

# Dietary long-chain fatty acids and carbohydrate biomarker evaluation in a controlled feeding study in participants from the Women's Health Initiative cohort<sup>1,2</sup>

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# ABSTRACT

**Background:** Biomarkers of macronutrient intake are lacking. Controlled human feeding studies that preserve the normal variation in nutrient and food consumption are necessary for the development and validation of robust nutritional biomarkers.

**Objective:** We aimed to assess the utility of serum phospholipid fatty acids (PLFAs) as biomarkers of dietary intakes of fatty acids, total fat, and carbohydrate.

**Design:** We used an individualized controlled feeding study in which 153 postmenopausal women from the Women's Health Initiative (WHI) were provided with a 2-wk controlled diet that mimicked each individual's habitual food intake. A total of 41 PLFAs were measured with the use of gas chromatography in end-of-feeding-period fasting serum samples and expressed in both relative and absolute concentrations.  $R^2$  values (percentages of variation explained) from linear regressions of (In-transformed) consumed fatty acids (individual, groups, and broad categories) on (In-transformed) corresponding measures of serum PLFAs alone and together with selected participant-related variables (age, race/ethnicity, body mass index, season of study participation, education level, and estimated energy intake from doubly labeled water) were used for evaluation against established urinary recovery biomarkers of energy and protein intake as benchmarks. Models to predict intakes of other nutrients were also explored.

**Results:** Intakes of eicosapentaenoic acid and docosahexaenoic acid achieved the benchmark of  $R^2 > 36\%$  with or without covariates. When all 41 serum PLFAs and participant-related covariates were initially included in the model for selection, cross-validated  $R^2$  achieved >36% for consumed total carbohydrate (grams per day), total saturated fatty acids (SFAs), percentage of energy from SFAs, and total *trans* fatty acids with serum PLFAs in both relative and absolute concentrations.

**Conclusions:** Serum PLFA biomarkers perform similarly to established energy and protein urinary recovery biomarkers in describing intake variations for several nutrients and, thus, appear suitable for application in this population of postmenopausal women. This approach represents an important methodologic contribution toward the utilization of nutritional biomarkers to assess macronutrient intake. This trial was registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2017;105:1272–82.

Keywords: carbohydrate, controlled feeding study, dietary biomarker, phospholipid fatty acids, total fat

# INTRODUCTION

Commonly used dietary assessment tools, food-frequency questionnaires, and dietary recalls are subject to substantial systematic and random measurement errors and recall bias (1-3). Therefore, studies that have relied on self-reported dietary intake data have had a limited ability to infer diet and disease associations, thereby leading to an inconsistency in the scientific literature about the association between nutrition and chronic disease risk. Objective biological measurements provide a critical approach to characterizing dietary exposures, and such biomarkers need to reflect the intake variation in the study population. Controlled feeding studies have long been used to evaluate the impact of the diet on biological and physiologic processes in humans (4, 5). Because such studies involve the consumption of known amounts of specific foods or nutrients, they also provide an opportunity to evaluate measures of various nutrients, metabolites, and other compounds in biological specimens as biomarkers of dietary exposure under controlled conditions (6, 7). However, controlled feeding studies usually provide the same standard diets to all study participants, thereby limiting the range of nutrient intakes and types and structures of foods. To overcome this limitation, we devised a novel feeding study in which we designed an individual menu for each female participant that mimicked her habitual diet as estimated with the

<sup>&</sup>lt;sup>1</sup> Supported by the National Cancer Institute (grant R01 CA119171). The Women's Health Initiative (WHI) is supported by the National Heart, Lung, and Blood Institute, NIH, US Department of Health and Human Services [contracts HHSN268201100046C (to the Fred Hutchinson Cancer Research Center), HHSN268201100001C (to State University of New York Buffalo), HHSN268201100002C (to The Ohio State University), HHSN268201100003C (to Stanford), HHSN268201100004C (to Wake Forest), and HHSN271201100004C (to the WHI Memory Study).

<sup>&</sup>lt;sup>2</sup> Supplemental Figures 1 and 2 and Supplemental Tables 1–4 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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Received January 18, 2017. Accepted for publication March 20, 2017. First published online April 26, 2017; doi: 10.3945/ajcn.117.153072.

use of a 4-d food record (4DFR),<sup>6</sup> which was further augmented through a discussion and documentation of food habits with a research dietitian. The goal was not to replicate precisely the diets of study participants but to approximate the diets so as to minimally perturb blood and urine measures by the end of the 2-wk controlled feeding period and to substantially preserve the normal variation in nutrient and food consumption in the study population while knowing the foods and quantity being consumed.

The composition of blood fatty acids changes in response to an alteration in dietary fatty acid intake and can potentially serve as a quantitative biomarker of dietary intake (8-11). Specifically, serum phospholipid fatty acids (PLFAs) reflect intake in the past days and weeks (9, 11). However, a biomarker of fat intake has been elusive. Most of the measured circulating fatty acids are not exclusively from dietary sources; they also represent de novo lipogenesis (fatty acid synthesis) and complex fatty acid metabolism. Dietary carbohydrate provides much of the substrate acetyl-CoA for the de novo lipogenesis pathway and has been shown to increase circulating concentrations of certain fatty acids (12). The goal of this article was to assess the utility of the serum PLFAs as biomarkers of intakes of dietary fatty acids, total fat, and carbohydrate in the context of a controlled feeding study with a range of amounts and types of macronutrient sources. Epidemiologic studies of circulating fatty acids have typically expressed each fatty acid as a relative concentration (i.e., as either the weight or molar percentage of total fatty acids analyzed). Although relative and absolute concentrations of most PLFAs are moderately to highly correlated (r > 0.6), for some PLFAs, such as palmitic acid (16:0), the correlation is low (13), and the use of relative compared with absolute concentrations may lead to different conclusions in association studies. Therefore, in the current study, we evaluated 41 serum PLFAs in both relative and absolute concentrations. We also examined whether an additional variation in intake could be explained by other participant-related variables [age, race/ethnicity, BMI (in  $kg/m^2$ ), season of study participation, education level, and estimated energy intake derived from doubly labeled water (Ein)] by entering these variables into the PLFA regression models for selection.

