**WHI Extension Study Nutrition and Physical Activity Assessment Study Protocol**

## I. INTRODUCTION

Nutritional and physical activity factors likely play a major role in determining the risk of major chronic diseases such as cardiovascular disease and cancer. However, the dietary and physical activity assessment instruments used in epidemiological investigations of diet and disease are subject to random measurement errors leading to bias in estimates of the relative risk of disease in relation to nutritional and activity factors. Moreover, certain population subgroups, such as obese individuals, may systematically underreport dietary intake or over report physical activity. These measurement errors can seriously affect study findings (usually by attenuating relative risk estimates) where diet is an important exposure variable (*1-3*). Data are needed on the measurement error properties (including the error variance) of diet and physical activity assessment instruments. An understanding of these measurement error properties and the subsequent development of statistical approaches that consider these factors will permit substantially improved estimates of the association of diet and physical activity with disease outcomes. Regression calibration is a statistical method that can correct for these biases in measurement error, provided suitable biomarkers of nutrient consumption are measured, and thus provide more reliable diet-disease analyses (*4*) (*3,5*). Therefore, the overall objective of this study is to collect objective (i.e., blood and urine) measures of both physical activity and diet to use in regression calibration models in the Women's Health Initiative Observational Study.

This protocol describes the procedures for this study of nutritional biomarkers among a subset of women in the Women's Health Initiative (WHI) Observational Study (OS) who have consented to participate in the WHI – Extension Study. The procedures described in this study will take an important step beyond the currently available literature that documents the phenomenon of dietary underreporting. This study will investigate and quantify the many variables that potentially contribute to measurement error. These data will then be applied to models of disease risk. We know of no other studies that have examined measurement properties of diet and physical activity assessment instruments with a direct application to disease association analyses in the WHI-OS.

## II. OBJECTIVES

This study has two principal aims, each having major implications for nutrition and physical activity epidemiologic research. These are:

**A.1** To produce calibrated estimates of total energy and specific nutrient consumption, and of total physical activity-related energy expenditure, for use in a wide range of disease risk analyses in relation to nutrition and physical activity in the 161,808 postmenopausal women of the Women's Health Initiative (WHI) Clinical Trial and Observational Study (OS).

**A.2** To assess and contrast the measurement error properties of prominent nutritional and physical activity assessment methods, and their combination, in a racially and ethnically diverse sample from the WHI OS. Nutritional methods will include a food frequency questionnaire (FFQ), a four-day food record (4DFR), and three 24-hour dietary recalls (24HR). Physical activity tools will include an activity frequency questionnaire (AAFQ), a 7-day physical activity recall (PAR), and a personal habits questionnaire (PHQ).

These two principal aims will be accomplished through five sub-aims as follows:

**A.3** To recruit 450 women from the WHI OS to participate in a Nutrition and Physical Activity Assessment Study (NPAAS), to be conducted at nine WHI Clinical Centers. Participants will be selected to yield an overrepresentation of racial/ethnic minority women and an overrepresentation of women in the extremes of body mass index.

**A.4** To obtain self-report measures: of energy and nutrient intake and physical activity using the measures listed in A.2 above.

**A.5** To obtain objective biomarker measures: of total and resting energy expenditure using a doubly-labeled water (DLW) protocol and indirect calorimetry; of protein, sodium and potassium expenditure from urinary recovery; and to obtain selected nutrient consumption-related blood concentrations, for all participating women.

**A.6** To develop calibration equations to relate self-report assessments of total energy, protein, sodium and potassium consumption and self-reported activity-related energy expenditure and the consumption of selected other nutrients to the corresponding actual consumption or expenditure. The biomarker measures (A.5) will 'anchor' this development, and the calibration procedure may depend on individual characteristics (e.g., body mass, ethnicity) as well as on person-specific and random measurement errors. Calibration equations for assessments based on each of the instruments listed in A.2 and their combination will be developed. The entire protocol will be repeated in a random 20% reliability subsample as an essential component of calibration equation development.

**A.7** To produce biomarker-calibrated estimates of energy and nutrient intakes, and of activity-related energy expenditure, for the baseline FFQ, 4DFR, and physical activity data in the WHI, using the pertinent calibration equations from A.6. These biomarker-calibrated estimates will enhance a range of analyses of weight change and disease risk in relation to nutrient consumption and activity-related energy expenditure in the WHI cohorts. Comparisons between disease risk associations based on calibrated and un-calibrated exposures will have direct implications for the reliability of the epidemiologic literature on nutrition and physical activity more generally.

## III. BACKGROUND AND SIGNIFICANCE

Ecological and migration studies support an association between diet and physical activity and cancer risk, with strong correlations, for example, between dietary fat intake and increased risk for cancer of the breast, prostate, and colon (*6,7*) and inverse associations of plant foods with cancer risk (*8-10*). Fat may act as a cancer initiator or promoter, while other nutrients such as carotenoids and antioxidant vitamins may decrease cancer risk by protecting cellular constituents from oxidative damage. This biological rationale is supported by both *in vitro* and animal model studies (*9*). However, data are less consistent from analytic epidemiologic studies of diet and cancer risk with many studies unable to detect hypothesized associations nutrients with cancer risk. Clearly, these inconsistencies pose a considerable challenge when making dietary or physical activity-related public health recommendations for chronic disease prevention.

Observational investigations of diet and cancer risk typically rely on self-report of diet by using standard dietary assessment tools such as food diaries, 24-hour dietary recalls or food frequency questionnaires. Similarly, studies of physical activity also rely on self report through diaries, checklists or other self-report tools. Many studies have failed to provide consistent information on the relationships between activity or dietary factors (particularly fat intake) and disease. A potential reason for this failure is the lack of reliability of self-reported physical activity or dietary intakes (*4*). Most notable is the fact that underreporting of dietary energy intake appears to be a common phenomenon and that the extent of underreporting likely varies by participant characteristics, such as obesity (*11,12*).

