

Dietary biomarker evaluation in a controlled feeding study in women from the Women's Health Initiative cohort^{1,2}

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ABSTRACT

Background: Controlled human feeding studies are necessary for robust nutritional biomarker development and validation. Previous feeding studies have typically evaluated single nutrients and tested relatively few diets.

Objectives: The objectives were 1) to simultaneously associate dietary intake with a range of potential nutritional biomarkers in postmenopausal women by using a controlled feeding study whereby each participant was provided a diet similar to her usual diet and 2) to evaluate serum concentrations of select nutrients as potential biomarkers with the use of established urinary recovery biomarkers of energy and protein as benchmarks for evaluation.

Design: Postmenopausal women from the Women's Health Initiative ($n = 153$) were provided with a 2-wk controlled diet in which each individual's menu approximated her habitual food intake as estimated from her 4-d food record and adjusted for estimated energy requirements. Serum biomarkers, including carotenoids, tocopherols, folate, vitamin B-12, and phospholipid fatty acids, were collected at the beginning and end of the feeding period. Doubly labeled water and urinary nitrogen biomarkers were used to derive estimates of energy and protein consumption, respectively.

Results: Linear regression of (ln-transformed) consumed nutrients on (ln-transformed) potential biomarkers and participant characteristics led to the following regression (R^2) values for serum concentration biomarkers: folate, 0.49; vitamin B-12, 0.51; α -carotene, 0.53; β -carotene, 0.39; lutein + zeaxanthin, 0.46; lycopene, 0.32; and α -tocopherol, 0.47. R^2 values for percentage of energy from polyunsaturated fatty acids and urinary recovery biomarkers of energy and protein intakes were 0.27, 0.53, and 0.43, respectively. Phospholipid saturated fatty acids and monounsaturated fatty acids and serum γ -tocopherol were weakly associated with intake ($R^2 < 0.25$).

Conclusions: Serum concentration biomarkers of several vitamins and carotenoids performed similarly to established energy and protein urinary recovery biomarkers in representing nutrient intake variation in a feeding study, and thus are likely suitable for application in this population of postmenopausal women. Further work is needed to identify objective measures of categories of fatty acid intake.

This trial was registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2017;105:466–75.

Keywords: carotenoids, doubly labeled water, energy, fatty acids, folate, human feeding study, nutrition assessment, protein, tocopherols, vitamin B-12

INTRODUCTION

Diet plays a major role in the etiology of chronic disease in the United States (1–7), and prevention is critical for reducing risk and health care costs (8). Nonetheless, public health promotion programs and policy initiatives have been hampered by a lack of accurate quantification of dietary intake. One barrier is that self-reported diet is subject to substantial random and systematic measurement error. Observational and interventional studies rely on self-report data for the determination of primary exposures and for assessment of intervention adherence, respectively. Thus, self-report assessment limits our ability to draw inferences concerning diet-disease associations. Consistent with the larger body of evidence (9–16), we showed previously in Women's

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²Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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Health Initiative (WHI)¹² cohorts that there was systematic bias related to underreporting (~30–50%) of energy intake among overweight and obese women, that younger postmenopausal women underreport energy more than do older women, and that reporting bias for protein is similar but less pronounced (17, 18). These data have been used to develop calibrated energy and protein consumption estimates in the WHI (17, 18). Disease associations are mostly absent without calibration in the WHI, but bias-corrected (calibrated) energy and protein are typically positively related to chronic disease risk (1, 19–21).

Objective biological measurements provide a useful approach to characterizing dietary exposures; however, to be acceptable for scientific use, the objective measure must provide a suitably accurate estimate of intake variation in the study population. Controlled feeding studies have long been used to evaluate the impact of diet on biological and physiologic processes in humans (22, 23). Feeding known amounts of specific foods or nutrients also provides an opportunity to evaluate measures of nutrients, metabolites, and compounds in biological specimens as biomarkers of dietary exposure under controlled conditions (24, 25). Typically, feeding studies use the same standardized menus for all participants, thereby reducing the variation in nutrient intakes as well as the variance introduced by food type, handling and storage, preparation, and processing, but they depart from participants' habitual diets. To apply a controlled feeding study to dietary biomarker assessment, but preserve the normal variation in nutrient and food consumption at the individual level in the study population, we designed an individual menu plan for each woman that mimicked her habitual food intake as estimated by using a 4-d food record (4DFR) and adjusted for energy requirements on the basis of calibrated energy estimates (17, 18) and standard energy estimating equations (26).

The aims of this report were as follows: 1) to evaluate to what degree serum concentrations of carotenoids, tocopherols, folate, vitamin B-12, and phospholipid fatty acids (PLFAs) explain, with the same confidence as established urinary recovery biomarkers of total energy intake (Ein) from doubly labeled water (DLW) and total protein intake from 24-h urinary nitrogen, the corresponding controlled intake variations in postmenopausal women who consumed the foods of their habitual diets, and 2) by using regression analysis, to examine what percentage of variation in intake can be explained by participant characteristics, such as BMI and age.

METHODS

Participants

Recruitment for the Nutrition and Physical Activity Assessment Study Feeding Study (NPAAS-FS) began in April 2011, and the study ended in October 2013. The NPAAS-FS was implemented in a subset of 153 women who had consented to participate in the WHI Extension Study (27). Women were eligible to participate in the NPAAS-FS if they 1) were currently enrolled

in the WHI Extension Study; 2) previously had been included in the Observational Study cohort, Dietary Modification Trial Comparison (nonintervention) group, or Hormone Therapy Trials [including having participated previously in the Nutrition Biomarker Study (17) and original Nutrition and Physical Activity Assessment Study (18)]; 3) had a deliverable address and a zip code in King or surrounding counties in Washington State (Seattle area); 4) were of full follow-up status within the WHI; 5) were ≤80 y of age as of April 2011 (when the list of eligible women was compiled); and 6) did not have medical conditions that would preclude successful completion of the protocol (including, but not limited to, diabetes, kidney disease, bladder incontinence requiring the use of special garments or medications, or routine use of oxygen). A list of 450 WHI participants eligible for the NPAAS-FS was provided by the WHI Clinical Coordinating Center (**Figure 1**).

Study design and diet formulation

We designed the feeding study to approximate the habitual diet of each participant so as to minimize perturbation of blood and urine measures that would otherwise be slow to equilibrate over the 2-wk controlled feeding period and to preserve substantially the normal variation in nutrient and food consumption at the individual level in the study population. The 450 eligible women received an invitation letter followed by telephone screening in which the study was described and initial eligibility and willingness to participate were determined. Next, eligible and willing participants attended a 1.5-h information meeting (study visit 1; **Figure 1**) at the Fred Hutchinson Cancer Research Center (Fred Hutchinson) Prevention Center during which they completed the consent process and signed the consent form before proceeding with study activities.

