

THE WOMEN'S INTERAGENCY HIV STUDY SECTION 37: MUSCULOSKELETAL (MSK) SUBSTUDY

A. STUDY PURPOSE

With the advent of successful antiretroviral therapy (ART), young HIV-infected women are now transitioning through menopause and surviving into middle and old age. In normal women, menopause is marked by declining estrogen production and accelerated loss of bone and muscle mass, changes that ultimately lead to clinical features of musculoskeletal senescence - frailty, falls and fractures. In the Women's Interagency HIV Study (WIHS) Metabolic Substudy (MS), we found significantly lower bone mineral density (BMD) in HIV+ than HIV- premenopausal women; however, serum pro-inflammatory cytokines, bone resorption markers, rates of bone loss¹ and incidence of fracture² did not differ significantly by HIV status. In contrast, in a separate study of postmenopausal minority women, we detected significantly lower BMD, and higher serum levels of bone resorption markers and rates of bone loss in HIV+ than HIV- women^{3,4}. Also in contrast to our findings in premenopausal HIV+ women, serum levels of TNF α were higher in HIV+ than HIV- postmenopausal women and attenuated the association of HIV and BMD in the model, suggesting that TNF α mediated the effects of HIV on BMD. Multiple animal/*in vitro* studies have demonstrated that acute estrogen deficiency upregulates T cell TNF α production, primarily by increasing the number of TNF α -producing T cells⁵. Conversely, estrogen downregulates T cell activation and mitigates the effects of T cell-derived, pro-inflammatory cytokines on osteoclast-mediated bone resorption. **We therefore hypothesize that estrogen attenuates the adverse effects of HIV infection on the skeleton in premenopausal women⁶ and that during the menopausal transition, the combined effects of declining estrogen levels and persistent T cell activation associated with HIV infection may accelerate bone remodeling and bone loss to a greater extent in HIV+ than HIV- women.**

A subset of WIHS participants will have more detailed musculoskeletal studies performed using DXA scanning, Quantitative CT (QCT), and functional performance tests. Details of these aspects of the study follow.

B. SPECIFIC AIMS

Specific Aim 1: To compare HIV+ and HIV- women undergoing the menopausal transition with respect to bone turnover markers and rates of trabecular and cortical bone loss.

Specific Aim 2: To determine the effects of estrogen deficiency on T cell activation, osteoclast and osteoblast precursors and osteoblast apoptosis in HIV+ and HIV- women.

Specific Aim 3: To compare HIV+ and HIV- women undergoing the menopausal transition with respect to changes in muscle mass, strength, and functional measures of fall risk.

C. HYPOTHESES

Hypothesis 1: During the menopausal transition, HIV+ women will have higher bone turnover and rates of trabecular and cortical bone loss than HIV- women, and also than premenopausal HIV+ women.

Hypothesis 2: During the menopausal transition, HIV+ women will have greater increases in activated T cells, pro-inflammatory cytokines, osteoclast formation and apoptotic osteoblast precursors than HIV- women, and these markers will be associated with increased bone turnover and decreased bone and muscle mass.

Hypothesis 3: During the menopausal transition, HIV+ women will have a greater rate of decline in hip and thigh muscle mass and muscle strength, and greater increase in measures of fall risk than HIV- women.

D. RESEARCH DESIGN AND METHODS

1. STUDY DESIGN

The proposed research is a longitudinal study of 330 WIHS women recruited from the Bronx, San Francisco, and Chicago WIHS sites that will begin during WIHS visit 35 (October 1, 2011). The 330 women (130 from Bronx, 120 from San Francisco, 80 from Chicago) will be studied over a two-year time period beginning at the baseline visit (held during WIHS visits 35-38), followed by a two-year follow-up visit (held during WIHS visits 39-42). Women seen for baseline at visit 35 should receive follow up at visit 39; women seen for baseline at visit 36 should receive follow-up at visit 40, and so on.

Recruitment for the prospective Musculoskeletal (MSK) Substudy will consist of all eligible women age 40-60 who weigh less than 264 pounds, are no taller than 6'1", and are either in late perimenopause or early postmenopause and have an anti-mullerian hormone (AMH) level < 0.1 ng/ml.

Approximately 2/3 of participants from each site should be HIV-positive and 1/3 HIV-negative, which would provide us with 200 HIV-positive and 100 HIV-negative participants for analysis after an anticipated 10% loss to follow-up. Each quarter, the number of enrolled women in each group will be assessed and recruitment at future visits will focus on women still needed to fulfill targets.

Enrollment will be tracked in Apollo via site completion and data entry of the *MSKNOTI* form. Enrollment statistics will be available to sites in real time by running an Apollo substudy enrollment report.

2. INCLUSION CRITERIA

A list of potential eligible recruits at each site will be provided by WDMAC prior to each CORE visit. Inclusion criteria for the MSK Substudy will include:

- Enrollment and participation in the WIHS core study at Bronx, San Francisco or Chicago
- Age 40-60 at baseline MSK visit
- Are either in **late perimenopause** (no bleeding in 3-11 of the last 12 months) based upon the SWAN study definitions⁷ or **early postmenopausal** (no bleeding for >1 but <5 years)
- Have an Anti Mullerian Hormone AMH level <0.1 ng/ml at any point prior to study entry
- HIV-seropositive women must have current CD4>100 cells/ul (at last CORE visit) and be on ART (any regimen) for at least 1 year prior to enrollment (may not have missed >2/12 months during last year of ART therapy)

NOTE: Participants may be considered eligible if they have a history of hysterectomy or hysterectomy and unilateral ovariectomy if both of the following conditions are met:

- Have one or more symptoms of menopause such as hot flashes, vaginal dryness, or loss of breast fullness
- Are over age 45

3. EXCLUSION CRITERIA

Exclusion criteria for the prospective MSK Substudy will include:

- Hysterectomy with bilateral oophorectomy
- History of bisphosphonate use (ever)
- Estimated GFR by MDRD of <60ml/min
- Use of hormone replacement therapy or use of hormonal birth control in three months prior to MSK visit
- Regular use of growth hormones or steroids in the 12 months prior to the MSK visit
- Study participants over 264 pounds or more than 6'1" tall, the limits imposed by the manufacturer of the DXA scanner
- Women who have metal rods in their spines
- Participant is unable to walk without use of an assistive device (e.g., cane, walker, wheelchair, crutches), is missing lower extremities or limb(s), or uses a lower extremity prosthesis (i.e., artificial limb)
- HIV-seropositive participants must not have a documented history of AIDS wasting (ever) or an opportunistic infection within the last 3 months

4. SCHEDULING THE MUSCULOSKELETAL (MSK) VISIT

Women will be informed of the MSK Substudy at their core WIHS visit. An informational sheet describing the purpose, benefits, risks, eligibility, and procedures is in the **Appendix F**. If she expresses interest in participating and meets the eligibility criteria, she should be consented and the extra blood drawn at the CORE visit. (A Consent Form template is included in **Appendix G**.) Then, she should be called back within 63 days (if HIV-positive) or 94 days (if HIV-negative) of her CORE WIHS visit in order to schedule a MSK visit. If the potential participant is interested and eligible but either has no anti-mullerian hormone (AMH) data or has an AMH>0.1ng/ml in the database, then sera will be requested from the repository to test for AMH. If the resultant AMH is <0.1ng/ml then the potential participant should be consented at the following CORE visit. The AMH results will be included in the eligibility list sent to each site by WDMAC.

When calling a participant to confirm her MSK appointment the night before her MSK visit, the interviewer should verify that the participant is not taking hormones that would disqualify her from participation in the substudy and has not had a barium swallow procedure or nuclear medicine procedure in the week preceding her MSK visit. In addition, remind the participant that she must not take any calcium supplements the morning of her DXA scan.

The MSK visit will last up to 2.25 hours (approximately 0.25 hour for questionnaires, 0.5 hour for the DXA, 0.75 hour for the QCT, 0.75 hour for functional performance tests, including set up time). In addition, Bronx participants will have an additional blood draw and peripheral QCT, which will last up to 0.75 hour (approximately 0.25 hour for blood draw and 0.50 hour for peripheral QCT, including set up time).

E. SERA AND PBMCs

Participants will have additional blood drawn at their CORE visit of 40 ml: 10 ml SST; and 3x10 ml in CPT with sodium citrate. The procedures for processing the sera and PBMCs drawn in CPT with sodium citrate will be exactly the same as for all CORE visits (see WIHS

Manual of Operations, Sections 10.II.H and 10.II.I). Sera from the 10 ml SST should be aliquoted into 1.2 ml aliquots.

All sera and PBMCs will be stored at -70°C to -80°C for no more than 3 months and sent to the central repository on dry ice. In Year 3, once the full MSK cohort has completed their baseline visit, sera from the central repository will be sent to the laboratory of Dr. Serge Cremers, and PBMCs will be sent to the laboratories of Dr. Sanil Manavalan (1 vial, 10 million cells) and Dr. Alan Landay (2 vials, 10 million cells each).

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F. FUNCTIONAL PERFORMANCE TESTS

1. OVERVIEW

The majority of fragility fractures occur as a consequence of falling. Although HIV-infected individuals have multiple risk factors that may increase the risk of falling, fall risk has not been systematically assessed in older HIV-infected women. Men and women from the MACS and WIHS will be compared by HIV-status on their performance on several, well-validated, clinically applicable performance assessments that have been extensively used to evaluate the risk of falling. Balance will be evaluated using a static balance test (Standing Balance Test) and a dynamic balance test (Functional Reach Test). Strength will be assessed in both the lower extremities (Repeated Chair Stands) and upper extremities (Grip Strength). Lastly, walking speed and endurance will be assessed with a 4-meter and 400-meter walk tests. The battery of tests that tap into the risk dimensions described above was selected with the assistance of Dr Luigi Ferrucci based on his experience in the InCHIANTI study and in the Baltimore Longitudinal Study on Aging (BLSA). Dr. Ferrucci is working with the MACS and WIHS investigators to implement the tests, train the testers, and ensure quality control. The approximate time for completion of the battery of tests is 30-45 minutes. The battery of tests should be performed in the following order:

1. Standing Balance Test
2. Functional Reach Test
3. Repeated Chair Stands
4. Grip Strength
5. 4-meter walk
6. 400-meter walk

a. Rationale of performance based assessments.

Direct assessment of physical performance has become standard practice in epidemiologic observational studies of health and disease processes. The most commonly used assessments were initially designed to differentiate function in older adults,^{8,9} but modifications in administration and scoring¹⁰ can improve the utility of these assessments to discriminate meaningful differences and change in functional capacity in most middle-age persons. Although the measurement ceiling of these tests may be low for young and some middle-age adults (i.e., they can easily achieve the maximum possible performance on all tests), repeat assessment following standardized procedures over subsequent visits can aid in identifying the approximate point at which meaningful loss of functional capacity begins to emerge.

The recommended battery of tests constitutes a modification of the physical performance battery originally used in the Established Populations for Epidemiologic Studies of the Elderly (EPESE)⁹ and Women's Health and Aging Study (WHAS), developed for and used in the Health, Aging and Body Composition (Health ABC) study.¹¹ The test battery is commonly administered and has demonstrated reliability and predictive validity in older adults.^{9,10,12-14} To further improve discrimination of functional capacity at the higher end of the functional spectrum, the duration in which a position is held in the standing balance test has been increased to 30 seconds and the number of repeated chair stands is increased from 5 to 10, as has been done in a few other studies.^{15,16} By taking a split time after 5 completed chair stands, comparability with previous batteries will be maintained.

b. Safety Issues and Exclusions

The vast majority of participants should be able to attempt each performance test. Walking aids may not be used for the chair stand or standing balance tests. Participants unable to walk without an assistive device are excluded from participation in the MSK Substudy. Exclusion from any performance test shall be based on examiner assessment or participant concerns that the test would be unsafe. In the latter case, the examiner should describe the test and discuss with the participant his/her specific concerns about attempting the test including physical problems and known disabilities. Refusal, reason for not attempting a test, or inability to perform a test should be recorded on the data form (see instructions below).

The detailed protocols describe how to administer the tests safely, including instructions on how to support the participant, if required. For the walking and balance tests, the examiner should stand next to and slightly behind the participant and position his/her hands very close to either side of the participant's trunk at the hip or waist level without touching the participant. The examiner should be ready to place both hands on the participant to stabilize her if necessary. If the participant loses balance, the examiner should grab/catch the participant with both hands at the trunk to stabilize her. If the participant begins to fall, the examiner should reach under the participant's shoulders from behind and slowly ease her down to the floor,

rather than try to catch the participant while standing still. This strategy should protect both the participant and examiner from injury.

If the participant falls and is not injured, the examiner should have the participant get on her knees or on all fours, place a chair next to the participant, and have the participant support herself on the chair as he/she helps lift the participant under the shoulders. The examiner should not try to lift the participant from the floor by him/herself.

c. Participant and Exam Space Preparation

- Footwear: To reduce effects of different footwear on test performance, the participant should wear tennis shoes or comfortable walking shoes with minimal or no heels. The participant may perform the tests in stocking or bare feet if appropriate footwear is not available.
- The standard chair should be placed on a non-skid surface (e.g., low pile carpeting) with the back of the chair against a wall for stability. There should be adequate room in front and on the sides of the chair for the examiner and participant to move freely.
- The standing balance test should be performed with the participant standing a little less than an arm's length from a wall to provide an additional source of support if a loss of balance does occur.
- Walking course: The 9-meter path for the 400-meter walk should be laid out in an uncarpeted, unobstructed, low traffic corridor of at least 122 cm wide.

d. General Instructions

Since motivation and level of understanding can have a significant impact on performance, each component of the exam should be administered strictly according to the protocol and in the following sequence:

- Explain the procedure to the study participant making sure to convey key points from the suggested script.
- Demonstrate the procedure using the suggested script.
- Ask the participant if she has any questions.
- Re-explain the procedure briefly using the suggested script.
- Ask the participant to perform the procedure.
- Begin all timed procedures with the words, "Ready? Go!"

Use the script provided to assure that all key points are covered when you describe each test and how to perform it properly. Do not provide additional description or encouragement beyond the key points provided by the standard scripts.

Demonstrate each maneuver correctly. Experience has shown that participants follow more closely what the examiner does rather than what he/she says. If the participant indicates she does not understand the test maneuver, demonstrate it again rather than solely relying on repeating the verbal instructions.

Limit practice trials for each test to those described in the individual measurement procedures.

Allow the participant to rest between tests if out of breath or fatigued during the assessments. If a test is not attempted because the participant refuses or cannot understand the instructions, record "participant refused." If you or the participant

considers the test unsafe, record “Not attempted/unable” on the scoring form (*PBM*). If a test is attempted, but cannot be completed or scored, record “Attempted, unable” on the scoring form.

e. Equipment and Supplies

- 1) Digital stopwatch (standing balance, repeated chair stands, walk tests)
- 2) Meter-stick (functional reach test)
- 3) Leveler (functional reach test)
- 4) Tape (functional reach, walk tests)
- 5) Standard chair, straight back, flat, level, firm seat; seat height 45 cm at front (repeated chair stands)
- 6) Jamar dynamometer (grip strength)
- 7) Measuring tape (walk tests)
- 8) Cones (400 m walk)

f. Recording Results and Scoring

Results for all functional performance tests should be captured on Form *PBM*, and scoring of results will be calculated centrally.

2. PERFORMANCE TESTS

a. Standing Balance Test

This is a series of timed, progressively more difficult, static balance tests. The level of difficulty increases as the lateral base of support decreases. The time (up to 30 seconds) the participant can hold each position (side-by-side, semi-tandem, tandem, and single-leg stands) is recorded. Walking aids such as a cane, may not be used. Use Form *PBM* to record results for each section of the standing balance test.

Script: “I’m going to ask you to stand in several different positions that test your balance. I’ll demonstrate each position and then ask you to try to stand in each position for up to 30 seconds. I’ll be near you to provide support, and the wall is close enough to prevent you from falling if you lose your balance. Do you have any questions?”

For each stand, describe the position to the participant and then demonstrate it while facing the participant. After demonstrating, approach the participant from the front and off to the side away from the wall. Offer her your arm (the one away from the wall) for support while she gets in position.

If the participant feels it would be unsafe to try, probe for the reason, and reassure the participant that you will help them into the position and that she can use the wall for additional support. If she still feels she should not attempt it, record, “participant refused” or “Not attempted, unable” (whichever is appropriate) for this and the more difficult stands, and go on to the next test.

If the participant attempts the stand incorrectly, demonstrate it again. Time each stand. After 30 seconds, tell the participant to stop. If the participant loses balance before the designated time (30 seconds), record the number of seconds for which the stand was held. See figures for placement of feet for each type of stand.

i. Side-by-side stand

- a) Describe the position.

Script: "First I would like you to try to stand with your feet together, side-by-side, for about 10 seconds. Please watch while I demonstrate."

- b) Demonstrate and say:

Script: "You may use your arms, bend your knees, or move your body to maintain your balance, but try not to move your feet. Try to hold your feet in this position until I say stop."

- c) Begin the test. Allow the participant to hold onto your arm to get balanced.
Say:

Script: "Hold onto my arm while you get in position. When you are ready, let go."

Start timing when the participant lets go.

(If the participant does not hold onto your arm, start timing when she is in position. Optional script: "Ready? Begin.")

Stop the stopwatch if she takes a step or grabs for support.

Record to 0.01 second the time the participant could hold this position.

Say, "STOP" after 30 seconds.

ii. Semi-tandem stand



- a) Describe the position.

Script: "Now I would like you to try to stand with the side of the heel of one foot touching the big toe of the other foot for about 30 seconds. Please watch while I demonstrate."

- b) Demonstrate and say:

Script: "You may put either foot in front, whichever is more comfortable. You can use your arms and body to maintain your balance. Try to hold your feet in position until I say stop. If you lose your balance, take a step like this."

- c) Begin the test. Allow the participant to hold onto your arm to get balanced.
Say:

Script: "Hold onto my arm while you get in position. When you are ready, let go."

Start timing when the participant lets go.

(If the participant does not hold onto your arm, start timing when she is in position. Optional script: "Ready? Begin.")

Stop the stopwatch if she takes a step or grabs for support.

Record to 0.01 second how long participant is able to hold this position.

Say, "STOP" after 30 seconds.

- d) If the participant is unable to hold the semi-tandem stand for at least 10 seconds, do not attempt the other standing balance tests (the tandem or single-leg stand). Go to the walking tests.

Based on the results from the semi-tandem stand, if you reason it would be unsafe for the participant to proceed to the more difficult positions, record "not attempted" on the form for the more difficult stands and continue to the walking tests.

iii. Tandem stand



- a) Describe the position.

Script: "Now I would like you to try to stand with the heel of one foot in front of and touching the toes of the other foot. I'll demonstrate."

- b) Demonstrate, and say:

Script: "Again, you may use your arms and body to maintain your balance. Try to hold your feet in position until I say stop. If you lose your balance, take a step, like this."

- c) Begin the test. Allow the participant to hold onto your arm to get balanced.
Say:

Script: "Hold onto my arm while you get in position. When you are ready, let go."

Start timing when the participant lets go.

(If the participant does not hold onto your arm, start timing when they are in position. Optional script: "Ready? Begin.")

Stop the stopwatch if she takes a step or grabs for support. Record to 0.01 second how long the participant is able to hold this position.

Say, "STOP" after 30 seconds.

- d) If the participant holds the position for 30 seconds, go to the Single Leg Stand. If the participant attempts the Tandem Stand and is unable or cannot hold it for at least one second, perform a second trial of the Tandem Stand.

Script: "Now, let's do the same thing one more time. Hold onto my arm while you get into position. When you are ready, let go."

iv. Single leg stand

- a) Describe the position.

Script: "For the last position, I would like you to try to stand on one leg for 30 seconds. You may stand on either leg, whichever is more comfortable. I'll demonstrate."

- b) Demonstrate the single leg stand by lifting the opposite leg so that the toes are about 2 inches off the floor. The knee should be flexed. While demonstrating say:

Script: "Try to hold your foot up until I say stop. If you lose your balance put your foot down."

- c) Allow the participant to hold onto your arm to get balanced. Say:

Script: "Hold onto my arm while you get in position. When you are ready, let go."

Start timing when the participant lets go.

Stop the stopwatch if she takes a step or grabs for support. Record to 0.01 second how long participant is able to hold this position.

Say, "STOP" after 30 seconds.

- d) If the participant holds the position for 30 seconds, then stop. Otherwise, perform a second trial of the Single Leg Stand.

Script: "Now, let's do the same thing one more time."

v. Scoring

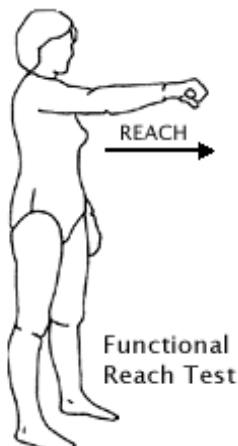
If the participant refuses to do the test or cannot understand the instructions, score "participant refused."

If you do not allow the participant to attempt a stand for safety reasons, score "Not attempted, unable."

If the participant cannot attain the position at all or cannot hold it for at least one second, score "Unable to attain position or cannot hold for at least one second."

Record to 0.01 second how long participant is able to hold each position, up to 10 seconds for the side-to-side and 30 seconds for the other positions.

b. Functional Reach Test



a) Preparation

1. Have participant stand next to the wall free of obstructions with the feet hip-width apart. The dominant arm should be closest to the wall.
2. Tape or Velcro a yardstick to the wall at the level of the shoulder joint. Have high values of yardstick closest to the subject.
3. Use leveler to make sure that the yardstick is level
4. Have participant extend his/her arm to the front at approximately 90 degrees of shoulder flexion (at shoulder height). The participant MAY NOT touch the yardstick or wall. Instruct the participant to simply lift the arm, but not to reach forward hand fist. Watch for any scapular protraction and correct the participant.
5. Take note of the starting position by determining what number the Metacarpophalangeal (MCP) joints (knuckles) line up with on the yardstick.

b) Performance

1. Provide the following instructions to the participant:
Script: "Reach forward as far as you can at the level of the yardstick by shifting your weight forward on your feet. Hold your endpoint until I tell you to stop. Do not step forward and keep your heels on the floor. You may not touch the yardstick. Keep the other arm at your side."
2. Take note of the end position of the MCP joints against the rule, and record the starting and end position numbers
3. If the feet move, that trial must be discarded and repeated.
4. Guard the subject as the task is performed to prevent a fall.
5. Practice for 2 trials, provide feedback.
6. Record results for 3 official trials. The final score will be calculated as the mean distance from the 3 trials.

Trial 1: Starting Point: _____" Ending Point: _____" Total: _____"

Trial 2: Starting Point: _____" Ending Point: _____" Total: _____"

Trial 3: Starting Point: _____" Ending Point: _____" Total: _____"

c. Repeated Chair Stands

This is a test of lower extremity strength in which the participant stands up from a seated position ten times as quickly as possible. The time it takes to stand five times and ten times is recorded.

If the participant can arise from the chair without using arms, attempt ten stands.

a) Explain the test.

Script: "This time, I want you to stand up ten times as quickly as you can, keeping your arms folded across your chest."

b) Demonstrate the test.

Cross your arms over your chest and then rise while emphasizing “full standing position,” and sit while emphasizing “all the way down.”

Script: “When you stand up, come to a full standing position each time, and when you sit down, sit all the way down each time. I’ll demonstrate two chair stands to show you how it is done.”

Rise two times as quickly as you can, counting as you stand up each time.

c) Begin the test

Script: “When I say ‘Go’ stand ten times in a row, as quickly as you can, without stopping. Stand up all the way, and sit all the way down each time.”

Ready? Go!”

Start timing as soon as you say “Go.” Count: “1, 2, 3, 4, 5, 6, 7, 8, 9, 10” as the participant stands up each time. After the participant stands up for the fifth time, glance at the time and depress the split button on the stopwatch (see instructions on stopwatch in **Appendix I**).

- d) If the participant is unable to complete the chair stands correctly (e.g., is not coming to a full stand), stop the procedure, repeat the demonstration, wait 1 minute, and begin the procedure again.

- e) If the participant stops before completing five stands, confirm that she cannot continue by asking:

Optional script: “Can you continue?”

If she says yes, continue timing. Otherwise, stop the stopwatch.

- f) If the participant stops before completing ten stands, confirm she cannot continue by asking:

Optional script: “Can you continue?”

If she says yes, continue timing. Otherwise, stop the stopwatch.

g) Scoring:

If the participant refuses to do the test or cannot understand the instructions, score “participant refused.”

If the procedure was not attempted because the participant was unable to perform the test, score “Not attempted/unable,” and comment about why the participant was unable to perform the test.

If participant attempted but was unable to complete five stands without using her arms, score as “Attempted, unable to complete five stands without using arms” and record the number completed without using arms.

If five chair stands were completed, record the number of seconds, to a hundredth of a second, required to complete five stands.

If participant completed five stands but was unable to complete ten stands without using her arms, score as “Attempted, unable to complete ten stands without using arms” and record the number completed without using arms.

If ten chair stands were completed, record the number of seconds, to a hundredth of a second, required to complete ten stands.

d. Grip Strength



The grip strength examination is used to test how strong the participant's hands are. We will be using the same Jamar Dynamometer and the same protocol utilized at each WIHS site in 2005.

a) Tester Instructions: The key points.

- The participant should be seated in an armless chair
- Her elbow should be bent at a 90 degree angle
- The dynamometer should be set at "2" strength for testing of all participants.
- Do not allow the participant to squeeze the dynamometer before testing.
- The tester must coach the participant by saying "squeeze, squeeze, squeeze" while the participant is squeezing.
- Tell the participant to stop when you see the arrow starting to go down.
- Record the results of each trial before the next attempt.
- Repeat the examination three times in the dominant hand.

b) Preparation

Participants with one or more of the following conditions that affect their DOMINANT HAND should not be tested: Do NOT switch to the other hand:

- Acute flare-up of wrist/hand; for example, arthritis, tendonitis or carpal tunnel syndrome.
- Less than 13 weeks after surgery for fusion, arthroplasty, tendon repair or synovectomy of the upper extremity.
- If the technician has concerns that this test may exacerbate symptoms of heart disease (e.g., angina), the situation should be investigated. Ask the participant if she is currently having symptoms from heart problems. This does NOT exclude the participant from the grip strength test. Local procedures may be developed in this situation to assure safety for the participant.

Use the following questions to assess whether the participant can complete the Grip Strength Test. Script: "In this exercise, I am going to use this instrument to measure the strength in your dominant hand."

1. *“Have you had any recent pain in your wrist or any acute flare-up in your hand or wrist from conditions like arthritis, tendonitis or carpal tunnel syndrome?”*
2. *“Have you had any surgery on your hands or arms during the last 13 weeks?”*
3. *“Which hand is your dominant hand?”*
4. *“Do you think you could safely squeeze this instrument as hard as you can with your dominant hand?”*

In subsequent tests, perform the hand grip test only on the dominant hand that was established at her first test.

c) Testing

Script: “I’d like you to take your dominant arm, bend your elbow at a 90 degree angle, press your arm against your side and grab the two pieces of metal together like this.”

Examiner should demonstrate at this point. “When I say ‘squeeze,’ squeeze as hard as you can. The two pieces of metal will not move but I will be able to read the force of your grip on the dial. I will ask you to do this three times. If you feel any pain or discomfort, tell me and we will stop.”

d) Performance and Scoring

Face the participant and squeeze the dynamometer so that the participant can see the dial rotate.

Script: “Now you should bend your elbow at a 90 degree angle, press your arm against your side and grip the two pieces of metal with your dominant hand. Your wrist should be straight. “Ready? Go! Squeeze, squeeze, squeeze!” (Tell the participant to “stop” when the arrow starts going down.)

Record whether or not the grip strength test was completed. If unable, indicate why she was unable to complete the grip strength test and STOP TESTING. If attempted, but unable physically, STOP TESTING.

Record the strength for the **first** attempt in kilograms. The Dynamometer should be read at eye level. Round down to the nearest line on the dynamometer (will always be an even number). Be sure to set the dynamometer dial to zero prior to each attempt. A minimum of three attempts with the dominant hand must be made. Record the strength for the **second** attempt in kilograms. Record the strength for the **third** attempt in kilograms.

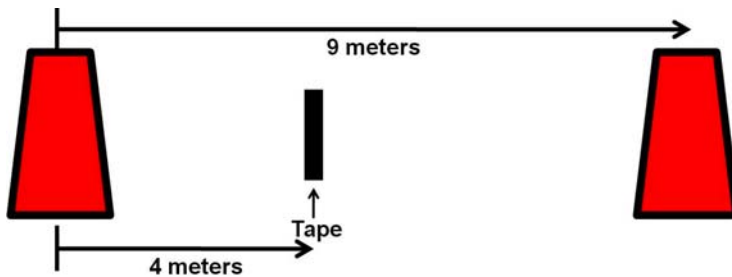
e. 4-meter walk test

a) Preparation

The walking course for both the 4-meter and 400-meter walk test should be marked out in advance of the participant’s test in an unobstructed corridor of at least 12 meters (approximately 39 feet) in length. Using the measuring tape, place a cone at 0 and a cone at 9 meters (the participant will walk approximately 1 meter rounding the cones, yielding a 10 meter walking course). Using the measuring tape, place a piece of tape 4 meters from the starting cone.

