Research Article

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An Exploratory Study of Respiratory Quotient Calibration and Association with Postmenopausal Breast Cancer

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Abstract

Background: The respiratory quotient (RQ), defined as the ratio of carbon dioxide exhaled to oxygen uptake, reflects substrate utilization when energy is expended. Fat and alcohol have RQ values of approximately 0.7, compared with 1.0 for carbohydrate, and approximately 0.8 for protein. Here, the association between RQ and postmenopausal breast cancer risk is studied.

Methods: Paired RQ measurements were obtained, separated by approximately 6 months, for women in the reliability subset of a Women's Health Initiative (WHI) Nutrition and Physical Activity Assessment Study. Linear regression of the average of the paired log RQ assessments on a corresponding log food quotient (FQ) average and other study subject characteristics, including age, body mass index, race, and education, yielded calibration equations for predicting RQ.

Results: Calibration equations, using any of food frequency, food record, or dietary recall data, explained an appreciable fraction of measured log RQ variation, and these were used to compute calibrated RQ estimates throughout WHI cohorts. Calibrated RQ estimates using 4-day food record (4DFR) data related inversely ($P = 0.004$) to (invasive) breast cancer risk in the WHI Dietary Modification trial comparison group, and corresponding RQ estimates using food-frequency data related inversely ($P = 0.002$) to breast cancer incidence in this cohort combined with the larger WHI observational study.

Conclusion: Although preliminary, these analyses suggest a substantially higher postmenopausal breast cancer risk among women having relatively low RQ.

Impact: RQ elevation could provide a novel target for breast cancer risk reduction. *Cancer Epidemiol Biomarkers Prev; 22(12); 2374–83. ©2013 AACR.*

Introduction

There is little reliable information on energy expenditure patterns in relation to chronic disease risk. Studies relating self-reported macronutrient consumption to risk reveal few established or probable associations (1, 2), perhaps because of dietary assessment measurement issues. The incorporation of objective measures of energy consumption and expenditure in epidemiologic studies provides an attractive strategy to advance epidemiologic research in this area.

Biomarker substudies within Women's Health Initiative (WHI) cohorts include objective measures of short-term total energy expenditure using a doubly-labeled water (DLW) technique, and protein consumption from 24-hour urinary nitrogen (3, 4). Linear regression of log-biomarker values on corresponding log self-report dietary assessments in combination with such study subject characteristics as body mass index (BMI), age, and race led to "calibrated" total energy and protein consumption assessments that make an adjustment for measurement error in self-reported dietary assessment. Associations of calibrated total energy and protein estimates with risk were found for several diseases (5–9), even though mostly not evident when using uncalibrated dietary data.

The Nutrition and Physical Activity Assessment Study (NPAAS) in WHI also included indirect calorimetry (IC), which assesses oxygen uptake and carbon dioxide exhalation. Under controlled conditions, IC provides a noninvasive assessment of resting energy expenditure (REE) during the assessment period (10). Recently, the REE data were used, in conjunction with DLW and dietary self-report data, to develop calibrated

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estimates of activity-related energy expenditure (11) in WHI cohorts. Included in the IC instrument's data output is a respiratory quotient (RQ) assessment, which reflects substrate oxidation during the IC testing period.

The RQ is defined as the ratio of carbon dioxide exhaled to oxygen inhaled as the body expends its energy reserves. For carbohydrate oxidation the RQ is 1.0, whereas for fat and alcohol it is approximately 0.7, and for protein it is close to 0.8, depending on the specific amino acids undergoing oxidation during the test (12). Therefore, RQ estimation provides the opportunity to contrast fat and carbohydrate (stored as glycogen) utilization in relation to chronic disease risk.

Here, we develop RQ calibration equations based on corresponding food quotients (FQ), defined (13) as the sum of 0.7, 0.8, and 1.0 times the estimated percentage of energy from fat plus alcohol, protein, and carbohydrate respectively, with dietary data from each of three assessment procedures, in combination with pertinent study subject characteristics. RQ estimates using each dietary assessment procedure are then developed for women throughout WHI cohorts, and these estimates are associated with subsequent invasive breast cancer incidence during WHI cohort follow-up.