These measurement error issues are important for the interpretation of the Women's Health Initiative Observational Study. The principal results from this large study will examine in cohort and nested case-control designs dietary and physical activity exposures in relation to breast cancer, colorectal cancer, coronary heart disease, and a range of other secondary outcomes. Aside from blood concentrations for selected vitamins from baseline and year 03, we have only dietary self-report data from which to "estimate" individual consumption of specific nutrients. Food frequency questionnaires (FFQs), the primary dietary assessment tool in WHI, are available at baseline and year 3 for all WHI OS women. However, the measurement properties of FFQs, as well as food records and recalls, in populations like the WHI OS cohort are substantially unknown, in part due to the typical absence of adequate objective marker substudies in nutritional epidemiologic studies to date (*13*). As noted above these measurement error issues may strongly influence the primary results of nutritional epidemiology studies, and the interpretation of the WHI OS. For example, a recent nutrient biomarker study conducted by the National Cancer Institute and a substudy in the European Prospective Investigation of Cancer suggest systematic biases in nutritional self-report data of a magnitude that could greatly affect both the validity of observational studies and the interpretation of intervention trials in the nutritional epidemiology areas (*7,14*). It is therefore critical to collect objectives measures of dietary intake whose sources of error are independent of the sources of error in self-reported measures of diet. But since it is neither economically feasible nor practical to collect certain key biological markers of diet from all participants in large studies, these measures should be collected on a subset of participants and the objective data used to "calibrate" the self-reported data from the entire sample (*5*).

This protocol describes a study of physical activity and nutrient biomarkers in a subset of about 450 women who have consented to participate in the WHI Observational Study Extension Study. This study will be conducted in nine WHI Field Centers (FCs), three of which will each enroll 70 women and the remaining six centers will enroll 40 women. Three centers will enroll minority women exclusively in order to have a final sample whose minority composition is reasonably representative of the entire WHI OS cohort. The principal nutritional biomarkers to be obtained in this study are: 1) energy expenditure; 2) nitrogen (as a measure of protein intake); 3) fatty acids; and 4) micronutrients (i.e., vitamins and minerals). To complete the protocol, participants will attend two visits to their local WHI Field Center where they are enrolled as WHI Extension Study participants; the visits will be two weeks apart. The first visit will last approximately five hours. The second visit will last about three hours and will be conducted two weeks after the first visit. The biomarkers will be obtained from blood and urine specimens collected at the two clinic visits as well as from a urine collection completed in the participant's home and brought to the clinic.

Measure of total energy expenditure: doubly labeled water

Briefly, total energy expenditure is the amount of energy that an individual uses throughout the day. In weight stable individuals, energy expenditure is approximately equivalent to energy intake such that if we can measure the former with some degree of precision, it gives an unbiased estimate of a person's caloric intake. The method of choice to measure energy expenditure is the doubly labeled water (DLW) technique, which measures energy expenditure over a period of approximately two weeks (*15,16*). The principle is that after a loading dose of water labeled with deuterium (a stable isotope of hydrogen) and the stable isotope $^{18}O$, these tracers rapidly equilibrate in body water. The deuterium is eliminated from the body as water and the elimination rate is thus proportional to water turnover. The $^{18}O$ is eliminated as water and carbon dioxide and thus its elimination is proportional to the sum of water and carbon dioxide production. The difference between these two elimination rates is proportional to carbon dioxide production, which is the end product of energy metabolism (*15*).

The actual measurement involves administration of the labeled water and collection of physiologic specimens (urine and blood) on the day of administration and two weeks later. The participant arrives at the clinic after a four hour fast, provides a baseline urine specimen, is weighed and takes the DLW in a single dose of 10 atom percent $^{18}O$ labeled water and 0.12 grams of 99.9% deuterium labeled water per kilogram body weight. Participants remain in the clinic for at least four hours post-dosing and provide spot urine specimens at 2, 3, and 4 hours after administration of the isotopes (*14,16*). Women receive a meal replacement beverage one hour after ingesting the DLW and additional fluids if they have difficulty producing a urine specimen. Women aged 60 years and above will provide a blood sample three hours after consuming the DLW to compensate for post-void urine retention that is often experienced by older women (*17*). This will be collected in a standard 10 ml vacutainer. A complementary meal or snack is provided after collection of the 4 hour post DLW dosing void. Women return to the clinic

for a shorter visit on about Day 15, at which time they will return a 24-hour urine collection (see below; this 24-hour urine specimen will be used to measure urinary nitrogen as an estimate of protein intake) and their weight will be measured. Two urine specimens, collected one hour apart will be obtained. They will also complete the indirect calorimetry protocol (see below). A complementary meal will be provided to all participants after collection of the final urine void.

The first spot urine, which is collected prior to the DLW dosing, provides data on the participant's natural isotopic abundance. The subsequent spot urines collected during the first visit are necessary to determine the equilibration of the tracer with body water. The two specimens collected at visit 2 give a reliable estimate of isotope turnover.

The isotopes are measured in the biological specimens by mass spectrometry and total energy expenditure is then calculated from carbon dioxide production using standard equations (*14,15*). The isotopes, deuterium and $^{18}O$ are nontoxic and safe. They are naturally occurring isotopes. The DLW solution is tasteless, odorless and colorless. DLW has been used in studies with babies as small as four pounds (Dr. Dale Schoeller, personal communication). About 1% of all participants experience transient vertigo after drinking the DLW solution that lasts only few minutes (*16*). While the vertigo is rare, as a precaution all participants remain seated for at least 15 minutes after drinking the DLW.

