Women’s Health Initiative Dietary Modification Trial: Update and Application of Biomarker Calibration to Self-Report Measures of Diet and Physical Activity

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78

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78.1 Rationale for Biomarker Calibration of Self-Report Measures of Diet

Evidence has been accruing that self-reported dietary intake underestimates actual intake. Particularly noteworthy is that overweight and obese persons may systematically underreport energy intake [1-3]. Women who, at Women’s Health Initiative (WHI) enrollment during 1993-1998, were in their 50s underreported energy intake to a greater degree than women who were in their 70s, and there is some evidence that Black or Hispanic women may underreport energy intake to a greater degree than White women [1, 2]. Protein intake was also under-reported although to a lesser extent than energy, which resulted in a modest overestimation of protein density (% energy from protein). Numerous reasons may exist for this underreporting including social desirability [4,5], bias relating to recall or potentially an inadequate representation of regional or cultural foods when limited choice food frequency questionnaires (FFQs) are used as the assessment instruments.

Given this background and impetus from the Observing Protein and Energy Nutrition Study (OPEN) study [3], WHI investigators launched two biomarker studies: the Nutrient Biomarker Study (NBS) and the Nutrition & Physical Activity Assessment Study (NPAAS) to study measurement error properties of the WHI dietary assessment tools. The NBS and NPAAS compared self-report measures of consumption
with biomarkers such as a doubly-labeled water (DLW) assessment of energy and a urinary nitrogen assessment of protein. Within the WHI, we had the opportunity to investigate the measurement characteristics of three dietary assessment self-report instruments: the FFQ, the 4-day food record (4DFR) and 24-hour dietary recalls (24HRs). Energy expenditure by DLW served as a surrogate of energy intake among weight stable women. Intake of protein was assessed by 24-hour urine excretion of nitrogen, with excretion adjusted to provide surrogate of intake in weight stable women. Utilizing the data from self-report and objective markers we developed calibration equations, described below, that have been applied to self-report dietary data from all WHI participants for several investigations of diet-disease associations utilizing biomarker-calibrated estimates of energy and protein intakes.

In this section we describe the calibration studies and the subsequent efforts to relate calibrated intakes to disease risk specifically cardiovascular disease, diabetes and cancer as well as aging-related indicators such as frailty and renal function. We conclude by describing future avenues of investigation.

78.2 Nutrient Biomarker Study Energy and Protein Calibration

Our first study to better understand the measurement properties of self-reported dietary intake was called the WHI Nutritional Biomarkers Study (WHI-NBS), which was conducted in 2004–2007 [1]. The overall objectives of WHI-NBS were to: (1) use objective biomarkers of total energy and protein consumption to characterize the error distribution from a measure of self-reported diet (i.e., food frequency questionnaire); (2) examine whether the measurement error distribution varied by participant characteristics such as age, race/ethnicity, or BMI and (3) develop equations using the biomarker data that could be used to calibrate or correct for the error in the self-report.

Briefly, we recruited 544 weight stable, healthy postmenopausal women who had been participants in the Women’s Health Initiative Dietary Modification Trial (WHI-DMT). Participants attended two clinic visits where they completed a doubly labeled water (DLW) protocol to assess total energy expenditure (approximately equivalent to total energy intake in weight stable persons), collected a 24-hour urine specimen for assessment of consumption of protein and other nutrients, such as potassium and sodium. Participants also completed a food frequency questionnaire and height and weight were measured.

We compared the self-report to biomarkers and our results confirmed that systematic measurement error existed in self-report. On average, energy intake was underreported by 27% and 32% by WHI-DMT participants in comparison and intervention groups, respectively. Protein intake was underreported by 10% and 15% in the comparison and intervention groups, respectively. Further, we regressed the difference between self-report and objective biomarker on participant characteristics and found that women with higher BMI had more underreporting compared to women of normal BMI as did women who were Black or Hispanic compared to White women. Younger women had more underreporting compared to older women.

We then regressed the biomarkers on the self-report and participant characteristics and used the coefficients from the regression to create regression calibration equations where the calibrated estimates of energy and protein become predicted values that are corrected for the systematic measurement error.

