

MUSCLE TISSUE SAMPLE COLLECTION AND PROCESSING

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MUSCLE TISSUE SAMPLE COLLECTION AND PROCESSING

1. Background and rationale

This year of the Health ABC study involves a bench to bedside opportunity to implement a procedure that has been used in the exercise physiology laboratory for years, but never yet in a large epidemiologic study. During this examination, we are asking eligible participants to donate approximately 150 mg (size of a few grains of rice) of muscle tissue from the thigh, specifically the vastus lateralis muscle. About 75 mg of this tissue will be used by Dr. Goodpaster's lab to determine mitochondrial energetics (high-resolution respirometry), oxidative stress (H₂O₂ emission), expression of proteins related to muscle growth and atrophy signaling, mitochondrial dynamics, and histochemistry for muscle fiber type and fiber size; these will be shared with the Health ABC investigators and will be available for use by others in the scientific community. The rest of the muscle tissue, along with any adipose or connective tissue that is collected, will be put into a special repository so that investigators of muscle physiology and structure can use it to relate properties of the muscle to the unique information gathered in Health ABC.

The percutaneous muscle biopsy technique was first described in 1962 and is recognized as an essential tool in clinical practice and biomedical research. In 1982, the addition of suction through a cutting trocar allowed increased sample yield that made possible a wider variety of analytic assays. This technique has been instrumental in understanding human skeletal muscle physiology and will provide an unmatched understanding of human muscle tissue in older adults.

Every effort must be made to make the entire procedure as easy and painless as possible for the participants. This manual of operations includes materials pertaining to the preparation of the participants and the actual muscle tissue collection procedure. Additionally, directions for processing the muscle specimens (and any fat or connective tissue collected) are provided.

1.1 Overview

A brief overview of the steps involved in completing the muscle tissue collection is outlined here. More detail regarding each step is provided in the sections below.

Participants will be screened initially over the telephone to determine eligibility for the muscle tissue collection, using the Muscle Tissue Collection Screening Form (Appendix 1). Major exclusion criteria include the use of medications that affect bleeding, bruising, and platelets, such as Coumadin, Aggrenox, Ticlid, Plavix, Agrylin, and Xagrid. Participants' status will be assessed again at an in-person clinic visit, and they will be asked to sign a consent form for the tissue collection. They will also receive a sheet with Frequently Asked Questions (Appendix 2) and the Muscle Tissue Collection Instructions (Appendix 3). Participants will be instructed, unless taking aspirin regularly as prescribed by their physician, to avoid the use of aspirin and aspirin-containing products (such as alka-seltzer) and other NSAIDS (such as Aleve, Ibuprofen, or other anti-inflammatories), for 5 days before the procedure which will

take place approximately 7 to 10 days after their screening interview. Participants will be told that it is okay to take Tylenol. The muscle tissue will be collected in the morning after a fast of at least 8 hours. Participants will be instructed to avoid eating or drinking anything except prescription medications and water after midnight on the day before the tissue collection appointment.

Participants who agree to the muscle tissue collection will be asked to sign a consent form. The consent statement will inform study participants that muscle sampling with a needle may cause pain, discomfort, bleeding, and bruising at the time of the sampling and that soreness for the next several days is common. Additional risks are indicated in the appropriate section of this manual. The procedure will take 30 to 60 minutes.

At the time of the muscle tissue collection, the Muscle Tissue Collection and then the Muscle Tissue Processing forms (Appendix 4) will be completed. All participants who have muscle tissue collected will be given instructions for post-procedure care in the Muscle Tissue Collection Discharge Instructions (Appendix 5). Lists of aspirin-containing and ibuprofen-containing medications that should not be taken are included in Appendix 5 as well. Two to three days after the muscle tissue collection, participants will be contacted via telephone by field center personnel, and the Muscle Tissue Collection Follow-up Telephone Contact Form (Appendix 6) will be completed. In the rare situation that an adverse event occurs, the Muscle Tissue Collection Adverse Event Form (Appendix 7) will be completed.

PARTICIPANT PRESCREEN AND PROCEDURE INSTRUCTIONS

1.2 Screening

1.2.1 Screening (Muscle Tissue Collection Eligibility Pre-Screener)

An example of the Muscle Tissue Collection Eligibility Pre-Screener is in Appendix 1.

Participants will be introduced to the muscle tissue collection component of the Health ABC study. If the participant is interested in participating in this part of the study, following the questionnaire format on the Muscle Tissue Collection Eligibility Pre-Screener, each participant is asked a series of questions regarding eligibility for the muscle tissue collection.

Participants who are regularly taking medications that strongly affect bleeding, bruising, or platelets will be excluded. Also, participants who regularly take medications for cognitive problems will be excluded. These medications include:

Coumadin (also known as Warfarin, Jantoven, Marevan, and Waran)

Plavix (also known as Clopidogrel, Clopilet, and Ceruvin)

Aggrenox (also known as aspirin with Dipyridamol and Persantine)

Ticlid (also known as ticlopidine)

Agrylin or Xagrid (also known as Anagrelide)

Aricept (also known as Donepezil)

Namenda (also known as Memantine)

Exelon (also known as Rivastigmine)
Razadyne (also known as Galantamine)

If a participant indicates that they do not know what medications they take, this may be a sign of cognitive problems. If the participant has cognitive problems, they will be excluded from the procedure.

Those with any allergies to lidocaine will be excluded from the procedure. Those who have been told that they have a bleeding disorder or clotting problem will also be excluded from the procedure. A blood sample will be obtained at the participant's first Year 16 clinic visit. Those whose platelet count is not at least 50,000 or is insufficient will also be excluded from this procedure.

If a participant reports taking aspirin or an aspirin-containing medication such as Excedrin or Alka-seltzer, several questions will be asked to ascertain if use of this medication is secondary to a health provider's order or just taken occasionally for pain. The responses will be noted on the Muscle Tissue Collection Eligibility Pre-Screener.

Participants taking aspirin or aspirin-containing medications will be included as potential muscle tissue collection participants and should be asked to participate. The physician performing the tissue collection should be made aware of the participant's aspirin usage.

If the participant is deemed eligible and indicates that they are now taking any of the medications that affect bleeding or for treating cognitive problems (Coumadin, Plavix, etc.; see above), they will be excluded from the procedure.

1.2.2 Participant instructions for procedure

Participants should receive the Muscle Tissue Collection Instructions sheet either at their first Year 16 clinic visit or by mail, about 1 week before the muscle tissue collection procedure. The Muscle Tissue Collection Instructions can be found in Appendix 3. Participants will also receive a sheet with frequently asked questions about muscle tissue collection, and they can share this with family members who may be concerned about the procedure (Appendix 2).

The instructions provide a brief description of the muscle tissue collection procedure. Participants are instructed to call the clinic with any questions. The participants should also complete the informed consent at the beginning of the visit.

The participant instructions for preparation for muscle tissue collection are described below.

The participant should be told to only take Tylenol for pain or cold symptoms for 1 week before the procedure.

A brief description of the procedure will also be provided, and instructions will be given to limit their activity for the rest of the day and to not take a shower for 24 hours or bathe in a tub for 72 hours after the procedure.

1.3 Preparation of participants for muscle tissue collection

This study requires the voluntary cooperation of the participants and, as with all procedures, participants may refuse to cooperate at any time. As with any other procedure in Health ABC, we should strive to make this experience as pleasant as possible. Ask participant if they have any questions before beginning the procedure.

1.4 Muscle tissue collection procedure

1.4.1 General

The technician briefly describes the procedure. Sample script: *"I am going to be collecting some tissue about the size of a few grains of rice. This tissue will be used to examine how muscle changes as people age. We hope to be able to use the results of these tests to better understand health and disease in older people."*

1.4.2 Handling participants who are extremely apprehensive about having tissue collected

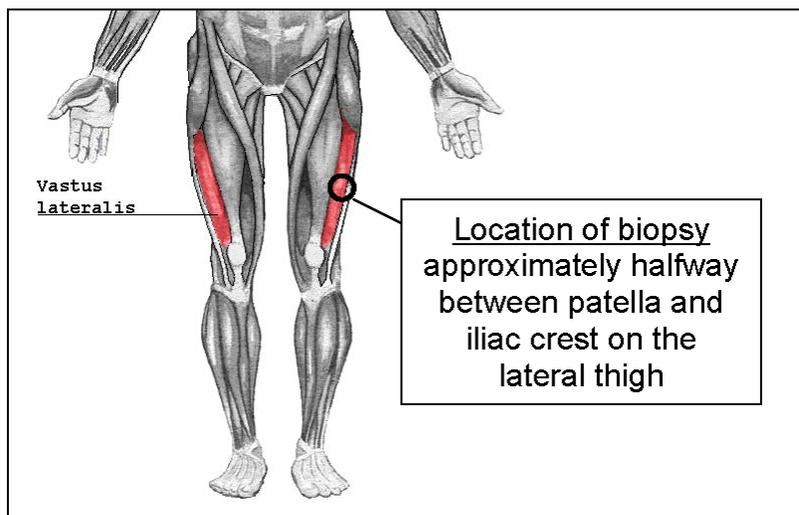
Remember that participants can opt out of the muscle tissue collection at any time. If a participant has concerns, it may help to explain to the participant that every effort will be made to make sure they are as comfortable as possible. If a participant expresses concerns that cannot be easily addressed at the time of the tissue collection, please call the person who will be obtaining the tissue collection to speak with them.

Do not under any circumstances force the participant to have a muscle biopsy.

1.4.3 Tissue collection procedure**BEFORE PROCEDURE**

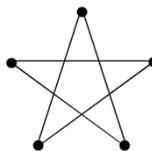
- Make sure the participant is feeling well. If the participant is not feeling well, reschedule the muscle tissue collection procedure. Try to reschedule the visit within 1 or 2 weeks, but, if necessary, the visit can be rescheduled for up to 45 days after their originally scheduled visit keeping the Year 16 visit schedule parameters in mind. Note that it will be especially important to ask the participant if they have started to take a new medication since their first visit.
- Ask participant if they have started to take a new prescription since their screening call about 1 week before. Make sure that this new prescription is not Coumadin, Plavix, Aggrenox, Ticlid, Agrylin, Xagrid, Aricept, Namenda, Exelon, or Razadyne. If they are taking any of these medications, they are no longer eligible for muscle tissue collection.
- Take participant's seated blood pressure. If systolic is greater than 180 mm Hg or diastolic greater than 110 mm Hg, the participant cannot have the muscle tissue collection.
- Ask the participant if they have taken aspirin or aspirin-containing products or Aleve, Ibuprofen, Motrin, or any other anti-inflammatory medications in the past 7 days. If so, alert the physician who will be obtaining the muscle tissue. Depending on how much aspirin or other NSAID the participant may have taken, the physician may decide to reschedule the muscle tissue collection. See Appendix 5 for lists of aspirin-containing and ibuprofen-containing medications.
- Ask participant the date and time they last ate anything. If they did not fast, this will be noted and the tissue collection procedure can still occur. The participant will NOT be rescheduled.
- Check the Year 16 Pittsburgh Clinic Visit Workbook (page P15/M23) to see which leg was tested during the isokinetic strength (Kin-Com) measurement. The tissue collection should be performed on this same leg. If the isokinetic strength (Kin-Com) measurement was not done, perform the tissue collection on the right thigh, if possible. If not, perform the tissue collection on the left thigh.
- Be sure to note which side you need to access before asking the participant to lie down on the table and position the participant accordingly.
- Have the participant change into a gown and lie comfortably in a reclined position with both legs outstretched.
- Ask participant if they are allergic to latex, and if so, use alternative material gloves.