Materials and Methods

WHI cohorts

The design of the WHI clinical trial (CT) and observational study (OS) and enrollee characteristics have been presented previously (14–16). Briefly, all women were postmenopausal and in the age range of 50 to 79 when enrolled at 40 U.S. clinical centers between 1993 and 1998. The CT enrolled 68,132 women to either or both of the Dietary Modification (DM) trial (48,835 women) or to 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 (DM-C). The companion WHI OS is a prospective cohort study that enrolled 93,676 postmenopausal women in the age range of 50 to 79 years from 1994 to 1998. The OS and DM cohorts were drawn from essentially the same catchment populations, with substantial overlap in baseline data collection and in outcome-ascertainment procedures (17) during cohort follow-up. The WHI food-frequency questionnaire (FFQ; ref. 18) was administered at baseline and 1 year in the DM trial, and approximately every 3 years thereafter during the trial intervention period (ended April 8, 2005), and was administered at baseline and 3 years in the observational study. A 4DFR was also provided at baseline by women in the DM trial as a part of eligibility determination, and 24-hour dietary recalls (24HRs) were obtained for certain subsets of DM trial participants throughout the trial intervention period.

The NPAAS enrolled 450 weight-stable postmenopausal women from the observational study between 2007 and 2009 (4). These women were recruited from observational study enrollees at nine WHI clinical centers. Black and Hispanic women were oversampled as were women in the extremes of body mass index (BMI) and relatively younger postmenopausal women.

Women were excluded from the NPAAS for having any medical condition precluding participation, weight instability in 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. All women provided written informed consent for their WHI participation, and for their participation in the NPAAS.

NPAAS protocol and procedures

The NPAAS study protocol involved two clinical center visits separated by a 2-week period, along with at-home activities. The first visit included eligibility confirmation, informed consent, DLW dosing for short-term energy expenditure assessment, completion of FFQ and other questionnaires, collection of a blood specimen and spot urines after DLW dosing, and training in 4DFR completion. Between the two clinic visits, participants completed a 4DFR and collected 24-hour urine on the day prior to the second clinic visit.

At the second clinic visit, participants provided additional spot urine specimens and a fasting blood sample, 4DFRs were reviewed, and participants completed an IC protocol. The first of three 24HRs was obtained in the 1 to 3 weeks after visit 2, and then monthly thereafter for the other two.

IC was carried out using a standard protocol (19), with either a DeltaTrac II Respiratory Gas Analyzer (Datex-Ohmeda Inc.) or a SensorMedics VMAX metabolic cart (SensorMedics Inc). All metabolic carts were calibrated each day according to the manufacturer's instructions, and gases were monitored during each test. Participants arrived on day 15 after a 12-hour fast and rested in a semireclined position in a thermally neutral room for 30 minutes followed by a 30-minute test under a canopy (eight clinics) or using a non–rebreathing-fitted mouthpiece (one clinic). Data points were obtained every minute, but the first 10 minutes of collected data were not included in subsequent data analysis as 10 minutes are needed to achieve steady-state conditions. Steady state was defined as 10 minutes during which the oxygen consumption, minute ventilation, and the respiratory exchange ratio did not vary by more than 10% (19). Sixteen participants who did not reach a steady state or did not have at least 10 minutes of usable data were not included in data analysis.

Specimen handling and quality assurance procedures have been described previously (4). Blinded duplicates (5%) were included in all biomarker assessments.

Recovery biomarkers

Total energy expenditure was objectively estimated during the 2-week protocol from relative urinary elimination rates of oxygen-18 and deuterium (3, 4).

Dietary assessment in the NPAAS

Participants completed the self-administered WHI-FFQ (18) in English or Spanish. This FFQ includes 122 foods or food groups, 19 adjustment questions, and four summary questions, and was designed to assess typical dietary habits over the preceding three months in a multiethnic and geographically diverse population.

Participants viewed a 25-minute instructional video and received a food-record instruction booklet, in English or Spanish, at the first clinic visit. In addition, participants received a 12-page serving size booklet with photographs and other measuring devices. They completed 4 days of recording on alternate days (Sunday through Saturday) over the 2-week period between visits 1 and 2.

The three 24-hour dietary recalls for each participating woman were telephonically conducted by trained and certified study staff. Interviews targeted all food and beverages consumed during the previous 24 hours (midnight to midnight) using the U.S. Department of Agriculture (USDA)'s multiple pass method.