Measurement of Resting Energy Expenditure: Indirect Calorimetry

Participants will also complete studies of Resting Energy Expenditure using Indirect Calorimetry.
The resting energy expenditure measurements will serve multiple purposes:

- Identify the physiologic determinants of total energy expenditure
- Use in conjunction with total energy expenditure to provide a measure of physical activity by difference

**Resting Energy Expenditure Measurement**

Resting metabolic rate will be measured with a Deltatrac II Respiratory Gas Analyzer, a Sensormedics 29 or similar instrument. These are semi-portable units that measure the concentrations of oxygen and carbon dioxide in air streams entering and exiting a clear plastic hood 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 30 minutes to complete. A 30-minute rest period is required prior to the start of the indirect calorimetry test to ensure that resting energy expenditure is measured (*18*). At Chapel Hill, a nose clip and mouthpiece, rather than a clear plastic hood, will be used with the gas analyzer.

Measure of protein intake: urinary nitrogen:

Urinary nitrogen has been used for decades to assess whether or not patients in the clinical setting (i.e., trauma, burn and surgical patients) are in nitrogen balance.

Controlled experimental studies show that the correlation between controlled protein intake and 24-hour urinary nitrogen is 0.8-0.9 (*19*). More recently, urinary nitrogen has been used as a biomarker in epidemiologic studies (*14,20*).

Urinary nitrogen is an excellent biomarker of protein intake because over 80% of the nitrogen in protein is recovered in the urine. Because approximately 16% of protein is nitrogen:

Nitrogen/0.8 x 6.25 = Estimated Protein Intake (*19*)

In this WHI substudy participants will be asked to collect all of their urine for a 24-hour period and bring the specimens to the clinic when they return on Day 15 to complete doubly labeled water part of the protocol (see above). A collection kit and instructions will be provided. Participants will be instructed to discard the first void on the day of collection and begin the collection with the next void. The 24-hour period ends with the first void of the next day.

Women will also take a B- vitamin (Para amino benzoic acid) the day of their 24-hour urine collection. In order to assess completeness of the 24-hour urine collections, participants will take 80 mg PABA (para amino benzoic acid tablets, which is a B-vitamin and is available for purchase over the counter) at each main meal on the day of the urine collections. This is standard protocol for research studies collecting 24-hour urine specimens (*14*). Because this B-vitamin, PABA, is nearly completely recovered in the urine, its measurement functions as an excellent measure of compliance. This is important because incomplete urine collections would result in a miscalculation of the urinary nitrogen measure. A packet of the vitamins will be placed in the urine kit (they will be purchase as a single lot from a single manufacturer).

There are no known risks to collection of urine specimens. We will use a modified Kjeldahl method to measure the urinary nitrogen. Urine remaining after the urinary nitrogen is estimated will be used to assess urinary calcium, sodium and potassium, nutrients for which consumption cannot be validly measured using blood specimens.

<u>Measure of fatty acids and micronutrients:</u>

A fasting blood specimen will be collected at the second visit on Day 15 to conduct laboratory measures of fatty acids and micronutrients. We will collect three 7-ml vacutainers of serum and one 10-ml vacutainer of plasma from all participants. Bloods will be processed and aliquoted at the Field Centers and all specimens shipped to the WHI Central Specimen Repository at Fisher BioServices in Rockville, MD. All specimens will be stored at –70 º C until analysis. Details about specimen handling, processing and shipment will be identical to standard WHI procedures, which are specified in the NPAAS manual, Section 7 (taken from the WHI manuals, Volume 2, Section 11: Blood and urine collection, processing and shipment.

Plasma phospholipid fatty acids will be analyzed by gas-liquid chromotography (*21*). Total, HDL and LDL cholesterol will be measured by an enzymatic method (necessary for statistical adjustments of the fat- soluble nutrients.) Retinol, tocopherols and carotenoids will be analyzed using high performance liquid chromatography (*22*). Folate and vitamin $B_{12}$ will be measured by radioimmunoassay (*23*). Selenium and zinc will be measured by atomic absorption spectrometry (*22*).

Other Measures:

The following standard WHI forms will be completed as part of this study: Form 60 (food frequency questionnaire), Form 45 (dietary supplement use), and Form 35 (personal habits update). We will also measure height and weight. WHI participants are familiar with these measures as they are part of routine data collection in WHI and are approved as part of the primary IRB approval (see IR File # 3467). Women will also complete a four day food record, a physical activity recall, the Arizona Activity Frequency Questionnaire, NPAAS Form 171 (Viewpoints, regarding attitudes, eating habits, and body image), and three 24-hour dietary recalls.

Other Details:

A sample size of 450 will be sufficient to accommodate selected sources of systematic bias in this calibration procedure. We plan to include about 120 women from racial/ethnic minorities to ensure adequate minority representation. Participants from the nine selected WHI FCs will be invited to participate in this substudy via a personal letter and follow-up phone call. The invitation to participate will come from the FC where they have completed previous WHI activities. Willing participants will be consented at the clinic visit and complete all the procedures described above. For a subset of about 90 women (a "reliability sample"), the protocol will be repeated about 6 months later.

All participants who complete the 'entire' protocol will receive $100.00 to compensate for time and travel. The entire protocol includes: the DLW procedures, completion of all forms and questionnaires, the four day food record, fasting blood draw, indirect calorimetry, 24-hour urine collection and three 24-hour dietary recalls. Participants who are willing to attend both clinic visits, but are unable to produce sufficient urine or for whom the trained clinic phelebotomists are unable to obtain a sufficient quantity of blood will receive the $100.00. Participants who do not return for the second visit will not receive the $100.00. Women who become part of the reliability sample and repeat the protocol six months after the first completion of the protocol will receive an additional $100.00. All participants will receive a complementary meal at the end of each clinic visit. This study will be conducted between 2006 and 2008.