We aimed to recruit a feeding study sample of ~150 women. This sample size target was based on sample variation in the fraction of ln-transformed consumption explained by a potential

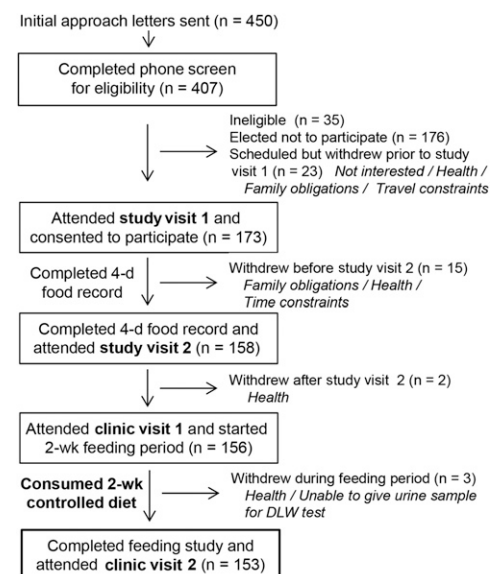


FIGURE 1 Recruitment and study activities of the NPAAS-FS. (Details on study visits 1 and 2 and clinic visits 1 and 2 activities are presented in the Methods section.) DLW, doubly labeled water; NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.

¹² Abbreviations used: DLW, doubly labeled water; Ein, energy intake (derived from doubly labeled water); HNL, Human Nutrition Laboratory; NDS-R, Nutrition Data System for Research; NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study; PLFA, phospholipid fatty acid; QC, quality control; TEE, total energy expenditure; WHI, Women's Health Initiative; 4DFR, 4-d food record.

biomarker. With an R^2 value of 0.5 (correlation of $0.5^{0.5} = 0.7$) for the ln-transformed total energy intake biomarker as a benchmark, we chose a sample size of 150, so that there would be a high probability (power >88%) that a biomarker having an actual $R^2 \geq 0.5$ would have a sample R^2 of ≥ 0.36 (sample correlation of ≥ 0.6) in the context of our feeding study.

This study was approved by the Fred Hutchinson Institutional Review Board in accordance with the Declaration of Helsinki. The WHI Observational Study Monitoring Board provides additional oversight to all such ancillary studies in which participants complete study procedures. The WHI program was registered at clinicaltrials.gov as NCT00000611.

After consent, women received instruction on keeping a 4DFR and were asked to consume their usual diet while completing the record. Between the information session and food record review visit, women recorded all foods and beverages consumed for 4 d with the use of a standardized 4DFR booklet. At study visit 2, women provided information about current dietary supplement use (multivitamins with or without minerals, specialty mixtures including but not limited to B-complex and antioxidant mixtures, and single supplements) and medication use (prescription and over-the-counter) with the use of standardized protocols (28, 29). The study dietitian reviewed the 4DFR and conducted a standardized, in-depth interview to assess usual food choices and patterns that may not have been captured on the 4DFR. Questions included food likes, dislikes, brands, meal patterns, recipes, snacks, and alcohol use; this additional information was used to design the individual planned diets for the feeding study. Food records were entered into the Nutrition Data System for Research (NDS-R; Nutrition Coordinating Center, version 2010; University of Minnesota) software by trained technicians for nutrient analysis and menu planning. Other data collected during this study visit included measured height and weight with the use of standardized equipment and protocols, and self-reported usual physical activity.

Study diet energy needs were established on the basis of self-reported 4DFR energy intake together with standard energy estimating equations (26) and data from previous WHI calibration equations (17, 18) that include a woman's BMI, race-ethnicity, and age. For women whose food record energy intake results were less than the correction value [111 of 153 (73%) of the women], food prescriptions were increased proportionally to reach the correction energy value. On average (\pm SD), an additional 335 ± 220 kcal/d were added. For those with food record results greater than the correction value ($\sim 27\%$ of the women), calories were not changed, because we wanted to ensure that we were providing sufficient food to discourage women from supplementing their controlled diets with nonstudy foods. Each woman's study menu and meals were prepared in the Fred Hutchinson Prevention Center Human Nutrition Laboratory (HNL). When designing the diets, we selectively sourced foods for which the nutrient database values were likely to be most complete. ProNutra (version 3.4.0.0; Viocare) was used to create the menus, recipes, production sheets, and labels and to record both planned and consumed intake data. The final planned and consumed diets were analyzed for nutrient content by using the NDS-R.

Approximately 2 wk after study visit 2, women returned to the Prevention Center to begin the feeding study with the first feeding intervention visit, which lasted ~ 5 h (clinic visit 1). Women

initially provided a fasting blood specimen and a spot urine specimen before receiving a single oral dose of DLW (30). Height, weight, and waist circumference were measured by trained staff. Over the next 4 h, women provided 4 additional spot urine specimens in accordance with the DLW protocol. An additional blood sample was drawn at hour 3 as part of the DLW protocol for adults aged >60 y. During the visit, women completed questionnaires on diet (food-frequency questionnaire) (31) and leisure physical activity, reported by 2 methods with the use of a questionnaire previously used in the WHI to capture recreational physical activity (29, 32) and the Arizona Activity Frequency Questionnaire (33). Finally, the Nutrition and Physical Activity Assessment Study Viewpoints questionnaire was administered to collect data on body image, social desirability, and eating habits (34–36). Women were instructed to maintain their habitual physical activity schedules and medication and dietary supplement use during the feeding period.

Women returned 2–3 times/wk to the HNL during the 2-wk feeding period to consume one study meal on site, have their body weight measured, and pick up the remainder of their food to take home for the following days. Modest diet adjustments were made if a woman lost weight from 1 visit to the next, but no adjustments to the food provided were made with weight gain. A daily menu checklist, which was used to record consumption of study and nonstudy (when applicable) foods and beverages, also was collected. Although all of the participants were advised to eat only foods prepared by the HNL, occasional circumstances dictated the intake of other foods. In these cases, study staff provided guidance on suitable foods to substitute for the planned foods, and these were detailed on the checklist. Women who consumed alcohol were required to provide their own alcohol during the study, recording the type (including added items such as flavorings, condiments, etc.) and amount on each day's menu checklist. Uneaten study foods were returned to the HNL (when applicable) and weighed and recorded. Quantities of consumed foods were then re-entered into the NDS-R for use in nutrient analysis. On the penultimate day of the feeding study, women collected a 24-h urine sample with the use of a provided kit and instructions. On the last day of the feeding period, women returned to the clinic for a 2-h visit (clinic visit 2), at which time they were weighed, their first-void urine and completed 24-h urine specimen were processed, and a fasting blood specimen and 2 spot urine specimens (per DLW protocol) were collected.