### METHODS

## **Participants**

The Nutrition and Physical Activity Assessment Study Feeding Study (NPAAS-FS) is a substudy of the Women's Health Initiative (WHI). The WHI, which was launched in 1993, is a long-term, national health study that is focused on strategies for the prevention of heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women (14). At the end of the initial study period in 2005, WHI Extension Studies (in 2005–2010 and 2010–2020) continued to follow up all women who consented. Women were selected to participate

in the NPAAS-FS on the basis of several selection criteria. Specifically, eligible women 1) were currently enrolled in the WHI Extension Study (15), 2) had previously participated in the Observational Study, Dietary Modification Trial comparison arm, or Hormone Trial (16), 3) had a deliverable US postal address, 4) had full follow-up status in the WHI, 5) had a zip code in King County, Washington, or surrounding counties, 6) were  $\leq 80$  y of age as of April 2011 (when the list of eligible women was compiled), and 7) had no medical conditions that would preclude the successful completion of the protocol (including, but not limited to, diabetes, known kidney disease, bladder incontinence that required the use of special garments or medications, or the routine use of oxygen). Of 450 Seattle-area WHI women who were approached, 174 women (39%) were eligible and consented to participate in the feeding study. Of these women, 21 participants withdrew (18 women withdrew before starting the feeding period), which left a total of 153 women who completed the protocol and were included in the analyses of the current study (Supplemental Figure 1). The NPAAS-FS was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board. All participants provided informed written consent before participating in the study. WHI trials are registered at clinicaltrials.gov as NCT0000611.

## Study procedures

The study design and diet formulation for the NPAAS-FS have been described in detail (17). Briefly, participants were asked to eat their usual diets while keeping a 4DFR. After a review of the 4DFR and a record of dietary supplement and medication use, the research dietitian conducted an in-depth interview to assess usual food choices and patterns that may not have been captured on the 4DFR. 4DFRs were analyzed with the use of Nutrition Data System for Research software (version 2010; Nutrition Coordinating Center, University of Minnesota). Energy needs were estimated with the use of the Mifflin-St Jeor equation (18) and data from previous WHI calibration equations (3, 19) that estimated energy needs according to a woman's BMI, race/ethnicity, and age. A weight-maintenance 4-d-rotation menu was individually designed for each participant to mimic her usual food intake on the basis of the 4DFR and food choices that were not captured in the 4DFR with the use of ProNutra software (version 3.4.0.0; Viocare). During their first consumption-intervention visit, participants provided a fasting blood specimen and a spot urine specimen before receiving a single oral dose of doubly labeled water (20). Other blood and urine specimens were collected according to the protocol throughout the study to measure urinary doubly labeled water and nitrogen and other potential biomarkers. Study meals over the 2-wk feeding period were prepared in the Fred Hutchinson Cancer Research Center Prevention Center Shared Resource Human Nutrition Laboratory. Participants returned to the Human Nutrition Laboratory 2-3 times/wk to consume the study meal on site and to pick up the remainder of their food to take home for the following days. A daily menu checklist, which was used to record the consumption of study and nonstudy foods and beverages, was collected during study visits. Uneaten study foods were returned and weighed

<sup>&</sup>lt;sup>6</sup>Abbreviations used: BIC, Bayesian information criterion; Ein, energy intake derived from doubly labeled water; NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study; PLFA, phospholipid fatty acid; WHI, Women's Health Initiative; 4DFR, 4-d food record.

and recorded. The final consumed diets (intake values for the current analyses) were analyzed for nutrient contents with the use of the Nutrition Data System for Research software.

## Serum PLFA measurements

Fasting serum samples at the end of the 2-wk feeding period were used for the current study. The serum PLFA assay was described in detail previously (13). Briefly, total lipids were extracted from serum with the use of the method of Folch et al. (21). The internal standard 1,2-dihenarachidoyl-sn-glycero-3phosphocholine (21:0 PC; Avanti Polar Lipids Inc.) was added to each serum sample before lipid extraction. Phospholipids were separated from other lipids with the use of one-dimensional thin-layer chromatography (22). Fatty acid methyl esters of the phospholipids were prepared via direct transesterification (23) and separated with the use of gas chromatography [Agilent 7890 Gas Chromatograph with flame-ionization detector (Agilent); Supelco fused-silica 100-m capillary column SP-2560 (Supelco)]. This gas chromatography method was used to quantify 41 known fatty acids for the study. The relative concentration of each fatty acid was expressed as a weighted percentage of the total PLFAs analyzed (i.e., the sum of the 41 PLFAs was 100%). The absolute concentration (expressed as  $\mu$ g/mL) of each fatty acid was calculated by comparing its peak area to that of the internal standard as follows

absolute concentration = 
$$\mu g/mL_{21:0}$$
  
  $\times$  (peak area count<sub>fatty acid of interest</sub>  
  $\div$  peak area count<sub>21:0</sub>) (I)

A laboratory quality-control sample (pooled plasma) was included with each batch of study samples. The interbatch CV for the laboratory quality-control sample was <12.7% (median: 2.6%) for all PLFAs except for the very minor fatty acid, 11,14,17-eicosatrienoic acid (20:3n–3; <0.1% by weight percentage), which had CVs of 27.1% and 29.0% for relative and absolute concentrations, respectively.

## Statistical analyses

Our analyses used In-transformed dietary intakes of fatty acids and selected broader categories of nutrients [total fat (grams per day), percentage of energy from fat, total carbohydrate, percentage of energy from carbohydrate, total starch, total sugar, total SFAs, percentage of energy from SFAs, total MUFAs, percentage of energy from MUFAs, total PUFAs, percentage of energy from PUFAs, total trans fatty acids, and percentage of energy from trans fatty acids (Supplemental Table 1)] and In-transformed measurements of PLFAs after 2 wk of consumption (in both relative and absolute concentrations). Dietary intake (mean of the 2-wk intake during the feeding period) was determined from the daily menu checklist and returned food plus self-reported intakes of dietary supplement and nonstudy foods, the latter of which was consumed by only a small fraction of participants. These transformed variables were each approximately normally distributed. Values that fell outside the 25th and 75th percentiles by >3 times the IQR (i.e., values greeter than the 75th percentile plus 3 times the IQR and values less than the 25th percentile minus 3 times the IQR) were excluded

as outliers (<2% for all variables). For each measured PLFA, the geometric mean and 95% confidence range of the sample were reported.

