Biomarker-calibrated dietary energy and protein intake associations with diabetes risk among postmenopausal women from the Women's Health Initiative[1–4]

Lesley F Tinker, Gloria E Sarto, Barbara V Howard, Ying Huang, Marian L Neuhouser, Yasmin Mossavar-Rahmani, Jeannette M Beasley, Karen L Margolis, Charles B Eaton, Lawrence S Phillips, and Ross L Prentice

ABSTRACT

Background: Self-report of dietary energy and protein intakes has been shown to be systematically and differentially underreported.

Objective: We assessed and compared the association of diabetes among postmenopausal women with biomarker-calibrated and uncalibrated dietary energy and protein intakes from food-frequency questionnaires (FFQs).

Design: The analyses were performed for 74,155 participants of various race-ethnicities from the Women's Health Initiative. Uncalibrated and calibrated energy and protein intakes from FFQs were assessed for associations with incident diabetes by using HR estimates based on Cox regression.

Results: A 20% increment in uncalibrated energy consumption was associated with increased diabetes risk (HR) of 1.03 (95% CI: 1.01, 1.05), 2.41 (95% CI: 2.06, 2.82) with biomarker calibration, and 1.30 (95% CI: 0.96, 1.76) after adjustment for BMI. A 20% increment in uncalibrated protein (g/d) resulted in an HR of 1.05 (95% CI: 1.03, 1.07), 1.82 (95% CI: 1.56, 2.12) with calibration, and 1.16 (95% CI: 1.05, 1.28) with adjustment for BMI. A 20% increment in uncalibrated protein density (% of energy from protein) resulted in an HR of 1.13 (95% CI: 1.09, 1.17), 1.01 (95% CI: 0.75, 1.37) with calibration, and 1.19 (95% CI: 1.07, 1.32) with adjustment for BMI.

Conclusions: Higher protein and total energy intakes (calibrated) appear to be associated with a substantially increased diabetes risk that may be mediated by an increase in body mass over time. Diet-disease associations without correction of self-reported measurement error should be viewed with caution. This trial is registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2011;94: 1600–6.

INTRODUCTION

To improve diabetes-prevention strategies, a better understanding of nutrition-related risk is needed. For example, despite the role of energy balance in weight management and the role of weight management in preventing type 2 diabetes, prospective studies have not shown energy intake to be associated with the incidence of diabetes (1–3). NBS[5] (4) and the OPEN Study (5) have shown a comparatively larger underreporting of dietary energy intake by women who are overweight than by nonoverweight women. In the WHI-NBS, a modest additional underreporting of energy intake was found among racial and ethnic minorities compared with white participants. Because overweight and minority race and ethnicity are risk factors for diabetes, measurement error from self-reports of energy intake may impede the ability to investigate these factors in diabetes incidence.

In this study, we evaluated the association of diabetes with biomarker-calibrated energy and protein intake among participants from the WHI DM-C group and the WHI OS and compared these associations with those for uncalibrated FFQ measures. Because the WHI-NBS also found protein intake to be underreported (4), we further examined the effect of biomarker-calibrated estimates of protein consumption on diabetes incidence. We hypothesized that uncalibrated estimates of energy and protein intakes may lead to distorted estimates of energy intake association with diabetes risk among postmenopausal women.

SUBJECTS AND METHODS

The WHI Dietary Modification Trial and Observational Study

The design and baseline descriptions of the WHI studies have been published (6–8). Briefly, 48,835 and 93,676 generally

[1] From the Fred Hutchinson Cancer Research Center, Seattle, WA (LFT, RLP, YH, MLN, and JMB); University of Wisconsin, Madison, WI (GES); Medstar Research Institute and Georgetown University, Washington, DC (BVH); Albert Einstein College of Medicine, Bronx, NY (YMR); HealthPartners Research Foundation, Minneapolis, MN (KLM); Memorial Hospital of Rhode Island, Pawtucket, RI (CBE); and Emory University, Atlanta, GA (LSP).

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[4] Address correspondence to LF Tinker, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, PO Box 19024–Mailstop M3-A410, 1100 Fairview Avenue North, Seattle, WA 98109-1024. E-mail: ltinker@whi.org.

[5] Abbreviations used: DM, Dietary Modification; DM-C, Dietary Modification Comparison; FFQ, food-frequency questionnaire; NBS, Nutritional Biomarkers Study; OPEN, Observing Protein and Energy Nutrition; OS, observational study; WHI, Women's Health Initiative.