For the 4-meter walk, the participant will walk from the starting cone to the tape. Participants needing to use a walking aid, or with prosthetics, are excluded from participating in the MSK Substudy. Participants should be asked to wear

comfortable footwear (sneakers, comfortable walking shoes) and not footwear that impedes their walking (high heels).



b) Explain the test

Script “In this test, I would like you to walk **at your usual pace** from this line to the line at the end of the hall. Do you think you could do that? Good. Can you see the tape? Good. Let me demonstrate what I want you to do.” (Demonstrate for the participant.)

“To do this test, place your feet with your toes behind, but touching the cone. I will time you. When I say ‘**Ready, go!**’ walk at your usual pace to the line. I will walk with you.”

c) Performing the Test

When the participant is properly at the cone, say “Ready, go!” and start the stopwatch as the participant begins walking; keep the stopwatch behind the participant so she can’t see it. Your arm can provide support if the participant loses balance. Stop the stopwatch when the participant’s first foot is completely across the finish line.

The participant will then be asked to perform the measured walk a second time.

Script: “Now, I’d like you to try this test a second time. When I say “Ready, go!” walk at your usual pace to the line. I will walk with you.”

When the participant is properly at the line, say “Ready, go!” and start the stopwatch as the participant begins walking; keep the stopwatch behind the participant so she can’t see it. Your arm can provide support if the participant loses balance. Stop the stopwatch when the participant’s first foot is completely across the finish line.

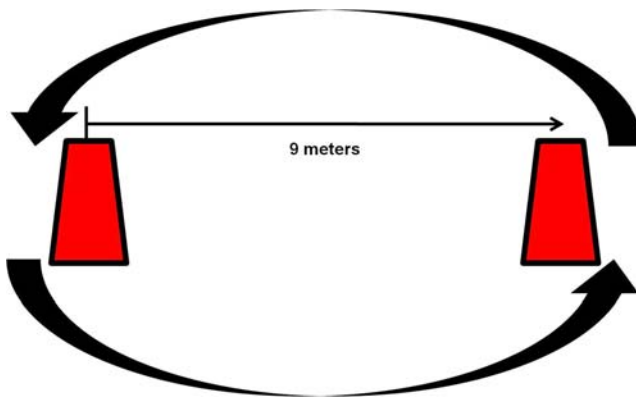
The participant will be allowed only two attempts at completing the measured walk unless there is external influence that interrupted her during the walk. For example, if the participant trips on her own during the first attempt, it will be recorded as such on the PBM form and she will then go on to perform her second attempt. However, if during either of her attempts, she trips due to interference from another person, she may repeat that attempt.

Record the time in seconds (rounded to the first decimal point) of the two trials and take the average of two trials. Please see **Appendix I** for instructions on how to use the Stopwatch for this test. If the participant did not attempt or complete the trial, record the reason.

f. 400-meter walk test

a) Preparation

Preparation for the 400-meter walk is detailed in the 4-meter walk section, above. For the 400-meter walk, the participant will walk counter-clockwise around each cone. Each lap consists of rounding the far cone and returning to the starting cone, a total of 20 meters. To complete the 400-meter walk, each participant will complete 20 laps.



b) Explain the test

Script: “We would like you to attempt to walk 400 meters (about a ¼ mile) **as quickly as possible** as a measure of physical function. If you have had any of the following medical conditions or surgical procedures in the last **3 months**, you should not do this test.

1. Been hospitalized for myocardial infarction or heart attack?
2. Had angioplasty or heart surgery, major thoracic (chest), abdominal or joint surgery?
3. Seen a health professional or thought about seeing a health professional for new or worsening symptoms of angina or chest pain?
4. Have a systolic blood pressure greater than 200 mmHg or a diastolic blood pressure greater than 110 mmHg.

If, at any time during the test, you feel any chest pain, tightness or pressure in your chest, you become short of breath or if you feel faint, lightheaded or dizzy, or you feel knee, hip, calf, or back pain, please tell me. If you feel any of these symptoms, you may slow down or rest. You may also choose to stop the walk.”

c) Performing the test

Script: “When I say ‘GO’ start walking. Ready, Go.”

Start timer with participant’s first footfall. For every lap, offer standard encouragement and call out the numbers of laps completed and the number remaining. Record each lap on the form.

If the participant feels she needs to stop in one place and rest, after 30 seconds of rest, ask her if she can continue walking. If she can, continue the walk and record the rest on the form. If she needs to rest longer, have her continue to stand. After another 30 seconds, ask her if she can continue walking. If she can, continue the walk and record the rest stop on the form. If the participant reports a significant degree of any of the following symptoms, then stop the test: chest

pain, tightness or pressure; trouble breathing or shortness of breath; feeling faint, lightheadedness or dizzy; leg pain; needed to sit down.

Record the split time on each of 20 laps and the total amount of time to completion of the 20 laps (400 meters). Please see **Appendix I** for instructions on how to use the Stopwatch for this test. If the participant does not complete the 20 laps, record the split times for the laps completed (do not include the incomplete lap if participant stopped between cones) and then record the reason why the participant stopped the test.

g. Quality Assurance

The examiner requires no special qualifications or experience to perform this assessment. Training should include:

1. Read and study Manual of Operations.
2. Attend MACS/WIHS training session on performance test administration techniques (or receive training by experienced examiner).
3. Practice on at least two other staff or volunteers.
4. Discuss problems and questions with local expert or QC officer.
5. The primary examiner/trainer for each WIHS site will be certified by Dr. Ferrucci before starting the study and re-certified twice yearly (below). After certification, the primary examiner/trainer can train and certify other examiners at his/her site.

h. Certification Requirements

1. Complete training requirements.
2. Able to recite exclusions.
3. Conduct exam on two volunteers, according to protocol. A **video** of the performance of the full battery of performance measures (standing balance, functional reach, repeated chair stands, grip strength, 4-meter, and 400-meter walk) should be performed on two volunteers by each examiner. The video should be recorded by site staff on low resolution and sent to Dr. Ferrucci by site staff. Dr. Ferrucci will review the performance video and provide comments. Following is the contact information for Dr. Ferrucci:

Dr. Luigi Ferrucci
ATTN: Ms. Leslie Vuncannon
National Institute on Aging at Harbor Hospital
3001 S. Hanover Street, 5th Floor
Baltimore, MD 21225

Phone: 410-350-7330

Fax: 410-350-7304

Email: yuncannonla@mail.nih.gov

4. The certified primary examiner at each site should also be the trainer for other examiners at the site and the QC officer. He/she at each site can certify other examiners by evaluating the performance of the trainee on two participants, demonstrating the successful completion of QC checklists and checking to see that the times/measurements agree with the QC officer.
5. There will be a twice-yearly (every 6 months) recertification of the primary examiner that will be performed through uploading a video of the functional performance tests on one subject for Dr. Ferrucci's review.

Quality Assurance/Certification Checklists will be in the **Appendix A**.

3. FRAILITY

A phenotypic assessment of frailty (Fried) has been implemented in many studies of aging in the general population^{17,18} as well as in the MACS^{19,20} and WIHS²¹. It consists of five components:

1. **Physical shrinking:** Defined as unintentional weight loss of ≥ 10 lbs in the prior year or, at follow-up, of $\geq 5\%$ of body weight in the prior year (by direct measurement of weight). Using core WIHS data, we will calculate $(\text{weight}_{\text{previous year}} - \text{weight}_{\text{current}}) / (\text{weight}_{\text{previous year}}) = K$. If $K \geq 0.05$ and the subject does not report that he/she was trying to lose weight (i.e., unintentional weight loss of at least 5% of previous year's body weight), then the participant will be categorized as frail by weight loss criterion.
2. **Poor endurance and energy:** This will be measured by self-report of exhaustion, identified by two questions from the CES-D scale. Self-reported exhaustion is associated with stage of exercise reached in graded exercise testing, as an indicator of VO₂ max. Using the CES-D Depression Scale, the following statements are read: "I felt that everything I did was an effort." "I could not get going." And then, "How often in the last week did you feel this way?", which is coded as: 0 = rarely or none of the time (<1 day) 1 = some or a little of the time (1-2 days) 2 = a moderate amount of the time (3-4 days) 3 = most of the time. Participants answering "2" or "3" to either of these questions will be categorized as frail by the exhaustion criterion.
3. **Low physical activity level:** A weighted score of kilocalories expended per week will be calculated based on each participant's response to the FRAM (Fat Redistribution and Metabolic Changes in HIV Infection Study) Physical Activity Questionnaire (PAQ). Kcals per week expended will be calculated using a standardized algorithm. For women, those with Kcals per week <270 will be considered frail by the physical activity criterion.
4. **Slowness:** This will be measured by the time to walk 4 meters, adjusting for gender and standing height.
5. **Weakness:** Defined as grip strength in the lowest 20% at baseline, adjusted for gender and body mass index. Handgrip strength will be measured by a hand dynamometer such as the JAMAR dynamometer (Model BK-7498, Fred Sammons Inc, Brookfield, IL). Grip strength will be measured three times for the participant's dominant hand only. The best measure in the dominant hand will be used. The following table shows the cutoff for frailty categorization, defined by BMI:

BMI	Cutoff for grip strength (Kg): criterion for frailty
≤23	≤17
23.1-26	≤17.3
26.1-29	≤18
>29	≤21

Scoring Frailty:

Positive for frailty phenotype: ≥ 3 of above criteria present

Intermediate (pre-frail): 1 or 2 criteria present

Robust (non-frail): 0 criteria present.

Among the 5 criteria above, 2 criteria (physical shrinking and poor endurance) are available from the WIHS database, 2 criteria (slowness and weakness) are assessed as part of the functional performance tests; therefore, only low physical activity remains to be assessed. The PAQ questionnaire will take approximately 5 minutes to administer, was utilized WIHS-wide in 2005, and is in **Appendix E**.

G. DXA and VFA PROTOCOL

1. INTRODUCTION TO QUALITY ASSURANCE

This protocol serves as an adjunct to the official GE/Lunar Prodigy DXA Operator's Guide for the WIHS. The purpose of this protocol is to standardize the dual xray absorptiometry (DXA) and vertebral fracture analysis (VFA) scanning acquisition procedures between the three clinical centers participating in the WIHS MSK Substudy. The success of the DXA and VFA portion of the MSK Substudy will depend on several factors, including the qualifications and dedication of the scanner operators, clear specification and understanding of the study requirements as set forth in this protocol, and good lines of communication between the clinical sites, the Image Reading Center (IRC), the WIHS Data Management and Analysis Center (WDMAC), and participants in the WIHS MSK Substudy.

This protocol is intended to serve as the DXA operator's guide for the WIHS and builds upon (rather than replaces) the operator training and documentation provided by the GE/Lunar Corporation. It is expected that each technologist performing scan acquisition for the WIHS is familiar and competent with the scanning system employed at his/her study site. In addition, the material in this protocol should be read and understood before beginning to scan participants for the MSK Substudy. The IRC will certify that each DXA technologist is qualified via expert review of five DXA scans each of AP spine, proximal femur, forearm and whole body acquired and analyzed by each technologist. Upon receipt of the above scans, a four-digit certification number will be assigned to each technologist participating in the substudy.

Other requirements for DXA and VFA in this substudy are as follows:

- No scanner hardware changes – without prior approval from IRC
- No scanner software upgrades – without prior approval from IRC
- No scanner relocation – without prior notification to IRC

Requests for the above can be made by completing and submitting the *Request for Software/Hardware Upgrade Form (Appendix B)* to IRC, and approval or denial will be issued using IRC-specific forms.

All scan analysis will be done by IRC.

Any questions or comments concerning the DXA and VFA procedures of the MSK Substudy should be directed to the Image Reading Center (IRC):

Phone/Fax: 877-472-5227 (877-IRC-LABS)
Email: WIHS@imagereadingcenter.com

Other questions regarding the MSK Substudy should be directed to your local Project Director. Questions that cannot be resolved by the local Project Director may be addressed to the WDMAC Project Director, Christine Alden (calden@jhsph.edu).

2. INTRODUCTION TO THE DXA AND VFA PORTION OF THE WIHS MSK SUBSTUDY

The dual x-ray absorptiometry (DXA) scans to be performed at the MSK Substudy visits will be AP spine, left hip, forearm, and whole body and VFA. DXA and VFA scans should NOT be analyzed by the sites. **All scans will be analyzed by IRC.** All unanalyzed scans should be forwarded to the IRC for review and processing. This protocol will describe the procedures to be followed for participant scanning and longitudinal calibration.

NOTE: PLEASE READ THIS PROTOCOL CAREFULLY BEFORE SCANNING ANY PARTICIPANTS. THESE INSTRUCTIONS ASSUME YOU ARE FAMILIAR WITH CORRECT SCANNING AND ANALYSIS PROCEDURES.

Table 1.0 Schedule of DXA and VFA scans to be performed on each participant.

Exams	Baseline Visits 35-38 Code 00	2- year Follow-up Visits 39-42 Code 24
DXA AP Spine	X	X
DXA Left Hip	X	X
DXA Forearm	X	X
DXA Whole Body	X	X
VFA	X	X

DXA scan data will be submitted on a monthly basis to:

Shipping: IMAGE READING CENTER
419 Lafayette Street
New York, NY 10003

3. CREATION OF NEW DATABASE

Create a WIHS MSK database (see Figure 1, New Database), which will allow you to easily locate and compare all future scans of the same participant to her original scans. Please note the name of the working directory for future reference. This will be different for each site.

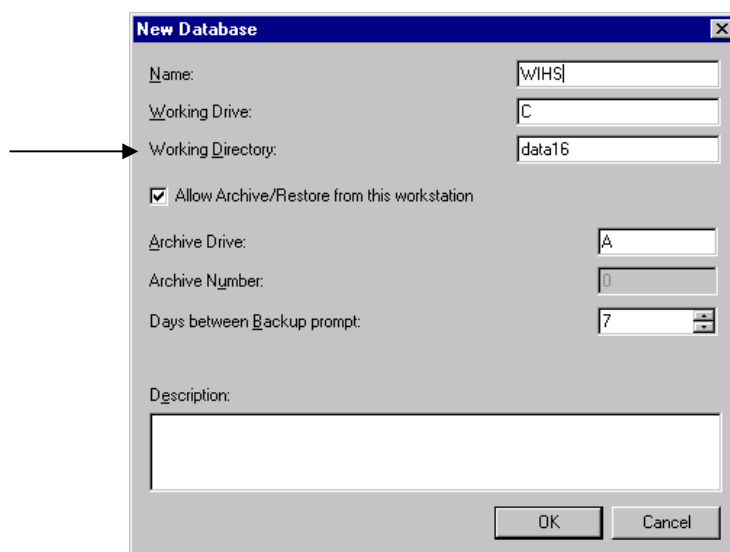


Figure 1. New Database.

4. NEW PARTICIPANT REGISTRATION

This section describes in detail the specific procedures to be conducted for the DXA and VFA portion of the MSK Substudy with respect to entering the participant's biography. Be sure to read and understand the material in this section before scanning any participants for the WIHS. Any questions you may have regarding the acquisition of DXA and VFA scans should be directed to the IRC.

Participant Name and ID Fields

Select the WIHS database and New (patient). The Mandatory Information screen appears. Enter the following data in the information fields. At the end of each field, press Tab to move the cursor to the next field (see Figure 2, New Patient Directory).

Enter **WIHS** in the *First Name* field. Enter the participant's **eight-digit WIHSID** in the *Last Name* field **exactly** as it is entered on the MSK Substudy Participation Notification Form (MSKNOTI). Enter the **site number** (Bronx 001; San Francisco 005; Chicago 006 – Sites may wish to use the WIHS numbers. Bronx=1 San Fran=5, Chicago=6) plus the participant's **eight-digit WIHSID** in the *Patient ID* field. This is not the same information as the other protocol. For this reason, you should always obtain the WIHSID number, DOB, and race/ethnicity from the Project Director to ensure accuracy. An example of the Prodigy Biography screen is shown in Figure 2A.

Enter the date of birth, sex, race/ethnicity, height and weight in the appropriate fields. The visit number and visit code (baseline 00; follow-up 24) should be entered into the *Exam ID* field on the Secondary page (shown in Figure 2B) and updated at the follow-up visit. Please enter the Operator's initials and ID code in the *Attendant* field.

Fields should be completed as follows:

First Name:	WIHS
Middle Initial:	Leave blank
Last Name:	Participant's eight-digit WIHSID (e.g., 1-23-4567-8; dashes included)
Patient ID:	Site number (Bronx 001; San Francisco 005; Chicago 006) plus participant's WIHSID (e.g., 0031-23-4567-8)

- Birth Date: Participant's date of birth (MM/DD/YYYY)
- Height: Participant's height in inches (round to nearest 1.0 inch)
- Weight: Participant's weight in pounds (round to nearest 1.0 pound)
- Sex: Female
- Ethnic Group: Enter as appropriate
- HIPAA: To prevent the letters and numbers in the *Name* field from becoming # signs (secure HIPAA report), please use the following directions:
1. Go to Tools
 2. User Options
 3. Reports
 4. HIPAA Secure report – **Do not check**

This may be a desired feature for your clinical scans, so please remember to switch it back (shown in Figure 2C).

Note from this example that all leading zeroes must be included. Be sure to use zeroes (Ø) and not the letter "O" when entering the WIHSID into the *Last Name*, and *Patient ID* fields.

The screenshot shows a 'New Patient' window with the following data entered:

- Primary tab selected
- First: WIHS
- Middle Initial: (empty)
- Last: 01234567
- Patient ID: 00201234567
- Physician: VISIT18,00
- Birth Date: 1 / 1 / 1950 (Age: 53.2 years)
- Height (in.): 65 (5 feet 5 inches)
- Weight (lbs.): 180
- Gender: Female, Male
- Ethnicity: Asian, Black, Hispanic, White, Other
- Buttons: OK, Cancel

Figure 2A. New Patient Directory.

Figure 2B. Secondary Page.

Figure 2C. HIPAA Field.

5. DXA and VFA SCAN ACQUISITION

In order to get the most consistent participant results, it is important to follow consistent procedures in acquiring all scans. These include the following:

1. Use the same scan mode for each participant for the baseline and each subsequent scan throughout the study (Standard or Thick).
2. The participant must be dressed in a hospital gown or pajamas, wearing only underpants and thin socks, if necessary. Remove all radiopaque objects from the scan areas.

3. Ensure correct positioning of the participant on the screening exam.
4. Keep a print-out of the baseline scan at the imaging facility to use as a reference for the follow-up scans.
5. Monitor the scan during acquisition. If positioning is not correct, or the participant moves, etc., interrupt the scan, reposition the participant, if necessary, and restart the scan.
6. For participants that cannot fit within the scan field, please perform a hemi-scan. Please see Section e. Whole Body Hemi-scans and Figure 7, below.

Please note any problems with scan acquisition in the comment field of the *Participant Data Log* (WIHS Form *MSK01*) and on the *DXA Data Transmittal Form*.

a. AP Spine scans

The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper AP Lumbar Spine scanning procedures. Clarifications for the WIHS are noted below.

1. Use the **Standard** scan mode unless the **default** scan mode goes to Thick.
2. Keep the scan width and length set to their defaults.
3. Make sure that each AP scan includes both iliac crests at the bottom of the image and at least one vertebra with ribs at the top. Also, make sure the spine is straight and centered in the scan-field. Ensure optimal participant positioning by consistently using the positioning cushions for each scan.
4. When performing the follow-up scan, refer to a printout of the baseline scan to assure duplicate positioning and scan parameters. If the positioning or starting point do not match the baseline scan, interrupt the scan, reposition the participant, if necessary, and restart the scan.
5. Save the UNANALYZED scan to diskette and make an additional copy, one for the site's files and one to send to the IRC.

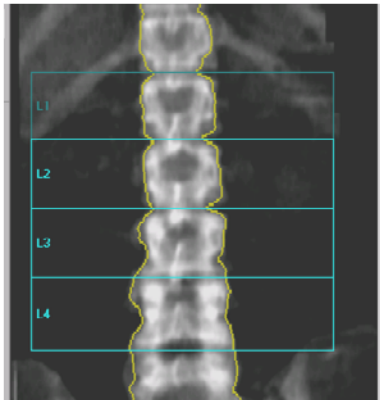


Figure 3. Properly Acquired AP Spine Scan

b. Hip scans

The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper hip scanning procedures. Clarifications and exceptions for the WIHS are noted below.

1. Scan the **LEFT** hip. If you scan the right hip, then note on the *Participant Data Log* (WIHS Form *MSK01*) and on the IRC DXA Data Transmittal Form the reason(s) why the left hip was not evaluable.

2. Use the **Standard** scan mode unless the **default** scan mode goes to Thick. Keep the scan width and length set to their defaults.
3. Make sure the leg to be scanned is properly rotated and abducted. Ensure optimal participant positioning by consistently using the hip scan positioning fixture for every scan. Always position the participant's leg by applying rotating force **above the knee**. This will ensure that the hip itself is rotated, not just the lower leg. Proper rotation and abduction will alleviate many analysis problems. With optimal positioning, the lesser trochanter will be barely visible.
4. When performing follow-up scans, refer to a printout of the baseline scan to assure duplicate positioning and scan parameters. Follow-up scans should be of the same side scanned at baseline. If the positioning/starting point does not match the baseline scan then interrupt the scan, reposition the participant, if necessary, and restart the scan.
5. Save the UNANALYZED scan to diskette and make an additional copy, one for the site's files and one to send to the IRC.

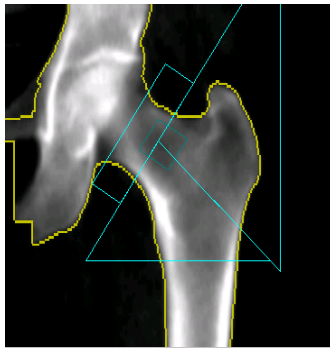


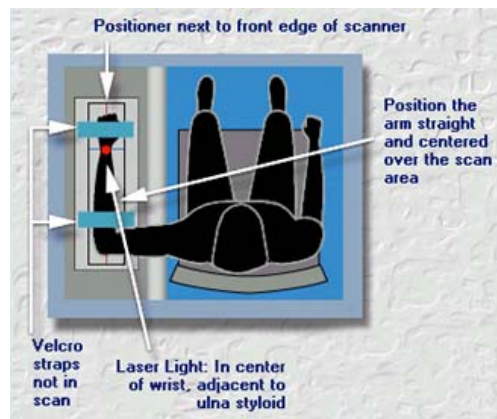
Figure 4. Properly Acquired Hip Scan

c. Forearm Scans

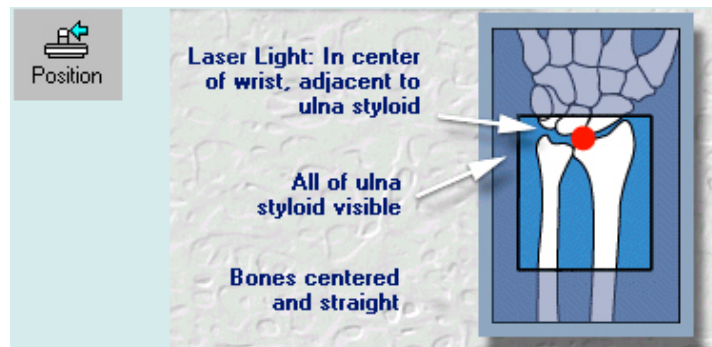
The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper forearm scanning procedures. Clarifications and exceptions for the WIHS are noted below.

1. Ask the participant if any metal implants are present and note presence on *Participant Data Log* (WIHS Form *MSK01*) and on *IRC DXA Data Transmittal Form*. The subject should remove any article that might create an artifact on the forearm scan.
2. The Forearm to be scanned will be the **non-dominant** forearm. If the subject has fractured the non-dominant forearm, please scan the dominant forearm and indicate the change in the comments section of the *IRC DXA Data Transmittal Form*.
3. Position the forearm positioner in the center of the scanner table against the front edge of the table with the GE logo in the direction of the subject's fingers. Seat the subject in a low-seated, high back chair with **no wheels or arms** next to the positioner.
4. Instruct the subject to sit in the chair as follows:
 - 1) Left Forearm scan: Subject faces the head (right end) of the table.
 - 2) Right Forearm scan: Subject faces the bottom (left end) of the table.

- Instruct the patient to lean into the table so that their rib cage is pressing against the edge of the table.



- Place the laser crosshairs:
 - For Left Forearm: $\frac{3}{4}$ of the length of the entire forearm below the carpal bones.
 - For Right Forearm: Right above the ulna styloid process and under the first row of carpal bones.
- Have the subject make a loose fist. Secure the velcro straps over the hand and over the arm just below the elbow.



- Make sure for:
 - Left forearm scan. The scan is straight and centered in the scan field. Forearm and first row of carpal bones are flat and not rotated. Scan image includes forearm and first row of carpal bones.
 - Right forearm scan. The scan is straight and centered in the scan field. Forearm and first row of carpal bones are flat and not rotated. Scan image includes forearm and first row of carpal bones.
- When performing follow-up scans, refer to a printout of the baseline scan to assure duplicate positioning and scan parameters. If the positioning does not match the baseline scan, interrupt the scan, reposition the participant, if necessary, and restart the scan.
- Save the UNANALYZED scan to diskette and make an additional copy, one for the site's files and one to send to the IRC.



Figure 5. Properly Acquired Forearm Scan

d. Whole Body scans

The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper whole body scanning procedures. Clarifications and exceptions for the WIHS are noted below.

1. Ask the participant if any metal implants are present and note presence on *Participant Data Log* (WIHS Form *MSK01*) and on *IRC DXA Data Transmittal Form*.
2. Position the participant in the center of the table aligned with the long axis of the table with the head towards the head end of table and within the scan limit line. The participant's head should look straight up, not be turned left or right. If required for participant comfort, use only radio-lucent pillows. If pillows are used, make a note to use the same pillows again during follow-up measurements.
3. Feet and legs should be placed together with no internal rotation of the hips. Use a velcro strap around the ankles to avoid movement. **Do not** strap the knees.
4. Position hands with palms flat against the scan table. If necessary, with larger or heavier participants, the hands may be placed in a lateral position next to the hips. **Do not** tuck the hands under the hips to keep them in the scan field. If necessary, wrap the participant around the torso and arms with a sheet or perform a hemi-scan. For participants who are too tall to fit within the scanning limits, it is acceptable for the head to extend beyond the upper scan limit line. **Do not** bend the knees to keep the feet in the scan field.
5. Use the **Standard** scan mode unless the **default** scan mode goes to **Thick**. Keep the scan width and length set to their defaults.
6. Monitor the scan during acquisition. If the participant moves, the scan may be interrupted and restarted.
7. When performing follow-up scans, refer to a printout of the baseline scan to assure duplicate positioning and scan parameters. If the positioning does not match the baseline scan, interrupt the scan, reposition the participant, if necessary, and restart the scan. If the participant does not fit within the scan field at the follow-up scan, perform a hemi-scan. Please see Section d. Whole Body Hemi-scans and Figure 7, below.
8. Save the UNANALYZED scan to diskette and make an additional copy, one for the site's files and one to send to the IRC.

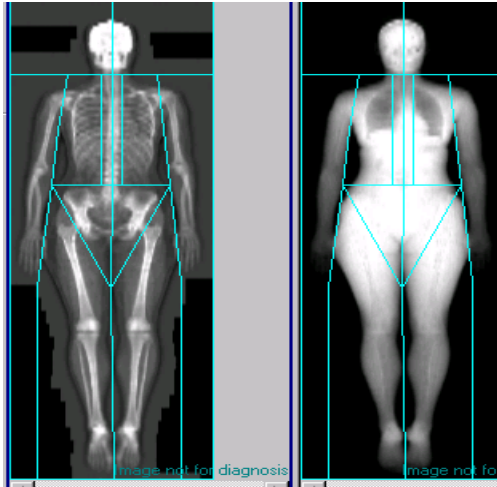


Figure 6: Properly Acquired Whole Body Scan

e. Whole Body Hemi-scans

This is an exception to the official GE/Lunar Prodigy Operator's Manual for the proper whole body scanning procedure.

1. Ask the participant if any metal implants are present and note presence on *Participant Data Log* (WIHS Form *MSK01*) and on *IRC DXA Data Transmittal Form*.
2. Position the participant on the table. Ask the participant to move closer to the edge of the table on the side free of the scanning arm (the side free of the scanning arm is usually on the left of the participant). The participant's head should look straight up, not be turned left or right. If required for participant comfort, use only radio-lucent pillows. If pillows are used, make a note to use the same pillows again during follow-up measurements.
3. Feet and legs should be placed together with no internal rotation of the hips. Use a velcro strap around the ankles to avoid movement. **Do not** strap the knees.
4. Position hands with palms flat against the scan table leaving sufficient room between the arms and the torso (see Figure 7). **Do not** tuck the hands under the hips to keep them in the scan field. For participants who are too tall to fit within the scanning limits, it is acceptable for the head to extend beyond the upper scan limit line. **Do not** bend the knees to keep the feet in the scan field.
5. Use the **Standard** scan mode unless the **default** scan mode goes to **Thick**. Keep the scan width and length set to their defaults.
6. Monitor the scan during acquisition. If the participant moves, the scan may be interrupted and restarted (see Figure 7). The entire right side of the participant should be visible and only the left arm and/or part of the left trunk and leg should be excluded from the scan.
7. When performing follow-up scans, refer to a printout of the baseline scan to assure duplicate positioning and scan parameters. If the positioning does not match the baseline scan, interrupt the scan, reposition the participant, if necessary, and restart the scan.
8. Save the UNANALYZED scan to diskette and make an additional copy, one for the site's files and one to send to the IRC.

