

ENERGY EXPENDITURE

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ENERGY EXPENDITURE**1.1 Background and rationale**

Significance of Energy Expenditure: The first law of thermodynamics states that energy cannot be created or destroyed. As applied to the human body, this means that energy expenditure must equal metabolizable energy intake plus change in body energy stores. Weight changes therefore may result from either changes in energy expenditure or energy intake. Measurement of energy expenditure thus permits one to partition any change in weight between either input or expenditure. Furthermore, energy expenditure can be used to determine the energy needs of the elderly.

By combining measures of total energy expenditure with those of resting energy expenditure further information can be obtained. The difference between total energy expenditure and resting energy expenditure is the energy expenditure in physical activity and the thermic effect of meals. Because the latter is relatively small and similar across individuals, the difference also provides an objective measure of the energy costs of physical activity. This will provide insight into the quality of life and provide a baseline measure of physical activity.

1.2 Introduction to energy expenditure measurements

The University of Wisconsin serves as the Energy Expenditure Reading Center (EERC) and will provide centralized analysis for the measurement of total energy expenditure by doubly-labeled water and reading of the resting metabolic rate data. Tasks of the Center include protocol design; training of field center personnel on the use of the energy expenditure equipment and supplies; preparation of loading doses of doubly-labeled water for use by field centers; analysis of physiological specimens for deuterium and oxygen-18 to determine energy expenditure; collating, analyzing and storing energy expenditure data; providing quality assurance of energy expenditure data; transmitting energy expenditure data analysis to the Coordinating Center at specified intervals; providing technical support to the field centers; and participating in the analysis and publication of results.

1.3 Total energy expenditure

Total energy expenditure is the amount of energy that an individual uses throughout the 24-hour day. It includes resting energy expenditure, the thermic effect of meals, and the energy expended during physical activity.

Total energy expenditure will be measured by the elimination of water labeled with two heavy atoms, deuterium and oxygen-18. After a loading dose of the labeled water, deuterium is eliminated from the body as water and oxygen-18 as water and carbon dioxide. The difference between the elimination rates is therefore a measure of carbon dioxide production. Carbon dioxide is the end product of energy metabolism and thus provides a measure of energy expenditure. The measurement involves the administration of the labeled water and collection of physiologic specimens on two days separated by 12 to 16 days. The measurement of total energy expenditure therefore requires two clinic visits separated by 12 to 16 days.

1.4 Resting energy expenditure measurement

Resting metabolic rate will be measured with a /+ II Respiratory Gas Analyzer. This is a portable unit that measures the concentrations of oxygen and carbon dioxide in air streams entering and exiting a clear plastic bubble placed over the participant's head. Oxygen consumption and carbon dioxide production are calculated from the change in concentration and flow rate. The measurement must be made under standard conditions and requires about 40 minutes to complete.

It is vital that the methods used to measure energy expenditure be consistent between the field centers to insure that the data is uniform across the study cohort. Standard techniques for instrument calibration, data collection, and specimen handling must be followed to insure comparability between sites. This requires that all field center personnel responsible for performing the energy expenditure protocol be trained and certified by the EERC. Training and certification of field center personnel will be performed prior to initiation of the energy expenditure protocol. Monthly reevaluation will be confirmed after review of energy expenditure calibration data and participant data by the EERC.

2. Training and certification

2.1 Training of energy expenditure technicians

A representative of the EERC will travel to the field center to conduct the initial training session. Training for measurement of resting energy expenditure and total energy expenditure will be combined. Energy expenditure technicians will be trained in the administration of the loading dose, specimen collection, specimen handling, and use of the Deltatrac metabolic monitor. Energy expenditure technicians will also be trained to accurately complete the questionnaires and participant transmittal forms. After completion of training energy expenditure technicians will be certified as described below. New personnel will be trained by certified energy expenditure technicians or other trained personnel such as the local QC Coordinator. The new personnel will require certification as described below. The use of the Health ABC staff ID number will provide tracking information so the EERC can perform technician-specific quality control assessment on a monthly basis.

2.2 Certification of energy expenditure technicians

Energy expenditure technicians must be certified before they can be involved in the Energy Expenditure protocol. The certification procedure is as follows.

1. Five methanol calibrations will be performed as described in section 3.7.
2. Three resting energy expenditure determinations will be performed in succession on one individual as described in section 5, except that an overnight fast is not required of this individual. The test participant is required to have fasted at least 2 hours, and the time of the last meal should be noted on the submission form.

3. A sample questionnaire will be completed.
4. The methanol calibration and resting energy expenditure will be sent to the EERC along with questionnaires and certification form. Information will be transmitted as specified in section 5.
5. The EERC will evaluate the data using the quality control criteria cited in section 5.
6. If the data grades as high quality, certification will be issued.

Indicators of poor quality will be:

1. Excessive variation between methanol burns
2. Excessive variation between resting energy expenditure determinations
3. Excessive variation during a single resting metabolic rate determination
4. Incomplete transmittal forms

Upon satisfactory completion of the certification procedure, the EERC will issue a certification for the energy expenditure technician. The EERC will use the Health ABC staff ID number as provided by the Coordinating Center for tracking. This number must be entered on subsequent transmittal forms and will be used for quality control throughout the Health ABC Energy Expenditure Protocol.

3. Energy expenditure equipment

3.1 Total energy expenditure supply information

Doubly-labeled water	EERC
Screw capped urine cups, 6/participant	Local hospital supply
Red cap blood collection tubes 1/participant (7mL)	Local hospital supply
Corning Cryotubes 6/participant (5 mL), p/n 430656	Fisher, Sci Prod, etc
Avery clear labels	Staples.com or local
Ultra-fine Sharpie black-ink pens	Staples.com or local
Ziplock type plastic sandwich bags	Local grocery store
Sustical or Ensure, 8 oz	Local hospital supply
Styrofoam shipping containers.	Fisher, Sci Prod, etc
Cool Packs (shipping refrigerant)	Fisher, Sci Prod, etc

3.2 Total energy expenditure monthly supply check

At the beginning of each month, the energy expenditure technician should check their supply inventory. If the inventory is less than a 6-week supply of any material, supplies should be ordered. An exception can be made in the last month of the energy expenditure study.

Assuming that one participant is enrolled in the energy expenditure protocol each working day, then a 6-week supply is:

Doubly-labeled water	20 of each of the four sizes
Red cap, blood collection tubes	30
Syringes, needles and other blood draw supplies	30
Urine cups	150
Pasteur pipettes	150
Corning Cryotubes (5 mL)	150
Avery clear labels	330
Ultra-fine Sharpie black-ink pens	5
Ziplock type plastic sandwich bags	30
Sustical or Ensure, 8 oz	30
Hospital gown	60
Styrofoam shipping containers	6
Cool Packs (shipping refrigerant)	12

3.3 Resting energy expenditure equipment list

Deltatrac II Metabolic Monitor, SensorMedics, Corp. 22705 Savi Ranch Parkway, Yorba Linda, CA 92687. Phone: 800-231-2466, FAX: 714-283-8471.

This unit is a portable device for the measurement of gas exchange across the lungs. The device measures 42 x 34 x 46 cm and weighs 21 kg. It comes with a plastic bubble that is placed over the participant's head and an InkJet printer. The operating temperature is 50 – 95°F with a relative humidity between 10 and 90%. The unit runs on 110 volt line current. **WARNING. A technician must be present at all times after the bubble has been placed over a participant's head to confirm proper delivery of fresh air.**

3.3.1 Equipment support and troubleshooting

The equipment should be purchased with a two-year warranty/service contract. All equipment failures should be reported to the SensorMedics service facility and repair arranged as soon as possible.