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healthy postmenopausal women aged 50–79 y were randomly assigned to the DM trial or the OS at 40 clinical centers across the United States between 1993 and 1998. The DM trial investigated a low-fat dietary pattern with increased vegetables, fruit, and grains on the incidence of breast and colorectal cancers and heart disease over an average of 8.1 follow-up years. Results have been published for the principal trial outcomes for breast (9) and colorectal cancers (10), for other cancers (11, 12), and for the secondary outcomes of cardiovascular disease (13) and diabetes (14). The OS offered opportunities for investigating a broad range of epidemiologic research questions.

Dietary intake for the WHI was monitored primarily by an FFQ designed for the WHI (15). The FFQ was administered to all DM trial participants during screening (baseline), 1 y after randomization, and thereafter annually to one-third of the participants on a rotating basis. The FFQ was administered to OS participants during screening (baseline) and during the third year after enrollment.

Diabetes in the WHI at baseline was documented by self-report during prerandomization screening by asking each woman if she had ever been told by a physician that she had "sugar diabetes" when not pregnant. Incident diabetes during follow-up was documented by self-report at each semiannual contact when participants were asked, "Since the date given on the front of this form, has a doctor prescribed any of the following pills or treatments?" Choices included "pills for diabetes" and "insulin shots for diabetes." Data from a WHI diabetes confirmation study showed that prevalent and incident diabetes was consistent with medication inventories of oral agents or insulin (16).

The WHI-NBS

With the aim of facilitating the diet-disease association assessment, the WHI-NBS substudy compared self-reported intakes of energy and protein assessed by FFQs completed during the WHI-NBS with recovery biomarkers of energy (doubly labeled water as deuterium and oxygen-18) and protein (total urinary nitrogen excretion). The WHI-NBS recruited 544 weight-stable postmenopausal women from the DM trial comparison and intervention groups with 20% of WHI-NBS participants repeating the protocol 6 mo later as a repeat reliability substudy. Women were excluded from the WHI-NBS for having any medical conditions precluding participation, such as diabetes (because of concerns of meal conflicts) or history of colonoscopy (because of receiving intravenous fluids), weight instability, or plans to travel during the study period. Self-reported energy (kcal/d) was underreported by 33%, protein (g/d) was underreported by 15%, and protein density (% of energy from protein) was overreported by 25%. Energy underreporting was greater as BMI increased ($P = 0.001$) and with race-ethnicity (global $P = 0.0009$), with greater underreporting among black and Hispanic women than in white women (4).

The associated biomarker measurement error plausibly adheres to a simple classic measurement model (4):

$$W = Z + e \qquad (1)$$

where Z is the targeted (log-transformed) nutrient consumption, W is the (log-transformed) biomarker measured consumption, and e is measurement error that is assumed to be independent of Z and of all other study subject characteristics. The measurement model for the self-report data typically needs to be more complex than the classic measurement model (Equation *1*): other factors such as body mass, race-ethnicity, and age may affect the assessment, and measurement errors may be correlated if the assessment is repeated for specific study subjects. Thus, we considered a measurement model (4, 17):

$$Q = S_o + S_1 Z + S_2 V + S_3 V Z + r + u \qquad (2)$$

for the (log-transformed) self-report nutrient assessment Q, where V is a set of characteristics listed above that may relate to systematic bias in the assessment, r is a person-specific error variable that will be present in each self-report assessment for a study subject, and u is an independent measurement error term. Also, S_0, S_1, S_2, and S_3 are constants to be estimated and all variables on the right sides of Equations *1* and *2* are assumed to be independent, given V.

Calibration equations for use in disease-risk association studies were developed by using linear regression models that predicted true intakes of energy and protein given the self-reported intakes and data on study subject characteristics (4). In weight-stable persons, urinary recovery of metabolites produced when energy and protein are expended leads to objective estimates of energy and protein consumption. Backward selection ($P = 0.10$) served to identify model covariates from a fuller list. Retained covariates for energy included BMI, age, race-ethnicity, income, and physical activity as metabolic equivalents per week; for protein, the retained covariates were BMI, age, race-ethnicity, income, education, and an interaction term for FFQ × BMI; and for percentage of energy from protein, the retained covariates were BMI, age, and current smoking status.

Uncalibrated and biomarker-calibrated nutrient estimates

FFQ self-reported intakes from DM-C year 1 and OS year 3 for energy, protein, and protein density served as the uncalibrated baseline nutrient consumption estimates for the analyses herein. These, rather than the baseline FFQs, were used to avoid distorted estimates due to the DM trial exclusionary criterion of consuming a low-fat diet as assessed by baseline FFQ <32% energy from fat (7). For the calibrated estimates, logs of nutrient consumption were obtained directly from the biomarker measurements for the 276 DM-C women included in the WHI-NBS. For women not in the WHI-NBS, the WHI-NBS calibration equations (4) were applied.