Biomarker measurements

Recovery biomarkers: DLW and urinary nitrogen

Biomarker-derived Ein was calculated from total energy expenditure (TEE) by using DLW as in our previous biomarker studies (17, 18) and taking into consideration any weight change over the 2-wk controlled feeding period. The mean CV for DLW assessment for 9 blinded duplicate pairs was 5.1%. DLW data were not available for 5 of the 153 women (3.3%), because the dose-day urine enrichment failed to reach a plateau that indicated equilibrium. For each participant, we calculated the weight change and converted that to grams per day. We used a 2-compartment model to estimate the change in body energy stores for each woman, with 12% of weight change as fat mass and the other 88% as fat-free mass (37). The energy density of fat mass was assumed to be 9.5 kcal/g and that of fat-free mass was

assumed to be 1.0 kcal/g. Thus, the energy density of weight change was $(0.12 \times 9.6) + (0.88 \times 1.0) = 2.0$ kcal/g, which was then multiplied by weight change (grams per day) to determine change in (Δ) body energy stores. Ein was then calculated as TEE + Δ body energy stores. Thus, a woman who lost weight would have an Ein less than TEE because she was contributing energy from body energy stores to meet energy expenditure.

Urinary total nitrogen was measured in the 24-h urine samples collected at the end of the 2-wk feeding period. Urine (1 mL) was diluted 1:100 and analyzed by using an assay based on the Kjeldahl method (38). The CV for 8 blinded duplicate pairs was 2.2% for urinary nitrogen.

Concentration biomarkers: serum measures

Serum aliquots from fasting blood samples were stored at -80°C until analysis. Assays were completed in the Fred Hutchinson Public Health Sciences Biomarker Laboratory. For all assays, serum samples from baseline and post-controlled feeding period were run in the same batch for each woman. Serum folate and vitamin B-12 concentrations were determined in duplicate by radioimmunoassay, as recommended by the manufacturer (SimulTRAC-SNB Vitamin B12/Folate RIA Kit; MP Biomedicals). Mean interbatch CVs for laboratory quality control (QC) samples were 2.8% for folate and 4.1% for vitamin B-12. Serum carotenoids and tocopherols were measured by HPLC, as described previously (39). Lutein, zeaxanthin, and lycopene were detected at 476 nm; α -carotene and β -carotene at 452 nm; retinol at 325 nm; and α -tocopherol and γ -tocopherol at 292 nm. Interbatch CVs for laboratory QC samples were $<6.0\%$ for all analytes. Carotenoid and tocopherol concentrations were adjusted for serum cholesterol. Serum PLFAs (as relative weight percentages of all PLFAs analyzed) were measured by gas chromatography with flame ionization detection, as previously described (40). Interbatch CVs for laboratory QC samples were $<10.0\%$ for all PLFA analytes, except for 1 very minor fatty acid, 20:3n-3 (eicosatrienoic acid; $<0.1\%$), which was 27.1%.

Statistical analysis

We used mean daily ln-transformed dietary intakes (consumed, planned, and 4DFR) and biomarker measurements, including carotenoids, tocopherols, PLFAs, folate, vitamin B-12, total energy, and protein (at baseline and/or after the 2-wk feeding period) in the analyses. Statistical analyses were carried out by using SAS (version 9.4; SAS Institute). Consumption (mean of 14-d of intake during the feeding period) was determined by using the information from the daily menu checklist and returned food (when applicable) and adjusting the provided menus and associated nutrient intakes accordingly (see previous section entitled "Study design and diet formulation"). The data had a missing rate of $<1\%$ for the majority of nutrient biomarker and dietary data (sample sizes indicated accordingly). Values that fell outside the IQR by >3 times their width were excluded as outliers ($<2\%$ for all transformed nutrient variables). For each nutrient variable, geometric means and 95% CIs (2.5th–97.5th percentiles) were reported. Ratios of geometric means were computed to characterize differences between 2 measures from the same participant. In particular, we compared the following: 1) the distribution of nutritional biomarkers measured in blood at baseline with their values post-feeding study and 2) the

distributions of self-reported dietary intake measured by 4DFR, the planned dietary intake, and the consumed dietary intake. Pearson correlation coefficients were computed to characterize the strength of association between paired measures.

The principal data analyses regressed ln-transformed mean daily nutrient consumption (from the prescribed controlled diet consumed plus dietary supplements used) over the 2-wk feeding period on the corresponding ln-transformed potential biomarker assessed at the end of the feeding period. The percentage of variation explained, denoted as R^2 , provides the criterion for biomarker evaluation, with R^2 values for the established energy and protein biomarkers offering benchmarks for comparison. Because the measurement properties of some candidate biomarkers may depend on participant characteristics, the linear regression analyses were extended by considering the following additional explanatory variables: race-ethnicity and educational level (collected at WHI enrollment), age, BMI, dietary supplement use (yes or no), leisure physical activity (total metabolic equivalent task hours/wk), season (measured at NPAAS-FS enrollment), and weight variability (absolute difference in kilograms over the 2-wk feeding period). A backward-selection procedure ($P = 0.10$) was applied to eliminate noncontributing participant characteristics. The percentage of variation in the (ln-transformed) diet consumed explained (R^2) by the regression model was computed to characterize the potential of a biomarker specification that included both biospecimen measures and participant characteristics. Comparison of these R^2 values with R^2 values based on the biospecimen measure alone evaluates the contribution of participant characteristics to the composite biomarker. Here, we limited our characterization of fatty acid intakes to broad classes of fatty acids; a more detailed analysis of PLFAs and intakes of specific fatty acids and other nutrients will be reported elsewhere.

RESULTS

A total of 450 Seattle-area WHI women were approached to enroll in the study, with a target sample size of 150; 173 (39%) women consented to participate in the NPAAS-FS. Of these, 20 participants dropped out (17 before starting the 2-wk feeding period), leaving a total of 153 women who completed the feeding period (Figure 1). Three women who started the feeding period did not complete it because of health issues unrelated to the study ($n = 2$) or being unable to provide urine for DLW measures ($n = 1$). Baseline demographic and lifestyle characteristics of these 153 women who participated in the NPAAS-FS are presented in **Table 1**. Overall, the women were well educated (82.9% college degree or higher) and nonsmokers (98%), with a median recreational physical activity level of 12.25 metabolic equivalent task hours/wk. Most (95%) of the NPAAS-FS women were white, and the other 5% ($n = 7$) were African American, Hispanic, American Indian or Alaska Native, or Asian or Pacific Islander. On the basis of BMI, 39.2% were overweight (in kg/m^2 ; 25.0 to <30.0) and 20.9% were obese (≥ 30.0). In comparison, among the 450 women approached by letter, 95% were white, 45.8% had a college degree or higher, and the percentages in each of the 3 age categories (60–69, 70–79, and 80–85 y) were 11%, 81%, and 8%, respectively.