- The muscle tissue collection will be obtained approximately midway between the patella and iliac crest on the lateral thigh. See figure below. This distance was chosen so each participant will receive the tissue collection at the same relative length of their lower extremity.
- Do not allow the participant to watch the procedure.
- Record procedure start and stop time on Muscle Tissue Collection Form.



PROCEDURE

- The skin is cleaned with ChloroPrep solution to create a clean surgical field. The study physician may decide to shave the skin if necessary. Place sterile drape over cleaned skin.
- Inject 2 to 5 cc 2% lidocaine HCL subcutaneously, and ensure that the fascia lata – but not below the fascia – is well infiltrated. Wait 3 to 5 minutes and then inject more lidocaine into the fascia around the muscle in a star pattern. See figure below:



- Wait 10 to 15 minutes after injection of the lidocaine before proceeding to ensure maximal participant comfort.

- Using a no. 11 blade scalpel, make a small (5 mm) incision in the skin through the subcutaneous fat and through the muscle fascia.
- If it is deemed that there is an adequate subcutaneous adipose layer available, a subcutaneous adipose sample should be obtained. Note that in some very lean individuals there will be insufficient subcutaneous adipose to obtain a sample.
- Insert the trocar just below the skin to obtain a sample of subcutaneous adipose tissue.
- Once the physician opens the trocar, the assistant will immediately apply suction through a 60 cc syringe, drawing tissue into the cutting chamber as the trocar is simultaneously advanced. Place tissue on sterile Petri dish that is sitting on ice.
- Re-insert the trocar through the incision, and through the fascia, advancing the trocar into the muscle belly.
- Again, once the physician opens the trocar, the assistant will immediately apply suction through a 60 cc syringe, drawing tissue into the cutting chamber as the trocar is simultaneously advanced.
- To maximize tissue yield, rotate the trocar 90 degrees without removing from the leg and repeat the suction procedure two more times for a total of three separate muscle samples. Record number of rotations on the Muscle Tissue Collection Form.
- If the yield is insufficient you may re-insert the trocar up to three times, as long as the participant is comfortable. Place tissue on sterile Petri dish that is sitting on ice. Record number of insertions on the Muscle Tissue Collection Form.
- Close the incision with a sterile liquid bonding agent (such as Dermabond) or with steri-strips. This will help prevent bleeding and scarring.
- Place Tegaderm and wrap with elastic wrap. This wrap should serve as a compression device and should be wrapped tightly, but not too tightly, for at least 10 minutes. Then place an ice bag on the incision site for 15 to 30 minutes to reduce swelling and pain.
- Record procedure stop time on Muscle Tissue Collection Form.
- Record approximate amount of 2% lidocaine injected on Muscle Tissue Collection Form.
- Ask the participant to rate their pain at the muscle tissue collection site and record their response on the Muscle Tissue Collection Form (Page P19 in Year 16 Pittsburgh Clinic Visit Workbook).
- Record whether or not there were any adverse reactions during or post procedure.

- Record whether or not any subcutaneous fat was collected.
- Record whether or not any muscle tissue sample was collected.
- The total amount of time for this procedure is approximately 45 minutes to 1 hour. This includes about 15 minutes of interaction with the study physician for the site preparation and the tissue collection itself; 10 to 15 minutes to wait for the lidocaine to take effect, about 15 minutes for icing the area after the tissue collection; and 15 minutes for a snack/recovery. Be sure to offer the snack as soon as possible after the tissue collection.

1.4.4 PRECAUTIONS WHEN A PARTICIPANT FEELS FAINT OR LOOKS FAINT FOLLOWING THE MUSCLE TISSUE COLLECTION

- One of the most common adverse events for muscle tissue collection is a vasovagal episode where a participant faints or feels faint.
- If not already lying down, have the participant lie down with their legs raised onto a pillow or blanket.
- Provide the participant with a basin if they feel nauseated.
- Have the participant stay lying down until the color returns and they feel better.
- Place a cold wet cloth on the back of the participant's neck.
- If the participant faints, use smelling salts to revive by crushing the ampule and waving it under the participant's nose for a few seconds. If you need to use smelling salts, be sure to complete a Muscle Tissue Collection Adverse Event Form (Appendix 7).
- If the participant continues to feel sick, contact a medical staff member who will advise you on further action.
- The participant should be evaluated by a physician if they faint.
- Always make sure there is a call button in the room to be used to alert the emergency team if necessary.

1.4.5 Muscle tissue collection discharge instructions

Before the participant leaves the clinic, the participant who received the biopsy will be instructed about care of the biopsy site and made aware of any complications that might occur.

The following instructions are provided to the participant on the Muscle Tissue Collection Discharge Instructions (Appendix 5).

- To prevent infection, participants should keep the site clean and dry for at least 24 hours.
- Participants should not shower for 24 hours and should not take a bath for 72 hours.

- Participants should not experience excessive bleeding or bruising. A few drops of blood on the bandage are expected. If excessive bleeding occurs, the participant should call the clinic immediately.
- To prevent bleeding and/or bruising, the bandage should be kept on for 72 hours.
- Participant should not do any strenuous activity or exercise for 48 hours after the procedure.
- The participant should not take aspirin or other NSAIDS for at least 3 days after the procedure unless prescribed by their health care provider. The participant may take Tylenol for any discomfort as needed.
- If there is pain at the tissue collection site, the participant may apply ice every two hours for 20 minutes until the pain subsides.
- Although it is very rare, infection (presenting as redness, pus from the wound, increasing pain) can occur with muscle tissue collection. If the participant develops any of these symptoms, they should call the clinic immediately.
- If the participant has a problem during office hours, they should be instructed to call the field center staff. If there is an emergency related to this procedure after normal office hours, the participant should go to the hospital emergency room, and indicate to the hospital staff that a muscle tissue collection was completed.

Note that in Appendix 5, after the Muscle Tissue Collection Discharge Instructions, is a comprehensive list of aspirin-containing and ibuprofen-containing medications that should not be taken.

2. Adverse events and follow-up

We anticipate that adverse events will be rare. The most common adverse events are likely to be ecchymosis/hematoma, bleeding/oozing at tissue collection site, vasovagal episodes at the time of tissue collection, and pain more than 3 days after the tissue collection. We do not anticipate infection of the tissue collection site to occur in any participants, although this is a remote possibility.

2.1 Discharge instructions

Participants will be given post-procedure instructions for care of the tissue collection site, what activities to avoid, and when to report problems (Appendix 5). Also, participants should be told that no results will be made available to the participant but we will contact them to see how they are doing.

2.2 Follow-up contact

Although complications after the muscle tissue collection are rare, a follow-up call will be made to assess how the participant is feeling and to assess if there are any complications. The Muscle Tissue Collection Follow-Up Telephone Contact Form (Appendix 6) should be completed by telephone 2 to 3 days after the muscle tissue collection procedure.

At this contact participants will be queried about pain, whether they took pain relieving drugs for pain related to the procedure; problems related to the bandage/Dermabond (rash, excess itching); bruising or bleeding that concerns the participant; signs of infection (redness, pus, or warmth); and whether there were any other problems or concerns with the procedure.

If the participant reports pain or answers “Yes” to any of the questions on the Muscle Tissue Collection Follow-up Telephone Contact Form, a Muscle Tissue Collection Adverse Event Form must be completed (Appendix 7).

2.3 Adverse events

If an adverse event occurs, complete the Muscle Tissue Collection Adverse Event Form (Appendix 7) and notify the PI and the Coordinating Center.

Possible adverse events include:

- Infection (redness, pus, warmth)
- Pain
- Bruising or bleeding
- Problem related to bandage or Dermabond
- Reaction to lidocaine
- Fainting
- Other_____

For each adverse event, the clinic staff, with input from the study physician, will determine the severity of the event, the relationship of the event to the muscle tissue collection procedure, and the action taken at the time the Muscle Tissue Collection Adverse Event Form was completed. The outcome of the event will be determined after the participant is contacted 3 days after the event.

Note that not all events recorded on the Muscle Tissue Collection Adverse Event Form will be reported to the IRB.

Any sign of infection is not expected and will be considered an adverse event. Any infection should be reported to the IRB.

Some pain, bruising, bleeding, and/or problems with the bandage/Dermabond are expected. Mild cases do not need to be reported to the IRB; moderate, severe, or worse cases, i.e., cases that are significant enough to cause the participant concern, are not expected and should be reported to the IRB.

Any allergic reaction to lidocaine is not expected and will be considered an adverse event and should be reported to the IRB. In the event of a reaction to lidocaine, the physician present will use best clinical judgment to treat the participant based on symptoms and severity.

Fainting (vasovagal episode) is occasionally expected and will be considered an adverse event if smelling salts were needed to revive the participant. Mild cases in which the participant is easily revived without smelling salts do not need to be reported to the IRB; moderate and severe cases, e.g., when reviving the participant is difficult, are not expected and should be reported to the IRB. If a participant faints they should be examined by a study physician in clinic.

Other adverse events are not expected. Any moderate or worse adverse events that do not fit the categories above should be reported to the IRB. List as many “other events” as needed.

If an event requires follow-up, call the participant 3 days after the event is reported and complete the “Outcome” section of the Muscle Tissue Collection Adverse Event Form.

LABORATORY COLLECTION AND PROCESSING INSTRUCTIONS

3. Equipment and supplies

3.1 Sample ID labels

You will be supplied with sheets of sample ID barcode labels to use for labeling forms and cryovials. A sample sheet of barcode labels can be found in Appendix 8. All labels on each sheet have the same 6-digit sample ID number (the first digit identifies the clinic: Pittsburgh = 2).

Each cryovial label also has a 2-digit extension (21 to 32) that serves as a unique identifier for each cryovial within a sample ID. See Appendix 8 for proper orientation of the barcode label.

Beneath the human-readable ID number, cryovial labels also have one to three lines of text intended to increase accuracy in labeling and filling the cryovials. The text corresponds to the type of processing and sample to be stored in the cryovial, e.g., histology, respirometry, EM, Muscle BCHM, etc.

There are 2 barcoded labels with the ID number, one with the words “Muscle Tissue Collection Form,” which is placed on the Muscle Tissue Collection Form, and the other with the words “Muscle Tissue Processing Form,” which is placed on the Muscle Tissue Processing Form (Appendix 4). *This process of matching the participant-specific Health ABC Enrollment ID# (already on the form brought to the lab by the participant) to the sample-specific ID barcode is crucial to being able to use the data collected from muscle tissue.*

4. Exam room preparation

4.1 Tissue collection room

The muscle tissue collection should take place in an isolated room or participants should be separated by room dividers. The room should be equipped with all of the necessary supplies and an emergency call button. The processing of the specimen can take place either in the same room as the tissue collection or another room in the clinic that is close by. A separate counter or work table should be equipped with all of the materials and vials that are used in the tissue handling and processing. The -80 degree C freezer and 4 degree C refrigerator should be nearby.