Dietary data from each of the three methods were analyzed for nutrient content using the University of Minnesota's Nutrition Data Systems for Research (NDS-R).

Outcome ascertainment and breast cancer cases and controls

Breast cancer and other clinical outcomes were reported semiannually in the clinical trial through the end of the intervention period, and annually in the observational study, by self-administered questionnaires (17). Invasive breast cancer occurrences were confirmed by review of medical records and pathology reports by physician–adjudicators at local clinical centers, and classified centrally using the National Cancer Institute (NCI)'s Surveillance, Epidemiology, and End Results (SEER) coding system.

Study cases and controls

A dietary fat and breast cancer report by Freedman and colleagues (20) included the initial 603 (invasive) breast cancer cases from the DM comparison group, along with 1,206 controls, 2:1 matched to the cases on baseline age, enrollment date, and clinical center. An additional 469 breast cancer cases had arisen in the DM comparison group by the end of the trial intervention period (April 8, 2005). 4DFRs for these additional cases were analyzed for use in case-only analyses in DM trial reporting (21). Here, we report analyses that associate estimated RQ from 4DFRs, using these combined 1,072 cases and 1,206 controls. Following exclusions for missing values for variables used in breast cancer risk modeling, there were 898 cases and 1,057 controls for these association analyses, in which 4DFRs were used to develop FQ estimates, and thus calibrated RQ estimates.

At the end of the clinical trial intervention period, all participating WHI women were invited to re-enroll for an additional 5 years of noninterventional follow-up, and 81% of women chose to do so. Association analyses of calibrated RQ using FFQ data include follow-up through September 30, 2010, in both the DM-C and the observational study. The baseline FFQ% energy from fat assessments in the DM trial are distorted by the use of the FFQ in eligibility screening (22). Therefore, the DM-C component of analyses uses FFQ data collected at 1-year following enrollment, and only cases occurring after the 1-year data collection are included. Following missing data exclusions, a total of 5,059 invasive breast cancer cases and 92,298 noncases are included in combined DM-C and observational study cohort analyses, in which FFQs were used to develop FQ and calibrated RQ estimates.

Statistical analysis

Measurement error analysis used a classical additive measurement model for the log-transformed RQ with "errors" that are independent of the underlying actual log RQ, and independent of other study subject characteristics.

Linear regression of log RQ on log FQ and other study subject characteristics was used to model IC-based RQ variation and to estimate the fraction of the total variance (R^2) in the log RQ that could be explained by log FQ and other pertinent variables. In line with the regression calibration approach to addressing measurement error in disease-association analyses (23–25), all variables included in the disease-association model (see following sections) for confounding control, are also included also in the calibration equations. The calibration equations include all variables used to oversample subsets of women for NPAAS participation, and they are expected to be applicable to the observational study cohort more generally, and also to the DM-C cohort.

Case–control analyses of calibrated log RQ, with dietary data from 4DFRs, used unconditional logistic regression analyses with baseline 5-year age categories, enrollment year (to control for duration of follow-up), race/ethnicity, education, smoking status, 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), first-degree relative with breast cancer, BMI, the Gail model 5-year risk score (26), alcohol consumption, and an estimate of recreational physical activity, included in the disease risk model as control variables in all analyses. Log-total energy was also included in the regression model, and separate analyses were carried out with and without biomarker calibration of total energy. A sandwich-type variance estimator was used for the regression parameter estimate, to acknowledge the regression calibration approach to estimation (24).

Combined observational study and DM-C cohort analyses of calibrated log RQ, with dietary data from the FFQ, used the Cox model (27) with these same primary exposure and control variables except that the follow-up time for the DM-C component of the data was time since the 1-year visit following randomization. In addition, the Cox