The device is equipped with a computer and CRT display screen. Most errors are detected by the data checking software and reported to the user on the display screen. There is a troubleshooter's guide to the error reports contained in the Operators Manual (P/N 77002).

3.4 Resting energy expenditure supply information

<u>Item</u>	<u>Vendor</u>
Printer paper for Deltatrac II	Local office supply depot
Inkjet cartridges for Deltatrac II	Local office supply depot
Glutaraldehyde disinfecting solution	Hospital supply
Methanol, reagent grade, 500mL	Local chemical supply

3.5 Initial set-up of Deltatrac II

Calibration will involve five Methanol burns (see below, section 3.7) done over 3 or more days. The CO₂ needs to be within 2% of each burn. The coefficient of variation of those five burns needs to be <3%. Continue calibration until unit meets these requirements.

Connect the water trap, inspiratory gas sampling line, and air flow hose as directed for canopy mode (Figure 3-3 on page 3-7 of Deltatrac II manual – see below). Be sure that neither the inspiratory inlet line nor the hose are located in a dusty place.

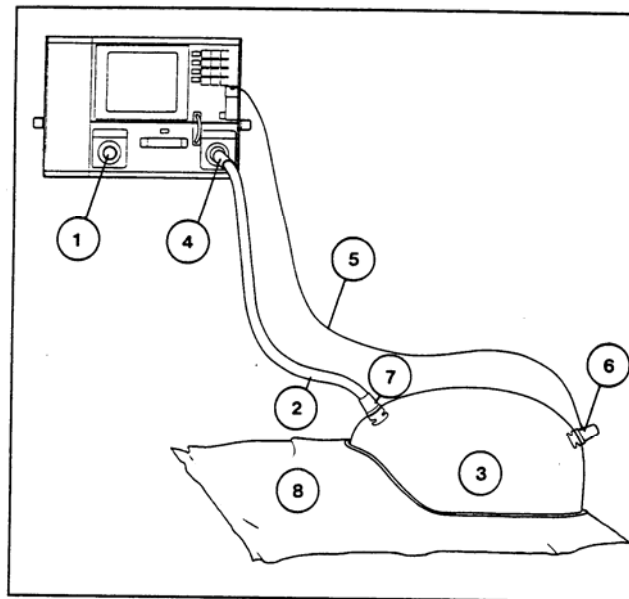


Figure 3-3 Patient Connections in Canopy Mode with Room Air

Plug the unit into line voltage as directed and turn on the unit. The switch is located on the rear. The unit will perform a self-test. If any errors are detected, an error message will appear on the screen. Check against the error list in the SensorMedics Operator's Manual. If the cause of the problem is not obvious, contact your service representative.

Press the CHANGE MODE key on the front panel for 2 seconds and place the unit in the CANOPY MODE. Manually turn the Flow Selector Wheel on the rear of the unit to the adult position. Be sure that the flow outlet is not blocked or otherwise obstructed by printer paper, walls, or other items.

Enter the PERMANENT SETUP. These settings are entered in memory and are retained even if the power is turned off.

If not already in MEASUREMENT OFF state, press the START/STOP key to interrupt the measurement.

Press the SETUP key and the PERMANENT SETUP menu will appear on the screen.

Use the NEXT key to move the cursor to the parameter to edit.

Use the INCREASE or DECREASE keys to edit the parameter.

The SETUP conditions should be:

1. TIME=local time
2. START DELAY, CANOPY=10 minutes (does not include first 10 minutes in average if artifact suppression is on).
3. UNITS = KJ, CM, KG
4. DATA DUMP= off
5. POWER ON SETTING = Canopy
6. ARTIFACT SUPPRESSION = on
7. AVERAGING = off
8. VT ALARM is not active in the canopy mode. Either setting.
9. PRINTER OPERATION = Num (minute by minute printer op)

Set the printer options.

Press DISPLAY/PRINTER key

Press PRINTER OPTIONS

Select NUMERIC using the SELECT key to move cursor.

Press NORMAL SCREEN

This completes the initial set-up. In addition to these items, the service representative will perform a series of quality control tests. If the unit has performed appropriately during set-up, then it will be the energy expenditure technician's responsibility to run a series of five methanol burns to check the calibration. See section 3.7 for directions.

3.6 Semi-monthly equipment check

Drain the water trap. Note: as with all physiologic fluids, the contents of the water trap should be treated as a potential biohazard and handled with care.

Inspect the inspiratory gas sampling line and small metal line connector for blockage by dust or other debris.

Inspect all tubing for leaks and insure that all connections are secure.

Clean the air filter on the cooling fan at the rear of the unit. Remove and knock the dust and dirt from the filter. Wash with warm water, drain on a paper towel and let air dry. Reinstall filter.

Clean the exterior of the unit with a rag dampened with a mild detergent. Avoid excess water that might seep into the unit. Avoid contact with plugs and connectors.

Inspect cords for fraying or other damage.

Confirm that all connectors are secure.

Check supplies and order as needed.

Calibrate the pressure transducer

Call the local weather bureau for the current atmospheric pressure
Convert to mmHg = 25.4 x pressure in inches of Hg (usually between 720-750 mmHg)

Press CAL key to call the CALIBRATION MENU

Select PRESSURE (key 2)

Adjust the pressure to equal atmospheric pressure as calculated above
Perform a methanol burn to check calibration as described in section 3.7.

3.7 Methanol calibration burn

The methanol calibration burns are performed to calibrate the flow rate and the respiratory ratio (a.k.a. respiratory quotient). Methanol is preferred over ethanol because it is easier to obtain at high purity. Methanol burns must be performed as part of the initial testing of equipment performance and energy expenditure technician certification. Additional burns must be performed every two weeks to track any drift in the equipment and recalibrate should such drift be observed.

1. If the metabolic monitor is off, turn it on and let it warm-up for at least 30 minutes.
2. Check that the unit is in the MEASUREMENT OFF mode.

3. Place the unit in the CANOPY MODE and set the flow wheel to adult mode.
4. Press the Cal key and the CALIBRATION MENU will be displayed.
5. Press GAS (key 1, next to screen) to start the calibration.

The unit will begin a baseline check.

Avoid long term breathing within 18" of the unit as it is measuring room air and it may detect your breath as baseline.

6. The BASELINE CHECKING screen should appear. You will be requested to wait until stable readings are obtained. O₂ should read 21.0% and CO₂ should read 0.03%.

If the ambient CO₂ level is below 0.03%, then the internal soda-lime water absorber is exhausted. Contact the local service representative for replacement.

7. The unit will automatically proceed to the GAS CALIBRATION screen. The unit will perform a calibration by admitting gas automatically from the internal calibration gas can.

It is assumed that the calibration gas is Datex 95.0% O₂ and 5% CO₂. Other calibration gases should not be used for the Health ABC.

If this does not proceed, press START/STOP to interrupt. Return to the CALIBRATION MENU. Press GAS SETUP and select automatic mode. Return to step 5.

8. The unit will automatically proceed to the ADJUSTING VALUES screen. If all proceeds within fail-safe limits, the unit will display "Calibration completed." The O₂ concentration should read 95.0% and the CO₂ concentration 5.00%.

If the Datex calibration gas bottle is low in gas, the unit will detect a decreasing gas concentration. It will display the message BOTTLE EMPTY and NO CALIBRATION GAS. Replace the bottle with a fresh Datex bottle with 95.0% O₂ and 5% CO₂. The bottle is located behind the right back (front in some cases) panel. It is attached via a threaded connector. Twist the new can into place and be sure that the connection is tight. If not the calibration gas will leak out and need to be replaced again.

