

THE WOMEN'S INTERAGENCY HIV STUDY

SECTION 25: METABOLIC SUBSTUDY (PROSPECTIVE)

A. STUDY PURPOSE

To examine the effects of HIV infection, antiretroviral therapy, and family history of diabetes, hyperlipidemia or cardiovascular disease on glucose tolerance, diabetes, hyperlipidemia, fat redistribution and osteopenia/osteoporosis in the WIHS cohort.

A subset of WIHS participants will have more detailed metabolic studies performed using DXA scanning, to quantify regional body fat and bone mineral density, and performance of an oral glucose tolerance test (OGTT). Details of these aspects of the study follow.

B. SPECIFIC AIMS

1. To determine the prevalence and incidence of abnormalities in fat distribution and bone density, using whole-body and regional (hip and spine) dual-energy x-ray absorptiometry (DXA) scanning in HIV-infected and HIV-uninfected women.
2. To evaluate glucose and insulin responses to a 75-gram glucose load in this same group of women over a two-hour time period.

C. HYPOTHESES

- **Hypothesis 1a:** Limb fat will be decreased in HIV-infected women before the initiation of antiretroviral therapy; limb fat will be decreased in HIV-infected women due to both HIV infection and antiretroviral therapy use; limb fat will decrease over time in HIV-infected women on antiretroviral therapy.
- **Hypothesis 1b:** Menopausal women not on hormone replacement therapy (HRT) will have higher arm fat, but not leg fat, than pre-menopausal women.
- **Hypothesis 2a:** Truncal fat will increase over time due to menopause in HIV-infected women on antiretroviral therapy.
- **Hypothesis 2b:** After controlling for menopausal status, truncal fat will be decreased in HIV-infected women compared to HIV-uninfected women.
- **Hypothesis 2c:** Effective antiretroviral therapy will lead to increased truncal fat.
- **Hypothesis 3a:** Fasting hyperglycemia, insulin resistance, and diabetes will be more prevalent in HIV-infected women than in HIV-uninfected women due to protease inhibitor and, to a lesser extent, NRTI use when controlled for truncal obesity and menopause.
- **Hypothesis 3b:** Fasting hyperglycemia, insulin resistance, and diabetes will be more prevalent in HIV-infected women in the presence of truncal obesity, when controlled for antiretroviral therapy use and menopause.
- **Hypothesis 4a:** Risk factors for osteopenia will include inflammatory markers, past use of corticosteroids, menopause and, in HIV-infected women, HIV itself and antiretroviral therapy use.
- **Hypothesis 4b:** Peripheral fat loss will be associated with osteopenia and osteoporosis.

D. RESEARCH DESIGN AND METHODS

1. STUDY DESIGN

The proposed research is a longitudinal study of 400 fasting WIHS women recruited from the San Francisco and Bronx WIHS sites that will begin during WIHS visit 18 (April 1, 2003, to September 30, 2003). The 400 women (200 women each from San Francisco and Bronx sites) will be studied over a five-year time period beginning at the baseline visit (held during WIHS visits 18, 19, 20 and 21), followed by a two-year follow-up visit (held during WIHS visits 22, 23, 24 and 25) and a five-year follow-up visit (held during WIHS visits 29, 30, 31). Women seen for baseline at visit 18 should receive follow up at visit 22; women seen for baseline at visit 19 should receive follow-up at visit 23, and so on.

An additional 100 women will be recruited from the Chicago WIHS site during visits 22 and 23 to increase the number of women studied at baseline to 500. The two-year follow-up of these women will occur at visit 27, and five-year follow-up will begin at visit 32.

Recruitment for the prospective Metabolic Substudy will consist of all eligible women up to 65 years who weigh less than 264 pounds, are no taller than 6'1", have not been pregnant or breast feeding in the past six months, and have not taken female hormones, anabolic steroids, or growth hormone in the past 12 months. Recruited women should also not have regularly taken catabolic steroids such as prednisone in past 12 months. All women who participated in the cross-sectional Metabolic Toxicity Substudy will be asked to participate in this study.

At the baseline visit, enrolled women will be categorized into one of the four groups listed below. Each quarter, the number of enrolled women in each targeted group will be assessed and recruitment at future visits will focus on women still needed to fulfill targets. We anticipate that the highest priority women to target will be HIV-positive pre-menopausal women not receiving ART, due to the overall smaller number of women available in this group. Efforts will be made to enroll all eligible Caucasian women.

During visit 19, an interim evaluation of enrollment status demonstrated under-enrollment of HIV-positive pre-menopausal women not on antiretroviral therapy. As a result, the criterion was changed to include women who had been on antiretroviral therapy in the past, but not in the past 36 months. It was decided that, if under-enrollment in this group continued, the criterion would be further relaxed to include HIV-positive pre-menopausal women who had been on antiretroviral therapy in the past, but not in the past 18 months.

The target number for each group at the Bronx and SF sites is listed below:

- a. HIV-infected, pre-menopausal, not receiving any antiretroviral therapy in the past 18 months (N=50)
- b. HIV-infected, pre-menopausal, receiving HAART (N=50)
- c. HIV-uninfected, pre-menopausal (N=50)
- d. HIV-infected, post-menopausal (N=50)

The Chicago site will contribute 25 women to each of the four groups.

TOTAL: 200 each from the Bronx and SF sites; 100 from the Chicago site.