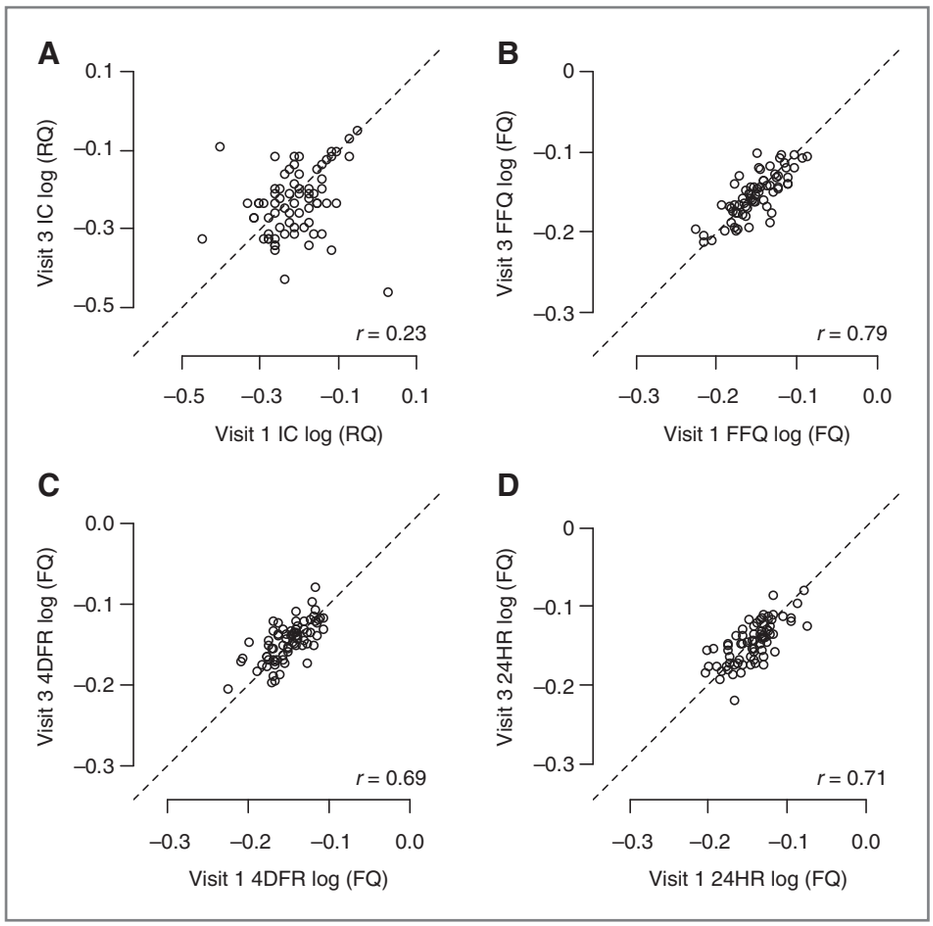

Figure 1. Correlation between primary (visit 1) and reliability (visit 3) samples among 76 reliability subsample women in the WHI NPAAS. A, log (RQ) from IC; B–D, log (FQ) from FFQs, from 4DFRs, and from three 24HRs, respectively.

model hazard rate was stratified on age at baseline (enrollment, or 1-year visit) in 5-year categories, cohort (observational study or DM-C), hormone therapy treatment assignment if randomized in the hormone therapy trials, and on whether or not the women consented to postintervention follow-up. Censoring time for noncases was defined as the earliest of April 8, 2005 for women not consenting to postintervention follow-up; September 30, 2010 for women consenting to additional follow-up, or date of last follow-up contact. A sandwich-type variance estimator was again employed for the regression parameter estimate.

All P values are two-sided, and P values less than 0.05 were considered significant.

Results

NPAAS data quality control analyses showed that the RQ data from one of the nine participating clinical centers were systematically lower than expected, and lower than that from the other centers, possibly due to faulty equipment. The data from this center were excluded from further RQ analyses, leaving 380 women. For the measured RQ to characterize a woman's typical substrate oxidation pattern, it should track strongly across repeated measures over time. Therefore, the correlation of the paired log RQ values was examined for the 76 of these 380 women who were in the 20% repeatability subsample. As shown in Fig. 1A, the paired log RQ assessments from the IC correlated only weakly (correlation of 0.23), with a few extreme discrepancies that are suggestive of occasional measurement difficulties. Figure 1B–D also show the repeatability of the paired log FQ estimates (correlation 0.68–0.79) from each of the three dietary assessment procedures.