If the adjustment to the calibration gases exceeds 3%, but less than 5%, the unit will display ADJUSTMENT OVER 3% OF O₂ (or CO₂). PLEASE REPEAT CALIBRATION. Please do so. If the calibration remains unstable, contact your local service representative.

If the adjustment exceeds 5%, the unit will not adjust the concentration. The unit will display MAXIMUM ADJUSTMENT FOR O₂ (or CO₂) 5%. PLEASE REPEAT

CALIBRATION. Check the calibration gas bottle to be sure the connection is tight and the correct gas is in use. If not, contact the local service representative.

9. Connect the methanol burn kit as shown in figure 5-7 on page 5-9 of Deltrac II operations manual chapter. See below.

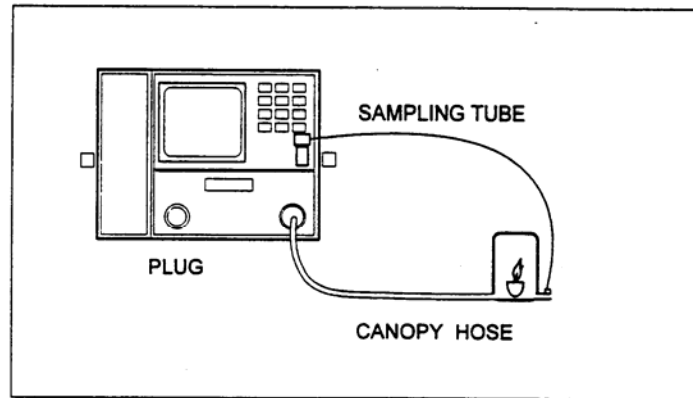


Figure 5-7 Alcohol Burning Test Setup

10. Select the following:

Clear possible old results using the END menu.

Go to the DISPLAY OPTIONS menu and select AVERAGING OFF.

Select ARTIFACT SUPPRESSION OFF.

Go to PRINTER OPTIONS and select NUMERIC PRINTER OUTPUT.

Using the pipette from the burn kit, fill the alcohol burn vessel with **exactly** 5.0 mL ($\pm 1\%$) of methanol.

Between uses the methanol should be stored in a sealed jar with desiccant.

If the methanol bottle is less than 1/3 full, purchase a new bottle and properly dispose of the old methanol.

11. Using a pipette bulb or pipette wheel (do not mouth pipette), fill the pipette to above the mark. There should be no air bubbles in the pipette.
Slowly release methanol until the base of the meniscus is level with the 5.0 mL mark.

Touch the drop at the tip to the methanol bottle.

Let the methanol drain into the burn vessel.

Touch the drop at the tip to the burn vessel. Do not blow out the pipette.

Recap the methanol bottle and return to storage.

Do not wait more than 2 minutes after filling the vessel to start the burn in order to minimize evaporative loss.

12. Start the measurement by pressing START/STOP. The measurement sequence will begin after a 30 second delay, and the measurement time on the display will be reset to zero.
13. After the measurement time is set to zero, light the methanol. Use appropriate caution for working with a burning fluid in order avoid burning yourself or starting a larger fire.
14. Place the cover over burn vessel immediately after lighting the methanol.
15. The flame will burn out after about 20 minutes and the no breathing alarm will sound. Suppress the alarm and wait until the V_{CO_2} has been below 10 mL/min for two consecutive minutes. Press START/STOP to end the measurement.
16. Visually confirm that all of the methanol has burned. If not, cool and dry the vessel and repeat the test.
17. Calculate the results after completion of the methanol burns.
To assess the flow rate calibration, sum the minute by minute V_{CO_2} values across the entire measurement.

To assess the RQ, sum the minute by minute V_{O_2} values across the entire measurement.
Calculate the respiratory ratio = V_{CO_2} / V_{O_2}
If the calibration was successful the V_{CO_2} should be between 2778-2891 mL and the respiratory ratio between 0.65-0.69 (see Appendix 1 for details).

If the calibration was unsuccessful (one burn outside the above ranges), perform a third methanol burn.

If two of the methanol burns were unsuccessful, contact the EERC (608-263-5477 or 262-1082).
18. Enter the V_{CO_2} and respiratory ratio values in the Deltatrac maintenance log (Appendix 3).
19. After cooling, pack the burn kit away in a manner that protects it from breakage.
20. Complete the methanol burn transmission form and transmit the data to the EERC via fax:
Dale Schoeller
Nutritional Sciences
University of Wisconsin – Madison
608-262-5860

3.8 Adjustment of flow rate

Adjustment of flow rate calibration should only be performed in collaboration with the EERC. The adjustment is made to bring the Deltatrac II back into calibration if the methanol burn data indicates that the flow unit has drifted relative to the factory setting. Incorrect adjustment will produce inaccuracies in the resting metabolic rates.

1. The Metabolic Monitor power should be off.
2. Hold down key 1 and turn on the power to the unit (switch is on the rear panel).
3. The display should be the FACTORY SETTINGS menu.
4. Move the cursor to Flow Calibration (key 1 or 2) and press key 4.
5. Adjust the ADULT flow constant to the new value.
6. Write the date and new flow value on the calibration label on the rear panel.
7. Enter the new flow value in the maintenance log (Appendix 3).
8. Repeat the methanol burn calibration.

4. Measurement of total energy expenditure

Total energy expenditure will be measured during the Year 10 clinic visits. The measurement will require two clinic visits separated by 12 to 16 days. During the first visit, the participants will receive the oral loading dose of doubly-labeled water and produce the physiologic specimens for measurement of tracer equilibration. During the second visit, they will produce the final specimens for measurement of tracer turnover and have their resting metabolic rate measured. Both visits require that the participants arrive at the field center in the fasted state.

4.1 Participant safety and exclusions

Total energy expenditure will be measured on the same subset of the Health ABC cohort in which resting energy expenditure is measured.

There are five general exclusion criteria for participants in the energy expenditure protocol. These are:

1. Insulin using (dependent) diabetes mellitus.
2. A requirement for supplemental oxygen.
3. Blood transfusions, administration of blood products, or administration of intravenous fluids in excess of 500 mL in the week previous to the first energy expenditure visit or an

expectation of same during the period between the first and second energy expenditure visit.

4. Travel in excess of 200 miles away from home during week prior to energy expenditure visit 1 or an expectation of same during the period between visits 1 and 2.
5. Claustrophobia which would cause the participant to become anxious when the plastic RMR hood is placed over their head.

The primary safety concern is that the loading dose of doubly-labeled water may cause temporary vertigo. This, however, is rare at the small tracer dosage used for this study. As a precaution, however, the participants should sit down when drinking the loading dose and should remain seated for 15 minutes after drinking the loading dose. Because the participants are arriving at the center in the fasted state, there is also a potential for hypoglycemia. Care should be exercised when the participants stand up to ensure that their balance is sound.

4.2 Pre-examination procedures

Script: Hello. My name is <insert interviewer's name>. May I please speak to <insert participant's name>? I am calling from the <insert name of institution>. The interview will take about 5 minutes. Is this a good time to talk?

If this is not a good time to talk, reschedule the interview. If participant agrees to interview, thank them and complete the interview.

Script: Eight years ago you participated in a special study of energy use as part of your Health ABC clinic visit. This special study measured how many calories you burn at rest and during your normal daily activities. We would like you to participate in the special study again except this time we would like you to wear an activity monitor called a pedometer. This information will help us know about changes in energy use, including exercise and diet, over the past 8 years. The procedures are not painful and can't hurt you. You will need to come back to the Health ABC clinic about 2 weeks after your regular visit. This extra visit will take about 90 minutes and you will receive \$20 and learn how many calories you use.