Both biomarkers and corresponding self-
report assessments were log-transformed in developing these equations. Predicted, or calibrated, values from these equations throughout WHI cohorts are then used in disease association models to provide better estimates of diet-disease associations. Calibration equations were extended to include potential confounding factors for use in these association analyses.

**78.3 Measurement Error Properties of 4DFR, 24HR, and FFQ**

Prentice et al. [2] considered measurement error sources and correction for the estimation of energy, protein and percent of energy from protein, based on data from 450 postmenopausal women drawn from the WHI Observational Study, a prospective cohort study among 93,676 women, in the Nutrition and Physical Activity Assessment Study (NPAAS). The NPAAS expanded upon NBS by not only evaluating the ubiquitous food frequency questionnaire (FFQ), but also four-day food records (4DFRs) and three 24-hour dietary recalls (24HRs), each of which provided a dietary assessment approach that may be practical in large epidemiologic cohorts, if warranted by measurement properties. As with the WHI-NBS biomarker calibration study analyses, regression calibration equations were developed [2]. Through comparison with the DLW biomarker of energy consumption and the urinary nitrogen biomarker of protein consumption, the food record was shown to provide a relatively stronger estimate of energy and protein than does the FFQ, with 24HRs mostly intermediate. Differences were smaller and non-significant for protein density. Food frequencies, records, and recalls were respectively able to “explain” 3.8, 7.8, and 2.8 percent of biomarker variation for energy; 8.4, 22.6, and 16.2 percent of biomarker variation for protein; and 6.5, 11.0, and 7.0 percent of biomarker variation for protein density, with all variables log-transformed. However, calibration equations that include body mass index, age, and ethnicity substantially improve these numbers to 41.7, 44.7, and 42.1 for energy; 20.3, 32.7, and 28.4 for protein; and 8.7, 14.4, and 10.4 for protein density. Calibration equation coefficients from the NPAAS study, for energy, protein and percent of energy from protein, for each of the three dietary assessment procedures, and their combination are shown in Table 1.

Note that the adjusted $R^2$ values in this Table allow for temporal variation in the biomarker assessments by dividing the unadjusted $R^2$ values by the correlation between repeat (log-transformed) biomarker assessments in a 20% reliability subsample from NPAAS, and can be viewed as targeting average daily consumption over a period of about a year. Calibration equations using any of the assessment procedures appear to yield suitable consumption estimates for epidemiologic study purposes in this cohort. Furthermore, comparison of calibration equations from NBS and NPAAS did not reveal differences of practical importance, so that calibration equations from either biomarker study are evidently suitable for application throughout WHI cohorts. As noted above, these equations may need to be augmented in specific applications by including confounding factors for the diet-disease association. Calibration equations from NBS [1] that include several additional potential confounding variables have been used in most WHI applications to date.

**78.4 Calibration of Self-Report Measures of Physical Activity**

We were also interested in measurement error in self-reported physical activity assessment in the Nutrition and Physical Activ-
Table 1: Calibration Equation Coefficients ($\beta$), Standard Errors (SE), and Percent of Biomarker Variation Explained ($R^2$) From Regression of Log(biomarker) on Log(self-report), Body Mass Index, Age, and Ethnicity in the Women’s Health Initiative Nutrition and Physical Activity Assessment Study (NPAAS) Among 450 Postmenopausal Women. Adjusted $R^2$ Values that Correct for Biomarker Measurement Error are also Provided.

