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Regression Calibration in Nutritional Epidemiology: Example of Fat Density and Total Energy in Relationship to Postmenopausal Breast Cancer

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Regression calibration using biomarkers provides an attractive approach to strengthening nutritional epidemiology. We consider this approach to assessing the relationship of fat and total energy consumption with postmenopausal breast cancer. In analyses that included fat density data, biomarker-calibrated total energy was positively associated with postmenopausal breast cancer incidence in cohorts of the US Women's Health Initiative from 1994–2010. The estimated hazard ratio for a 20% increment in calibrated food frequency questionnaire (FFQ) energy was 1.22 (95% confidence interval (CI): 1.15, 1.30). This association was not evident without biomarker calibration, and it ceased to be apparent following control for body mass index (weight (kg)/height $(m)^2$), suggesting that the association is mediated by body fat deposition over time. The hazard ratio for a corresponding 40% increment in FFQ fat density was 1.05 (95% CI: 1.00, 1.09). A stronger fat density association, with a hazard ratio of 1.19 (95% CI: 1.00, 1.41), emerged from analyses that used 4-day food records for dietary assessment. FFQ-based analyses were also carried out by using a second dietary assessment in place of the biomarker for calibration. This type of calibration did not correct for systematic bias in energy assessment, but may be able to accommodate the "noise" component of dietary measurement error. Implications for epidemiologic applications more generally are described.

bias; biological markers; breast cancer; dietary assessment; dietary energy; dietary fat; postmenopausal women

Abbreviations: 24HR, 24-hour dietary recall; 4DFR, 4-day food record; BMI, body mass index; CI, confidence interval; DM, Women's Health Initiative Dietary Modification; DM-C, Women's Health Initiative Dietary Modification Trial comparison group; DLW, doubly labeled water; FFQ, food frequency questionnaire; HR, hazard ratio; NPAAS, Nutrition and Physical Activity Assessment Study; OR, odds ratio; WHI, Women's Health Initiative.

The hypothesis that a low-fat diet may reduce breast cancer risk was stimulated by rodent feeding experiments, the results of which support a tumor-enhancing role for both total energy consumption and the fraction of energy from fat (1). The dietary fat hypothesis is consistent with international correlations that use food supply data (2) and with early case-control studies that used a variety of dietary assessment approaches, including food records, dietary recalls, and food frequency questionnaires (FFQs) (3). The hypothesis, however, was not supported by a 1996 analysis of several cohort studies (4), each of which used a FFQ for dietary assessment.

By 1996, randomized intervention trials of a low-fat eating pattern, with breast cancer as a primary outcome, were well underway. The largest of these was undertaken by the Women's Health Initiative (WHI). The Women's Health Initiative Dietary Modification (DM) Trial included 48,835 postmenopausal women in the United States, 40% of whom were assigned to a low-fat eating pattern intervention with the goal of reducing energy from fat (hereafter, "fat density") to 20% from baseline levels that averaged 35% percent. The intervention also included goals of increasing daily fruit and vegetable intake and grain servings to 5 and 6, respectively, primarily

to achieve a low-fat eating pattern. There were no specific goals for total energy consumption. By the end of the trial intervention period (on April 8, 2005), the breast cancer hazard ratio comparing the intervention with the usual diet comparison group (5) was 0.91 (95% confidence interval (CI): 0.83, 1.01) overall, with a significant interaction ($P = 0.04$) of hazard ratio with baseline fat density as measured by 4-day food records (4DFRs). The hazard ratio in the upper quartile of baseline fat density was 0.78 (95% CI: 0.64, 0.95). The hazard ratio reduction in the DM Trial was approximately 70% of that projected in the trial design (6), and the fat density difference between the intervention and control groups was also approximately 70% of that targeted.

In comparison, an intervention trial of a low-fat, high-carbohydrate diet among 4,690 women having extensive mammographic densities, most of whom were premenopausal at enrollment, did not produce results suggestive of any benefit (7) with a breast cancer hazard ratio of 1.19 (95% CI: 0.91, 1.55). Also relevant, an intervention trial among 2,437 women (age range, 48–79 years) who had early-stage breast cancer diagnoses yielded a breast cancer recurrence reduction with a hazard ratio of 0.76 (95% CI: 0.60, 0.95) for a low-fat diet intervention (8).

Although limited, these intervention trials suggest some breast cancer benefit among postmenopausal women. However, their interpretation is affected by issues of statistical power, owing to moderate dietary differences between intervention and control groups and to interventions that have dietary goals and influences beyond fat reduction. Hence, observational studies have a continuing role in providing evidence on the long-standing dietary fat and postmenopausal breast cancer hypothesis.

Observational studies of diet and disease encounter a number of challenges. Most important is measurement error in dietary assessment. Specifically, the measurement properties of fat density are not known for any available dietary assessment methodology, nor is there an established objective measure that can be used to elucidate and accommodate such properties. We do know from repeat application of the same assessment procedure at different time points that there is a major random measurement error component to fat density assessment, but the question of systematic measurement error as a function of such subject characteristics as body mass, age, and ethnicity remains elusive in the absence of a fat consumption biomarker.

