4/15/89

STUDY OF OSTEOPOROTIC FRACTURES (V2)

Protocol for Urine Specimens (Bone Loss Cohort)

1. Introduction

The purpose of urine samples is to identify women who lose bone most rapidly. The amount of calcium in the urine may be a measure of the rate of bone loss. Kidney function can also be measured from the amount of creatinine excreted into the urine over a measured amount of time. Decreased kidney function may lead to rapid bone loss and osteoporosis. Other markers of bone loss are being developed; we will preserve the samples for several years until the best potential markers have been developed.

2. Equipment

The coordinating center will provide the following items:

- 150 urine hats: "American Specipan" from Baxter Hospital Supply, catalogue # 134465.

- 3 graduated cylinders: Pyrex brand calibrated "to contain" graduated cylinders. Fisher Supply, catalogue # 08-562-5D.

- Brushes for cleaning graduated cylinders: Graduated cylinder brushes, Fisher Supply, catalogue # 03-560.

- Color cap inserts (green) for the cryotubes containing urine.

3. Preparation of participants

Food changes the amount of calcium in the urine. Therefore, all participants must not eat anything after dinner until the urine specimen has been collected in the clinic. Water is permitted. If the participant breaks her fast before collection is complete, collect the urine and keep record the lack of fasting.

Participants taking insulin are excluded from this test.

To increase the amount of urine all participants should drink at least 16 oz of water within the first hour of the clinic visit. They should drink 8 oz as soon

as they come into the clinic and another 8 oz during the first hour of the collection period. There is no limit on the amount of water they can drink; this does not interfere with the tests.

4. Collecting the timed specimen

Timing is very important. The participant starts the collection time by completely emptying her bladder. This time should be recorded to the nearest minute. Ideally, this first voiding is done in the clinic. Alternatively, some subjects can do it at home **if they write down the exact time of voiding and bring the time with them.** Enter the exact time on the"Urine Specimen" form.

Urine will be collected in "urine hats" set in the toilet. **After at least 2 hours,** a staff member should place the hat in the toilet and the participant should go to the bathroom and empty her bladder into the hat. She does not need to clean her urethra and should **not** let any of the urine spill into the toilet. If urine is spilled, the collection should be repeated. After the participant has voided, a staff member retrieves the hat and measures the urine by pouring it into the graduated cylinder.

A few participants may need to urinate more than one time during the 2 hour collection period. Each time they urinate, the urine must be collected in a hat and the volume measured and recorded. Keep the urine from each collection and combine at the end of the collection period. When they have urinated after 2 hours, the collection period may stop. The urine volume recorded on the form is the total for all collections during the 2 hour collection period.

The time of the end of the collection period must be recorded to the nearest minute on the Urine Specimen form. Figure the total number of minutes (at least 120 minutes) and enter on the form as "Total collection time." If a collection period lasts longer than 2 hours. That is fine, so long as the exact amount of time is recorded on the form.

5. Measuring, freezing and shipping the urine specimen

The amount of urine voided should be measured in the graduated cylinder to the nearest 2 ml. The total amount collected during the collection period should be entered on the "Urine Specimen" form. Most participants will have 50-200 mls. If a participant has more than 250 ml, measure the first 200-250 ml in the cylinder, write down the volume and discard and then measure the remaining urine.

Take a 4 ml sample for storage in the cryotube. For participants who void more than one time during the collection period, be sure the sample is taken after combining all the collected samples for that person. Pipette about 3.6 ml of urine into same cryotubes as used for serum and fill them in the same way, leaving at least 10% unfilled to prevent breaking the tubes during freezing. Use a green cap insert to mark the urine tube.

If more than about 5cc of urine is spilled in handling prior to taking the sample, throw out the urine and begin the collection period again.

The urine sample in the cryotube should be frozen at -20° C as soon as possible (within 4 hours) to prevent growth of bacteria that could interfere with measurements. The urine tubes should be labelled, stored at -20° C, just as you do with serum, and shipped with regular shipments to BRI. (Altogether, each participant in the "bone loss" study will have four tubes: 2 serum, one white cell, and one urine).

We are not using preservatives in the urine (to prevent growth of bacteria) because we will freeze it within 4 hours of collection. The urine needs no special treatment or preservation for the measurements we plan.

Rinse the cylinders with water several times between uses in a single day. Scrub the cylinders in a fast acting cleaner and solvent, such as Kler-Ro powder, at the end of each day.

Urine Specimen Form

Participant ID # _____

Name code _____

Time of first voiding: ___ ___ : ___ ___

Time of last voiding: ___ ___ : ___ ___

TOTAL COLLECTION TIME: ___ ___ ___ minutes

URINE VOLUME: ___ ___ ___ mls

SOF MEMORANDUM 187

To: Osteon Operators
From: Peter Steiger
Date: May 2, 1989
Re: OSTEON SCAN PROCEDURE FOR BONE LOSS STUDY

Please use the following procedure when performing Osteon scans for the bone loss study. Refresh your memory by reviewing memos 25,43,55,61,88 and 136.