Analytic data set

We excluded from analysis participants with prevalent diabetes, ie, those who reported diabetes at enrollment or during the first year of follow-up for the DM-C ($n = 1380$) or the first 3 years for the OS ($n = 4064$) to correspond with the FFQ analysis time points. To align the participant characteristics of the DM-C and OS for these analyses, the following DM trial exclusionary criteria were applied to the OS: breast or colorectal cancer or other cancer (except nonmelanoma skin cancer) within 10 y preceding enrollment ($n = 8677$), stroke or acute myocardial infarction 6 mo before enrollment ($n = 271$), BMI <18 ($n = 678$), hypertension (>200/>105 mm Hg) ($n = 244$), FFQ

reported daily energy intake of <600 kcal or >5000 kcal) (n = 3571), ≥10 meals prepared away from home per week (n = 3598), special low-fiber diet (n = 568), special diet due to malabsorption (n = 514), unintentional weight loss of >15 lb (6.8 kg) in the 6 mo preceding baseline (n = 594), and self-report of diabetes diagnosed at age ≤21 y at baseline (n = 95). After the above exclusion criteria were applied and the participants with complete data were selected, the analytic data set included 19,111 DM-C and 55,044 OS participants.

The WHI and NBS protocol and consent forms were approved by the Institutional Review Board for each participating institution and the Clinical Coordinating Center (Fred Hutchinson Cancer Research Center, Seattle, WA).

Statistical analysis

The association of uncalibrated and calibrated dietary energy, protein, and protein density with incident diabetes was assessed by HR estimates based on Cox regression (18), with all analyses computed by using Statistical Software R version 2.11.1 (http://cran.r-project.org/) (19). Follow-up times started with the DM-C at year 1 or the OS at year 3 and continued to the earliest of treated diabetes, death, loss to follow-up, or 31 March 31 2005, when the WHI intervention ended. The Cox model was stratified on age (year 1 for DM-C, year 3 for OS) in 5-y categories, on hormone therapy trial participation for those in the DM-C also participating in the hormone trials (active estrogen, estrogen placebo, active estrogen plus progestin, estrogen plus progestin placebo, and not randomized), and for DM or OS cohort membership. To control for confounding factors, the diabetes risk models were additionally adjusted by standard risk factors, including physical activity in units of metabolic equivalent tasks per week, race-ethnicity, education, income, history of cardiovascular disease, smoking status, alcohol consumption, hypertension, family history of diabetes, hormone use, glycemic index, and glycemic load.

Because the effects of dietary intake on diabetes incidence may be substantially mediated by body fat accumulation over many years, it is important, for assessing the full dietary exposure association to include analyses that do not include BMI among the control variables in the diabetes risk model. However, exclusion of BMI may lead to undercontrol because of the effect of body mass on energy expenditure and hence energy intake needs. Hence, the HRs of the calibrated estimates were computed without and with the inclusion of BMI in the diabetes risk models.

The challenge of an over- or undercontrolled model relative to dietary effects and BMI exists with or without biomarker calibration. With calibration, however, there is the possibility of some bias when the disease risk model excludes BMI and the calibration equations include BMI. To examine this issue, we first applied an HR analysis that includes both the dietary consumption under study as well as BMI and then formed a linear combination of the respective HR coefficients to assess the dietary association without control for BMI (20). The linear combination involves the correlation between measurement errors between the initial and repeat biomarker assessments in the WHI-NBS reliability subsample about 6 mo apart, relative to the long-term consumption average that may be associated with diabetes risk. This correlation cannot be estimated directly based on assessments 6 mo apart, so sensitivity analyses were conducted. For example, we considered reliability subsample measurement error correlations of 0.0 and 0.2 for log-transformed protein and protein density and for (log-transformed) energy, which involves lesser day-to-day variation compared with protein or protein density. For energy, we also considered a correlation of 0.5. *See* Prentice and Huang (20) for further discussion of these correlations and for a detailed description of the related HR estimation procedure.

The log-HR was modeled linearly on log-nutrient consumption (uncalibrated and calibrated), which resulted in an HR for a fractional increase in the nutrient that is independent of intake. For demonstration, HRs are presented for a 20% increment in dietary intakes. On the basis of mean intakes, these 20% increments correspond to ~283 kcal, 12 g protein, and 3.4% of energy from protein (uncalibrated) and 415 kcal, 15 g protein, and 2.9% of energy from protein (calibrated).