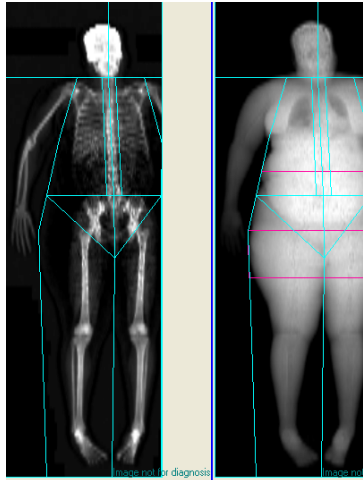
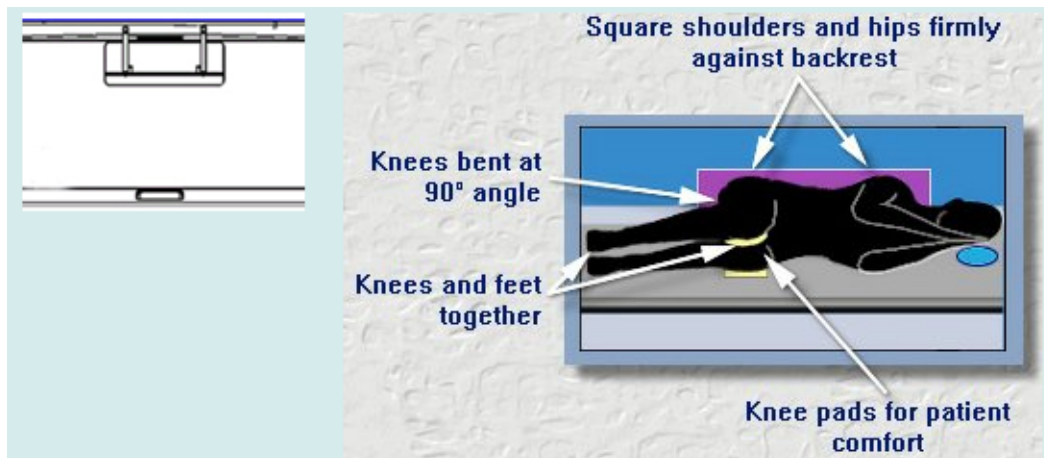


Figure 7: Properly Acquired Whole Body Hemi Scan

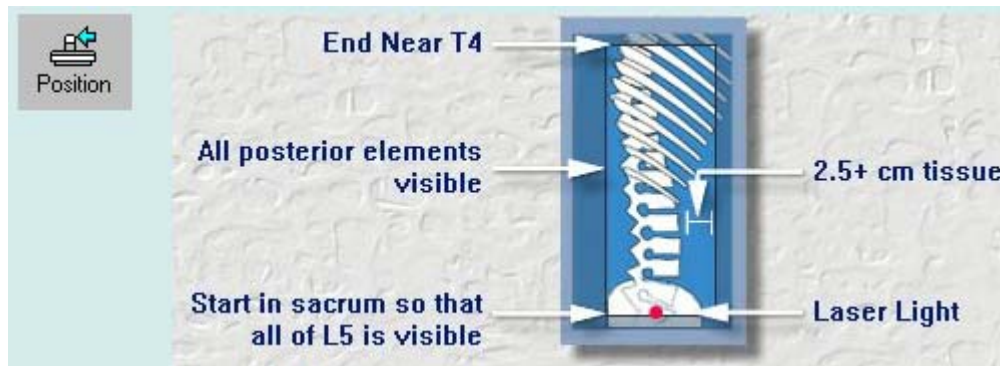
f. VFA scans

The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper VFA scanning procedures. Clarifications and exceptions for the WIHS are noted below.

1. Ask the participant if any metal implants are present and note presence on *Participant Data Log* (WIHS Form *MSK01*) and on *IRC DXA Data Transmittal Form*.
2. Pre-position the subject so that the spine is kept straight in all directions. Use all necessary positioning pads and the positioner to position properly (shoulder, pelvis, and legs should be square and centered). The positioner should unfold and set against the back rail of the table.



3. Select position from the Measurement toolbar. The scan arm will move to the approximate starting position in the middle of the table. **Align the positioning laser in the top of the iliac crest.**



4. An ideal VFA scan measurement ensures the spine is centered and all of L5 is visible. The software will automatically save the measurement once the designated scan length is achieved (to T4).
5. When performing follow-up scans, refer to a printout of the baseline scan to assure duplicate positioning and scan parameters. If the positioning does not match the baseline scan, interrupt the scan, reposition the participant, if necessary, and restart the scan.
6. Save the UNANALYZED scan to diskette and make an additional copy, one for the site's files and one to send to the IRC.



Figure 8. Properly Acquired VFA scan

6. DATA SHIPMENT AND SCAN ARCHIVAL OF PARTICIPANT DATA

When preparing to send participant scan files to the IRC, use the below procedure to transfer participant scan files to diskettes or CD-R. Do not use the **Archive** command.

a. To copy scan images to the diskette/CD-R

- Within the database, choose the participant for whom you want to copy scan data.
- Click on the first scan in the series. Hold down the Shift key and click on the last scan.

- All scans for a selected participant should then be highlighted. **Right** click on the shaded area. (See Figure 9, Participant Scan Directory.)
- A small command box will appear.
- Select “Send Image File To → Disk...”
- At “My Computer,” select A:\ (3 ½ Floppy A): (see Figure 10, Browse for Folder) to send scans to the diskette. Repeat until all scans for all desired participants have been copied to the diskette. If you are using CD-R, at “My Computer,” select the CD drive to send scans to the CD-R. Repeat until all scans for all desired participants have been copied to the CD-R (D:Drive in Figure 10).

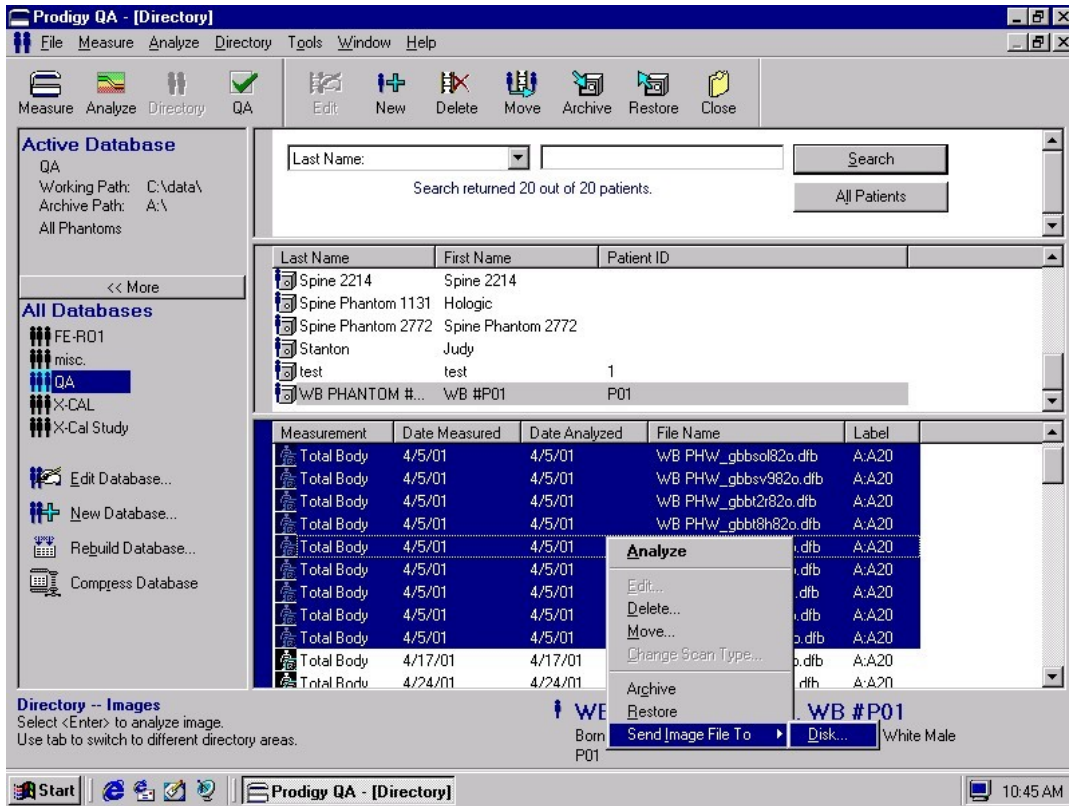


Figure 9. Directory – Example of Choosing Scans from the Database.



Figure 10. Browse for Folder.

b. Labeling copy diskettes

Each diskette/SuperDisc/CD-R must be labeled (see Figure 11), using the labels provided by the Project Directors, prior to mailing to IRC. Enter “YES” or “NO” into the QC Data field to indicate whether or not the disk contains QC data.

WIHS Data Transfer Label	
Number of Participants:	_____
QC Data	_____
Date:	__ __ / __ __ / __ __
	D D M M M Y Y
Diskette #	_____ of _____

Figure 11. Data Label for Diskettes/CD-R.

c. Baseline and follow-up participant data

Participant data for all visits will be copied onto diskette/CD-R, batched, and sent by the Project Director to IRC **monthly via FedEx**. . Each diskette/CD-R must be labeled with the appropriate study label provided by the Project Directors (see Figure 11).

d. Archive

The **Archive** function on the Prodigy scanner is used to store scan files onto floppy diskettes, or CD-R. **Archive your scan files on a regular basis**. See your Prodigy Operator's Manual for instructions. **Do not** use the archive command to transfer participant scan files to diskette/CD-R to be sent to the IRC.

7. LONGITUDINAL QUALITY CONTROL

Use the existing QC database for the GE/Lunar Phantom (either the water bath or the Lucite). This phantom must be scanned every day a participant is scanned, or at least three times per week.

a. Scanner quality control

Scanner quality control procedures are used to monitor scanner performance throughout the course of the study. QC procedures consist of:

- The study site technologist uses the Lunar Aluminum Phantom to monitor the performance of a single scanner over time. A copy of the Master Database (MDB) will be made to diskette/CD-R and will be collected from the technologist by the Project Director and sent to IRC monthly.
- Scanner cross-calibration procedures (coordinated by IRC with the study site) will be used to monitor scanner variation from machine to machine.
- The *QC Spine Phantom Form* and *Maintenance Record Form* will be completed by the study site technologist monthly. This will be collected from the technologist by the Project Director and mailed to IRC monthly.

b. QC Spine Phantom Form and Maintenance Record Form

The *QC Spine Phantom Form* is filled out monthly by the technologist and submitted to the Project Director. It provides a record of all the Aluminum Phantom scan acquisitions. The *Maintenance Record Form* tracks scanner maintenance and repairs, which are then reviewed by the IRC. Two very important pieces of identifying information are the Prodigy scanner serial number and the GE/Lunar Spine Phantom serial number. Blank *QC Spine Phantom* and *Maintenance Record Forms* are supplied in **Appendix B**. Make copies of this form for future use.

IMPORTANT: Changes in scanners, software, or location of scanners can have a large impact on the integrity of study data. For this reason, such changes are NOT ALLOWED for the duration of this study without prior approval from IRC and notification of WDMAC. Requests for changes may be made by completing and submitting the *Request for Software/Hardware Form (Appendix B)* to IRC. Approval or denial will be issued using IRC-specific forms.

c. Operator qualification

It is the responsibility of the study site to ensure that all technologists are qualified (trained, licensed, certified) to scan study participants. Please complete the GE/Lunar Prodigy *DXA Operator's Log (Appendix B)* and submit five scans each of AP spine, left hip and whole body acquired and analyzed by each prospective technologist to IRC. In addition, a copy of the log (with IRC-assigned certification numbers) should be sent to WDMAC for tracking and archiving.

d. Copying MDB (Master Database Files) to transfer media

QC MDB file may be copied to the same media as the subject scans. Do not copy the **subject** MDB. At the Main Menu, click on the WIHS database containing the QC scans. Note in the "Active Database" field, the "Working Path"; i.e., C:\data2\ (see Figure 12, Database Directory). This will appear as a folder when you open the "C" drive.

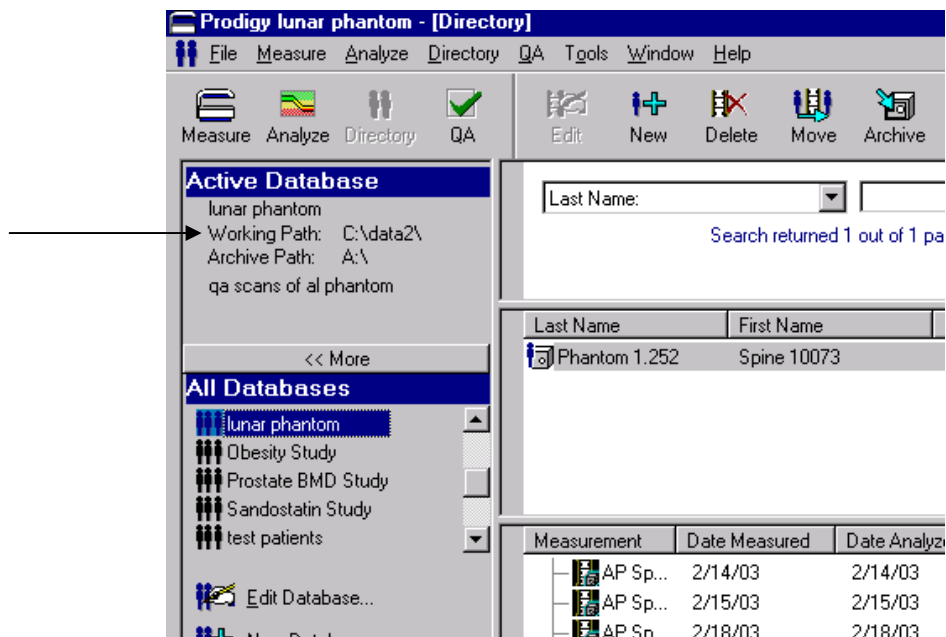


Figure 12. Database Directory.

- Exit from Prodigy to Windows.
- Place diskette/CD-R in the “A” drive.
- Go to “My Computer” – double click to open.
- Select C: Drive – double click to open – several folders will appear (see Figure 13, Folders in the C: Drive).
- Highlight the appropriate data set folder, i.e., data2 – double click to open.
- The lunar.mdb document will appear (see Figure 14, Lunar MDB Document).
- Right click on document (do not open document). A menu will appear.
- Scroll down to “Send.” Choose “3 ½ inch Floppy (A)” – files will be sent to the diskette in the “A” drive.
- When the send procedure is complete, remove diskette from “A” drive.
- End procedure by going to “File” menu and selecting “Close.”

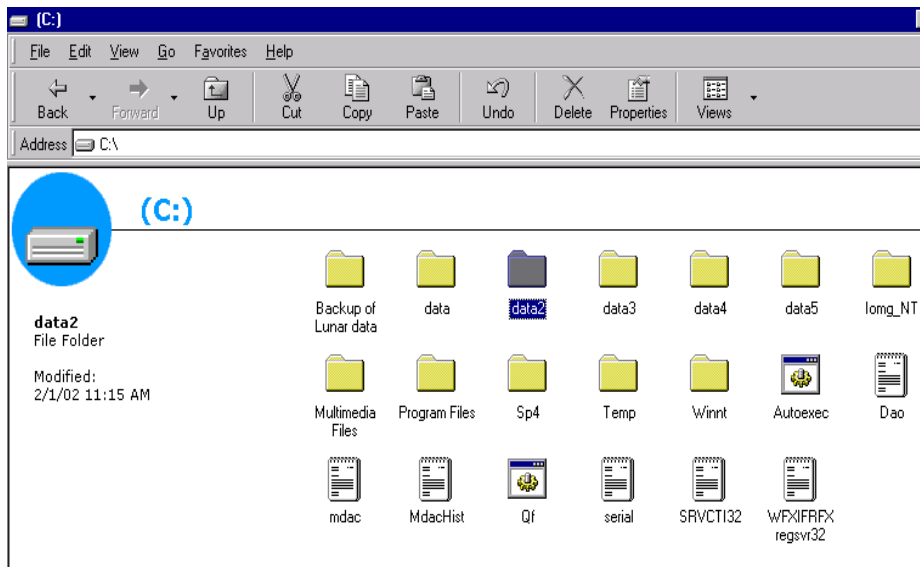


Figure 13. Folders in the C:\ Drive.

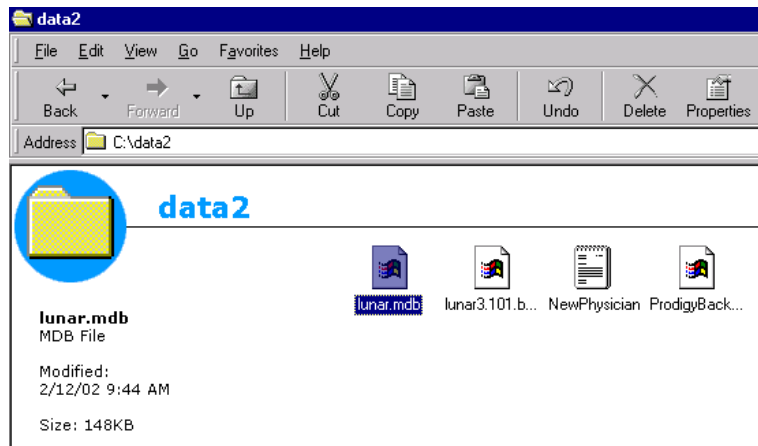


Figure 14. Lunar MDB Document.

e. Archive and data shipments

After the MDB has been copied, please label (see Figure 15) the disk. This will be collected from the technologist by the Project Director and mailed to IRC monthly along with the *QC Spine Phantom* and *Maintenance Record Forms*.

Archive the scans to the system archive disk only after they have been copied for shipping.

WIHS Data Transfer Label	
Number of Participants:	_____
QC Data	_____
Date:	___/___/___
	D D M M M Y Y
Diskette #	_____ of _____

Figure 15. QC Data Transfer Label.

Table 2.0 Frequency of data transfers from the site to IRC

	Baseline	2-Year Follow-up Visit
Participant Data	Weekly (batched)	Weekly (batched)
Quality Control Data	Monthly copy of <i>QC Spine Phantom</i> and <i>Maintenance Record Forms</i> , and copy of the Master Spine Phantom MDB	

H. QUANTITATIVE COMPUTED TOMOGRAPHY (QCT) OF THE SPINE AND HIP, AND ABDOMINAL AND THIGH SECTION AQUISITION

NOTE: A brief overview of technical aspects of the QCT procedure is also available in **Appendix C** for the QCT technicians.

1. BACKGROUND

Quantitative Computed Tomography, or QCT Densitometry, is a method used to measure bone mass. It is one of the three methods cited by the National Osteoporosis Foundation as useful and safe in the evaluation of osteoporosis. A QCT bone mass measurement is used to assess osteoporosis in the same way a cholesterol measurement is used for coronary heart disease or blood pressure for stroke. The data are used to measure an important risk factor and determine the necessity, choice and efficacy of therapy.

The principle underlying QCT Densitometry and other bone mass measurements (SPA, DPA, DXA/QDR) is that calcified tissue will absorb more x-rays than surrounding tissue so that the CT density measurement can be used to measure total bone mass within a sample of tissue. QCT and DXA both measure spinal bone mass, a significant advantage over methods, e.g., ultrasound, which measures bone in the peripheral skeleton where bone density is slow to change in response to disease therapy. Spinal bone is a mixture of high-turnover trabecular (spongy) bone and slowly-changing cortical (compact) bone. DXA/QDR measures the sum of these two compartments but also includes aortic calcification and osteophytes in the calculation of bone mineral in the spine. Only QCT isolates the metabolically-active trabecular bone for analysis. Lateral DXA, a newer approach, has recently been shown to have a sensitivity intermediate between the high sensitivity of QCT and the somewhat lower one of conventional DXA for detection of osteoporosis, but it uses 4 to 10 times the radiation exposure, is less precise, and the study time is increased compared to conventional DXA/QDR. New ultrasound methods for the knee or the heel are being used for osteoporosis screening, but unlike QCT or DXA they do not measure bone mass, and cannot be used to measure the spine where most osteoporotic fractures occur.

This study is being conducted at three WIHS clinical sites (Bronx NY, San Francisco, and Chicago). Each participant will have a QCT scan of the spine and hip at baseline and 2 years. Each scan takes approximately 30 minutes to complete. QCT scan data must be transmitted to the designated central reader for this study, which is the Image Reading Center.

Image Reading Center, Contact Information:

Shipping: IMAGE READING CENTER
 419 Lafayette Street
 New York, NY 10003

Phone/Fax: 877-472-5227 (877-IRC-LABS)
 Email: WIHS@imagereadingcenter.com

2. CT TECHNOLOGIST QUALIFICATION

- All technologists acquiring scans for the study must read and understand this manual thoroughly prior to acquiring scans for study participants. If any questions arise, please call 877-472-5227 (877-IRC-LABS).
- **The technologist(s) must submit a *Phantom Log Form* to the IRC for review before the start of the study and monthly during the course of the study.**
- Any **new** technologist assigned to the study is required to submit the *Site Technologist Change Notification Form* before being involved in the study.

3. RESPONSIBILITIES

a. QCT Site Responsibilities

- Monitor CT scanner performance.
- Ensure that QCT scans are acquired for each participant following appropriate procedures
- Transmit data to IRC within the specified timeframe and archive scans and documents on site.

b. IRC Responsibilities

- Conduct a screening performance of CT scanner and review any upgrades thereafter.
- Central analysis of all scans according to IRC standard operating procedures.
- Generate, maintain and deliver data to the Sponsor/Principal Investigator.

4. INSTRUMENT QUALITY CONTROL

a. Responsibility

The site has the full responsibility to maintain the quality of the scanner to monitor and control all influences that affect its performance. A Mindways QA/QC phantom will be used for your routine QA/QC and will be scanned before any study participants are scanned. It will be scanned in the same mode as the scan type to be used within this study. IRC will review the phantom scan data before the study.

b. Reporting QC data to IRC

- **Prior to scanning study participants:** Submit a phantom scan as described above. IRC will review the materials to determine if the scanner is working properly before scanning the study participants.
- **Yearly:** WIHS will circulate a single Mindways QA/QC phantom for scanning at all sites for cross-calibrating each scanner. Please scan the phantom accordingly and submit the scans to the IRC.

c. Documenting Upgrades

- Before upgrading software or hardware on the scanner or its system, notify IRC in writing with a copy of the maintenance notes to be sure the upgrade will not affect acquisition and analysis of the scans.
- IRC will review the notes and let the site know if the upgrade is permissible with regards to the study.

- After permission to upgrade is given by IRC, record software upgrades onto the *Maintenance Record Form*. Be sure to include the old and new software version numbers.

d. Hardware Upgrades

- Notify IRC immediately of any proposed hardware upgrades and contact information of the maintenance team. IRC will contact the maintenance team to be sure the upgrade will not affect acquisition QCT data.
- Record all preventative maintenance on the *Maintenance Record Form*.

e. Re-establishing the Phantom Screening Measurement

The site must scan the phantom before and after the following:

- The CT scanner requires a hardware or software upgrade.
- Any manufacturer service.
- The scanner is participant to a dramatic environmental fluctuation (e.g., natural disasters, power failures, temperature/humidity changes, etc.).
- Report the scans as “New Phantom Baseline” on the top of the CD used to send the QA phantom data.

5. SCANNING SCHEDULE

Where possible, all QCT scans for an individual participant should be performed with the same scanner. QCT scanning of the Spine and Hips will be performed at baseline and 2 years. All QCT scans will be sent to the IRC for review and analysis. The same slice thickness at which scans are acquired at screening must be used for all scans at later time-points.

6. SPINE QCT SCAN ACQUISITION

a. Setup and Scanning

- Place the cutout pad and extenders on the CT table.
- Place phantom in the cutout pad with the “head” end of the phantom toward the same end of the table as the participant’s head will be positioned.



- Bolus bags are intended to fill the void between the participant's spine and the CT calibration phantom to reduce the possibility of image degradation in the CT images in the region of the CT calibration phantom.
- Place 1 or 2 bolus bags along the center of the CT calibration phantom.

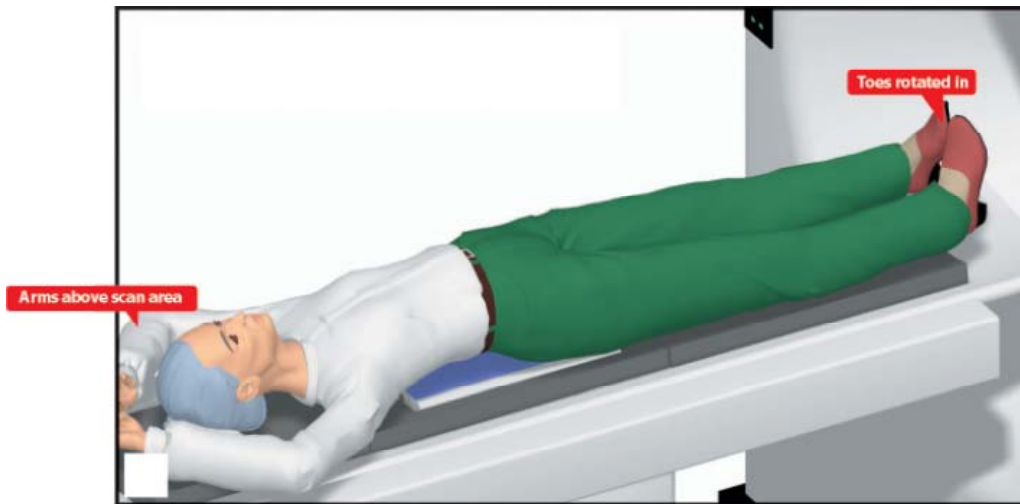
b. Participant Preparation

- Remove ALL metal objects that will be in the scan field.
- DO NOT scan a participant that:
 1. Has had intravenous contrast administered within the past week.
 2. Has high density barium contrast in the bowel.
 3. Is pregnant or may be pregnant.
- If a participant has Harrington Rods or other metal in the spine, scanning through those objects will cause artifacts that will affect the accuracy of the BMD results.
- Participants weighing more than 160 kg (352 lbs) will be excluded from the CT exam secondary to technical difficulties related to imaging individuals of this size and greater.

c. Participant Positioning

- Sit the participant at the foot of the phantom.
- Place a bolus bag on the phantom.

- Carefully recline the participant onto the phantom without displacing the bolus bag.
- The top of the phantom should be at the axilla.
- Legs should be straight with the heels out and the toes pointed inward.
- Have participant place arms over head.
- Set the Table Height to the proper height for your site.



d. Participant Information

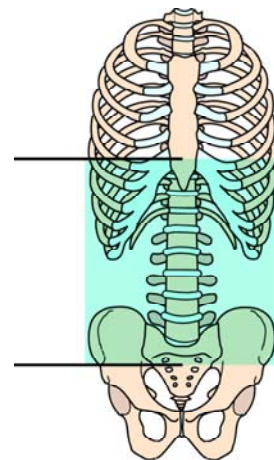
Include the following information for study purposes:

- The participant WIHSID
- Date of birth
- Gender
- Additional identifiers may also be utilized for site-specific tracking requirements (e.g., name or hospital ID number)

NOTE: Full name or other personal identification details should NOT be entered into the scan.

e. Scan Localizer

- Start the lateral localizer scan 2-3 cm above the xyphoid.
- Use a scan length of 30 cm or more to make sure the L5/S1 joint can be visualized.
- Allow the participant to breathe normally during the scan; do not use full inspiration.
- Localizer should start above the xyphoid and cover the region down to and including the L5/S1 joint.



f. Prescribing and Taking the Axial Scans

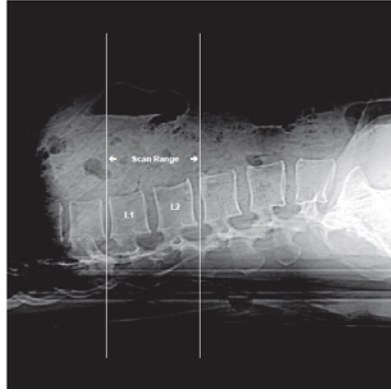
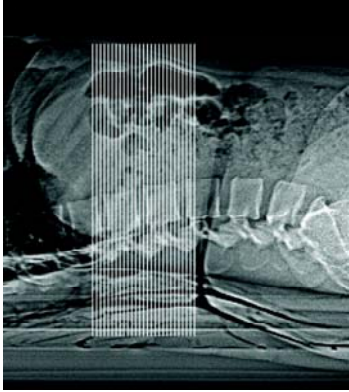
If possible, scan two vertebral bodies in range T11-L4. **L1 and L2 are preferred.** If desired, up to three vertebrae may be analyzed simultaneously. Scan intervening vertebrae if vertebrae to be analyzed are not contiguous.