Pearson correlation coefficients were calculated to measure the association between the In-transformed mean daily fatty acid consumption over the 2-wk feeding period and the corresponding In-transformed potential biomarker (serum PLFAs) that were measured at the end of the feeding period. The squared Pearson correlation coefficient was equivalent to the  $R^2$  (the percentage of variation explained) from the linear regression of dietary fatty acids on measured serum PLFAs.  $R^2$  provided a major criterion for the biomarker evaluations with  $R^2$  values for the established WHI-NPAAS energy and protein biomarkers (17) (urinaryrecovery biomarkers of total Ein from doubly labeled water and total protein intake from 24-h urinary nitrogen) serving as benchmarks for comparison. Because the measurement properties of some candidate biomarkers may have depended on other participant-related covariates, linear regression analyses were extended by considering race/ethnicity and education level (collected at WHI enrollment), age, BMI, and season (collected at NPAAS-FS enrollment) as well as the mean Ein (17) over the 2-wk feeding period as potentially additional explanatory variables. All of these participant-related covariates except for Ein, which was In transformed, were on their original scale as presented in Table 1. Age, BMI, and In-transformed Ein were continuous variables, and race/ethnicity, education level, and season were categorical variables with Caucasian, college degree or higher, and fall season as reference groups, respectively. Each continuous covariate in the regression models was centered by its mean value as presented in Supplemental Table 2. A stepwise-selection procedure with the use of the Bayesian information criterion (BIC) (24) was applied to eliminate noncontributing covariates. The percentage of variation in the (ln) diet consumed that was explained  $(R^2)$  by the regression model was computed to characterize the potential of a biomarker specification that included biospecimen measures and other participant covariates. Comparisons of these  $R^2$  values that included participant covariates with  $R^2$  values that were based on the biospecimen measure alone were used evaluated the contributions of the covariates to the composite biomarker.

In addition, selected broader categories of nutrients [total fat (grams per day), percentage of energy from fat, total carbohydrate, percentage of energy from carbohydrate, total starch, total sugar, total SFAs, percentage of energy from SFAs, total MUFAs, percentage of energy from MUFAs, total PUFAs, percentage of energy from PUFAs, total trans fatty acids, and percentage of energy from trans fatty acids) were regressed on all 41 measured serum PLFAs and the participant covariates previously listed with the use of the BIC to build prediction models. Each continuous covariate in the regression models was centered by its mean value as presented in Supplemental Table 2.  $R^2$  values that were based on the selected model were computed from the data. In addition, random cross-validated  $R^2$  values were computed to minimize potential model overfitting that could have been caused by the high-dimensional predictors that entered into the model (25). To compute the cross-validated  $R^2$ , the data were randomly split into 5 equally sized groups with 4 groups for training and the remaining one group for testing. Linear regression models that were selected with the BIC were derived

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Baseline demographic and lifestyle characteristics of the 153 women who participated in the NPAAS- $\mathrm{FS}^1$ 

Variable	Value
Age, <sup>2</sup> y, n (%)	
60–69	10 (7.0)
70–79	127 (83.0)
80-85	16 (10.0)
Race/ethnicity, $n (\%)$	
Caucasian	146 (95.4)
Non-Caucasian <sup>4</sup>	7 (4.6)
Height, <sup>2</sup> cm	162 (157–166) <sup>5</sup>
Weight, <sup>2</sup> kg	69.0 (60.8-76.4)
BMI, <sup>2</sup> kg/m <sup>2</sup> , $n$ (%)	
Normal (<25.0)	61 (39.9)
Overweight (25.0–29.9)	60 (39.2)
Obese $(\geq 30)$	32 (20.9)
Season of study participation, <sup>2</sup> $n$ (%)	
Spring	38 (24.8)
Summer	51 (33.3)
Fall	31 (20.3)
Winter	33 (21.6)
Education, $n (\%)$	
High school/General Educational	10 (6.5)
Development diploma	
Schooling after high school	16 (10.5)
College degree or higher	126 (82.3)
Missing	1 (0.7)

<sup>1</sup>NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study.

<sup>2</sup>Measured at the time of enrollment in the NPAAS-FS.

<sup>3</sup> Collected at the time of enrollment in the Women's Health Initiative. <sup>4</sup> Included 3 African Americans, 2 Hispanics, 1 American Indian/Alaska

Native, and 1 Asian/Pacific Islander.

<sup>5</sup>Mean; IQR in parentheses (all such values).

from the training subset and applied to the test subset for the prediction of consumed nutrients on the basis of the covariates previously mentioned.  $R^2$  was computed in the test subset as the squared correlation between the actual consumed nutrient and its predicted value. The procedure was repeated 100 times with the mean  $R^2$  computed. All statistical analyses were performed with the use of R version 3.3.1 software (https://cran.r-project.org/).

# RESULTS

The 153 women who completed the feeding study were mostly aged 70–79 y (83.0%), well educated (82.3% had a college degree or higher), and Caucasian (95.4%) (Table 1). Many women were overweight [BMI  $\geq$ 25 but <30 (39.2%)] or obese [BMI  $\geq$ 30 (20.9%)]. Only 2% of participants were current smokers (data not shown); therefore, smoking status was not considered in the analysis.

Because we In transformed the PLFA data for statistical analyses, we present the geometric mean of the relative and absolute concentrations of the 41 PLFAs and the 95% confidence range of the sample (**Table 2**). The arithmetic mean  $\pm$  SD and correlations that were derived with the use of the original scales are presented in **Supplemental Table 3** to facilitate the comparison of the data to those in previous studies (13, 26). Correlations between the relative weight-percentage concentration and the absolute concentration were high for most PLFAs (>0.6

for 36 of 41 PLFAs). However, palmitic acid, stearic acid (18:0), arachidic acid (20:0), tricosylic acid (23:0) and vaccenic acid (18:1n–7 *cis*) had lower correlations between the 2 measurement metrics. Palmitic and stearic acid were 2 of the PLFAs that showed the most differential correlations with other PLFAs when measured in relative concentrations compared with in absolute concentrations (**Supplemental Figure 2**).

Correlations between fatty acids that were consumed and corresponding potential biomarker measures of serum PLFAs are presented in **Table 3**. The  $R^2$  on the basis of the biomarker alone (squared Pearson correlation) ranged from 0.2% for consumed cis MUFA 16:1 to 47.1% for PUFA 22:6 (DHA) when the relative concentration of PLFAs was used as a potential biomarker and ranged from <0.1% for PUFA 22:5 and the percentage of energy from PUFAs to 38.3% for PUFA 20:5 (EPA) when the absolute concentration of PLFAs was used. These linear regression models were further enriched by other important participant-related covariates that were selected with the use of the BIC (Table 3). EPA and DHA, in either relative or absolute concentrations, were the only fatty acids that achieved the benchmark of  $R^2 > 36\%$  with or without additional covariates; the incorporation of the additional covariates increased the  $R^2$ values from 44.1% to 46.5% and from 47.1% to 50.3% for EPA and DHA, respectively, with the use of their relative serum PLFA concentrations and from 38.3% to 41.2% and from 35.0% to 39.8%, respectively, with the use of their absolute serum PLFA concentrations (Table 3). The contributions of participantrelated variables to the estimated intake of fatty acids with an overall  $R^2 > 25\%$  are presented in **Table 4**. Because the outcome variable (nutrient intake) was ln transformed, the  $\beta$  coefficient was interpreted in terms of the fold change. With the use of the model of SFA myristic acid (14:0)-intake prediction that was based on the relative concentration of PLFA myristic acid (presented in Table 4) as an example, a 1-unit change in BMI corresponded to a 2% [i.e., exp(0.02) - 1] increase in geometric mean of SFA myristic acid intake; a 20% increase in the relative concentration of myristic acid corresponds to an 18% increase [i.e.,  $(1 + 0.2)^{0.89} - 1$ ] in the geometric mean of SFA myristic acid intake.