## IV. STUDY PROCEDURES

### A. Recruitment

Nine WHI Field Centers (FCs) have been selected to participate in this substudy (Seattle, Oakland, New York City, Chicago-Northwest, Worcester, Madison, Chapel Hill, Arizona and Memphis). These FCs were selected based on their overall performance in the WHI and their stated willingness and ability to complete this protocol. Seattle. Oakland and Chicago-Northwest will each recruit 70 participants and the remaining six centers will each recruit 40 participants. Three centers, Memphis, New York City and Tucson will recruit minority participants exclusively. Each Field Center will enroll about 20% of these participants in the reliability sample.

IRB approval will be secured at the Fred Hutchinson Cancer Research Center (for the Coordinating Center) and at each institution having a participating FC.

**A.1. Participant Eligibility and Exclusionary Criteria**

A.1.1. Participants will be recruited from WHI-OS women who have consented to participate in the WHI-Extension Study.

A.1.2. The total number of participants will be 450. All 450 women will complete the protocol once and about 90 women will repeat the protocol six months later to obtain measures of reliability.

A.1.3. Exclusion criteria:

Women will be excluded who

1. Do not have a FFQ (Food Frequency Questionnaire) at baseline (SV1) and year 3.
2. Did not participate in the OS at the full follow-up level.
3. Take insulin or oral hypoglycemic agents to manage diabetes.*
4. Require supplemental oxygen.*
5. Have had blood transfusions, administration of blood products or administration of intravenous fluids in excess of 500 mL in the week prior to the first clinic visit for this study or an expectation of same during the period between visits 1 and 2 (including IV fluids administered as part of any administered anesthesia such as during a screening colonoscopy).*
6. Travel more than 200 miles from home during the week prior to the study and throughout the two week study period.*
7. Have lost or gained more than 10% of their body weight in the previous month.*
8. Have bladder control problems that would make collection of a 24-hour urine specimen difficult.
9. Women who have self-reported claustrophobia, which could cause the participant to become anxious when the plastic hood is placed over their head for the indirect calorimetry procedures. These procedures require that the participant lie quietly for 30 minutes, followed by the approximate 30 minute test period. Anxiety caused by claustrophobia would produce inaccurate test results. At Chapel Hill, women who have any medical issues that would

prevent wearing a nose clip and mouthpiece for up to 30 minutes will be excluded.

* These factors interfere with the doubly labeled water measurements of energy expenditure (Dr. Dale Schoeller, personal communication).

**A.2. Recruitment**

A.2.1. The nine participating WHI Field Centers will receive a list of eligible participants based on exclusions #1, #2, and # 3 (information that is in the WHI Extension Study database [WHIX]). These lists will be provided by the WHI Clinical Coordinating Center at the Fred Hutchinson Cancer Research Center.

A.2.2. These lists of participants will be generated to include women across the age and race/ethnicity distribution of the WHI OS. The lists for the three minority recruitment FCs will be comprised of minority women only.

A.2.3. FCs will mail letters of invitation to potential participants in a rolling recruitment fashion.

A.2.4. FCs will telephone potential participants within one week of mailing the letter. FC staff will conduct telephone screening and recruitment script. During this telephone screening, staff will determine eligibility based on exclusions # 4-9 (above) to assess current status in relation to eligibility. Up to five attempts will be made to contact potential participants by telephone. In the event that clinic staff cannot reach a participant after five attempts, the participant will be classified as "ineligible/unreachable" and no further inquiries will be made by phone. Disposition of the telephone screening call will be noted, and the status result will be recorded on Form 174 - NPAAS Status Update.

After completion of the telephone screening interview, should a potential participant decline the offer to be part of the study, any information collected during the telephone screening interview will be destroyed.

Clinic staff may refer to the "Frequently Asked Questions" document when answering questions about the study protocol during the telephone screening.

A.2.5. FCs will schedule eligible participants.

**B. Study Procedures**

B.1 Pre-examination procedures – one day prior to visit 1.

B.1.1. Telephone participant to confirm appointment and remind participant to refrain from eating any food or drink any beverages for four hours prior to the appointment time. If participant is scheduled for Monday, the call may be made on the preceding Friday.

Remind participant to bring dietary supplements to the clinic for the Form 45 (current supplements) completion.

B.1.2. Confirm that all supplies are available for the participant visit.

B.2.1 Day 1

1. Greet participant
2. Complete questions on NPAAS Visit 1 Eligibility Worksheet
3. Complete informed consent (The Frequently Asked Questions may also be used here) and give participant "Study at a Glance"
4. Obtain participant height and weight and record on Form 175 - NPAAS Visit 1.
5. Obtain baseline urine specimen. Place hat over toilet and ask participant to void into hat. Record time of void. Fill 5 mL corning cryotube with 4.0 mL urine specimen, label with participant ID and date and time of collection and store in freezer. Discard remaining urine. Rinse and dry hat. (It is critical that the hat be thoroughly dried.)
6. Select the appropriate doubly labeled water (DLW) bottle appropriate for the participant's weight:

≤ 65 kg – Bottle A
66 to ≤ 80 kg – Bottle B
81 to ≤ 105 kg – Bottle C
≥ 106 kg – Bottle D

The DLW will be a dose of approximately 2 g of 10 atom percent $^{18}O$ labeled water and 0.12 g of 99.9 atom percent deuterium labeled water per kilogram of measured body weight. The total volume to drink is about ¼ cup.