If a participant was not originally part of the energy expenditure cohort, use the following script:

Script: This year Health ABC is doing a special study of energy use as part of your clinic visit. This special study measures how many calories you burn at rest and during your normal daily activities. We would like you to participate in the special study and wear two monitors; one is an activity monitor called a pedometer, and the other is a heart rate monitor. This information will help us learn about your energy use, including exercise and diet. The procedures are not painful and can't hurt you. You will need to come back to the Health ABC clinic about 2 weeks after your regular visit. This extra visit will take about 90 minutes and you will receive \$20 and learn how many calories you use.

Script: Would you like to hear more about this special study?

If no, probe to determine why they don't want to participate. If, after probing, the participant still does not want to participate, go to Question #9: "Is participant eligible and interested in the energy expenditure study?," choose "No" and schedule a regular Year 10 follow-up clinic visit.

If yes, proceed with the script:

Script: The tests for measuring energy and using the activity monitor are really quite simple. At your regular clinic visit you will be given four ounces of a special kind of water to drink that will allow us to measure how many calories you burn during the next 2 weeks. This special water is tasteless and harmless. During this visit you will be asked to provide four urine samples and one blood sample. You will also be taught how to wear three activity monitors. One will be placed on your waist, one will be placed on your arm, and another on your chest that will monitor your heart rate. You will also be asked to return to the clinic 12 to 16 days later to give two more urine samples and return the monitors. At this return visit you will have a test to measure how many calories you use at rest. For this test you will be asked to lie in bed and a clear plastic bubble will be placed over your face for about 40 minutes. A Health ABC researcher will be with you to measure the amount of oxygen you breathe while resting. This return visit will take about 90 minutes. You will receive a printout from the activity monitors that will tell you how much activity you did over the 12 to 16 days.

Script: Would you like to participate again?

If no, schedule regular visit.

If yes, continue with pre-screener:

Script: Are you currently using insulin by injection for diabetes?

No - Note: oral agents are not exclusionary

Yes - thank them for their interest and tell them they are ineligible. Go to Question #9, choose the "No," response option and schedule a regular Year 10 follow-up clinic visit.

Script: Are you using supplemental oxygen?

No - proceed

Yes - thank them for their interest and tell them they are ineligible. Go to Question #9, choose the "No," response option and schedule a regular Year 10 follow-up clinic visit.

Script: Have you traveled more than 200 miles from your home in the past 7 days?

No - proceed

Yes – if the participant is otherwise eligible, schedule first energy expenditure clinic visit more than 7 days after they traveled.

Script: Are you planning a trip that is more than 200 miles from your home in the next 4 weeks?

No - proceed

Yes - thank them for their interest and tell them they are ineligible. Go to Question #9, choose the "No," response option and schedule a regular Year 10 follow-up clinic visit.

Script: Are you scheduled for major surgery in the next 4 weeks?

No - proceed

Yes - thank them for their interest and tell them they are ineligible. Go to Question #9, choose the "No," response option and schedule a regular Year 10 follow-up clinic visit.

Script: Have you received a blood transfusion or any intravenous (IV) fluids in the past 7 days?

No - proceed

Yes - thank them for their interest and tell them they are ineligible. Go to Question #9, choose the "No," response option and schedule a regular Year 10 follow-up clinic visit.

Answer Question 9. Is participant eligible and interested in the energy expenditure study? If you have not been directed to choose "No," the participant is eligible.

Schedule eligible and interested participants for **two clinic visits**: 1) The regular Year 10 visit and 2) the second visit 12 to 16 days after the regular Year 10 visit. Although scheduling is easier if the second visit is exactly two weeks later than the first, the second visit can be plus or minus two days relative to the preferred date.

4.3 Pre-examination procedures-workday before first EE visit

(Regular Year 10 exam)

On the workday prior to the first visit, the participant should be contacted and reminded they should not eat after 10:00 pm the night before their clinic visit.

Script: You are scheduled for a clinic visit at (time and date). During this visit, you will have a fasting blood sample collected and undergo a test to measure the number of calories that you burn. In order to obtain accurate test results, you must not eat or drink any calorie containing beverages after 10 pm on the night before your visit.

4.4 Pre-examination procedures-day of exam

(Regular Year 10 Clinic Visit)

Prior to the arrival of the participants, check the following supplies:

- A preweighed bottle of doubly-labeled water appropriate for the weight of the participant.
- Hospital gown.
- Rinse water (50 mL)
- A can of Sustical or Ensure and cup for drinking
- 1 red top blood collection tube, needles and syringes for blood draws
- 4 screw capped urine cups
- 5 Cryotubes for specimen storage
- 5 Pasteur pipettes
- 10 clear Avery labels for cups, tubes, and cryovials

- Black ultra-fine Sharpie pen for labeling cups, tubes, and cryovials
- Ziplock bag
- Year 10 Energy Expenditure Visit 1 form
- Actiheart monitor is charged and ready to be initialized – see Accelerometry chapter about pre-entering participant data
- Actigraph monitor is charged and ready to be initialized
- SenseWear armband is ready to be initialized – see SenseWear Armband chapter
- New AAA battery
- Year 10 Prescreener for SenseWear Armband
- Year 10 SenseWear Armband Distribution form

The questions for energy expenditure visit 1 are as below. Mark the answers on the Year 10 Energy Expenditure Visit 1 data collection form.

First record the participant's weight. You will find this weight on page 37 in the Year 10 clinic Visit Workbook.

Script: Have you traveled more than 200 miles from your home in the past 7 days?

No - proceed

Yes – If the participant is otherwise eligible, schedule first energy expenditure clinic visit more than 7 days after they traveled.

Script: Have you received a blood transfusion or any intravenous fluids in the past 7 days?

No - proceed

Yes - The participant is not eligible for energy expenditure measurements. Go to Question #4: "Is participant eligible for the energy expenditure measurements?" and mark "No." STOP

Record on Question #4 whether or not the participant is eligible for the energy expenditure measurements. Then ask:

Script: How many hours ago did eat or drink anything (do not include water)?

Was it less than 4 hours since the participant had something to eat or drink (do not include water).

If not, then proceed.

If less than 4 hours ago, ask the participant to wait until 4 hours has elapsed since last food/drink was consumed before proceeding.

4.5 Examination procedures-first EE visit

(Regular Year 10 Visit)

- Obtain a baseline urine specimen.
- Cap, label the specimen with HABC Enrollment ID#, acrostic, and specimen number (U0), record date and time, and place in temporary storage.
- Enter the time on the Year 10 Energy Expenditure Visit 1 data collection form.
- Select a doubly-labeled water bottle appropriate for the participant's weight (see table below).