Mean levels of urine biomarkers measured over the course of (for Ein) or at the end of (for protein) the 2-wk feeding period



TABLE 1

Baseline demographic and lifestyle characteristics of the 153 postmenopausal women who participated in the NPAAS-FS¹

Variable	Value
Age, ² n (%)	
60–69 y	10 (7.0)
70–79 y	127 (83.0)
80–85 y	16 (10.0)
Race-ethnicity, ³ n (%)	
White	146 (95.4)
African American	3 (2.0)
Hispanic	2 (1.3)
Other ⁴	2 (1.3)
Height, ² cm	162 (157–166) ⁵
Weight, ² kg	69.0 (60.8–76.4)
BMI (kg/m ²), ² n (%)	
Normal (<25.0)	61 (39.9)
Overweight (25.0–29.9)	60 (39.2)
Obese (≥30)	32 (20.9)
Medication use, ^{2,6} n (%)	
No	14 (9.2)
Yes	120 (78.4)
Missing	19 (12.4)
Dietary supplement use, ^{2,7} n (%)	
No	23 (15.0)
Yes	130 (85.0)
Current smoking, ² n (%)	
No	150 (98.0)
Yes	3 (2.0)
Physical activity ² (MET-h/wk), n (%)	
0–5.5	39 (25.5)
5.6–12.25	38 (24.8)
12.3–24.0	39 (25.5)
>24	37 (24.2)
Season of study participation, ² n (%)	
Spring	38 (24.8)
Summer	51 (33.3)
Fall	31 (20.3)
Winter	33 (21.6)
Education, ³ n (%)	
High school or General Educational Development diploma	10 (6.5)
Schooling after high school	16 (10.5)
College degree or higher	126 (82.3)
Missing	1 (0.7)

¹ MET-h, metabolic equivalent task hour; NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.

² Measured at time of enrollment in the NPAAS-FS.

³ Collected at time of enrollment in the Women's Health Initiative.

⁴ "Other" race included 1 American Indian/Alaska Native and 1 Asian/Pacific Islander.

⁵ Mean; IQR in parentheses (all such values).

⁶ Self-reported prescription and over-the-counter medications.

⁷ Self-reported dietary supplement use from the NPAAS-FS baseline 4-d food record.

and potential serum biomarkers measured at baseline and at the end of the feeding period are shown in **Supplemental Table 1**. We found correlations [Pearson correlations (*r*)] between baseline and post-feeding study serum biomarker measures that ranged from 0.47 for the PLFA 18:2n–6tc (*trans*, *cis* linolelaidic acid, a low relative percentage abundance fatty acid) to 0.97 for vitamin B-12. Of the 49 variables measured at baseline and the end of feeding, only 9 (18%), all of them PLFAs, were correlated at <0.60.

Supplemental Table 2 shows geometric means and 95% CIs for energy and nutrient intakes as consumed by the women during the 2-wk feeding period and compared with the planned feeding period menus and 4DFRs kept at baseline. There were strong correlations between the nutrient intakes of diets as consumed compared with the diets as planned, except for alcohol (*r* = 0.27), which was not part of the provided food and beverages but was left to the participants to provide if it was part of their typical diet. Women who consumed alcoholic beverages during the 2-wk feeding period recorded these on the daily menu checklist, and nutrient intakes from these beverages were included in overall estimates. A total of 111 (72.5%) women consumed alcohol on approximately half of the study days for a mean (median) percentage of energy from alcohol of 4.5% (2.7%). Correlations were slightly weaker for planned and consumed menus compared with 4DFRs, reflecting modifications to the menus to allow for consumption over the 2-wk period (see "Study design and diet formulation"). Mean energy intakes as consumed on the controlled diets tended to be, on average, ~200 kcal/d higher than those estimated from the reported 4DFRs; however, percentages of energy from carbohydrate, protein, and fat were similar on both the feeding study diet and 4DFRs. Overall, nutrient intakes as consumed were lower than the diets as planned, because the planned study diet energy levels were established to be equal to or greater than the correction equation value for each woman. Furthermore, geometric mean energy intake as estimated from 14-d reported intakes and biomarker-derived Ein were 1904 and 1790 kcal/d, respectively. Self-reported data from the daily check-off forms and food returned to study staff indicated that participants adhered to the study protocol. Over the 2-wk controlled period, only 6 women (4%) reported the consumption of nonstudy foods providing >100 kcal/d over ≥3 d. Among these 6 participants, nonstudy foods accounted for a mean of 3.9% of total daily energy. The exclusion of these women from the analysis did not substantially alter the correlations between intakes and biomarkers (data not shown).

Correlations between energy and nutrients consumed and potential biomarker measures are presented in **Table 2**. *R*² (percentage) based on the biomarker alone (squared Pearson correlation) ranged from 0.45 for serum α -carotene to 0.01 for phospholipid MUFAs. Overall, ln-transformed serum carotenoid, folate, vitamin B-12, and α -tocopherol concentrations, along with Ein and protein, explained ≥25% of the variation in ln-transformed intake of these nutrients, whereas PLFAs and plasma γ -tocopherol were weakly associated with intake. The ln-transformed intake of α -tocopherol correlated with ln-transformed serum α -tocopherol adjusted for cholesterol when α -tocopherol intake from supplements was included (*R*² = 0.42); however, when intake was limited to food sources, the correlation was weak (*R*² = 0.06).

The contribution of various participant-related variables, including weight variability during the 2-wk feeding period, to the estimate of intakes of nutritional variables is presented in **Table 3**. The study protocol allowed for 3% weight variability over the 2-wk period, an amount of variability consistent with normal day-to-day weight fluctuations and within the limits of the requirements for accuracy of TEE from DLW (30). Women's weights were monitored throughout the feeding period, and although efforts were made to minimize weight variation, the



TABLE 2Correlations between ln-transformed nutrient intake over the 2-wk controlled feeding period and ln-transformed post-feeding period biomarker measures in the postmenopausal women in the NPAAS-FS¹