A second challenge relates to distinguishing an association of breast cancer with a high-fat diet from that with a high-energy diet, given the high energy concentration of fat compared with other macronutrients. Fortunately, a doubly labeled water (DLW) technique allows objective measurement of short-term total energy expenditure, which can be used to develop calibrated (corrected) estimates of energy consumption, as has been done in WHI cohorts (9, 10).

A third issue is the overcorrection that could arise through the inclusion of factors that may mediate an association of dietary fat with disease. For example, studies in WHI cohorts relating calibrated energy consumption to various cancers (11) and to vascular diseases (12, 13) revealed positive associations that were not evident by using uncalibrated FFQ energy consumption estimates. However, the breast cancer association appeared to be rather completely mediated by body mass index (BMI) (weight (kg)/height $(m)^2$). Inclusion of BMI data in the disease risk model could lead to overcontrol in total energy and fat density association analyses.

Cohort studies more recent than those included in the report by Hunter et al. (4) have continued to yield mixed results. A 2003 report from the European Prospective Investigation of Cancer-Norfolk cohort reported a positive association between fat consumption and breast cancer when analyses were based on food diaries (records) but not when FFQ data were substituted (14). A similar finding arose from a 2006 analysis from the Women's Health Initiative Dietary Modification Trial comparison group (DM-C) (15). However, a later analysis from 4 United Kingdom cohorts, including European Prospective Investigation of Cancer-Norfolk, did not find an association between dietary fat and breast cancer when using either food diaries or FFQs (16). Other recent reports from cohorts having large numbers of breast cancer cases found either a positive association (17) or no evidence of association (18, 19) when using FFQs for dietary assessment.

Here we report observational data analyses from WHI cohorts on dietary fat and total energy in relationship to breast cancer incidence, with emphasis on the influence of the biomarker calibration of energy and the comparison of associations that use 4DFRs with those that use FFQs. Because established biomarkers are unavailable for many nutrients and foods, Freedman et al. (20) recommended the use of a second self-report, such as multiple-day food records or recalls, as a reference instrument in place of a biomarker to calibrate a main self-report assessment. This approach is applied here to fat density and total energy by using either 4DFRs or three 24-hour dietary recalls (24HRs) as reference instruments in a WHI nutritional biomarker study context. The presentation includes updated case-control analyses by using food records in the DM-C, as well as cohort analyses by using FFQ dietary data in this comparison group and in the larger WHI observational study.

MATERIALS AND METHODS

Participants

The design of the WHI clinical trial and observational study and corresponding baseline enrollee characteristics have been presented (5, 6, 21, 22). Briefly, all subjects were postmenopausal women 50–79 years of age when they were enrolled at 40 US clinical centers during 1993–1998. The clinical trial enrolled 68,132 women in either or both the DM Trial (48,835 women) or overlapping postmenopausal hormone therapy trials (27,347 women). The DM Trial randomly assigned 40% of enrollees (19,541 women) to a low-fat eating pattern intervention and 60% (29,294 women) to a usual diet comparison group. The WHI observational study enrolled 93,676 women from essentially the same catchment populations in a companion prospective cohort study, similar to the clinical trial in data collection and clinical outcome ascertainment.

Dietary assessment

The provision of a completed 4DFR was an eligibility criterion for the DM Trial, as was an estimated fat density of

0.32 or greater assessed by using a WHI FFQ. Major exclusion criteria for the DM Trial included any prior breast cancer, colorectal cancer, or other cancer except nonmelanoma skin cancer within the last 10 years or a predicted survival of less than 3 years (6). The analyses presented here are based on the 29,294 women from the DM-C, along with 83,101 women from the WHI observational study who satisfied the DM Trial eligibility criteria just listed. Women in the observational study also provided a WHI FFQ as part of their enrollment process. There was no exclusion based on estimated fat density in the observational study.

The WHI FFQ collects frequency of intake and portion size information for the past 3 months of 122 foods or food groups, along with 19 adjustment questions focusing on dietary fat and 4 summary questions (23). Women providing 4DFRs were given a recording booklet along with instruction in accurate recording by means of a 15-minute videotape and 15–30 minutes of personal instruction from certified staff. The 4DFR booklets are stored centrally, with nutrient analysis taking place on a case-control basis at the Fred Hutchinson Cancer Research Center (Seattle, Washington).

Nutrition biomarker study

The WHI Nutrition and Physical Activity Assessment Study (NPAAS) enrolled 450 weight-stable postmenopausal women from the WHI observational study during 2007–2009 (10). These women were recruited from observational study enrollees at 9 WHI clinical centers. Black and Hispanic women were oversampled, as were women with extreme BMI values and relatively younger postmenopausal women.

Women were excluded from NPAAS for having any medical condition precluding participation, weight instability in the preceding months, or travel plans during the study period. A 20% reliability subsample repeated the entire biomarker study protocol at approximately 6 months after the original protocol application.