When all 41 PLFAs and the other covariates [age, race/ethnicity, BMI, season of study participation, education level, and mean Ein over the 2-wk feeding period (mean  $\pm$  SD: 1802  $\pm$  282 kcal/d; 95% confidence range: 1296, 2357 kcal/d)] were initially included in the model for selection with the use of the BIC, the crossvalidated  $R^2$  achieved  $\geq$ 36% for consumed total carbohydrate (grams per day), total SFA, the percentage of energy from SFA, and total *trans* fatty acids with potential PLFA biomarkers in both relative and absolute concentrations (**Table 5**). In addition, crossvalidated  $R^2$  achieved  $\geq$ 25% for the consumed percentage of energy from carbohydrate, total starch (grams per day), total sugar, percentage of energy from MUFAs, total PUFAs, percentage of energy from PUFAs, and percentage of energy from *trans* fatty acids (**Supplemental Table 4**).

# DISCUSSION

In the current study of 153 postmenopausal women who were enrolled in the WHI, PLFA biomarkers were examined for their associations with women's diets and to generate biomarker equations that included serum PLFA concentrations and

Relative and absolute concentrations of serum phospholipid fatty acids after 2 wk of controlled individualized feeding in the NPAAS-FS  $(n = 153)^1$ 

	P	LFA <sup>2</sup>		
	Weight percentage	Concentration, $\mu$ g/mL	Pearson correlation	Spearman correlation
ω-3 Fatty acids				
18:3n-3	0.19 (0.10, 0.36)	2.8 (1.33, 6.16)	0.9	0.9
20:3n-3	0.03 (0.02, 0.06)	0.52 (0.31, 0.94	0.85	0.85
20:5n-3	1.21 (0.48, 3.75)	18.22 (6.27, 59.48)	0.96	0.95
22:5n-3	0.92 (0.65, 1.29)	13.83 (8.75, 22.07)	0.74	0.73
22:6n-3	4.04 (2.06, 6.10)	60.72 (30.82, 99.70)	0.86	0.82
ω-6 Fatty acids				
18:2n-6	19.2 (13.44, 23.91)	288.59 (175.75, 420.14)	0.7	0.65
18:3n-6	0.07 (0.03, 0.17)	1.04 (0.38, 2.86)	0.96	0.97
20:2n-6	0.33 (0.22, 0.46)	5.01 (3.25, 7.86)	0.75	0.74
20:3n-6	2.62 (1.50, 4.18)	39.36 (19.64, 70.90)	0.89	0.88
20:4n-6	11.16 (7.43, 17.52)	167.76 (105.95, 293.85)	0.78	0.75
22:2n-6	0.09 (0.05, 0.19)	1.36 (0.76, 3.17)	0.91	0.91
22:4n-6	0.31 (0.17, 0.50)	4.67 (2.51, 8.75)	0.87	0.86
22:5n-6	0.22 (0.09, 0.42)	3.25 (1.33, 6.98)	0.94	0.93
SFAs				
14:0	0.26 (0.17, 0.44)	3.97 (1.95, 7.97)	0.89	0.89
15:0	0.17 (0.11, 0.25)	2.59 (1.67, 4.19)	0.76	0.75
16:0	25.82 (23.46, 28.12)	388.12 (281.78, 553.03)	0.45	0.41
17:0	0.42 (0.28, 0.55)	6.35 (4.34, 9.15)	0.62	0.61
18:0	13.74 (12.24, 15.38)	206.51 (145.79, 294.75)	0.36	0.35
20:0	0.57 (0.43, 0.69)	8.56 (6.02, 11.49)	0.48	0.47
22:0	1.63 (1.11, 2.24)	24.57 (15.44, 38.44)	0.69	0.67
23:0	0.82 (0.56, 1.08)	12.39 (8.21, 18.10)	0.59	0.55
24:0	1.45 (0.92, 1.98)	21.78 (13.36, 33.81)	0.73	0.72
cis MUFAs				
16:1n-9c	0.1 (0.07, 0.15)	1.56 (1.03, 2.65)	0.76	0.77
16:1n-7c	0.47 (0.29, 0.88)	7.02 (3.77, 13.92)	0.91	0.89
17:1n–9c	0.29 (0.17, 0.46)	4.43 (2.63, 7.43)	0.81	0.82
18:1n-8c	0.12 (0.06, 0.21)	1.85 (0.94, 3.49)	0.87	0.86
18:1n-9c	7.62 (5.93, 10.44)	114.52 (72.49, 182.35)	0.76	0.75
18:1n-7c	1.22 (0.94, 1.63)	18.37 (12.86, 26.65)	0.54	0.55
18:1n-5c	0.1 (0.04, 0.23)	1.49 (0.72, 3.83)	0.93	0.9
20:1n-9c	0.14 (0.10, 0.22)	2.08 (1.42, 3.34)	0.73	0.73
24:1n-9c	2.51 (1.74, 3.65)	37.51 (25.65, 54.85)	0.65	0.65
trans Fatty acids				
16:1n-9t	0.03 (0.02, 0.04)	0.44 (0.26, 0.72)	0.79	0.78
16:1n-7t	0.19 (0.11, 0.29)	2.83 (1.57, 4.89)	0.82	0.79
18:1n-10-12t	0.06 (0.03, 0.11)	0.84 (0.43, 1.80)	0.89	0.87
18:1n-9t	0.06 (0.03, 0.13)	0.93 (0.52, 2.07)	0.89	0.87
18:1n-8t	0.07 (0.03, 0.14)	0.98 (0.44, 2.19)	0.92	0.9
18:1n-7t	0.15 (0.08, 0.25)	2.22 (1.19, 4.10)	0.86	0.85
18:1n-6t	0.15 (0.08, 0.24)	2.24 (1.31, 4.42)	0.85	0.83
18:2n-6tt	0.05 (0.03, 0.07)	0.68 (0.43, 1.15)	0.78	0.76
18:2n-6ct	0.03 (0.02, 0.05)	0.49 (0.30, 0.78)	0.76	0.75
18:2n-6tc	0.03 (0.02, 0.05)	0.44 (0.24, 0.86)	0.89	0.88

<sup>1</sup>Correlations were determined between the ln-transformed weight-percentage relative concentration and the ln-transformed absolute concentration. NPAAS-FS, Nutrition and Physical Activity Assessment Study Feeding Study; PLFA, phospholipid fatty acid.