Consumed nutrients	Biomarker measure	<i>n</i> ²	Pearson correlation	Pearson correlation squared
Total energy, kcal/d	Ein, kcal/d	147	0.71	0.51
Protein, g/d	Protein, g/d	149	0.61	0.38
Vitamin B-12, µg/d	Vitamin B-12, pg/mL	152	0.63	0.40
Dietary folate equivalents µg/d	Folate, ng/mL	151	0.66	0.44
α-Carotene, µg/d	α-Carotene (adj), µg/mL	152	0.67	0.45
β-Carotene, µg/d	β-Carotene (adj), µg/mL	151	0.53	0.28
Lutein + zeaxanthin, µg/d	Lutein + zeaxanthin (adj), µg/mL	152	0.61	0.37
Lycopene (<i>trans</i> + <i>cis</i>), µg/d	Lycopene (<i>trans</i> + <i>cis</i>) (adj), µg/mL	151	0.54	0.30
α-Tocopherol, mg/d	α-Tocopherol (adj), µg/mL	152	0.65	0.42
γ-Tocopherol, mg/d	γ-Tocopherol (adj), µg/mL	151	0.14	0.02
Fatty acids	Phospholipid fatty acids, relative concentration (%)			
Total SFAs, g/d	SFAs, ³ %	149	0.25	0.06
SFAs, % of energy	SFAs, %	149	0.25	0.06
Total MUFAs, g/d	MUFAs, ⁴ %	150	0.12	0.01
MUFAs, % of energy	MUFAs, %	150	0.18	0.03
Total PUFAs, g/d	PUFAs, ⁵ %	152	0.37	0.13
PUFAs, % of energy	PUFAs, %	152	0.43	0.19

¹ adj, serum nutrient concentration adjusted for serum cholesterol; Ein, energy intake (derived from doubly labeled water); NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.² Number of pairs of participant diet and biomarker data used to compute the Pearson correlation.³ SFAs = 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0 + 23:0.⁴ MUFAs = 16:1n-9c + 16:1n-7c + 17:1n-9c + 18:1n-8c + 18:1n-9c + 18:1n-7c + 18:1n-5c + 20:1n-9 + 24:1n-9.⁵ PUFAs = 18:3n-3 + 20:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3 + 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:2n-6 + 22:4n-6 + 22:5n-6.

mean (median) weight change and percentage weight change from clinic visit 1 to clinic visit 2 was -0.75 (-0.70) kg [-1.1% (-1.0%)] and ranged from -3.6 to 2.4 kg (-4.6% to 3.8%). The model R^2 value was 0.53 for the energy intake calibration model; the energy-related biomarker, Ein, had the highest partial R^2 at 0.51 , with race (partial $R^2 = 0.02$) the only other variable that reached significance for inclusion ($P < 0.1$) in the model. Thus, the model R^2 explained 53% of the variation in ln-transformed energy intake, with ln-transformed Ein derived from DLW after correction for weight change, explaining 51% of the variance, and race-ethnicity explaining an additional 2%, which indicated that carefully prescribed energy intake as consumed may not have perfectly matched the participants' habitual energy intakes. For protein intake, the urinary nitrogen-derived estimate (biomarker partial R^2) was 0.37 , with BMI, supplement use, physical activity, and weight variability contributing modestly to the overall R^2 of 0.43 . Similarly, for the serum biomarkers, the biomarkers themselves contributed most to the model, whereas the contribution of participant characteristics varied by nutrient and were modest. For example, race-ethnicity, BMI, and dietary supplement use were included in the model for several of the nutrients, but physical activity only reached significance for inclusion ($P < 0.1$) for vitamin B-12.

DISCUSSION

In this study in 153 postmenopausal women enrolled in the WHI, recovery and concentration biomarkers were used to characterize women's diets and to generate biomarker calibration equations that included the pertinent biospecimen measure and

participant characteristics. The equations for ln-transformed intake of folate, vitamin B-12, various carotenoids, α-tocopherol, and percentage of energy from PUFAs, constructed in the relevant study population under carefully controlled dietary conditions, provide useful biomarkers for the further evaluation of diet-disease associations in the WHI, and possibly for similar populations of postmenopausal women. Analyses of this type have previously been restricted to energy and protein based on recovery biomarkers and a small number of other nutrient variables having an associated potential biomarker (e.g., carotenoids, B vitamins). Our approach of using 4DFRs, further qualified to enhance accuracy in reflection of habitual intake by using standardized dietary interviews conducted by registered dietitians, was effective in maintaining similar nutrient intakes during the 2-wk controlled feeding period compared with reported habitual intake (Supplemental Table 2). Furthermore, serum biomarkers measured at baseline and at the end of the 2-wk feeding period were highly correlated (82% at $r > 0.6$; Supplemental Table 1), suggesting that this approach resulted in minimal short-term perturbation of the biomarkers of intake.

We used the urinary recovery biomarkers of energy and protein as benchmarks for the evaluation of the serum nutrient biomarkers we measured in this sample of postmenopausal women. The recovery biomarkers ln-transformed Ein and ln-transformed urinary nitrogen—established approaches to assess energy (41) and protein (30) intakes—were correlated with feeding study consumed ln-transformed energy intake ($r = 0.71$) and ln-transformed protein intakes ($r = 0.61$), respectively (Table 2). Ein (partial $R^2 = 0.51$) and race-ethnicity (partial $R^2 = 0.02$) explained 53% of the variation in ln-transformed energy intake. Urinary nitrogen (partial $R^2 = 0.37$), BMI, and dietary supplement use explained



TABLE 3

Regression estimates and R^2 regression values of ln-transformed nutrient intake on corresponding ln-transformed nutrient biomarker and study participant characteristics in the NPAAS-FS ¹

Variable ²	$\beta \pm SE$	R^2
Total energy intake, kcal/d		
Intercept	7.454 \pm 0.039	
Biomarker: Ein	0.680 \pm 0.055	0.51
Race-ethnicity (white)	0.102 \pm 0.040	0.02
Overall		0.53
Total protein intake, g/d		
Intercept	4.302 \pm 0.034	
Biomarker: protein	0.437 \pm 0.046	0.37
BMI	0.010 \pm 0.003	0.04
Supplement use ³	0.062 \pm 0.036	0.01
Overall		0.43
Vitamin B-12, $\mu\text{g/d}$		
Intercept	2.190 \pm 0.286	
Biomarker: vitamin B-12	1.869 \pm 0.192	0.40
BMI	0.081 \pm 0.027	0.05
Physical activity	-0.016 \pm 0.008	0.01
Supplement use	1.227 \pm 0.312	0.05
Overall		0.51
Dietary folate equivalents, $\mu\text{g/d}$		
Intercept	6.730 \pm 0.147	
Biomarker: folate	0.808 \pm 0.091	0.43
Supplement use	0.380 \pm 0.123	0.04
College degree or higher	-0.244 \pm 0.107	0.02
Overall		0.49
α -Carotene, $\mu\text{g/d}$		
Intercept	6.362 \pm 0.160	
Biomarker: α -carotene ⁴	1.241 \pm 0.101	0.45
BMI	0.082 \pm 0.019	0.06
Spring	-0.325 \pm 0.219	0.0001
Summer	-0.534 \pm 0.207	0.02
Fall	-0.258 \pm 0.232	0.004
Overall		0.53
β -Carotene, $\mu\text{g/d}$		
Intercept	8.478 \pm 0.040	
Biomarker: β -carotene ⁴	0.624 \pm 0.066	0.28
BMI	0.050 \pm 0.010	0.10
Overall		0.39
Lutein + zeaxanthin, $\mu\text{g/d}$		
Intercept	7.426 \pm 0.228	
Biomarker: lutein + zeaxanthin ⁴	1.101 \pm 0.100	0.37
Age	-0.028 \pm 0.014	0.01
BMI	0.049 \pm 0.012	0.05
Race-ethnicity (white)	0.593 \pm 0.233	0.02
Overall		0.46
Lycopene, $\mu\text{g/d}$		
Intercept	8.262 \pm 0.060	
Biomarker: lycopene ⁴	1.052 \pm 0.127	0.30
BMI	0.035 \pm 0.015	0.03
Overall		0.32
α -Tocopherol, mg/d		
Intercept	2.885 \pm 0.162	
Biomarker: α -tocopherol ⁴	2.077 \pm 0.231	0.43
Supplement use	0.510 \pm 0.177	0.03
Weight variability ⁵	0.117 \pm 0.060	0.01
Overall		0.47
Percentage of energy from PUFAs		
Intercept	1.940 \pm 0.054	
Biomarker: PUFAs	4.284 \pm 0.707	0.19
Age	-0.012 \pm 0.0058	0.02