7. Ask participant to drink all of the DLW from the pre-weighed bottle. Participant should remain seated for 15 minutes to minimize the small chance for vertigo that may occur upon drinking the loading dose.
8. If any DLW water spills, mop up with the pre-weighed tissue in the ziplock bag. Return to the bag and seal. Note any spillage**.**
9. Fill the DLW dose bottle with 50 mL of tap water. Ask the participant to push the straw down into the bottle. Replace the cap (with straw inside the bottle) and mix gently. Provide the participant with a second (new) straw, and ask her to drink the rinse water. Record the time.
10. One hour post-dose, provide participant with 8 oz of Sustacal or other meal replacement beverage and allow them to drink it completely. Record the time and volume consumed.
11. Give participant Form 60 (Food Questionnaire), Form 35 (Personal Habits Update), Form 45 (Dietary supplement use), Form 171 (Viewpoints) and either the Arizona Activity Frequency Questionnaire (AAFQ) or the physical activity recall (PAR) to complete. (Note that NPAAS staff administer the PAR; it is not self-administered. Staff will also administer Form 45, Current Supplements. To minimize response bias, the activity questionnaire (AAFQ or PAR) to be administered at Visit 1 and Visit 2 will be

determined by random assignment. The CCC will provide the randomization scheme.) The questionnaires may be completed over the next few hours while the participant waits for her next urine specimen collection time. Participant will also need to view the WHI video, "Keeping Track of What you Eat," and staff should review correct completion of the food record with participants.
12. Two hours post-DLW dose obtain a urine specimen. Place hat over toilet and ask participant to void into hat. Record time of void. Fill 5 mL corning cryotube with 4.0 mL urine specimen, label with participant ID and date and time of collection and store in freezer. Discard remaining urine. Rinse and dry hat.
13. Three hours post DLW dose, obtain a urine specimen. Place hat over toilet and ask participant to void into hat. Record time of void. Fill 5 mL corning cryotube with 4.0 mL urine specimen, label with participant ID and date and time of collection and store in freezer. Discard remaining urine. Rinse and dry hat. <u>If participant is over 60 years of age, draw 1 Lavender Dry EDTA tube of blood.</u> Use the standard WHI blood handling and processing procedures in NPAAS manual, section 7 (taken from WHI manual Volume 2, section 11) and NPAAS visit forms. Label specimen with ppt ID and date of collection and place in freezer.
14. Four hours post DLW dose, obtain a urine specimen. Place hat over toilet and ask participant to void into hat. Record time of void. Fill 5 mL corning cryotube with 4.0 mL urine specimen, label with participant ID and date and time of collection and store in freezer. Discard remaining urine. Rinse and dry hat.
15. After collection of the fourth urine specimen, provide participant with meal/snack.
16. Collect completed Form 60 (FFQ), Form 35 (Personal Habits Update), Form 45 (Dietary Supplements) and Form 171 (Viewpoints).
17. Give participant the "Between NPAAS Visits At-a-Glance" sheet.
18. Instruct participant on 24-hour urine collection procedures. Give participant 24-hour urine collection kit (including the PABA B vitamins). Answer questions participant may have.
19. Give participant Four Day Food Record and portion size booklet and review instructions using the Four Day Food Record Guidelines and Talking Points. Participant should record all foods and beverages (including recipes) for four non-consecutive days over the next two weeks. Staff should pre-record the days on the booklet. At least one week-end day should be included.
20. Schedule/confirm appointment for visit # 2 (two weeks later).
21. Complete any other remaining tasks.
22. Thank participant.
23. Complete Form/Task 174 – NPAAS Status Update.

<u>Note:</u> 250 mL of beverages may be provided between hours 2-4 of the DLW protocol. All volumes and time of consumption must be recorded.

**<u>Protect urine specimens from all sources of water during the collection and storage procedures</u>. <u>If hat is rinsed between urine collections, it MUST be dried thoroughly prior to re-use.</u>**

C.2.2. Day 13.

1. Telephone participant to remind her to collect all of her urine the next day in the specially provided urine collection kit. Remind her to take the PABA pill (the B-vitamin) at each main meal of the day, and ask her to please return the empty PABA packet. Remind her to refrain from using acetaminophen or taking any dietary supplements besides the PABA vitamins.
2. Remind participant of her appointment at the clinic two days later (Day 15). Remind participant to fast for 12 hours. A 12 hour fast is required prior to measuring resting energy expenditure because some energy is expended in digestion and metabolism of food (the "thermic effect of food"); 12 hours of fasting ensures that we are measuring resting energy expenditure ONLY and not any residual thermic effect of food from the last meal.) If clinic visit is scheduled for a Monday, call may be made the preceding Friday.
3. Remind participant to bring her completed Four Day Food Record.

C.2.3. Day 15.

1. Greet participant.
2. Collect 24-hour urine specimens and completed four day food record.
3. Obtain participant weight and record on Form 176 - NPAAS Visit 2
4. Complete NPAAS Visit 2 Participant Update Worksheet.
5. Obtain blood specimen. Draw 3 Royal Blue and 1 Lavender (EDTA) tubes of blood. Use the standard blood handling and processing procedures as described in the NPAAS manual. See also NPAAS Visit Forms. Label specimens with participant ID label. Place in freezer.
6. Obtain urine specimen. Place hat over toilet and ask participant to void into hat. Record time of void. Fill 5 mL corning cryotube with 4.0 mL urine specimen, label with participant ID and date and time of collection and store in freezer. Discard remaining urine. Rinse and dry hat.
7. Administer either the Arizona Activity Frequency Questionnaire (AAFQ) or the PAR (whichever was not completed at Visit 1).
8. One hour after the first urine collection, obtain a second and final urine specimen. Place hat over toilet and ask participant to void into hat. Record time of void. Fill 5 mL corning cryotube with 4.0 mL urine specimen, label with participant ID and date and time of collection and store in freezer. Discard remaining urine. Rinse and dry hat.
9. Provide participant with meal/snack (provided that indirect calorimetry is completed).
10. Provide 24 hour recall approach letter and inform participant that the WHI Clinical Coordinating Center will telephone her three times over the next 2-3 months to conduct a 20 minute dietary interview. She will be asked about all foods and beverages consumed over the previous 24 hours. The interviews will be unannounced, but if the call comes at an inconvenient time, it may be completed on another day. Upon completion of Visit 2, Field Center staff will need to complete Form 174- NPAAS Status Update so that the Clinical Coordinating Center (CCC) can schedule the 24-hour recall.
11. Thank participant for completing the protocol and give the $100.00.