Females	Body weight	Dose
	≤ 65 kg	A (54.0 g)
	65 kg to ≤ 80 kg	B (60.0 g)
	80 kg to ≤ 105 kg	C (66.0 g)
	> 105 kg	D (79.0 g)
Males	≤ 55 kg	A (54.0 g)
	55 kg to ≤ 70 kg	B (60.0 g)
	70 kg to ≤ 95 kg	C (66.0 g)
	> 95 kg	D (79.0 g)

- Ask the participant to drink all of the doubly-labeled water from the preweighed bottle.
- If any labeled water spills, mop up with the preweighed tissue in the ziplock bag. Return tissue to bag and seal.
- Mark the Year 10 Energy Expenditure Visit 1 form regarding spillage.
- Place about 50 mL of water in the dose bottle, cap and mix. Ask the participant to drink the rinse water.
- Enter the bottle number and dose information on the Year 10 Energy Expenditure Visit 1 form.
- 1 hour after the dose. Provide the participant with an 8 oz can of Sustical or Ensure and allow them to drink this. This is provided to minimize the discomfort of the fast. The Sustical or Ensure is provided for the participant's comfort, but is not required. They can drink water, tea, or coffee if they prefer.
- 1 hour after the dose. Obtain a urine specimen. Record time. Label the container with participant's HABC Enrollment ID#, acrostic, date, time, and specimen number (U1) in temporary storage.
- Offer the participant a 6-ounce drink of water, tea, or coffee to maintain urine production. Record time and volume consumed.
- 2 hours after the dose. Obtain a urine specimen. Record time. Label the container with participant's ID, date, time, and specimen number (U2) and place in temporary storage.
- Offer the participant a 6-ounce drink of water, tea, or coffee to maintain urine production at least one hour before the final urine collection. Record time and volume consumed.
- 3-4 hours after the dose. Obtain a urine specimen. Record time. Label the container with participant's ID, date, time, and specimen number (U3) and place in temporary storage. Obtain a 7 mL blood specimen in a red topped tube. Label the container with participant's HABC Enrollment ID#, acrostic, specimen number (S1), date and time, and place in temporary storage.
- Enter the time on the Year 10 Energy Expenditure Visit 1 form.

This completes the measurements for energy expenditure visit 1. Before the participant leaves, however, show the participant the Deltatrac metabolic monitor. Explain the procedure for measuring resting metabolic rate including the importance of an overnight fast. If not in use, allow the participant to sit in the bed and try on the bubble. Be sure that the metabolic monitor is on and that all hoses are connected so that there is airflow through the bubble. The

trial need not last longer than 1 or 2 minutes. The purpose of this is to reduce stress during the measurement that will be made in 12 to 16 days.

Be sure to download data from the Actiheart and Actigraph monitors. See Accelerometry operations manual for instructions.

4.6 Post-examination procedure-EE visit 1

(Regular Year 10 Visit)

Label the Cryotubes with the participant's HABC Enrollment ID# and acrostic, date and times for each of the urine and blood specimens.

Centrifuge the blood specimen to separate serum.

Being careful to prevent cross contamination, transfer each urine specimen and the serum specimen to the appropriate Cryotube. Seal the tube, dry the outside of the tube and place in a ziplock bag with the HABC Enrollment ID# and acrostic. Store the tubes in a freezer (about -10 degrees C).

Scan the Year 10 Energy Expenditure Visit 1 form to the Health ABC data base.

5. Measurement of resting energy expenditure

The resting energy expenditure will be measured in a return visit scheduled about 12 to 16 days after the Year 10 clinical exam.

5.1 Participant safety and exclusions

The primary safety concern is that airflow through the bubble is maintained while the bubble is in place over the participant's head. **Loss of flow due to a failure of the fan in the Deltatrac or due to a loose hose will cause discomfort and in an extreme case may cause asphyxiation. Although an alarm will sound if the unit does not detect breathing, the energy expenditure technician should remain with the participant throughout the measurement.** Care should also be exercised when the participant stands-up after the measurement should dizziness develop secondary to the fast.

5.2 Pre-examination procedures

Staggering the times of the RMR visit, e.g., scheduling visits at 8:00 am, 9:30 am, and 11:00 am is acceptable. On the workday prior to the visit for measurement of resting metabolic rate, the participant should be contacted the night before the scheduled clinic visit for measurement of resting energy expenditure and reminded they should not eat after 8 pm, 9:30 pm, or 11:00 pm depending on their RMR visit time.

Script: You are scheduled for a clinic visit at (time and date). During this visit, you will undergo a test to measure the number of calories that you burn at rest. In order to obtain accurate test results, you must not eat after [8:00] [9:30][11:00] pm the night before the visit or on the morning before the visit. You may not drink any calorie containing beverages on the morning of your visit. It is also important that you do not smoke or take any nicotine products (chew, nicotine gums or patches), any caffeine containing products (coffee or tea other than decaf, colas or caffeinated soft drinks, or drugs to stay awake) for 2 hours before the visit. Please also remember to bring your activity monitors.

5.3 Pre-examination procedures-exam day

On the morning of the visit, check the following:

- The Deltatrac metabolic monitor is plugged in and turned on so that it has “warmed-up” at least 30 minutes before calibrating prior to the measurement of resting metabolic rate.
- Check printer power and turn on if not already on.
- The printer is loaded with paper and Inkjet print head is writing clearly.
- Presence of a spare printer ink replacement unit.
- Set the top of the page. Position printer paper to top of page-press LF key on printer followed by pressing the blue button on the printer.
- The bubble and hoses are clean and clear and connected as Figure 3-3 on page 3-7 of the vendor’s manual.
- Check that the mixing chamber inlet plug is in place. Note this plug has a small hole in the center to let in a small flow of room air (Figure 3-3. Page 3-7 of vendor’s manual).
- The water trap is not more than half-full. Empty if more than half-full.
- The flow selector wheel (on rear) is in the adult mode (40 L/min)
- The bed is clean, functional and in the recumbent position with knees up.
- The Year 10 Energy Expenditure Visit 2 form is available.
- Actiheart software is up and running and supplies are available.
- InnerView software for the SenseWear armband is up and running.
- SenseWear armband USB cable is connected to the computer and ready to be connected to the armband.
- Year 10 SenseWear Armband Return and Reading form is available.
- A new AAA battery is available, if needed, for data retrieval from SenseWear armband.
- Check that the exam room is quiet, free of distracting activity, dimly lit, and temperature between 24-27°C (75-81°F).

Weigh the participant in a hospital gown and record weight in kilograms.

The preliminary questions for energy expenditure visit 2 (pages 1 through 2 in the Year 10 Energy Expenditure: Visit 2 Workbook) include the following:

Script: Since your last clinic visit several weeks ago, did you cut down on things that you usually do, such as going to work or working around the house, because of an illness or injury?

Record answer and proceed.

Script: Have you traveled more than 200 miles from home since your last clinic visit several weeks ago?

Record answer and proceed.

Script: Have you received a blood transfusion or any intravenous fluids since your last clinic visit several weeks ago?

Record answer and proceed

Script: How many hours ago did you eat or drink anything (do not include water)?

- If it is less than 6 hours since the participant had something to eat or drink (not including water), ask the participant to wait until 6 hours has elapsed since last food/drink was consumed before proceeding.
- If the participant would have to wait more than 2 hours, re-schedule a visit to occur during the next few days. Remind the participant to fast overnight before the visit.

Script: Have you smoked a cigarette, pipe, or cigar in the past 2 hours?

- If more than 2 hours ago, proceed.
- If less than 2 hours ago, ask the participant to wait until the interval is 2 hours and then proceed.

Script: Have you had a cup of coffee or another caffeinated beverage in the past 2 hours?

- If more than 2 hours ago, proceed.
- If less than 2 hours ago, ask the participant to wait until the interval is 2 hours and then proceed.

5.4 Measurement procedures

- Obtain first Year 10 energy expenditure visit 2 urine specimen (U4). Record time on page 2, Question #8 of Year 10 Energy Expenditure Visit 2 workbook.
- Remove the SenseWear armband from the participant's arm and place it beside the computer where you will download the data during the post- examination quality check.
- Apply two electrodes for Actiheart: Prepare the skin and use Actiheart placement positions from the initial walks.
- Ask participant to assume a recumbent position in the bed. To assure consistency between centers, the head should be raised 16 to 18” and the knee raised 8 to 10.” Ask the participant to rest quietly for about 30 minutes. If the participant feels cold, offer them a blanket. If the participant feels hot, alter the environment to insure that they do not sweat.
- Administer the Year 10 Energy Expenditure Visit 2 interview (pages 3 through 25 in the Year 10 Energy Expenditure: Visit 2 Workbook) during the 30 minute rest period.