- If the participant had a previous spine QCT on the same scanner, or is returning for their second QCT on study, scan the same vertebrae as before (baseline visit) unless one or more previously scanned vertebrae is damaged. One or more other intact vertebrae can be substituted.
- Count up from L5/S1 to standardize vertebra selection.
- On lateral localizer, set the superior (start) position for the scan series to include all of the disc space above the top vertebra to be analyzed.
- On lateral localizer, set the inferior (end) position for the scan series to include all of the disc space below the bottom vertebra to be analyzed.
- Instruct the participant to breath normally during scanning.

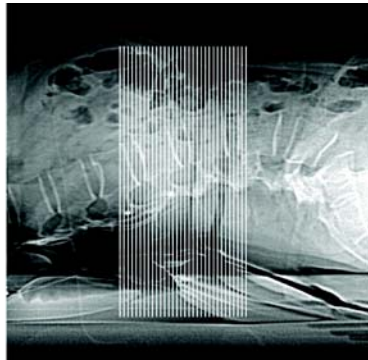
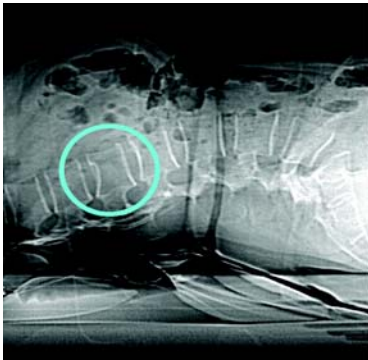
CT Technical Parameters for the Spine QCT Scans

CT System	Scan Field	Reconstruction Field of View	Reconstruction Center	Tube Potential	Tube Current	Slice Thickness	Pitch	Reconstruction
GE Light Speed VCT 64 (NY & SF)	Large Body	380 mm	Spine Center: X=0, Y=0	120 kVp	150 mA	2.5 mm	1	Standard Abdomen
Siemens Definition Dual Source 64 (CH)	Large Body	380 mm	Spine Center: X=0, Y=0	120 kVp	150 mA	2.5 mm	1	B40 s

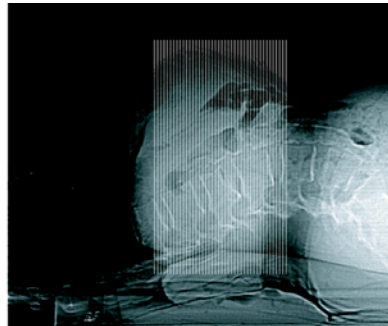
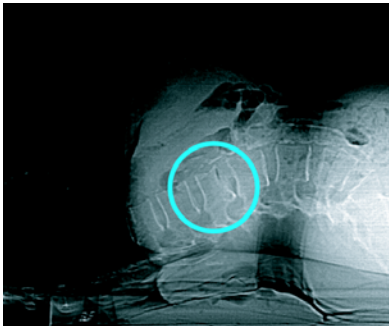
Localizer prescription examples:



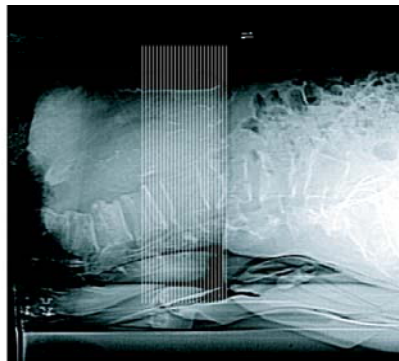
Ideal Case: Scan L1-L2



L1 Fracture: Scan L2-L3

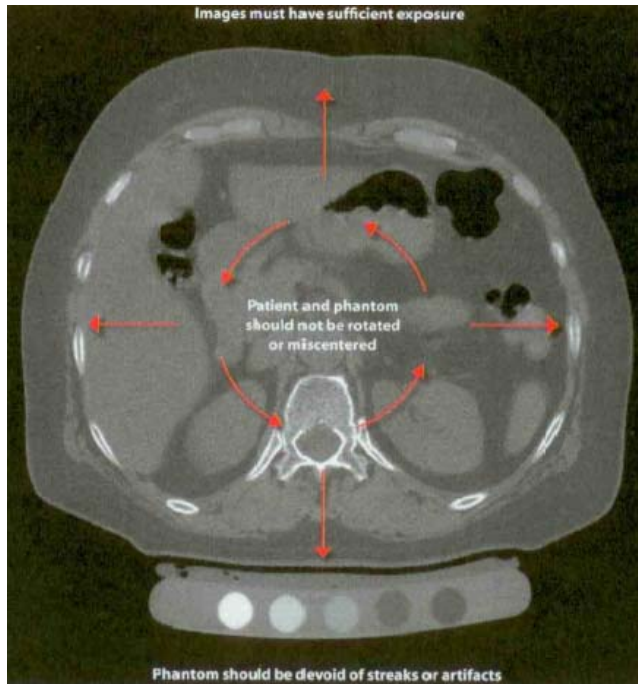


L2 fracture: Scan L1-L3.
Do NOT analyze L2.



Multiple Compression Fractures: Only L2 is uncompressed, and should be scanned.

Axial Scan Example



7. ABDOMINAL CT SCAN ACQUISITION

a. Setup and Scanning

- Participant should remain supine prior to each scan.

b. Participant Preparation

- Remove ALL metal objects that will be in the scan field.

c. Participant Positioning

- Body should be straight on the table pad using the centerlines at the head and foot ends of the table pad as the gauge with the feet pointing up.
- Body and should be positioned entirely within the scanning area.
- Arms may rest comfortably on upper chest.

d. Participant Information

Include the following information for study purposes:

- The participant WIHSID
- Date of birth
- Gender
- Additional identifiers may also be utilized for site-specific tracking requirements (e.g., name or hospital ID number)

e. Scan Localizer

- Coronal scout images should initially be obtained to determine the orientation of the skeletal landmarks, especially the **L4-L5 Intervertebral Space**.

- Metallic implants cause artifacts during the acquisition of CT images and interfere with the analysis.

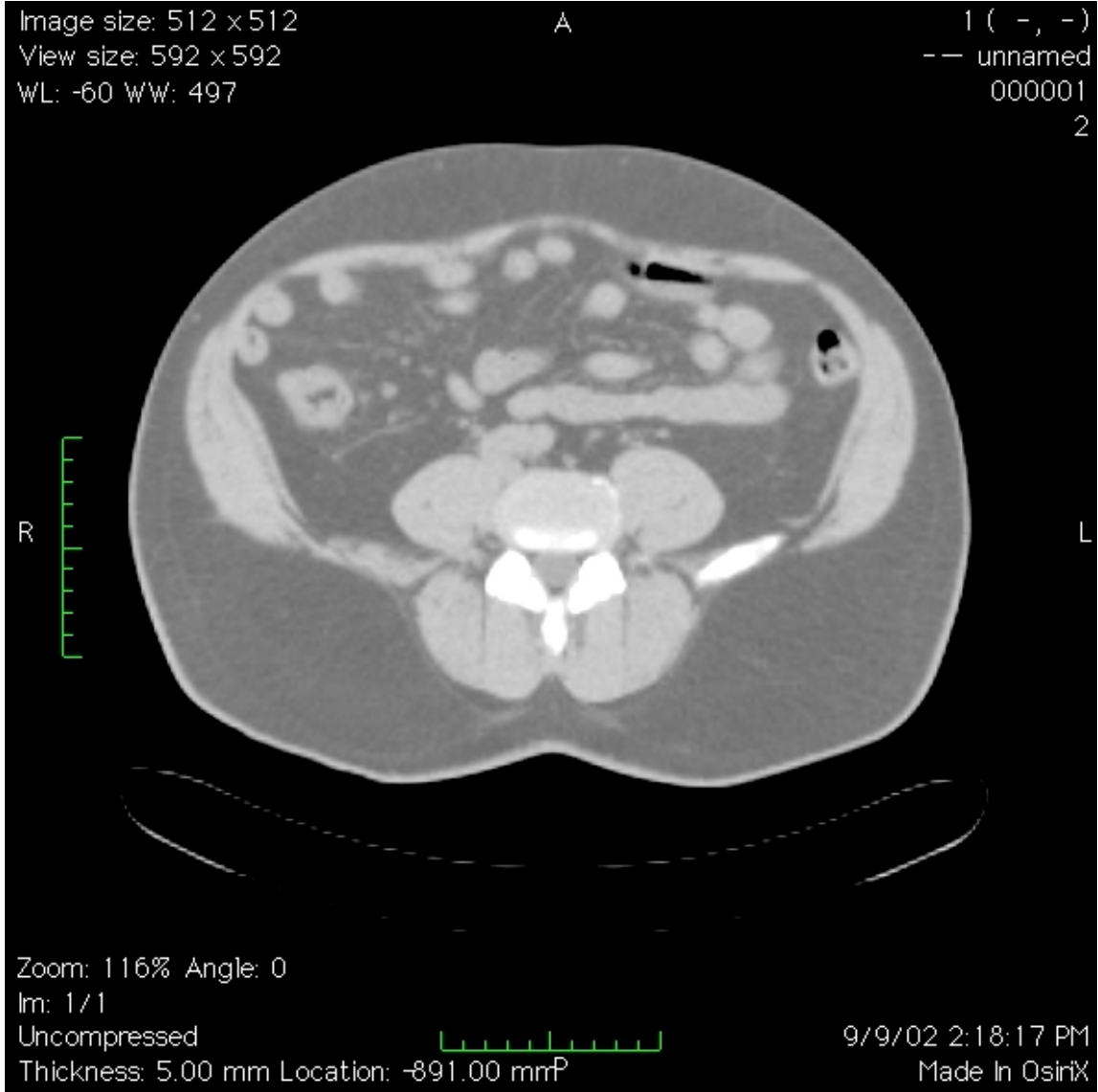
f. Prescribing and Taking the Axial Scans

- The axial slice positions and the ruler markings should be saved and embedded in the scout scan. Scout images should be submitted to the IRC along with all axial images.
- The **slice thickness should be 10 mm** with a matrix size of 512 X 512.
- The entire cross-section of the abdomen must be within the Field of View, from skin to skin. If any tissue falls outside of the Field of View please rescan with a larger Field of View.

CT Technical Parameters for the Abdominal CT Scans

CT System	Scan Field	Reconstruction Field of View	Reconstruction Center	Tube Potential	Tube Current	Slice Thickness	Pitch	Reconstruction
GE Light Speed VCT 64 (NY & SF)	Large Body	500 mm	X=0, Y=0	120 kVp	150 mA	10 mm	1	Standard Abdomen
Siemens Definition Dual Source 64 (CH)	Large Body	500 mm	X=0, Y=0	120 kVp	150 mA	10 mm	1	B40 s

Axial Scan Example



8. HIP QCT SCAN ACQUISITION

a. Setup and Scanning

- Place the cutout pad on the CT table.
- Place phantom in the cutout pad with the “head” end of the phantom toward the same end of the table as the participant’s head will be positioned.



- **Bolus Bags** are intended to fill air gaps between CT calibration phantom and the participant’s lower back and thighs to reduce the possibility of image degradation in the CT images in the region of the CT calibration phantom. They may be positioned, as needed, before or after participant positioning on the phantom. Bolus material should not extend out significantly beyond the lateral extent of the CT calibration phantom. Bolus bag placement should not result in elevating the participant’s thighs such that positioning them with legs flat, toes pointed in, as discussed later, would not be possible.

b. Participant Preparation

- Remove ALL metal objects that will be in the scan field.
- Exclusion criteria are detailed in Spine QCT section.

c. Participant Positioning

- The participant should be positioned so that the phantom is under the participant and placed from 1-2 cm below the lesser trochanter to the mid-back.
 - The lower edge of the phantom should be aligned approximately with the V in the intersection of the participant’s legs and pelvis.
 - The bolus bags should fill any air gaps between the participant and the CT calibration phantom.
- Position the participant’s knees flat and toes pointed inward.



- If participant cannot put her knees flat, make her comfortable but try to keep her knees at an angle less than 30 degrees above the table.
- The participant's hands and arms should be placed over the participant's head or as high on the chest as is comfortable to avoid interfering with the scan area.
- Set Table Height to the same height as established during the QA process.

d. Participant Information

Include the following information for study purposes:

- The participant WIHSID
- Date of birth
- Gender
- Additional identifiers may also be utilized for site-specific tracking requirements (e.g., name or hospital ID number)

e. Scan Localizer

- Obtain an AP localizer from the iliac crest to 2-3 centimeters below the base of the lesser trochanter.
- Use a scan length of 25 cm or more.



f. Prescribing and Taking the Axial Scans

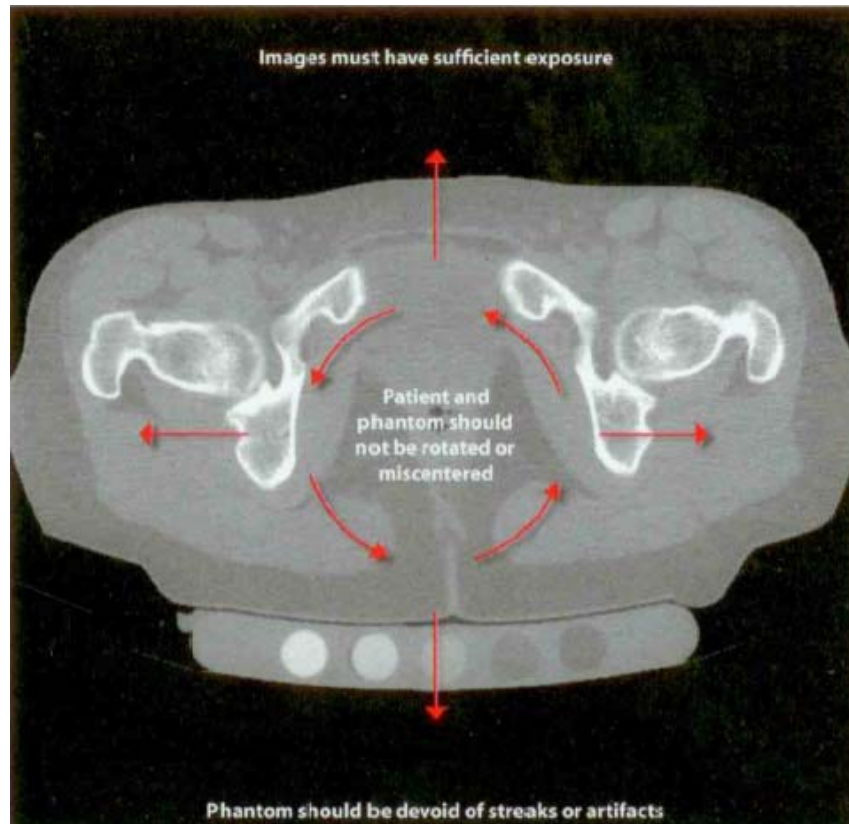
On the localizer:

- Set the superior (start) scan position near the top of the femoral head.
- Set the inferior (end) scan position such that at least one axial image is acquired below the lesser trochanter.
 - The most inferior image must not include any pelvic bone.
 - It may be helpful to set the start scan position such that only one or two axial images are acquired above the greater trochanter if the participant has a hip implant.
- Instruct the participant to breath normally during scanning

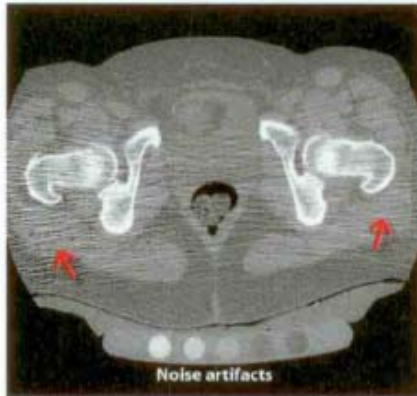
CT Technical Parameters for the Hip QCT Scans

CT System	Scan Field	Reconstruction Field of View	Reconstruction Center	Tube Potential	Tube Current	Slice Thickness	Pitch	Reconstruction
GE Light Speed VCT 64 (NY & SF)	Large Body	380 mm	X=0, Y=center both hips in FOV	120 kVp	150 mA	2.5 mm	1	Standard Abdomen
Siemens Definition Dual Source 64 (CH)	Large Body	380 mm	X=0, Y=center both hips in FOV	120 kVp	150 mA	2.5 mm	1	B40 s

Axial Scan Example



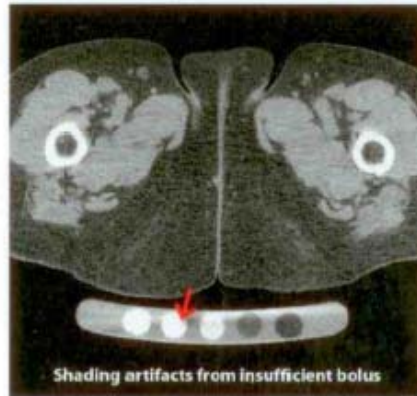
Examples of scans that cause analysis failures



Noise artifacts

Insufficient Exposure

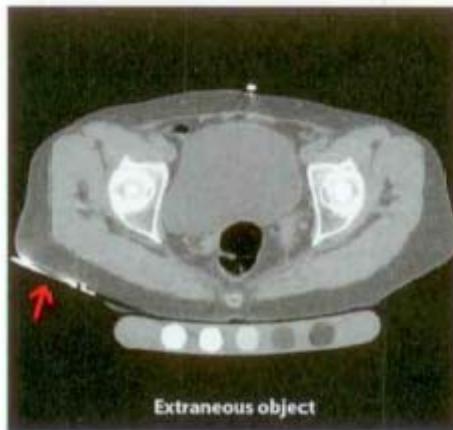
Insufficient exposure resulted in high noise, bad phantom data, and hip isolation difficulties. Solution: Use exposure tables to determine patient-specific exposure.



Shading artifacts from insufficient bolus

Insufficient bolus

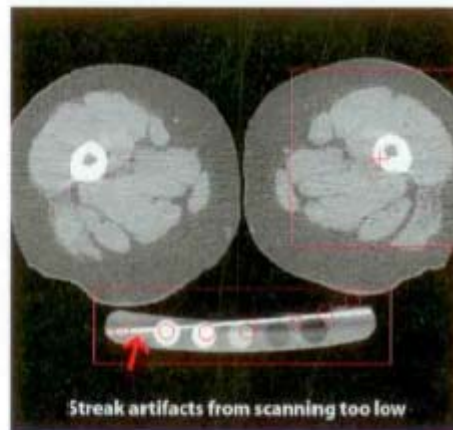
Insufficient bolus, particularly in the area under the upper thigh can produce artifacts preventing successful analysis. Be mindful of shading or streak artifacts in the phantom.



Extraneous object

Extraneous high density object in scan field

A high density object in the patient's hip pocket was interpreted as "bone" by the CTXA analysis software. Depending on circumstances this false bone may prevent analysis or bias the bone density estimates.



Streak artifacts from scanning too low

Scanning too low

Scanning below the bolus can produce artifacts preventing successful analysis. Solution: Position bolus properly under thighs and do not scan more than 2 cm below the base of the lesser trochanter.

9. THIGH CT SCAN ACQUISITION

g. Setup and Scanning

- Participant should rest supine for 30 minutes prior to each scan in order to minimize muscle size changes and fluid shift changes.

h. Participant Preparation

- Remove ALL metal objects that will be in the scan field.
- Exclusion criteria are detailed in Thigh CT section.

i. Participant Positioning

- Body should be straight on the table pad using the centerlines at the head and foot ends of the table pad as the gauge with the feet pointing up.
- Consistent positioning of the legs for all time points during the study is very important.
- Body and the thighs should be positioned entirely within the scanning area.
- The legs should be placed parallel and straight. Feet should be together and not angled. Use a Velcro strap or tape to hold the feet together.

j. Participant Information

Include the following information for study purposes:

- The participant WIHSID
- Date of birth
- Gender
- Additional identifiers may also be utilized for site-specific tracking requirements (e.g., name or hospital ID number)

k. Scan Localizer

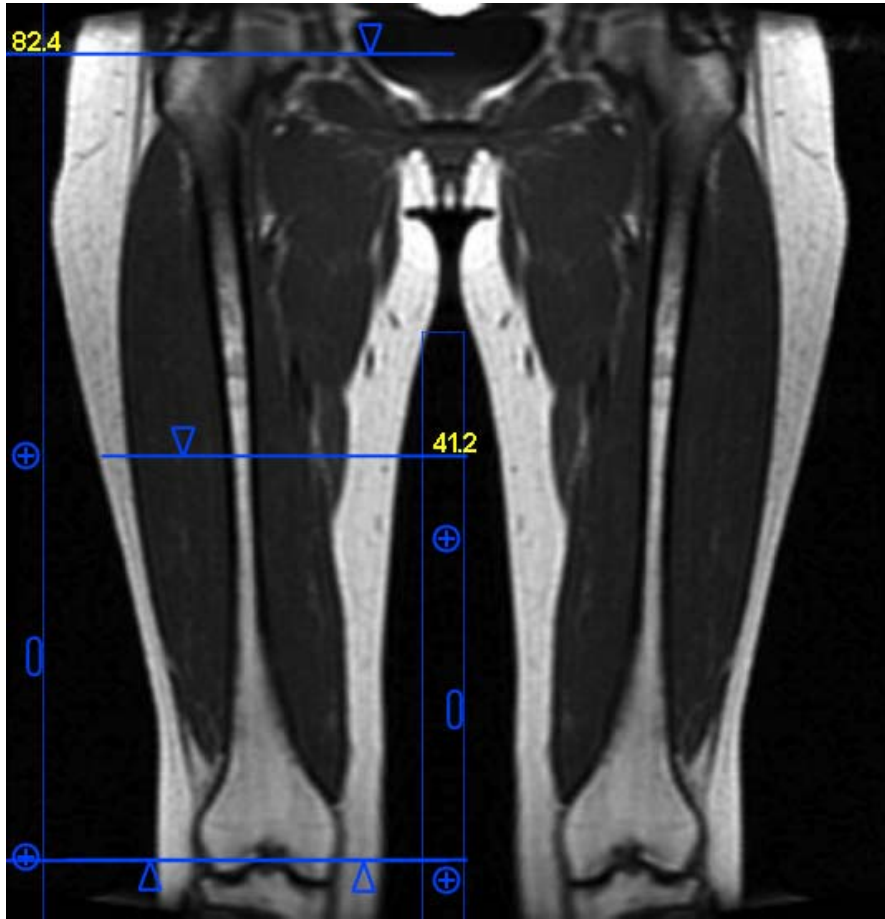
- Coronal scout images should initially be obtained to determine the orientation of the skeletal landmarks, especially the pelvic bone and the distal end of the femur. The scout will be used to measure the distance between medial edge of the greater trochanter and the intercondyloid fossa of the right leg, and to locate the position of the mid-thigh slice.
- Metallic implants cause artifacts during the acquisition of CT images and interfere with the analysis performed at the Image Reading Center. Knee implants at the distal end of the femur should not interfere with the axial slices.

i. For Subjects **WITHOUT** Artificial Hip Replacements:

The mid-thigh is defined as the total distance from the medial edge of the greater trochanter and the intercondyloid fossa of the right leg, divided by 2. The single axial slice should be obtained at this mid-thigh location.

ii. For Subjects **WITH** Artificial Hip Replacements:

If implants are present, the axial slice should be obtained 10 mm below the tip of the longest implant.



I. Prescribing and Taking the Axial Scans

- The axial slice positions and the ruler markings should be saved and embedded in the scout scan. Scout images should be submitted to the IRC along with all axial images.
- The **slice thickness should be 10 mm** with a matrix size of 512 X 512.
- The entire cross-section of the thigh must be within the Field of View, from skin to skin. If any tissues falls outside of the Field of View please rescan with a larger Field of View.

CT Technical Parameters for the Thigh CT Scans

CT System	Scan Field	Reconstruction Field of View	Reconstruction Center	Tube Potential	Tube Current	Slice Thickness	Pitch	Reconstruction
GE Light Speed VCT 64 (NY & SF)	Large Body	380 mm	X=0, Y=center both thighs in FOV	120 kVp	150 mA	10 mm	1	Standard Abdomen
Siemens Definition Dual Source 64 (CH)	Large Body	380 mm	X=0, Y=center both thighs in FOV	120 kVp	150 mA	10 mm	1	B40 s

Axial Scan Example



10. SENDING PARTICIPANT QCT SCANS TO IRC

- The participant scan should be sent uncompressed on a CD-R along with the signed and completely filled *QCT Data Transmittal Form*.
- The preferred format for transmitting the data is DICOM3.
- The CDs should be packaged in a foamy material to avoid damage.
- CD-R should be labeled with a permanent marker or a label with **all** of the following information:
 - **Study Number:** WIHS MSK
 - **Site Number:** (001 Bronx, 005 San Francisco, 006 Chicago)
 - **Scan Date:** Date of scanning
 - **Participant ID:** Participant's eight-digit WIHSID
 - **Study Timepoint:** 0, 2 years

11. EARLY TERMINATION PARTICIPANTS

A participant might decide to drop out of the study after the baseline scan. If the participant has agreed to an Early Termination scan, the study coordinator will make an appointment or might bring in the participant for the scan with little notification. In the case of an Early Termination scan, **the imaging technologist or study coordinator must notify** the IRC immediately prior to scanning the participant. To do so, please call 877-572-5227 (877-IRC-LAB)

APPENDIX A: Quality Assurance/Certification Checklist

1. Standing balance

Side-by-side stand

- Script correctly and clearly delivered
- Correctly demonstrates position
- Timing started coincident with participant release and stopped when participant takes a step or holds on
- If task was not performed, codes/records reasons

Semi-tandem stand

- Script correctly and clearly delivered
- Correctly demonstrates position
- Timing started coincident with participant release and stopped when participant takes a step or holds on
- If task was not performed, codes/records reasons

Tandem stand

- Script correctly and clearly delivered
- Correctly demonstrates position
- Timing started coincident with participant release and stopped when participant takes a step or holds on
- If task was not performed, codes/records reasons
- Repeat (second trial), if necessary

Single-leg stand

- Script correctly and clearly delivered
- Correctly demonstrates position
- Timing started coincident with participant release and stopped when participant takes a step or holds on
- If task was not performed, codes/records reasons

2. Functional Reach

- Yardstick placed on wall at proper height and leveled
- Script correctly and clearly delivered
- Correctly demonstrated position and testing procedure
- Says "ready? Go" for each test
- Measures reach correctly (end position of MCP joints against the rule)
- Records and explains unusual values
- If task was not performed, codes and explains reasons

3. Chair stands

- Back of chair against a wall
- Script correctly and clearly delivered
- Correctly demonstrates single stand, emphasizing keeping arms tight across chest
- Correctly demonstrates two stands, emphasizing full stand and return to complete sit
- Says “ready? Go” for each test
- Counts each chair stand, takes a split time after participant stands upon the fifth stand and records final time after participant stands up on the tenth stand
- Records and explains unusual values
- If task was not performed, codes and explains reasons

4. Grip Strength

- Participants properly positioned
- Script correctly and clearly delivered
- Correctly demonstrates measurement technique
- Says “ready? Go” for each test
- Has participant maintain proper positioning during 3 attempts
- Records and explains unusual values
- If task was not performed, codes and explains reasons

5. 4-meter walk

- Script correctly and clearly delivered
- Toes touching start line
- Time stopped with first footfall over finish line
- Records and explains unusual values
- If task was not completed, codes and explains reasons

6. 400-meter walk

- Script correctly and clearly delivered
- Correctly demonstrates the test
- Toes touching start line
- Timing started coincident with participant’s first footfall
- Time stopped with first footfall over finish line
- Records and explains unusual values
- If task was not completed, codes and explains reasons

APPENDIX B: DXA FORMS

The following forms are available in Appendix B:

- GE/LUNAR PRODIGY DXA OPERATOR'S LOG
- WIHS FEDEX TRANSFER LOG
- DXA DATA TRANSMITTAL FORM (IRC-DXA-01)
- QC SPINE PHANTOM FORM (IRC-DXA-02)
- MAINTENANCE RECORD FORM (IRC-DXA-03)
- REQUEST FOR SOFTWARE/HARDWARE UPGRADE FORM (IRC-DXA-04)
- SITE TECHNOLOGIST CHANGE NOTIFICATION FORM (IRC-DXA-05)

You may also log on to www.imagereadingcenter.com/WIHS to download additional copies of the following forms:

- DXA DATA TRANSMITTAL FORM (IRC-DXA-01)
- QC SPINE PHANTOM FORM (IRC-DXA-02)
- MAINTENANCE RECORD FORM (IRC-DXA-03)
- REQUEST FOR SOFTWARE/HARDWARE UPGRADE FORM (IRC-DXA-04)
- SITE TECHNOLOGIST CHANGE NOTIFICATION FORM (IRC-DXA-05)

GE LUNAR PRODIGY DXA OPERATOR'S LOG

Dear DXA Technologist and Project Director,

IRC and the WIHS require that all technologists using the GE/Lunar Prodigy densitometer read and fully understand the GE/Lunar Prodigy DXA Operator's Guide for the WIHS.