(Continued)

TABLE 3 (Continued)

Variable ²	$\beta \pm SE$	R^2
Physical activity	0.003 \pm 0.0014	0.02
Supplement use	0.111 \pm 0.058	0.02
Weight variability	0.035 \pm 0.021	0.01
Overall		0.27

¹ Presented nutrients are restricted to those with overall $R^2 > 0.25$. Included were study participant characteristics that remained significant for inclusion in the model on the basis of a backward-selection procedure ($P = 0.1$). Ein, energy intake (derived from doubly labeled water); NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.

² Age was centered at 75.36 y; BMI (in kg/m^2) was centered at 26.39; physical activity was centered at 15.78 metabolic equivalent task-hours/wk; weight variability was centered at 1.33 kg. For biomarker measures, ln Ein was centered at 7.48 ln kcal/d; ln protein was centered at 4.33 ln g/d; ln vitamin B-12 was centered at ln 6.27 pg/mL; ln folate was centered at ln 2.99 ng/mL; ln %PUFAs was centered at 3.71 ln % relative abundance. Serum cholesterol-adjusted biomarkers were residuals from the model of biomarker and cholesterol; these residuals were entered into the calibration equation without centering.

³ Self-reported use of any dietary supplements from the NPAAS-FS baseline 4-d food record.

⁴ Serum cholesterol-adjusted.

⁵ Weight variability over the 2-wk controlled feeding period.

43% of the variation in ln-transformed protein intake. Bingham and Cummings (42) showed that, with multiple 24-h urine collections under metabolic ward conditions, urinary nitrogen and nitrogen intakes were highly correlated ($r = 0.90$). This association was stronger than we observed; however, our participants were older and free-living even though consuming controlled diets, and estimates relied on a single 24-h urine collection. Inaccuracies in the women's estimated consumption of alcohol and foods that were departures from planned intakes and differences between actual and estimated individual digestibility and absorption factors for foods (43, 44) may also explain the lower biomarker-intake agreement. Given that recovery biomarkers have a known quantitative relation to intake, in the context of the controlled feeding study, we were able to establish that in our sample personal characteristics of the participants did not contribute substantially to the overall model R^2 (Table 3).

The capacity to generate regression calibration equations by using concentration biomarkers has been limited by the availability of published feeding studies with sufficient ranges of intake and a variety of food sources (45). In the NPAAS-FS, we found that, similar to the recovery biomarkers, serum concentrations of folate, vitamin B-12, α -tocopherol, and the major groups of carotenoids were well correlated with the nutrients consumed for 2 wk (Table 2). There was no association between γ -tocopherol intake and serum concentrations. Two-thirds of the participants (102 women) used dietary supplements that contained vitamin E, and α -tocopherol intake from supplements contributed substantially to the strength of the correlation. Reported decreases in plasma γ -tocopherol concentrations with α -tocopherol supplementation (46) may, in part, explain the lack of association between γ -tocopherol intake and circulating concentrations in our study. Thus, our work supports findings from observational studies of tocopherols as biomarkers, which suggest that circulating



α -tocopherol concentrations are a useful biomarker of intake when supplement users are included in the analysis (47, 48).

Overall, PLFAs were less effective in capturing ln-transformed intake (grams per day) of the major classes of fatty acids (Table 2). Correlations (r) between PLFAs and SFA, MUFA, and PUFA intakes were 0.25, 0.12, and 0.37, respectively—associations similar to those reported in observational studies with self-reported diet (reviewed in reference 49). This likely, in part, reflects endogenous synthesis and the dynamic effects of fatty acid and carbohydrate metabolism on PLFAs (49), as well as the limitations of the fatty acid data for some foods in the dietary database. Nonetheless, ln-transformed phospholipid PUFAs accounted for 19% of the variation in the estimate of ln-transformed percentage of total energy from PUFAs. Including age, physical activity, dietary supplement use, and weight variability in the model accounted for a total of 27% of the variation in the estimate, suggesting the potential utility of phospholipid PUFAs as a biomarker of intake. Linoleic acid (18:2n-6) was the most commonly consumed PUFA in the participants' diets (Supplemental Table 2), contributing to 84% of PUFA intake, and few fatty acids beyond 18:2n-6 and 20:4n-6 (arachidonic acid) contributed substantially to phospholipid PUFA concentrations (Supplemental Table 1); this more direct comparison may have improved the estimate. Contrasting our PLFA data with those of other feeding studies is difficult. Most feeding studies of dietary fatty acid composition are intervention trials designed to change blood lipid fatty acid fractions rather than characterize associations between intake and PLFAs (reviewed in reference 49); in contrast, we aimed to reconstruct habitual diets and to minimize changes in fatty acid profiles. Even so, in cross-sectional studies, dietary PUFA content correlates well with the fatty acid composition in all blood lipid fractions (49). Further evaluation of the utility of specific PLFAs as markers of fatty acid intake will be reported elsewhere.

There are several strengths of this study. The NPAAS-FS is, to our knowledge, the first of its kind to compare a panel of potential serum dietary biomarkers with established recovery biomarkers under controlled conditions among persons consuming diets designed to be similar to their habitual diets. The variety of food sources of nutrients fed provides a robust estimate of the utility of these serum biomarkers under controlled conditions. The work was conducted in postmenopausal women participating in WHI cohorts that have been extensively characterized in terms of chronic disease risk factors and that have been carefully followed for a range of clinical outcomes for the past 2 decades. The NPAAS-FS provides an important resource for furthering the use of biomarkers in nutritional epidemiologic studies.