 $^{2}$  All values are geometric means; 95% confidence range of the sample (2.5% to 97.5% percentiles in the biomarker distribution).

participant-related covariates (age, race/ethnicity, BMI, season of study participation, education level, and estimated Ein from doubly labeled water). The equations for ln-transformed intakes of EPA, DHA, total carbohydrate (grams per day), total SFA, percentage of energy from SFA, and total *trans* fatty acids may be useful biomarkers for a further evaluation of diet-disease

associations in the WHI and possibly also in other similar populations of postmenopausal women.

This investigation corroborated previous observations (13) that correlations between the relative weight-percentage concentration and the absolute concentration were high for most PLFAs. The PLFAs with the lowest correlation between the 2 metrics

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				μ	FA <sup>1</sup>				
			Weight percenta	ge		μg/mL			
Consumed nutrient	Biomarker measure	Pearson correlation	Squared Pearson $\times 100$	$R^2$ of calibration equation, <sup>2</sup> %	Pearson correlation	Squared Pearson $\times 100$	$R^2$ of calibration equation, <sup>2</sup> %	$n^3$	$n^4$
SFA a/d									ĺ
14:0	14:0	0.48	22.9	27.3	0.42	17.5	28.3	152	147
16:0	16:0	0.14	1.9	21.3	0.18	3.2	22.0	147	147
17:0	17:0	0.13	1.8	8.8	0.23	5.2	12.2	148	148
18:0	18:0	0.05	0.3	10.1	0.20	4.0	13.8	147	147
20:0	20:0	0.16	2.6	2.6	0.24	5.7	5.7	150	152
22:0	22:0	0.49	24.1	24.1	0.45	19.8	19.8	152	152
cis MUFA, g/d									
16:1	16:1n-9c + 16:1n-7c	-0.04	0.2	5.7	-0.04	0.2	5.7	145	145
18:1	18:1n-9c + 18:1n-8c + 18:1n-7c + 18:1n-5c	0.15	2.2	10.0	0.15	2.2	10.0	147	147
18:1	18:1n-9c	0.18	3.2	11.2	0.18	3.3	10.5	147	147
20:1	20:1n-9c	0.17	3.0	8.3	0.16	2.6	7.0	147	147
PUFA, g/d									
18:2	18:2n–6	0.39	15.1	22.0	0.27	7.1	14.8	147	147
18:3	18:3n-3 + 18:3n-6	0.25	6.3	10.5	0.14	2.1	5.9	151	151
20:4	20:4n-6	0.18	3.1	3.1	0.26	6.5	6.5	152	152
20:5	20.5n-3	0.66	44.1	46.5	0.62	38.3	41.2	150	150
22:5	22:5n-3 + 22:5n-6	-0.08	0.6	10.0	0.01	<0.1	6.2	143	148
22:6	22:6n–3	0.69	47.1	50.3	0.59	35.0	39.8	147	152
trans, g/d									
18:1 1	18:1n-10-12t + 18:1n-9t + 18:1n-8t + 18:1n-7t + 18:1n-6t	0.47	21.7	21.7	0.50	25.3	28.1	153	153
18:2	18:2n-6tt + 18:2n-6ct + 18:2n-6tc	0.35	12.0	17.5	0.31	9.8	15.1	147	148
16:1	16:1n-9t + 16:1n-7t	0.25	6.3	11.0	0.28	8.0	13.4	153	153
SFA									
Total, g/d	SFA <sup>5</sup>	0.27	7.5	21.9	0.18	3.1	19.3	147	147
Percentage of energy	SFA	0.28	7.8	7.8	0.11	1.2	1.2	152	152
cis MUFA									
Total, g/d	cis MUFA <sup>6</sup>	0.14	2.0	1.11	0.17	2.8	11.3	147	147
Percentage of energy	cis MUFA	0.20	3.8	3.8	0.11	1.2	1.2	152	152
PUFA	L					1		ļ	ļ
Iotal, g/d	PUFA	0.30	12.9	8.62	0.07	C.U	10.3	14/	14/
Percentage of energy	PUFA	0.43	18.5	18.5	-0.01	<0.1	<0.1	152	152
Total, g/d	Total trans <sup>8</sup>	0.47	22.0	24.9	0.49	23.7	27.8	153	153
Percentage of energy	Total trans	0.47	22.1	22.1	0.47	21.6	21.6	152	152
<sup>1</sup> PLFA, phospholipid	l fatty acid.								
<sup>2</sup> Committee in the line	tion momentum and DI EA monoming that compensation to i	intelse nonticies	the second s	ated in Table 1 (eac -	aca/othnicity D	MIT according to the start of t	the burn of the second second		

isted in table 1 (age, race/ethnicity, BMI, season of study participation, and education level), Covariates in the linear regression are PLFA measures that corresponded to intake, participant character and mean daily energy intake over the 2-wk feeding period that was estimated from doubly labeled water.

<sup>3</sup>Number of participants for deriving the calibration equation on the basis of PLFAs in relative concentrations.

<sup>4</sup>Number of participants for deriving the calibration equation on the basis of PLFAs in absolute concentrations.

514:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 23:0 + 24:0.

 $^{6}16:1n-9c+16:1n-7c+17:1n-9c+18:1n-8c+18:1n-9c+18:1n-7c+18:1n-5c+20:1n-9c+24:1n-9c.$ 

 $\frac{7}{8}18:3n-3+20:3n-3+20:5n-3+22:5n-3+22:6n-3+18:2n-6+18:3n-6+20:2n-6+20:3n-6+20:3n-6+20:4n-6+22:2n-6+22:4n-6+22:5n-6, 8=18:1n-9t+18:1n-9t+18:1n-7t+18:1n-7t+18:2n-6t+18:1n-7t+18:2n-6t+28:2n-6t+18:2n-6t+28:2n$ 

Regression estimates and  $R^2$  regression values of ln-transformed fatty acid intake on corresponding ln-transformed serum phospholipid fatty acid measures and other study-participant variables that remained in the model on the basis of BIC<sup>1</sup>