Please print your name, sign, and date this letter to confirm completion of this requirement. In the box below (to be filled out by the DXA technologist), please leave the column under ID number blank. For IRC's documentation, please send a copy of this information, including the DXA scans of each anatomical site as described in Section A1. Please keep the original at your site to document any future changes in personnel. **A four-digit ID number will be assigned to each DXA technologist after review of their scans by IRC. A copy of this form with the number assigned will be faxed back to the site, along with comments regarding the certification scans.** Sites should then send a copy of the log (with IRC-assigned certification numbers) to WDMAC for tracking and archiving.

Site Principal Investigator: _____

Site Project Director: _____

Site ID#: ____ ____ ____ (Bronx 001; San Francisco 005; Chicago 006)

Site Personnel (please complete with names of any personnel who might be directly connected to the study):

Printed Name	Signature	MM/DD/YY	Initials

DXA Technologists (IRC will assign the ID number and return by fax):

Printed Name	Signature	MM/DD/YY	Initials	ID Number

WIHS MSK FEDEX TRANSFER LOG

Complete this form and place it on top of the contents in the FedEx package. Send an email to wihis@imagereadingcenter.com with the expected delivery date and tracking # for the package. In addition, a copy of this log should be sent to WDMAC for tracking and archiving.

FedEx to: Image Reading Center
 419 Lafayette Street
 New York NY 10003
 Phone/Fax: 877-472-5227 Email: WIHS@imagereadingcenter.com

1. Subject Data Enclosed in this FedEx:

PARTICIPANT ID	DATA ENCLOSED <i>Check appropriate boxes</i>
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log
_ - _ _ - _ _ _ _ - _	<input type="checkbox"/> DXA ___ Tot. # transfer media (CD's, diskettes, etc.) <input type="checkbox"/> Participant Data Log

Note: For each participant ID listed above, a Participant Data Log (form MSK01) must be included in the FedEx; all CD's, diskettes, SuperDiscs, etc., must be labeled!

2. Quality Control Data Enclosed in this FedEx:

DXA: (ALL OF THE FOLLOWING SHOULD BE INCLUDED):

- Copy of QC MDB
- Copy of QC Spine Phantom and Maintenance Record Forms

PROJECT DIRECTOR SIGNATURE: _____ **SITE#** _____

DATE: ___ ___ / ___ ___ / ___ ___
 M D Y

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
DXA Data Transmittal Form



Site Information

PI Name: _____ Imaging Site Name: _____ Site Number:

Subject Information

WIHSID:

Subject DOB: ____/____/____
(month) (day) (year)

Scan Information

Study Visit: Baseline (00) Visit Code (35, 36, 37, 38)

2-year follow-up (24) Visit Code (39, 40, 41, 42)

Scan Type: Whole Body Mode: _____

AP Spine Mode: _____

Total Hip Mode: _____ Left Right

Forearm Mode: _____ Left Right

VFA Mode: _____

Scan Date: ____-____-____
(month) (day) (year)

DXA Technical Information

Scanner Manufacturer: GE Lunar Hologic Model: _____ Software Version: _____

Comments:

DXA Technologist: _____ Phone: _____ Date: _____

For IRC Use Only: Received by: _____ Date: _____



QC Spine Phantom Form – Lunar & Hologic Machines

Use this form to record daily quality control spine phantom scans performed on your DXA scanner. Submit this form monthly to the Image Reading Center.

PI Name: _____ Site Number: _____

Study: The Women’s Interagency HIV Study (MSK Substudy)

QC Phantom Type: Hologic Phantom Lunar Aluminum Phantom
 (check one) Lunar Phantom – Lucite Bona Fide Phantom

Spine Phantom Serial # _____ BMD Mean _____

Acceptable Low BMD: _____ Acceptable High BMD: _____

QC Scan Filename	Scan Acquisition Date mm/dd/yy	Scan Mode	Total BMD g/cm ² (L1-L4)	Within Range? Yes or No

Completed by: _____ Date: _____



Maintenance Record Form

Use this form to record the maintenance, service, and software upgrades performed on your DXA scanner. Submit this form monthly to IRC **even if** no service was performed. If applicable, **include a copy** of the maintenance report for work or any upgrades performed on the DXA scanner.

PI Name: _____ Site Number: _____

Sponsor/Protocol: The Women's Interagency HIV Study (MSK Substudy) _____

Scanner ID # _____ Month/Year: _____ / _____

Hardware Changes Yes _____ (describe below) No _____

Date	Problems - Maintenance

Software Changes Yes _____ (describe below) No _____

Date	Old Version	New Version

Completed by: _____ Date: _____

IMAGE READING CENTER
Request for Software/Hardware Form



Process:

1. Complete this form PRIOR to implementing any software or hardware changes.
2. Fax form to IRC at 877-472-5227 (877-IRC-LABS) Attn: DXA Manager
3. Spine Phantom must be scanned 10 times before and 10 times after an upgrade.
4. Send electronic data to IRC immediately for review along with a copy of this form.

Study: The Women's Interagency HIV Study (MSK Substudy)

PI Name: _____ **Site Number:** _____

Current

System Manufacturer: _____ Model: _____

System S/N: _____ Software version: _____

New Upgrade

System Manufacturer: _____ Model: _____

System S/N: _____ Software version: _____

Date of Anticipated Upgrade: _____

Form completed by: _____ Date: _____

IRC Response: _____ Upgrade Approved _____ Upgrade Denied

Comments: _____

IRC Reviewer: _____ Date: _____

Site Technologist Change Notification Form



Study: The Women's Interagency HIV Study (MSK Substudy)

PI Name: _____ Site Number: _____

To ensure consistency throughout the study, it is important that one Primary DXA Technologist scans all the study participants. A Secondary (Back-up) DXA Technologist should be familiar with the protocol.

Use this form to identify all DXA Technologists and to report any changes with DXA staff. Please complete and fax this form to 877-472-5227 (877-IRC-LABS)
Attn: DXA Manager

New Primary DXA Technologist: _____

E-mail address: _____

Phone number: _____

How many years of DXA experience do you have? _____

What DXA scanner(s) do you have experience with? _____ Hologic _____ Lunar

Certification or formal training: _____

What types of scans do you regularly perform?

___ AP Spine ___ Femur ___ Forearm ___ Total Body BMD ___ Total Body Comp

Do you have any DXA Clinical Trial experience? _____ How many trials? _____

New Secondary DXA Technologist: _____

E-mail address: _____

Phone number: _____

How many years of DXA experience do you have? _____

What DXA scanner(s) do you have experience with? _____ Hologic _____ Lunar

Certification or formal training: _____

What types of scans do you regularly perform?

___ AP Spine ___ Femur ___ Forearm ___ Total Body BMD ___ Total Body Comp

Do you have any DXA Clinical Trial experience? _____ How many trials? _____

Completed by: _____ Date: _____

APPENDIX C: Form for QCT technicians

WIHS MSK Study QCT Form

Site PI:

Study coordinator:

Study coordinator contact number:

Patient Name (optional): _____

Patient ID: _____

Date of Visit: _____

Patient should be dressed in hospital gown or pajamas with no metal in field of view. Clinic should complete this QCT form and provide phantom and study-specific MOD.

1) QC phantom

QC phantom imaged each day a patient is imaged

- KVp:80
- MAs: 280
- Slice thickness 2.5mm
- FOV 48 cm
- Standard recon kernel
- Table height 168.5
- 3 images in identical location in middle of torso

2) Patient Positioning

- Hands behind back of head or on forehead
- Please make sure that the patient's pelvis is aligned with the knee
- Invert feet (toes touching)

3) Spine scanning

- Do not scan spine of patient if all vertebrae between T12-L3 are fractured
- If L1 is fractured → scan L2 and T12
- If L2 is fractured → scan L1 and T12
- If both L2 and L1 are fractured → scan L3 and T12

Instructions for spine scan:

- Patient supine, inferior aspect of phantom aligned with anterior superior iliac crest
- Gelbag under lumbar spine of patient
- Lateral scoutview extending from xyphoid process to 40 cm inferior to determine L1,L2 levels
- L1,L2 Helical series:
- Range = 3-5 mm superior to L1 superior endplate to 3-5 mm inferior to L2 inferior endplate
- Tube Voltage = **120 kVp**
- Tube Current = **150 mAs**
- Slice thickness = **2.5 mm**
- Pitch = **1**
- FOV = **38 cm**
- Reconstruction = **Standard recon kernel**
- Table height = **standard**

4) Abdomen scanning

- Coronal scout to determine the **L4-L5 Intervertebral Space**.
- The **entire cross-section of the abdomen must be within the FOV**, from **skin to skin**.
- Tube Voltage = **120 kVp**
- Tube Current = **150 mAs**
- Slice thickness = **10 mm**
- Pitch = **1**
- FOV = **50 cm**
- Matrix size = **512 X 512**
- Reconstruction: **Standard recon kernel**
- Table height = **standard**

5) Hip Scanning

- Do not scan hip of patient if patient has hip replacement or hardware in hip

Instructions for Hip scan:

Phantom between hips of patient. Superior aspect of phantom aligns with iliac crest
AP scout view to determine proximal femur location

- Hip Helical series:
- Range: 5mm superior to acetabulum to 5 mm inferior to lesser troch
- Tube Voltage = **120 kVp**
- Tube Current = **150 mAs**
- Slice thickness = **2.5 mm**
- Pitch = **1**
- FOV = **38 cm**
- Reconstruction = **Standard recon kernel**
- Table height = **standard**

6) Thigh Scanning

- Coronal scout view to determine the **entire femur length**, from pelvic bone to distal end of the femur.
- Measure the distance between **medial edge of the greater trochanter** and the **intercondyloid fossa** of the **right leg**, to locate position of the **mid-thigh slice**.
- The **entire cross-section of both thighs must be within FOV**, **skin to skin**.
- The **axial slice positions** and the **ruler markings** should be **saved and embedded in the scout scan**.
- Scout images should be submitted to the IRC along with all axial images.
- Tube Voltage = **120 kVp**
- Tube Current = **150 mAs**
- Slice thickness = **10 mm**
- Pitch = **1**
- FOV = **50 cm**
- Matrix size = **512 X 512**
- Reconstruction: **Standard recon kernel**
- Table height = **standard**

APPENDIX D: Additional QCT forms

Form #	FORM	FILLED BY	SENT TO	PURPOSE
IRC-QCT-01	QCT Data Transmittal Form	Imaging site	Image Reading Center	To record the details of a participant scan sent along with the scan.
IRC-QCT-02	Phantom Data Transmittal Form	Imaging site	Image Reading Center	To record the details of a Phantom scan sent along with the scan.
IRC-QCT-03	Maintenance Record Form	Imaging site	Image Reading Center	To record any maintenance or upgrade on the CT scanner.
IRC-QCT-04	Site Technologist Change Notification	Imaging site	Image Reading Center	To identify any new technologist involved in the study.
IRC-QCT-05	QCT Study startup Questionnaire	Imaging site	Image Reading Center	To provide technical details about the scanner and the site before the start of study.

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
QCT Data Transmittal Form



Site Information

PI Name: _____ Site Number: _____

Subject Information

Subject ID: _____ Subject DOB: ____/____/____
(month) (day) (year)

Study Timepoints

- Screening 2 Years
 Repeat Timepoint _____ Early Termination

Scan Date: ____/____/____
(month) (day) (year)

Comments: _____

Technologist Initials: _____ Phone: _____ Date: _____

FOR IRC USE:
Date Received: _____ CD#: _____

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
Phantom Data Transmittal Form



Site Information

PI Name: _____ Site Number: _____

Phantom Information

Phantom Name/ ID: _____ Date of Scan: _____

Study Timepoint

Screening Other

Specify if other: _____

Comments: _____

Technologist

Technologist Initials: _____ Phone: _____ Date: _____

FOR IRC USE:

Date Received: _____ CD # _____

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
Maintenance Record Form



Use this form to record the maintenance service, and software upgrades performed on your CT scanner. If applicable, include a copy of the maintenance report for work or any upgrades performed on the CT scanner.

PI Name: _____ **Site Number:** _____

Scanner Serial Number: _____ **Month/ Year:** _____ / _____

Hardware Changes	
Date	Maintenance Description

Software Changes	
Date	Maintenance Description

Maintenance performed by: _____ Phone: _____

Completed by: _____ Date: _____

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
Site Technologist Change Notification Form



To ensure consistency throughout the study, it is important that one Primary CT Technologist scans all the study participants. A Secondary (Back-up) CT Technologist should be familiar with the protocol. Use this form to identify all CT Technologists and to report any changes with CT staff.

Please fax this form to the IRC @ 877-472-5227 (877-IRC-LABS)
Attn: QCT Manager

PI Name: _____ **Site Number:** _____

Technologist Information:

New Primary CT Technologist: _____

E-mail address: _____

Phone number: _____

Years of CT experience _____

Certification or formal training: _____

New Secondary CT Technologist: _____

E-mail address: _____

Phone number: _____

Years of CT experience: _____

Certification or formal training: _____

Completed by: _____ **Date:** _____

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
QCT Study Start-up questionnaire



PI Name: _____ Site Number: _____

Scanner Information

Manufacturer: _____ Model Name: _____

Software Version: _____ Scanner Movements: _____

Console Model No: _____ Console Serial No: _____

Scanner Capabilities

- Can multiple slices be obtained by the scanner? _____
- This protocol requires 7-10 mm thick slices. What slice thickness do you plan to use for subject scans? _____
- Can the scanner obtain mid-thigh slices? _____
- Field of View: _____ Matrix: _____

Contact Information

Name of Imaging/Radiology Facility: _____

Mailing Address: _____

City/Town: _____ State: _____ Zip: _____

Name and Title of Management Contact: _____

Email: _____ Phone: _____ Fax: _____

Name and Title of Technical Contact: _____

Email: _____ Phone: _____ Fax: _____

IMAGE READING CENTER
The Women's Interagency HIV Study
MSK Substudy
QCT Study Start-up questionnaire



Quality Control

Do you have an established routine QC protocol? _____

If Yes, Please explain: _____

Phantom Model, if any: _____ Manufacturer: _____

Frequency of the Quality Control: _____

Did you have scanner problems in the last 3 months? _____

If yes, Please explain: _____

Regular maintenance contract and service: _____

Technologist Information

Primary CT technologist: _____

Email: _____ Phone: _____

Experience as CT technologist: _____ years _____ months

Secondary CT technologist: _____

Email: _____ Phone: _____

Experience as CT technologist: _____ years _____ months

Data Transfer

The methods of data transfer, your site is capable of:

- FTP CD-ROM DICOM CD
 Magnetic Optical Disc Pioneer MOD

Completed by: _____ Signature: _____ Date: _____

APPENDIX E: Physical Activity Questionnaire (PAQ)

B3. Did you do vigorous racket sports in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B4)**

- a. How many months did you do this activity? |_|_| months
- b. How many of these months did you do this activity for at least three (3) hours per week? |_|_| months

B4. Did you bicycle faster than 10 miles/hour or exercise hard on an exercise bicycle in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B5)**

- a. How many months did you do this activity? |_|_| months
- b. How many of these months did you do this activity for at least two (2) hours per week? |_|_| months

B5. Did you swim in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B6)**

- a. How many months did you do this activity? |_|_| months
- b. How many of these months did you do this activity for at least two (2) hours per week? |_|_| months

B6. Did you do a vigorous exercise class or vigorous dancing in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B7)**

- a. How many months did you do this activity? |_|_| months
- b. How many of these months did you do this activity for at least three (3) hours per week? |_|_| months

B7. Did you do any vigorous job activities such as lifting, carrying or digging in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B8)**

- a. How many months did you do any of these activities? |_|_| months
- b. How many of these months did you do any of these activities for at least five (5) hours per week? |_|_| months

B8. Did you do any home or leisure activities such as snow shoveling, moving heavy objects, or weight lifting in the past 12 months for at least one hour total time in any month?

YES..... 1
 NO..... 2

(B9)

- a. How many months did you do any of these activities? |__|__| months
- b. How many of these months did you do any of these activities for at least three (3) hours per week? |__|__| months

B9. Did you do other strenuous sports such as basketball, football, skating, or skiing in the past 12 months for at least one hour total time in any month?

YES..... 1
 NO..... 2

(B10)

- a. How many months did you do any of these activities? |__|__| months
- b. How many of these months did you do any of these activities for at least three (3) hours per week? |__|__| months

B10. Now I'd like to ask you about more leisurely activities. Did you do non-strenuous sports such as softball, shooting baskets, volleyball, ping pong, or leisurely jogging, swimming or biking, which we haven't included above, in the past 12 months for at least one hour total time in any month?

YES..... 1
 NO..... 2

(B11)

- a. How many months did you do any of these activities? |__|__| months
- b. How many of these months did you do any of these activities for at least three (3) hours per week? |__|__| months

B11. Did you take walks or hikes or walk to work in the past 12 months for at least one hour total time in any month?

YES..... 1
 NO..... 2

(B12)

- a. How many months did you do any of these activities? |__|__| months
- b. How many of these months did you do any of these activities for at least four (4) hours per week? |__|__| months

B12. Did you bowl or play golf in the past 12 months for at least one hour total time in any month?

YES..... 1
 NO..... 2

(B13)

- a. How many months did you do either of these activities? |__|__| months
- b. How many of these months did you do either of these activities for at least three (3) hours per week? |__|__| months

B13. Did you do home exercise or calisthenics in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B14)**

- a. How many months did you do any of these activities? |__|__| months
- b. How many of these months did you do any of these activities for at least three (3) hours per week? |__|__| months

B14. Did you do home maintenance or gardening, including carpentry, painting, raking or mowing in the past 12 months for at least one hour total time in any month?

YES1
 NO2 **(B15)**

- a. How many months did you do any of these activities? |__|__| months
- b. How many of these months did you do any of these activities for at least five (5) hours per week? |__|__| months

B15. SHOW PARTICIPANT RESPONSE CARD PAQ #3.

During leisure time do you watch television?

Never1 **(B16)**
 Seldom.....2
 Sometimes3
 Often.....4
 Very often.....5

- a. On the average, about how many hours per day do you watch television? |__|__| hours

B16. The next questions concern household activities outside your occupation.

How many months in the past year did you do major household chores such as cleaning the garage, car or rugs, or scrubbing floors? |__|__| months **(If 0, skip to B17)**

- a. How many times per month did you do such major household chores? |__|__| times

B17. How many months in the past year did you do light household chores including light cleaning, making beds, shopping, doing laundry, preparing meals or washing dishes?

|__|__| months **(If 0, skip to B18)**

- a. How many hours per week in a typical month did you do such light household chores? |__|__| hours

B18. How many months in the past year did you take care of a child 10 years of age or less? Taking care of a child includes activities such as feeding, dressing, bathing, playing and carrying.

|__|__| months **(If 0, skip to B19)**

- a. How many days per week in a typical month did you do such childcare? |__|__| days
- b. How many hours per day in a typical day did you do such childcare? |__|__| hours

B19. TIME MODULE ENDED: |__|__| : |__|__| AM..... 1
 PM 2

Appendix F: WIHS Musculoskeletal (MSK) Substudy Information Sheet

Introduction: We are doing this research study to look at the effect of estrogen deficiency during menopause on bone strength and muscle and immune function. You are being asked to participate in this research study because you enrolled in the WIHS and are nearing or have transitioned into menopause.

Who can enter the study? About 330 women from the WIHS will be enrolled in this substudy. Your eligibility will be determined based upon data already collected for the WIHS study and questions about your menstrual bleeding. If you are eligible you will be given additional information about the substudy, and given a chance to ask questions. Then you can decide whether or not you would like to participate.

What will be done? You will continue to come to the WIHS semi-annual (6 month) visits and have two additional visits (now and in 2 years) for measurements of bone and muscle health. We will also utilize blood specimens already collected during WIHS study visits. You will undergo the following tests and procedures at each of the two additional visits:

1. **Dual energy xray absorptiometry, (DXA; 30 minutes)** – DXA is a routine, painless x-ray procedure during which you will lie on a table and the amount of bone mineral per area at the spine, wrist, and hip and amount of fat and muscle in your entire body will be measured.
2. **Quantitative Computed Tomography (QCT; 30 minutes)** of the spine and hip will be performed in an open machine, which gives very thin pictures of several vertebrae in your spine and bones in your hips. Participants at the Bronx site will also have an additional peripheral QCT (30 minutes), which gives thin pictures of the bones in the forearm and lower leg.
3. **Muscle strength and functional performance tests (30-45 minutes)** – This includes measuring your balance while standing and reaching, handgrip strength, measuring your ability to stand from a seated position on a chair, walking speed, and endurance walking a ¼ mile.
4. **Blood tests (15 minutes)** – 4 additional tubes of blood (3 tablespoons) will be drawn at your WIHS CORE visit in order to measure your bone and immune function. Participants at the Bronx site will have an additional 3 tubes of blood (2 tablespoons) for bone cell studies performed on a separate study visit day.
5. **Physical activity questionnaire (15 minutes)** will be administered to see what kind of activities you usually do.

What are the risks? Potential risks of the study are related to the blood draw, radiation exposure from the DXA and QCT, and performance of the muscle strength and function tests. You can ask the research staff to review these potential risks with you in greater detail.

APPENDIX G: Consent Form Template

WIHS Musculoskeletal (MSK) ROI Consent Template

Research Purpose

The purpose of this research study is to determine the effect of menopause on bone and muscle in HIV-infected and uninfected women.

Information on Research

Study Purpose

We are doing this research study to look at the effect of estrogen deficiency during menopause on bone mass and structure, muscle mass and strength and immune function. You are being asked to participate in this research study because you enrolled in the Women's Interagency HIV Study (WIHS) and are nearing or have transitioned into menopause.

In our previous studies, we found that the rate of bone loss was greater in HIV-infected than in -uninfected postmenopausal women. Some studies have shown that fractures (broken bones) occur more frequently in HIV-infected individuals, especially those who are older. Conditions associated with aging, such as osteoporosis and frailty, may occur earlier in HIV-infected individuals, which may lead to more falls and fractures. In this study, we will investigate whether loss of estrogen during menopause affects bone and muscle strength differently in HIV-infected and -uninfected women.

About 330 women are expected to be enrolled in this study. **Approximately, 130 women will participate at Columbia University Medical Center.** The study will last for 5 years and will include two additional visits for measurements of bone and muscle health. This study will also utilize blood specimens already collected during WIHS study visits. Your eligibility for this study will be determined based upon data already collected for the WIHS study.

If you are eligible to join the study, you will be invited to obtain additional procedures to better define your bone mass and structure, as well as your muscle mass, strength, function and endurance. The same procedures will be repeated at the end of the study. A description of each procedure is described in the sections that follow.

Study Procedures

You will undergo the following tests and procedures at the baseline and follow-up visit.

1. DXA Scan: your bone mass will be measured by dual energy xray absorptiometry (DXA). DXA is a routine, painless xray procedure during which you will lie on a table for 10 to 15 minutes and the amount of bone mineral per area at the spine, wrist, and hip will be measured. These values are then compared to the average for normal women aged 30 and to women of your age. During your DXA scan, you will have a vertebral fracture assessment to evaluate whether there may be a fracture in your spine. The DXA will also measure your total body composition, which gives us information about the ratio of fat to muscle in your body.
2. cQCT: A central QCT of the spine and hip will be performed. Central QCT involves very thin pictures of several vertebrae (bones) in your lumbar spine and bones in your hips.

3. Muscle strength and function measures: This includes measuring your handgrip strength, measuring your ability to stand from a seated position on a chair, measuring your balance, and measuring your walking speed.
4. Blood tests: 5 tubes of blood will be drawn in order to measure numbers cells that can potentially turn into bone cells. **This study will be performed only for participants from the Bronx site.**
5. pQCT: A peripheral quantitative computed tomography (pQCT) will be performed of the wrist and ankle to get a 3-dimensional picture of your bones, which gives more detail about your bone geometry and the quality of your bones than the DXA scan. The pQCT machine has not yet been approved by the FDA for commercial use. **This study will be performed only for participants from the Bronx site.**

Use of Data/Specimens for Future Research

We may want to use or share your data and/or blood sample(s) with other investigators at **Columbia University** and/or with investigators at another institution so that other research studies can be done now or in the future. If you agree to let us keep your samples for future research, they may be kept for an indefinite amount of time. Data and specimens sent to other institutions will be sent with all identifying information removed.

Some research using blood or tissue allows the researchers to make medical tests or treatments that may have commercial value. If this happens, there are no plans to pay you for any products or treatments that are made, or for using your samples.

Please initial below to indicate whether or not you give permission for your data/specimens to be used for future research.

____ (initial) I agree to have my data/specimens stored for future research by the investigators who are conducting this study.

____ (initial) I agree to have my data/specimens stored and shared with other investigators who are doing research that is related to this study or my condition.

____ (initial) I agree to have my data/specimens stored and shared with other investigators who are doing different kinds of research that is not related to this study or my condition.

____ (initial) Please do not use my data/specimens for future research or share them with other researchers.

Risks

Your participation in this study involves risks related to the blood draw, radiation exposure, and muscle function tests.

1. Risk of Drawing Blood: The needle used to collect blood may cause a bruise at the insertion site, and in very rare circumstances, an infection may develop. Other risks related to the blood draw include pain, bleeding, blood clots, discomfort, swelling, lightheadedness, and fainting.
2. Radiation Risk of the DXA scan, pQCT scan, cQCT scan: If you take part in this research, you will have 2 DXA scans and 2 cQCT scans over the course of 5 years, all of which involve radiation. **Study participants at the Bronx site will also have 2 pQCT scans.** To give you an idea about how much radiation you will get, we will make a comparison with an everyday situation. Everyone receives a small amount of

unavoidable radiation each year. Some of this radiation comes from space and some from naturally occurring radioactive forms of water and minerals.

The imaging scans that you receive as part of this study will result in about the same radiation dose that you would get from normal living with natural background radiation in about 2 years. The risks associated with this radiation dose are very small; estimates of the cancer risk associated with this radiation dose vary from zero to about one chance in 5,000.

The radiation dose we have discussed is what you will receive from this study only and does not include any exposure you may have received or will receive from other tests. Radiation exposure is cumulative throughout life and any additional exposure should be considered carefully.

3. The muscle function tests may cause fatigue or muscle soreness, and very rarely dizziness and shortness of breath. You can stop anytime you feel discomfort or that you cannot continue. During the balance tests, there is a small risk of falling. The Study Personnel performing the muscle function tests have been trained to administer the test as safely as possible and to prevent injury with falls.

Benefits

If you take part in this study, there is no guarantee of a direct benefit to you. Benefits to you may include learning about your bone health. You will be provided with results of the DXA scans. Benefits to society include information gained from these tests that may also be helpful to patients with HIV.

Alternative Procedures

You have the option not to participate in this study at all.

Confidentiality

Any information collected during this study that can identify you by name will be kept confidential. We will do everything we can to keep your data secure, however, complete confidentiality cannot be promised.

Despite all of our efforts, unanticipated problems, such as a stolen computer may occur, although it is highly unlikely. Any information or specimens pertaining to you will be associated with a unique code number, and all computerized databases used in this study will only be accessible by a special password determined by the study investigators.

The following individuals and/or agencies will be able to look at and copy your research records: (1.) The investigator, study staff and other medical professionals who may be evaluating the study; (2.) Authorities from Columbia University and New York Presbyterian Hospital, including the Institutional Review Board ('IRB'); (3.) The National Institutes of Health and/or the Office of Human Research Protections ('OHRP').

The investigator, regulatory authorities, IRB and study sponsor may keep the research records indefinitely. If the results of the study are published or presented at a medical or scientific meeting, you will not be identified.

Research Related Injuries

If you are hurt or become ill during the course of this study, you should contact Michael Yin, MD at (212) 305-7185.

Although compensation for injury that results from participation in this research is not available, Columbia University will assist you in obtaining medical treatment, including emergency treatment, hospital care and follow-up care as needed.

Your insurance carrier will be billed for the cost of such treatment and will be charged in the usual way. If your carrier denies coverage, Columbia University is under no obligation to pay for the treatment, but may do so at its sole discretion.

By providing financial or other assistance, neither Columbia University nor the researchers are stating that they are legally responsible for the injury. Further information regarding compensation for injured research subjects may be obtained from the IRB Office.

Compensation

You will receive a customary participant compensation fee of \$50.00 to help with transportation costs for visits.

Additional Costs

There is no cost to you for participating in this study.

Additional Information

If you have any questions or concerns about the study, you may contact **Dr. XX at XX**. If you have any questions about your rights as a subject, you may contact:

**Institutional Review Board
Address**

An Institutional Review Board is a committee organized to protect the rights and welfare of human subjects involved in research.

Voluntary Participation

Your participation in this study is completely voluntary. You can refuse to participate or withdraw from the study at any time and such a decision will not affect your care at CUMC now or in the future.

Statement of Consent

I voluntarily consent to participate in the study. I have read this consent form and understand the nature and the purpose of the study.