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

If the target for any particular group is not met, WIHS participants from the Brooklyn site may be asked to enroll in the substudy to help attain the target number of enrollees. These women will have their DXA scans and GTT performed at the Bronx site. If the target number for a particular

group still cannot be met, we will over-recruit in the other three groups to reach the target number of a total of 500 women studied at baseline.

2. INCLUSION CRITERIA

Inclusion criteria for the prospective Metabolic Substudy will include:

- Enrollment and participation in the WIHS core study.
- Inclusion in one of the four groups described in Section D1, above.
- Willingness to undergo a DXA scan and OGTT at baseline and two years later.

3. EXCLUSION CRITERIA

Exclusion criteria for the prospective Metabolic Substudy will include:

- Women who have been pregnant or breastfeeding in the past six months.
- Women who have received exogenous female hormone therapy, including estrogen, progesterone, birth control pills, norplant, depo provera, birth control patch (Ortho Evra), and hormone replacement therapy for menopause in the past 12 months.
- Women who have received growth hormone and steroids on a regular basis in the past 12 months.
- 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.

4. SCHEDULING THE METABOLIC VISIT

Women will be informed of the Metabolic Substudy at their core WIHS visit and, if they express interest in participating and meet the eligibility criteria, will be called back within 63 days (if HIV-positive) or 94 days (if HIV-negative) of their core WIHS visit in order to schedule a Metabolic visit.

When calling a participant to confirm her appointment the night before her Metabolic 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 Metabolic visit. In addition, remind the participant that she must not take any calcium supplements the morning of her DXA scan.

The Metabolic visit will last up to three hours (approximately 2.5 hours for the OGTT, including set up time, and 0.5 hour for the DXA scan, including set up time). If the OGTT is to be performed in close proximity to the DXA scanner, the DXA scan can be performed between the 60-minute and 120-minute OGTT time points, thereby shortening the visit to 2.5 hours.

E. GTT PROTOCOL

Participants will be asked to fast for at least eight hours prior to coming in for the oral glucose tolerance test (OGTT). Therefore, every effort should be made to have the participant come in for her OGTT in the morning after an overnight fast.

Participants who are on antiretroviral therapy should take their usual scheduled antiretroviral dose, with water only, if the time to take the antiretroviral medication dose falls within the eight-hour fasting period. For example, participants who are prescribed antiretroviral medications to be taken in the morning should take them in the morning before their OGTT.

When scheduling participants for the OGTT visit, they should be told not to take **any other medications** during the eight-hour fasting period, **especially medications for diabetes**. If a participant is taking insulin, metformin or thiazolidinediones [such as avandia (rosiglitazone) or actos

(pioglitazone)] for her diabetes, she will not be eligible to participate in the OGTT portion of the Metabolic Substudy. Women who are taking insulin, metformin or thiazolidinediones should still fast overnight in order to have a single blood draw for glucose and insulin. If the participant is taking oral sulfonylureas for diabetes, she can participate in the OGTT, but should not have taken the medication during the eight-hour fast period.

Therefore, women who have diabetes, but are not on insulin or oral hypoglycemic agents including metformin or thiazolidinediones, can have an OGTT performed.

1. OGTT PROCEDURE

The OGTT procedure will consist of:

- a. Placement of a peripheral IV catheter for multiple blood draws over a two-hour period. A nurse will place a small intravenous catheter about 2 cm long into the vein on the dorsum of the participant's hand or arm. All subsequent blood samples will be drawn from this catheter.
- b. At the time of placement of the peripheral IV catheter, baseline bloods will be drawn for glucose and insulin. 2 ml of blood for glucose should be collected aseptically in a 3 ml sodium fluoride, potassium oxalate gray-top tube. Tubes should be filled completely, if possible. Gently invert the tube approximately five times after filling to make sure that anticoagulant and blood are well mixed.

Additionally, 4 ml of blood for insulin should be collected aseptically in a serum separator red-gray top tube (SST). Gently invert the tube approximately five times after collection to activate the clotting process. Place the tube in a vertical position for 30 minutes to allow for the clotting process to conclude. (See Manual of Operations, Section 10, for a detailed description of WIHS blood collection procedures.)

Beginning with visit 22 and concluding at visit 26, the serum used for insulin assay will also be used to perform a lipid panel. No extra blood need be collected.

Also beginning at visit 22 and concluding at visit 26, an extra tube will be collected one time only per participant for NMR Lipoprofile. Collection kits will be supplied by Liposcience, Inc.

- c. After the baseline bloods are drawn, the participant will be asked to drink a 75-gram glucose solution in its entirety. If the participant is unable to drink the glucose solution in its entirety, please reschedule the OGTT for another day.
- d. 30 minutes after the glucose solution is consumed, 2 ml of blood will be drawn for glucose in a gray top tube, and 4 ml for insulin in a serum separator tube. Please follow the same collection procedures for glucose and insulin as above.
- e. 60 minutes after the glucose solution is consumed, 2 ml of blood will be drawn for glucose only in a gray top tube. Please follow the same collection procedures for glucose as above.
- f. 120 minutes after the glucose solution is consumed, 2 ml of blood will be drawn for glucose in a gray top tube, and 4 ml for insulin in a serum separator tube. Please follow the same collection procedures for glucose and insulin as above.
- g. Record the actual time of each blood draw on the **MS01** (Specimen Collection Form).