	PLFA						
	Weight perce	ntage	µg/mL				
Variable	$\beta \pm SE$	$R^2, \%$	$\beta \pm SE$	$R^2, \%$			
SFA 14:0 intake, g/d							
Intercept	$0.89 \pm 0.03$		$0.89 \pm 0.03$	_			
Biomarker: 14:0	$0.89 \pm 0.13$	22.9	$0.56 \pm 0.10$	20.5			
BMI	$0.02 \pm 0.01$	4.4	$0.02 \pm 0.01$	4.7			
Ein	_	_	$0.58 \pm 0.24$	3.0			
Overall	_	27.3		28.3			
PUFA 20:5 intake, g/d							
Intercept	$-2.22 \pm 0.09$	_	$-2.22 \pm 0.10$				
Biomarker: 20:5n-3	$1.78 \pm 0.16$	44.1	$1.64 \pm 0.17$	38.3			
Age	$-0.07 \pm 0.03$	2.4	$-0.07 \pm 0.03$	3.0			
Overall	_	46.5	_	41.2			
PUFA 22:6 intake, g/d							
Intercept	$-1.79 \pm 0.07$		$-1.78 \pm 0.07$	_			
Biomarker: 22:6n-3	$2.61 \pm 0.23$	46.2	$2.09 \pm 0.23$	35			
Age	_		$-0.05 \pm 0.02$	1.8			
Race (non-Caucasian)	$-0.79 \pm 0.31$	2.3	$-0.94 \pm 0.34$	3.0			
Ein	$0.97 \pm 0.43$	1.8	_	_			
Overall	—	50.3	—	39.8			
Total PUFA intake, g/d							
Intercept	$2.79 \pm 0.02$	_	_	_			
Biomarker: total PUFA <sup>2</sup>	$4.15 \pm 0.75$	13.7	_				
Ein	$0.70 \pm 0.15$	12.1	_	_			
Overall	—	25.8	—				
18:1 trans intake, g/d							
Intercept	—	_	$0.22 \pm 0.04$				
Biomarker: total 18:1t <sup>3</sup>	—	_	$0.88 \pm 0.12$	25.3			
BMI	—	_	$0.02 \pm 0.01$	2.8			
Overall	—	_	—	28.1			
Total trans, g/d							
Intercept	—	_	$0.50 \pm 0.03$				
Biomarker: total trans <sup>4</sup>	_	—	$0.92 \pm 0.13$	23.7			
BMI	—		$0.02 \pm 0.01$	4.1			
Overall		_		27.8			

<sup>1</sup>Other study-participant variables in the linear regression were age, race/ethnicity, BMI, season of study participation, education level, and Ein (daily mean over the 2-wk feeding period estimated from doubly labeled water). Only overall  $R^2$  values >25% are shown. BIC, Bayesian information criterion; Ein, energy intake derived from doubly labeled water; PLFA, phospholipid fatty acid.

 $^2$ 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.  $^3$ 18:1n–10–12t + 18:1n–9t + 18:1n–8t + 18:1n–7t + 18:1n–6t.

 $^{4}$ Total 18:1t + 16:1n-9t + 16:1n-7t + 18:2n-6tt + 18:2n-6tt +

18:2n-6tc.

showed the most-differential correlation with other PLFAs. For example, in absolute concentrations, palmitic and stearic acid were highly correlated with a number of  $\omega$ -6 PLFAs [linoleic acid (18:2n–6),  $\gamma$ -linolenic acid (18:3n–6), eicosadienoic acid (20:2n–6), and dihomo- $\gamma$ -linolenic acid (20:3n–6)] (Supplemental Figure 2); however, in relative concentrations, palmitic and stearic acid were not correlated, or were weakly correlated, with these  $\omega$ -6 PLFAs. The biological relevance of this difference is unknown. In the nutrition science and biomedical literature, circulating fatty acids are mostly expressed as either molar- or weightpercentage relative concentrations. Certain journals require the use of molar percentages but not weight percentages. The current study (Supplemental Table 3) as well as 3 independent data sets that were used in a previous study (13) (data not shown) showed a perfect correlation between molar- and weight-percentage concentrations for all PLFAs investigated. Therefore, in association studies of circulating PLFAs with disease outcomes, results should be the same whether molar- or weight- percentage units are used.

We used the established urinary recovery biomarkers of energy (20, 27) and protein (28) as benchmarks to evaluate the serum PLFA biomarkers that we measured in this sample of postmenopausal women. The ln Ein and ln urinary nitrogen were correlated with consumed ln energy intake (r = 0.71, partial  $R^2 = 0.51$ ) and ln protein (r = 0.61, partial  $R^2 = 0.37$ ), respectively, in the feeding study as was previously shown in this sample of participants (17), thereby providing support for setting a benchmark of r = 0.6 ( $R^2 = 0.36$ ) for other nutrients that were evaluated in this context. Only intakes of EPA and DHA with their corresponding serum PLFA concentrations passed the threshold of r = 0.6. This result was not surprising because of the de novo lipogenesis and complex fatty acid metabolism with circulating concentrations that reflect the combined effects of intake, endogenous synthesis, and metabolism for most PLFAs. The conversion of EPA and DHA from  $\alpha$ -linolenic acid (18:3n-3), which is an essential fatty acid, is largely inefficient in humans (29), and circulating EPA and DHA concentrations follow a dose-response curve of intake (30). Therefore, EPA and DHA were the PLFAs that met the threshold of  $r \ge 0.6$  and most reflected dietary intake in our study with a partial  $R^2$  of 44.1% (EPA) and 47.1% (DHA) in relative PLFA concentrations and 38.3% and 35.0%, respectively, in absolute PLFA concentrations. Age, race/ethnicity, and Ein further explained <5% of the variation in ln EPA and DHA intakes. Our conclusion that serum phospholipid EPA and DHA are robust biomarkers of their intake strengthens the findings of previous studies (31). Previous cross-sectional studies (9, 32) of PLFAs as potential biomarkers of dietary fatty acid intake have used food-frequency questionnaires or food records to estimate intake; and dietary fatty acid variables were expressed as the percentage of total fat intake, percentage of total energy intake, g/kg body weight, g/d, or not specified, which makes a direct comparison of correlation coefficients difficult. However, except for EPA and DHA, correlations between dietary fatty acids and circulating PLFAs were weak or inconsistent in these studies.