I have discussed the study with the investigator or study staff, have had the opportunity to ask questions and have received satisfactory answers. The explanation I have been given has mentioned both the possible risks and benefits to participating in the study and the alternatives to participation.

I understand that I am free to not participate in the study or to withdraw at any time. My decision to not participate, or to withdraw from the study will not affect my future care or status with this investigator.

I understand that I will receive and may keep a copy of this signed and dated consent form. By signing and dating this consent form, I have not waived any of the legal rights that I would have if I were not a participant in the study.

Signature

Study Participant

Print Name _____ Signature _____ Date _____

Person Obtaining Consent

Print Name _____ Signature _____ Date _____

Printed On:

APPENDIX H: IRB Protocol

Title: HIV and the menopausal transition: effects on musculoskeletal health

Abstract: With effective antiretroviral therapy, HIV infected women are transitioning through menopause and surviving into middle and old age. The adverse musculoskeletal effects of estrogen deficiency may be potentiated in HIV infection, since T cells play a fundamental role in the mechanisms by which estrogen deficiency causes early postmenopausal bone loss. There is increasing evidence that HIV+ women may be at higher risk for clinical features of musculoskeletal senescence - frailty, falls and fracture. We hypothesize that in premenopausal women, estrogen attenuates the adverse effects of HIV infection on the skeleton and muscle, and that the combined effects of declining estrogen levels associated with menopause and persistent T cell activation associated with HIV infection may accelerate bone remodeling and loss of bone and muscle mass to a greater extent in HIV+ than HIV- women. We will address this hypothesis in a longitudinal study of 330 HIV+ and HIV- women currently participating in the Women's Interagency HIV Study (WIHS). by extending the WIHS Metabolic Substudy to follow HIV+ and HIV- women as they transition through menopause. We will use state-of-the art and novel imaging, cell biology, and biochemical methodologies to determine the effects of the menopausal transition on bone turnover, trabecular and cortical bone density and muscle mass, functional measures of muscle strength, balance and endurance, gonadal and calciotropic hormones, pro-resorptive cytokines and T cell activation, osteoblasts and osteoclasts.

1. Study Purpose and Rationale

The face of the AIDS epidemic is changing in this country. Women, particularly minority women, are increasingly represented in the ranks of the HIV-infected. With the advent of successful antiretroviral therapy (ART), young HIV-infected women are now transitioning through menopause and surviving into middle and old age. In normal women, menopause is marked by declining estrogen production and accelerated loss of bone and muscle mass, changes that ultimately lead to clinical features of musculoskeletal senescence - frailty, falls and fractures. Menopause is associated with increased T cell activation and production of bone resorbing cytokines such as receptor activator of NF κ B ligand (RANKL) and tumor necrosis factor alpha (TNF α)[1] and T cells are now thought to play a fundamental role in the mechanisms by which estrogen deficiency causes early postmenopausal bone loss[2, 3]. As HIV infection is associated with persistent T cell activation and increased production of RANKL and TNF α , the adverse skeletal effects of estrogen deficiency may be potentiated in HIV+ women. Another concern is the association between estrogen deficiency and loss of muscle mass and strength [4, 5]; this process may also be accelerated in HIV+ women during the menopausal transition, thus increasing the risk of falls. In this regard, a recent study found that spine, hip and wrist fractures were 2-3 times higher in HIV+ postmenopausal women than HIV- controls[6].

In the Women's Interagency HIV Study (WIHS) Metabolic Substudy (MS), we found significantly lower bone mineral density (BMD) in HIV+ than HIV- premenopausal women; however, serum pro-inflammatory cytokines, bone resorption markers, rates of bone loss[7] and incidence of fracture[8] did not differ significantly by HIV status. In contrast, in a separate study of postmenopausal (PM) minority women, we detected significantly lower BMD, and higher serum levels of bone resorption markers and rates of bone loss in HIV+ than HIV- women[9, 10]. Also in contrast to our findings in premenopausal HIV+ women, serum levels of TNF α were higher in HIV+ than HIV- postmenopausal women and attenuated the association of HIV and BMD in the model, suggesting that TNF α mediated the effects of HIV on BMD. Multiple animal/*in vitro* studies have demonstrated that acute estrogen deficiency upregulates T cell TNF α production, primarily by increasing the number of TNF α -producing T cells[3]. Conversely, estrogen downregulates T cell activation and mitigates the effects of T cell-derived, pro-inflammatory cytokines on osteoclast-mediated bone resorption. We therefore hypothesize that estrogen attenuates the adverse effects of HIV infection on the skeleton in premenopausal women [11] and that during the menopausal transition, the combined effects of declining estrogen levels and persistent T cell activation associated with HIV infection may accelerate bone remodeling and bone loss to a greater extent in HIV+ than HIV- women.

We will address these hypotheses in a cohort of mainly minority women (reflective of the HIV epidemic in women) currently participating in the WIHS MS. Building upon five years of already acquired longitudinal data, this value-added proposal will delineate the effects of the menopausal transition on bone turnover, rates of bone loss, muscle mass, strength and function in these women. We will also determine whether, and by what mechanisms, estrogen deficiency modifies relationships between the musculoskeletal and immune systems in the setting of HIV infection. To a major extent, we will use biomarkers, repository specimens and other data from previous WIHS studies. We will apply novel imaging technologies, such as quantitative CT to assess trabecular and cortical volumetric BMD (vBMD), hip and thigh muscle mass, and intramuscular fat content, and

apply cell biology techniques to peripheral blood mononuclear cells (PBMCs) to assess osteoclast/osteoblast precursors and apoptosis. Elucidation of the dominant and modulatory pathways associated with excess bone loss in aging HIV+ women is critical to the development of mechanistic, interventional studies to prevent of the deleterious effects of menopause on their musculoskeletal health.

Specific Aim 1: To compare HIV+ and HIV- women undergoing the menopausal transition with respect to bone turnover markers and rates of trabecular and cortical bone loss.

Hypothesis 1: During the menopausal transition, HIV+ women will have higher bone turnover and rates of trabecular and cortical bone loss than HIV- women, and also than premenopausal HIV+ women.

Specific Aim 2: To determine the effects of estrogen deficiency on T cell activation, osteoclast and osteoblast precursors and osteoblast apoptosis in HIV+ and HIV- women.

Hypothesis 2: During the menopausal transition, HIV+ women will have greater increases in activated T cells, pro-inflammatory cytokines, osteoclast formation and apoptotic osteoblast precursors than HIV- women, and these markers will be associated with increased bone turnover and decreased bone and muscle mass.

Specific Aim 3: To compare HIV+ and HIV- women undergoing the menopausal transition with respect to changes in muscle mass, strength, and functional measures of fall risk.

Hypothesis 3: During the menopausal transition, HIV+ women will have a greater rate of decline in hip and thigh muscle mass and muscle strength, and greater increase in measures of fall risk than HIV- women.

2. Study Design and Statistical Procedures

2.a. Study Design: This is a longitudinal study to investigate the contribution of HIV infection and estrogen deficiency during the menopause transition to musculoskeletal health in 330 HIV+ and HIV- women. We will build upon data from the WIHS MS. WIHS is an ongoing, multicenter observational study of 3,766 women recruited from 6 US cities during 2 enrollment periods (1994-5, 2001-2)[12, 13] A key scientific priority of the 4th 5-year cycle of WIHS has been to understand the pathogenesis of long-term comorbidities. In the WIHS MS, 440 women (122 HIV-, 318 HIV+) with a median age of 42 from the WIHS San Francisco (SF), Bronx and Chicago sites were enrolled from April 2003 through October 2006 and subsequently followed 2 and 5 years later. In this study, we will extend the WIHS MS by repeating dual energy xray absorptiometry (DXA) tests in WIHS participants at Years 8 and 10, and add hormonal and cytokine measures, bone turnover markers (BTMs), quantitative CT (QCT) for bone and muscle, measures of muscle strength and function, as well as novel *in vitro* immune and bone cell assays. Columbia University Medical Center will serve as the site for both central QCT and High-Resolution peripheral QCTs (HR-pQCT) for participants from Bronx, NY.

Table 1 shows the proposed data to be collected in relation to the WIHS MS. At each visit in the proposed study, serum will be analyzed for gonadal and calciotropic hormones, cytokines (TNF- α , IL6, RANKL, osteoprotegerin), and bone turnover markers (osteocalcin, C-telopeptide) and compared to banked sera from the WIHS repository from a premenopausal timepoint. DXA will be utilized to measure areal BMD (aBMD), body composition, and vertebral fracture assessment (VFA), QCT will be utilized to measure vBMD. A subgroup (Bronx) will also have HR-pQCT to examine vBMD of the radius and tibia to correlate central and peripheral CT findings.

Table 1. Study Procedures	Metabolic Substudy (MS)			Proposed study	
	Premenopause			Late peri-/Early PM	
	Year 0 (2003-6)	Year 2 (2005-8)	Year 5 (2009-11)	Year 8 (2012-14)	Year 10 (2014-16)
WIHS Semiannual Visit #	V18-V23	V22-V28	V29 –V33	V35 – V38	V39 – V42
Aim 1					
Hormones, cytokines, BTMs	a			X	X
BMD & Body comp by DXA	A	A	A	X	X
VFA by DXA				X	X
Central QCT of spine and hip				X	X
HR-pQCT (Bronx only)				X	X
Aim 2					
Immunological assays	a			X	
Osteoblast Assays	a			X	
Osteoclast Assays				X	

Aim 3					
Lean mass by DXA	A	A	A	X	X
Pelvic & thigh muscle by QCT				X	X
Strength/ fall assessment				X	X
Grip strength assessment		A		X	X
A= available data; a= to be performed on stored sera/PBMCs; X= data to be collected					

2.b. Statistical Procedures (Specific Aim 1):

Design Overview: We will enroll only participants in late perimenopause or early PM (late peri/early PM). The majority will be enrolled as part of an extension of the WIHS MS. This strategy will enable us to utilize BMD data and stored specimens already obtained in WIHS MS to compare premenopause to late peri/early PM stages. Participants will undergo DXA to measure aBMD of the spine, hip and forearm. New studies in women at the proposed Year 8 and Year 10 exam of the WIHS MS will include performance of central QCT to measure vBMD of hip and spine and HR-pQCT to measure vBMD of the radius and tibia. Vertebral fracture prevalence will be assessed by VFA. Sera collected at the semi-annual CORE visits will be analyzed to assess calcitropic and gonadal hormones, BTMs, and cytokines. We will perform a cross sectional analysis comparing baseline aBMD, central (spine and hip) and peripheral (radius and tibia) trabecular and cortical vBMD, VFA, and BTMs in HIV+ and HIV- late peri/early PM women using data from Visit 8 and a longitudinal analysis comparing rate of change in aBMD and trabecular and cortical vBMD between HIV+ and HIV- late peri/early PM using data from Visit 8 and 10. In both analyses, we will examine associations between aBMD and vBMD with hormone and cytokine levels, body composition and other known correlates of BMD. We will also compare rate of change in aBMD in HIV+ women at the premenopause and late peri/early PM stage in participants previously enrolled in WIHS MS, and examine associations between hormones, cytokine, body composition and other correlates.

Covariates of Interest: At each WIHS substudy visit, data are acquired through questionnaires, physical examination and collection of biological specimens. The following variables will be assessed in their relationship to bone and muscle parameters: demographic and behavioral variables (age, race, smoking, alcohol use, opiate use), anthropomorphic measures (weight, BMI, fat and lean mass by DXA and QCT both at baseline and annualized change), comorbid conditions (HBV/HCV infection, diabetes), calcitropic and gonadotropic hormones, cytokines. Analyses restricted to HIV+ women will include HIV-related variables (CD4 count, nadir and current, HIV plasma RNA, AIDS-defining illness) and ARV exposure (cumulative and current exposure to ART class and individual ARVs).

Expected results: We expect that BTMs and cytokines will be higher in HIV+ than HIV- late peri/early PM women, and associated with lower aBMD and vBMD in HIV+ women at baseline. Declines in aBMD and trabecular and cortical vBMD will also be greater in HIV+ than HIV- late peri/early PM women. In participants with premenopausal data, we expect that BTMs and rate of change in aBMD and vBMD will be greater in the late-peri-/early PM than premenopausal stage, and between group (HIV+ vs HIV-) differences will be greater at the late peri-/early PM stage.

Statistical Plan: Determination of the cumulative amount of time spent in each menopausal stage will follow the algorithm utilized in SWAN [14]. The rate of change in aBMD and trabecular and cortical vBMD during each menopausal stage will be estimated using separate linear mixed models for each outcome, as outlined above under general analytic strategies. Repeated measures of each outcome will be modeled as a function of two separate time variables, one for the cumulative amount of time spent in each menopausal stage (premenopause and late peri/early PM). We will use the models developed for each outcome to compare rates of change in HIV+ and HIV- women within and between menopausal stages.

Power and Sample Size: All power calculations were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina, USA). Separate calculations were performed for 80% and 90% power, with all calculations assuming a two-tailed $\alpha=0.05$. A previous study of premenopausal women[7] reported an annual decrease in lumbar spine BMD of $-0.8\% \pm 2.1\%$ for HIV+ and $-0.4\% \pm 1.9\%$ for HIV- women and an annual decrease in femoral neck BMD of $-0.8\% \pm 3.4\%$ for HIV+ and $-0.6\% \pm 1.6\%$ for HIV- women. Because we anticipate 10% loss to follow-up, we will recruit 330 participants, which will yield 100 HIV- and 200 HIV+ late peri/early PM women. Assuming a pooled SD of 2% for lumbar spine, we will have 80% power to detect a difference of 0.7% and 90% power to detect a difference of 0.8% between HIV+ and HIV- women in annual lumbar spine decrease. Assuming a pooled SD of 2.8% for femoral neck, we would have 80% power to detect a difference of 1.0% and 90% power to detect a difference of 1.1% between HIV+ and HIV- women in annual femoral

neck decrease. This is within the range of the 0.5-0.9% between-group difference that we saw in the postmenopausal study[9]. We anticipate that controlling for demographic and other factors in multivariable repeated measures analysis will substantially reduce the residual SD, giving us power to detect an even smaller difference between HIV+ and HIV- women in BMD measures.

2.c. Statistical Procedures (Specific Aim 2):

Design Overview: We will analyze PBMCs from 200 subjects (140HIV+, 60HIV-) at premenopause (defined by regular menses and anti-mullerian hormone>0.1ng/ml) using PBMCs from the WIHS repository and late peri/early PM using PBMCs collected during the proposed study (Table 1). Since the osteoclast assays are labor-intensive and require fresh PBMCs, we will compare osteoclast activity only in HIV+ and HIV- late peri/early PM participants from the Bronx site (Table 1), and will not have premenopausal specimens for comparison. As an additional comparator group, we will examine immunological and bone cell measures in HIV+ women receiving exogenous estrogen by using PBMCs from the WIHS repository in 30 HIV+ women aged 45-55 who were receiving HRT. We will also examine associations between immunological and bone cell measures and change in BTMs, TNF α /RANKL/IL6, and aBMD and vBMD, muscle mass and function.

Covariates of interest: Age, race, HIV-related variables (CD4 count, nadir and current, HIV RNA, antiretroviral exposure), calciotropic and gonadotropic hormones (PTH, 25OHD, estradiol, estrone, SHBG), and cytokines (TNF α , IL6, RANKL, OPG). Given the known association between immune activation and chronic cytomegalovirus (CMV) infection, we will also adjust for CMV serostatus in the analyses.

Expected results: Studies of immune phenotypes in the general population have demonstrated increased proportions of activated (CD8+ cells co-expressing TNF- α and IFN- γ) and senescent (CD8+CD57+) T cells from circulating PBMCs[15] and lower numbers of memory CD4+ T cells (CD4+/CD27+/CD45RA-) expressing RANK and CD28 in PM women with osteoporotic fractures[16]. Senescent T cells are an important source of TNF α production in the bone marrow in an estrogen deficient state[3], and may also be an important mechanism of aging-related bone loss. We expect that the proportion of CD8+ T cells with markers of immune activation and senescence will be higher in late peri/early PM HIV+ than HIV- women and associated with increased numbers of osteoclast and osteoblast precursors, total and % free estradiol, BTMs, and Tb and Ct bone loss as well as increased loss of lean body mass, hip and thigh CSA, and muscle strength and function. Circulating osteoclast and osteoblast precursors and apoptotic osteoblast precursors will be similarly higher in late peri/early PM HIV+ than HIV- women, and associated with increased BTMs and Tb and Ct bone loss. Among HIV+ women, we expect that the proportion of activated T cells will be higher in PBMCs from the late peri/early PM stage than the premenopause stage. Finally, we expect that in HIV+ participants receiving exogenous estrogens, immune and bone cell parameters will be similar to those of premenopausal HIV+ women.

Statistical Analysis Plan: The general analytic approach described for Aim 1 will apply to Aim 2. Outcome measures will include proportions of CD4+, CD8+ T cells with markers of immune activation (CD38\HLA DR), senescence (CD57\CD28), cytokine expression after stimulation, and CD4+ and CD8+ T cell memory\naive (CD45RA\CCR7) subsets with markers of immune activation (CD38\HLA DR) and RANK/RANKL expression. Outcomes also include osteoclast and osteoblast cell precursor numbers and proportion with apoptosis markers. For Aim 2, each participant will have data from a premenopausal (WIHS MS repository) and late peri/early PM stage (visit Year 8). Repeated measures will be modeled for each outcome to compare differences in proportions in HIV+ and HIV- women within and between menopausal stages. We will also use models developed to examine associations between immunological and bone cell measures, and between immunological and bone cell measures and BTMs, TNF α /RANKL/IL6, BMD and muscle outcomes in Aim 1 and Aim 3, respectively.

Sample Size and Power: A WIHS cardiovascular substudy (n=159) demonstrated significantly higher activation and senescence markers in HIV+ than HIV- women[17] and the Immunos study found significant correlations between T cell subsets and FN BMD in 26 PM women with osteoporotic fracture and 24 PM controls[16]. In a study comparing circulating osteogenic cell number in patients with hypoparathyroidism (n=19) and controls (n=19), Rubin et al. found fewer OCN+ (Mean \pm SEM: 0.7 \pm 0.1 vs. 2.0 \pm 0.1%; p<0.0001) and more OCN+/CD34+ cells (Mean \pm SEM: 11.0 \pm 1.0 vs 5.6 \pm 0.7%) in subjects than controls [18]. Assuming a pooled SD of 1.4% for OCN+ and 11.5% for OCN+/CD34+ cells, 140 HIV+ and 60 HIV- subjects provide 80% power to detect a difference of 0.61% and 90% power to detect a difference of 0.70% in OCN+ cells and 80% power to detect a difference of 5.0% and 90% power to detect a difference of 5.8% in OCN+/CD34+ cells between HIV+ and HIV- women. Based on the Immunos study, we have 80% power to detect a correlation of 0.197 between T cell subsets and femoral neck BMD and 90% power to detect a correlation of 0.227.

2.d. Statistical Procedures (Specific Aim 3):

Design Overview: Our goal is to determine whether there are differences muscle mass and quality, and functional measures of strength, balance, and endurance in HIV+ and HIV- women in late peri/early PM at baseline (visit Year 8) and whether there are group differences in rate of change in these measures over two years of early PM (visit Year 8 to Year 10). We will determine whether change in muscle function is better correlated with declines in mass or quality (increased intramuscular fat). And through modeling, we will assess whether T cell activation and inflammation mediate declines in muscle mass and function. Aim 3 will utilize the same approach as Aim 1. In a subgroup of women with grip strength data collected from a previous WIHS substudy[19], we will also compare annualized change in grip strength during premenopause and late peri/early PM.

Covariates of Interest: Same as in Aim 1

Expected results: We expect that lean mass, pelvic and thigh CSA will be slightly lower in HIV+ than HIV- late peri/early PM women at baseline, but rate of decrease in muscle mass will be much greater in HIV+ than HIV- women. We also expect that decreased muscle mass will be associated with lower total and % free estradiol, higher levels of T cell activation and TNF α /RANKL/IL-6 in HIV+ women, and associated a more rapid decline of muscle strength, balance and endurance.

Statistical Analysis Plan: Baseline differences and rate of change in muscle mass (appendicular lean mass by DXA, pelvic and thigh muscle CSA by QCT) and quality (attenuation coefficient), and functional measures of strength, balance and endurance during late peri/early PM will be estimated using separate linear mixed models for each outcome, as outlined above under general analytic strategies. Covariates utilized in this analysis are similar to Aim 1.

Sample size and Power: One study of early PM women (n=77), demonstrated a $-1.17 \pm 2.03\%$ (mean \pm SD) annual decrease in appendicular lean mass by DXA[20]. Another study (n=530) demonstrated a -0.2 kg/yr annual decrease in grip strength[21]. Assuming a pooled SD of 2.03%, 300 subjects (200 HIV+ and 100 HIV-) will provide 80% power to detect a difference of 0.69% and 90% power to detect a difference of 0.81% in change in muscle mass between HIV+ and HIV- women. Based on the observed significant decrease of -0.2 kg/yr ($p < 0.005$) in grip strength, we estimate a SD of 0.07. Assuming a pooled SD of 1.2, 300 subjects will provide 80% power to detect a 0.41 kg/yr difference and 90% power to detect a 0.48 kg/yr difference between HIV+ and HIV- women in change in grip strength.

3 Study Procedures

3.a. Biochemical Assays: All WIHS specimens were collected fasting in the morning, aliquoted, frozen immediately, stored at -80° , and will be run in batches at the Columbia University Medical Center Irving Institute for Clinical and Translational Research (CUMC IICTR). Interassay precision is given in brackets after each test. **OCN** by ELISA (N-mid Osteocalcin, IDS Ltd, Fountain Hills, AZ; 12.7% at 26.2 ng/ml). **CTX** by ELISA (IDS Ltd, Fountain Hills, AZ; 10.7% at 0.121 ng/ml). **FSH** by chemiluminescent immunometric assay (Immulite, Deerfield, IL; <5%) **Estradiol** by gas chromatography/mass spectrometry (Agilent 7000 series; <10%); **Estrone** by RIA (DSL, Webster, TX; 9.0%,). **SHBG** by ELISA (DSL, Webster, TX; 3.7% at 26 nmol/L). Free estradiol (%) will be calculated by the formula of Sodergard et al.[22] **PTH** by total intact PTH IRMA (Scantibodies Laboratory, Inc., Santee, CA; 5%) **25-OHD** is measured by RIA (Diasorin RIA, Stillwater, MN; 15% at 17 ng/mL and 15% at 57 ng/mL). **OPG** by ELISA (Immundiagnostik, Bensheim, Germany; 8% at 19.5 pg/mL). Total soluble **RANKL** by ELISA (Immundiagnostik, Bensheim, Germany; 7.1% at 2300 pg/mL). **TNF α** by high sensitivity ELISA (R&D Systems, Inc. Minneapolis, MN; 16.7% at 2.4 pg/mL). **IL-6** by chemiluminescent immunometric assay (Immulite 1000, Siemens, Deerfield, IL; <5%)

3.b. aBMD, Body Composition and Vertebral Fracture Assessment (VFA) by DXA: aBMD of the LS (L1-4), proximal femur and non-dominant forearm, lean body mass (total and appendicular) and fat mass (total, trunk and appendicular), and VFA will be measured on Lunar Prodigy densitometers (GE Medical Systems, Madison WI) at all 3 sites. Dedicated technologists, certified by the International Society of Clinical Densitometry, with long-term research experience perform all scans. Phantoms are scanned daily to check for detector drift; the results are appended to a quality control database. A phantom will also be circulated among the 3 sites biannually. Results are downloaded to specific project databases and sent electronically without identifiers to be read centrally by the Image Analysis Lab (IAL), a leading imaging analysis center within the Obesity Research Center at St. Luke's Roosevelt Hospital, NY. IAL performed the DXA analysis for the WIHS MS and has performed other imaging studies for WIHS. VFA demonstrates good agreement with conventional radiographs (96.3%, $k=0.79$) in classifying vertebrae as normal or deformed[23] and has good sensitivity (91.9%) in identi-

cation of moderate to severe deformities and excellent negative predictive value (98%)[24].

3.c. vBMD and Muscle Mass by cQCT: Volumetric QCT acquisitions of the L1-L2 vertebrae and the hips (80 kVp, 140-300 mAs, 2.5 mm slice thickness, pitch=1, standard reconstruction algorithm, Image Analysis QCT Calibration Phantom) and a single slice through the mid-thigh will be performed on GE Light Speed VCT 64 (New York, SF) or Siemens Definition dual source 64 (Chicago) using a standardized protocol. Scan data are forwarded to IAL for scan quality assurance and analysis using QCT Pro (Mindways, Austin, TX) to quantify Tb and Ct vBMD of the spine and hip. In order to ensure comparability of data obtained on different CT scanners, CT data are cross-calibrated using the Image Analysis Torso phantom which will be circulated to all 3 sites bi-annually. Our collaborator and consultant, Thomas Lang, PhD (see letter of support) will provide additional guidance to the IAL. He has vast experiences with QCT analyses that have demonstrated correlation of not only between hip vBMD parameters and fracture [25-29] but also between cross sectional area (CSA) of the thigh muscle[30] and pelvic musculature and hip fracture[31]. Attenuation of muscle will also be assessed since increased fat accumulation in muscle with aging is an important aspect of sarcopenia[32]. Mean attenuation coefficients by QCT correspond with histological assessments of fat accumulation within and between thigh muscles[33] and predict incident mobility limitations in older persons[34].

3.d: HRpQCT is performed on the XtremeCT (Scanco Medical AG, Switzerland) at CUMC on only participants from the Bronx WIHS. The nondominant distal radius and tibia are immobilized in a carbon fiber shell[35-37]. The region of interest is defined on a scout film by manual placement of a reference line at the endplate of the radius or tibia. A stack of 110 parallel CT slices is acquired at the distal end of both sites (effective energy 40 keV, slice thickness 82 μm , image matrix size 1024x1024, nominal voxel size 82 μm . This provides a 3D image of 9 mm in the axial direction. Attenuation data are converted to equivalent hydroxyapatite (HA) densities. The European Forearm Phantom is scanned regularly for quality control. The analysis methods have been described, validated[38-41]^[42] and applied in recent clinical studies[35-37, 43-47]. Briefly, the volume of interest (VOI) is automatically separated into Ct and Tb regions using a threshold-based algorithm set to 1/3 the apparent cortical bone density (D_{cort}). Mean Ct thickness (Ct.Th) is defined as the mean cortical volume divided by the outer bone surface. Tb bone density (D_{trab}) is the average bone density within the Tb VOI. BV/TV (%) is derived from D_{trab} assuming a density of fully mineralized bone of 1,200 mg HA/cm³ ($\text{BV/TV} = 100 \times D_{\text{trab}}/1200 \text{ mg HA/cm}^3$). The resolution of the XtremeCT approximates the width of individual trabeculae; therefore, Tb structure is assessed using a semi-derived algorithm[40].

3.e. Characterization of T cell immune activation, senescence, cytokine production and RANK/RANKL expression will be performed by polychromatic flow cytometry on frozen/thawed PBMCs in the laboratory of Dr. Alan Landay (Rush Medical Center). Frozen PBMC will be thawed rapidly and incubated overnight at 37°C 5% CO₂. Cytokine assays will be performed *in vitro* on PBMC after no stimulation (media alone) or stimulation with phorbol 12-myristate 13-acetate (PMA, 10 ng/ml) and Ionomycin (1 $\mu\text{g/ml}$) for 6 hours at 37°C in the presence of brefeldin A. To assess cell viability, PBMC will be stained with Aqua Live/Dead cell stain kit (Invitrogen) prior to cell surface staining and intracellular cytokine staining. Cells in the cytokine assay will be stained with fluorochrome-conjugated monoclonal antibodies to CD3, CD4, CD8, CD57, CD28, HLA DR, and CD38. For intracellular cytokine detection, cells will be fixed and permeabilized and stained with fluorochrome conjugated IL-6 and TNF α cytokine antibodies. Phenotypic analyses will be performed to assess CD4⁺ and CD8⁺ memory/naïve cell subsets for markers of immune activation and RANK/RANKL expression. Cells will be stained with fluorochrome-conjugated monoclonal antibodies to CD3, CD4, CD8, CD45RA, CCR7, HLA DR, CD38, and RANK/RANKL, washed, fixed in 2% formaldehyde, and analyzed on a LSR2 flow cytometer (BD) using FlowJo software. Immune activation (CD38⁺HLA DR⁺), senescence (CD57⁺/CD28⁻) and cytokine (TNF- α , IL-6) analyses will be performed on CD4⁺ or CD8⁺ T cells. We will also evaluate the immune activation and RANK/RANKL expression on central memory (CD45RA⁻CCR7⁺), naïve (CD45RA⁻CCR7⁻), effector memory (CD45RA⁺CCR7⁻), and terminal effector (CD45RA⁺CCR7⁻) CD4 and CD8 T cells.