4.2 Tissue collection tray

A Mayo stand will contain the material needed for the instrumentation for the tissue collection. The instrumentation will be located on a sterile field. The needles and 60 cc syringe and tubing will be sterilized.

Tray contents:

- ChloraPrep
- Band-Aids
- Gauze (4x4)
- Tegaderm dressing
- Scissors
- 5 mm Bergstrom biopsy needle - have at least one for back up for each procedure
- 2% buffered lidocaine
- Scalpel (#11 blade)
- 2-10 cc syringe
- 22 g and 25 g needles
- 60 cc syringe and tubing for suction
- Dermabond
- Ziplock bag for ice (or alternative)
- Cork
- Kimwipes
- #9 filter paper
- Sterile drape (fenestrated)
- Plunger for biopsy needle
- Forceps
- Tweezers

Other materials should be available in the room (but should not be placed on the sterile field):

- Smelling salts
- Dissecting microscope
- Isopentane
- Container for isopentane
- Liquid nitrogen
- Needle/sharps container
- Pencils/pens
- Sterile gloves of various sizes. Latex gloves should be used unless the participant is allergic to latex. If the participant is allergic to latex gloves, alternative material gloves should be available.
- Extra set of tweezers
- Glass Petri dish on ice
- Glass Petri dish with section of parafilm
- EM vial with ~1 mL 3.7% glutaraldehyde on ice

- 3 to 4 mm x 6 mm sections of Kimwipe for sample preparation

4.3 Tissue collection rack: labeling and setup

A separate cryovial rack containing the necessary storage cryovials is set up for each participant. They are arranged according to the priority of the tissue acquisition.

The remaining tissue containers should be pre-labeled with barcodes from the sheet described in section 3.1:

Tissue container #21 is a plastic container that will hold between 15 to 20 mg of muscle for histology.

Cryovial #22 will hold about 10 mg of muscle.

Cryovial #23 will hold between 2 to 5 mg of muscle.

Cryovials #24 through #29 will hold muscle (in varying amounts).

Cryovial #30 will hold intermuscular fat (in varying amounts).

Cryovial #31 will hold connective tissue (in varying amounts).

Cryovial #32 will hold subcutaneous fat (in varying amounts).

4.4 Preparation for tissue collection

Preparation for tissue collection is done in the following manner. Early morning, before any participants arrive:

- Check to make sure that tissue collection trays are properly equipped, including sterilized needles. Every item on the checklist (Appendix 9) must be ready before proceeding.
- Check that each tissue container is properly labeled with sample ID labels.
- Check that the sample processing station is properly equipped.
- Make sure the tissue collection room is tidy and stocked with extra smelling salts, basin, and disposable wash cloths.

Before performing tissue collection:

- Under a dissecting microscope, place a glass Petri dish with a section of parafilm.
- Cut several sections (3 to 4 mm X 6 mm) of Kimwipe for histology sample preparation.

- Fill the electron microscopy vial with ~1 mL 3.7% glutaraldehyde on ice.
- Prepare liquid nitrogen and isopentane for flash (quick) freezing of muscle specimen:
 - a. Place liquid nitrogen in container as illustrated in Figure 1.
 - b. Pour isopentane into the metal cup as also shown in Figure 1. A similar container as shown will be adequate.
 - c. The liquid nitrogen is used to cool the isopentane to near its freezing point. Enough liquid nitrogen is used so that the container of isopentane is sitting atop of the liquid nitrogen (refill with nitrogen as the tissue collection is taking place). By the time the muscle is flash frozen, the isopentane should be near the freezing point as evidenced by some freezing (ice in the bottom). There should, however, still be enough liquid isopentane in the metal cup.

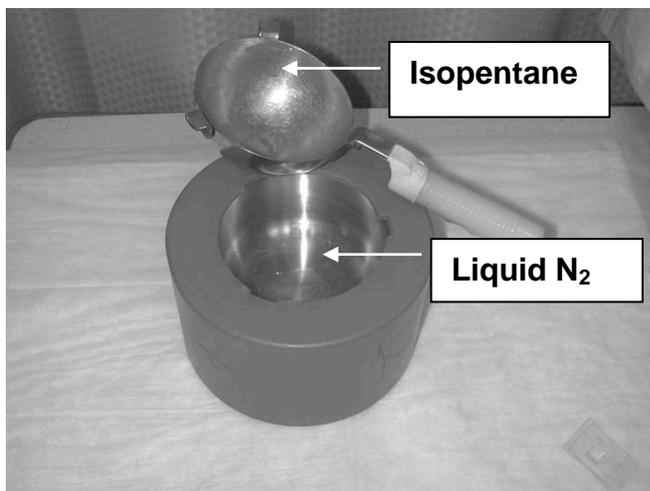
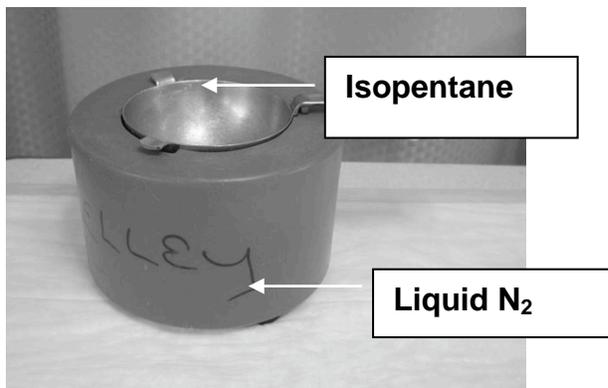


Figure 1. Dewar containing isopentane and liquid N₂ for flash freezing



5. Detailed procedures for tissue collection

5.1 Precautions for handling tissue collection specimens

In accordance with the OSHA regulations on blood borne pathogens (see OSHA regulations that are kept in the laboratory), the central lab recommends the following laboratory safety protocol for the field center laboratories:

- Non-permeable lab coats, latex gloves, and face shields should be used when handling any blood in any situation where splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.
- Universal Precautions should be followed when handling any tissue products.
- Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak proof container. Never recap or break needles.
- Hepatitis B vaccine should be offered to all unvaccinated technicians handling tissue, and documentation of vaccination or technicians' declining to be vaccinated should be kept.

6. Tissue specimen processing

An example of the Muscle Tissue Collection and Muscle Tissue Processing form is in Appendix 4. The collection must be done in an efficient manner, with maximum protection for the participant. All forms must be completed in ink.

The Muscle Tissue Collection and Muscle Tissue Processing forms have the following purposes:

1. Assure the most efficient and safest possible muscle tissue collection for participants
2. Allow the monitoring of the quality of the above procedures

The participant will arrive at the muscle tissue collection station with their Health ABC participant ID# already filled in on their Muscle Tissue Collection and Processing forms.

The expected range for the sample yield is 50 to 200 mg (average: 150 mg).

As many as 12 samples will be stored: one 15 to 20 mg muscle tissue sample for histology, one 10 mg muscle tissue sample for respirometry (*on fresh tissue; this will not be stored!*), one 2 to 5 mg muscle tissue sample for electron microscopy, up to five muscle tissue aliquots of 15 to 20 mg for other assays; one extra muscle tissue aliquot (various amounts); one sample of any intermuscular fat collected (various amounts); one sample of any connective tissue collected (various amounts); and one sample of any subcutaneous fat (various amounts).

Despite the best efforts of the clinic staff, occasionally a limited amount of tissue will be collected. Not all types of tissue (subcutaneous fat, connective tissue) will be available in every specimen. *Always process the tissue in the order specified.* Make notations of difficulties on the Muscle Tissue Collection and Muscle Tissue Processing forms. Due to the extremely time sensitive nature of freezing the tissue, the priority order for tissue collection is somewhat different than the processing

order. The priority and processing order is listed below, with “A” having the highest priority and “K” the lowest.

<i>Priority collection order</i>		<i>Priority processing order</i>	
A	Histology sample	A	Muscle – 10 mg for Oroboros, H2O2
B	EM sample	B	Muscle – 15-20 mg
C	Muscle – 10 mg for Oroboros and H2O2	C	Muscle – 15-20 mg
D	Muscle – 15-20 mg	D	Muscle – 15-20 mg
E	Muscle – 15-20 mg	E	Muscle – 15-20 mg
F	Muscle – 15-20 mg	F	Muscle – varying amounts
G	Muscle – 15-20 mg	G	Histology sample
H	Muscle – varying amounts	H	EM sample
I	Intermuscular fat – varying amounts	I	Subcutaneous fat – varying amounts
J	Connective tissue – varying amounts	J	Intermuscular fat – varying amounts
K	Subcutaneous fat – varying amounts	K	Connective tissue – varying amounts

6.1.1 General

Personal protective equipment (non-permeable lab coats and double-gloves with at least one latex pair) **MUST BE** worn for processing.

It is possible that not all containers will be filled due to problems with the tissue collection or absence of fat or connective tissue in the muscle. During processing, work in the order specified and make as many aliquots as possible while meeting the weight requirement of each cryovial. On the Muscle Tissue Processing Form, record the estimated weight of the tissue samples in each container. Timing: Process the samples immediately, no later than 5 minutes after the specimen is collected.

6.1.2 Description of muscle tissue cryovials and processing

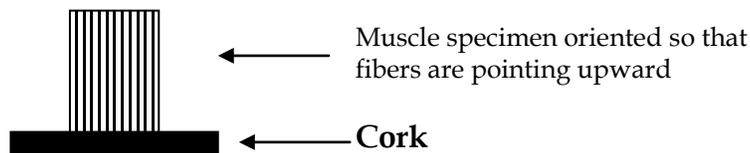
1. Upon receiving the biopsy tissue, dissect out any visible adipose tissue within the muscle tissue and place this adipose tissue in cryovial #30.
2. Dissect out any visible connective tissue and place in cryovial #31.
3. Place subcutaneous fat into cryovial #32.
4. Blot the muscle sample to dryness using a Kimwipe, careful to keep the fibers intact.
5. Remove a small (~10 mg) of muscle, paying careful attention to obtaining intact muscle fibers to be placed in ice-cold Biops media. This sample will be transferred within 15 min to the Goodpaster lab for high-resolution respirometry and H2O2 measurements.

Separate a 15 to 20 mg section for histology and place on a piece of Kimwipe. Process this piece in step #8.

6. Remove a small section (~2-5 mg) for electron microscopy (EM). It's best if fibers are intact longitudinally and only 3 to 4 fibers deep. Place on a piece of Kimwipe. Process this piece in step #9.
7. Separate approximately five sections of tissue, 15 to 20 mg each and place in cryovials #24 to #28. If there is more muscle tissue, place in cryovial #29. Do not discard any muscle tissue. More than 20 mg may be placed in cryovial #29. Aliquots #24 to #28 should be a minimum of 15 mg each; aliquot #29 may be of any weight.