To avoid undue influence from log RQ outliers, further RQ analyses were based on 64 women in the reliability sample for whom the difference between the paired log RQ values was less than 0.15 (ratio of RQ values between 0.86 and 1.16). Following further exclusions for missing data on modeled covariates, 59 reliability sample women remained. The left side of Table 1 shows distributional characteristics for these women. The right side of Table 1 shows characteristics for 370 NPAAS women, following missing data exclusions, whose data were used to develop calibration equations for total energy expenditure, formed by regressing log DLW energy on corresponding dietary log energy and log FQ along with other participant characteristics included in the breast cancer risk model.

Table 1. Characteristics of subjects of biomarker study at the time of NPAAS participation

	NPAAS subset used for RQ calibration ($n = 59$)		NPAAS subset used for total energy calibration ($n = 370$)	
	n	%	*n*	%
Age, y				
≤55	0		0	
>55–60	0		0	
>60–65	9	15.25	70	18.92
>65–70	26	44.07	143	38.65
>70–75	15	25.42	87	23.51
>75	9	15.25	70	18.92
Race/ethnicity				
White	41	69.49	236	63.78
Black	8	13.56	72	19.46
Hispanic	9	15.25	51	13.78
Other/unknown	1	1.69	11	2.97
Education				
≤High school graduate/GED	19	32.20	47	12.70
Post high school	10	16.95	135	36.49
College graduate or higher	30	50.85	188	50.81
Body mass index (kg/m^2)[a]				
<25	27	45.76	122	32.97
25–<30	11	18.64	103	27.84
≥30	21	35.59	145	39.19
Smoking status[a]				
Never	32	55.17	209	57.10
Past	24	41.38	147	40.16
Current	2	3.45	10	2.73
Alcohol intake				
None	25	42.37	150	40.54
<1 drink/wk	10	16.95	84	22.70
1–<7 drinks/wk	20	33.90	88	23.78
>7 drinks/wk	4	6.78	48	12.97
Total recreational physical activity (MET h/wk)				
≤2.6	6	10.17	72	19.51
>2.6–9.3	10	16.95	66	17.89
>9.3–19.0	20	33.90	105	28.46
>19.0	23	38.98	126	34.15
Family history (first-degree relative)	8	13.56	39	10.54
Gail 5-year risk (%)				
<1.26	28	47.46	195	52.70
1.27–1.80	16	27.12	110	29.73
>1.80	15	25.42	65	17.57
Estrogen-alone use ever[a]				
Never	31	52.54	192	51.89
Current/former	28	47.46	178	48.11
Estrogen plus progestin use ever[a]				
Never	33	55.93	202	54.59
Current/former	26	44.07	168	44.41
Study component				
DM-C	0		0	
OS	59	100.00	370	100.00

(Continued on the following page)

Table 1. Characteristics of subjects of biomarker study at the time of NPAAS participation (Cont'd)

	NPAAS subset used for RQ calibration ($n = 59$)		NPAAS subset used for total energy calibration ($n = 370$)	
	n	%	*n*	%
Enrollment year				
≤1995	22	37.29	104	28.11
1996	22	37.29	131	35.41
1997	9	15.25	86	23.24
1998	6	10.17	49	13.24

Abbreviations: DM-C, dietary modification trial comparison group; GED, general educational development; MET, metabolic equivalent unit.

[a]At visit 1 in the NPAAS sample, baseline in the case–control sample and OS cohort; at year 1 in the DM-C cohort.

Table 2 presents coefficients (SEs) from linear regression of the average of the paired log RQ values on the corresponding average of paired log FQ values and paired total energy values, along with the other personal characteristics listed.

The log FQ coefficients in Table 2 are positive for each assessment method, but not significantly so with these modest sample sizes. Noteworthy RQ variation is explained by educational achievement. Approximately 30% of the log RQ variation is accounted for by these simple equations. Similar regression coefficients and R^2 values arose from more stringent outlier exclusion criteria (e.g., difference of ≥0.10 between paired log RQ values). The lack of ability to identify RQ outliers precluded the development of calibration equations that would make use of the single RQ assessments in the nonreliability component of NPAAS.