- If the participant weighs in excess of 120 kg, change the Flow Selector Wheel to the obese range (80 L/min).
- Check that the Deltatrac is in canopy mode. Change if needed by pressing mode key for 1 to 5 seconds.
- Check that the Deltatrac is in the artifact suppression mode with a 10 minute start delay.
- Check that the hoses from the bubble to the Deltatrac are connected and that the unit is turned on.
- Perform an autocalibration of the Deltatrac.
- Press PATIENT DATA.
- Use the SELECT key to indicate the participant's gender.
- Use NEXT key to select other variables (birth date, height, weight)
- Use the INCREASE/DECREASE keys to adjust value. Check that units are correct.
- Nitrogen excretion: use the default value which is about 0.2g/kg/24h.
- Press SAVE to enter the participant data in memory
- Press NORMAL SCREEN to return.
- Remind the participant that the measurement will last 40 minutes and they must lie relatively still and not talk, but remain awake for the measurement.
- Write the participant's HABC Enrollment ID#, acrostic, and date on two sheets of paper and place in printer.
- Set up Actiheart in 'step test' mode using the test ID 'rest', start time to begin at 15 minutes into the RMR measurement. To achieve this, look at computer time, decide exact start that RMR measurement will begin, allowing a couple minutes for placing monitor on and getting the participant under the hood. Set up Actiheart monitor 15 minutes ahead of this start time.
- Place Actiheart on participant – remember to first touch one of the electrodes with a finger (grounding).
- Just before you want to start RMR measurement at the predicted start time (when the Actiheart software reaches a countdown of 15:00 minutes). Place the bubble over the participant's head and spread the plastic drape as flat as possible to minimize leakage.

- Press the START/STOP key to begin the RMR measurement.

(Note: The software will continue counting down, indicating the Actiheart has not started collecting data yet. This is fine as we are only capturing 13 minutes of raw Actiheart data during minutes 15 to 28 of the RMR measurement). This time synchronization is important as it ensures consistency in the time the HRV measure is captured during the RMR measurement.

- Record room temperature on page 2, Question #12 in Year 10 Energy Expenditure Visit 2 workbook.
- The printer should be reporting data on a minute by minute basis. Check connections, printer power or see PRINTER SETUP if it is not printing.

The measurement will proceed for 40 minutes.

The technician must remain with the participant and monitor gas flow alarms and visually check for labored breathing to insure that gas flow does not fail.

The participant must remain at rest but not sleep.

The participant must not talk, except when necessary to communicate a potential problem. If the participant does talk, lift their arms to scratch an itch, shift their weight to prevent stiffness etc, indicate the time and movement on the printout using a pen or pencil.

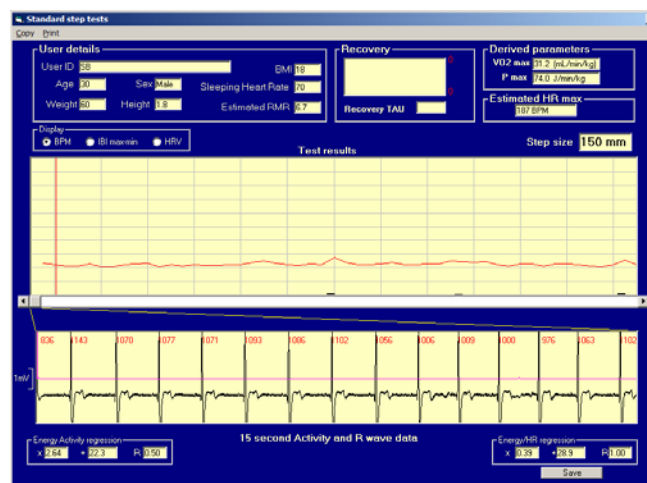
- Confirm that the participant is still thermally comfortable.

If the participant has to get up because they need use the bathroom, then the measurement can be interrupted. The measurement sequence, however, needs to be extended by 10 minutes. Press start to restart the measurement after the participant returns to bed and the bubble has been replaced as above. The next 10 minutes of data will be automatically left out of the calculation of mean RMR.

If the participant needs to go to the bathroom and you have not been able to capture 13 minutes of Actiheart data, you may need to set up the Actiheart again to capture the last 13 minutes of the resting measurement. In this case, use the test ID 'rest 2'.

- At 40 minutes, check the display data printout for a stable reading.
- If the oxygen consumption (or energy expenditure) have been stable (+/- 10% for 30 minutes, press START/STOP to end the measurement.
- If the data indicates a downward trend of 400 kJ/day or more during the final 30 minutes (not including the initial 10 minutes after putting on the bubble), or if the participant was moving for more than a few minutes, extend the measurement for 5 to 10 minutes.

- Press START/STOP to end the measurement.
- Press END REPORT to output the final portion of the report to the printer.
- Remove the bubble from over the participant's head.
- Ask the participant to sit upright.
- Help the participant to their feet and be sure that they steady. Remember that they have fasted and there is a small risk of hypoglycemia.
- Obtain second Year 10 energy expenditure urine specimen (U5). Record time on page 2, Question #9 of Year 10 Energy Expenditure Visit 2 workbook.
- On page 2 also record, energy expenditure (MJ/d) and Standard Deviation (SD); Resp. Quotient and SD, Room temperature, and if the start time of the heart rate variability (Actiheart measurement) was not exactly 15 minutes into the resting energy expenditure protocol.
- Download the data from the Actiheart monitor, and, if the start time of the heart rate variability (Actiheart measurement) was not exactly 15 minutes into the resting energy expenditure protocol, note this on the computer in the "Comments" section. Review trace.



- Click through the test by clicking in the large middle window and review the ECG waveform in the lower window (zoomed 15-sec epochs). If the data looks very noisy, you may try downloading the Actiheart again, otherwise 'Power Down' the Actiheart unit via the software.
- Measurement complete - Remove electrodes and place the Actiheart unit on the multicharger.

5.5 Post-examination quality check

Check that the printout is legible. If not, correct problem and reprint by pressing END REPORT. This will reprint the averages, but not minute by minute data.

Check that the average RQ is between 0.75 and 0.9. Values outside of this range may indicate that the participant fasted longer than 15 hours (<0.75), ate within the last 6 hours (>0.93), or hyperventilated during the measurement (>0.93). Other possible explanations are very high fat diets (<0.75), a weight loss diet (<0.75) or very high carbohydrate diets (>0.93). If the participant admits to a recent meal, reschedule the test.

Check that the coefficient of variation is less than 10%. Possible explanations are excessive participant movement, irregular breathing pattern, failure to suppress the first 10 minutes of the measurement, or instrument maintenance problems. If the first 10 minutes of the measurement were not deleted, manually calculate the average and SD without the first 10 minutes. If the revised coefficient of variation less than 10%, record these values. If not, repeat the measurement of resting metabolic rate.

Record Mj/d and standard deviation, Question #10, and Resp. Quotient and standard deviation, Question #11 on page 2 of Year 10 Energy Expenditure Visit 2 workbook.

Retrieve the data from the SenseWear armband (see SenseWear Armband Operations Manual for detailed instructions).

Complete the Year 10 SenseWear Armband Return and Reading form.

5.6 Post-examination data check

Always review all Energy Expenditure forms before scanning and before photocopying.

5.7 Post-examination equipment care

Turn off the printer power.

Confirm that the metabolic monitor is the measurement OFF mode.

Leave the metabolic monitor power on.

While wearing gloves, wipe the inside of the bubble with a towel dampened with 2% Glutaraldehyde solution. Invert and allow to air dry or wipe dry if a second use is imminent.