2. PROCESSING OF GLUCOSE AND INSULIN

Blood samples collected at the SF GCRC will be processed on site at the GCRC lab and then sent to the UCSF AIDS Specimen Bank (Contact: Yvonne DeSouza: 415-476-2513; email: Yvonne.DeSouza@ucsf.edu) for storage and eventual shipping. Processed samples will be stored in standard boxes that allow for storage of 100 cryovial samples at -70° C at the GCRC lab, and then transported in the box on dry ice to the AIDS Specimen Bank quarterly by study personnel. Glucose and insulin samples will be stored at the AIDS Specimen Bank in separate boxes and

then sent to Quest Diagnostics-Baltimore quarterly. (See Section E3 below for shipping procedures.)

Blood samples collected at the Bronx site will be shipped immediately to Quest Diagnostics-Baltimore for real-time processing. (See Section E3 below for shipping procedures.)

Blood samples collected at the Chicago GCRC will be processed on site (i.e., centrifuged for 15 minutes) and then FedExed overnight to Quest Diagnostics-Baltimore for aliquoting and testing. (See Section E3 below for shipping instructions.)

a. Processing plasma from gray-top tubes for glucose testing:

- 1) Blood should be collected aseptically in a 3 ml sodium fluoride, potassium oxalate gray-top tube. Tubes should be filled completely, if possible.
- 2) Gently invert the tube approximately five times after filling to make sure that anticoagulant and blood are well mixed.
- 3) Tubes do not need to be centrifuged immediately, but instead can be transported overnight as whole blood to another lab for processing. If the ambient temperature is above 75° F, samples should be transported to the processing lab on -20° C frozen cold packs. When using cold packs, make sure that the blood does not come into direct contact with the cold pack.
- 4) If the blood is not sent to a processing lab, centrifuge the blood with a horizontal rotor (swing-out head) at 1100 x g for 15 minutes.
- 5) Aliquot plasma (minimum needed for assay: 1x0.2ml for each timepoint of plasma) into a plastic 2 ml screw cap cryovial tube. (Use cryovials that have a white writing space on the tube in order to insure that the labels stick better to the tubes at -70° C.) Standardize labels according to the LDMS format. (Please see Section 31 of the WIHS Manual of Operations for details.) If plasma is sent batched to Quest Diagnostics, then store the labeled cryovial tubes in standard white sample boxes that can hold 100 samples and label the box: "WIHS Metabolic Substudy Glucose samples."
- 6) Freeze specimens at -70° C.

b. Processing serum from serum separator tubes for insulin testing:

- 1) Blood should be collected aseptically in an SST (serum separator) tube.
- 2) Gently invert the tube approximately five times after collection to activate the clotting process.
- 3) Place the tube in a vertical position for 30 minutes to allow for the clotting process to conclude.
- 4) Filled SST tubes should be kept at room temperature and centrifuged within six hours of collection in a horizontal rotor at 1100 x g for 15 minutes.
- 5) If the ambient temperature is above 75° F, the samples should be transported to the processing lab on -20° C frozen cold packs. When using cold packs, make sure that the blood does not come into direct contact with the cold pack.
- 6) Aliquot serum (minimum needed for assay: 1x0.5ml for each timepoint of serum to run the insulin tests; however, at baseline, at least 2-1.0ml aliquots are needed to allow processing of the lipid panel – direct LDL from one aliquot and processing of insulin from the other aliquot) into plastic 2 ml screw cap cryovial tubes. (Use cryovials that have a white writing space on the tube in order to insure that the labels stick better to the tubes at -70° C.) Standardize labels according to the LDMS format. (Please see Section 31 of the WIHS Manual of Operations for details.) If plasma is sent batched to Quest

Diagnostics, then store the labeled cryovial tubes in standard white sample boxes that can hold 100 samples and label the box: “WIHS Metabolic Substudy Insulin samples.”

7) Freeze the specimens at -70° C.

3. SHIPMENT OF GLUCOSE AND INSULIN SAMPLES

Plasma for **glucose** processing and serum for **insulin** processing will be shipped in dry ice using an overnight carrier to Quest Diagnostics-Baltimore:

Quest Diagnostics, Inc.
ATTN: Department of Virology
1901 Sulphur Spring Road
Baltimore, MD 21227

Phone: (410) 536-1713

Fax: (410) 536-1474

Contacts: William Meyer, Larry Hirsch, Denise Bopst

Boxes sent to Quest Diagnostics should be labeled with “[Site name], MS, [Box number, increasing consecutively across ALL shipments for Metabolic Substudy samples]” on the top and side of the box lid, in addition to the side of the box bottom holding vials. MS boxes can be sent with Cardiovascular Substudy monthly shipments. Be sure that the boxes are marked with “MS” to ensure proper billing. It is also advisable to send box-specific manifests because the manifest format for these is slightly different than the manifest for the Cardiovascular Substudy specimens.

The following fields must be included in the manifest:

- 1) Unique vial identifier
- 2) WIHSID
- 3) WIHS visit number
- 4) Date of collection
- 5) Time point of collection
- 6) WIHS Specimen code (Plasma should be “50” for 00 timepoint; “51” for 30 minute timepoint; “52” for 60 minute timepoint; “54” for 120 minute timepoint. Serum should be “55” for 00 timepoint; “56” for 30 minute timepoint; “59” for 120 minute timepoint.)
- 7) Volume
- 8) Quest Diagnostics, Inc., test number (Glucose plasma: 67777W; Insulin serum: 15701E)
- 9) Protocol Number (“MS”)
- 10) Box
- 11) Row
- 12) Column

The following fields can be provided in the manifest, but are not required:

- 13) LDMS primary, additive and derivative codes
- 14) Custom, site-specific identifier

4. RESULTS OF LABORATORY DATA (GLUCOSE, INSULIN)

Laboratory data from Quest Diagnostics-Baltimore will be sent to the Bronx, Chicago and San Francisco sites. Data will be transcribed onto WIHS form MS02 and entered into Apollo.

