

<p style="text-align: center;">WOMEN'S INTERAGENCY HIV STUDY ARTERIAL BRACHIAL INDEX SUBSTUDY</p>

A. STUDY PURPOSE

To measure peripheral arterial disease (PAD) using the ankle brachial index (ABI) in WIHS women 40 years of age or older (i.e., ≥ 40 yo) every two years to determine the factors associated with PAD and its progression in HIV-infected and uninfected women.

We will also study how PAD might affect physical function in women who are participating in the Musculoskeletal (MSK) Study, because MSK women have detailed measures of limb fat, limb muscle, inflammatory markers and physical function performed.

B. SPECIFIC AIMS

Aim 1: To determine the association of HIV and metabolic factors with PAD in HIV-infected and HIV-uninfected women, and to determine the association of HIV-related factors with PAD among HIV-infected women.

Aim 2: To examine the association of HIV infection and PAD with physical function.

C. HYPOTHESES

- **Hypothesis 1a:** HIV infection will be independently associated with lower ABI values and increased symptomatic and asymptomatic PAD, after controlling for demographic and behavioral factors.
- **Hypothesis 1b:** The association of HIV infection with lower ABI values and increased symptomatic and asymptomatic PAD will be only partially explained by metabolic factors associated with HIV infection.
- **Hypothesis 1c:** Among HIV-infected women, the severity of immunosuppression and elevations in markers of inflammation will be associated with lower ABI values and increased symptomatic and asymptomatic PAD after controlling for demographic, behavioral, and metabolic factors.
- **Hypothesis 2a:** HIV infection will be associated with decreased physical function due to the catabolic effects of HIV on muscle mass in the lower extremities.
- **Hypothesis 2b:** Lower ABI values will be associated with decreased physical function due to decreased perfusion of lower extremity muscles by peripheral arteries.
- **Hypothesis 2c:** HIV infection, sarcopenia, and PAD will be associated with greater declines in physical function; PAD and sarcopenia may be more important predictors than HIV status itself because the negative effects of HIV infection on physical function will be ameliorated by effective HAART use.

D. RESEARCH DESIGN AND METHODS

1. STUDY DESIGN

The proposed research is a longitudinal ABI study to begin at WIHS core visit 39 (October 1, 2013). ABI will serve as both a predictor and an outcome in analyses.

For studies related to ABI as the outcome (Aim 1), all WIHS participants ≥ 40 years will undergo an ABI measurement every two years (because significant changes from a normal ABI to a low ABI were demonstrated over an average of three years in an HIV-uninfected cohort). We

estimate that if there are 2,500 active women that are seen at any given visit and about 80% are ≥ 40 , then we will study 2,000 women (approximately 1,350 HIV-infected and 650 HIV-uninfected) every two years.

For studies related to ABI as the predictor (Aim 2), we will leverage the ongoing MSK data collection which has targeted 330 WIHS participants (approximately 220 HIV+ and 110 HIV- between the ages of 40 and 60 years) for enrollment into MSK from three WIHS study sites (Bronx, San Francisco, Chicago).

The proposed study will add ABI measurements to all MSK participants at their baseline and two-year follow up visit (that will be obtained either at the WIHS CORE visit or at the MSK visit, if the MSK visit does not coincide with the every two-year visit proposed in the entire WIHS cohort ≥ 40 years). For women who have already completed their MSK visit, bring back MSK women for ABI measurement if the MSK visit is within one year of the initiation of the ABI study, since progression of PAD is slow.

2. INCLUSION CRITERIA

WIHS women 40 years of age or older (i.e., ≥ 40 yo) will be asked to take part in this study.