We did not find suitable biomarkers for total fat intake or for the percentage of energy from fat. A previous study by King et al. (10) showed that PLFAs 18:1 *trans*, linoleic acid, vaccenic acid, and stearic acid almost perfectly distinguished the consumption of 34% of fat from that of 17% of fat. These PLFAs contributed little to the explanation for the variations of fat intake in our study (data not shown). The study by King et al. (10) was a traditional feeding trial in which all participants in each group consumed the same foods, whereas our study participants consumed their usual diets in a controlled manner with a continuum of fat content and different types of fat, which could be the reason that these markers were not confirmed in our study. Although biomarkers for total fat intake remain elusive, we have

Regression estimates and  $R^2$  regression values of ln-transformed nutrient intake on 41 ln-transformed serum phospholipid fatty acids and study-participant variables that remained in the model on the basis of the BIC<sup>1</sup>

		PLFA					
		Weight per	centage	_	μg/m	L	
Variable/biomarker	$\beta \pm SE$	<i>R</i> <sup>2</sup> , %	Cross-validated $R^2$ , %	$\beta \pm SE$	$R^2, \%$	Cross-validated $R^2$ , %	
Total carbohydrate intake, g/d							
Intercept	$5.37 \pm 0.01$	_		$5.37 \pm 0.01$		_	
22:6n-3	$0.18 \pm 0.06$	0.21	_	$0.20 \pm 0.06$	0.02	_	
14:0	$0.24 \pm 0.06$	15.48	_	$0.24 \pm 0.06$	6.75	_	
17:0	$0.49 \pm 0.12$	1.21	_	$0.57 \pm 0.12$	1.45	_	
17:1n–9c	$-0.13 \pm 0.06$	0.39	_	$-0.14 \pm 0.06$	0.69	_	
18:1n-8c	$-0.18 \pm 0.07$	0.34	_	$-0.17 \pm 0.07$	1.10	_	
18:1n-5c	$-0.22 \pm 0.06$	0.25	_	$-0.20 \pm 0.06$	0.59	_	
16:1n-7t	$-0.35 \pm 0.09$	6.67	_	$-0.31 \pm 0.09$	5.71	_	
18:1n-6t	$0.33 \pm 0.09$	5.65	_	$0.36 \pm 0.09$	6.59	_	
18:2n-6tc	$0.17 \pm 0.06$	1.57	_	$0.14 \pm 0.06$	1.11	_	
Race (non-Caucasian)	$-0.21 \pm 0.06$	3.61		$-0.23 \pm 0.06$	4.34	_	
Ein	$0.68 \pm 0.08$	18.38	_	$0.65 \pm 0.08$	16.16	_	
20:5n-3	$-0.14 \pm 0.03$	0.00		_		_	
22:5n-3	$0.23 \pm 0.08$	8.70		_		_	
18:1n-10-12t	$0.18 \pm 0.06$	5.14		_		_	
20:0	$0.24 \pm 0.11$	0.09		_	_	_	
16:0	_	_		$-0.87 \pm 0.14$	12.81	_	
20:4n-6	_	_	_	$-0.20 \pm 0.08$	2.04	_	
22:4n-6	_	_		$0.36 \pm 0.08$	6.98	_	
Overall	_	67.68	41.63	_	66.34	37.06	
Total SFA intake, g/d							
Intercept	$3.26 \pm 0.02$	_	_	$3.27 \pm 0.02$		_	
15:0	$0.55 \pm 0.14$	20.40		$0.49 \pm 0.13$	19.42	_	
16:0	$-1.67 \pm 0.55$	2.71	_	$-1.02 \pm 0.29$	0.63	_	
17:0	$-1.04 \pm 0.20$	5.45		$-0.86 \pm 0.19$	7.71	_	
16:1n-9c	$-0.37 \pm 0.11$	11.59	_	$-0.25 \pm 0.10$	6.36	_	
16:1n-7c	$-0.20 \pm 0.08$	0.51		$-0.20 \pm 0.09$	0.07	_	
18:1n-9c	$0.35 \pm 0.15$	0.88		$0.47 \pm 0.16$	1.43	_	
16:1n-7t	$0.66 \pm 0.12$	8.15		$0.60 \pm 0.12$	7.77	_	
18:1n-9t	$-0.25 \pm 0.07$	1.45		$-0.20 \pm 0.07$	0.18		
18:2n-6tc	$0.23 \pm 0.08$	3.55		$0.25 \pm 0.08$	3.30	_	
BMI	$0.01 \pm 0.00$	4.75		$0.01 \pm 0.00$	4 41		
High school	$-0.12 \pm 0.06$	2.56		$-0.13 \pm 0.06$	2.57		
Race (non-Caucasian)	$-0.17 \pm 0.07$	1.45		$-0.17 \pm 0.07$	1.16		
Ein	$0.46 \pm 0.11$	4.35		$0.43 \pm 0.11$	3.88	_	
24:1n-9	$-0.21 \pm 0.10$	0.77		_			
20:5n-3				$0.13 \pm 0.05$	0.52		
18·2n-6				$0.37 \pm 0.14$	1.76		
22:5n-6	_	_		$0.17 \pm 0.06$	5.78		
20:0				$0.22 \pm 0.13$	1.60		
Overall	_	68.57	39.82		68.55	39.87	
% Energy from SFAs							
Intercept	$2.50 \pm 0.01$	_		$2.50 \pm 0.01$	_	_	
15:0	$0.34 \pm 0.11$	22.08	_	$0.33 \pm 0.11$	22.52	_	
16:0	$-1.13 \pm 0.44$	3.03		$-1.12 \pm 0.26$	0.37	_	
17:0	$-0.83 \pm 0.14$	1.65		$-0.82 \pm 0.12$	2.75	_	
22:0	$0.71 \pm 0.19$	0.06	_	$0.80 \pm 0.19$	0.10	_	
24.0	$-0.65 \pm 0.17$	2.59		$-0.74 \pm 0.17$	3.32	_	
16:1n-9c	$-0.46 \pm 0.08$	16.25		$-0.39 \pm 0.08$	12.46	_	
18·1n-8c	$0.17 \pm 0.06$	3 39	_	$0.20 \pm 0.06$	3 91	_	
18:1n-9c	$0.46 \pm 0.11$	4.98	_	$0.72 \pm 0.03$	7.08	_	
16:1n-7t	$0.62 \pm 0.10$	7 88	_	$0.61 \pm 0.12$	8.59	_	
18·1n-10-12t	$-0.19 \pm 0.06$	3.28	_	$-0.22 \pm 0.06$	3.69	_	
High school	$-0.04 \pm 0.05$	2 78	_	-0.04 + 0.05	2 39	_	
24·1n_9	-0.24 + 0.07	0.90	_			_	
= <i>y</i>	$0.27 \pm 0.07$	0.70					

(Continued)