Specimens #21, and #24 to 32 should also be placed into liquid nitrogen for flash freezing (about 60 seconds) either using forceps holding onto the cap portion, or the tube can be "floated" in the liquid nitrogen bath and retrieved with forceps. Immediately store at -80 degrees C for long-term storage before shipping.

8. Histology processing: Wrap the section in a small piece of one-ply extra low lint wipe (i.e., Kimwipe) (like sushi) and place it in a spot of mounting medium in the center of the cork. Mount the muscle specimen in the cryomatrix so that the muscle fibers are completely embedded and oriented on a small piece of cork as shown below.



Submerge the cork with cryomatrix and muscle specimen into cooled isopentane. Hold submerged for 5 to 10 seconds until cryomatrix is frozen. Turn cork over and keep in the isopentane for approximately 1 to 2 minutes. After freezing in isopentane, place the sample in a labeled plastic cryovial (Container #21), close container, and submerge in liquid nitrogen until longer-term storage in at -80 degree C freezer.

9. Place the EM section into 2.5% glutaraldehyde/PBS and place back into ice. EM samples are stored indefinitely in a 4 degree C refrigerator (Container #23).

6.2 Analysis

Skeletal Muscle Biopsy Measurements

- Muscle Fiber Type and Fiber Cross-Sectional Area.** Histochemistry: Serial transverse sections (10 μm) of mounted biopsy samples will be generated using a cryostat at $-20\text{ }^{\circ}\text{C}$ and placed on a cleaned glass slide. Analysis of fiber type, fiber size, intramyocellular lipid (Oil Red O staining), oxidative capacity (SDH staining), capillary density, and mitochondrial DNA damage will be performed. Images will be visualized using a Leica microscope, digitally captured, and semi-quantitative image analysis will be performed using specialized software.
- Protein expression and activation using Western blot quantification** will be conducted on a targeted set of proteins associated with protein synthesis and breakdown, mitochondrial dynamics and metabolism from the table below.
Western Blot: Frozen muscle sample (15-25mg) will be homogenized in ice-cold cell lysis buffer with protease inhibitor cocktail tablets. Protein concentration will be determined in triplicate using the bicinchoninic acid assay (BCA). Aliquots of the sample will be mixed with 5x Laemmli buffer and denatured by heating. Samples will be kept in -80°C until western blot analysis. Proteins will be separated by gel electrophoresis using a 4-20% gel and transferred onto polyvinylidene difluoride membranes. Membranes will be blocked for 2 hours with 5% non-fat milk, and incubated with primary antibodies. On following day, membranes will be washed, and incubated in appropriate species-specific HRP-conjugated secondary antibodies. Protein band will be visualized using a chemiluminescence detection kit and gel documentation system. Protein bands of interest will be quantified by densitometry. Protein loading will be controlled by normalizing bands of interest to α -Tubulin and a control samples loaded in all the gels.

Proteins Associated with Protein Synthesis. Reductions in skeletal muscle protein synthesis due to aging or inactivity have been extensively characterized. One focus of investigation has been on mTOR signaling and downstream targets due to their regulation of muscle protein synthesis.

Proteins Associated with Protein Breakdown. It is not clear whether or not protein breakdown is elevated with aging or disuse. It is well accepted that the ubiquitin-proteasome system functions as the primary degradative system for most muscle proteins. We will examine the signaling leading to the activation of the ubiquitin-proteasome system, and inflammatory signaling that is known to influence this pathway.

Proteins Associated with Mitochondrial Dynamics. To date measurements of mitochondrial quality control processes (i.e., fission, fusion, autophagy, mitophagy), and quantity with aging or inactivity have not been fully elucidated. Skeletal muscle disuse is associated with altered mitochondrial morphology, mitochondrial respiratory dysfunction, and increased ROS production. Emerging evidence indicates mitochondrial fission, fusion, autophagy and mitophagy are essential for maintenance of a healthy mitochondrial pool by preventing mtDNA mutations and removing damaged mitochondria. Our laboratory has experience performing measurements of mitochondrial fission, fusion, autophagy, mitophagy and content in human skeletal muscle biopsy samples.

Muscle Biopsy Protein Markers

Protein Synthesis	Protein Breakdown	Mitochondrial Dynamics
S6K1	FOXO1/3A	Fis1
eEF2	MuRF-1	DRP1
mTOR	Atrogin-1	OPA1
	Myostatin	Mfn2
	IL-6	Beclin-1
	IL-1b	LC3B I+II
	TNF-a	Bnip3
		PGC-1a
		OXPHOS

3. **High Resolution Respirometry and ROS Production.** Mitochondrial respiratory capacity will be measured in permeabilized muscle fibers using an Oroboros Oxygraph 2K. The instrument has two chambers, which permits high-resolution respiration measurements to be made in duplicate with low amounts of muscle tissue (2mg). The O2K assay allows a comprehensive evaluation of mitochondrial respiratory function upon titration with ETC complex-specific inhibitors and substrates. Measurement of H₂O₂ emission by mitochondria will be measured in from permeabilized muscle fiber bundles by real time monitoring of Amplex Red oxidation using HPLC detection.

7. Quality assurance**7.1 Training requirements**

Clinical experience with muscle tissue collection is mandatory. Additional training should include:

- Read and study manual
- Attend Health ABC training session on techniques (or observe procedure by experienced examiner)
- Discuss problems and questions with local expert or QC officer
- Appropriate medical license as needed
- Physician and assistant should practice together on store-bought meat

7.2 Certification requirements

- Completes training requirements
- Successfully performs tissue collection on store-bought meat five times
 - According to QA checklist
- Successfully processes store-bought meat five times
 - According to QA checklist
- Conducts tissue collection on volunteers according to requirements on checklist

7.3 Quality assurance checklist

Collection: Physician

Tissue collection properly carried out:

- Non-permeable lab coats and gloves used
- Script properly delivered
- Participant in a supine position with both legs outstretched, unable to view procedure
- Preparation of tissue collection site correctly done
- Tissue collected from leg that was tested for strength (Kin-Com)
- 2% lidocaine HCL subcutaneously injected in fascia lata, but not below fascia – in a star pattern
- Waits 10 to 15 minutes
- 5 mm incision made through muscle fascia, using no. 11 blade scalpel
- Trocar inserted through incision to collect subcutaneous fat
Assistant immediately applies suction through 60 cc syringe, drawing subcutaneous fat tissue as trocar is simultaneously advanced.
- Trocar inserted through incision, through the fascia, to muscle belly
Assistant immediately applies suction through 60 cc syringe, drawing muscle tissue as trocar is simultaneously advanced.
- Trocar rotated 90 degrees without removing from leg, and up to three separate muscle samples collected
- Sterile liquid bonding agent applied
- Pressure applied for at least 10 minutes

Collection: Assistant

Preparation:

- Tissue collection trays properly prepared
- Questions on Muscle Tissue Collection Form asked

Tissue collection properly carried out:

- Non-permeable lab coats and gloves used
- Script properly delivered
Trocar inserted through incision to collect subcutaneous fat (physician)
- Assistant immediately applies suction through 60 cc syringe, drawing subcutaneous fat tissue as trocar is simultaneously advanced.
Trocar inserted through incision, through the fascia, to muscle belly (physician)
- Assistant immediately applies suction through 60 cc syringe, drawing muscle tissue as trocar is simultaneously advanced.
- Scalpel and trocar appropriately prepared for sterilization

Muscle Tissue Collection Forms properly filled out:

- Time at start of tissue collection entered
- Amount of 2% lidocaine infused recorded
- Correct number of insertions of needle recorded
- Correct number of rotations recorded
- Adverse reaction question answered

Discharge:

- Participant instructed about care of tissue collection site

Tissue Processing

Preparation

- Glass Petri dish with section of parafilm available
- EM vial with ~1 mL 3.7% glutaraldehyde on ice available
- 3 to 4 mm x 6 mm sections of Kimwipe for sample preparation available
- Prepare liquid nitrogen and isopentane for flash freezing of specimen
- Aliquot racks correctly set up
- Containers correctly labeled

Processing tubes

- Time at start of processing recorded
- Visible adipose tissue dissected
- Visible connective tissue dissected
- Sample blotted to dryness using Kimwipe
- Small muscle section (2 to 5 mg) removed for electron microscopy and placed in 2.5% glutaraldehyde/PBS (container #23)
- 15 to 20 mg histology section separated
- Containers #21, #24-32 placed into liquid nitrogen and snap frozen (about 60 seconds)
- 15 to 20 mg histology section wrapped, mounted, placed in isopentane and labeled plastic storage container (container #21)
- Container fill information completed for all 12 containers

Freezing

- Aliquots checked to ensure they are not wet
- Container #21 placed in 5 x 5 freezer box and stored in -80° C freezer
- Container #22 brought to Goodpaster lab for high-resolution respirometry and H2O2 measurements
- Containers #24-32 placed in 9 x 9 freezer box and stored in -80° C freezer
- Container#23 placed in 4° C refrigerator

End of day procedure

- Muscle Tissue Collection and Processing forms placed in daily work folder
- Frozen aliquots removed from rack and placed in appropriate freezer boxes
- Freezer boxes correctly labeled

Appendix 1 Muscle Tissue Collection Screening Form

	HABC Enrollment ID #	Acrostic	Date Form Completed			Staff ID #	
	<input type="text" value="H"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>			
		Visit Year: <input checked="" type="radio"/> 16					

MUSCLE TISSUE COLLECTION ELIGIBILITY PRE-SCREENER

SCRIPT: "As part of the Health ABC clinic exam this year we will be collecting muscle tissue samples from participants. Understanding muscle is very important in the Health ABC study. During past clinic visits we have done CT scans to look at your muscle. We have also done muscle strength measurements, such as the chair stands and leg strength tests. We will continue to administer some of these tests, but now we would like to look directly at your muscle tissue. The muscle sample will be taken from the middle of your thigh. There may be some pain and discomfort during this procedure, and we will make every attempt to make sure you are as comfortable as possible. We plan to collect around 150 mg of muscle tissue, which is about the size of 5 to 6 grains of rice. This tissue will undergo testing that we hope will help us better understand health and disease in older people."

1. Are you interested in participating in this part of the study?

- Yes
 No

↓

STOP. Complete Box A on Page 4. Mark "NOT INTERESTED."

SCRIPT: "Thank you for your interest in this procedure. Now, I'm going to ask you some questions to see if you are eligible."

2. Are you allergic to lidocaine or any other "caine" anesthesia?

(Examiner Note: These include Lidocaine [Xylocaine, Alphacaine, Lignospan, Octocaine], Mepivacaine [Carbocaine, Arestocaine, Isocaine, Polocaine, Scandonest], Prilocaine [Citanest], Articaine [Septocaine, Septanest, Astracaine, Ultracaine], and Bupivacaine [Marcaine].)

- Yes
 No
 Don't know
 Refused

↓

STOP. NOT ELIGIBLE. Go to Box A on Page 4.

3. Have you ever been told you have a bleeding disorder or clotting problem?

- Yes
 No
 Don't know
 Refused

↓

STOP. NOT ELIGIBLE. Go to Box A on Page 4.