12. Inquire about interest in returning in six months to repeat all procedures by asking her to read and sign the "consent for future contact form." If she indicates willingness to complete the study again, please schedule the six month return appointment.

C.2.4. Indirect Calorimetry

On the workday prior to the visit for measurement of resting metabolic rate, the participant should be contacted and reminded that she should not eat after 8 PM the night before the scheduled clinic visit for measurement of resting energy expenditure (12 hour fast).

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 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."

C.2.4.a Indirect Calorimetry Measurement Procedures- The Indirect Calorimetry may be conducted either before or after the rest of the NPAAS Visit # 2 procedures. The participant must remain fasted until the conclusion of the indirect calorimetry.

1. Ensure that the equipment has been properly calibrated at the start of each day.
2. Ensure that the equipment is turned on and warmed up for at least 30 minutes prior to using it with the participant.
3. Ask the participant to lay down and rest quietly for about 30 minutes.
4. If the participant feels cold, offer her a blanket. If the participant feels hot, alter the environment to insure that she does not sweat.
5. Check that the monitor is in canopy mode. Change if needed (This may differ between instruments). At Chapel Hill, the monitor should be in the appropriate mode for use of the nose clip and mouthpiece.
6. Check that the monitor is in the artifact suppression mode with a 10 min start delay (This may differ between instruments).
7. Check that the hoses from the hood (or mouthpiece) to the metabolic monitor are connected and that the unit is turned on.
8. Perform a calibration of the metabolic monitor.
9. After the initial 30 minute rest period, measure resting metabolic rate as per instrument instructions.
10. 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.
11. The measurement will proceed for 30-40 min.

a. 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.
b. The participant must remain at rest but not sleep.
c. 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.
d. Confirm that the participant is still thermally comfortable.
e. If the participant has to get up because they need use the bathroom, then the measurement can be terminated. The measurement sequence, however, needs to be repeated beginning with the 10 min rest in place of the 30 min called for in the basic protocol.

12. At 30-40 minutes, check the display data printout for a stable reading.
13. End the measurement.
14. Obtain the output data from the metabolic cart.
15. Remove the hood (or noseclip and mouthpiece) from over the participant's head.
16. Ask the participant to sit upright.
17. 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.
18. Give the participant their snack.
19. Offer the participant the letter with their resting energy expenditure results. Answer any questions.

**Note:** The primary safety concern is that airflow through the hood is maintained while the hood is in place over the participant's head. Loss of flow due to a rare failure of the fan in the metabolic cart 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 EE 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.

C.2.4.b Post-Indirect Calorimetry Quality Check

1. Check that the printout/electronic record is legible. If not, correct problem and reprint.
2. 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 h (<0.75), ate within the last 6 h (>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.
3. 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 min of the measurement, or instrument maintenance problems. If the first 10 min of the measurement were not deleted, manually

calculate the average and SD without the first 10 min. If the revised coefficient of variation is less than 10%, record these values. If not, repeat the measurement of resting metabolic rate.

C.2.4.c. Data Management

1. Save all data at the end of each test. All raw data should be sent to the Coordinating Center in an ASCII file format.

**Six month visit procedures will be identical to visits 1 and 2**

**D. Study Timeline and Tasks**

| **3-4 weeks prior to NPAAS visit** | **(Day 1)** | **At home (Day 2-13 and Day 14)** | **Visit 2 (Day 15)** |
|---|---|---|---|
| Invitation letter | Informed consent | Participant completes four day food record collects 24 hour urine at home | Receive food record and 24 hr urine at FC |
| Telephone recruitment and screening | Height, weight, FFQ, DLW protocol, blood draw (if over 60 years of age), physical activity questionnaires, Form 45 (Dietary Supplement Use), NPAAS Form 171 Viewpoints. Give complementary meal. Give Between-Visits-At-A-Glance | | Weight, fasting blood draw, complete activity questionnaires, 2 spot urines to complete the DLW protocol, indirect calorimetry. Give $100.00 when protocol completed. Give complimentary meal. Give resting energy expenditure results letter and 24-hour recall notification letter |
| Schedule appointments | Confirm visit # 2, give four day food record and 24-hour urine collection kit and instructions | | Inquire about repeat visit in 6 months Notify CCC to schedule 24-hr recalls |

**E. Supplies**

1. Participant recruitment letters, envelopes, postage
2. Participant telephone recruitment script*
3. Informed consent document

4. “Study at a Glance” Instruction Sheet*
5. Seven Day Physical Activity Recall (PAR) Script
6. Form 171 – Viewpoints Guidelines/Talking Points
7. Food Frequency Questionnaire (FFQ) Guidelines/Talking Points
8. Four Day Food Record Guidelines/Talking Points
9. Arizona Activity Frequency Questionnaire (AAFQ) Guidelines/Talking Points
10. Resting Energy Expenditure Results Letter
11. “Between Visits At-a-Glance” Instruction Sheet
12. 24-Hour Recall Approach Letter
13. 24-Hour Recall Thank-You Letter
14. Forms, Worksheets and Questionnaires:

- Form 174 – NPAAS Status Update
- NPAAS Visit 1 Eligibility Worksheet
- Visit 2 Participant Update Worksheet
- NPAAS Visit 3 (Reliability Study) Eligibility Worksheet
- NPAAS Visit 4 (Reliability Study) Participant Update Worksheet
- Form 175 – NPAAS Visit 1
- Form 176 – NPAAS Visit 2
- Form 177 – NPAAS Visit 3 (Reliablity Study)
- Form 178 – NPAAS Visit 4 (Reliability Study)Form 35 (Personal Habits Update)
- Form 45 (Dietary Supplements)
- Form 60 (Food Frequency Questionnaire)
- Arizona Activity Frequency Questionnaire (AAFQ)
- Form 172- 7-day physical activity recall (PAR)
- Form 171 – Viewpoints