The geometric means (10th and 90th percentiles) for the FQ values used in the Table 2 RQ calibration equations were 0.88 (0.84–0.93), 0.89 (0.85–0.93), and 0.89 (0.85–0.93),

Table 2. Calibration equation coefficients from linear regression of log RQ on log FQ and personal characteristics, using data from 59 NPAAS reliability sample women[a]

	Food quotient source								
	FFQ			4DFR			24HR		
Characteristic	Coefficient	(SE)	R^2	Coefficient	(SE)	R^2	Coefficient	(SE)	R^2
Log (FQ)	0.048	(0.338)	0.01	0.345	(0.367)	1.03	0.264	(0.348)	0.44
Log (total energy)	0.012	(0.031)	0.26	0.030	(0.047)	0.69	0.025	(0.049)	0.42
Age, y	−0.003	(0.002)	5.26	−0.002	(0.002)	4.38	−0.002	(0.002)	4.53
BMI	0.001	(0.002)	1.67	0.000	(0.002)	0.65	0.000	(0.002)	0.62
Black (vs. White)	−0.027	(0.041)	1.77	−0.032	(0.038)	2.43	−0.032	(0.040)	2.53
Hispanic/mixed (vs. White)	−0.019	(0.032)		−0.019	(0.031)		−0.018	(0.031)	
School after HS (vs. HS or less)	−0.028	(0.032)	14.40	−0.026	(0.031)	15.36	−0.031	(0.030)	15.84
College degree or higher (vs. HS or less)	−0.070	(0.033)[b]		−0.073	(0.032)[b]		−0.073	(0.032)[b]	
Current cigarette smoking (yes vs. no)	−0.027	(0.061)	0.31	−0.022	(0.055)	0.10	−0.013	(0.055)	0.08
Recreational physical activity (METs/wk)	−0.001	(0.001)	1.70	−0.001	(0.001)	1.23	−0.001	(0.001)	1.00
Enrollment date (years)	0.012	(0.011)	3.33	0.013	(0.010)	3.45	0.013	(0.010)	3.20
Gail model 5-year risk (%)	0.006	(0.029)	0.25	−0.000	(0.027)	0.30	−0.000	(0.028)	0.31
Breast cancer, first-degree relative (yes vs. no)	0.010	(0.050)	0.06	0.023	(0.049)	0.13	0.024	(0.049)	0.29
Alcohol use (drinks/wk)	−0.000	(0.003)	0.02	0.001	(0.002)	0.15	0.000	(0.002)	0.04
Estrogen-alone therapy (ever vs. never)	−0.006	(0.023)	0.17	−0.002	(0.022)	0.01	−0.000	(0.022)	0.00
Estrogen plus progestin use (ever vs. never)	0.015	(0.023)	1.09	0.017	(0.023)	1.21	0.016	(0.023)	0.99
Total			30.29			31.11			30.28

Abbreviations: 24HR, three 24-hour dietary recalls; METs, metabolic equivalent units; R^2, percentage of variation explained; School after HS, school after high school but no college degree.

[a]Following missing data exclusions, analyses are based on 57, 59, and 59 observations for FFQ, 4DFR, and 24HR analyses, respectively.

[b]Significance level (*P*) less than 0.05.

respectively, when based on FFQs, 4DFRs, or 24HRs. The corresponding values for total energy in kilocalories were 1,581 (954–2,509), 1,638 (1,186–2,222), and 1,586 (1,187–2,015).

The corresponding total energy calibration equations explained approximately 45% of the log DLW energy variation, and regression coefficients are given in Supplementary Table S1, for each of the three dietary assessment approaches. The geometric means (10th and 90th percentiles) for the total energy values (kilocalories) used in these equations were 1,471 (845–2,458), 1,628 (1,184–2,193), 1,573 (1,119–2,168), respectively, when based on FFQs, 4DFRs, and 24HRs. The corresponding FQ values were 0.86 (0.83–0.88), 0.86 (0.83–0.89), and 0.86 (0.83–0.89) respectively.

Supplementary Table S2 shows characteristics of the cases and controls from the DM-C for calibrated RQ association analyses that use 4DFRs to develop FQ and total energy estimates, and characteristics of cases and noncases from the combined DM-C and observational study cohorts for calibrated RQ association analyses that use FFQs to develop FQ and total energy estimates. Geometric means (10th and 90th percentiles) are also included for FQ, total energy, calibrated RQ, and calibrated total energy.