For uncalibrated nutrient consumption, the SEs of the log-HR estimates were estimated on the basis of standard Cox procedure. For the calibrated SE estimates, where sampling variation in the calibration coefficient estimates needs to be taken into account, a bootstrap procedure (500 bootstrap samples) was applied with bootstrap sampling stratified on cohort (DM-C compared with OS), participation in the WHI-NBS, and membership in the WHI-NBS reliability subset. Bootstrapping allows computation of estimated SEs, CIs, and hypothesis testing under circumstances of estimate uncertainty, as with calibration equation coefficient estimates (17), and may confer less bias than the split-sample technique, especially with relatively small sample sizes (21). The 95% CIs for uncalibrated or calibrated HRs were calculated as the exponential of log-estimated HR ± 1.96 SE estimate. Two-sided P values, based on a Wald test assuming normality of log-HR estimate, were reported throughout this manuscript (with model-based SE estimate for uncalibrated analysis and bootstrap-based SE estimate for calibrated analysis). The 95% CIs including ranges ≥1.00 or <1.00 correspond to $P \leq 0.05$. The 95% CIs crossing the null value of 1.00 correspond to $P > 0.05$. The equality of HRs between the DM-C and OS was tested with a bootstrap variance estimate.

RESULTS

Incident treated diabetes was reported in 3319 participants (4.5%) within the analytic cohort. The tests of equality of the HRs between the DM-C and OS were not statistically different ($P \geq 0.05$; data not shown), which indicated the acceptability of combining HR estimates from the DM-C and OS, as is done in the sequel. The P value was based on the difference between log-HRs from the DM-C and OS cohorts, with bootstrap estimate of SD for the difference between the calibrated log-HRs. The data were consistent with the modeling assumption that log HR was a linear function of the log-dietary factor.

The baseline characteristics of the analytic cohort (**Table 1**) were similar to those of the full cohorts (6, 7), except that the participants were slightly older in the analytic cohort because year 1 and year 3 data were used as baseline.

Compared with biomarker-calibrated measures, the uncalibrated (self-reported) measure of energy intake was considerably lower, protein was slightly lower, and protein density was higher (**Table 2**). The decrease in protein density on calibration reflects the greater underreporting of energy intake and lesser underreporting of protein intake. HRs based on uncalibrated consumption were significantly, but only weakly,

TABLE 1
Participant characteristics for the Women's Health Initiative analytic cohort (n = 74,155)[1]

Characteristic	Participants
	n (%)
Age	
50–59 y	16,196 (21.8)
60–69 y	32,764 (44.2)
70–79 y	23,105 (31.2)
80–89 y	2090 (2.8)
BMI	
Normal, <25.0 kg/m^2	29,485 (39.8)
Overweight, 25.0 to <30 kg/m^2	26,006 (35.1)
Obese, ≥30 kg/m^2	18,664 (25.2)
Race-ethnicity	
White	64,351 (86.8)
Black	4517 (6.1)
Hispanic	2114 (2.9)
Other	3173 (4.3)
Annual income	
<$20,000	9623 (13.0)
$20,000–34,999	17,176 (23.2)
$35,000–49,999	15,513 (20.9)
$50,000–74,999	15,876 (21.4)
≥$75,000	15,967 (21.5)
Education	
<High school diploma	2509 (3.4)
High school diploma/GED	11,785 (15.9)
School after high school	27,204 (36.7)
College degree or higher	32,657 (44.0)
Smoking	
Current	4235 (5.7)
Past	31,845 (42.9)
Never	38,075 (51.3)
Recreational physical activity	
0 < METs/wk ≤ 3.125	18,548 (25.0)
3.125 < METs/wk ≤ 9.833	18,491 (24.9)
9.833 < METs/wk ≤ 19.5	18,762 (25.3)
19.5 < METs/wk ≤ 142.3	18,354 (24.8)

[1] Number of participants meeting the analytic criteria and for whom there were no missing values for the energy regression calibration or the treated diabetes HR analysis. Characteristics are for year 1 for the Dietary Modification Trial Comparison group participants and year 3 for the Observational Study participants. METs, metabolic equivalent tasks.

elevated with increasing energy or protein consumption, and these elevations were little modified by the inclusion of BMI in the disease risk model. In contrast, HRs were highly elevated as a function of calibrated energy or protein consumption, and these elevations were substantially attenuated toward the null when BMI is added to the disease risk model (**Table 3**). The footnotes to Table 3 provide HR estimates without BMI adjustment as a function of reliability subsample measurement error correlations (*see* Subjects and Methods). These corrected estimates were somewhat attenuated relative to those in the body of Table 3, but there was considerable robustness to this measurement error correlation specification, and these analyses support substantial positive associations between both energy and protein intakes and diabetes risk and with the likelihood of a protein-specific association that goes beyond the contribution of protein to total energy consumption.