F. NMR LIPOPROFILE

NOTE: NMR LipoProfile WILL BE PERFORMED ONLY AT THE BASELINE VISIT FOR CHICAGO SITE AND AT THE FIRST FOLLOW UP VISIT FOR THE BRONX AND SF SITES. SAMPLES WILL BE COLLECTED AT THE 5-YEAR FOLLOW UP AND STORED FOR TESTING.

At the time the baseline blood is drawn for the OGTT, please also draw additional blood in the plasma separator tube provided in the kit from Liposcience, Inc. The Liposcience kit has everything you will need to successfully submit samples for testing. All sites will be provided eight kits initially. Each kit includes instructions, specimen bags, EDTA tube, an ice brick, insulation and protective packaging, and preprinted prepaid shipping labels and bags. **You must use the EDTA gel barrier collection tube provided by Liposcience, Inc.** If additional kits are needed, please send an email to: Claudia Ponath at cponath@itsa.ucsf.edu.

Please note that at the five-year follow-up visit, blood should be drawn in regular EDTA collection tubes.

1. PROCESSING OF NMR LipoProfile

Remove ice brick from kit and freeze for 12 hours.

Complete a requisition form for each sample.

a. Processing plasma from purple and yellow-topped vacuette EDTA gel barrier collection tube:

- 1) Blood should be collected aseptically in the purple and yellow-topped vacuette EDTA gel barrier collection tube provided by Liposcience, Inc. Tubes should be filled completely, if possible in order for the preferred plasma volume of 500µl to be obtained. Please note that plasma specimens drawn in any other gel barrier collection tube will be rejected, due to interfering substances that make them unsuitable for NMR analysis.

At the five-year follow-up, use regular purple-topped EDTA collection tubes.

- 2) Gently invert the tube to mix contents.
- 3) EDTA specimens should be centrifuged immediately or kept refrigerated and centrifuged within 4 hours. Centrifuge specimens at 3,000 rpm for 10-15 minutes. Centrifuged collection tubes can be kept refrigerated for up to 4 days before shipping.

At the five-year follow-up, plasma should be aliquoted and stored for future testing.

- 4) Prepare collection tubes for shipping using the specimen bags provided by Liposcience. Place each collection tube in its own bag with the corresponding requisition placed in the front pouch of the bag. Only one requisition and patient collection tube should be included per bag.

At the five-year follow-up, plasma should be aliquoted and stored for future testing.

- 5) Lay specimen bags in Styrofoam box and cover with a frozen ice brick. Replace the Styrofoam top, place the Styrofoam box in the cardboard box, and tape the cardboard box shut, place the entire unit inside the plastic shipping bag. Call FedEx for pickup 1-800-463-3339, option 1.

At the five-year follow-up, plasma should be aliquoted and stored for future testing.

2. SHIPMENT OF NMR LipoProfile

The EDTA specimen should be shipped refrigerated using an overnight carrier **on the day of collection** to Liposcience Clinical Research Services.

At the five-year follow-up, frozen plasma will batch shipped to Liposcience and stored for future testing.

Liposcience Clinical Research Services
2500 Sumner Boulevard
Raleigh, NC 27616
Phone: 919-212-1999

Ship samples Monday through Thursday only. Blood collected on a Friday should be refrigerated, but must be shipped within four days of collection. The research contact person at Liposcience, Inc., is: Shannon Lancaster at slancaster@liposcience.com.

However, for any questions regarding resupply of kits, please contact Claudia Ponath at cponath@itsa.ucsf.edu.

3. RESULTS OF LABORATORY DATA

Hard copy results of the NMR Lipoprofile will be sent to sites by standard mail within one week of processing the blood. Electronic laboratory data from Liposciences will be sent to WDMAC every six months.

G. DXA 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 DXA scanning acquisition procedures between the three clinical centers participating in the WIHS Metabolic Substudy. The success of the DXA portion of the Metabolic 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 Metabolic 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 Metabolic Substudy. The IRC will certify that each DXA technologist is qualified via expert review of five DXA scans each of AP spine, proximal femur 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 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 A) 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 procedures of the Metabolic Substudy should be directed to the Image Reading Center (IRC):

Elli Joannidou, CDT (ATTN: WIHS)
Image Reading Center
1090 Amsterdam Avenue, Room 14K
New York, NY 10025

Tel: (212) 523-2098 or (646) 736-2979

e-mail: elli@imagereadingcenter.com

Other questions regarding the Metabolic 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 PORTION OF THE WIHS METABOLIC SUBSTUDY

The dual x-ray absorptiometry (DXA) scans to be performed at the Metabolic Substudy visits will be AP spine, left hip and whole body. DXA 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 scans to be performed on each participant.