The NPAAS protocol involved 2 clinical center visits separated by a 2-week period, along with at-home activities. The first visit included eligibility confirmation; informed consent; measurement of height and weight by using a standardized protocol; DLW dosing for short-term energy expenditure assessment; completion of a FFQ and dietary supplement and other questionnaires; and collection of blood and urine specimens after DLW dosing. Between the 2 clinic visits, participants completed a 4DFR and collected urine over a 24-hour period on the day prior to the second clinic visit.

At the second clinic visit, the 24-hour urine samples were delivered to clinic staff, participants provided additional urine specimens and a fasting blood sample, and 4DFRs were reviewed. The first of three 24HRs was obtained in the 1–3 weeks after visit 2 and monthly thereafter.

Dietary data from each of the 3 methods were analyzed for nutrient content by using the University of Minnesota's (St. Paul, Minnesota) Nutrition Data Systems for Research (http://www.ncc.umn.edu/products/ndsr.html), which derives from the US Department of Agriculture's (Washington, DC) Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/) and its periodic revisions, as well as information from food manufacturers.

Total energy expenditure during the 2-week protocol was estimated from relative urinary elimination rates of oxygen-18 and deuterium. In weight-stable persons, total energy consumption over a 2-week period is objectively estimated by this procedure. Similarly, protein consumption was objectively estimated by 6.25 × 24-hour urinary nitrogen / 0.81.

Outcome ascertainment and breast cancer cases

Clinical outcomes (24) were reported semiannually in the clinical trial through the end of the intervention period and annually thereafter, and they were reported annually in the observational study by self-administered questionnaire. Invasive breast cancer occurrences were confirmed by review of medical records and pathology reports by physician-adjudicators at local clinical centers and were classified centrally, including coding of histology, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) overexpression by using the National Cancer Institute's (Bethesda, Maryland) Surveillance Epidemiology and End Results coding system.

A report by Freedman et al. (15) included the initial 603 (invasive) breast cancer cases from the DM-C, along with 1,206 controls, matched 2-to-1 to the cases on baseline age, enrollment date, and clinical center. An additional 469 breast cancer cases had arisen in the DM-C by the end of the trial intervention period. For these later cases, 4DFRs were analyzed for use in case-only analyses in DM Trial reporting (5). Here, we report 4DFR-based case-control analyses by using the combined 1,072 cases and 1,206 controls. After exclusions for missing values for modeled variables in breast cancer association analyses, there were 902 cases and 1,059 controls for 4DFR-based association analyses.

At the end of the clinical trial intervention period, all participating WHI subjects were invited to re-enroll for an additional 5 years of nonintervention follow-up, and 81% chose to do so. FFQ-based association analyses include follow-up through September 30, 2010, in both the DM-C and the observational study. The baseline FFQ fat density assessments in the DM Trial are distorted by the use of the FFQ in eligibility screening. Women who meet the 0.32 FFQ fat density DM Trial eligibility criterion tend to have a positive random error component to their assessed fat density, leading to fat density overestimation that averages approximately 3%. Therefore, the DM-C component of analyses presented here uses FFQ data collected 1 year after enrollment, and only cases occurring after the 1-year data collection are included. Following exclusions for missing data, the combined cohort FFQ-based analyses included 5,061 invasive breast cancer cases and 98,365 noncases.

Statistical methods

Case-control analyses of 4DFR-based dietary data used unconditional logistic regression analyses with baseline 5-year age category, enrollment year, race/ethnicity, current smoking status, education, prior postmenopausal hormone therapy use (ever prior use of estrogen alone, ever prior use of estrogen plus progestin); randomization assignment for women in hormone therapy trials, Gail model 5-year risk score (25), and

Table 1. Characteristics of Breast Cancer Cases and Controls in 4DFR-Based Case-Control Analyses and FFQ-Based Cohort Analyses in the WHI, 1994–2010

Characteristic	DM-C Case-Control Sample				DM-C/Observational Study Cohort			
	Cases (n = 902)		Controls (n = 1,059)		Cases (n = 5,061)		Controls (n = 98,365)	
	No.	%	No.	%	No.	%	No.	%
Age, years[a]								
≤55	142	15.74	150	14.16	713	14.09	15,597	15.86
>55–60	205	22.73	246	23.23	966	19.09	19,720	20.05
>60–65	236	26.16	285	26.91	1,212	23.95	22,119	22.49
>65–70	190	21.06	246	23.23	1,216	24.03	21,588	21.95
>70–75	102	11.31	107	10.10	708	13.99	14,195	14.43
>75	27	2.99	25	2.36	246	4.86	5,146	5.23
Race/ethnicity								
White	776	86.03	886	83.66	4,504	88.99	82,528	83.90
Black	69	7.65	97	9.16	280	5.53	7,805	7.93
Hispanic	22	2.44	26	2.46	96	1.90	3,455	3.51
Other/unknown	35	3.88	50	4.72	181	3.58	4,577	4.65
Education								
≤ High school	159	17.63	231	21.81	874	17.27	20,900	21.25
School after high school	346	38.36	434	40.98	1,823	36.02	36,589	37.20
College degree or higher	397	44.01	394	37.20	2,364	46.71	40,876	41.56
Body mass index[a,b]								
<25 (or college degree)	215	23.84	291	27.48	1,853	36.61	37,406	38.03
25–<30	303	33.59	368	34.75	1,714	33.87	33,733	34.29
≥30	384	42.57	400	37.77	1,494	29.52	27,226	27.68
Smoking status[a]								
Never	442	49.00	533	50.33	2,460	48.61	50,425	51.26
Past	404	44.79	466	44.00	2,305	45.54	41,850	42.55
Current	56	6.21	60	5.67	296	5.85	6,090	6.19
Total recreational physical activity, METs/week								
≤2.6	294	32.59	333	31.44	1,258	24.86	24,837	25.25
>2.6–9.3	273	30.27	301	28.42	1,241	24.52	24,545	24.95
>9.3–19.0	219	24.28	253	23.89	1,334	26.36	24,537	24.94
>19.0	116	12.86	172	16.24	1,228	24.26	24,446	24.85