15. 24-hour urine collection instructions
16. Four Day Food Record and portion size booklet
17. Consent for future contact
18.Blood and Urine handling and shipping supplies, per institutional safety guidelines, supplied by each FC
19. Doubly labeled water (supplied by University of Wisconsin) – 1 bottle per participant on each protocol application.
20. Urine collection “hats” – 3 per participant; 1 to use at each clinic visit, 1 to send home with participant for 24 hour urine collection.
21. Corning cryotubes with O-ring seal for spot-urine collections (5 mL) – 6 per participant
22. Cryovials (5 mL) for 24-hour urine collections – 2 per participant
23. Cryovials (2 mL) for 24-hour urine collections – 2 per participant
24. Royal blue vacutainers – 3 per participant
25. Lavender EDTA vacutainers – 2 per participant
26. Syringes, needles, gloves, cryovials needed for blood draws
27. Graduated cylinders to measure total volume of urine from 24 hr urine collection
28. Preweighed tissue (provided by University of Wisconsin) and Ziplock-type storage bags

29. Sustacal, 8 oz – 1 for each participant
30. Participant nourishment/meal
31. 24 hour urine collection kits – 1 per participant – hat, urine collection bottles, PABA the B-vitamin pills, instructions
32. $100.00 per participant to for time and travel expenses; $100.00 additional for women who repeat the protocol in six months).
33. Participant ID labels for forms and specimens
34. Frequently Asked Questions and Answers about DLW Sheet
35. Metabolic cart (and appropriate staff who can conduct the measures)
36. Top-loading scale (for measuring 24-hour urine collections)

**F. Specimen Handling, Mailing and Storage**

1. All specimens will be handled and processed in accordance with WHI Manual Volume 2, Section 11. All specimens will be shipped by Fed Ex to Fischer BioServices in Rockville, MD per the standard WHI procedures. The CCC will provide a shipping schedule.

**V. Data Management**

Data management and quality control will be the responsibility of the WHI Clinical Coordinating Center. Quality control will be achieved by adherence to the WHI Manuals of Operations for procedures that are already used in WHI (i.e., the collection of the body measurements, blood, urine, and self-report data). For aspects of this substudy that are not already part of WHI, such as the Doubly Labeled Water protocol, quality control measures will be provided to all clinic staff by our Doubly Labeled Water collaborator, Dr. Dale Schoeller at the University of Wisconsin. Dr. Schoeller is the nation's leader the in the DLW protocol. Third, all clinic staff will receive training prior to conducting this study and there will be on-going support from the WHI Clinical Coordinating Center. All data will be managed at the WHI CCC.

The Clinical Coordinating Center will provide specimen aliquot labels for all biospecimens. Neither the specimen repository nor the laboratories conducting the assays will have access to any participant identifiers. All specimens will be logged and tracked in the primary WHIX database. The Clinical Coordinating Center will be responsible for verifying that all samples are sent to the correct laboratories at the correct time, ensuring security and confidentiality of all data, quality assurance and coordination of necessary data for analysis.

The standard WHI forms (Form 35, Form 45, Form 60) will either be scanned or directly data-entered into the WHIX database. The physical activity recall data and the psychosocial questionnaire data will be entered at the WHI CCC. Data collected on visit forms, such as the time of void for the spot urine collections, will be data entered by CCC staff into the WHIX database. The Clinical Coordinating Center will be responsible for daily backups of the WHIX database. The completed Arizona Activity Questionnaires will be sent to the University of Arizona where they will be scanned and datasets

prepared. The datasets will be sent back to the WHI Clinical Coordinating Center. The Four Day Food Records will be coded and data entered at the Fred Hutchinson Cancer Research Center's Nutrition Assessment Shared Resource. The 24-hour recall data are data entered in real time during the interview by trained interviewers at the FHCRC Nutrition Assessment Shared Resource. Datasets of the food record and recall data will be prepared by the Shared Resource and delivered to the WHI CCC.

Quality Control (QC)

Laboratory quality control will be assessed for all blood and urine analyses. QC procedures will be implemented at the Seattle, Chicago, and Oakland Field Centers.

For the nutritional biomarkers from blood analyses (i.e., vitamins and minerals) a 5% blind duplicate testing rate will be added using blood samples from the banked WHI QC reserve.

For the urine analyses (DLW and urinary nitrogen), samples collected at each time point from 5% of the women will be randomly assigned to have one extra aliquot prepared. These 5% samples (for DLW and urinary nitrogen) will receive blinded identification numbers and become the QC samples. We will randomly select any remaining serum aliquots from the 3-hour post dose blood draw for participants $\geq$ 60 years of age as blind duplicates.

## VI. DATA ANALYSIS

The measurement error assessment and the calibration goals of the project will use the statistical model developed by Dr. Ross Prentice (PI of this study) and other colleagues (see references 1,3 and 4).
These measurement model and calibration approaches will be used to address the specific aims of the project. Typically, these measurement models will be applied to the logarithm of the nutritional or physical activity assessment and associated biomarkers. Here we list specific planned analyses. The first three involve measurement model assessments and contrasts (Aim A1, while the remaining three involve applications to disease risk and weight change in the WHI cohorts (Aim A.1):

1. Measurement Error Properties of Major Assessment Methods Using Recovery Biomarkers

Nutritional analyses in this category will focus on total energy consumption, protein, potassium and sodium consumption, for which established recovery biomarkers are available. Measurement model (i) - (iii) will be applied for each of the FFQ, 4DFR, and (3) 24-hour dietary recalls using data collected in this study. Assessment instruments will be evaluated and compared in terms of the coefficients $a_0$ to $a_3$, the measurement error (b + e) variance, and the correlation among measurement errors between replicates. For example, departures of $a_1$ from unity suggest a lack of sensitivity of the assessment, departures of $a_2$ from zero suggest a lack of specificity, a large measurement error variance implies a loss of efficiency for disease association studies, while correlated

measurement errors indicate that the assessment procedures incorporate residual 'person-specific' bias. Statistical tests to compare these model parameter estimates between assessment tools will be carried out, and recommendations concerning the use of these instruments in a population like WHI will be made. Similar analyses will be carried out for the ratios of protein, potassium and sodium, to energy.