*Be careful not to drip or otherwise allow any fluid to enter the metabolic monitor between panel edges as this may cause an electrical shock and/or electrical failure.

*Work in a ventilated area and allow the parts to vent before the next use.

*The air flow is always away from the participant and the inside of the metabolic monitor does not generally require sterilization. If, however there is concern about heavy contamination of the unit, then a factory-trained technician must perform the procedure. Field center personnel should not open the electrical panels. There are no self-service parts inside the electrical cabinet.

Attend to bedding.

5.8 Data logging and transmission

On a bi-weekly basis, send the following to the EERC:

Photocopy of resting energy expenditure printout, minute by minute and end report.

Photocopy of Energy Expenditure Forms (Visit 1 and Visit 2)

Visit 1

1. Scan the Energy Expenditure (Visit 1) into the Health ABC database.
2. Bi-weekly, mail a photocopy of the Year 10 Energy Expenditure Visit 1 form and Energy Expenditure Specimen Collection and Processing Form (Visit 1) to the EERC.

Dale Schoeller
Department of Nutritional Sciences
1415 Linden Dr.
University of Wisconsin
Madison, WI 53706

Visit 2

1. Scan the Year 10 Energy Expenditure Visit 2 Form into the Health ABC database.
2. Bi-weekly, mail a photocopy of the Year 10 Energy Expenditure Visit 2 form and the Energy Expenditure Specimen Collection and Processing Visit 2 form to the EERC (see address above).

5.9 Specimen shipping to EERC

Specimens should be shipped to the EERC every two weeks.

1. Place frozen specimens in a Styrofoam shipping box. Specimens from a single participant should be in the same ziplock bag.
2. Place a frozen Cool Pack in Styrofoam shipping box (two during months of May through October).
3. Close shipping container. Send Mon-Wed only.
4. Enclose or FAX a copy of the participant's Visit 1 and Visit 2 scannable forms, and the RMR printout to Dale A. Schoeller.

5. Send shipping box by overnight delivery to the EERC.

Dale Schoeller
Department of Nutritional Sciences
1415 Linden Dr.
University of Wisconsin
Madison, WI 53706

6. Fax or e-mail the EERC regarding shipping date and tracking number. E-mail address:
EERC@nutrisci.wisc.edu. Fax 608-262-5860

5.10 Confirmation of data transmission

EERC personnel will confirm receipt of data and specimens (for doubly-labeled water) by email. Any missing material will highlighted and completion requested.

Appendix 1 Energy Expenditure Technician Certification Document

Field Center
 ABC Tech #
 Date of request:

Methanol burns date(s) performed:

	MeOH, mL	VO2	VCO2	RQ
1	5.00			
2	5.00			
3	5.00			
4	5.00			
5	5.00			

recovery
0
0
0
0
0

mean: 0
 sd: 0

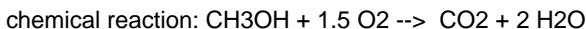
Replicate Resting Energy Expenditure Determinations on the Same Person

date(s) performed:

	0-10m, MJ/d	11-20min, MJ/d	21-30min, MJ/d	31-40min, MJ/d	11-40min, MJ/d	SD,11-40min
1						#DIV/0!
2						#DIV/0!
3						#DIV/0!

mean: #DIV/0!
 sd: #DIV/0!

Information on the combustion of methanol.



Carbon dioxide production (VCO2): $5.00mL * 0.787g/mL / 32.04 g/mole = 0.1228 moles MeOH$
 density at 25 C

1 mole of MeOH produces 1 mole of CO2.
 $0.1228molesCO_2 * 22,400 mL/mole = 2751 mLCO_2$
 3% correction for methanol burn kit flow restriction
 $2751/1.03 = 2834mL$

range (2%)=2778-2891mL

Respiratory quotient (RQ) = $VCO_2/VO_2 = 1/1.5 = 0.67$

range (2%)=0.65-0.69

to be completed by EERC only

Action:
 By:
 Date:

Appendix 4 Energy Expenditure Protocol Checklist**ENERGY EXPENDITURE VISIT 1-DOUBLE LABELED WATER DOSING**

Day before participant arrives:

Phone reminder regarding appointment and morning fast.

Before participant arrives:

- Preweighed bottles of doubly-labeled water for each participant weight range.
- Rinse water (50 mL)
- A can of Sustical or Ensure and cup for drinking
- 4 screw capped urine cups
- 1 red top blood collection tubes, needles and syringes for 3 blood draws
- 5 Cryotubes for specimen storage
- 5 Pasteur pipettes
- Ziplock bag.
- Year 10 Energy Expenditure Visit 1 form
- SenseWear armband is ready to be initialized
- New AAA battery
- Year 10 Pre-screener for SenseWear Armband
- Year 10 SenseWear Armband Distribution form

After participant arrival:

- Ask exclusionary questions on data form
- Explain procedure
- Obtain informed consent
- Retrieve participant's weight from examine station and record on Year 10 Energy Expenditure Visit 1 form.
- Obtain a urine specimen. Cap and label the container with participant's HABC Enrollment ID# and acrostic, specimen number (U0), date and time and place in temporary storage.
- Ask the participant to drink all of the doubly-labeled water from the preweighed bottle.
- If any labeled water spills, mop up with the preweighed tissue in the ziplock bag. Return tissue to bag and seal.
- Place about 50 mL of water in the dose bottle, cap and mix. Ask the participant to drink the rinse water.
- 1 hour after the dose. Provide the participant with an 8 oz can of Sustical or Ensure and allow them to drink this. The Sustical or Ensure is provided for the participant's comfort, but is not required. They can drink water, tea, or coffee if they prefer.
- 2 hours after the dose. Obtain a urine specimen. Cap and label the container with participant's HABC Enrollment ID#, acrostic, specimen number, date and time, and place in temporary storage.

- Offer the participant 6 ounces of water, tea, or coffee. Record time and volume consumed.
- 3-4 hours after the dose. Obtain a urine specimen. Cap and label the container with participant's HABC Enrollment ID# and acrostic, specimen number, date and time and place in temporary storage. Obtain a 7 mL blood specimen in red top tube. Label the container with participant's HABC ID# and acrostic, specimen number (S1) date and time and place in temporary storage.
- Place the SenseWear armband on the participant at the same time you attach the three other monitors (Actigraph, Actiheart, and Polar heart rate monitor) and complete the Year 10 SenseWear Armband Distribution form.
- Check that all specimens are labeled and that the Year 10 Energy Expenditure Visit 1 form has been filled out.

After participant leaves:

- Aliquot 4 to 4.5 mL of each urine specimen to the appropriately labeled cryotube (i.e., HABC Enrollment ID#, specimen number). NOTE: A NEW CLEAN AND DRY PIPETTE MUST BE USED FOR EACH SPECIMEN TO AVOID CROSS-CONTAMINATION. Seal the tube, dry the outside of the tube and place in a ziplock bag. Store the tubes in a freezer (about -10 degrees C).
- Centrifuge the blood specimen to separate serum.
- Carefully transfer each serum specimen to the appropriate Cryotube. Seal the tube, dry the outside of the tube and place in a ziplock bag . Store the tubes in a freezer (about -10 degrees C).
- Bi-weekly, send the Year 10 Energy Expenditure Visit 1 forms and specimens to the EERC.

ENERGY EXPENDITURE VISIT 2-RESTING METABOLIC RATE AND SPECIMEN COLLECTION

Day before participant arrives:

Phone reminder about appointment and overnight fast.