Table continues

estimated recreational physical activity as control variables in all analyses.

The principal exposure measures considered were log-transformed fat density and log-transformed total energy, the latter with and without biomarker calibration by using the calibration equation described below. For convenient interpretation, odds ratios are given for a 40% increment in fat density and a 20% increment in total energy. These are rather large increments (e.g., 35% vs. 25% for fat density and 2,100 vs. 1,750 kcal/day for total energy), but are well within the range of estimated values in WHI cohorts.

To explore potential mediation of these breast cancer associations, we conducted additional analyses that added BMI to the disease risk model, with and without biomarker calibration of total energy consumption.

Cohort analyses of the FFQ-based dietary data used the Cox model (26) and included these same primary exposure and control variables, except that follow-up time for the DM-C component was time since the 1-year visit following randomization. Also, the Cox model baseline hazard rate was stratified on cohort (DM-C or observational study), age at FFQ completion (at either enrollment or 1-year visit) in 5-year categories, hormone therapy treatment assignment if randomized in the hormone therapy trial, and whether or not the women consented to postintervention follow-up in a time-dependent fashion. Censoring time for noncases was defined as the earliest of either September 30, 2010, or the date of last follow-up contact.

Standard errors for odds ratios and hazard ratios from analyses that included calibrated exposures used a bootstrap estimation procedure (1,000 bootstrap samples).

Table 1. Continued

Characteristic	DM-C Case-Control Sample				DM-C/Observational Study Cohort			
	Cases ($n = 902$)		Controls ($n = 1,059$)		Cases ($n = 5,061$)		Controls ($n = 98,365$)	
	No.	%	No.	%	No.	%	No.	%
Family history of breast cancer (first-degree relative)	169	20.51	141	14.42	982	21.10	13,615	15.11
Gail 5-year risk, %[c]								
>1.26	250	27.72	322	30.41	1,171	23.14	30,543	31.05
1.27–1.80	295	32.71	379	35.79	1,645	32.50	33,740	34.30
>1.80	357	39.58	358	33.81	2,245	44.36	34,082	34.65
Postmenopausal estrogen use[a]								
Never	572	63.41	655	61.85	3,214	63.51	60,630	61.64
Current/former	330	36.59	404	38.15	1,847	36.49	37,735	38.36
Postmenopausal estrogen plus progestin use[a]								
Never	579	64.19	739	69.78	3,167	62.58	69,608	70.77
Current/former	323	35.81	320	30.22	1,894	37.42	28,757	29.23
Study component								
DM-C	902	100.00	1,059	100.00	1,139	22.51	21,913	22.28
Observational study	0		0		3,922	77.49	76,452	77.72
Enrollment year								
1995 or earlier	236	26.16	291	27.48	1,332	26.32	23,104	23.49
1996	300	33.26	338	31.92	1,633	32.27	31,071	31.59
1997	274	30.38	314	29.65	1,205	23.81	24,655	25.06
1998	92	10.20	116	10.95	891	17.61	19,535	19.86

Abbreviations: 4DFR, 4-day food record; DM-C, Women's Health Initiative Dietary Modification Trial comparison group; FFQ, food frequency questionnaire; METs, metabolic equivalent units; WHI, Women's Health Initiative.

[a] At baseline in the case-control sample and observational study cohort; at year 1 in the DM-C cohort.

[b] Body mass index is weight (kg)/height $(m)^2$.

[c] The model of Gail et al. (25) assesses 5-year breast cancer risk as a function of age, race, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and prior benign breast biopsy history.

Energy calibration equations that update those of Prentice et al. (10) by including all variables in the disease risk model (27–30) were developed by linear regression of log(DLW energy). For comparison, corresponding energy calibrations were also developed by using 4DFR and 24HR total energy in place of the DLW biomarker. These food record and recall sources were also used to develop calibrated FFQ estimates of log(fat density) and were used in the breast cancer association analyses presented here. All *P* values are 2-sided.

All women provided written informed consent for their various components of WHI participation, and all procedures were reviewed and approved by the institutional review board of each participating institution.