More explicitly, and with an emphasis on race-ethnicity, with a 20% increment in uncalibrated energy consumption, estimated diabetes risk was larger by only 3% overall and 4% in white participants (P values ≤ 0.05), whereas the HRs were not statistically significant ($P > 0.05$) for black or Hispanic women (Table 3). The HRs for uncalibrated energy were similar with and without adjustment for BMI for each ethnicity group. In comparison, after calibration, a 20% increment in energy was associated with more than a doubling of the estimated risk overall and in white women, although it was somewhat lower in black and Hispanic women than in white women. After adjustment for BMI, the association of higher calibrated energy intake with diabetes risk was no longer statistically significant ($P > 0.05$).

For protein, a 20% increment in uncalibrated intake (Table 3) was associated with a 5% higher risk of diabetes (HR: 1.05; 95% CI: 1.03, 1.07), whereas a 20% increment in calibrated protein was associated with an 82% higher risk (HR: 1.82; 95% CI: 1.56, 2.12). The HRs for uncalibrated protein were similar with and without adjustment for BMI. On adjustment for BMI, the effect of calibrated protein was much reduced, although still significant (HR: 1.16; 95% CI: 1.05, 1.28).

An increment of 20% uncalibrated protein density (Table 3) was positively associated with diabetes risk (HR: 1.13; 95% CI: 1.09, 1.17). The HRs for uncalibrated protein were similar with and without adjustment for BMI. The association was no longer apparent after biomarker calibration (HR: 1.01; 95% CI: 0.75, 1.37), but was again statistically significant after adjustment of calibrated intake for BMI (HR: 1.19; 95% CI 1.07, 1.32). The results were similar by race and ethnicity.

DISCUSSION

We found that the association of energy intake with diabetes risk was obscured by the underreporting of energy intake; however, body mass, as an indicator of energy balance, appeared to be the dominant risk factor mediating the association. The race-ethnicity results were similar to the overall findings. The small sample sizes for the black and Hispanic groups limited the ability to precisely assess risk, as demonstrated by the wide CIs around the calibrated energy intake HRs. Recent research by Olendzki et al (22) provides supporting evidence of underreporting energy intake among Hispanic women with a higher BMI.

The best method to adjust for BMI in the risk models was not clear because BMI may influence diet-disease associations in more than one way. Adjustment of dietary self-report by BMI can assist with understanding the effects of long-term excess energy intake (regardless of accuracy of the self-report) on chronic diseases that are related to body mass, as are many of today's chronic diseases of overweight and obesity, such as diabetes, heart disease, and some cancers. Indeed, inclusion of dietary self-report of energy intake may not even be necessary when investigating disease associative risk factors of overweight or obesity (23). However, having a higher body mass commands a greater energy need to carry out activities of daily living and recreational pursuits, with potential confounding of the association of energy intake with disease by dietary intake mismeasurement. Furthermore, failure to adjust for BMI in the HR analyses may overestimate the influence of energy consumption on the risk of diabetes. Inclusion of BMI in the diabetes risk models may obscure the influence of a high-energy diet on body fat deposition (presumably predominantly fat in this situation)

TABLE 2
Uncalibrated dietary intakes as estimated by the WHI FFQ and calibrated intakes derived from nutritional biomarker data in the combined analytic WHI cohort[1]

	Energy		Protein		Protein	
Sample	Uncalibrated	Calibrated	Uncalibrated	Calibrated	Uncalibrated	Calibrated
	kcal/d	*kcal/d*	*g/d*	*g/d*	*% of energy*	*% of energy*
Total (n = 74,155)	1416 (662, 3025)	2073 (1737, 2474)	59.2 (25.8,137.4)	75.2 (55.8,101.5)	16.8 (11.5, 24.6)	14.4 (11.9, 17.5)
White (n = 64,351)	1432 (686, 2988)	2079 (1757, 2461)	60.6 (27.0, 136.0)	76.3 (57.3, 101.5)	16.9 (11.7, 24.6)	14.5 (12.0, 17.5)
Black (n = 4517)	1308 (522, 3276)	2132 (1756, 2588)	51.0 (18.7, 139.3)	67.8 (49.4, 93.0)	15.6 (10.2, 24.0)	13.9 (11.2, 17.2)
Hispanic (n = 2114)	1327 (530, 3321)	2109 (1764, 2520)	55.1 (20.4, 148.4)	74.9 (55.1, 101.9)	16.6 (11.1, 24.8)	14.5 (11.8, 17.7)