HABC Enrollment ID #	Acrostic	Visit Year
H [] [] [] [] [] [] [] []	[] [] [] [] [] [] [] []	● 16

MUSCLE TISSUE COLLECTION ELIGIBILITY PRE-SCREENER

4. Do you regularly take any of the following medications?

<p>a. Coumadin (also known as Warfarin, Jantoven, Marevan, and Waran)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>b. Plavix (also known as Clopidogrel, Clopilet, and Ceruvin)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>c. Aggrenox (also known as Dipyridamole and Persantine)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>d. Ticlid (also known as Ticlopidine)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>e. Agrylin or Xagrid (also known as Anagrelide)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>f. Aricept (also known as Donepezil)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>g. Namenda (also known as Memantine)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>h. Exelon (also known as Rivastigmine)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>
<p>i. Razadyne (also known as Galantamine)?</p> <p><input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Don't know <input type="radio"/> Refused</p> <p>STOP. NOT ELIGIBLE. Go to Box A on Page 4.</p>



HABC Enrollment ID #	Acrostic	Visit Year
H [] [] [] [] [] []	[] [] [] [] [] []	● 16

MUSCLE TISSUE COLLECTION ELIGIBILITY PRE-SCREENER

5. Is there any other reason why this participant would not be eligible for the muscle tissue collection procedure?

- Yes
- No

a. What is the reason? Please describe below.

STOP. NOT ELIGIBLE. Go to Box A on Page 4.

6. Do you regularly take aspirin (also known as Bayer, Ascriptin, Excedrin, Bufferin, Anacin, and Ecotrin)?

- Yes
- No
- Don't know
- Refused

a. Have you been told by a doctor or other health care provider to take aspirin every day for reasons other than pain?

- Yes
- No
- Don't know

Examiner Note: Instruct participant to NOT TAKE aspirin for 5 days prior to the tissue collection.

Examiner Note: Inform the study physician who is doing the biopsy that the participant regularly takes aspirin.

Complete Box A on Page 4. Mark "Eligible."



HABC Enrollment ID #	Acrostic	Visit Year
H <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 16

MUSCLE TISSUE COLLECTION ELIGIBILITY PRE-SCREENER

Box A

○ ELIGIBLE FOR MUSCLE TISSUE COLLECTION PROCEDURE

"Thank you for your answers. At this point you appear to be eligible for a clinic visit that includes the muscle tissue collection procedure. The next step is to schedule a time for you to come to our clinic. This will be at no cost to you. During the clinic visit, we will tell you more about this procedure and about the other clinic exams, similar to ones you've had in the past, such as blood pressure, chair stands, and the 20-meter walk."

- Appointment scheduled **Date:** _____ **Time:** _____
- Call back for appointment **Date:** _____ **Time:** _____

○ NOT ELIGIBLE

"Thank you very much for this information. At this point we will not be asking you to come to the clinic. Only a limited number of people are being asked to return for a study visit, but we greatly appreciate your time and effort in answering questions for us."

○ NOT INTERESTED

"Can you tell me the reason why you are not interested in participating?"
(Examiner Note: Do NOT read response options. Mark all of the responses that apply.)

- No time/too busy
- Too much trouble
- Illness
- Concerned about the muscle tissue collection procedure/pain/discomfort during clinic visit
- No specific reason given
- Refused
- Other **(Please specify: _____)**

"Thank you for taking the time to answer these questions. If you should change your mind about participating, please call us at the Health ABC clinic."

Appendix 2 Frequently Asked Questions**Frequently Asked Questions****What is a muscle sample?**

A muscle sample is a small piece of muscle tissue about the size of a few grains of rice, taken from your thigh muscle.

Why are you doing this study?

Understanding how muscle tissue changes as we age may someday help us to know how to intervene to improve muscle tissue and, therefore, improve the quality of life of people as they age.

How exactly will the collection be done?

You will be asked to lie down on a table and the physician will thoroughly clean a small area in the middle of your thigh. After the numbing procedure, similar to what a dentist might do to numb your tooth, the physician will make a small cut in the skin in the middle of your thigh. The physician will then insert a needle into the muscle tissue. You should not feel anything except the pressure of the needle and perhaps a sharp pinch during the procedure.

Muscle tissue will be removed through this needle. When the procedure is finished, either Dermabond or a steristrip and bandage will be placed over the site.

What will you do to make me comfortable?

The physician will inject some numbing medication into the area of your thigh from where the tissue will be taken. You will feel a burning sensation from this medication for a short period of time. This medicine is very similar to the medicine a dentist uses to numb your teeth and gums.

Will I have to do anything special after the procedure?

You should plan to limit your activity for the rest of the day after the procedure. You shouldn't take a shower for 24 hours or bathe in a tub for 72 hours after the procedure.

How long will each visit take?

Visit 1- Your regular HABC clinic visit– about 2 hours.

Visit 2- Your muscle sample visit -about 1 hour.

Can I eat or drink before I come in to clinic?

We will ask that you only drink water and take your usual medications before you come in. We would like you to fast for at least 8 hours.

Appendix 3 Muscle Tissue Collection Instructions

Muscle Tissue Collection Instructions

The muscle tissue collection procedure is done to obtain a sample of muscle cells from the thigh area. This procedure will take approximately 1 hour, and will be completed by a physician or physician assistant.

Below are instructions that you should follow to prepare for the muscle tissue collection procedure. If you have any questions about the information below, please call our clinic staff at (xxx) xxx-xxxx.

BEFORE THE MUSCLE TISSUE COLLECTION:

1. **DO NOT STOP** taking your prescription medications.
2. Only take Tylenol for pain or cold symptoms for 1 week before the procedure. However, if you are taking aspirin under the direction of a doctor, keep taking it.
3. Do not engage in strenuous exercise for 48 hours before the procedure.
4. Do not eat or drink anything except water from midnight the night before your procedure until after the tissue collection has taken place. However, you should take your prescription medications.

DURING THE MUSCLE TISSUE COLLECTION PROCEDURE:

1. Your blood pressure will be taken before the test.
2. The physician will inject some numbing medication into the area of your thigh from where the tissue will be taken. You will feel a burning sensation from this medication for a short period of time. This medicine is very similar to the medicine the dentist uses to numb your teeth and gums.
3. Once the area is numb, the physician will make a small cut in the skin and then insert a needle into the muscle tissue. You should not feel anything except the pressure of the needle and perhaps a sharp pinch during the procedure.
4. Muscle tissue will be removed through this needle.
5. When the procedure is finished, either Dermabond or a steristrip and bandage will be placed over the site.

AFTER THE MUSCLE TISSUE COLLECTION PROCEDURE:

Plan to limit your activity for the rest of the day and do not shower until the next day. You should not take a bath for 72 hours after the procedure.

Appendix 4 Muscle Tissue Collection and Processing Forms

HABC Enrollment ID #	Acrostic	Visit Year	Date Form Completed	Staff ID#
H		● 16	/ / 2 0 1 3 Month Day Year	



**MUSCLE TISSUE COLLECTION
ELIGIBILITY CONFIRMATION**

1. Did laboratory results indicate that platelet count was normal ($\geq 50,000$)?
(Examiner Note: Check lab report. Platelet count should be $\geq 50,000$, or, if clumped, labeled sufficient.)

Yes No

NOT ELIGIBLE for muscle tissue collection. Go to Page P20, Question #17.

2. Is the participant taking Coumadin (Warfarin, Jantoven, Marevan, Waran), Plavix (Clopidogrel, Clopilet, Ceruvin), Aggrenox (Dipyridamole, Persantine), Ticlid (Ticlopidine), Agrylin (Anagrelide), Xagrid, Aricept (Donepezil), Namenda (Memantine), Exelon (Rivastigmine), or Razadyne (Galantamine)?
(Examiner Note: Look at medications that participant brought in to confirm that participant is not taking any of the medications that would exclude them from having the muscle tissue collection procedure.)

Yes No Don't know Refused

NOT ELIGIBLE for muscle tissue collection. Go to Page P20, Question #17.

3. **Take seated blood pressure and record:**

a. Systolic mm Hg b. Diastolic mm Hg

4. Is participant's systolic blood pressure greater than 180 mm Hg or diastolic blood pressure greater than 110 mm Hg?

Yes No

NOT ELIGIBLE for muscle tissue collection. Go to Page P20, Question #17.

5. Is there any other reason why this participant should not have the muscle tissue collection procedure?

Yes No

NOT ELIGIBLE for muscle tissue collection. Please describe below and go to Page P20, Question #17.

Ask participant:

6. Have you taken aspirin or aspirin-containing products including Aleve, Ibuprofen, Motrin or other anti-inflammatory medications in the past 5 days?

Yes No Don't know Refused

Examiner Note: Alert study physician performing muscle tissue collection.

HABC Enrollment ID #	Acrostic	Visit Year
H [] [] [] [] [] []	[] [] [] [] [] []	● 16

MUSCLE TISSUE COLLECTION

7. **Examiner Note:** If participant already had their blood drawn get this information from the Phlebotomy Form, Page 26, Question #5. Otherwise ask:

What is the date and time you last ate anything?

a. Date of last food: [] [] / [] [] / [] [] [] []
 Month Day Year

b. Time of last food: [] [] : [] [] am pm
 Hours Minutes

Bar Code Label

8. From which leg was sample taken?
 (Examiner Note: Refer to Clinic Visit Workbook, Page P16/M24, Question #11. Choose same leg as tested during Isokinetic Quadriceps Strength [Kin-Com] test. If Kin-Com test not administered, obtain sample from right thigh, unless contraindicated.)

Right Left

9. **Ask participant:** Are you allergic to latex?
 Yes No

Examiner Note: Use non-latex gloves.

10. Procedure start time: [] [] : [] [] am pm
 Hours Minutes

11. Procedure stop time: [] [] : [] [] am pm
 Hours Minutes

12. Amount of 2% lidocaine injected: [] [] mL

After muscle tissue collection, ask participant:

13. Please rate the pain that you have at the muscle tissue collection site by picking a number from 0 to 10 where "0" means "No pain" and "10" means the "Worst pain you can imagine."

0 1 2 3 4 5 6 7 8 9 10



HABC Enrollment ID #	Acrostic	Visit Year
H <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 16

MUSCLE TISSUE COLLECTION

14. How many times was the trocar inserted through the incision?

times

15. How many times was the trocar rotated for suction?

times

16. Were there adverse reactions during or after procedure?

Yes No

a. What adverse events were reported?

Examiner Note: Mark all that apply.