3. PREDICTOR AND OUTCOME VARIABLES:

ABI is calculated as the ratio of the highest systolic blood pressure (SBP) in the brachial artery divided by the lowest SBP of either the dorsalis pedis or posterior tibial arteries. It is classified into one of four categories:

Normal	$1.00 \leq \text{ABI} \leq 1.40$
Abnormal	$\text{ABI} < 0.9$
Borderline	$\text{ABI} 0.9 - 0.99$
High	$\text{ABI} \geq 1.40$ in at least 1 leg (indicates incompressibility and possible arterial calcification)

E. ABI PROTOCOL

1. PREPARATION

1. Make sure to have a full kit containing:

- Nicolet ABI Kit (EN5A)
 - Nicolet Elite 100 Doppler
 - 5 MHz Probe
 - Sphygmomanometer
 - Four 12 cm (blue cuff) quick disconnect vascular cuffs
- Two 16 cm (black cuff) quick disconnect vascular cuffs (Welch Allyn Large Adult #5082-44)
- Two 20 cm (black cuff) quick disconnect vascular cuffs (Welch Allyn Thigh Cuff #5082-77)
- Ultrasound transmission gel

- Tissue or wash cloth to remove ultrasound contact gel
 - Non-toxic dry erase marker
 - T-Spray (Pharmaceutical Innovations, Inc.) and/or Clorox Disinfecting Wipes
2. If the ABI does not precede the blood draw, ensure that at least one hour passes between phlebotomy and the ABI, and that the needle site has clotted. For efficiency's sake, it may be best to do the ABI right after BIA has been completed because the participant will already be in a gown and lying supine on her back.
 3. Explain the procedure to the participant and allow him/her to ask questions.
 4. Conduct the examination in a quiet, warm, and comfortable room. If the room is cool, a blanket may be used to cover the participant (including arms, hands, and feet), except while the actual measurements are being made.
 5. Have the participant lie supine on a comfortable horizontal examination table. The head and heels must be at the same level, and therefore the table must be long enough so that for each participant, the entire head and both feet must be on the table, not overhanging. Because having the feet even slightly lower than the rest of the body will produce an invalid ABI measurement, an oversized examination table must be available at the field center for tall study participants.
 6. Arms below the shoulder and legs below the knee should be bare.
 7. Inspect all four blood pressure (BP) cuffs before placement and use only cuffs that are clean and dry. Do not place blood pressure cuffs over any lesion that could be a potential source of contamination. If a lesion is visible that could be a potential source of contamination, then do not perform the measurement in the affected extremity.
 8. Have the participant rest quietly for at least five minutes before beginning the measurement procedure. The participant may be sitting or supine while resting.
 9. While the participant is resting, place an appropriate BP cuff around both arms, based on arm circumference at midpoint. The general rule is that the cuff width must be at least 40% of the arm circumference. The two cuff sizes should be employed as follows:
 - Adult cuff (12 cm blue cuff) for arm circumference of < 32 cm
 - Large adult (16 cm black cuff) cuff for arm circumference of 32-42 cm
 - Thigh (20 cm black cuff) cuff for circumference of \geq 43 cm
 10. Place a cuff on each ankle, so that the tube is facing the torso, not the toes, and the lower portion rests 3 cm proximal to the greatest protuberance of the medial malleolus (ankle bone). Adult-sized cuffs (12cm) should almost always be able to fit around the ankles.
 11. Once all four cuffs are in place and the five minutes of resting are complete, you may begin the measurements as described below.

2. PROCEDURE

Before you begin the procedure, instruct the participant to remain relaxed and to refrain from helping you (e.g. lifting the arm to facilitate placement of the cuff). Once you begin the procedure, explain the steps to the participant as you proceed.

By palpation, locate the brachial artery on both arms (antecubital fossa), and the dorsalis pedis

(dorsum of the foot and often in direct line with the second toe) and posterior tibial (medial ankle just behind the medial malleolus) arteries on both legs. Mark the location of each artery with a marker. Sometimes an arm or ankle pulse will not be palpable but can be found with the Doppler.

Using the procedure below, measure SBPs in the following order (same as on the form):

1. Right brachial artery
2. Right dorsalis pedis artery (*top of the right foot, in line with the second toe*)
3. Right posterior tibial artery (*inside right ankle*)
4. Left posterior tibial artery (*inside left ankle*)
5. Left dorsalis pedis artery (*top of the left foot, in line with the second toe*)
6. Left brachial artery

Place an appropriate amount of ultrasound conducting gel over the end of the Doppler.

On occasion, there may be skin lesions on the arms, legs or at the insonation site that are of concern for performing the measurement of the SBP. In these instances, ABI should not be assessed in the affected extremity and reason for not calculating ABI should be recorded on form.