<table>
<thead>
<tr>
<th>Variable</th>
<th>FFQ</th>
<th>4DFR</th>
<th>24HR</th>
<th>All SelfReports</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>SE</td>
<td>Adj $R^2$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Intercept</td>
<td>7.614*</td>
<td>0.009</td>
<td></td>
<td>7.597*</td>
</tr>
<tr>
<td>FFQ</td>
<td>0.054*</td>
<td>0.017</td>
<td>3.8</td>
<td>6.5</td>
</tr>
<tr>
<td>4DFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.013*</td>
<td>0.001</td>
<td>26.9</td>
<td>45.9</td>
</tr>
<tr>
<td>Age</td>
<td>-0.009*</td>
<td>0.001</td>
<td>9.7</td>
<td>16.5</td>
</tr>
<tr>
<td>Black</td>
<td>-0.023</td>
<td>0.019</td>
<td></td>
<td></td>
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<tr>
<td>Hispanic</td>
<td>-0.062*</td>
<td>0.021</td>
<td>1.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Other minority (Total)</td>
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<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>41.7</td>
<td>71.1</td>
<td></td>
<td>44.7</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.263</td>
<td>0.017</td>
<td></td>
<td>4.235</td>
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<tr>
<td>FFQ</td>
<td>0.135*</td>
<td>0.021</td>
<td>8.4</td>
<td>16.4</td>
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<tr>
<td>4DFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.012*</td>
<td>0.002</td>
<td>5.8</td>
<td>11.4</td>
</tr>
<tr>
<td>Age</td>
<td>-0.012*</td>
<td>0.002</td>
<td>4.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Black</td>
<td>-0.120*</td>
<td>0.038</td>
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<tr>
<td>Hispanic</td>
<td>-0.078</td>
<td>0.040</td>
<td>2.0</td>
<td>3.9</td>
</tr>
<tr>
<td>Other minority (Total)</td>
<td>-0.018</td>
<td>0.076</td>
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</tr>
<tr>
<td></td>
<td>20.3</td>
<td>39.7</td>
<td></td>
<td>32.7</td>
</tr>
</tbody>
</table>

(continued)
Log-energy (kcal/day) is centered by 7.27; log-protein (grams/day) is centered by 4.14; log-protein density (g/kcal) is centered by 2.85; BMI is centered by 28.2 kg/m²; and age is centered by 70.9 years, in these analyses. BMI, body mass index; FFQ, food frequency questionnaire; 4DFR, four-day food record; 24HR, 24-hour dietary recall (3); Adj R², adjusted R² values (R² divided by log-biomarker correlation in reliability subsample).

Coefficient differs from zero at \( p = 0.05 \) significance level.

Total percentage of variation explained by all variables. R² values for specific variables arise from analyses with only these regression variables, with subsequent rescaling so that these R² values add to the total regression R². R² values for race/ethnicity pertain to comparisons among the four groups (White, Black, Hispanic, other minority).


<table>
<thead>
<tr>
<th>Variable</th>
<th>FFQ</th>
<th>4DFR</th>
<th>24HR</th>
<th>All Self-Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta )</td>
<td>SE</td>
<td>( R^2 )</td>
<td>Adj ( R^2 )</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.652*</td>
<td>0.017</td>
<td>2.671*</td>
<td>0.017</td>
</tr>
<tr>
<td>FFQ</td>
<td>0.344*</td>
<td>0.068</td>
<td>6.5</td>
<td>38.5</td>
</tr>
<tr>
<td>4DFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.002</td>
<td>0.002</td>
<td>0.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Age</td>
<td>-0.002</td>
<td>0.002</td>
<td>0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>Black</td>
<td>-0.100*</td>
<td>0.037</td>
<td>-0.130*</td>
<td>0.036</td>
</tr>
<tr>
<td>Hispanic</td>
<td>-0.043</td>
<td>0.041</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Other minority</td>
<td>-0.030</td>
<td>0.078</td>
<td>-0.006</td>
<td>0.075</td>
</tr>
</tbody>
</table>

| Protein Density   |       |      |       |        |       |      |       |        |       |      |       |        |
|                   | \( \beta \) | SE  | \( R^2 \) | Adj \( R^2 \) | \( \beta \) | SE  | \( R^2 \) | Adj \( R^2 \) | \( \beta \) | SE  | \( R^2 \) | Adj \( R^2 \) |
| (Total)b          | 8.7   | 52.1 | 14.4  | 86.1   | 10.4  | 62.3 | 15.5  | 93.4   |

Table 1 (continued)
Women’s Health Initiative Dietary Modification Trial

For this study we recruited 450 women who were part of the WHI Observational Study [6]. Women completed the same DLW and 24-hour urine collection protocols as was done for the WHI NBS, but we augmented the protocol to also include indirect calorimetry in the full NPAAS sample to assess resting energy expenditure (REE). Our objective measure of physical activity was defined as \( \text{AREE} = \text{TEE} \) (from the DLW) – REE (from the indirect calorimetry). Participants completed three self-report measures of physical activity; these include the Arizona Activity Questionnaire (AAFQ) the 7-day Physical Activity Recall (PAR) and a physical habits questionnaire (PHQ) on recreational physical activity that had been used throughout WHI.