Exams	Baseline	2- year Follow-up	5- year Follow-up
	Visits 18, 19, 20, 21, 22 Code 00	Visits 22, 23, 24, 25, 27, 28 Code 24	Visits 29, 30, 31, 32 Code 60
DXA AP Spine	X	X	X
DXA Left Hip	X	X	X
DXA Whole Body	X	X	X

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

Elli Joannidou, CDT (ATTN: WIHS)
Image Reading Center
1090 Amsterdam Avenue, Room 14K
New York, NY 10025

Tel: (212) 523-2098 or (646) 736-2979

e-mail: elli@imagereadingcenter.com

3. CREATION OF NEW DATABASE

Create a WIHS 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.

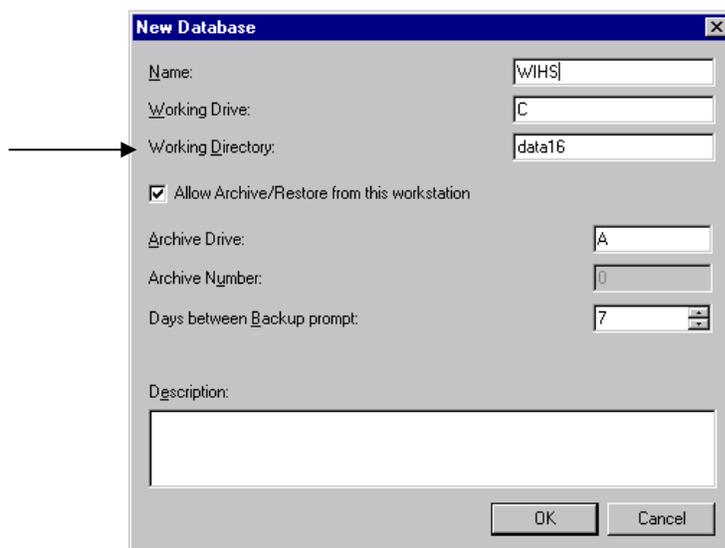


Figure 1. New Database.

4. NEW PARTICIPANT REGISTRATION

This section describes in detail the specific procedures to be conducted for the DXA portion of the Metabolic 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 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 Metabolic Substudy Participation Notification Form (MSNOTI). Enter the **site number** (Bronx 001; San Francisco 002; Chicago 003) plus the participant’s **eight-digit WIHSID** in the *Patient ID* field. For this reason, you should always obtain the WIHSID number 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, 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; please note the dashes)
Patient ID:	Site number (Bronx 001; San Francisco 002; Chicago 003) plus participant’s WIHSID (e.g., 0031-23-4567-8)
Birth Date:	Participant’s date of birth
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 (0) and not the letter "O" when entering the WIHSID into the *Last Name*, and *Patient ID* fields.

Figure 2A. New Patient Directory.

Figure 2B. Secondary Page.

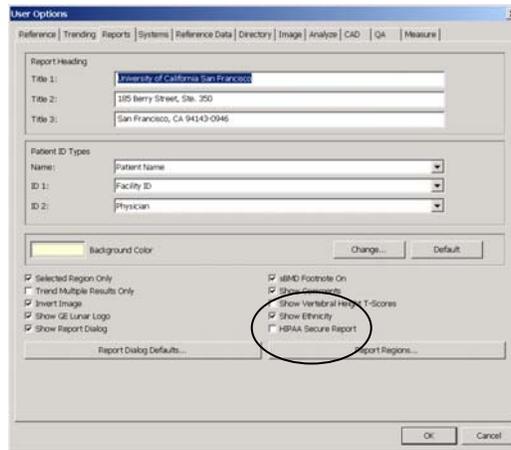


Figure 2C. HIPAA Field.

5. DXA 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 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 d. Whole Body Hemi-scans and Figure 6, below.

Please note any problems with scan acquisition in the comment field of the Participant Data Log (WIHS Form MS03) 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 and analysis 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 (see Figure 3).
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.

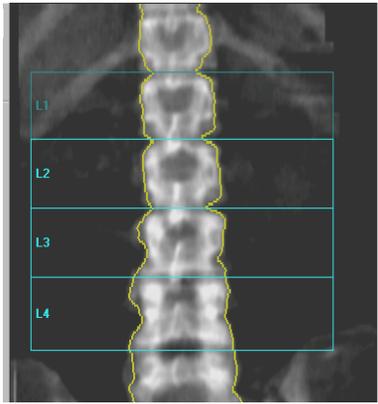


Figure 3. Properly Acquired and Analyzed AP Spine Scan.

b. Hip scans

The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper hip scanning and analysis 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 (Appendix A) 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 (see Figure 4).
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.

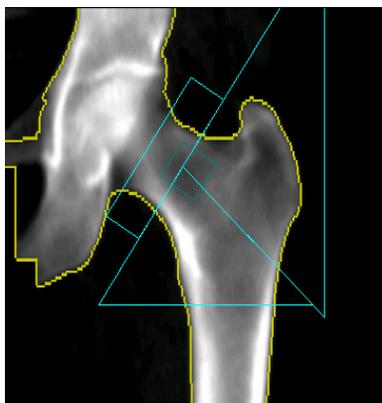


Figure 4. Properly Acquired and Analyzed Proximal Femur.

c. Whole Body scans

The official GE/Lunar Prodigy Operator's Manual should be consulted for the proper whole body scanning and analysis 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 MS03) 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 (see Figure 5).
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 6, 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.