3.f. Osteoblast and Osteoclast studies will be performed in the laboratory of Dr. Manavalan and Kousteni at Columbia University Medical Center. **Quantification of Peripheral Blood Osteogenic Cells (Osteoblasts):** Frozen PBMCs will be thawed as above and immunostained for flow cytometry analysis[18, 48-50]. PBMCs will be resuspended in flow-staining buffer (PBS plus 2% FBS) and the primary unconjugated goat polyclonal anti-human osteocalcin antibody (Santa Cruz Biotechnology) is added. After incubation at 4°C, the cells will be washed twice and fluorochrome-conjugated primary APC conjugated anti-CD15, and anti-PE-Cy7 conjugated

CD34 (both from Becton Dickinson, San Diego, CA) antibodies and secondary FITC –conjugated donkey anti-goat antibody (Jackson ImmunoResearch) will be added. The cells are then incubated at 4°C and washed twice before flow cytometry analysis. Three-color flow cytometry acquisition is performed using a LSR II flow cytometer (Becton Dickinson, San Diego, CA) and analysis using FLO-JO software. During acquisition/analysis, cells are gated for size, shape and granularity using forward and side scatter parameters. CD15 positive granulocytes are excluded prior to gating for specific osteogenic precursor populations, OCN+CD34+ and OCN+/CD34-. Osteoblast precursor number will be examined by determining the numbers of osteogenic precursors in the periphery. Apoptosis will be quantified by measuring caspase 3 activity using the Ac-DEVD-AFC fluorogenic substrate (AFC) (Biomol) at the FLUOstar Omega (IMGENT) fluorometer as we have previously described[51]. **Quantification of Osteoclastic Cells:** Osteoclast precursors are isolated from freshly isolated PBMC by magnetic bead sorting using anti-CD14 magnetic beads (Miltenyi Biotec). CD14+ cells are cultured for 3 days in MEM medium (Invitrogen) supplemented with 10% FBS (HyClone) and 30 ng/ml rhM-CSF (Peprotech) at a density of 6×10^4 cells per well in a 96 well plate and then supplemented with 40 ng/ml human soluble RANKL (Peprotech) for an additional 6 days. Cytokines will be replenished every 3 days. On day 9, cells will be fixed and stained for TRAP using an acid phosphatase leukocyte diagnostic kit (Sigma-Aldrich) as recommended by the manufacturer. Multinucleated (greater than three nuclei) TRAP-positive osteoclasts will be counted in triplicate wells.

3.g.. Muscle strength, balance, endurance: This battery of muscle strength, balance and endurance measures was developed in consultation with Dr. Luigi Ferrucci based upon his extensive experiences in the Baltimore Longitudinal Study on Aging (BLSA) and other aging cohorts[52-54]. Dr. Ferrucci and his staff at BLSA will train the WIHS investigators and personnel in performance of these measures at the semi-Annual WIHS meeting, and help to ensure quality control. **Muscle strength** will be assessed in two ways: Grip Strength as performed previously in a cross section of WIHS[19]. Participants will squeeze a hand-held Jamar dynamometer with maximum force using their dominant hand, the best of three attempts will be utilized in analysis. Grip strength has been shown to identify mobility problems with equivalent precision to measures of lower extremity strength and power[54] and to be as predictive of fracture as quadriceps strength in PM women[55]. Sirola et al. demonstrated that a change in grip strength over a 5-year interval predicted future falls in perimenopausal women[56, 57]. The Repeat Chair Stand is a functional test of lower extremity performance that has been proposed as a proxy measure of strength in the clinical setting. By measuring time to completion of 10 chair stands instead of the standard 5, we will minimize the ceiling effect for this higher functioning cohort. **Static balance** will be assessed with the Standing Balance Test. Subjects are asked to stand in several different positions with progressive narrowing of the base of support, namely the side-by-side stand, the semi-tandem stand (i.e. heel of one foot touching the great toe of the other foot), the tandem stand (i.e. heel-toe position), the single leg stand (i.e. opposite foot in knee flexed position with toes about 2 inches from the floor). The duration for which the participant can hold the stance without taking a step or grabbing for support is recorded. In order to reduce the possibility of a ceiling effect, we will add the one-leg stand and increase the duration that subjects will be asked to hold each position to 30 seconds [58]. **Dynamic Balance** will be assessed with the Functional Reach Test, which is widely used in geriatric populations, has been prospectively validated, and has a high test-retest and inter-rater reliability[59, 60]. This test measures the distance the subject can reach in front of him from a standing position without losing balance. **Endurance** will be assessed by a 6 Minute Walk, which measures the maximum distance a person can walk in 6 minutes. It is easy to administer and has been validated as an integrated measure of mobility in the older adults[61].

4. Study Drugs or Devices

(N/A)

5. Study Instruments

Muscle performance measures are attached

6. Study subjects

The proposed study extends follow-up of women in the Women's Interagency HIV Study (WIHS) Metabolic Substudy (MS) through the menopausal transition, and adds novel imaging tests and in vitro assays to understand the changes in musculoskeletal health. The WIHS MS originally enrolled 440 women from 2003 to 2006 and performed DXA scans to measure BMD and body composition at the baseline visit, and 2 and 5 years af-

ter enrollment. Of the 440 women, we estimate that 227 participants will agree to participate in a year 8 and 10 exam based on the study inclusion criteria and prior retention rates. An additional 100 WIHS participants (not originally enrolled in the WIHS MS) will be targeted for enrollment from WIHS MS sites (San Francisco, Bronx, Chicago). We do not anticipate any problems with enrolling 100 more participants into the WIHS MS extension, because WIHS will be open for new enrollment beginning in January 2011.

Inclusion criteria: WIHS participant between ages 40-60 from the three WIHS MS sites who are (1) either in late perimenopause (no bleeding in 3-11 of the last 12 months) based upon the SWAN study definitions[14] or early postmenopausal (no bleeding for >1 but <5 years) and (2) have an Anti-Mullerian Hormone level <0.1 ng/ml. **Exclusion criteria:** (1) history of bisphosphonate use, (2) estimated glomerular filtration rate (GFR) <60ml/min, (3) weight >264 lbs, the maximum weight of the densitometer (4) current hormone replacement therapy (5) current glucocorticoid use (oral or inhaled), (5) If HIV-infected, participants must be on ART for > 1 year in order to avoid the acute bone loss associated with ART initiation, and have current CD4>100 cells/ μ l, no history of AIDS wasting, and no opportunistic infections within the last 3 months to limit the variability of uncontrolled HIV on immunologic and musculoskeletal parameters.

Also specifically excluded are the following: 1) protected individuals (institutionalized); 2) prisoners; 3) any other prospective participant who, for any reason, might not be able to give voluntary informed consent.

7. Recruitment

Most of the potential participants are already enrolled in WIHS MS and will be asked by the principal investigators at each site (Drs. Tien, Co-PI of the Northern California WIHS at San Francisco; Dr. Anastos, PI of the Bronx WIHS; Dr. Cohen, PI of the Chicago WIHS) and/or their staff whether they would like to participate in the extension study. Additionally, participants who are enrolled in WIHS but had not previously been in WIHS MS will be approached to enroll in the proposed study.

8. Informed Consent Process

The site principal investigators will be directly responsible for enrolling subjects and obtaining consent for the study. Written consent will always be obtained according to appropriate Informed Consent forms that will be reviewed and approved by the institutional review board at each WIHS site. Potential participants are assured that participation is voluntary and that refusal to participate will not influence their care. Statements to this effect will be included in all Informed Consent forms, which will be signed by the investigator obtaining consent and by the subject in the presence of a witness. All investigators have completed courses in Good Clinical Practices and HIPAA compliance. Written informed consent will be obtained for every subject by the investigators after an explanation of the purpose, risks and benefits of the study. Confidentiality will be guarded with the use of computers that are password protected and storing questionnaires with sensitive information within a locked file. All subjects will be provided with instructions on how to contact the investigative team if any problems or concerns arise.

9. Confidentiality of Study Data

Personal Identifying Health Information (PHI) of participants will be kept only on paper in secure files accessible to the WIHS site PIs and project coordinators. Data will be recorded on case report forms on which the only identifier is a research ID code. Only the WIHS site PIs and project coordinator have access to the link between the research ID code and PHI. No names or identifying information will be included in research reports. Subjects' names will not appear on questionnaires. All computers housing research data have passwords and timed screen savers requiring a password for access.

10. Privacy Protections

Only a select group of study personnel will have access to patient study files. We are fully committed to safeguarding an individual's expectation that the information they offer will be held in confidence. As we are dealing with particularly sensitive information with this patient population, we will take all necessary precautions to ensure that a subject's HIV status is kept confidential and secure including signing a certificate of confidentiality; once we have received IRB approval for this protocol, we will apply to the NIH's Office of Extramural Research to issue the Certificate of Confidentiality for this study. The subject has the right to revoke the authorization for us to access her health information at any time, as is stated in the HIPAA form that each subject will sign prior to participation.

11. Potential Risks

The potential risks are related to venipuncture; radiation exposure from measurement of areal bone mineral density, vertebral fracture assessment (VFA) and Body Composition by dual x-ray absorptiometry (DXA), volumetric BMD of the spine and hip by central QCT (cQCT) and of the radius and tibia by high resolution peripheral QCT (HRpQCT).

ci. Venipuncture: The risks of venipuncture for blood drawing include pain, bleeding, bruising, and a remote possibility of infection or inflammation at the site. Additionally, there is a possible risk of syncope in individuals who are prone to vasovagal responses. To minimize these risks, trained phlebotomists who follow proper technique perform all venipunctures.

cii. Radiation: Radiation exposure for DXA of the spine, hip and forearm with the Lunar Prodigy is 1.4 μSv , for VFA 2.0 μSv and for Body Composition is 2.6 μSv . This is about the amount the average person receives from background radiation in 3 days. Radiation exposure from a central QCT scan of the spine, hip, and midhigh is 2180 μSv , which is equivalent to 8-10 months of background radiation. For HRpQCT of the forearm and leg, the estimated effective whole body dose is below 3 μSv per scan, since only a very small fraction of the distal forearm or leg is irradiated.

Based on these data, we estimate the following radiation exposure for Aims 1 and 3 for the entire study (two DXA and two cQCT scans). Participants at the Bronx WIHS site will have an additional two HRpQCT scans performed at Columbia University Medical Center:

Aim 1 and Aim 3 (Bronx participants): 4378 μSv = 16-20 months of background radiation

Aim 1 and Aim 3 (San Francisco and Chicago): 4372 μSv = 16-20 months of background radiation

For purposes of comparison, this amount of radiation exposure is similar to that associated with many other x-ray procedures: 450 μSv for a mammogram, 7,000-10,000 μSv for a standard abdominal/pelvic or chest CT scan, 60 μSv for a round-trip transcontinental plane flight and 2400-3600 μSv natural background radiation in a year. Expressed as equivalencies to background radiation, a standard mammogram, often obtained annually, is associated with radiation exposure equivalent to approximately 2 to 3 months of natural background radiation. A standard abdominal or chest CT scan is associated with radiation exposure equivalent to approximately 24 to 36 months of natural background radiation. Thus, the maximum amount of radiation received by participation in this study is less than a standard CT scan of the chest or abdomen. We will counsel all study subjects about the total amount of radiation that they will receive as a result of participation as part of Informed Consent procedures of that particular study. In addition, they will be counseled that radiation exposure is cumulative throughout life and any additional exposure should be considered carefully.

12. Data and Safety Monitoring

Since this an observational study, there are no specific plans for a data safety and monitoring plan

13. Potential Benefits

The potential benefits to individual subjects relate to the additional information regarding their musculoskeletal health (assessment of vitamin D and calcium intake, bone density testing, muscle function testing) that will become available for their care. Participants are not guaranteed, however, to receive personal benefits. On a wider scale, the information gained from this research project may be helpful to all HIV+ women undergoing the menopausal transition. The information gained may directly impact upon the diagnosis and management of osteoporosis in HIV+ postmenopausal women. In contrast, the risks of the study are minor in comparison to the information to be gained on each individual subject and on the disorder as a whole.

14. Alternatives

The alternative is not to participate in this study.

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APPENDIX I: How to use Stopwatch

You will be provided an Oslo/Robic 30 lap memory stopwatch.

- A: LAP/RESET
- B: RECALL
- C: MODE
- D: START/STOP
- E: EL LIGHT



Press button **C** to go into lap mode (the word lap should be visible on the left upper corner of the LCD screen)

- A. For **Standing Balance Tests**, you will use a simple time measurement to mark the beginning and end of 10 or 30 seconds (depending on the position being tested).
 - 6. To start measurement, press **D** once, and say "GO."
 - 7. After 10 or 30 seconds, press **D** again, and tell participant to "STOP."
 - 8. To reset timer, press **A** once. You can begin at step 1 for the next position.
- B. For the **Chair Stand Test**, you will use a lap measurement to get time to completion of the fifth chair stand and the tenth chair stand.
 - 1. To start measurement, press **D** once, and say "GO."
 - 2. When participants stands up for the **fifth** time, press **A** once.
 - 3. When participant stands up for the **tenth** time, press **D** once.
 - 4. Record the time to completion of five stands by recording the split for lap 1. Press **B** (small round black button) once until the lap number in the parentheses in the left upper corner of the LCD screen reads 1. Record the time on the upper line of the display.
 - 5. Next record the time for completion of 10 stands. Press **B** again, and the lap number should read 2. Now record the time on the lower line of the display, this is the cumulative time, which represents the time from start to the completion of 10 stands.
 - 6. Once you have recorded all the information, you can reset the stopwatch and erase the lap memory by pressing **B** once then **A** once.

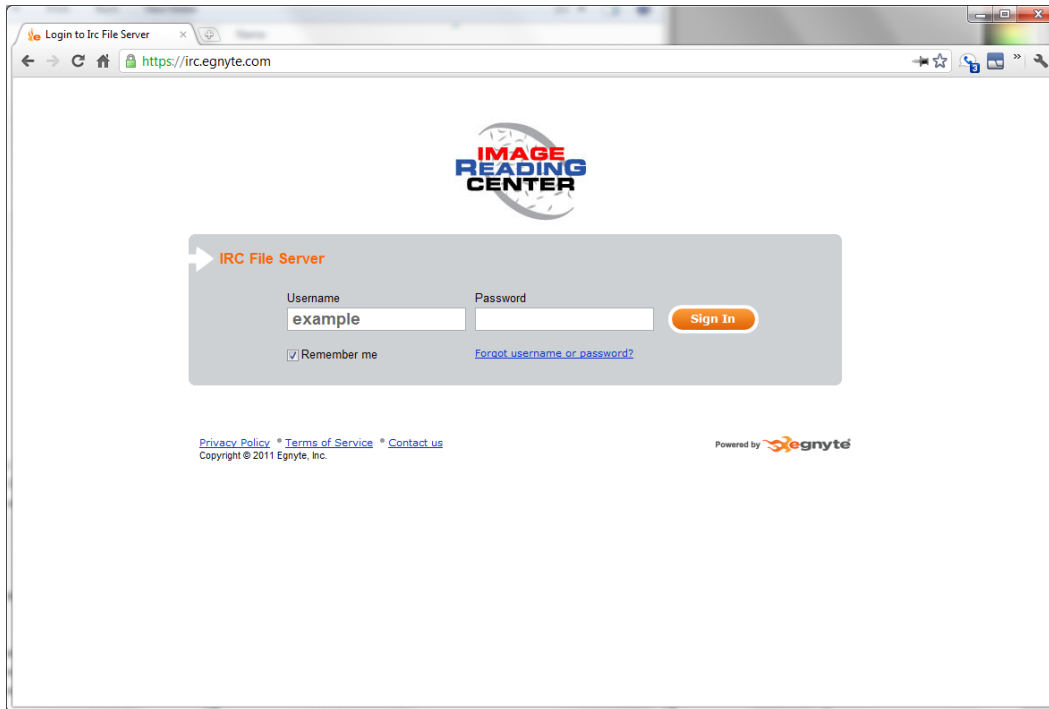
- C. For the **4-meter walk**, you will use a simple time measurement.
1. To start measurement, press **D** once, and say “GO.”
 2. At the first footfall past the 4 meter line, press **D** again.
 3. Record time on lower line of display.
 4. To reset timer, press **A** once.
- D. For the **400-meter walk**, you will use a lap measurement to get time for each individual lap (around the far cone and back to starting cone) and for completion of the 400 meters (completion of 20 laps).
1. To start measurement, press **D** once, and say “GO.”
 2. When participant completes the first lap, press **A** once.
 3. When participant completes the second lap, press **A** once. Continue to press **A** at the completion of each subsequent lap (19 times in total).
 4. At the completion of the final (20th) lap, press **D** once. The pattern for test should be: **D-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-A-D.**
 5. Record time for lap 1. Press **B** (small round black button) once until the lap number in the parentheses in the left upper corner of the LCD screen reads 1. Record the time on the upper line of the display for lap 1.
 6. Record the time for laps 2-20. Press **A** once. The lap number in the parentheses should now read 2. Record the time on the upper line of the display for lap 2. Press **A** again, and record the time for lap 3. Repeat until you have recorded splits for each of 20 laps.
 7. Lastly, record time for completion of 400-meters (20 laps). The cumulative time will be on the lower line of the display when the display is on lap 20 (parenthesis in left upper hand corner). Record this time for the time for completion of 400 meters.
 8. Once you have recorded all the information, you can reset the stopwatch and erase the lap memory by pressing **B** once then **A** once.

APPENDIX J: EGNYTE INSTRUCTIONS

IMAGE READING CENTER EGNYTE INSTRUCTIONS



1. Login to “IRC File Server” (<https://irc.egnyte.com>) using the credentials provided to you.



2. Setup Personal Information

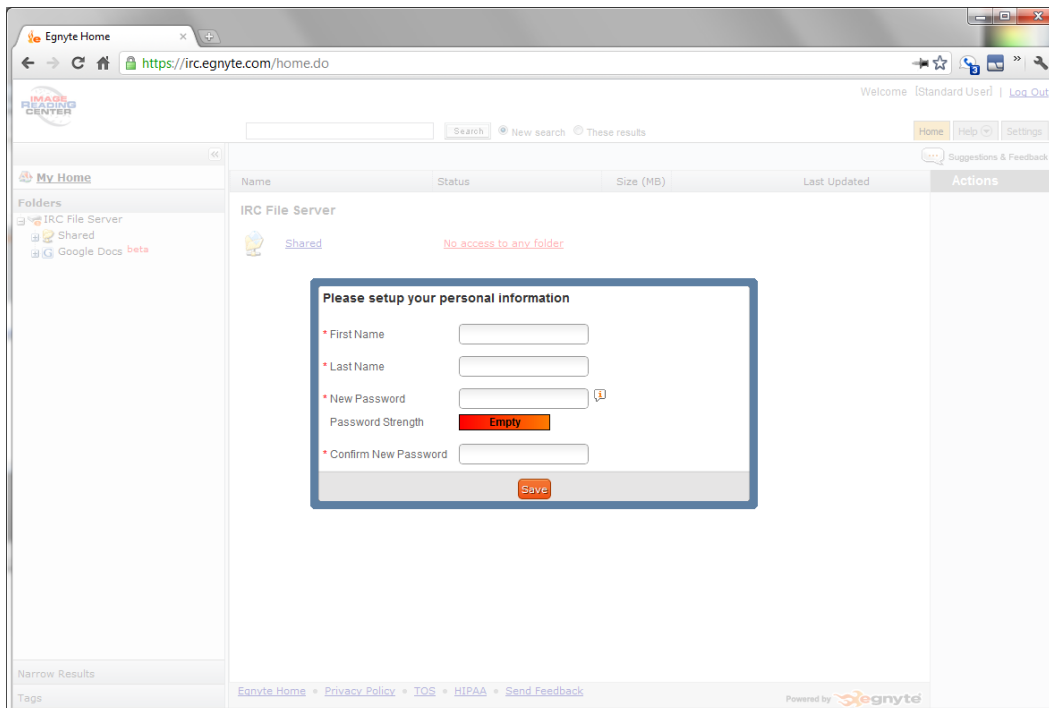
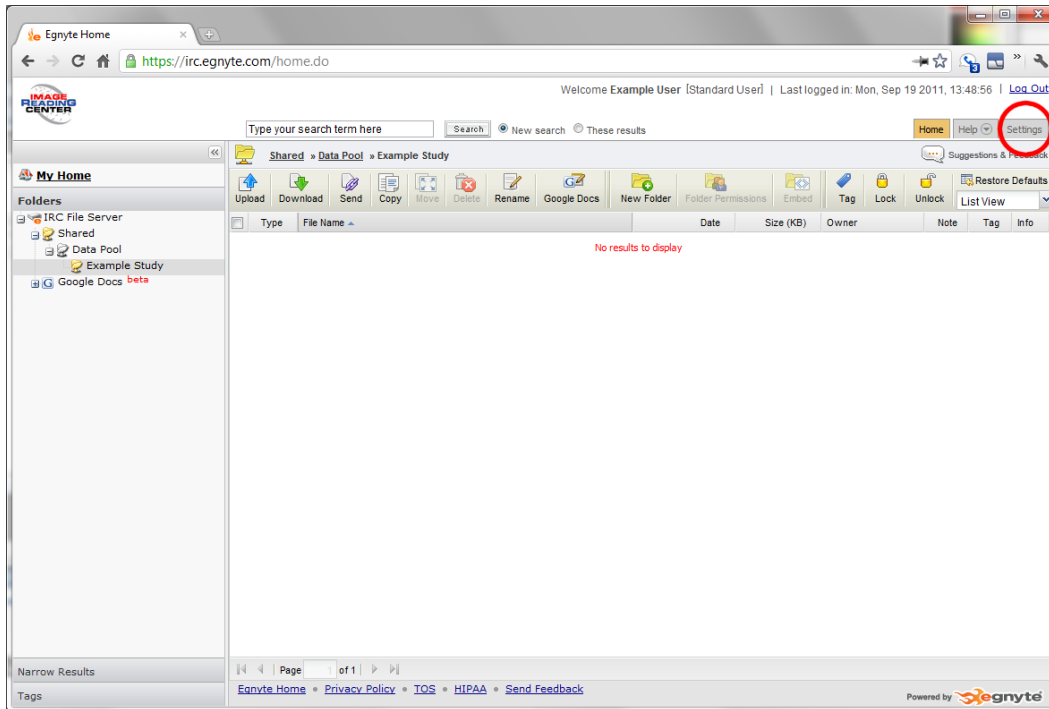


IMAGE READING CENTER EGNYTE INSTRUCTIONS



3. Click on “Settings”



4. Under the “File Management” Section, select “Use Multi-file & Folder Uploader as default for all file uploads” by ensuring there is a “check mark” in the selection box.

NOTE: You must have **Java** (<http://java.com/en/download/>) installed in order to use the **Multi-file & Folder Uploader**.

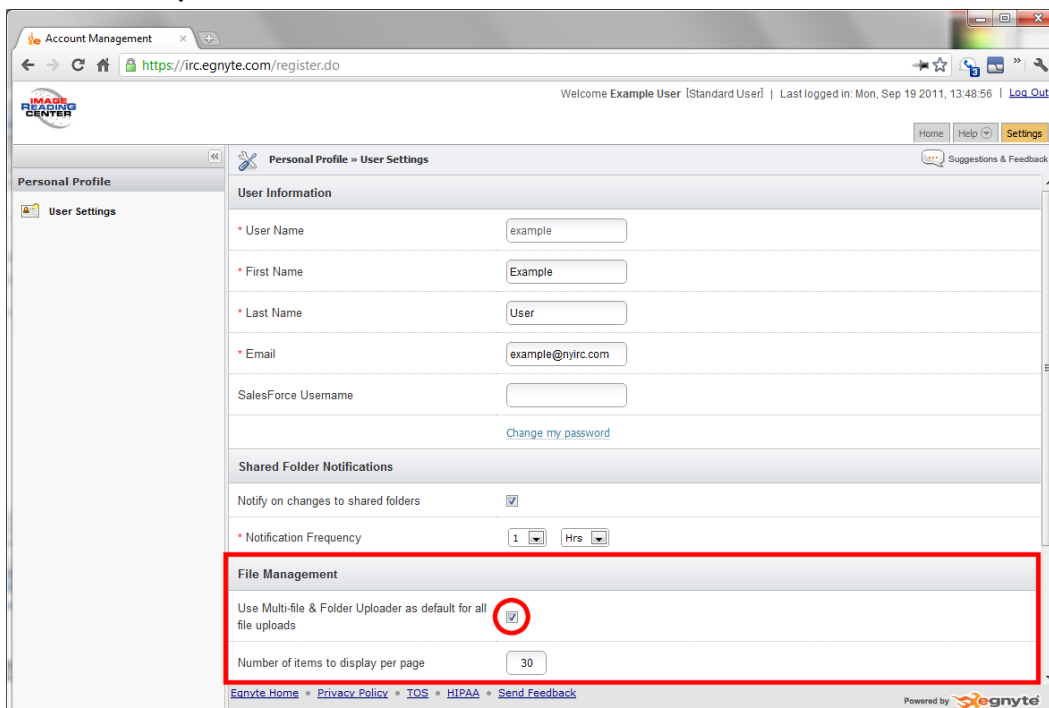
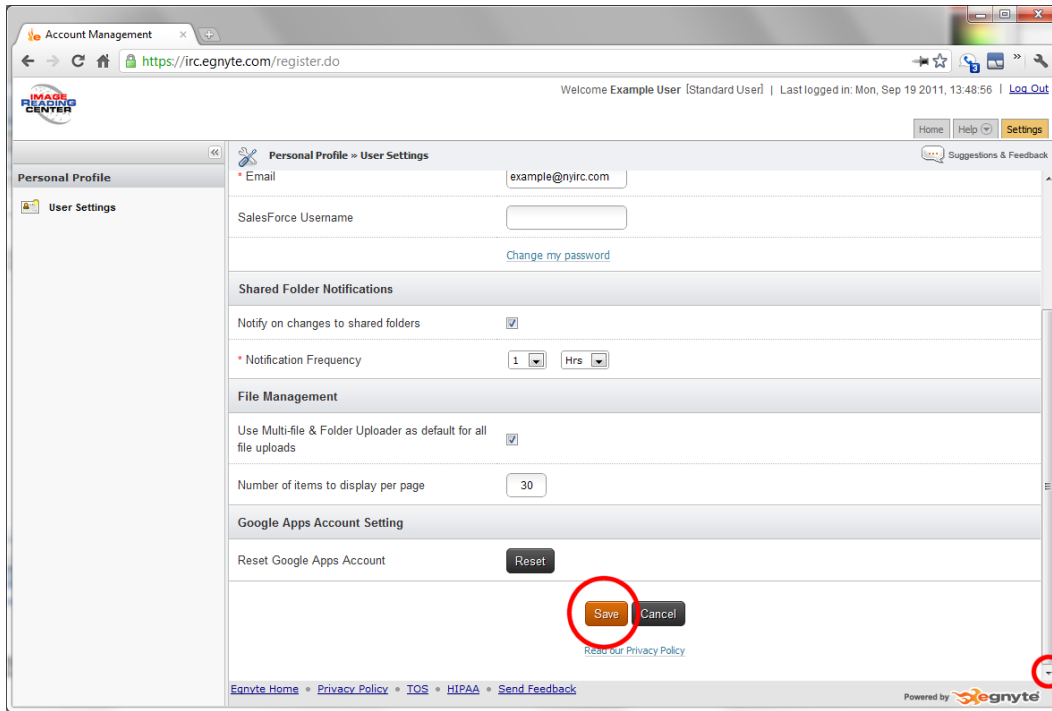