We used the same linear regression procedures as were used in the WHI-NBS to understand the participant characteristics’ influence on measurement error. We found that body mass index was associated with underreporting of physical activity on the AAFQ and PHQ but overreporting on the PAR. Black and Hispanic participants underreported activity on the AAFQ and PAR in comparison to the objective AREE biomarker. We found that using the self-report alone explained very little of the variation in the AREE biomarker (< 10% for all instruments), but biomarker calibrated measures substantially improved the variance explained to 21–26%. Adjusted for biomarker temporal variation over a one-year period using reliability subsample data, the variance explained by biomarker calibrated measures further increased to 68–79%. These results suggest that, as with energy and protein consumption self-report, physical activity self-report is subject to systematic measurement error. Calibrated estimates of the self-report improve the estimates substantially and are likely to be more reliable measures when used in physical activity-disease association studies.

78.5 Psychosocial Measures and Biomarker-Calibrated Intake

We examined the contribution of psychosocial and diet behavior factors that affect self-report measures of diet in 450 postmenopausal women in the WHI Observational Study who participated in NPAAS [5]. Using regression calibration we estimated bias of self-reported dietary instruments including psychosocial factors from the Stunkard-Sorensen Body Silhouettes for body image perception [7, 8], the Crowne-Marlowe Social Desirability Scale [9], and the Three Factor Eating Scale (R-18) [10] for cognitive restraint for eating, uncontrolled eating, and emotional eating. Social desirability is the tendency to respond to questionnaires or interviews with what is perceived to be a socially appropriate response as opposed to an objective or accurate response. We included a diet behavior factor on number of meals eaten at home using the 4DFR.

Three categories were defined for each of the six psychosocial and diet behavior variables (low, medium, high). Overall the sample scored high in social desirability with 60% of NPAAS women scoring high, 39% as medium and 0.9% as low according to the Crowne-Marlowe Scale classification (20–33 as high, 9–19 as medium and < 9 as low), with a mean (SD) social desirability score of 21.07 (5.35). Participants with high social desirability scores were more likely to under-report on the FFQ for energy (\( \beta = -0.174, \ SE = 0.054, \ p < 0.05 \)) and protein intake (\( \beta = -0.142, \ SE = 0.062, \ p < 0.05 \)) compared to participants with low social desirability scores. Participants consuming a high percentage of meals at home were less likely to under-report on the FFQ for energy (\( \beta = 0.181, \ SE = 0.053, \ p < 0.05 \)) and pro-
tein ($\beta = 0.127, SE = 0.06, p < 0.05$) compared to participants consuming a low percentage of meals at home. In the calibration equations combining FFQ, 4DFR, 24HR with age, body mass index, race, and the psychosocial and diet behavior variables, the six psychosocial and diet variables explained 1.98%, 2.24%, and 2.15% of biomarker variation for energy, protein, and protein density respectively. The variations explained are significantly different between the calibration equations with or without the six psychosocial and diet variables for protein density ($p = 0.02$), but not for energy ($p = 0.119$) or protein intake ($p = 0.077$). The results for protein density are presented in Figure 1.

The addition of psychosocial and diet behavior factors to calibration equations modestly but significantly increased the amount of total variance explained for protein density and their inclusion would be expected to strengthen the precision of calibration equations correcting self-report for measurement error. The contribution of these variables was generally small compared to that of BMI, age and race/ethnicity indicating that calibrated energy and protein consumption estimates based on self-report in conjunction with BMI, age and ethnicity only are likely to be the principal variables to include, along with contributing confounding factors, for most epidemiologic purposes. However for protein density consumption estimates, psychosocial and diet behavior variables seem to play a larger role. This finding points to the constellation of factors that differentially affect reporting of dietary components. In summary, the study of the psychosocial factors considered here provides valuable additional insight into the dietary reporting practices and provides additional precision in estimates of intake in postmenopausal women in the United States.