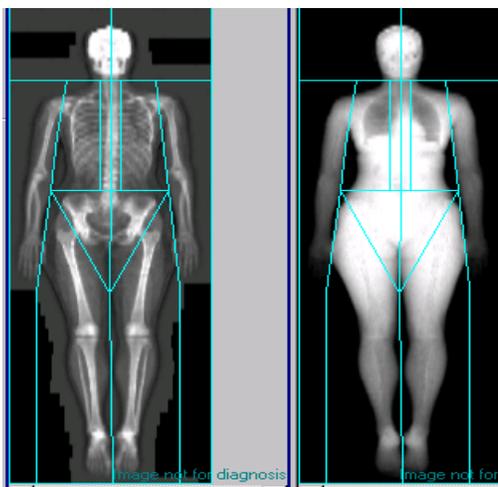


Figure 5: Properly Acquired and Analyzed Whole Body Scans.

d. Whole Body Hemi-scans

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

1. Ask the participant if any metal implants are present and note presence on Participant Data Log (WIHS Form MS03) 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 6.). **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 6). 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.

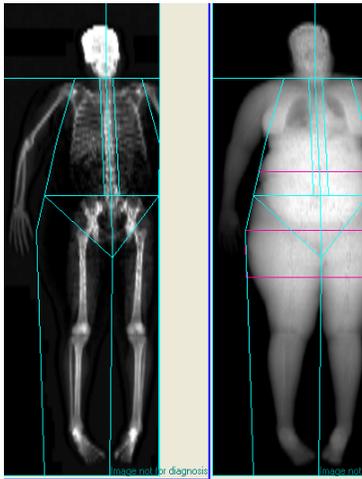


Figure 6: Properly Acquired and Analyzed Whole Body Scans.

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 diskette, SuperDisc or CD-R. (SuperDisc is a high capacity storage medium, recommended because of the volume of participants and QC data. Both participant and QC data can be copied to this disc.) Do not use the **Archive** command.

a. To copy scan images to the diskette/SuperDisk/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 7, 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 8, Browse for Folder) to send scans to the diskette/SuperDisk. Repeat until all scans for all desired participants have been copied to the diskette/SuperDisk. 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 8).

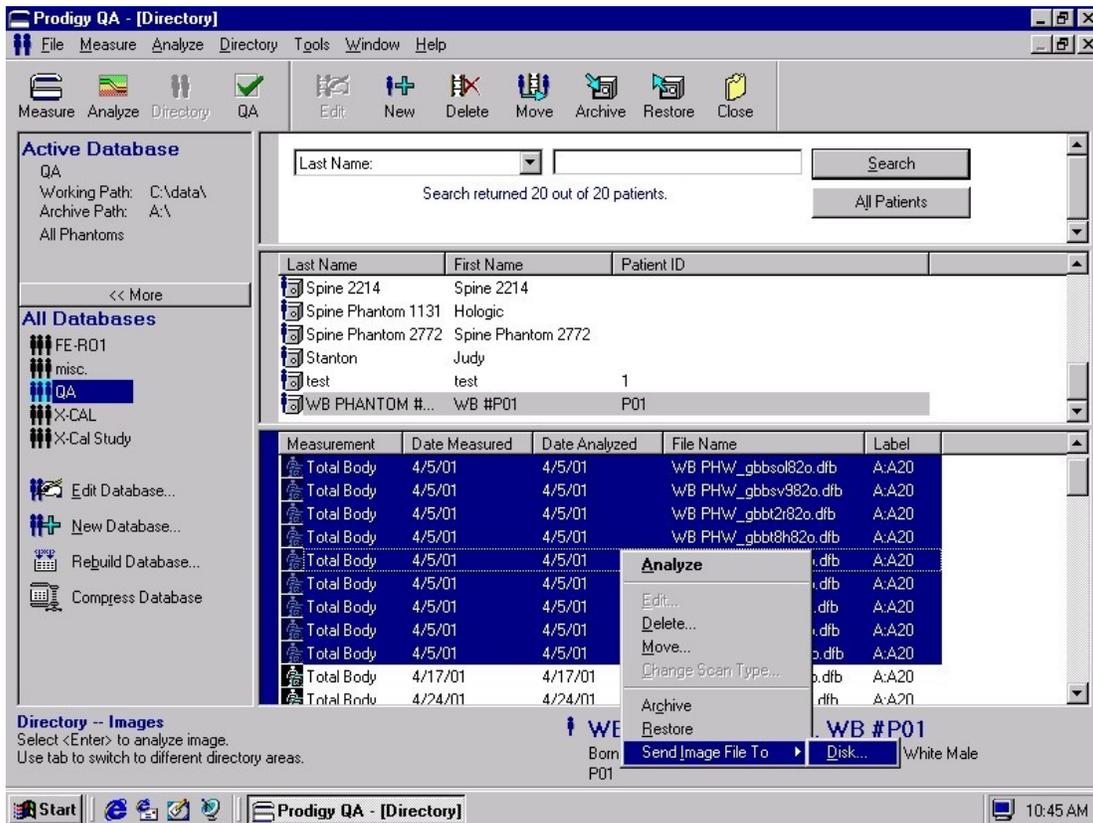


Figure 7. Directory – Example of Choosing Scans from the Database.