[1] All values are geometric means (95% CIs). Values were calibrated by using biomarkers only for women in the Nutrition Biomarker Study, otherwise by using equations developed on the basis of FFQ nutrients measure and other factors (4): calibrated log-energy (kcal) was calculated as 7.61 + 0.062(log FFQ energy − 7.27) + 0.013(BMI – 28.2) – 0.005(age – 70.9) – 0.016(black ethnicity) – 0.004(Hispanic ethnicity) – 0.093(other minority ethnicity) – 0.019(annual household income <$20,000) + 0.037(income $20,000–34,999) + 0.013(income $50,000–74,999) + 0.019(income ≥$75,000); calibrated log-protein (g) was calculated as 4.28 + 0.211(log FFQ protein – 4.14) + 0.012(BMI – 28.2) – 0.008(age – 70.9) – 0.130(black ethnicity) – 0.021(Hispanic ethnicity) – 0.100(other minority ethnicity) + 0.065(high school, GED, or less education) + 0.033(college degree or more) – 0.053(income <$20,000) – 0.009(income $20,000–34,999) + 0.042(income $50,000–74,999] + 0.067[income ≥$75,000] – 0.009(log FFQ protein – 4.14)(BMI); log-calibrated % of energy from protein was calculated as 2.66 + 0.439(log FFQ % of energy from protein – 2.85) – 0.004(BMI – 28.2) – 0.005(age – 70.9), where BMI is defined by weight (in kg)/height^2 (in m), and square brackets denote indicator variables. Included are data from participants who met the analytic criteria and for whom there were no missing values for the energy regression calibration or the treated diabetes HR analysis. Dietary intakes are for year 1 for the WHI Dietary Modification Trial Comparison group participants and year 3 for the WHI Observational Study participants. FFQ, food-frequency questionnaire; WHI, Women's Health Initiative.

and an increase in BMI over the lengthy time period that may be relevant to diabetes risk (20). The relative lack of change in the HRs after adjusting for BMI in the uncalibrated nutrient estimates, which could be interpreted as BMI not being a mediator of diet-disease associations, reinforces the possibility of misinterpreting results that rely on dietary self-report.

Underreporting among those who are overweight and at risk of diabetes may make it difficult to discern the effect of energy intake as a risk factor for diabetes (1, 24). Whereas the concept may be intuitive that a higher energy intake (in the absence of a counterbalance of physical activity expenditure) would be associated with higher adult body weight, mismeasurement can reduce power in epidemiologic studies (25), and biomarker-calibrated estimates of nutrient intake may assist in developing a more complete risk association model (26). The development and use of calibrated estimates of dietary intake are in their infancy, and application currently resides within the cohort where developed. However, efforts are underway to harmonize biomarker-calibrated estimates of several nutrients (energy, protein, and potassium) across multiple cohorts, with the ultimate goal of being able to apply these estimates more broadly.

Our research found a potentially positive association of protein intake (absolute amount and that proportional to diet) with risk of diabetes in postmenopausal women after calibration and BMI-adjustment, which suggests that protein consumption could contribute to diabetes risk through mechanisms other than body fat deposition. Higher animal protein intakes have been reported to be associated with increased risk of diabetes (27, 28), which suggests utility in considering animal protein intake when counseling persons at risk of developing diabetes. Biomarkers of meat intake, a novel area of biomarker development, appear promising (29) and may help to further examine associations of animal and meat protein intakes with diabetes.

The strengths of our analysis include WHI being among the largest studies of postmenopausal women's health with a multitude of data for participant characteristics and disease outcomes. In the WHI, 18% of participants were nonwhite, representing nearly 9000 women from racial and ethnic minority populations. In the WHI-NBS of 544 women from the WHI DM trial, age, race, ethnicity, and BMI were well represented and protocol adherence was high. The ability to estimate energy and protein intakes with nutrient biomarkers is rare in large epidemiologic studies. Although diabetes status, both prevalent and incident, was assessed by self-report without adjudication or confirmation by clinical measures, the WHI self-report data for diabetes have been found to be highly complementary to medication use inventories provided by participants (16).

This study also had limitations, including that energy expenditure and urinary nitrogen excretion in the weight-stable WHI-NBS participants were assumed to represent intakes of energy and protein rather than being true biomarkers of intake. Self-report of physical activity, a risk factor for diabetes and hence an adjustment factor in the analyses, may have been subject to systematic bias (30), and we did not have biomarker measures for calibration. The sample-wide calibration equation was applied to each race (white, black) and ethnicity (Hispanic) group because of the relatively small sample sizes for nonwhite racial and ethnic groups. With larger biomarker sample sizes, uniquely estimated calibration equations could be constructed for each race and ethnicity group.

Considering that substantially different HRs for risk of diabetes resulted from uncalibrated estimates of energy and protein intakes compared with calibrated measurement error corrections, diet-disease associations without correction of self-report measurement error should be viewed with caution. Despite the expense of biomarker studies, it may be possible to conduct such studies with a subset of participants within larger research programs. In the current study, BMI and energy intake were tightly intertwined, and their unique influences on the development of diabetes were difficult to assess. In the face of systematically biased underreporting of energy intake among overweight persons, together with overweight being a risk factor for diabetes, BMI may be the preferred surrogate to control for energy intake, compared with dietary self-report, in epidemiologic studies of diabetes.