After palpating the location of the pulse, turn on the Doppler and place the probe over the artery. With this large probe, careful angulation is not necessary. Place the probe in line with the artery and move it from side to side until the strongest pulse is heard. Don't press too hard on the artery with the probe. Rest your hand comfortably so that the probe is secured in place once a strong pulse is heard. Explain the procedure to the participant and ask if the participant has any questions before the measurements begin. If applicable, suggest to the participant to rest comfortably and to try to be quiet and still.

In a small percentage of participants, you may not be able to find the posterior tibial or dorsalis pedis pulse. If you are having trouble, be patient and continue to search for at least three minutes. If you are still unable to locate a pulse, enter a systolic pressure of "000" for that artery.

Inflate the cuff until the pulse is no longer audible. Inflate to 20 mm Hg above the level at which the pulse sound disappears. (If the pulse cannot be obliterated, you may raise pressure to a maximum of 300 mm Hg. If not obliterated at that point, record "300"). Deflate the cuff slowly allowing the pressure to drop at a rate of 2 mm Hg per second. Record the pressure at which the first sustained (more than one beat) pulse reappears. This is the SBP at this location. Deflate the cuff completely.

Enter the measurement in the appropriate field on the ABI form immediately. If a given measurement was not done, be sure to list the specific reason why on the form.

If the signal remains faint as more pressure is released or if the probe moves off the artery, deflate the cuff completely, and then repeat the measurement.

After completing the ABI measurements, thoroughly clean the Doppler probe with T-Spray or a Clorox Disinfecting Wipe. Please note that the Doppler must be completely clean and dry between participants.

3. CALCULATION OF THE ABI

The ABI will be calculated from your measurements in the following manner:

The ABI denominator – There is only one ABI denominator per participant for both the left and right ABIs. This denominator is the higher arm SBP. The higher of the brachial artery SBP is used to avoid potential bias from subclavian stenosis (which could lead to a falsely low SBP).

The right ABI numerator is defined as the higher of (1) the right posterior tibial SBP or (2) the right dorsalis pedis SBP.

The left ABI numerator is defined as the higher of (1) the left posterior tibial SBP or (2) the left dorsalis pedis SBP.

We will also perform analysis where the lowest lower extremity SBP (of either dorsalis pedis or posterior tibial) will be used as the numerator as use of the lowest pressure has been associated with greater sensitivity in the detection of subclinical disease. The highest leg SBP is often used as the numerator, because a greater sensitivity in detecting ischemic leg pain has been suggested when using the highest leg pressure.

The right ABI is the right ABI numerator divided by the ABI denominator.

The left ABI is the left ABI numerator divided by the ABI denominator.

F. ABI QA ACTIVITIES

1. FIELD STAFF QUALIFICATIONS

No specific qualifications are necessary.

2. FIELD STAFF TRAINING

All technicians will be trained by the primary technician, Heather Freasier. An overview and video describing the procedure and the necessary equipment is provided. Technicians should practice the procedure on volunteer participants and perform the requirements necessary for certification (described in the next section).

3. FIELD STAFF CERTIFICATION

To become certified, technicians must

- Read the ABI protocol in the Manual of Operations, Section 38.
- Practice the procedure according to the ABI protocol on volunteers as necessary. If a technician has never previously performed ABI measurements, 10-20 practice readings are recommended before continuing with the certification process.
- Pass a practical examination administered by the central trainer. The practical examination involves performing the ABI procedure on one volunteer participant under observation of the central trainer and in accordance with the WIHS Supine ABI Certification checklist. As the trainee records the pressures, the trainer should verify that they are correct (i.e. the values the trainer would have recorded) and complete the checklist to document that the procedure is done according to the protocol.

4. MAINTAINING CERTIFICATION

To maintain certification, each Field Staff technician must

- Perform the ABI procedure on at least six participants every two months.
- Repeat the original certification process prior to each new examination cycle.

5. QC ACTIVITIES

Blood pressure cuffs:

Verify that the full range of blood pressure cuff sizes is available. Report any missing cuffs to the site Project Director immediately.

Nicolet Doppler apparatus:

Check to make sure the batteries for the Doppler are working. Replace batteries as needed.