# **TABLE 5** (Continued)

			PL	FA		
	Weight percentage			μg/mL		
Variable/biomarker	$\beta \pm SE$	$R^2, \%$	Cross-validated $R^2$ , %	$\beta \pm SE$	$R^2, \%$	Cross-validated $R^2$ , %
20:4n-6		_	_	$0.36 \pm 0.09$	0.01	_
20:5n-3	_	_		$0.11 \pm 0.03$	0.07	_
18:2n–6	_	_		$0.38 \pm 0.12$	0.11	_
20:3n-6	_	_		$0.12 \pm 0.05$	2.62	_
18:1n–7c	_	_		$-0.29 \pm 0.10$	1.24	_
Overall	_	68.88	45.19	_	71.24	47.23
Total trans fatty acid intake, g/d						
Intercept	$0.50 \pm 0.03$	_	_	$0.50 \pm 0.03$		—
22:2n-6	$0.13 \pm 0.08$	0.00		$0.16 \pm 0.08$	0.13	_
18:1n-7c	$-1.18 \pm 0.22$	5.38		$-1.05 \pm 0.17$	1.08	_
16:1n-7t	$0.49 \pm 0.19$	1.58	_	$0.15 \pm 0.12$	16.72	—
18:2n-6tc	$0.64 \pm 0.14$	7.38		$0.63 \pm 0.10$	13.43	_
18:1n-5c	$-0.14 \pm 0.14$	17.15	_	—		—
15:0	$-0.56 \pm 0.22$	8.08		_	_	_
16:1n-7c	$0.34 \pm 0.12$	6.04		_	_	_
18:1n–6t	$0.67 \pm 0.19$	4.85		_	_	_
24:0	_	_		$-0.30 \pm 0.13$	0.71	_
18:1n-10-12t	_	_	_	$0.46 \pm 0.11$	18.00	_
Overall		50.48	37.46	_	50.07	36.44

<sup>1</sup> Study-participant variables in the linear regression were age, race/ethnicity, BMI, season of study participation, education level, and Ein (daily mean over the 2-wk feeding period estimated from doubly labeled water). Only cross-validated  $R^2$  values >36% are shown. BIC, Bayesian information criterion; Ein, energy intake derived from doubly labeled water; PLFA, phospholipid fatty acid.

identified panels of PLFAs (in either relative or absolute concentrations) together with participant-related covariates as potential biomarkers for intakes of total SFA, the percentage of energy from SFA, and total *trans* fatty acids.

Dietary carbohydrate provides much of the substrate acetyl-CoA that is used in de novo lipogenesis. Previous studies have shown that de novo lipogenesis is increased by eucaloric low-fat, high-carbohydrate diets (33, 34). Our regression model with PLFAs and participant-related covariates explained 41.6% and 37.1% (cross-validated  $R^2$ ) of the variation in total carbohydrate intake with the use of relative and absolute PLFA concentrations, respectively. A previous study by Volk et al. (12) showed that increasing dietary carbohydrate across a range of intakes promoted an increase in palmitoleic acid (16:1n-7 cis) in plasma triglycerides and cholesteryl esters. In our study, the serum phospholipid palmitoleic acid explained <5% of the variation in total carbohydrate intake (grams per day) and did not remain in the model on the basis of the statistical criteria. Of the PLFAs that remained in the regression model were 14:0 (relative concentration of PLFA) and 16:0 (absolute concentration of PLFA), both of which are main products of de novo lipogenesis, explaining 15.5% and 12.8%, respectively, of the variation in total carbohydrate intake. The addition of physical activity, either with the use of an objective measure of activity-related energy expenditure (35) or a self-reported measure of leisure physical activity, as an independent variable did not materially alter the carbohydrate model or any other models (data not shown).

The NPAAS-FS is a unique feeding study in which participants consumed foods that were designed to be similar to their habitual diets instead of providing the same study diets to all participants.

The variety of food sources of nutrients and the range of macronutrient composition provide a robust estimate of the utility of these serum PLFA biomarkers under controlled conditions. Our study also had several limitations. A sample size of n = 153, although large by the standards of a controlled feeding study, may have been small for biomarker identification and evaluation. The controlled feeding period of 2 wk may not have been long enough for some biomarkers to reach equilibrium. However, we tried to mimic the usual diet of each individual participant, which should have minimized the changes in the PLFA profile. Only 8 of 41 PLFAs had a baseline and end-of-feedingperiod correlation coefficient <0.6 (17). These 8 PLFAs are of very low abundance (<0.1% in relative concentrations) and, therefore, are more challenging to measure, which also could have partly explained the lower correlation between the 2 time points. Participants were free-living, and the consumption of some nonstudy foods may not have been captured accurately despite the study protocol requirement to do so. The dietary database incompleteness of fatty acid data for some foods affected the reliability of the estimated consumption. A limited distribution in race/ethnicity, BMI, and education level of participating women limited the generalization of the results to a broader population of postmenopausal women. Few studies, to our knowledge, have Ein data available and, therefore, can apply our total carbohydrate intake-prediction model to which Ein contributed substantially. Last, genotype data of participants were not available. Previous studies have shown that the genotypes of key fatty acid metabolism genes are associated with circulating PLFA concentrations (36-41). The inclusion of genotype data may have improved the calibration equations but would also have required much-larger sample sizes.

In conclusion, our study approach represents an important methodologic contribution toward the utilization of nutritional biomarkers to assess macronutrient intake. The NPAAS-FS is a valuable resource for furthering the use of biomarkers in nutritional epidemiologic studies. Biomarkers that meet the established threshold criteria will be applied to stored specimens in WHI cohorts to generate calibration equations for self-reported nutrient intake for future diet-and-disease association analyses in postmenopausal women.

We thank Xiaojun Hu and Pamela Yang for their technical assistance with laboratory assays and the following short list of the WHI investigator groupprogram office (National Heart, Lung, and Blood Institute, Bethesda, Maryland): Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller; clinical coordinating center (Fred Hutchinson Cancer Research Center, Seattle, Washington): Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg; investigators and academic centers: JoAnn E Manson (Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts), Barbara V Howard (MedStar Health Research Institute/ Howard University, Washington, District of Columbia), Marcia L Stefanick (Stanford Prevention Research Center, Stanford, California), Rebecca Jackson(The Ohio State University, Columbus, Ohio), Cynthia A Thomson (University of Arizona, Tucson/Phoenix, Arizona); Jean Wactawski-Wende (University at Buffalo, Buffalo, New York), Marian Limacher (University of Florida, Gainesville/Jacksonville, Florida), Jennifer Robinson (University of Iowa, Iowa City/Davenport, Iowa), Lewis Kuller (University of Pittsburgh, Pittsburgh, Pennsylvania), Sally Shumaker (Wake Forest University School of Medicine, Winston-Salem, North Carolina), Robert Brunner (University of Nevada, Reno, Nevada), and Karen L Margolis (University of Minnesota, Minneapolis, Minnesota); and WHI Memory Study: Mark Espeland (Wake Forest University School of Medicine, Winston-Salem, North Carolina). A list of all investigators who have contributed to WHI science is available from: https://www.whi.org/researchers/Documents%20%20Write%20a %20Paper/WHI%20Investigator%20Long%20List.pdf.

The authors' responsibilities were as follows—JWL, MLN, LFT, and RLP: designed the research; XS: analyzed the specimens and data and had primary responsibility for the final content of the manuscript; XS and JWL: wrote the manuscript; YH: performed the statistical analyses; YH, RLP, MLN, LFT, and MZV: provided critical edits to and reviewed the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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