TABLE 3
HR estimates of incident diabetes for a 20% increased consumption of energy (kcal/d), protein (g/d), and protein density (% energy from protein/d) in the analytic cohort from the WHI[1]

	Total (n = 74,155)	White (n = 64,351)	Black (n = 4517)	Hispanic (n = 2114)
Energy (kcal/d)				
Uncalibrated HR (95% CI)	1.03 (1.01, 1.05)	1.04 (1.01, 1.06)	0.99 (0.95, 1.03)	1.05 (0.98, 1.13)
Uncalibrated HR (95% CI), adjusted for BMI	1.02 (1.00, 1.04)	1.02 (1.00, 1.04)	0.99 (0.95, 1.04)	1.04 (0.97, 1.12)
Calibrated HR (95% CI)[2,3]	2.41 (2.06, 2.82)	2.61 (2.21, 3.07)	1.78 (1.44, 2.19)	1.81 (1.29, 2.52)
Calibrated HR (95% CI), adjusted for BMI	1.30 (0.96, 1.76)	1.34 (0.92, 1.94)	0.82 (0.38, 1.81)	1.55 (0.42, 5.73)
Protein (g/d)				
Uncalibrated HR (95% CI)	1.05 (1.03, 1.07)	1.06 (1.04, 1.08)	1.02 (0.99, 1.06)	1.06 (0.99, 1.13)
Uncalibrated HR (95% CI), adjusted for BMI	1.03 (1.02, 1.05)	1.03 (1.01, 1.05)	1.02 (0.98, 1.06)	1.05 (0.98, 1.12)
Calibrated HR (95% CI)[2,3]	1.82 (1.56, 2.12)	1.96 (1.62, 2.37)	1.47 (1.24, 1.74)	1.54 (1.18, 1.99)
Calibrated HR (95% CI), adjusted for BMI	1.16 (1.05, 1.28)	1.15 (1.03, 1.29)	1.11 (0.91, 1.36)	1.30 (0.91, 1.86)
Protein density (% of energy from protein)				
Uncalibrated HR (95% CI)	1.13 (1.09, 1.17)	1.12 (1.08, 1.17)	1.14 (1.05, 1.24)	1.10 (0.95, 1.27)
Uncalibrated HR (95% CI), adjusted for BMI	1.08 (1.05, 1.12)	1.07 (1.03, 1.11)	1.11 (1.02, 1.20)	1.08 (0.94, 1.26)
Calibrated HR (95% CI)[2,3]	1.01 (0.75, 1.37)	0.96 (0.70, 1.32)	1.14 (0.86, 1.50)	1.09 (0.73, 1.62)
Calibrated HR (95% CI), adjusted for BMI	1.19 (1.07, 1.32)	1.16 (1.05, 1.28)	1.25 (1.00, 1.56)	1.24 (0.82, 1.87)

[1] Incident diabetes was reported in 3319 (4.5%) participants of the WHI analytic cohort. The HR was stratified on age (year 1 for the DM-C trial, year 3 for the observational study) in 5-y categories, on hormone therapy trial participation for those in the DM-C trial also participating in the hormone trials (active estrogen, estrogen placebo, active estrogen plus progestin, estrogen plus progestin placebo, and not randomly assigned), and for the Dietary Modification or observational study cohort membership. To control for confounding factors, the diabetes risk models were additionally adjusted for standard risk factors, including physical activity in units of metabolic equivalent tasks per week, race-ethnicity, education, income, history of cardiovascular disease, smoking status, alcohol consumption, hypertension, family history of diabetes, hormone use, glycemic index, and glycemic load. DM-C, Dietary Modification Comparison; WHI, Women's Health Initiative. The 95% CIs including ranges $\geq$1.00 or $<$1.00 were estimated as $P \leq 0.05$. The 95% CIs crossing the null value of 1.00 were estimated as $P > 0.05$. A 2-sided Wald test was used for the estimations assuming normality of the log-HRs with SEs estimated by bootstrap.

[2] 95% CIs for calibrated HRs are based on log-estimated HR $\pm$ 1.96 bootstrap SE.