IMAGE READING CENTER EGNYTE INSTRUCTIONS



5. Scroll downwards and Click on “Save”



6. Click on “Home”

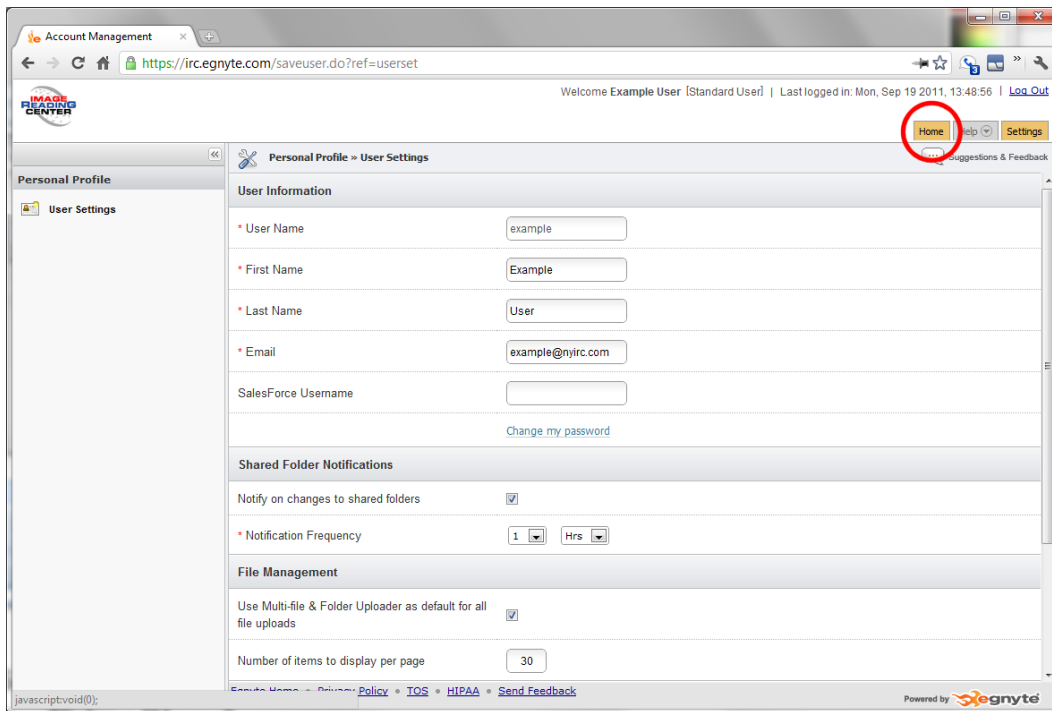
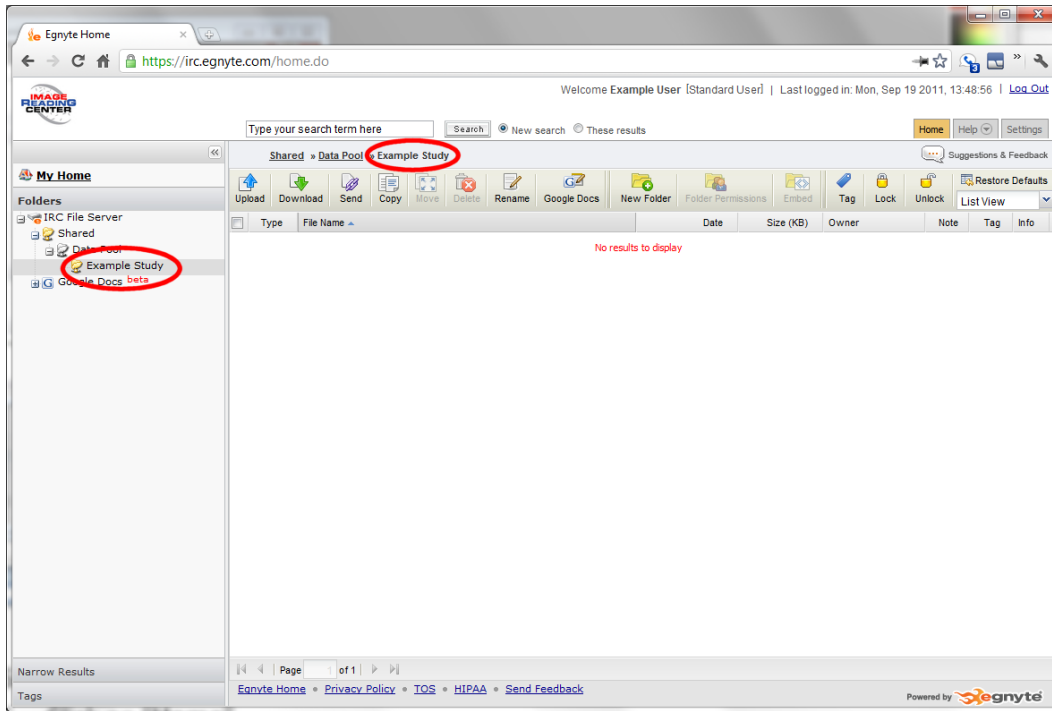


IMAGE READING CENTER EGNYTE INSTRUCTIONS



7. Make sure you are in the correct folder for your study.



8. Click on "New Folder"

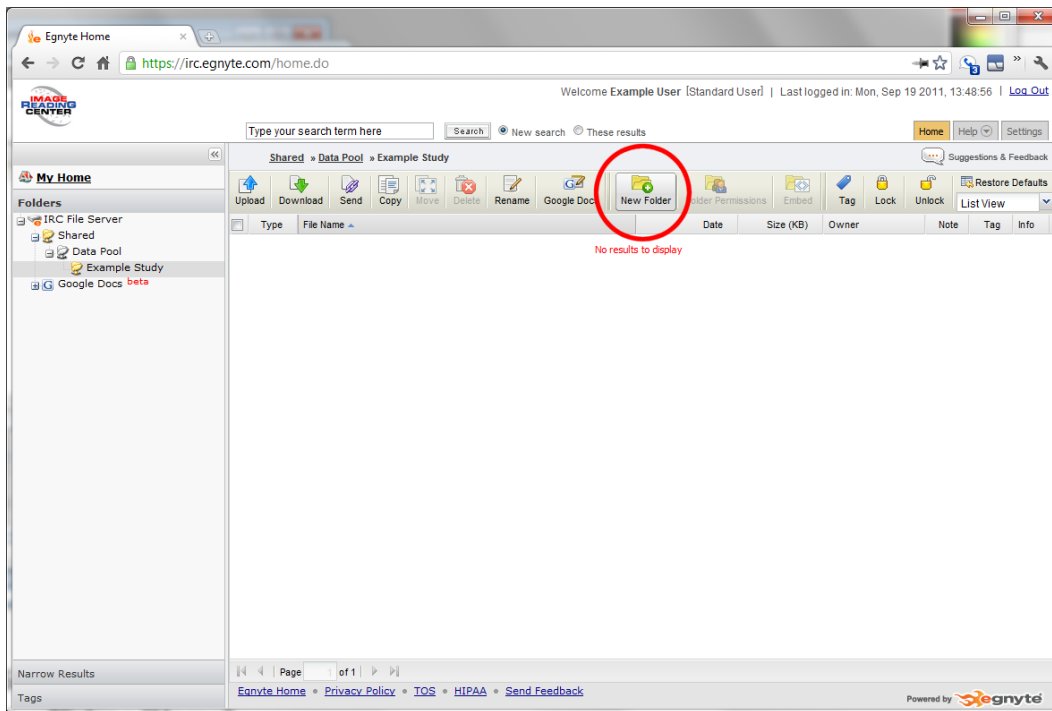
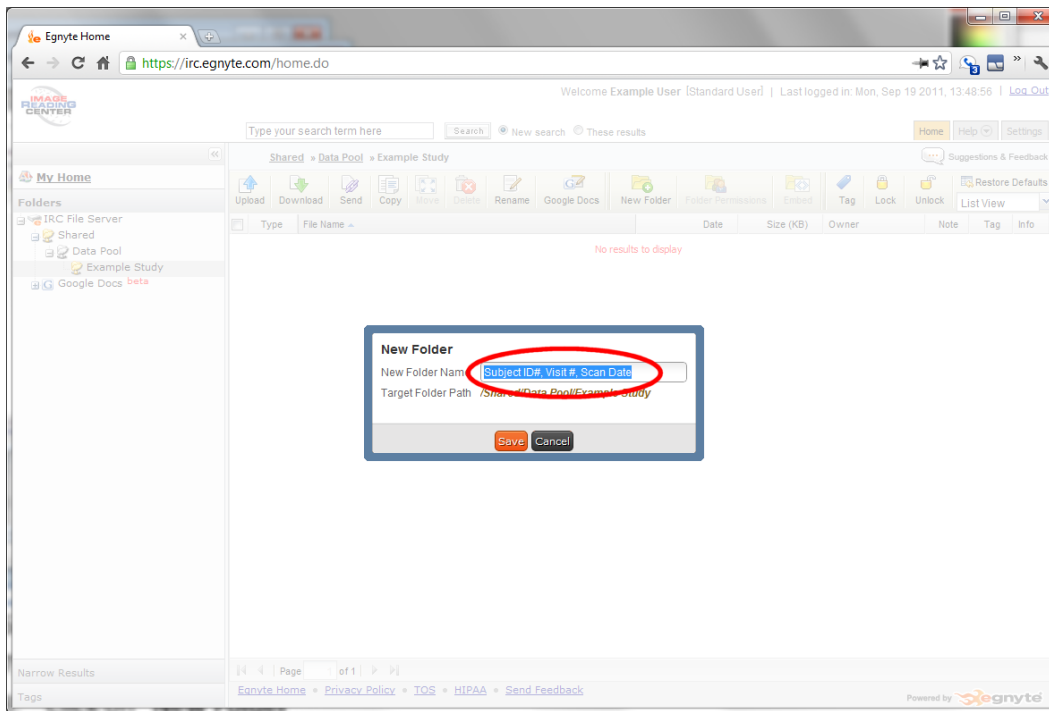


IMAGE READING CENTER EGNYTE INSTRUCTIONS



9. Enter the appropriate format of “Subject ID#, Visit #, Scan Date” for your study and Click on “Save”.



10. Navigate into folder of the scan that you are going to upload.
In this example: **001234-V12-20110919** (Subject: **001234**, Visit: **V12**, Scan Date: **20110919**)

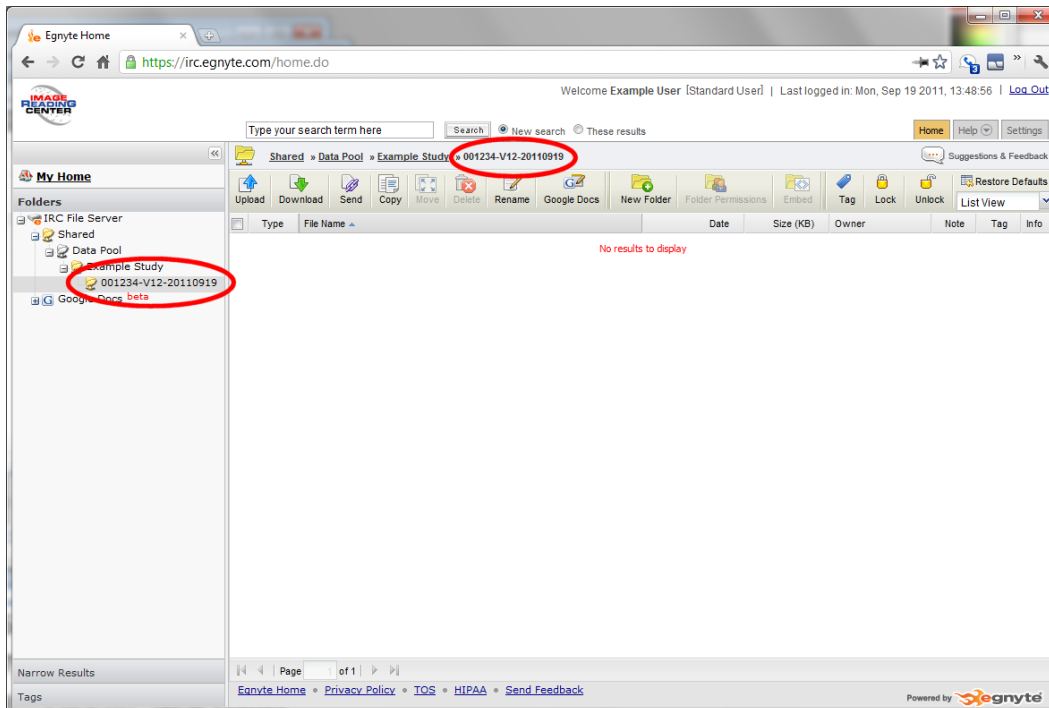
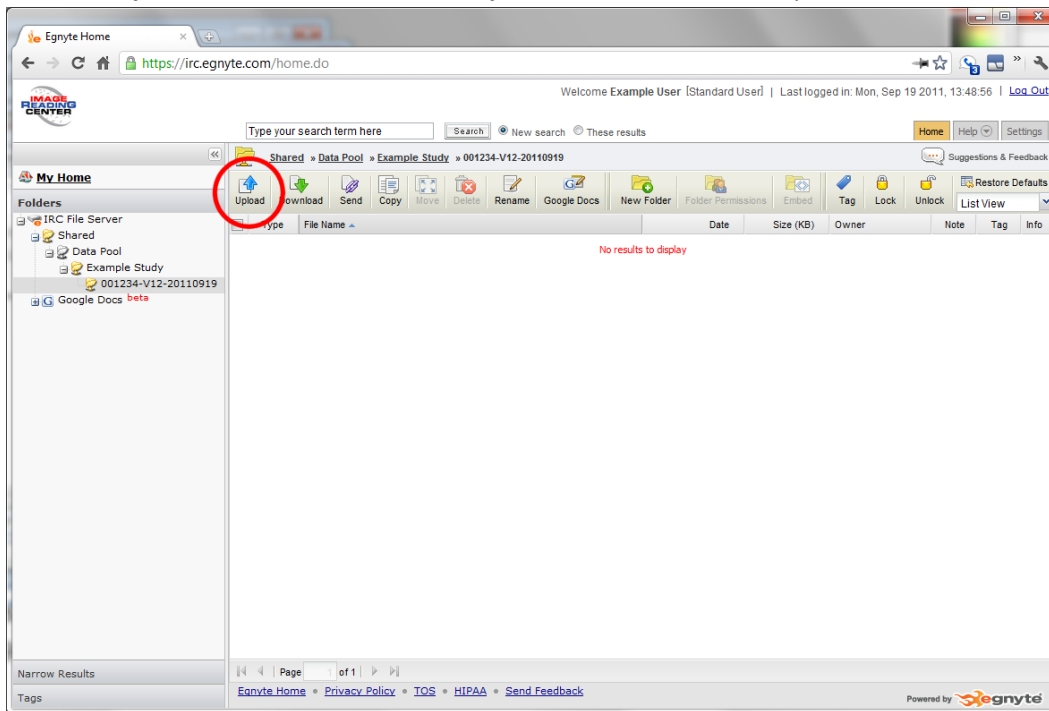


IMAGE READING CENTER EGNYTE INSTRUCTIONS



11. Click on **“Upload”**. **Multi-File & Folder Uploader** window should open.



12. Click on **“Select File(s)”**.

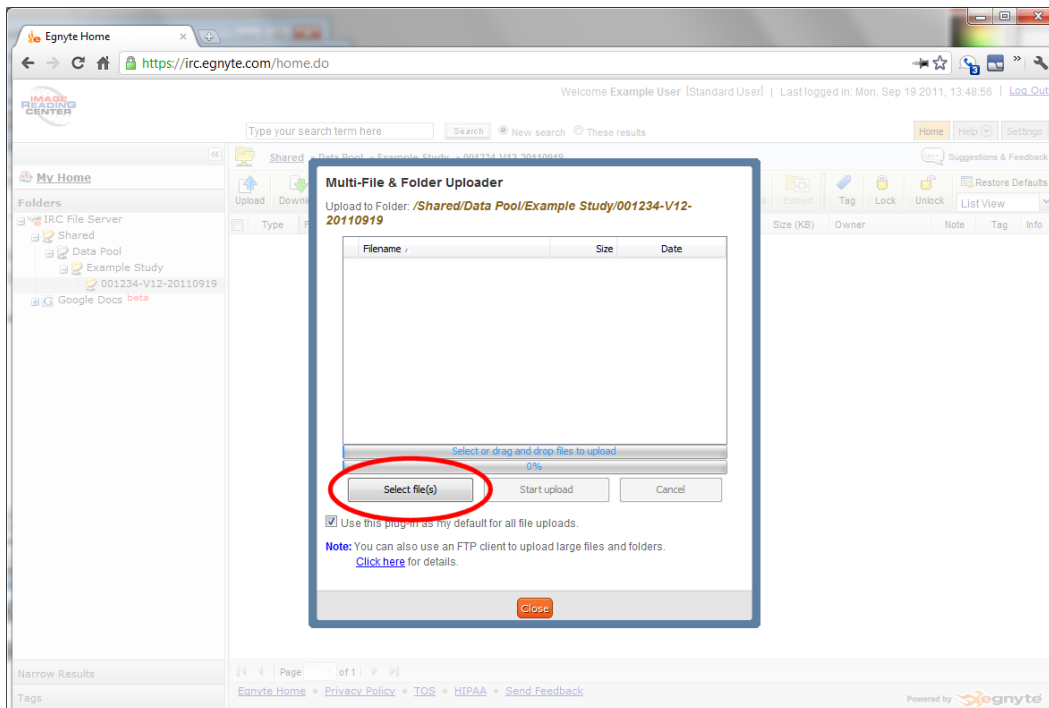
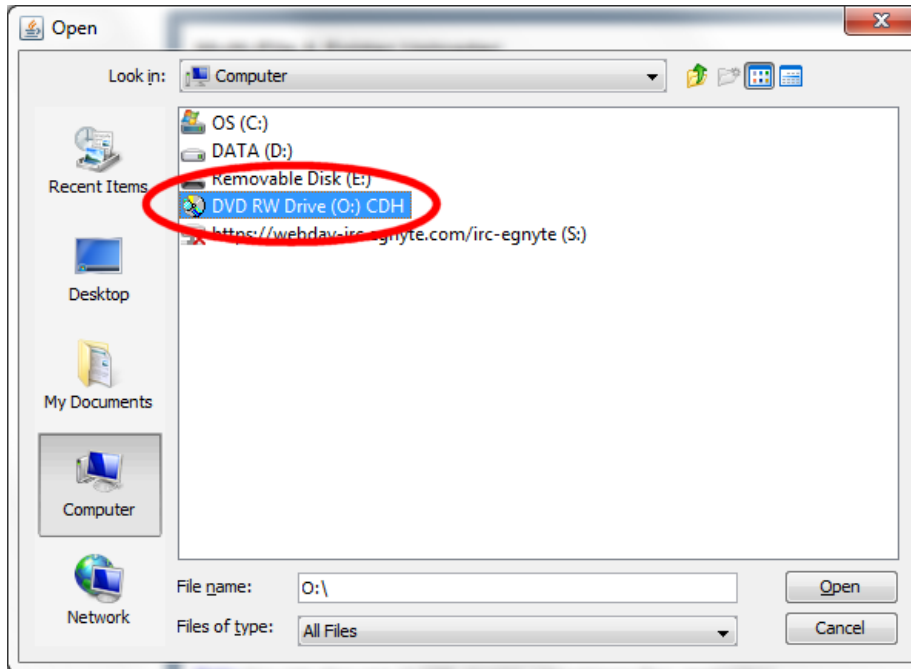


IMAGE READING CENTER EGNYTE INSTRUCTIONS



13. Select the appropriate Disk, Drive, or Folder where the scans are stored.



14. Select the appropriate Folder or Files that you would like to upload.
You may press and hold the "Ctrl" key to select multiple Folders and Files.

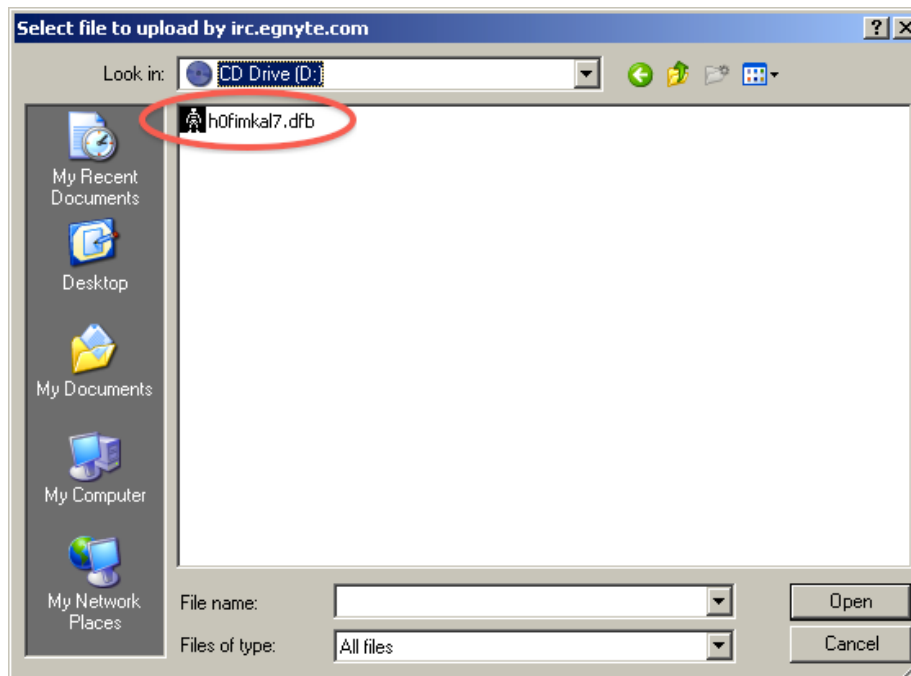
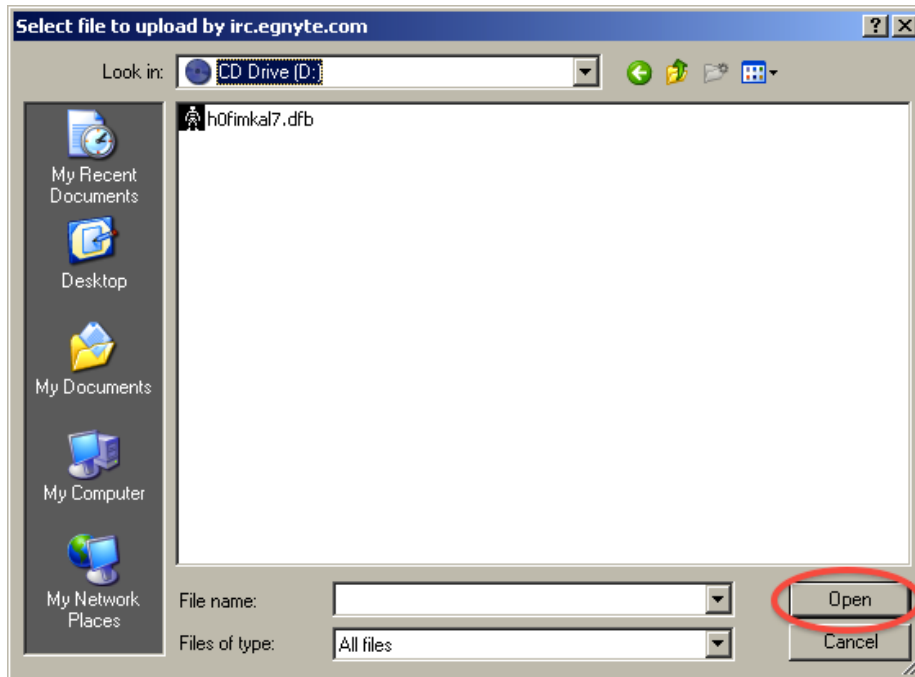


IMAGE READING CENTER EGNYTE INSTRUCTIONS



15. Click “Open”



16. Confirm that the Folder and Files that you would like to upload appear in the **Multi-File & Folder Uploader**.

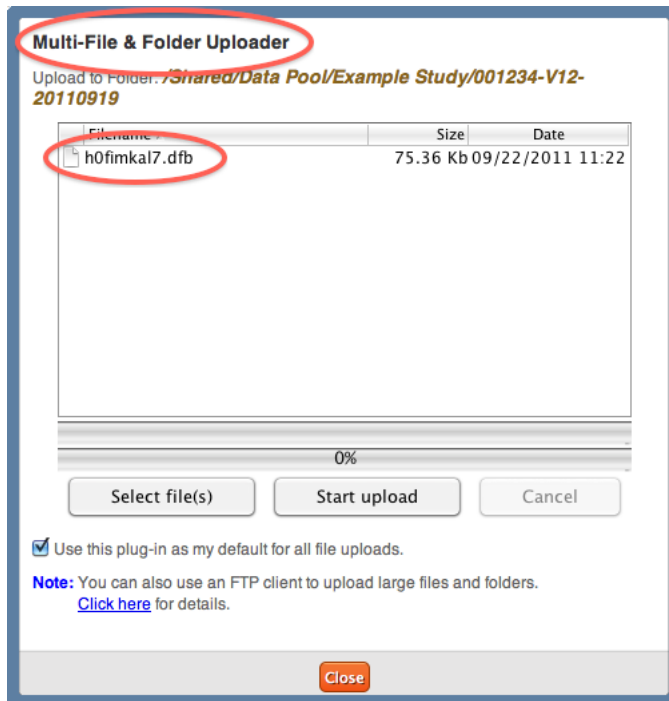
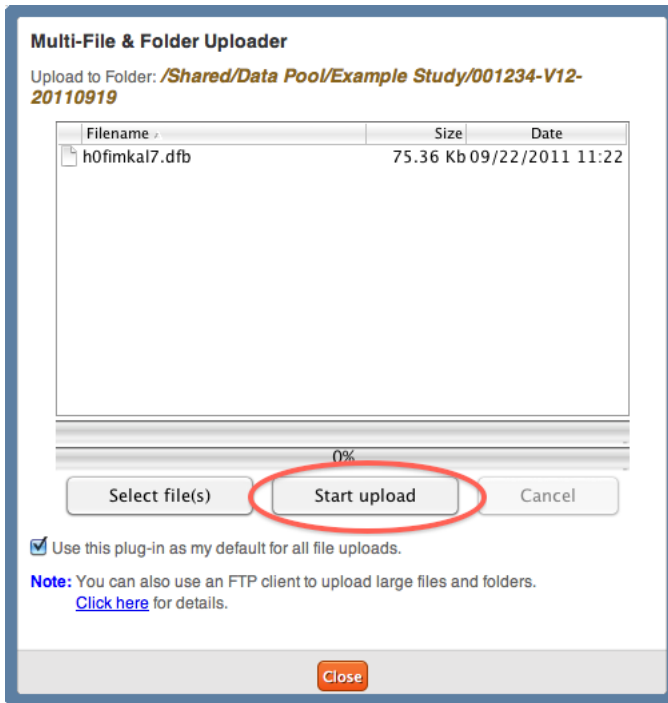


IMAGE READING CENTER EGNYTE INSTRUCTIONS



17. Click “Start Upload”



18. Confirm that “Upload progress” is proceeding.

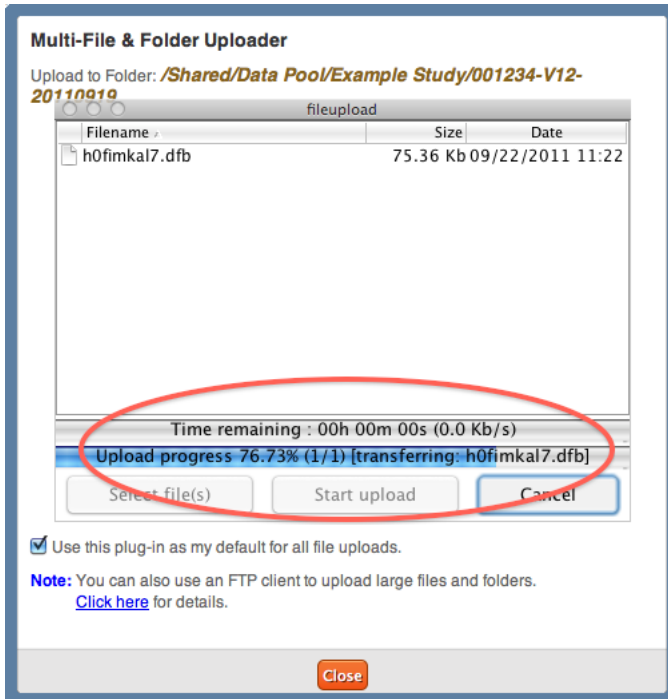
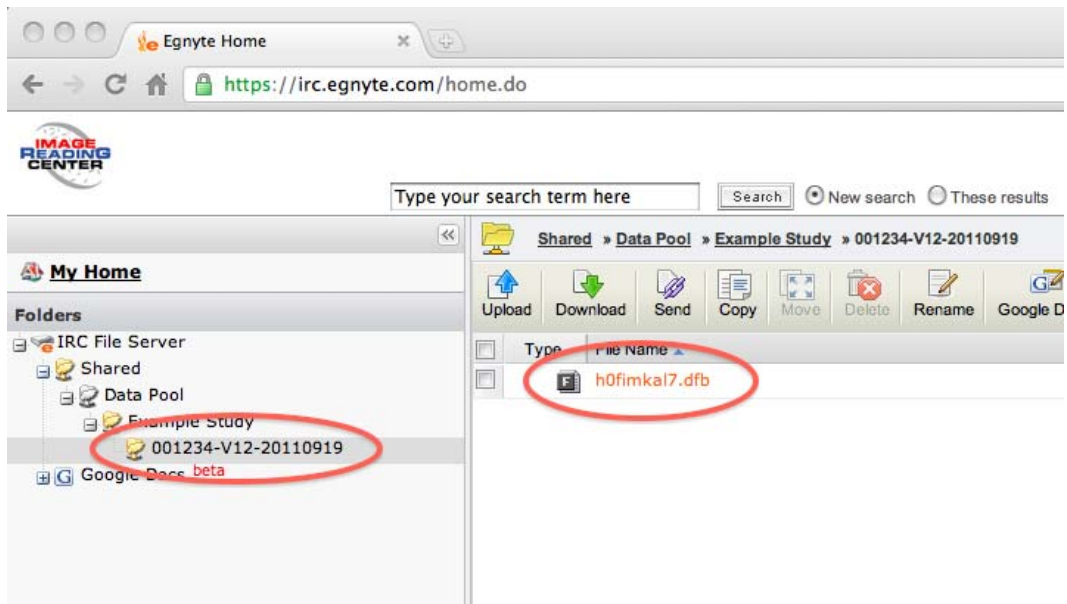


IMAGE READING CENTER EGNYTE INSTRUCTIONS



19. Once the upload is complete, confirm that the Folder and Files that you have uploaded appear in the Scan Folder



APPENDIX K: References

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