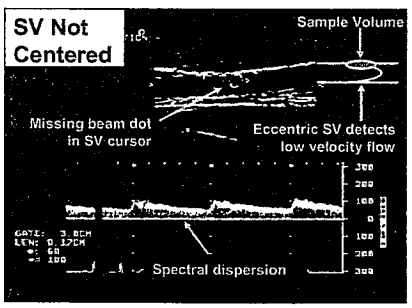


Brachial Reactivity

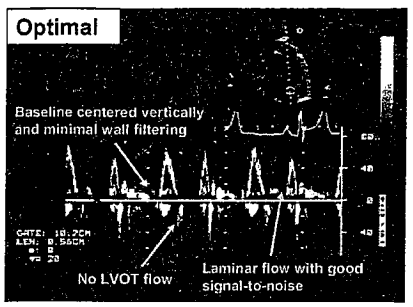


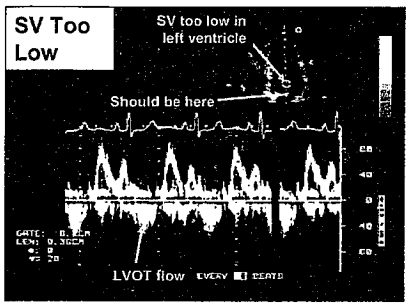


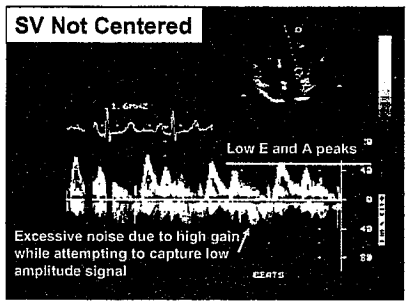


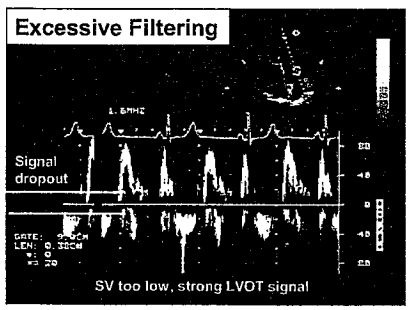


Mitral

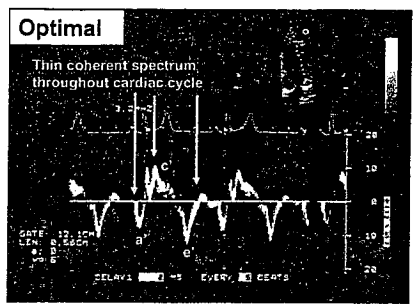


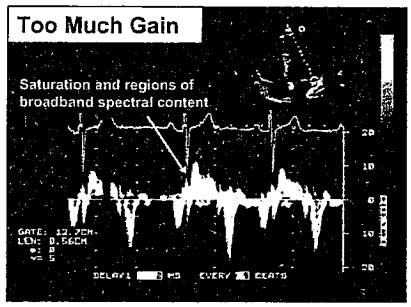


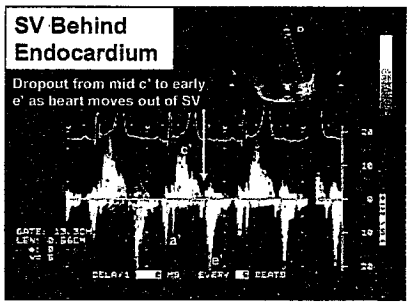




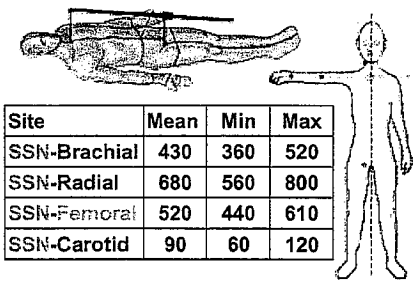
DTV







Typical transit distances (mm)



Site	Mean	Min	Max
SSN-Brachial	430	360	520
SSN-Radial	680	560	800
SSN-Femoral	520	440	610
SSN-Carotid	90	60	120

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