1. Identify all previous scans based on the information found in your file prior to a participant's visit.

2. Identify the Osteon archive disks that contain the previous scans. Print-out all previous scans using software version 1.3. This is necessary because version 1.3 generates different results for the radius than our previously used software version 1.2.

3. If you have multiple scans on a participant, select the one that is best according to the guidelines set forth in our earlier memos (see above) as the "baseline scan".

4. Review the print-outs of the baseline scan and verify the side scanned for radius and os calcis. Verify visually by looking at the distal radius print-out that the side scanned actually corresponds to the side logged (cf. Memo 88). Note that the ulnar length printed out on the report of the baseline scan is the number you have to enter on the current scan. This number does not have to be translated with the ulnar length table.

5. Perform the current scan following the guidelines set forth in our earlier memos (see above). Make sure you scan the same bones and use the same ulnar length.

6. Generate a detail report. Refer to the Osteon Manual to refresh your memory on how to generate reports.

7. Send all print-outs to me on a biweekly basis; the first mailing should be around May 7.

Please do not hesitate to call me at (415) 476-9805 if you have any questions.

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SOF Memorandum 180

Date: 4/13/89

To: Project Directors and Clinic Coordinators

From: Michael Nevitt, PhD

Re: **Bone Loss Study Update and Miscellaneous**

I. A few comments on protocols and procedures for the bone loss study.

1. Urine protocol: You should have received your urine collection supplies from us by now. If not, please call Diana Tanaka immediately.

After voiding to begin the two hour collection period, all urine must be collected until the collection period is over. If a ppt voids at home and records the exact time, but voids again before reaching the clinic, the collection period must be started over by having the ppt void at the clinic, record the time, and begin a new two hour collection period. If the ppt needs to void before the two hour collection period is over, that urine must be collected and saved!

Remember, if ppts begin the collection period by voiding at home before coming to the clinic, they must write down the exact time (to the minute) and bring the written time with them to the clinic. If the time is not written down, you must start the collection period again.

The overnight fast should last at least 10 hours. So, a ppt who begins her collection period at home at 7:30 AM can have a snack up to about 9 PM. Ppts whose collection period begins later in the morning can have an even later evening snack to help carry them through the morning.

To avoid spillage, do not have ppts carry the full urine hat from the toilet to your lab area. A staff member should retrieve the hat from the toilet. Use the covers to help prevent spillage.

2. Questionnaries: You should have received the new questionnaire for the bone loss study by now. If not, call Sarah. Use the same questionnaire for your morning (bone loss) and afternoon (regular protocol) participants. Someone mentioned that ppts might be confused by the box on the face page to check if the ppt is the "Bone Loss Study". If you are concerned about this, don't check the box until after reviewing the questionnaire in the clinic.

3. Serum protocol:. The red cryotube cap insert in the supplies that we sent you is for the tube containing the extra serum we are collecting for the bone loss study (using the baseline protocol). Put all four tubes from the bone loss ppts together in the storage box and ship to BRI as usual.

We have a supply of cryotubes here and will ship them to you toward the end of Visit Two as needed.

4. Flexicurve: Please stop doing the flexicurve measurements when you start the bone loss study. We should have enough data by then.

5. List of questionnaires, tests, and exams to include for bone loss study ppts.

Bone loss study take-home questonnaire
Clinic Interview
QDR
Osteon of heel and forearm
Urine Protocol (1cryotube)
Serum (2 cryotubes)
White cells (1 cryotube)
Height, weight, and wrist circumference
BIA
Reflexes
Reaction time (hand only)
Grip strength
Quad strength on BodyMaster
Measured Walk*
Trailmaker

*Measured Walk: Although you will not be doing the Video portion of the walks for bone loss ppts, **continue doing the timed and counted walks** (i.e. Usual Pace two trials and Rapid Walk one trial).

6. Afternoon ppts not in the bone loss study will receive all of the above plus the other items in the current protocol, excluding the flexicurve, urine, extra tube of serum, and Osteon.

7. If you have not received your source for the Osteon by Friday, April 14, call Peter Steiger.

8. Do not enter data for bone loss ppts now. You will receive another data system update for entry of bone loss data.

II. A few miscellaneous protocol issues

1. BIA: Starting now, please return to doing two sets of BIA measurements, just as you did at the beginning of the study, by putting electrodes on both right and left feet. Some examiners have continued to note discrepancies greater than 20 in resistance readings. Having an extra set of measurements as a check provides assurance that the measurement is being made correctly. Since it only adds a couple of minutes, it is worth it. Record the second set of values right next to the first set on the existing form. (The data system is set up to enter both sets of measurements. The form will be updated at the next printing.) For "Electrode position tested", continue to check the box for "Right hand/right foot=1", unless you have to use the left hand, in which case check box=3.