RESULTS

Table 1 shows the distribution of key characteristics for both the 4DFR- and FFQ-based analyses. Geometric means for fat density, total energy (in kcal/day), and biomarker-calibrated total energy (in kcal/day) from 4DFRs were 32.4 (10th–90th percentile range, 24.5–41.4), 1,654.0 (10th–90th percentile range, 1,221.9–2,238.7), and 2,188.0 (10th–90th percentile range, 1,902.3–2,515.1) for cases, respectively, and 31.7 (10th–90th percentile range, 24.1–40.8), 1,660.9 (10th–90th percentile range, 1,227.2–2,221.2), and 2,173.7 (10th–90th percentile range, 1,890.7–2,542.7) for controls, respectively. Corresponding geometric means for these same variables from FFQs were 30.1 (10th–90th percentile range, 20.6–42.2), 1,500.5 (10th–90th percentile range, 941.7–2,323.0), and 2,119.1 (10th–90th percentile range, 1,855.6–2,440.1) for cases, respectively, and 30.0 (10th–90th percentile range, 20.4–42.2), 1,476.5 (10th–90th percentile range, 901.9–2,343.9), and 2,110.4 (10th–90th percentile range, 1,843.7–2,429.3) for controls, respectively.

Table 2 shows coefficients and standard errors from linear regression of log(DLW energy) on log(self-reported energy) and the other factors shown, with self-reported energy data from either FFQs or 4DFRs. These calibration equations are based on data from the 450 women in the WHI NPAAS, and they augment those previously given (10) by including all variables modeled in the breast cancer analyses presented here. This augmentation turns out to have little influence on the calibrated energy estimates or on resulting breast cancer association analyses. Table 2 also includes coefficients and standard

Table 2. Calibration Equation Coefficients[a] for Total Energy Consumption and Fat Density for Various Dietary Measures and Reference Assessments Based on Data From 450 Women in the Women's Health Initiative Nutrition and Physical Activity Assessment Study, 2007–2010

Reference/Dietary Assessment Regression Variable	Total Energy								Fat Density			
	Doubly Labeled Water[b]/FFQ		Doubly Labeled Water/4-Day Food Record		4-Day Food Record /FFQ		Three 24-Hour Dietary Recalls/FFQ		4-Day Food Record /FFQ		Three 24-Hour Dietary Recalls/FFQ	
	Coefficient (SE)[c]	R^2	Coefficient (SE)	R^2	Coefficient (SE)	R^2	Coefficient (SE)	R^2	Coefficient (SE)	R^2	Coefficient (SE)	R^2
Intercept	756.07 (7.99)*		766.74 (8.00)*		703.62 (11.77)*		725.86 (13.16)*		232.40 (9.51)*		233.08 (10.7)*	
Dietary energy	5.26 (1.71)*	3.9	17.65 (2.93)*	7.4	19.55 (2.57)*	15.4	23.12 (2.90)*	15.0	−4.96 (2.08)*	0.0	−5.06 (2.36)*	0.1
Dietary fat density	2.09 (3.38)	0.4	−3.17 (3.41)	0.0	14.55 (5.04)*	1.6	1.80 (5.63)	0.0	52.30 (4.07)*	29.2	52.85 (4.58)*	24.5
Body mass index[d]	1.40 (0.13)*	25.8	1.45 (0.12)*	27.3	−0.13 (0.19)	0.0	−0.32 (0.21)	0.6	0.16 (0.15)	0.3	0.15 (0.17)	0.2
Age	−0.88 (0.13)*	9.6	−0.83 (0.13)*	8.3	−0.35 (0.20)	0.8	−0.25 (0.22)	0.2	−0.05 (0.16)	0.0	−0.32 (0.18)	0.1
Race/ethnicity												
Black	−2.69 (2.24)	1.8	−2.87 (2.12)	2.0	−1.69 (3.41)	0.2	−0.10 (3.82)	0.7	0.19 (2.75)	1.1	0.18 (3.11)	1.4
Hispanic	−7.51 (2.35)*		−8.10 (2.22)*		−0.07 (3.45)		0.59 (3.87)		−5.22 (2.78)		−7.97 (3.15)*	
Other/unknown	−3.40 (4.02)		−2.95 (3.87)		−4.21 (6.00)		−9.13 (6.70)		6.22 (4.85)		−0.63 (5.45)	
Enrollment year	0.39 (0.72)	0.0	0.48 (0.70)	0.1	−0.20 (1.07)	0.0	−1.59 (1.20)	0.5	0.33 (0.87)	0.0	−0.51 (0.98)	0.1
Gail risk score[e]	−0.49 (1.14)	0.0	−0.35 (1.11)	0.0	−0.38 (1.73)	0.0	0.13 (1.93)	0.0	−0.05 (1.39)	0.0	2.66 (1.57)	0.5
Prior postmenopausal estrogen use	0.30 (1.42)	0.0	0.66 (1.38)	0.0	−2.25 (2.11)	0.3	0.26 (2.36)	0.0	0.67 (1.70)	0.1	0.29 (1.92)	0.0
Prior postmenopausal estrogen plus progestin use	1.36 (1.48)	0.2	1.20 (1.44)	0.1	1.03 (2.21)	0.1	0.14 (2.47)	0.0	−1.64 (1.78)	0.2	−3.82 (2.01)	0.7
Current smoker	−5.71 (4.53)	0.2	−5.18 (4.38)	0.2	−0.44 (6.71)	0.0	−7.32 (7.49)	0.3	5.61 (5.42)	0.2	10.24 (6.09)	0.5
Physical activity (METs/week)	0.09 (0.05)	0.5	0.09 (0.05)	0.5	−0.06 (0.07)	0.1	−0.06 (0.08)	0.0	−0.04 (0.06)	0.1	0.02 (0.07)	0.0
Education												
School after high school	−0.63 (2.33)	0.0	−1.28 (2.26)	0.0	3.07 (3.36)	0.4	2.25 (3.76)	1.7	0.80 (2.71)	0.2	−2.89 (3.06)	0.2
College degree or higher	0.20 (2.33)		−0.99 (2.27)		4.76 (3.35)		8.81 (3.74)*		−1.20 (2.70)		−3.32 (3.05)	
Total[f]		42.6		46.0		18.9		19.0		31.4		28.4