There are limitations of this study. A sample size of 153, although large by controlled feeding study standards, is rather small for biomarker identification and evaluation and there may be imprecision in the equation-based biomarkers proposed here. Furthermore, the limited distribution in race-ethnicity, BMI (i.e., absence of class III obesity), and educational level of the participating women reduces generalization to a broader population of postmenopausal women. The controlled feeding period was 2 wk. Although some biomarkers respond quickly to dietary perturbations, others (e.g., some PLFAs) take longer (50) or are influenced by endogenous metabolism (49); thus, correlations between dietary intake and some serum biomarkers may not adequately reflect the relation and contribute to less stable estimates. The women

were free-living, and the consumption of some nonstudy foods may not have been captured accurately. Last, some women experienced weight change during the study period, which is not unusual in controlled feeding studies (51) but did contribute to the model R^2 for α -tocopherol and percentage of energy from PUFAs (Table 3).

In summary, among 153 postmenopausal who consumed foods reflecting their habitual diets as part of a controlled feeding study, serum concentration biomarkers of nutrient intakes correlated well with the provided diet as consumed, performing as well as established recovery biomarkers of energy and protein. This is consistent with the larger body of evidence for these biomarkers derived from observational studies. The availability of biomarker data specific to the WHI sample afforded the opportunity to use these data to develop novel biomarker equations and determined that, under controlled dietary conditions, personal characteristics contributed modestly, but still usefully, to biomarker specifications for some nutrients. Valid objective biomarkers identified in the current feeding study will be used in future WHI studies to correct for systematic measurement error in self-reported nutrient intake when assessing diet and disease associations.

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REFERENCES

1. Zheng C, Beresford SA, Van Horn L, Tinker LF, Thomson CA, Neuhauser ML, Di C, Manson JE, Mossavar-Rahmani Y, Seguin R, et al. Simultaneous association of total energy consumption and activity-related energy expenditure with risks of cardiovascular disease, cancer, and diabetes among postmenopausal women. *Am J Epidemiol* 2014;180:526–35.
2. Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, Brenneman AT, Brown-Friday JO, Goldberg R, Venditti E, et al; Diabetes Prevention Program Research Group. 10-Year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet* 2009;374:1677–86.
3. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346:393–403.



4. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, Lee IM, Lichtenstein AH, Loria CM, Millen BE, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2014;63:2960–84.
5. Catsburg C, Miller AB, Rohan TE. Adherence to cancer prevention guidelines and risk of breast cancer. *Int J Cancer* 2014;135:2444–52.
6. Hastert TA, Beresford SA, Sheppard L, White E. Adherence to the WCRF/AICR cancer prevention recommendations and cancer-specific mortality: results from the Vitamins and Lifestyle (VITAL) Study. *Cancer Causes Control* 2014;25:541–52.
7. Thomson CA, McCullough ML, Wertheim BC, Chlebowski RT, Martinez ME, Stefanick ML, Rohan TE, Manson JE, Tindle HA, Ockene J, et al. Nutrition and physical activity cancer prevention guidelines, cancer risk, and mortality in the Women's Health Initiative. *Cancer Prev Res (Phila)* 2014;7:42–3.
8. Diabetes Prevention Program Research Group. The 10-year cost-effectiveness of lifestyle intervention or metformin for diabetes prevention: an intent-to-treat analysis of the DPP/DPPOS. *Diabetes Care* 2012;35:723–30.
9. Millen AE, Tooze JA, Subar AF, Kahle LL, Schatzkin A, Krebs-Smith SM. Differences between food group reports of low-energy reporters and non-low-energy reporters on a food frequency questionnaire. *J Am Diet Assoc* 2009;109:1194–203.
10. Scagliusi FB, Ferrioli E, Pfrimer K, Laureano C, Cunha CS, Gualano B, Lourenco BH, Lancha AH Jr. Characteristics of women who frequently under report their energy intake: a doubly labelled water study. *Eur J Clin Nutr* 2009;63:1192–9.
11. Karelis AD, Lavoie ME, Fontaine J, Messier V, Strychar I, Rabasa-Lhoret R, Doucet E. Anthropometric, metabolic, dietary and psychosocial profiles of underreporters of energy intake: a doubly labeled water study among overweight/obese postmenopausal women—a Montreal Ottawa New Emerging Team study. *Eur J Clin Nutr* 2010;64:68–74.
12. Tooze JA, Subar AF, Thompson FE, Troiano R, Schatzkin A, Kipnis V. Psychosocial predictors of energy underreporting in a large doubly labeled water study. *Am J Clin Nutr* 2004;79:795–804.
13. Tooze JA, Krebs-Smith SM, Troiano RP, Subar AF. The accuracy of the Goldberg method for classifying misreporters of energy intake on a food frequency questionnaire and 24-h recalls: comparison with doubly labeled water. *Eur J Clin Nutr* 2012;66:569–76.
14. Burrows TL, Martin RJ, Collins CE. A systematic review of the validity of dietary assessment methods in children when compared with the method of doubly labeled water. *J Am Diet Assoc* 2010;110:1501–10.
15. Trabulsi J, Schoeller DA. Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake. *Am J Physiol Endocrinol Metab* 2001;281:E891–9.
16. Freedman LS, Commins JM, Moler JE, Arab L, Baer DJ, Kipnis V, Midthune D, Moshfegh AJ, Neuhaus ML, Prentice RL, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for energy and protein intake. *Am J Epidemiol* 2014;180:172–88.
17. Neuhaus ML, Tinker L, Shaw PA, Schoeller D, Bingham SA, Horn LV, Beresford SA, Caan B, Thomson C, Satterfield S, et al. Use of recovery biomarkers to calibrate nutrient consumption self-reports in the Women's Health Initiative. *Am J Epidemiol* 2008;167:1247–59.
18. Prentice RL, Mossavar-Rahmani Y, Huang Y, Van Horn L, Beresford SA, Caan B, Tinker L, Schoeller D, Bingham S, Eaton CB, et al. Evaluation and comparison of food records, recalls, and frequencies for energy and protein assessment by using recovery biomarkers. *Am J Epidemiol* 2011;174:591–603.
19. Prentice RL, Shaw PA, Bingham SA, Beresford SA, Caan B, Neuhaus ML, Patterson RE, Stefanick ML, Satterfield S, Thomson CA, et al. Biomarker-calibrated energy and protein consumption and increased cancer risk among postmenopausal women. *Am J Epidemiol* 2009;169:977–89.
20. Prentice RL, Huang Y, Kuller LH, Tinker LF, Horn LV, Stefanick ML, Sarto G, Ockene J, Johnson KC. Biomarker-calibrated energy and protein consumption and cardiovascular disease risk among postmenopausal women. *Epidemiology* 2011;22:170–9.
21. Tinker LF, Sarto GE, Howard BV, Huang Y, Neuhaus ML, Mossavar-Rahmani Y, Beasley JM, Margolis KL, Eaton CB, Phillips LS, et al. Biomarker-calibrated dietary energy and protein intake associations with diabetes risk among postmenopausal women from the Women's Health Initiative. *Am J Clin Nutr* 2011;94:1600–6.
22. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am J Clin Nutr* 1999;70:475S–90S.
23. Lampe JW. Nutrition and cancer prevention: small-scale human studies for the 21st century. *Cancer Epidemiol Biomarkers Prev* 2004;13:1987–8.
24. Kirkman LM, Lampe JW, Campbell DR, Martini MC, Slavin JL. Urinary lignan and isoflavonoid excretion in men and women consuming vegetable and soy diets. *Nutr Cancer* 1995;24:1–12.
25. Tasevska N, Runswick SA, Welch AA, McTaggart A, Bingham SA. Urinary sugars biomarker relates better to extrinsic than to intrinsic sugars intake in a metabolic study with volunteers consuming their normal diet. *Eur J Clin Nutr* 2009;63:653–9.
26. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* 1990;51:241–7.
27. Women's Health Initiative. Home page [Internet]. [cited 2016 Apr 8]. Available from: <https://www.whi.org/about/SitePages/WHI%20Extension%202005-2010.aspx>.
28. Patterson RE, Levy L, Tinker LF, Kristal AR. Evaluation of a simplified vitamin supplement inventory developed for the Women's Health Initiative. *Public Health Nutr* 1999;2:273–6.
29. Anderson GL, Manson J, Wallace R, Lund B, Hall D, Davis S, Shumaker S, Wang CY, Stein E, Prentice RL. Implementation of the Women's Health Initiative study design. *Ann Epidemiol* 2003;13:S5–17.
30. Schoeller DA. Recent advances from application of doubly labeled water to measurement of human energy expenditure. *J Nutr* 1999;129:1765–8.
31. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* 1999;9:178–87.
32. Women's Health Initiative. Women's Health Initiative, Form35 – Personal Habits Update [Internet]. [cited 2016 Apr 8]. Available from: https://www.whi.org/researchers/studydoc/_layouts/15/WopiFrame.aspx?sourceid=/researchers/studydoc/WHI%20Forms/F035%20v1.2.pdf&action=default.
33. Staten LK, Taren DL, Howell WH, Tobar M, Poehlman ET, Hill A, Reid PM, Ritenbaugh C. Validation of the Arizona activity frequency questionnaire using doubly labeled water. *Med Sci Sports Exerc* 2001;33:1959–67.
34. Crowne DP, Marlowe D. A new scale of social desirability independent of psychopathology. *J Consult Psychol* 1960;24:349–54.
35. Stunkard AJ, Sorensen T, Schulsinger F. Use of the Danish adoption register for the study of obesity and thinness. *Res Publ Assoc Res Nerv Ment Dis* 1983;60:115–20.
36. de Lauzon B, Romon M, Deschamps V, Lafay L, Borys JM, Karlsson J, Ducimetiere P, Charles MA; Fleurbaix Laventie Ville Sante Study Group. The Three-Factor Eating Questionnaire-R18 is able to distinguish among different eating patterns in a general population. *J Nutr* 2004;134:2372–80.
37. Thomas DM, Martin CK, Redman LM, Heymsfield SB, Lettieri S, Levine JA, Bouchard C, Schoeller DA. Effect of dietary adherence on the body weight plateau: a mathematical model incorporating intermittent compliance with energy intake prescription. *Am J Clin Nutr* 2014;100:787–95.
38. Hach CC, Brayton SV, Kopelove AB. A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. *J Agric Food Chem* 1985;33:1117–23.
39. Bauer SR, Richman EL, Sosa E, Weinberg V, Song X, Witte JS, Carroll PR, Chan JM. Antioxidant and vitamin E transport genes and risk of high-grade prostate cancer and prostate cancer recurrence. *Prostate* 2013;73:1786–95.
40. Reinders I, Song X, Visser M, Eiriksdottir G, Gudnason V, Sigurdsson S, Aspelund T, Siggeirsdottir K, Brouwer IA, Harris TB, et al. Plasma phospholipid PUFAs are associated with greater muscle and knee extension strength but not with changes in muscle parameters in older adults. *J Nutr* 2015;145:105–12.
41. Schoeller DA, Hnilicka JM. Reliability of the doubly labeled water method for the measurement of total daily energy expenditure in free-living subjects. *J Nutr* 1996;126:348S–54S.
42. Bingham SA, Cummings JH. The use of creatinine output as a check on the completeness of 24-hour urine collections. *Hum Nutr Clin Nutr* 1985;39:343–53.

43. Novotny JA, Gebauer SK, Baer DJ. Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets. *Am J Clin Nutr* 2012;96:296–301.
44. Baer DJ, Gebauer SK, Novotny JA. Walnuts consumed by healthy adults provide less available energy than predicted by the Atwater factors. *J Nutr* 2016;146:9–13.
45. Freedman LS, Midthune D, Carroll RJ, Tasevska N, Schatzkin A, Mares J, Tinker L, Potischman N, Kipnis V. Using regression calibration equations that combine self-reported intake and biomarker measures to obtain unbiased estimates and more powerful tests of dietary associations. *Am J Epidemiol* 2011;174:1238–45.
46. Baker H, Handelman GJ, Short S, Machlin LJ, Bhagavan HN, Dratz EA, Frank O. Comparison of plasma alpha and gamma tocopherol levels following chronic oral administration of either all-rac-alpha-tocopheryl acetate or RRR-alpha-tocopheryl acetate in normal adult male subjects. *Am J Clin Nutr* 1986;43:382–7.
47. Ascherio A, Stampfer MJ, Colditz GA, Rimm EB, Litin L, Willett WC. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J Nutr* 1992;122:1792–801.
48. Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol* 1988;127:283–96.
49. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* 2008;47:348–80.
50. Hodson L, Eyles HC, McLachlan KJ, Bell ML, Green TJ, Skeaff CM. Plasma and erythrocyte fatty acids reflect intakes of saturated and n-6 PUFA within a similar time frame. *J Nutr* 2014;144:33–41.
51. Dennis BH, Kris-Etherton PM. Designing, managing, and conducting a clinical nutrition study. In: Monsen E, editor. *Research: successful approaches*. 2nd ed. Chicago: American Dietetic Association; 2003. p. 87–105.