- Excessive bleeding
- Participant fainted (vasovagal episode)
- Excessive pain/discomfort
- Reaction to lidocaine
- Other **(Please specify:)** _____

b. Complete Adverse Event (AE) Form. Record AE Form #:

17. Was any subcutaneous fat collected?

Yes No

Please describe why not:

18. Was any muscle tissue sample collected?

Yes No

Please describe why not:

HABC Enrollment ID #	Acrostic	Visit Year	Staff ID#
H <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	● 16	<input type="text"/> <input type="text"/> <input type="text"/>



MUSCLE TISSUE PROCESSING

Bar Code Label

Time at start of processing: : am
 pm

Specimens	Container #	Weight	Type	Estimated weight (mg)*
Muscle	21	15-20 mg	histology embedding mold	<input type="text"/> <input type="text"/> mg
Muscle	22	10 mg	2 mL cryovial (for respirometry)	<input type="text"/> <input type="text"/> mg
Muscle	23	2-5 mg	2 mL cryovial (for electron microscopy)	<input type="text"/> <input type="text"/> mg
Muscle	24	15-20 mg	2 mL cryovial	<input type="text"/> <input type="text"/> mg
Muscle	25	15-20 mg	2 mL cryovial	<input type="text"/> <input type="text"/> mg
Muscle	26	15-20 mg	2 mL cryovial	<input type="text"/> <input type="text"/> mg
Muscle	27	15-20 mg	2 mL cryovial	<input type="text"/> <input type="text"/> mg
Muscle	28	15-20 mg	2 mL cryovial	<input type="text"/> <input type="text"/> mg
Muscle	29	variable	2 mL cryovial	<input type="text"/> <input type="text"/> <input type="text"/> mg
Intermuscular Fat	30	variable	2 mL cryovial	<input type="text"/> <input type="text"/> <input type="text"/> mg
Connective Tissue	31	variable	2 mL cryovial	<input type="text"/> <input type="text"/> mg
Subcutaneous Fat	32	variable	2 mL cryovial	<input type="text"/> <input type="text"/> <input type="text"/> mg

*If no specimen is obtained, record "0" mg for estimated weight.

Year 16 Clinic/Home Visit Workbook
EL

Appendix 5 Muscle Tissue Collection Discharge Instructions**Muscle Tissue Collection Discharge Instructions**

Complications after muscle tissue collection are rare. There may be bruising at the site of the muscle tissue collection. This should go away in a couple of weeks. There may be some mild discomfort after the biopsy.

POST-PROCEDURE INSTRUCTIONS:

1. To prevent infection, keep the site clean and dry for at least 24 hours.
2. Do not shower for 24 hours after the procedure. Do not take a bath for 72 hours after the procedure.
3. You should not experience excessive bleeding or bruising. A few drops of blood on the bandage is expected. If excessive bleeding occurs, call the clinic immediately.
4. To prevent bleeding and/or bruising, keep the bandage on for 72 hours.
5. You should not do any strenuous activity or exercise for 48 hours after the procedure.
6. You should not take aspirin or NSAIDS, such as Aleve, Ibuprofen, etc., for at least 3 days after the procedure, unless instructed by your doctor. You may take Tylenol for any discomfort during the first 3 days.
7. If there is pain at the tissue collection site, you may apply ice every two hours for 20 minutes until the pain subsides.
8. Although it is very rare, infection (presenting as redness, pus from the wound, increasing pain) can occur with muscle tissue collection. If this occurs contact the clinical center staff immediately. If this occurs outside of office hours, go to the hospital emergency room, and indicate to the hospital staff that a muscle biopsy was completed.

IF YOU HAVE A PROBLEM:

If you have a problem during office hours, call the clinical center staff at (xxx) xxx-xxxx.

If there is an emergency related to this procedure after normal office hours, you should go to the hospital emergency room, and indicate to the hospital staff that a muscle biopsy was completed.

MEDICATIONS THAT CONTAIN ASPIRIN (page 1 of 2)

<p>A.S.A. & CODEINE COMPOUND #2 CAP ACETAMINOPHEN AND ASPIRIN 250/25MG ACETAMINOPHEN/ASPIRIN 325/500MG ACETAMINOPHEN/ASPIRIN/CAFFIENE 250/250/65MG ACUPRIN 81MG ENTERIC COATED ACUPRIN TAB ALKA-SELTZER ALKA-SELTZER PAIN RELIEF ALKA-SELTZER PLUS ALKA-SELZER PM ALKA-SELZER PM PAIN RELIEVER & SLEEP AID ANACIN 400MG TAB ANACIN 500MG CAPS ANACIN 500MG TAB ANACIN CAPLET ANACIN MAXIMUM STRENGTH ANACIN TAB APC/CODEINE 15MG CAP APC/CODEINE 15MG TAB APC/CODEINE 16MG TAB APC/CODEINE 16MG-ZENITH-TAB -1/4GR #1- APC/CODEINE 30MG CAP APC/CODEINE 30MG TAB APC/CODEINE 30MG-COOPER-TAB APC/CODEINE 30MG-LEDERLE-TAB APC/CODEINE 32MG TAB APC/CODEINE 32MG-PARKE DAVIS-TAB -1/2GR #3- APC/CODEINE 32MG-ZENITH-TAB -1/2GR #3- APC/CODEINE 64MG TAB APC/CODEINE 8MG TAB APC/PSEUDOEPHEDRINE-20MG- TAB APC/PSEUDOEPHEDRINE-20MG/CODEINE PHOS-15MG- TAB ASA 163MG ASA 325MG ASA 325MG SR ASA 650MG ASA 800MG SR TABS ASA 81 MG ASA 975MG EC TAB ASA ENSEALS (LILLY) ASA ENTERIC-COATED, NS ASA TR TAB OTC ASA, AMINOBENZOIC ACID, Al(OH)3 ASA, APAP, CAFFEINE, Mg(OH), Al(OH), NS ASA, APAP, CAFFEINE, NS ASA, Mg(OH)2, Al, GLYCINATE, NS ASA, NaHCO3, CITRIC ACID, NS ASA, NONSPECIFIC ORAL ASA, PHENYLEPHRINE ASA/SALICYLIC ACID ASCODEEN-30 TAB ASCOMP WITH CODEINE ASCRIPTIN 325MG TAB ASCRIPTIN 81MG ASCRIPTIN A/D ASCRIPTIN EXTRA STRENGTH 500MG TAB ASCRIPTIN TABLETS, NOT OTHERWISE SPECIFIED ASPERGUM ASPERMIN 10MG TABLET ASPIRIN ASPIRIN (CHILDREN S) 1 1/4 GR (80 MG) TABLETS ASPIRIN (CHILDREN S) 1 1/4GR (81MG) TABS ASPIRIN (CHILDREN S) TAB ASPIRIN 1 1/4GR (80MG) ENTERIC COATED TAB ASPIRIN 1 1/4GR (80MG) TABLETS ASPIRIN 10MG ASPIRIN 162MG (2.5GRAIN) ASPIRIN 163MG & SALSALATE 488MG ASPIRIN 250 MG ASPIRIN 325MG TAB ASPIRIN 500MG ENTERIC COATED TAB ASPIRIN 500MG TAB</p>	<p>ASPIRIN 600MG TAB ASPIRIN 650MG ENSEALS OTC ASPIRIN 800MG TAB ASPIRIN 81MG ASPIRIN ALCOHOL RUB ASPIRIN AND CAFFEINE, NS ASPIRIN BUFFERED WITH MAALOX ASPIRIN ENTERIC COATED 200MG ASPIRIN ENTERIC COATED 325MG ASPIRIN ENTERIC COATED 500MG ASPIRIN ENTERIC COATED 81MG ASPIRIN PLUS ASPIRIN PLUS ANTACID ASPIRIN SUPP 10 GRAIN LILLY ASPIRIN SUPP 5 GRAIN LILLY ASPIRIN TAB BUFFERED 5 GRAIN ASPIRIN TABLETS 10 GRAIN EC ASPIRIN TABS 325MG (5 GRAIN) ASPIRIN TABS 5 GRAIN ASPIRIN TABS EC 5 GRAINS ASPIRIN W/CODEINE-1/2GR-TAB ASPIRIN W/CODEINE-1/4GR-TAB ASPIRIN W/CODEINE-1GR-TAB ASPIRIN, ENTERIC COATED 500MG TAB ASPIRIN, ENTERIC COATED, NOT OTHERWISE SPECIFIED ASPIRIN, TIME RELEASE ASPIRIN/ACETAMINOPHEN/SALICYLAMIDE/CAFFEINE ASPIRIN/ALCOHOL, TOPICAL ASPIRIN/BUTALBITAL TAB 325/50MG ASPIRIN/CAFFEINE/CODEINE ASPIRIN/CAFFEINE/MAGNESIUM SALICYLATE ASPIRIN/CALCIUM TABS/CAPLETS ASPIRIN/CODEINE/CAFFEINE ASPIRIN/MAALOX ASPRIMOX 325MG AXOTAL TAB BABY ASPIRIN BACK QUELL TABS BAYER ASPIRIN BAYER ASPIRIN 325MG BAYER ASPIRIN 650MG TABLET BAYER ASPIRIN PM BAYER ASPIRIN, MAXIMUM BAYER BABY ASPIRIN BAYER BABY ASPIRIN 81MG (1 1/4GR) TAB BAYER CHILDREN S ASPIRIN, NOS BAYER EC ADULT LOW STRENGTH TAB BAYER ENTERIC COATED ASPIRIN BAYER EXTRA STRENGTH BACK AND BODY PAIN BAYER S CHILDREN S ASPIRIN 81MG BAYER S EC ASPIRIN (1 1/4GR) TAB BAYER WOMENS CAPLETS ASPIRIN/CALCIUM 81/300MG BC ARTHRITIS PAIN BC PAIN RELIEF BC POWDER ARTHRITIS STRENGTH 722/222/65 BC SINUS COLD BEXOPHENE CAP BUFF-A CAP BUFF-A COMP BUFF-A-COMP CAPS BUFFERED ASPIRIN 325MG TAB BUFFERED ASPIRIN 500MG TAB BUFFERIN BUFFERIN 325MG TAB BUFFERIN 500MG TAB BUFFERIN, EXTRA STRENGTH 500MG TAB BUTALBITAL 50MG/APC/CODEINE 16MG CAP BUTALBITAL 50MG/APC/CODEINE 32MG CAP BUTALBITAL 50MG/APC/CODEINE 8MG CAP BUTALBITAL/ASPIRIN TAB 50/325MG CAFFEINE/CODEINE/ASPIRIN CALCIUM/ASPIRIN TABS/CAPLETS</p>
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MEDICATIONS THAT CONTAIN ASPIRIN (page 2 of 2)