Abbreviations: FFQ, food frequency questionnaire; METs, metabolic equivalent units; SE, standard error.

* $P = 0.05$.

[a] For display convenience, all linear regression coefficients and standard errors are multiplied by 100.

[b] Doubly labeled water used to assess total energy consumption.

[c] In these equations, age is centered by 70.93 years; body mass index is centered by 28.2; log(energy) is centered by 7.27 log(kcal); log(fat density) is centered by 1.26; Gail model (25) 5-year risk score is centered by 1.36%; enrollment year is centered by 1996.2; recreational physical activity is centered by 13.9 METs/week.

[d] Weight (kg)/height $(m)^2$.

[e] The risk score of Gail et al. (25) assesses 5-year breast cancer risk as a function of age, race, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and prior benign breast biopsy history.

[f] Tabular R^2 values have been rescaled to add to the total R^2 but may not do so exactly because of rounding.

errors for FFQ energy calibration using either 4DFR energy or 24HR energy data from NPAAS in place of the DLW biomarker. Table 2 also shows FFQ fat density calibration equation coefficients using either 4DFR or 24HR fat density data from NPAAS as the reference instrument for calibration. These equations arise from the linear regression of log(fat density) from the reference instrument on log(FFQ fat density) and the other factors listed.

The fraction of log(DLW energy) explained by the FFQ-based calibration equation is 42.6%, but it is only 18.9% or 19.0% when using 4DFRs or 24HRs, respectively, in place of the DLW biomarker (Table 2). Furthermore, the contributions of BMI, age, and race to the DLW-based energy calibration, which involves correction for systematic bias in the FFQ assessments in relationship to these variables, are not evident when using either of the self-reported assessments for calibration. The correlation in NPAAS between biomarker-calibrated log(energy) estimates and those based on 4DFRs or 24HRs as the reference instrument are 0.38 and 0.23, respectively. In contrast, the correlation between the log(energy) estimates from using the 2 self-reports as the reference assessment was 0.90, reflecting the previously noted (10) substantial measurement error correlations between 4DFR and 24HR estimates of log(energy). The log(fat density) calibration equations explain approximately 30% of the variation in log(4DFR fat density) or log(24HR fat density), with almost all of the signal from the corresponding log(FFQ fat density) assessment.

Table 3 shows estimated odds ratios for a 40% increment in 4DFR fat density and a 20% increment in 4DFR total energy, with and without biomarker calibration of energy, from the DM-C case-control study. A 40% increment in fat density is associated with a breast cancer odds ratio of 1.18 (95% CI: 0.99, 1.39) following control for calibrated energy. This odds ratio was 1.19 (95% CI: 1.00, 1.41) following further control for BMI in the disease incidence model. A 20% increment in calibrated energy is associated with a nonsignificant odds ratio of 1.08 (95% CI: 0.89, 1.31), which was not at all evident after controlling for BMI.

Table 4 shows corresponding hazard ratio estimates from combined DM-C and observational study cohorts and for these 2 cohorts separately by using FFQ data. In combined cohort analyses, a weaker association of FFQ fat density with breast cancer emerges, with a hazard ratio of 1.03 (95% CI: 0.99, 1.07) for a 40% increment in FFQ fat density following control for calibrated energy. This hazard ratio becomes 1.05 (95% CI: 1.00, 1.09) following control for BMI. A substantially larger hazard ratio of 1.22 (95% C: 1.15, 1.30) is associated with a 20% increment in calibrated energy consumption, which seems to be wholly mediated by BMI. These hazard ratio patterns evidently agree well between the DM-C and observational study cohorts in the separate analyses presented in Table 4.

Additional analyses were carried out to examine the robustness of the findings in Tables 3 and 4 to certain exclusions. For example, the Table 3 odds ratios were affected little by the exclusion of cases who had more than 2 years without a mammogram prior to diagnosis and the exclusion of controls who had more than 2 years without a mammogram prior to their selection as controls. Similarly, the odds ratios in Table 3 were essentially unchanged after exclusion of the 26 women who were taking tamoxifen or selective estrogen receptor modifiers prior to diagnosis (for cases) or control selection.