6. QC MONITORING OF TECHNICIAN QUALITY

To insure that technicians continue to perform the ABI procedure according to the WIHS protocol, each technician is observed in the clinic and evaluated according to the WIHS ABI Certification Checklist by Heather Freasier simultaneous to the Anthropometry recertification visit.

7. WDMAC QC ACTIVITIES

WDMAC periodically analyzes the available baseline blood pressure data and QC replicate data to insure that appropriate levels of measurement quality are maintained. The following variables are monitored:

- Systolic ankle pressures (posterior tibial and dorsalis pedis) in both legs
- Brachial systolic blood pressure in both arms
- Ankle Brachial Index

Age- and gender-adjusted means for these variables by WIHS site, by technician within WIHS site, and by increments in time are computed to examine trends, identify outlying or unusual values, quantify differences between the measurements recorded by different technicians at different WIHS sites, and monitor measurement drift. Technician- and WIHS site -specific measures of variability in these measurements will also be examined to assess measurement reliability and detect unusual discrepancies across WIHS sites. In addition, the blood pressure measurements obtained by each technician are analyzed for digit preference.

8. QC REPORTING

WDMAC and the Metabolic/Vascular WG will review results of these analyses and provide updates to the WIHS site PI so that appropriate action can be taken to improve measurements if needed in a timely fashion.

G. SUPPORTING DOCUMENTATION

WIHS Supine ABI Certification Checklist, adapted from the MESA Study:

WIHS Supine ABI Certification Checklist

DATE:
mo day year

WIHS site:

Technician:
Name/ID

Central Trainer:

Purpose of Evaluation:

Certification | Site Visit

Please check the appropriate box if technician performance is satisfactory (or unsatisfactory) for each line item. Please note any comments or remedial action taken in 'Comments' section if performance was not satisfactory.

General:

- | S | U | |
|--------------------------|--------------------------|---|
| <input type="checkbox"/> | <input type="checkbox"/> | Thoroughly explains the procedure to the participant. |
| <input type="checkbox"/> | <input type="checkbox"/> | Insures that the participant is relaxed and lying completely supine (legs straight and down with feet rolled outward) on the examination table. |
| <input type="checkbox"/> | <input type="checkbox"/> | Has participant rest quietly for at least 5 minutes prior to the procedure. |
| <input type="checkbox"/> | <input type="checkbox"/> | Informs participant just before inflating cuff to avoid startling the participant. |
| <input type="checkbox"/> | <input type="checkbox"/> | Records correct (i.e. pressure that observing trainer would record) pulse obliteration pressure. |
| <input type="checkbox"/> | <input type="checkbox"/> | Reads all pressure measurements at eye level . |
| <input type="checkbox"/> | <input type="checkbox"/> | Records correct (i.e. pressures that observing trainer would record) pressures for all arteries. |

Right Brachial Artery:

- | | | |
|--------------------------|--------------------------|---|
| <input type="checkbox"/> | <input type="checkbox"/> | Places blood pressure cuff of appropriate size over right brachial artery. |
| <input type="checkbox"/> | <input type="checkbox"/> | Locates brachial artery by palpation. |
| <input type="checkbox"/> | <input type="checkbox"/> | Marks the location of the artery with a black marker. |
| <input type="checkbox"/> | <input type="checkbox"/> | Applies ultrasound jelly over brachial artery. |
| <input type="checkbox"/> | <input type="checkbox"/> | Locates brachial artery using Doppler probe. |
| <input type="checkbox"/> | <input type="checkbox"/> | Inflates cuff quickly to at least 20 mm Hg above maximal pressure. |
| <input type="checkbox"/> | <input type="checkbox"/> | Deflates at 2 mm Hg/second until a sustained systolic pressure is audible. |
| <input type="checkbox"/> | <input type="checkbox"/> | Reads (at eye level) and records first systolic blood pressure at which a sustained pulse was first audible. |
| <input type="checkbox"/> | <input type="checkbox"/> | Deflates cuff quickly and completely after measurement is obtained. |

Right Dorsalis Pedis Artery:

19. Places blood pressure cuff of appropriate size to right ankle with midpoint of bladder over posterior tibial artery, with lower end of bladder approximately 3 cm above medial malleolus.
20. Locates right dorsalis pedis artery by palpation.
21. Marks the location of the artery with a black marker.
22. Applies ultrasound jelly over dorsalis pedis artery.
23. Locates right dorsalis pedis artery using Doppler probe.
24. Inflates cuff quickly to at least 20 mm HG above maximal pressure.
25. Deflates at 2 mm Hg/second until a **sustained** systolic pressure is audible.
26. Reads (at eye level) and records first systolic blood pressure at which a **sustained** pulse was first audible.
27. Deflates cuff quickly and completely after measurement is obtained.