Figure 8. Browse for Folder.

b. Labeling copy diskettes

Each diskette/SuperDisc/CD-R must be labeled (see Figure 9), 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:	___/___/___
	<small>DDMMYY</small>
Diskette #	_____ of _____

Figure 9. Data Label for Diskettes/SuperDisc/CD-R.

c. Baseline and follow-up participant data

Participant data for all visits will be copied onto diskette/SuperDisc/CD-R, batched, and sent by the Project Director to IRC **weekly via FedEx**. Each diskette/SuperDisc/CD-R must be labeled with the appropriate study label provided by the Project Directors (see Figure 9).

d. Archive

The **Archive** function on the Prodigy scanner is used to store scan files onto floppy diskettes, SuperDiscs 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/SuperDisc/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/SuperDisc/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 A. 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 A) to IRC. Approval or denial will be issued using IRC-specific forms.

c. Operator qualification

It is the responsibility of the study site and WDMAC 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 A) 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 10, Database Directory). This will appear as a folder when you open the "C" drive.

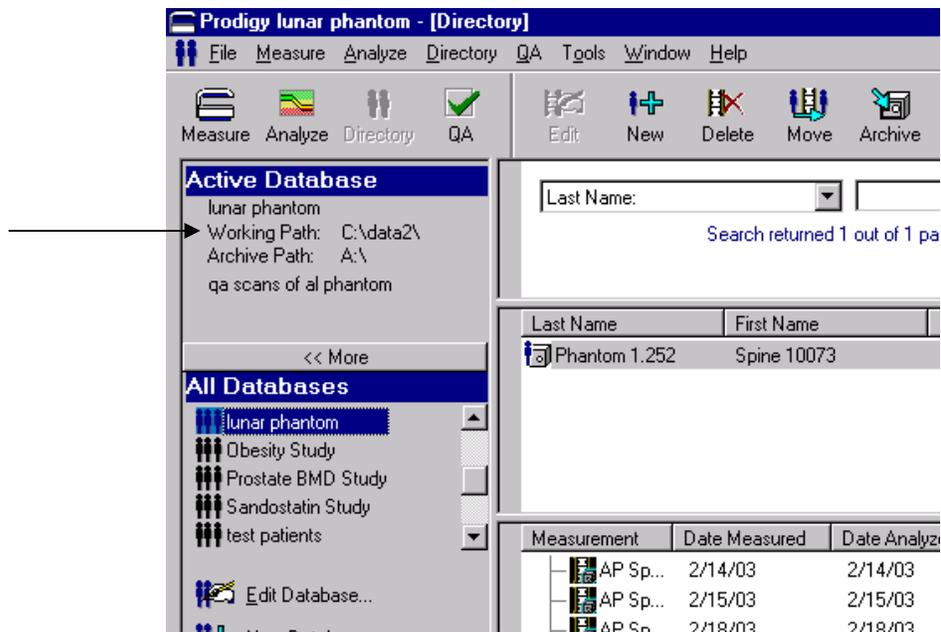


Figure 10. Database Directory.

- Exit from Prodigy to Windows.
- Place diskette/SuperDisc/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 11, 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 12, 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/SuperDisc in the “A” drive.
- When the send procedure is complete, remove diskette/SuperDisc from “A” drive.
- End procedure by going to “File” menu and selecting “Close.”

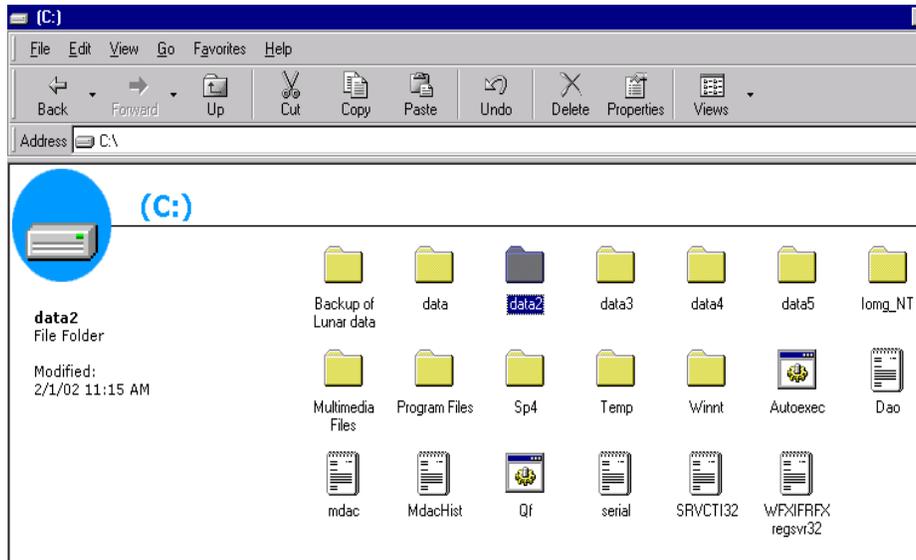


Figure 11. Folders in the C:\ Drive.

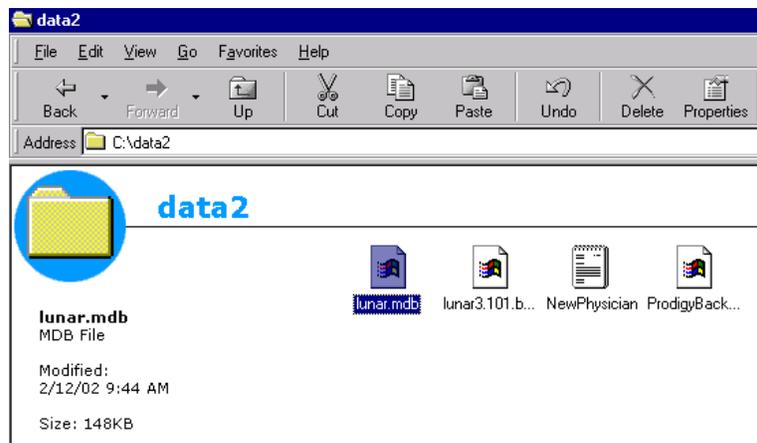


Figure 12. Lunar MDB Document.

e. Archive and data shipments

After the MDB has been copied, please label (see Figure 13) 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:	__ / __ / __
	<small>DDMMYY</small>
Diskette #	_____ of _____

Figure 13. QC Data Transfer Label.