### 78.6 Calibrated Energy, Protein, Protein Density, and Cardiovascular Disease Incidence

Prentice and colleagues [11] used FFQ-based calibration equations for energy, protein and protein density from the NBS to examine the association of these dietary factors with cardiovascular disease incidence among postmenopausal women in WHI cohorts. Nutritional epidemiology cohort studies primarily use FFQs, and since FFQs are more reliable for nutrient densities than for absolute nutrient consumption, association study reports typically present only nutrient density measures in relation to disease risk. In comparison, this study used objective biomarkers to correct FFQ assessments for measurement error, and examined absolute energy and protein consumption, as well as their ratio, in relation to cardiovascular disease incidence. Physician-adjudicated cardiovascular disease incidence was assessed for 80,370 postmenopausal women in the age range 50-79 yrs at enrollment in the comparison group of the WHI-DMT or the prospective WHI Observational Study. Urinary recovery biomarkers of energy and protein were obtained from the NBS subsample of 544 women, with concurrent FFQ. Following biomarker correction, energy consumption was positively associated with coronary heart disease incidence (HR 1.18, 95% CI: 1.04, 1.33 for 20% consumption increment) and protein density was inversely associated (HR 0.85, 95% CI: 0.75, 0.97). Ischemic stroke incidence was inversely associated with energy and protein consumption, but not with protein density.

A rather thorny issue arises in association analyses of this type that do not include all of the variables in the disease rate model that are used in calibration, as is the case with BMI in some of the analyses.
in this study. Such analyses are appropriate if BMI acts as a pure mediator, rather than confounder of the diet-disease association, as is a reasonable assumption if the targeted dietary variable applies to diet over the preceding years, or decades, that may be relevant to disease risk. Further insight into this issue can be obtained by first fitting a disease rate model that includes all calibration variables, and then “averaging” over the variables (e.g. BMI) that are excluded from certain analyses. This approach is detailed in Prentice and Huang [12] with application to these WHI cardiovascular disease analyses. This work reveals the need for longitudinal dietary and biomarker data for assessment of disease risk in relation to a preceding history of dietary consumption.

Analyses that included BMI in the disease rate model as a potential confounder, as is reasonable if the dietary variable targets short term consumption, do not show an association between energy consumption and coronary heart disease incidence, while the inverse association with stroke risk is strengthened by the BMI inclusion. In summary, a likely positive association between energy consumption and coronary heart disease risk can be attributed to body mass accumulation. The observed inverse association between ischemic stroke risk and both energy and protein consumption is unexplained, but possibly attributable to correlations between energy and protein intake and physical activity.

78.7 Diabetes and Calibrated Consumption

Much of the epidemiologic literature has not observed an association of type 2 diabetes risk with energy intake, despite the well-recognized role of energy balance
and weight management in preventing diabetes. The effect of systematic underreporting of energy and protein intake, particularly among overweight and obese persons, has not been studied in relationship to diabetes risk. We evaluated the association of diabetes with dietary intakes of energy, protein and protein density from self-reported dietary intakes of energy, protein and protein density using biomarker calibrated estimates of intake and uncalibrated estimates [13].

The WHI Observational Study and DMT Comparison group participants served as our study population. We excluded those who reported as ever having been told they had diabetes at baseline. The resulting sample size was 74,155 participants. A FFQ served as the self-report of dietary intake. To avoid baseline biases of self-reported intakes resulting from regression to the mean upon applying the WHI-DMT exclusion of consuming <32% energy from fat, we utilized Year 1 FFQs from the DMT comparison group as the analytic baseline for DMT participants. Consequently, in addition to excluding true baseline prevalent diabetes, cases of diabetes reported as diagnosed during the first year post-randomization were also excluded. Women who were ineligible for the DMT because they were already consuming a self-reported intake of <32% energy from fat often enrolled in the WHI OS, resulting in the possibility of a skewed distribution of % energy from fat at baseline. Using similar reasoning for utilizing the DMT year 1 as the analytic baseline, the OS year 3 (the next time after baseline when the FFQ was completed) was used as the analytic baseline for OS. Within the OS, cases of incident diabetes between baseline and year 3 were excluded. Biomarker calibration equations from NBS were applied to the uncalibrated estimates to compute calibrated estimates of dietary intake. Diabetes incidence was ascertained during WHI follow-up by self-report. Self-report consisted of a diabetes diagnosis with treatment by pills or insulin. Self-report of diabetes has been validated against medications inventory within the WHI [14]. Hazard ratios based on Cox regression were used to assess the association of incident diabetes with uncalibrated and calibrated intakes of energy, protein and protein density.