Right Posterior Tibial Artery:

28. Locates right posterior tibial artery by palpation.
29. Marks the location of the artery with a black marker.
30. Applies ultrasound jelly over posterior tibial artery.
31. Locates right posterior tibial artery using Doppler probe.
32. Inflates cuff quickly to at least 20 mm Hg above maximal pressure.
33. Deflates cuff at 2 mm Hg/second until a **sustained** systolic pressure is audible.
34. Deflates cuff quickly and completely.
35. Reads (at eye level) and records right posterior tibial systolic blood pressure at which a **sustained** pulse was first audible.

Left Posterior Tibial Artery:

36. Locates left posterior tibial artery by palpation.
37. Marks the location of the artery with a black marker.
38. Applies ultrasound jelly over posterior tibial artery.
39. Locates left posterior tibial artery using Doppler probe.
40. Inflates cuff quickly to at least 20 mm Hg above maximal pressure.
41. Deflates at 2 mm Hg/second until a **sustained** systolic pressure is audible.
42. Reads (at eye level) and records first systolic blood pressure at which a **sustained** pulse was first audible.
43. Deflates cuff quickly and completely after measurement is obtained.

Left Dorsalis Pedis Artery:

44. Places blood pressure cuff of appropriate size on left ankle with midpoint of bladder over posterior tibial artery, with lower end of bladder approx. 3 cm above medial malleolus.
45. Locates left dorsalis pedis artery by palpation.
46. Marks the location of the artery with a black marker.
47. Applies ultrasound jelly over dorsalis pedis artery.

- 48. Locates left dorsalis pedis artery using Doppler probe.
- 49. Inflates cuff quickly to at least 20 mm Hg above maximal pressure.
- 50. Deflates at 2 mm Hg/second until a **sustained** systolic pressure is audible.
- 51. Reads (at eye level) and records first systolic blood pressure at which a **sustained** pulse was first audible.
- 52. Deflates cuff quickly and completely after measurement is obtained.

Left Brachial Artery:

- 53. Places blood pressure cuff of appropriate size over left brachial artery.
- 54. Locates left brachial artery by palpation.
- 55. Marks the location of the artery with a black marker.
- 56. Applies ultrasound jelly over brachial artery.
- 57. Locates brachial artery using Doppler probe.
- 58. Inflates cuff quickly to at least 20 mm Hg above maximal pressure.
- 59. Deflates at 2 mm Hg/second until a **sustained** systolic pressure is audible.
- 60. Reads (at eye level) and records first systolic blood pressure at which a **sustained** pulse was first audible.
- 61. Deflates cuff quickly and completely after measurement is obtained.

Comments: _____

Corrective Action Taken: _____

Supervisor Site Visitor / Signature _____

H. QUESTIONNAIRE ADMINISTRATION

1. SAN DIEGO CLAUDICATION QUESTIONNAIRE (BRIEF VERSION)

The *San Diego Claudication Questionnaire (SDCQ)* consists of 12 questions that determine asymptomatic versus symptomatic lower extremity PAD (i.e., no pain, atypical pain, and ischemic pain).

Questionnaire will be administered at all visits where ABI is done.

2. PHYSICAL ACTIVITY QUESTIONNAIRE

The *Physical Activity Questionnaire (PAQ)* (adapted from the FRAM Study and CARDIA) will be administered at all visits where ABI is done. The same PAQ study has been used at earlier visits as part of other WIHS substudies and is currently being administered in the MSK Study.

Therefore, MSK participants do not have to repeat the questionnaire if performed at CORE visit.

If ABI is not done for any reason at a visit (e.g., participant is < 40 years old, bleeding at needle site, etc.), then do not complete either the SDCQ or the PAQ forms.