Table 2.0 Frequency of data transfers from the site to IRC

	Baseline	2-Year Follow-up Visit	5-Year Follow-up Visit
Participant Data	Weekly (batched)	Weekly (batched)	Weekly (batched)
Quality Control Data	Monthly copy of QC Spine Phantom and Maintenance Record Forms, and copy of the Master Spine Phantom MDB		

H. DONATION OF REMAINING ALIQUOTS FROM PK AND METABOLIC STUDIES TO ACSR

1. STORAGE AND SHIPMENT OF SPECIMENS

Any aliquots not sent for testing should be held locally until test results have been received in case back up specimens are needed at the testing lab. After test results have been received, extra aliquots not needed for testing may be donated to the ACSR. Specimens from the San Francisco and Chicago sites should be sent to the UCSF Specimen Resource. The UCSF ACSR address is the following:

Amy B. Narvaez
 Staff Research Associate
 AIDS Immunobiology Laboratory / AIRL
 AIDS and Cancer Specimen Resource / ACSR
 University of California San Francisco / SFGH
 SFGH
 1001 Potrero Avenue
 Bldg 100, Room 333 E
 San Francisco, CA 94110

Email: anarvaez@acsr.ucsf.edu

Tel: 415-206-5510

Fax: 415-206-3765

Prior to shipping specimens to UCSF, please call the lab at (415) 206-5510 and e-mail Amy Narvaez at anarvaez@acsr.ucsf.edu to inform the lab of the pending shipment and tracking number. In addition, an electronic manifest in Excel format should be e-mailed to Eileen along with the notification of shipment. See Section G.2 for details on manifest preparation. Also see Appendix B for notification format.

Specimens from the Bronx site should be sent to the East Coast AIDS and Cancer Specimen Bank at George Washington University Medical Center. The EC ACSB address is the following:

East Coast AIDS and Cancer Specimen Bank
Department of Pathology
George Washington University Medical Center
2300 I Street, NW

Phone: (202) 994-0434 or (202) 994-2530

No specimens should be shipped on Friday. Prior to shipping specimens to GWUMC, please call the Bank at (202) 994-0434 or (202) 994-2530 to inform them of the pending shipment and tracking number.

2. SHIPMENT MANIFESTS

Each shipment must be accompanied by a manifest indicating its contents (specimen list), date of shipment, conditions and destination. The specimen list should be prepared in the same format used for shipments to BBI, and printed out in a column format (e.g., Excel). Columns 4 through 9 (FREEZER and RACK) and 14 through 23 (FREEZER ID) should be left blank. Boxes should be numbered consecutively, starting with the first shipment and continuing in subsequent shipments. For example, if the first shipment consists of box 1 and box 2, the box number in the second shipment should start with 3. Additionally, a cover letter should be sent with the specimens stating that these specimens are being donated to the ACSR, the total number of specimens included in the shipment, and a contact person with phone and pager number.

3. SPECIMEN PACKING, BOX LABELING AND INVOICES

Samples should be sent on dry ice in a specimen storage box. The name of shipping site should be clearly marked on the outside of the specimen storage box.

All specimens should be packed with enough absorbent material to contain the sample volume in case of a leak. Each box should be rubberbanded. Box(es) should then be placed in a leak proof bag and then packed in dry ice. The fiberboard boxes containing the specimens should be labeled with the following information:

- the city of origin
- the date of the shipment
- the study name: WIHS PK specimens
- Box #

Specimens should be packed and shipped according to IATA regulation 650 for diagnostic specimens packed on dry ice.

APPENDIX A: DXA FORMS

The following forms are available in Appendix A:

- **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 the 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, to Elli Joannidou at IRC. 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 002; Chicago 003)

Site Personnel (please complete with the names of anyone 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



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 (Metabolic 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 (212) 749-2305 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 (Metabolic 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 (Metabolic 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 (212) 749-2305 Att. 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 B: NOTIFICATION OF SHIPMENT FORM FOR METABOLIC SUBSTUDY
DONATIONS TO ACSR**

WOMEN'S INTERAGENCY HIV STUDY

Notification of Shipment: Metabolic Substudy donations to ACSR

I. Sender Details

(Fax this form one day prior to shipment)

Site Name: _____
Contact Person: _____
Phone #: _____
Fax #: _____
Email address: _____
Project: _____

II. Recipient Details

Amy B. Narvaez
ACSR / SFGH
1001 Potrero Avenue
Bldg 100, Room 333 E
San Francisco, CA 94110

415-206-5510
415-206-3765 (FAX)
anarvaez@acsr.ucsf.edu

III. Shipment Details

(To be completed by sender)

Courier: _____
Airbill #: _____
Date shipped: _____
Number of Boxes: _____
Number of Vials: _____
Amount of Refrigerant: _____ kg

IV. Comments