A 20% increment in self-reported energy intake was associated with an increased diabetes risk of HR 1.03 (95% CI 1.01, 1.05) using uncalibrated dietary estimates, an HR of 2.41 (95% CI 2.06, 2.82) using calibrated dietary estimates without body mass index (BMI) adjustment, and an HR of 1.30 (95% CI 0.96, 1.76) with biomarker calibration and with BMI in the disease rate model. A 20% increment in protein (g/d) resulted in an HR of 1.05 (95% CI 1.03, 1.07) using uncalibrated estimates, an HR of 1.82 (95% CI 1.56, 2.12) using calibrated estimates without BMI adjustment, and an HR of 1.16 (95% CI 1.05, 1.28) with calibrated estimates and BMI adjustment in the disease rate model. A 20% increment in protein density (% energy from protein) resulted in an HR of 1.13 (95% CI 1.09, 1.17) using uncalibrated estimates, an HR of 1.01 (95% CI 0.75, 1.37) using calibrated estimates without BMI adjustment, and an HR of 1.19 (95% CI 1.07, 1.32) using calibrated estimates with BMI adjustment in the disease rate model. Underreporting of dietary intakes did attenuate the association of diabetes risk with energy intake. However, body fat deposition over time, and corresponding increasing body mass index appeared a major factor in mediating the energy consumption-diabetes association. The hazard ratios of energy intake in analyses that do not include BMI in the disease rate model may be somewhat overestimated because BMI may influence diet-disease relationships in more than one way.
There are the effects of long-term energy intake and also that higher body mass may call for greater fuel (energy). Exclusion of BMI in the diabetes risk models may over-estimate the influence of energy intake on diabetes risk, whereas inclusion may obscure the influence of higher energy intake on body fat deposition. See Tinker et al. [13] and Prentice and Huang [12] for further discussion of including or excluding BMI in the disease models investigating energy, protein, and protein density.

### 78.8 Cancer and Calibrated Intake

Prentice et al. [15] examined the relationship between cancer risk and total energy intake, protein and protein density, and provided a first application of urinary recovery markers to correct for systematic bias in self-reported consumption in an epidemiologic cohort setting. Over 5000 documented cancer outcomes were reported on 80,816 women from the Dietary Modification and Observational Trial cohorts [16]. These authors examined associations for total invasive cancer incidence (exclusive of non-melanoma skin) and incidence for eleven site-specific cancers, including breast, colon, rectal, ovarian, endometrial cancer, kidney and pancreatic cancers—for which obesity is thought to be an important risk factor [17]. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated from the Cox regression model, adjusted for standard risk factors to control confounding, and reported for a 20% increase in consumption.

In analyses that didn’t include BMI in the disease risk model, biomarker-calibrated energy intake was positively related to total cancer (HR 1.18, 95% CI: 1.10, 1.27), breast (HR 1.24, 95% CI: 1.11, 1.38), colon (HR 1.35, 95% CI: 1.06, 1.71), endometrial (HR 1.83, 95% CI: 1.49, 2.25), and kidney (HR 1.47, 95% CI: 1.00, 2.16) cancers, while uncalibrated energy was not significantly related to total or any specific cancer, with the possible exception of an inverse association with colon cancer. Associations with calibrated protein were significant for total cancer (HR 1.06, 95% CI: 1.01, 1.12), breast cancer (HR 1.09, 95% CI: 1.01, 1.19), endometrial cancer (HR 1.37, 95% CI: 1.16, 1.61), and leukemia (HR 1.39, 95% CI: 1.05, 1.83). These positive associations may largely be due to the correlation between protein and energy consumption, since the hazard ratio estimates for protein density were generally less than one, with a significant inverse association for total cancer (HR 0.92, 95% CI: 0.85, 0.99).