Physical activity analyses in this category will focus on activity-related energy expenditure. Model (i) - (iii) will be applied to each of the 7-day PAR, Arizona FFQ, and PHQ measures of activity-related energy expenditure (Q) along with the DLW/REE-derived recovery biomarker (W) to assess and compare measurement properties of these instruments.

For both the nutritional and physical activity measurement error models a range of factors (V) will be considered as potential sources of systematic bias, including age, body mass index and measures of body image, social desirability, and restrained eating (D.2). The 7-day PAR and AAFQ models will also include an indicator variable (in V) to assess whether the order of application of these instruments affects measurement properties. Also, pairwise averages of each of these nutritional and physical activity assessments will be analyzed using the same measurement model, to determine whether a combination assessment may have appreciably better measurement properties than does any of the dietary or physical activity assessments individually. The FFQ measurement error assessments just described will be repeated combining data from this study with that from the NBS to add precision to measurement model parameter estimates, and to contrast with other dietary assessments. Measurement error correlations between assessment instruments will also be estimated.

2. Measurement Error Properties of Major Assessment Methods Using Concentration Biomarkers

The measurement model (ii) - (iv) will be applied to FFQ, 4DFR, and 24HR assessments (Q) for carotenoids, tocopherols, and fatty acid consumption, and blood concentrations of these nutrients (W). Assessments will be compared in terms of the relative values of the measurement model coefficients $a_1$, $a_2$, and $a_3$, and by the relative values of corresponding measurement error variables and correlations. These comparisons are not expected to be sensitive to moderate correlations between errors (u) for replicates of the concentration measure, but sensitivity analyses will be conducted. The measurement properties of ratios of these nutrients to total energy will also be studied and contrasted, and the comparative properties of pairwise averages of the nutrient assessments will be evaluated. Systematic bias will be examined in relation to the same factors (V) listed above in the models for the dietary assessments, while the factors (V) considered for a specific blood concentration will focus primarily on other nutrient biomarker assessments.

3. Additional Macronutrient Measurement Error Assessment

The difference between the DLW and nitrogen biomarker assessments provides a recovery biomarker of non-protein energy. As an exploratory exercise, we propose to use the RQ from indirect calorimetry to yield separate biomarker estimates of fat plus alcohol

and carbohydrate consumption. The carbohydrate biomarker, using the RQ values previously given for the various energy sources is

$$(RQ - 0.7)\ T / 0.3 - P / 3$$

where T and P are respectively the DLW and nitrogen estimates of total and protein energy consumption. The biomarker of fat plus alcohol is then obtained by subtraction. The FFQ, 4DFR, and 24HR assessments of carbohydrate and fat (plus alcohol) consumption will then be assessed against these (likely noisy) biomarkers using models (i) - (iii) in the same manner described above for recovery biomarkers. Analyses will also be conducted restricting the sample to women who report little or no alcohol consumption to examine the comparative measurement error properties for fat consumption specifically. Corresponding analyses will also be carried out for percent energy from carbohydrate and fat, and for energy-adjusted carbohydrate and fat (from the regression of these nutrients on total energy). Once again, the comparative properties of pairwise combinations of the three dietary instruments will also be considered.

4. Nutritional Epidemiology Studies

The calibrated estimates of energy, protein, potassium, sodium, carbohydrate, and fat (plus alcohol), and the ratios of these quantities to total energy will be available for use in association studies with each of the clinical outcomes listed in Table C3 (and others). (These estimates will be based on a version of the above calibration procedure with V restricted to study subject characteristics included in the WHIX database.) Calibrated estimates for the FFQ will be available for both the Observational Study and Clinical Trial cohorts at baseline, and additionally at one year in the CT and three years in the OS. These estimates are also available approximately every three years after Year 1 in the DM trial. Joint analysis of CT and OS data will reduce biases arising from the use of the FFQ as a screening tool in the DM trial. Analyses in either cohort based on the Year 1 or later data will be free of this source of bias. The 4DFR data are available only at baseline in the DM trial. These records have been centralized at the CCC and records for women developing breast cancer, colorectal cancer, and coronary heart disease have been analyzed along with a sample of (1200) controls. These data provide a unique resource for nutritional epidemiology, and association studies based on suitably calibrated 4DFR data may yield nutritional epidemiology findings of substantial reliability.

5. Physical Activity Epidemiology Studies

PHQ estimates of activity-related energy expenditure are available at baseline and subsequently (Year 1 in the CT, Year 3 in the OS) for all women in the CT or OS. The use of corresponding biomarker-calibrated energy expenditure estimates will lead to association studies for a range of clinical outcomes (breast cancer, colorectal cancer, …) that are unique in including an adjustment toward removing systematic bias in relation to body mass and other factors from the assessment process. The resulting odds ratio or hazard ratio estimates are expected to be qualitatively more reliable than those based on

the un-calibrated assessments that have characterized the physical activity epidemiology literature to date.

6. Dietary and Physical Activity Predictions of Weight Change

Measured weight change over an average 10-year follow-up period for each CT woman will be studied in relation to (FFQ and 4DFR) calibrated nutrient consumption data, and (PHQ) calibrated activity-related energy expenditure data to identify dietary and activity patterns that are associated with weight maintenance. Once again, the calibration process can be expected to greatly enhance the reliability of these analyses. These analyses, along with simple regression analyses of the DLW data in relation to study subject characteristics, will also provide needed information on energy requirements for older women.

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