Prior to participant arrival:

- The Deltatrac metabolic monitor is "warmed-up."
- Check printer power and turn on if not already on.
- The printer is loaded with paper and Inkjet print head is writing clearly.
- Presence of a spare printer ink replacement unit.
- Set the top of the page.
- The plastic bubble and hoses are clean and clear and connected.
- Check that the mixing chamber inlet plug is in place
- The water trap is not more than half-full. Empty if more than half-full.
- The flow selector wheel (on rear) is in the adult mode (40 L/min)
- The bed is clean, functional and in the recumbent position with knees up.

- The Year 10 Energy Expenditure Visit 2 Form is available.
- Check that the exam room is quiet, free of distracting activity, dimly lit, and temperature between 24-27 °C (75-81 °F).
- 2 screw capped urine cups and related material for urine collection.
- Hospital gown
- 2 Pasteur pipettes
- 2 Cryotubes for specimen storage.
- InnerView software for the SenseWear armband is up and running.
- SenseWear armband USB cable is connected to the computer and ready to be connected to the armband.
- Year 10 SenseWear Armband Return and Reading form is available.
- A new AAA battery is available, if needed, for data retrieval from SenseWear armband.

After participant arrives:

- Ask compliance questions on Year 10 Energy Expenditure Visit 2 Form
- Weigh the participant in a hospital gown.
- Obtain a urine specimen. Cap, label (HABC Enrollment ID#, acrostic, specimen number [U4], date and time), and place in temporary storage.
- Ask the participant to rest quietly in bed for about 30 minutes.
- If the participant feels cold, offer them a blanket. If the participant feels hot, alter the environment to insure that they do not sweat.
- Administer the rest of the Year 10 Energy Expenditure Visit 2 interview during the 30 minute rest period.
- If the participant weighs in excess of 120 kg, change the Flow Selector Wheel to the obese range (80 L/min).
- Check the setting on the Deltatrac.
- Perform an autocalibration of the metabolic monitor.
- Remind the participant that the measurement will last 40 minutes and they must lie relatively still and not talk, but remain awake for the measurement.
- Write HABC ID#, acrostic and date on two sheets of paper and place in printer
- Set up Actiheart in 'step test' mode, start time to begin at 15 minutes into RMR measurement
- Place Actiheart on participant remembering to ground (touch electrode with finger)
- When Actiheart software reaching 15:00 minutes, place bubble over participant's head and spread plastic drape as flat as possible to minimize leakage
- Measure Resting Metabolic Rate
- Record room temperature in °C.
- The technician must remain with the participant and monitor gas flow alarms and visually check for labored breathing to insure that gas flow does not fail.
- If the participant does talk, lift their arms to scratch an itch, shift their weight to prevent stiffness etc, indicate the time and movement on the printout using a pen or pencil.
- Ask the participant to sit upright.
- Help the participant to their feet and be sure that they steady. Remember that they have fasted and there is a small risk of hypoglycemia.

- Obtain a second urine specimen. Cap, label (HABC Enrollment ID#, acronymic, specimen number [U5], date and time), and place in temporary storage.
- Check that the Year 10 Energy Expenditure Visit 2 form is complete and all specimens are labeled.
- Download the data from Actiheart monitor, noting in Comments section if not exactly 15 minutes into the resting energy expenditure protocol. Review trace.
- Remove electrodes and place Actiheart unit on multicharger.

After participant leaves:

- Carefully transfer each urine specimen to the appropriate cryotube. Seal the tube, dry the outside of the tube and place in a ziplock bag . Store the tubes in a freezer (about –10 degrees C).
NOTE: Use clean and dry pipettes for each specimen to avoid contamination.
- Check that Actiheart printout is legible. See Section 5.5 for details.
- Turn off the printer power.
- Confirm that the metabolic monitor is the measurement OFF mode.
- Leave the metabolic monitor power on.
- While wearing gloves, wipe the inside of the plastic bubble with a towel dampened with 2% Glutaraldehyde solution. Invert and allow to air dry or wipe dry if a second use is imminent.
- Attend to bedding.
- Bi-weekly, send the RMR printout and Year 10 Energy Expenditure Visit 2 Form to the EERC

Bi-weekly, send the urine and serum specimens to the EERC.

Appendix 5 Frequently Asked Questions

What is the special water?

This is heavy water. How quickly it leaves your body in your urine allows us to measure how much energy you use. There are no known hazards other than a few minutes of dizziness that affects about 1 person out of a hundred. It is considered so safe that it has been used in babies as small as 4 lbs.

Is the water radioactive?

No, the water contains safe, nonradioactive, stable atoms of oxygen and hydrogen. These are not toxic and pass completely from your body within a month.

Do I have to take anything?

Yes, you swallow the special water. See #1 for details

Why can't I eat breakfast?

This will increase your rate of metabolism and makes it difficult to compare results between participants.

Can I drink xxx before I come? (fill in the blank)

You may have a glass of water or a cup of DECAFFEINATED coffee. You may not add cream or sugar (artificial sweeteners are OK) or drink beverages that contain sugar or other calories.

But I can't function without my coffee (tea).

Most people do OK if they have a cup of DECAFFEINATED coffee or tea in place of their regular brew.

Can I eat xxx before I come in? (fill in the blank)

No, you can brush your teeth, but eating any foods during the time we tell you to fast will affect the tests.

Can I sleep during the measurement of resting metabolic rate?

No, this will cause a decrease in metabolic rate and make it difficult to compare results between participants.

How long will each visit take?

Visit 1-about the same time as your clinic visit – 4 hours.

Visit 2-about 1-2 hours.

How long will it take to get the results?

We tell you about your resting metabolic rate after visit 2. It may take 6-8 weeks to complete the analyses for the measurement of total energy expenditure.

Appendix 6 Specimen collection and tracking logs



HABC Enrollment ID #	Acrostic	Date Visit Completed			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"/>	<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	

**YEAR 10 ENERGY EXPENDITURE
SPECIMEN COLLECTION AND PROCESSING
VISIT 1**

Label each collection cup, collection tube, and cryovial with the following:

- ♦ HABC Enrollment ID#
- ♦ Date of specimen collection
- ♦ Time of specimen collection
- ♦ Specimen # (i.e., U0, U1, U2, U3, or S1)

1. Urine collection

Collection cup #	Volume in cup		
	50 mL or more	Less than 50 mL	None
U0	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U1	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U2	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U3	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

2. Urine processing

Cryovial #	Volume (Type: 5 mL)	To	Volume in Cryovial		
			4.0 to 4.5 mL	Less than 4.0 mL	None
U0	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U1	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U2	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U3	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

3. Serum

Collection tube #	Cryovial #	Vol. (Type: 5 mL)	To	Volume in Cryovial		
				4.0 to 4.5 mL	Less than 4.0	None
S1	S1	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

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Energy Expenditure Visit 1 Laboratory Processing
9/21/06



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

**YEAR 10 ENERGY EXPENDITURE
SPECIMEN COLLECTION AND PROCESSING
VISIT 2**

Label each collection cup and cryovial with the following:

- ♦ HABC Enrollment ID#
- ♦ Date of specimen collection
- ♦ Time of specimen collection
- ♦ Specimen # (i.e., U4 or U5)

1. Urine collection

Collection cup #	Volume in cup		
	50 mL or more	Less than 50 mL	None
U4	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U5	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

2. Urine processing

Cryovial #	Vol. (Type: 5 mL)	To	Volume in Cryovial		
			4.0 to 4.5 mL	Less than 4.0 mL	None
U4	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
U5	4.0 to 4.5 mL	EERC	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

EERC is the Energy Expenditure Reading Center at the University of Wisconsin in Madison.

*Energy Expenditure Visit 2 Laboratory Processing
9/21/06*