Calibrated energy and BMI were highly interdependent in this cohort; hence, as for the cardiovascular and diabetes analyses described above, the analyses could not reliably estimate separate associations for total energy and BMI, on risk of total or site-specific cancers. Principal results were reported without adjustment for BMI. When BMI was additionally included in the model, hazard ratios for both energy and BMI were not significant for most cancer sites and confidence intervals were wide. High BMI and increased energy consumption are likely inter-related and important aspects of total and site-specific cancer risk. The general lack of associations between uncalibrated intake and cancer risk suggests measurement error in the self-reported data obscured important associations between diet and cancer risk and highlight the importance of biomarker-calibrated analyses.

### 78.9 Associations Between Protein Intake, Frailty, and Renal Function

**Protein intake and frailty.** Whether the current Recommended Dietary Allowance (RDA) for protein is adequate...
for older adults is debatable [18-20]. WHI can inform this question using biomarker-calibrated protein intake and well-characterized aging indicators. For example “frailty” is a term that has been used clinically to describe a constellation of changes that occur in the very old such as impaired strength, endurance, and balance. Frail individuals are at higher risk for many adverse health outcomes such as falls, fractures, development of disabilities, hospitalizations, and death, even after accounting for demographic characteristics, health behaviors, disability, and co-morbidities [21,22].

After adjustment for confounders, a 20% increase in uncalibrated protein intake (% kcal) was associated with a 12% (95% CI: 8% to 16%) decrease in frailty risk, while a 20% increase in calibrated protein was associated with a 32% (95% CI: 23% to 44%) decrease in frailty risk (Figure 2) [23]. These data suggest that: (a) insufficient protein intake may be an intervention target for frailty prevention; (b) calibration using nutrient biomarkers deattenuates diet-disease associations.

**Protein intake and renal function.**

The effect of dietary protein restriction on kidney function among individuals with chronic kidney disease has been debated for the last 50 years, and several randomized controlled trials have addressed this question [24]. The American Dietetic Association and American Diabetes Association both recommend limiting protein intake to 0.8 to 1.0 g/kg/day (≈ 10–14% kilocalories from protein) if kidney function is impaired.

Of critical importance to public health is whether diets rich in protein have detrimental effects on kidney function among older adults without overt kidney disease, i.e., having an estimated glomerular filtration rate (GFR) above 60 mL/min per 1.73 m². To investigate this question using biomarker-calibrated protein, we used data from 2 nested case-control studies within the WHI Observational Study (n = 2419) [25]. We estimated protein intake using a FFQ and estimated glomerular filtration rate (eGFR) from cystatin C. Self-reported energy and protein were calibrated using biomarkers of energy and protein intake. Associations between protein intake and renal function were estimated by weighted linear and logistic regression models. Average calibrated protein intake (mean ± SD) was 1.1 ± 0.2 g/(kg body weight per day). Twelve percent (n = 292) of women had impaired renal function. The odds of impaired renal function, defined as eGFR < 60 mL/(min/1.73m²), was not associated with calibrated protein intake [25]. There was no evidence for effect modification by age, BMI, or general health status. These data suggest higher protein intake is not associated with impaired renal function among postmenopausal women without a diagnosis of chronic kidney disease.

### 78.10 Summary and Future Directions

This chapter provided an update on development of calibration equations based on biomarker-calibrated consumption as well as application of these equations to functional measures and disease outcomes. Future directions include analysis of calibrated measures of sodium and potassium intake using urinary measures as well as sugar biomarkers. Additional avenues include the development of novel biomarkers through feeding studies, and longitudinal biomarker studies in cohort subsamples that can weigh in on critical time periods in human development when diet might have a strong impact on health, and may help to elucidate the roles of dietary variables in health-related outcomes, in relation to body fat deposition and other mediating or confounding variables. Lastly biomarker

studies on diverse populations such as the one underway in four US cities—the Study of Latinos: Nutrition & Physical Activity Assessment Study (SOLNAS) in a Hispanic cohort can provide critical information that is currently not available.

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