CARISOPRODOL/ASPIRIN 200MG/325MG
CARISOPRODOL/CODEINE/ASPIRIN TABS 200/325/16MG
CHLORMEZANONE 100MG/ASPIRIN 300MG TAB
CHLORMEZANONE 50MG/ASPIRIN 300MG TAB
CHLORPHANIRAMINE, PHENYLEPHRINE, ASA, CAFFEINE,
CHLORPHENIRAMINE, PHENYLPROPANOLAMINE, ASA, CAF
CHLORPHENIRAMINE, ASA
CHLORPHENIRAMINE, PHENYLPROPANOLAMINE & ASA
CIRIN
CONGESPIRIN
DARVO TRAN CAP
DARVON 65 W/ASA
DARVON COMPOUND CAP
DARVON COMPOUND-65 CAP
DARVON W/ASA
DARVON-N W/ASA TAB
DASIN
DASIN CAP
DEMEROL/APC TAB
DOLENE COMPOUND-65
DRISTAN CAPSULES
DURADYNE
EASPRIN 975MG
ECASA TAB 81MG
ECOTRIN 325 MG
ECOTRIN 81MG ADULT STRENGTH TAB
ECOTRIN MAXIMUM STRENGTH 500 MG TAB
ECOTRIN TABS OTC
EFFERVESCENT COLD RELIEVER
EMPIRIN #1 WITH CODEINE
EMPIRIN COMP TAB OTC
EMPIRIN COMPOUND #1 TAB
EMPIRIN COMPOUND #2 TAB
EMPIRIN COMPOUND #3 TAB
EMPIRIN COMPOUND #4 TAB
EMPIRIN W/ CODEINE #2 TAB
EMPIRIN W/ CODEINE #3 TAB
EMPIRIN W/ CODEINE #4 TAB
EMPRAZIL TAB
EMPRAZIL-C TAB
ENTERIC COATED ASPIRIN TAB, NOS
EPRAGEN CAP
EQUAGESIC TAB
ETHOHEPATAZINE CITRATE 75MG/ASPIRIN 325MG TAB
ETHOHEPTAZINE CITRATE 100MG/APC TAB
EXCEDRIN
EXCEDRIN EXTRA STRENGTH CAPS/TABS/GELTABS
EXCEDRIN MIGRANE TABS
FIORINAL #1 CAP
FIORINAL #2 CAP
FIORINAL #3 CAP
FIORINAL CAP
FIORINAL TAB
FIORINAL W/ CODEINE CAPS 30/325
FIORINAL W/CODEINE #1 CAP
FIORINAL W/CODEINE #2 CAP
FIORINAL W/CODEINE #3 CAP
FIORINAL/CODEINE CAPS 30/40/50/325MG
FIORMOR TAB
GENACOTE 325MG
GENACOTE 500MG ENTERIC COATED TAB
GOODYS BODY PAIN
GOODYS EXTRA STRENGTH HEADACHE POWDER
GOODYS POWDER
HALFPRIN 165 MG
HALFPRIN 81MG
HISTADYL+ASA+CODEINE
ISOLLYL
MAALOX/ASPIRIN
MAGNAPRIN ARTHRITIS STRENGTH TABS 325/75/75MG
MAGNAPRIN COATED TABS 325/50/50MG
MARNAL
MEASURIN
MEPERIDINE 30MG/APC TAB
METHOCARBAMOL 400/ASPIRIN 325 MG TAB
MICRAININ
MIRACLE ASPIRIN SPRAY
MOMENTUM
NORGESIC FORTE TAB
NORGESIC TAB
ORPHENADRINE CIT 25MG/APC TAB
ORPHENADRINE CIT 25MG/APC-MOORE- TAB
OXYCODONE HCL 2.25MG/APC TAB
OXYCODONE HCL 4.5MG/APC TAB
OXYCODONE-4.5MG/TEREPH 0.38MG/HEXOBARB 100MG/
PABRIN ASA, AMINO BENZOIC ACID, Al(OH)₃
P-A-C W/CODEINE 1/2 -#3- TAB
P-A-C W/CODEINE 1/2GR -#3- CAP
P-A-C W/CODEINE 1/4GR -#2- TAB
PAIN AID TAB
PENTAZOCINE HCL-12.5MG-/ASPIRIN-325MG-TAB
PERCOBARB CAPS
PERCODAN TAB
PERCODAN-DEMI
PERSISTIN
PERSISTIN TAB
PHENACETIN-194MG-/ASPIRIN-162MG-/PHENOBARB-16MG-
PHENAGLYCODOL 150MG CAP
PHENAPHEN PLAIN CAP-BEFORE 3-1-76-
PHENERGAN COMPOUND TAB
PHENYLPROPANOLAMINE, ASA
PRAVASTATIN/ASPIRIN TAB 325MG/20MG
PRAVASTATIN/ASPIRIN TAB 325MG/40MG
PRAVASTATIN/ASPIRIN TAB 325MG/80MG
PRAVASTATIN/ASPIRIN TAB 81MG/20MG
PRAVASTATIN/ASPIRIN TAB 81MG/40MG
PRAVASTATIN/ASPIRIN TAB 81MG/80MG
PRAVIGARD TAB 325MG/20MG
PRAVIGARD TAB 325MG/40MG
PRAVIGARD TAB 325MG/80MG
PRAVIGARD TAB 81MG/20MG
PRAVIGARD TAB 81MG/40MG
PRAVIGARD TAB 81MG/80MG
PREDISAL TAB
PROMETHAZINE HCL 6.25MG CAP
PROMETHAZINE HCL 6.25MG TAB
PROPOXYPHENE HCL 65MG W/325M ASA
PROPOXYPHENE HCL 65MG/ASPIRIN 32KMG-ALLIANCE-
PROPOXYPHENE HCL COMP 32 MG
PROPOXYPHENE HCL COMP 65 MG
PROPOXYPHENE NAPSYLATE 100MG/ASPIRIN 325MG TAB
PYRILAMINE MALEATE, ASA, ACETAMINOPHEN
PYRROXATE & CODEINE
QUIET WORLD TABLETS
RHINEX D-LAY
ROBAXISAL TAB
ROXIPRIN TABS 4.5MG/325MG
SK-65 COMPOUND CAP
SOMA COMPOUND TAB
SOMA COMPOUND W/CODEINE TAB
ST. JOSEPH ADULT CHEWABLE ASPIRIN
ST. JOSEPH ASPIRIN
STANBACK POWDERS
SYNALGO D C CAPS
SYNALGOS CAP
TALWIN COMP TABS
TRANCOGESIC TAB
TRTANCOPRIN TAB
UNIGESIC-A TAB
VANQUISH
ZACTIRIN COMPOUND TAB
ZACTIRIN TAB
ZORPRIN TAB

MEDICATIONS THAT CONTAIN IBUPROFEN

ADVIL
ADVIL COLD & SINUS 200/30
ADVIL FLU & BODY ACHE
ADVIL PM 200/38 MG
ADVIL TAB 200MG
APAPW/COD/IBUPROFEN
ARTHRITIS FOUNDATION IBUPROFEN
BAYER SELECT PAIN RELIEF FORMULA
CLOTRIMAZOLE/IBUPROFEN/DMSO
CODEINE/APAP/IBUPROFEN
COMBUNOX TAB 5MG/400MG
EQUATE IBP 200MG
HYDROCODONE/IBUPROFEN 7.5/200mg
IBUPROFEN 200 MG TAB
IBUPROFEN 600MG TABLET
IBUPROFEN 800MG TABLET
IBUPROFEN LIQUID 100MG/5ML
IBUPROFEN TAB
IBUPROFEN TAB 300MG
IBUPROFEN TAB 400MG
IBUPROFEN/DIPHENHYDRAMINE
MOTRIN 800 MG
MOTRIN IB TAB 200 MG
MOTRIN MIGRAINE PAIN 200 MG IBUPROFEN
MOTRIN TAB
MOTRIN TAB 300MG
MOTRIN TAB 400MG
MOTRIN TAB 600MG
NUPRIN 200 MG TABS
NUPRIN TAB
OXYCODONE/IBUPROFEN TAB 5MG/400MG
RUFEN 400MG TAB
VICOPROFEN TABS 7.5/200MG

Appendix 6 Muscle Tissue Collection Follow-up Telephone Contact



HABC Enrollment ID #	Acrostic	Date Form Completed			Staff ID #
H [] [] [] [] [] []	[] [] [] [] [] []	[] / [] / [] [] [] []	Month	Day	Year
		Visit Year: ● 16			

MUSCLE TISSUE COLLECTION FOLLOW-UP TELEPHONE CONTACT

SCRIPT: "Hello, I'm _____ from the ____ Health ABC clinic. We'd like to know how you are doing after the muscle tissue collection procedure. I'd like to ask you a few questions. Is this a good time?"

1. Please rate the pain that you have at the tissue collection site by picking a number from 0 to 10 where "0" means "No pain" and "10" means the "Worst pain you can imagine."

0 1 2 3 4 5 6 7 8 9 10

Examiner Note: If participant reports any pain (1 through 10), complete the Adverse Event Form.

2. Did you need to take pain relieving drugs, such as Tylenol, for pain related to the procedure?
 Yes No Don't know Refused

Please explain: _____

3. Have you had any problems related to the bandage or Dermabond, such as a rash or itching?
 Yes No Don't know Refused

Please explain: _____

4. Do you have any bruising or bleeding around the tissue collection site?
 Yes No Don't know Refused

- a. Are you concerned about this bruising or bleeding?
 Yes No Don't know

Please explain: _____

5. Is there any sign of redness, pus, or warmth from the tissue collection site?
 Yes No Don't know Refused

Please explain: _____

6. Have you had any other problems or concerns with the procedure?
 Yes No Don't know Refused

Please explain: _____

7. Is an Adverse Event Form required?
(Examiner Note: If participant reports pain at the collection site (Question #1) or answers "Yes" to any of Questions #2, #3, #4a, #5, or #6 above, complete an Adverse Event Form.)

Yes No

Please explain: _____

Muscle Tissue Collection Followup Telephone Contact

Appendix 7 Muscle Tissue Collection Adverse Event Form



HABC Enrollment ID #	Acrostic	Date Form Completed	Staff ID #
<input type="text" value="H"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> / <input type="text"/> / <input type="text"/> Month Day Year	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>

Visit Year: 16

MUSCLE TISSUE COLLECTION ADVERSE EVENT FORM

1. Adverse Event (AE) Report # (AEs should be numbered, starting with #1 for each participant):
2. How was the clinic notified of the adverse event?
 - In clinic Interviewer-initiated Participant called clinic Proxy called clinic Hospital/doctor called clinic
 - follow-up phone contact

3. What adverse event was reported? (*Examiner Note: If any bubbles within a box are filled in, report AE to IRB.*)

Adverse Events Reported: (Mark <u>all</u> that apply.)	Severity	Relationship to Procedure	Type of Event	Action Taken	Outcome
a. <input type="radio"/> Infection of biopsy site (redness, pus, or warmth) →	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown
b. <input type="radio"/> Pain or discomfort →	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown
c. <input type="radio"/> Bruising or bleeding →	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown
d. <input type="radio"/> Problem related to bandage or Dermabond →	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown



HABC Enrollment ID #	Acrostic	AE Report #	Visit Year
H			16

MUSCLE TISSUE COLLECTION ADVERSE EVENT FORM

3. (con't) What adverse event was reported? (*Examiner Note: If any bubbles within a box are filled, report AE to IRB.*)

Adverse Events Reported: (Mark <u>all</u> that apply.)	Severity	Relationship to Procedure	Type of Event	Action Taken	Outcome
e. <input type="radio"/> Reaction to lidocaine →	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown
f. <input type="radio"/> Vasovagal episode →	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown
g. <input type="radio"/> Other → <i>Please specify:</i> _____ _____	<input type="radio"/> Mild <input type="radio"/> Moderate <input type="radio"/> Severe <input type="radio"/> Life-threatening <input type="radio"/> Unknown	<input type="radio"/> None <input type="radio"/> Unlikely <input type="radio"/> Possibly <input type="radio"/> Probably <input type="radio"/> Definitely	Report to IRB.	Describe: _____ _____ _____ _____ _____	<input type="radio"/> Recovered <input type="radio"/> Improved <input type="radio"/> Unchanged <input type="radio"/> Worsening <input type="radio"/> Unknown