Bert Hohman of Baltimore reports that switching the lead clips from electrodes on the right to left foot sometimes results in readings that are way out of line. Be sure to wait until the readings return to normal ranges and stabilize before recording the values. Press the "RUN" key to initialize the readings if you are concerned that the readings have not returned to normal ranges.

Debbie Medve of Pittsburgh has reported a case where a thin lady had an unusually high resistance reading, suggesting a high body fat content. This could be possible if this ppt experienced selective wasting of muscle tissue, so that her remaining tissue had a high fat content. However, in all cases where the resistance values are extreme (less than 400 or more than 700), or are contrary to your expectations based on body build, be sure to check your connections and equipment.

2. Body Master:

Pittsburgh, Minneapolis, and Baltimore have machines with an adjustable lever arm that is calibrated for length with numbered settings. These settings are continuously adjustable. However, set the lever arm so that it reads right on one number (usually 4, 5 or 6), not part way between two numbers. The most current form, dated 4/3/89 (which you should have), and the updated data system have a place to record this one digit setting. Portland should continue to record the measured distance between the two designated points on the lever arm.

Pittsburgh, Minneapolis, and Baltimore also have machines in which the range of motion limitation setting for testing at 125° is **G**, not **D** as stated in original protocol. D only applies in Portland.

3. Questionnaires:

You do not have to get the drivers license number of a ppt who is a nondriver. The purpose of the drivers licence number is to study risk factors for automobile accidents in drivers using motor vehicle accident data. However, if the person is nondriver but writes down her DL number, it should be entered.

Question 14 of Female History on the take-home incorrectly says skip to question 22 if ppt never had menstrual periods. This should say skip to 21. This will affect very few ppts, but be sure question 21 is answered when reviewing the take-home.

The latest version of the clinic questionnaire that was mass produced here in SF is missing the last page on Aids Used. Sarah is sending out a supply of these pages. Be sure the missing page is added to the interview form and the data are collected on all ppts.

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SOF Memorandum 179

Date: 4/4/89

To: Project Directors and Clinic Coordinators

From: Michael Nevitt, PhD

Re: **Bone Loss Study Update**

We have now received comments from someone at each clinic about the prospects for throughput of participants under the proposed boneloss study protocol. The consensus is that 3 in the morning is doable. Four in the morning is possible, but no promises. We will not really know our recruitment limits until we have some experience with real participants. I suggest that you schedule 3 participants the first few days, and 4 participants later in the week. (Some of us had talked about piloting a few subjects on the reduced protocol prior to the start of the study. On second thought, not having the Osteon up and running means the exam times from the pilot may not tell us much.)

A few comments on the protocols:

1. The urine protocol is attached. If you see any problems with it, let me know.

2. We will continue to do the BIA even though subjects will be fasting. This may affect the measurement, but we will know who is fasting.

3. Exclusions: a) subjects taking insulin and insulin dependent diabetics b) women who have had bilateral fractures of the wrist since baseline

4. Questionnaire: I have appended the questions that have been added to the take-home for the bone loss study. You may want to copy these and send them to your first bone loss participants to fill out. They are included in the bone loss study version of the questionnaire now at the printers. I am assuming that you are sending out the current take-home to bone loss participants until you receive the new questionnaire from us.

5. Fasting does <u>not</u> include medications. Subjects should take their medications as usual.

6. If all goes well, we will probably only need about 2 months worth of bone loss subjects.

7. Peter has made up a list of specific things your service rep should do when servicing your Osteon just after you receive your source. (See next page.)

8. Data from the Osteon scans will be entered at the Coordinating Center. Because the Osteon software update of last summer caused changes in values for bone density, we will also need a copy of the old scan printed out on the new software. If the participant had more than one scan of any site, we will need printouts of all scans. You will need to check your log and files to determine which subjects had more than one scan. You can start printing out new copies of the scans as soon as you know who is going to be in the study.

SOF MEMO 179 (Cont.)

To: SOF project directors and clinic coordinators

From: Peter Steiger

Date: 3/31/89

Re: **Preparation of OsteoAnalyzer for Bone Loss Study, Actions to be Performed by Field Service Engineer**

The following is a more specific list of things that your field service engineer should perform, when preparing your unit for the bone loss study:

1. Update the configuration file for the new standard.

2. Exercise the scanner and verify that all mechanical adjustments are up to specs, that all parts are lubricated as needed, that all belts are tight and no slipping is occurring.

3. Exercise the shutter and verify that it does not stick.

4. Test the multi-channel analyzer and adjust the peak.

5. Check the upper and lower window limits of the multi-channel analyzer

6. Run the standard at least 20 times (2 tenscans) and make sure variations of the standard values are up to specs.
7. Verify that the source activiity is as expected.

8. Test arm and heel holders for leaks and repair if necessary.

10. Run other tests and perform further adjustments as deemed necessary by the field service engineer at the time of the visit.

11 Check the power supply