Table 3. Odds Ratios[a] for a 40% Increment in Fat Density and a 20% Increment in Total Energy Consumption Based on 4-Day Food Records From 902 Invasive Breast Cancer Cases and 1,059 Controls From the Women's Health Initiative Dietary Modification Trial Comparison Group, 1994–2005

Energy Calibration	Control for BMI[b,c]	Fat Density		Total Energy	
		OR	95% CI	OR	95% CI
No	No	1.23	1.04, 1.44	0.95	0.88, 1.02
Yes	No	1.18	0.99, 1.39	1.08	0.89, 1.31
No	Yes	1.21	1.03, 1.43	0.94	0.87, 1.02
Yes	Yes	1.19	1.00, 1.41	0.72	0.44, 1.17

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

[a] From unconditional logistic regression of case versus control status on log(4-day food recall fat density), log(4-day food recall energy) with or without biomarker calibration, date of enrollment, age category at study entry, race/ethnicity, current smoking, education, postmenopausal hormone use (ever used estrogen alone, ever used estrogen plus progestin), randomization group in hormone therapy trials, Gail model risk score, and estimated recreational physical activity. The Gail model risk score (25) assesses 5-year breast cancer risk as a function of age, race, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and prior benign breast biopsy history.

[b] Weight (kg)/height $(m)^2$.

[c] This potential mediating variable was added to the logistic regression model.

Similarly, the hazard ratios in Table 4 for fat density or total energy were affected little by censoring the follow-up time for a woman when she was 2 years from her most recent mammogram, or by excluding the 83 women taking tamoxifen or selective estrogen receptor modifiers during follow-up and prior to diagnosis for cases.

Additional analyses examined the results in Tables 3 and 4 on breast cancer subsets defined by estrogen receptor, progesterone receptor, or HER2 expression. There was little evidence of important differences in the association strength of fat density or total energy with breast cancer risk according to these tumor characteristics.

Table 5 presents analyses corresponding to the combined cohort analyses of Table 4, when 4DFRs or 24HRs are used as reference instruments to calibrate FFQ fat density and, in some analyses, to calibrate total energy as well. Fat density hazard ratios are somewhat deattenuated compared with those in Table 4. Total energy hazard ratios are likewise somewhat deattenuated but do not yield the positive associations seen with DLW calibration.

DISCUSSION

Regression calibration provides a valuable approach to strengthening nutritional epidemiology research. This approach essentially involves replacing unmeasured dietary variables of interest with estimates of their conditional expectation given other modeled variables in the disease risk model (27–30).

Table 4. Hazard Ratios[a] for a 40% Increment in Fat Density and a 20% Increment in Total Energy Consumption Based on FFQ Data From 5,061 Invasive Breast Cancer Cases and 98,365 Noncases From the Women's Health Initiative Dietary Modification Trial Comparison Group and Observational Study Cohorts, 1994–2010

Energy Calibration by Group	Control for BMI[b,c]	Fat Density		Total Energy	
		HR	95% CI	HR	95% CI
Combined cohorts					
No	No	1.05	1.01, 1.09	1.01	1.00, 1.03
Yes	No	1.03	0.99, 1.07	1.22	1.15, 1.30
No	Yes	1.04	1.00, 1.08	1.01	0.99, 1.02
Yes	Yes	1.05	1.00, 1.09	0.94	0.73, 1.22
Dietary Modification Trial comparison group					
No	No	1.07	0.96, 1.18	1.00	0.97, 1.03
Yes	No	1.03	0.92, 1.14	1.33	1.17, 1.50
No	Yes	1.05	0.95, 1.16	0.99	0.96, 1.02
Yes	Yes	1.05	0.94, 1.17	0.92	0.54, 1.58
Observational study					
No	No	1.05	1.01, 1.09	1.02	1.00, 1.03
Yes	No	1.03	0.99, 1.08	1.19	1.11, 1.28
No	Yes	1.04	1.00, 1.08	1.01	0.99, 1.03
Yes	Yes	1.05	1.00, 1.10	0.95	0.70, 1.29

Abbreviations: BMI, body mass index; CI, confidence interval; FFQ, food frequency questionnaire; HR, hazard ratio.

[a] Based on Cox regression on log(FFQ fat density), log(FFQ total energy), with and without biomarker calibration, date of cohort enrollment, race/ethnicity, education, smoking, postmenopausal hormone use (ever use of estrogen alone, ever use of estrogen plus progestin), Gail model risk score, and estimated recreational physical activity with baseline hazard rate stratification on age category at FFQ completion, randomization group if in the hormone therapy trials, cohort (Dietary Modification Trial comparison group, observational study) and Women's Health Initiative Extension Study participation (time dependent). The Gail model risk score (25) assesses 5-year breast cancer risk as a function of age, race, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and prior benign breast biopsy history.

[b] This potential mediating variable was added to the Cox model regression vector.

[c] BMI is weight (kg)/height (m)^2.