4. Was study physician notified?
 Yes → Date of notification: / /
Month Day Year
 No → **Go to Question #8.** Name of physician notified:

5. Did study physician think participant should come to Health ABC clinic for follow up?
 Yes
 No

6. Was participant's physician notified?
 Yes → Date of notification: / /
Month Day Year
 No Name of physician notified:

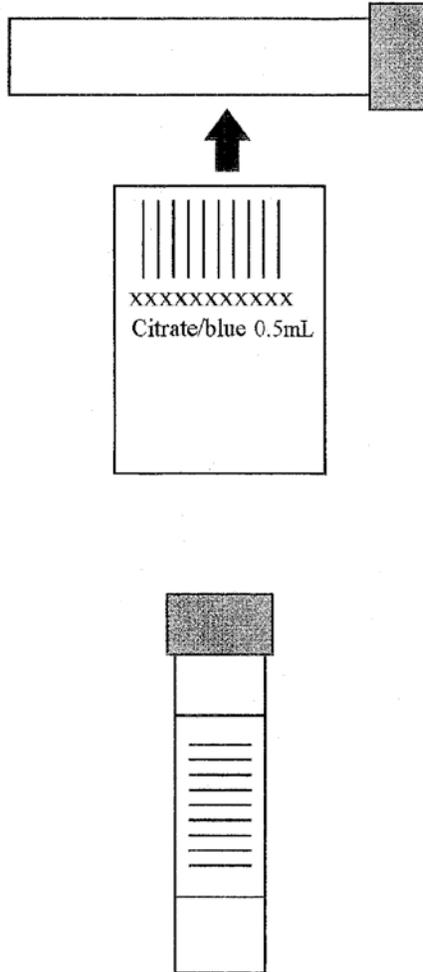
Appendix 8 Sample Label Sheet

 ##### Muscle Tissue Collection Form	 ##### Muscle Tissue Processing Form	
Place this end on vial first  #####-21 Histology	Place this end on vial first  #####-22 Respirometry	Place this end on vial first  #####-23 EM
Place this end on vial first  #####-24 Muscle BCHM #1	Place this end on vial first  #####-25 Muscle BCHM #2	Place this end on vial first  #####-26 Muscle BCHM #3
Place this end on vial first  #####-27 Muscle BCHM #4	Place this end on vial first  #####-28 Muscle BCHM #5	Place this end on vial first  #####-29 Muscle BCHM#6

<p>Place this end on vial first</p>  <p>#####-30 Intermuscular Fat</p>	<p>Place this end on vial first</p>  <p>#####-31 Connective Tissue</p>	<p>Place this end on vial first</p>  <p>#####-32 Subcutaneous Fat</p>
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HEALTH ABC STUDY

Label Orientation on Cryovial



Appendix 9 Muscle Tissue Collection Tray Checklist

Per tray:

- ChloraPrep
- Band-Aids
- Gauze (4x4)
- Tegaderm dressing
- Scissors
- Sterilized 5 mm Bergstrom biopsy needle (and at least one backup for each procedure)
- 2% buffered lidocaine
- Scalpel (#11 blade)
- 2-10 cc syringe
- 22 g and 25 g needles
- 60 cc syringe and tubing for suction
- Cork
- Kimwipes
- #9 filter paper
- Sterile drape (fenestrated)
- Plunger for biopsy needle
- Forceps
- Tweezers
- Antibiotic ointment

Other materials should be available in room (not placed in sterile field):

- Smelling salts
- Dissecting microscope
- Isopentane
- Container for isopentane
- Liquid nitrogen
- Needle/sharps container
- Timer/stopwatch
- Pencils/pens
- Sterile gloves of various sizes. Latex gloves should be used unless the participant is allergic to latex.

- Alternative gloves if participant is allergic to latex
- Extra set of tweezers
- Glass Petri dish on ice
- Glass Petri dish with section of parafilm
- EM vial with ~1 mL 3.7% glutaraldehyde on ice
- 3 to 4 mm x 6 mm sections of Kimwipe for sample preparation

Per participant:

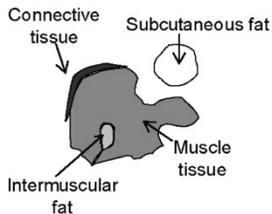
- Container for cryovial pouch and other tissue containers labeled and numbered.

At the muscle tissue collection station:

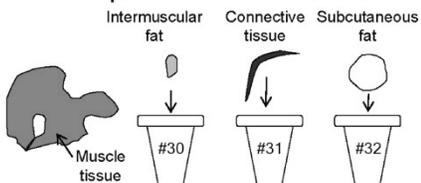
- Basin
- Cold cloth
- Biohazard containers
- Needle/Sharps container
- Paper towels

Appendix 10 Muscle Tissue Processing Steps

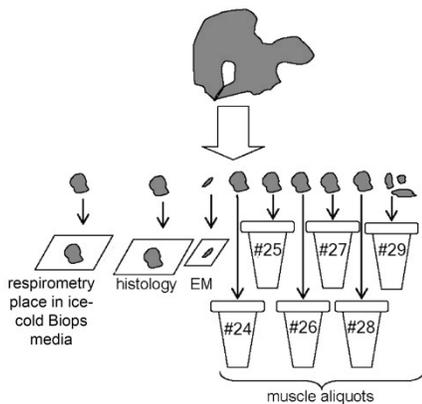
Sample collected:



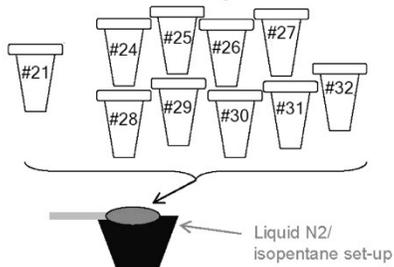
STEP 1: Separate fat and connective tissue



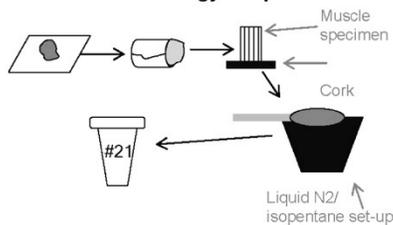
STEP 2: Divide muscle tissue



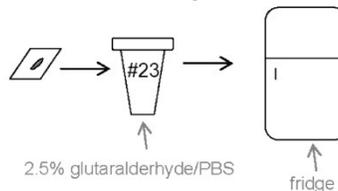
STEP 3: Flash freeze samples #21, #24-32



STEP 4: Process histology sample



STEP 5: Process EM sample



Appendix 11a Freezer Box Diagram for Pittsburgh Lab

Freezer Box Diagram for Histology Muscle Sample in Pittsburgh – cryovial #01

Numbers = cryovial #01 – one per participant

start #1

Top

Ppt #1 21	Ppt #2 21	Ppt #3 21	Ppt #4 21	Ppt #5 21	Ppt #6 21	Ppt #7 21	Ppt #8 21	Ppt #9 21
Ppt #10 21	Ppt #10 21	Ppt #12 21	Ppt #13 21	Ppt #14 21	Ppt #15 21	Ppt #16 21	Ppt #17 21	Ppt #18 21
Ppt #19 21	Ppt #20 21	Ppt #21 21	Ppt #22 21	Ppt #23 21	Ppt #24 21	Ppt #25 21	Ppt #26 21	Ppt #27 21
Ppt #28 21	Ppt #29 21	Ppt #30 21	Ppt #31 21	Ppt #32 21	Ppt #33 21	Ppt #34 21	Ppt #35 21	Ppt #36 21
Ppt #37 21	Ppt #38 21	Ppt #39 21	Ppt #40 21	Ppt #41 21	Ppt #42 21	Ppt #43 21	Ppt #44 21	Ppt #45 21
Ppt #46 21	Ppt #47 21	Ppt #48 21	Ppt #49 21	Ppt #50 21	Ppt #51 21	Ppt #52 21	Ppt #53 21	Ppt #54 21
Ppt #55 21	Ppt #56 21	Ppt #57 21	Ppt #58 21	Ppt #59 21	Ppt #60 21			

Bottom

End#81

continue to
next box....

Label outside of box: Histology / Muscle Box #1 Date: ____ / ____ / ____

Appendix 11b Freezer Box Diagram for Pittsburgh Lab

Freezer Box Diagram for Muscle/Fat/Connective Tissue in Pittsburgh – cryovials #24-#32

Numbers = cryovial #24-32

	start #1							Top	
Ppt #1 24	25	26	27	28	29	30	31	32	
Ppt #2 24	25	26	27	28	29	30	31	32	
Ppt #3 24	25	26	27	28	29	30	31	32	
Ppt #4 24	25	26	27	28	29	30	31	32	
Ppt #5 24	25	26	27	28	29	30	31	32	
Ppt #6 24	25	26	27	28	29	30	31	32	
Ppt #7 24	25	26	27	28	29	30	31	32	
Ppt #8 24	25	26	27	28	29	30	31	32	
Ppt #9 24	25	26	27	28	29	30	31	32	
	Bottom							End#81	

continue to
next box....

Label outside of box: Muscle and Fat Box #1 Date: ____ / ____ / ____

Appendix 11c Refrigerator Box Diagram – Pittsburgh – Specimen #3

Refrigerator Box Diagram for Muscle for Electron Microscopy in Pittsburgh – cryovial #23

Numbers = cryovial #23 – one per participant

start #1

Top

Ppt #1 23	Ppt #2 23	Ppt #3 23	Ppt #4 23	Ppt #5 23	Ppt #6 23	Ppt #7 23	Ppt #8 23	Ppt #9 23
Ppt #10 23	Ppt #10 23	Ppt #12 23	Ppt #13 23	Ppt #14 23	Ppt #15 23	Ppt #16 23	Ppt #17 23	Ppt #18 23
Ppt #19 23	Ppt #20 23	Ppt #21 23	Ppt #22 23	Ppt #23 23	Ppt #24 23	Ppt #25 23	Ppt #26 23	Ppt #27 23
Ppt #28 23	Ppt #29 23	Ppt #30 23	Ppt #31 23	Ppt #32 23	Ppt #33 23	Ppt #34 23	Ppt #35 23	Ppt #36 23
Ppt #37 23	Ppt #38 23	Ppt #39 23	Ppt #40 23	Ppt #41 23	Ppt #42 23	Ppt #43 23	Ppt #44 23	Ppt #45 23
Ppt #46 23	Ppt #47 23	Ppt #48 23	Ppt #49 23	Ppt #50 23	Ppt #51 23	Ppt #52 23	Ppt #53 23	Ppt #54 23
Ppt #55 23	Ppt #56 23	Ppt #57 23	Ppt #58 23	Ppt #59 23	Ppt #60 23			

Bottom

End#81

continue to next box....

Label outside of box: EM/Muscle Box #1 Date: ____ / ____ / ____