The top part of Table 3 shows invasive breast cancer ORs for a 10% increment in calibrated RQ and for a 20% increment in total energy, along with 95% confidence intervals (CI), and P values for tests of ORs equal to 1.0, from logistic regression of case–control data from the DM-C. Note that a 10% calibrated RQ increment contrasts values in the extremes of the calibrated RQ distributions. When total energy estimates from 4DFRs are included without calibration, a significant ($P = 0.003$) inverse association is evident between calibrated RQ and breast cancer incidence. The OR for RQ changed little (and $P = 0.004$) when calibrated total energy was substituted.

The bottom part of Table 3 shows corresponding HR estimates and P values from combined DM-C and OS cohort analyses, using FFQ data in the development of calibrated RQ and total energy values. The HR patterns are similar to, and more precise than, those in the top part of Table 3. Calibrated RQ is inversely associated with breast cancer risk ($P < 0.01$) whether or not total energy is calibrated, whereas there is a concurrent positive association of risk with calibrated total energy.

A sensitivity analysis was conducted that excluded women having weight instability (>10 kg weight change between baseline and 1 year in the DM-C, or more than 15 kg weight change between baseline and year 3 in the observational study; and excluded women known to have diabetes at baseline in the DM-C) in order to align these cohorts more closely with NPAAS eligibility criteria. The estimated OR (95% CI) for a 10% increment in calibrated RQ following these exclusions was 0.26 (0.09–0.79), based on 777 cases and 903 controls in 4DFR-based analyses; and the estimated HR (95% CI) was 0.23 (0.09–0.56), based on 4,275 cases and 79,897 noncases in FFQ-based analyses.

Table 3. Breast cancer ORs (top) and HRs (bottom) for a 10% increment in respiratory quotient and for a 20% increment in total energy, according to whether or not total energy estimates have been calibrated for measurement error

	ORs[a] (95% CI; *P* value; 898 cases; 1,057 controls)	
Total energy calibration	**Respiratory quotient (*P* value)**	**Total energy (*P* value)**
No	0.26 (0.11–0.65; 0.003)	1.03 (0.94–1.12; 0.524)
Yes	0.23 (0.08–0.64; 0.004)	1.27 (0.72–2.23; 0.400)
	HRs[b] (95% CI; *P* value; 5,059 cases; 98,298 noncases)	
	Respiratory quotient (*P* value)	**Total energy (*P* value)**
No	0.43 (0.24–0.78; 0.006)	1.02 (1.00–1.04; 0.017)
Yes	0.26 (0.11–0.60; 0.002)	1.66 (1.16–2.38; 0.006)

[a]From unconditional logistic regression of case versus control status on calibrated log (respiratory quotient) and log (total energy), date of study enrollment, age at study enrollment, body mass index, current smoking, race/ethnicity, education, postmenopausal hormone use (ever use of estrogens alone, ever use of estrogens plus progestin), randomization assignment in hormone therapy trials, first-degree relative with breast cancer, Gail model 5-year risk score, alcohol intake, and estimated recreational physical activity. Calibrated estimates are based on 4DFRs.

[b]From Cox regression of calibrated log (respiratory quotient) and log (total energy), date of cohort enrollment, body mass index, current smoking, race/ethnicity, education, postmenopausal hormone use (ever use of estrogens alone, ever use of estrogens plus progestin), first-degree relative with breast cancer, Gail model 5-year risk score, alcohol intake, and estimated recreational physical activity, and with baseline hazard rate stratification on age category at FFQ completion, randomization assignment in the hormone therapy trials, cohort (DM comparison, OS) and participation in WHI beyond the intervention period (time dependent). Calibrated estimates are based on food-frequency questionnaires.

Discussion

Consideration of substrate utilization may provide a novel approach to a more complete understanding chronic disease risk. The extent to which a person relies on fat, carbohydrate, or protein for energy oxidation may provide insights into physiology and metabolism that have evidently not been examined previously in chronic disease epidemiology.