There are a variety of other measurement error correction approaches, including some that yield estimates that are technically consistent for targeted parameters, but simple regression calibration tends to give parameter estimates that are essentially unbiased and considerably more efficient than many of these competitive estimators in the type of setting considered here (31).

Whether BMI should be regarded as a confounder, a mediator, or both in analyses that relate total energy consumption to disease risk likely depends strongly on the time period in question for the targeted energy consumption. If only a recent average daily energy consumption (e.g., over the past year) is targeted, then BMI will contribute to disease risk beyond short-term energy consumption, because it reflects energy consumption over earlier years or decades. On the other hand, if the underlying dietary exposure is, for example, average daily energy consumption over multiple years or even decades prior to cohort enrollment, it is plausible that BMI would not contribute further to disease risk determination, and BMI can be excluded from the disease risk model. Hazard ratio estimates can alternatively be obtained by beginning with a disease risk model that includes BMI and then "averaging out" BMI. The resulting hazard ratio estimates can depend rather strongly on biomarker measurement error patterns over time (13), emphasizing the need for longitudinal data on the biomarker and other variables for a satisfactory resolution of the joint energy and BMI association with risk.

Relative to the preceding paragraph, it is also interesting that the magnitude of the fat density association differed little according to whether BMI was or was not included in the disease risk model. The estimated odds ratios when using 4DFRs are considerably larger than corresponding hazard ratios when using FFQs, which could reflect more substantial coefficient attenuation due to measurement error with FFQs.

The utility of using a second self-report, rather than a biomarker, as a reference instrument (20) is another important topic in implementing a regression calibration approach. The energy calibration equations that use this approach (Table 2) do not align closely with that based on the strong DLW biomarker. In particular, the important dependencies on BMI, age, and ethnicity in the DLW-based calibration are not identified when using 4DFRs or 24HRs as reference instruments, presumably because of shared systematic biases related to these variables by each of the 3 self-reported energy assessments

Table 5. Hazard Ratios[a] for a 40% Increment in Fat Density and a 20% Increment in Total Energy Consumption Based on FFQ Data From 5,061 Invasive Breast Cancer Cases and 98,365 Noncases From the Women's Health Initiative Dietary Modification Trial Comparison Group and Observational Study Cohorts, 1994–2010

Reference Instrument for Calibration		Control for BMI[b,c]	Fat Density		Total Energy	
Fat Density	Total Energy		HR	95% CI	HR	95% CI
4-Day food record	Doubly labeled water	No	1.06	0.98, 1.15	1.22	1.14, 1.30
		Yes	1.08	1.00, 1.18	0.98	0.76, 1.25
24-Hour dietary recall	Doubly labeled water	No	1.06	0.98, 1.15	1.22	1.14, 1.30
		Yes	1.08	0.99, 1.18	0.98	0.76, 1.25
4-Day food record	4-Day food record	No	1.10	1.01, 1.21	1.03	0.94, 1.12
		Yes	1.06	0.97, 1.16	1.03	0.96, 1.11
24-Hour dietary recall	24-Hour dietary recall	No	1.12	1.04, 1.20	1.01	0.93, 1.09
		Yes	1.07	0.99, 1.16	1.03	0.96, 1.10

Abbreviations: BMI, body mass index; CI, confidence interval; FFQ, food frequency questionnaire; HR, hazard ratio.

[a] Based on Cox regression on log(FFQ fat density), log(FFQ total energy), with and without biomarker calibration, date of cohort enrollment, race/ethnicity, education, smoking, postmenopausal hormone use (ever use of estrogen alone, ever use of estrogen plus progestin), Gail model risk score, and estimated recreational physical activity with baseline hazard rate stratification on age category at FFQ completion, randomization group if in the hormone therapy trials, cohort (Dietary Modification Trial comparison group, observational study) and Women's Health Initiative Extension Study participation (time dependent). The Gail model risk score (25) assesses 5-year breast cancer risk as a function of age, race, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and prior benign breast biopsy history.

[b] Weight (kg)/height $(m)^2$.

[c] This potential mediating variable was added to the Cox model regression vector.

(10). Accordingly, the breast cancer associations with energy, identified by using the DLW biomarker, are not at all evident when substituting a self-reported energy assessment as a reference instrument (Table 5). It may be that calibration equations that use a second self-report as a reference assessment can correct for attenuation due to pure noise in a dietary assessment, because such noise components may be essentially independent among different assessment instruments but may not be so useful for systematic bias correction. Without a suitable, objective assessment, the researcher is not in a position to assess the importance of systematic biases. These considerations argue for high priority of the development of biomarkers for additional nutrients and foods in the future nutritional epidemiology research agenda.

In summary, the challenging measurement error considerations discussed here imply that there may be important public health implications related to dietary composition and energy imbalance that will be elucidated only when nutritional epidemiology methodologies are strengthened. With limited current opportunities for additional full-scale dietary intervention trials, it seems that observational studies that incorporate calibrated dietary exposure assessment, especially biomarker-based assessment, present an important research strategy for advancing this vitally important research area in the near term.

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