[3] Estimates of HRs (95% CIs) were computed without adjustment for BMI as a function of the reliability subsample measurement error correlations (20). For energy intake, the induced HR based on a biomarker measurement error correlation of 0.0 was 1.66 (1.36, 2.03), of 0.2 was 1.73 (1.48, 2.03), and of 0.5 was 2.36 (1.16, 4.89). For protein intake, the induced HR based on a biomarker measurement error correlation of 0.0 was 1.29 (1.18, 1.42) and of 0.2 was 1.34 (1.16, 1.55). For protein density intake, the induced HR based on a biomarker measurement error correlation of 0.0 was 1.23 (1.10, 1.37) and of 0.2 was 1.25 (1.10, 1.42). These calculations suggest that the HRs for calibrated energy and protein that were not adjusted for BMI in Table 3 may be somewhat overestimated and those for calibrated protein density may be somewhat underestimated, depending on the magnitude of these measurement error correlations.

Current guidelines for type 2 diabetes prevention or delay include body weight loss of 5% to 10% and increased moderate physical activity to $\geq$150 min/wk (31). On the basis of our research results, persons at risk of type 2 diabetes would clearly benefit from increasing attention to accurately monitoring their dietary intake and estimating portion sizes. Successful approaches to weight loss and dietary self-monitoring have been reported by the Diabetes Prevention Program (32), and community translation efforts of the Diabetes Prevention Program are under way (33).

Short list of WHI Investigators: Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller (Program Office, National Heart, Lung, and Blood Institute, Bethesda, MD).

Clinical Coordinating Center: Ross Prentice, Garnet Anderson, Andrea LaCroix, and Charles Kooperberg (Fred Hutchinson Cancer Research Center, Seattle, WA), Evan Stein (Medical Research Laboratories, Highland Heights, KY), and Steven Cummings (University of California at San Francisco, San Francisco, CA).

Clinical Centers: Sylvia Wassertheil-Smoller (Albert Einstein College of Medicine, Bronx, NY), Haleh Sangi-Haghpeykar (Baylor College of Medicine, Houston, TX), JoAnn E Manson (Brigham and Women's Hospital, Harvard Medical School, Boston, MA), Charles B Eaton (Brown University, Providence, RI), Lawrence S Phillips (Emory University, Atlanta, GA), Shirley Beresford (Fred Hutchinson Cancer Research Center, Seattle, WA), Lisa Martin (George Washington University Medical Center, Washington, DC), Rowan Chlebowski (Los Angeles Biomedical Research Institute at Harbor–UCLA Medical Center, Torrance, CA), Erin LeBlanc (Kaiser Permanente Center for Health Research, Portland, OR), Bette Caan (Kaiser Permanente Division of Research, Oakland, CA), Jane Morley Kotchen (Medical College of Wisconsin, Milwaukee, WI), Barbara V Howard (MedStar Research Institute/Howard University, Washington, DC), Linda Van Horn (Northwestern University, Chicago/Evanston, IL), Henry Black (Rush Medical Center, Chicago, IL), Marcia L Stefanick (Stanford Prevention Research Center, Stanford, CA), Dorothy Lane (State University of New York at Stony Brook, Stony Brook, NY), Rebecca Jackson (The Ohio State University, Columbus, OH), Cora E Lewis (University of Alabama at Birmingham, Birmingham, AL), Cynthia A Thomson (University of Arizona, Tucson/Phoenix, AZ), Jean Wactawski-Wende (University at Buffalo, Buffalo, NY), John Robbins (University of California at Davis, Sacramento, CA), F Allan Hubbell (University of California at Irvine, Irvine CA), Lauren Nathan (University of California at Los Angeles, Los Angeles, CA), Robert D Langer (University of California at San Diego, La Jolla/Chula Vista, CA), Margery Gass (University of Cincinnati, Cincinnati, OH), Marian Limacher (University of Florida, Gainesville/

Jacksonville, FL), J David Curb (University of Hawaii, Honolulu, HI), Robert Wallace (University of Iowa, Iowa City/Davenport, IA), Judith Ockene (University of Massachusetts/Fallon Clinic, Worcester, MA), Norman Lasser (University of Medicine and Dentistry of New Jersey, Newark, NJ), Mary Jo O'Sullivan (University of Miami, Miami, FL), Karen Margolis (University of Minnesota, Minneapolis, MN), Robert Brunner (University of Nevada, Reno, NV), Gerardo Heiss (University of North Carolina, Chapel Hill, NC), Lewis Kuller (University of Pittsburgh, Pittsburgh, PA), Karen C Johnson (University of Tennessee Health Science Center, Memphis, TN), Robert Brzyski (University of Texas Health Science Center, San Antonio, TX), Gloria E Sarto (University of Wisconsin, Madison, WI), Mara Vitolins (Wake Forest University School of Medicine, Winston-Salem, NC), and Michael S Simon (Wayne State University School of Medicine/Hutzel Hospital, Detroit, MI).

Women's Health Initiative Memory Study: Sally Shumaker (Wake Forest University School of Medicine, Winston-Salem, NC).

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