IC has been shown to have good accuracy in spontaneously breathing subjects, provided care is taken to avoid gas leakages (refs. 28–30), with most evaluation using the 30-year-old DeltaTrac II metabolic monitor. We used a well-developed IC protocol, but found that there was also considerable variation between paired RQ estimates from women who repeated the study protocol approximately 6 months after the original application. Although some such variation likely reflects actual changes in substrate utilization profile between the two time periods, there were some clear outliers that likely reflect occasional gas leakages or other measurement issues. These variations were sufficiently large, in relation to the between-subject RQ variations that we chose to develop RQ calibration equations based only on the data from 59 women whose paired RQ assessments were in reasonable agreement, and for whom other needed variables were available. Calibration equations provided an explanation for approximately 30% of the log RQ variation, although the FQ coefficients were not clearly different from zero with this small calibration equation sample size. An inverse association of educational achievement and log RQ was a major contributor to the calibration. Compared with women without post-high school education, women with a college degree had RQ values that were approximately 7% lower, whereas women with some post-secondary education, but no degree, had intermediate RQ values (Table 2). This inverse association could help explain higher postmenopausal breast cancer rates among women having higher socioeconomic status.

The (nonsignificant) log FQ regression coefficient estimates (Table 2) were largest for the 4DFR and smallest for the FFQ, with that for the three 24HRs being intermediate. Note, however, that the 4DFRs targeted a time period close to the IC application, whereas the FFQ targeted an earlier 3-month window, and the three 24HRs were spread over a subsequent 3-month period.

The estimated RQ values, whether FQ is based on 4DFRs or FFQs, related inversely to breast cancer risk in WHI cohorts (Table 3). Our previous analyses (5) found a positive relationship between DLW-calibrated total energy and breast cancer risk in these cohorts that ceased to be evident when BMI was added to the disease risk model. Here, there is some evidence (Table 3) for a positive association of total energy with risk, even with BMI in the disease risk model, after controlling for calibrated RQ. However, these analyses are not highly robust, due to the limited sample size for the RQ calibration. In additional analyses (not shown), when log-calibrated RQ in the Table 3 analyses is replaced by indicator variables for calibrated RQ quartiles, the association of calibrated energy with risk, seen in the bottom part of Table 3, is no longer significant, suggesting considerable sensitivity to the joint quantitative modeling of these variables. In addition, the OR pattern across calibrated RQ quartiles was consistent with that from Table 3 for the 4DFR-based case–control analyses, but was not so clear in the FFQ-based cohort analyses.

It seems that it may be metabolically advantageous in respect to breast cancer risk for women to be drawing substantially from carbohydrate or glycogen reserves, rather than fat reserves regardless of BMI. Doing so presumably reflects a diet that is relatively high in carbohydrate and hence not dense in calories. Glycogen reserves turn over quickly, during the course of each day, whereas body fat provides a continuously available source for energy metabolism. Reliance on fatty acid oxidation, as opposed to glycolysis and gluconeogenesis, results in the formation of ketone bodies. An overabundance of ketone body production can be induced by high-fat, low-carbohydrate weight loss data and can also occur with poorly controlled diabetes (31). Data linking diabetes to breast cancer suggest that this could be a contributing mechanism for the RQ associations reported here (32, 33).

A limitation of the study is that only short-term fasting RQ was assessed, whereas a 24-hour fed and fasting RQ may be more stable and correspond more closely to dietary assessment. An important limitation derives from the occasional rather extreme variability between paired fasting RQ values separated by approximately 6 months in time, and from the limited sample size ($n = 59$) for RQ calibration equation development.

These preliminary analyses suggest a role for the energy metabolism profile in determining breast cancer risk. This and corresponding associations with total energy consumption, in the absence of concurrent RQ modeling (5), may help in the development of recommended dietary and activity patterns for breast cancer prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. L. Prentice, M.L. Neuhouser
Development of methodology: R.L. Prentice, M.L. Neuhouser, Y. Huang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.L. Prentice, M.L. Neuhouser, L.F. Tinker, C.A. Thomson, Y. Mossavar-Rahmani
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. L. Prentice, L.F. Tinker, M. Pettinger, C.A. Thomson, Y. Mossavar-Rahmani, Y. Huang
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Decisions concerning study design, data collection and analysis, interpretation of the results, the preparation of the article, or the decision to submit the article for publication resided with committees comprised of WHI investigators that included NHLBI representatives.

A list of key investigators involved in the WHI research follows.

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Clinical Coordinating Center: Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg (Fred Hutchinson Cancer Research Center, Seattle, WA).

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For a list of all the investigators who have contributed to WHI science, please visit: https://cleo.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf

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