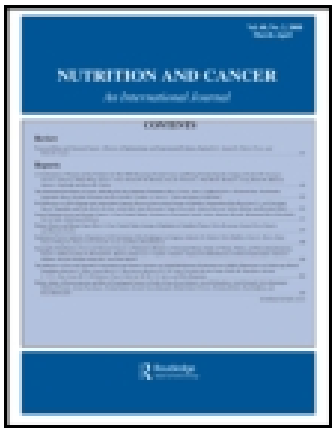

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Diet and Expression of Estrogen Alpha and Progesterone Receptors in Malignant Mammary Tissue

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Diet and Expression of Estrogen Alpha and Progesterone Receptors in Malignant Mammary Tissue

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Estrogen receptor (ER) and/or progesterone receptor (PR) expression has been associated with more favorable breast cancer prognosis. Results on the differential association of diet with ER and/or PR positive and negative tumors have been inconclusive. In a large case-control study conducted in Athens, Greece, we investigated whether diet is associated with the expression of ER-alpha or PR in mammary tumors of 421 women with histologically confirmed breast cancer. Diet was assessed through an extensive food frequency questionnaire and results were analyzed using multiple logistic regression. After controlling for non-nutritional variables and mutually adjusting for energy-generating nutrients and ethanol, carbohydrate intake was inversely associated with ER-alpha ($P = 0.04$) and PR ($P = 0.10$) expression. The odds ratios (OR) per one standard deviation increment were 0.69 with 95% confidence interval (95% CI) 0.48-0.98 for ER-alpha and 0.72 (95% CI 0.49–1.07) for PR expression. No consistent or statistically significant associations were noted for any of the other energy-generating nutrients or food groups examined. Although in these data no strong relations of qualitative aspects of diet with hormone receptor expression in breast cancer tumors were evident, the inverse association of carbohydrate intake with ER-alpha, and perhaps PR, expression merits further study in future investigations.

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INTRODUCTION

Approximately two thirds of breast cancer tumors are estrogen receptor (ER) positive. In Asian women, however, among

whom incidence of breast cancer is considerably lower, a larger fraction of postmenopausal breast cancer is ER negative compared to women in the western world (1–3). ER positivity has been positively associated with age, smaller tumor size, absence of nodal metastasis, and better histologic grade (4). Progesterone receptors (PRs) are the products of an estrogen-regulated gene, and their expression is considered to be an indication of a functional ER (5). Thus, PRs are generally expressed when ERs are also expressed and are detected in more than half of breast tumors (6). PRs can also be detected in ER negative tumors, but this could be due to a very low level of ER or the existence of ER variants that are not recognized by the antibody used for the determination of the ER level but that are still capable of inducing estrogen response (7).

In spite of intensive work for more than two decades, an association of diet with breast cancer risk has not been firmly documented, with the exception of a positive association with alcohol intake and weight gain among postmenopausal women (8,9). Investigators have also examined the association of diet with ER and/or PR positive breast cancer, given the generally worse prognosis of the receptor negative tumors, but the results, mostly focusing on fat intake, have been inconclusive (10).

We have investigated whether diet is associated with the expression of ER or PR in mammary tumors in the context of a large case-control study in Athens, Greece. In contrast to previous case-control studies, which have focused on the comparison between controls and breast cancer cases positive or negative for ER or PR with emphasis on fat intake, we opted for comparing directly breast cancer cases positive for receptors with breast cancer cases negative for receptors and examining several food groups as well as all energy-generating nutrients. In particular, we were interested in the possible role of carbohydrates, which have been reported to be inversely associated with the simultaneous expression of ERs and PRs (11).

SUBJECTS AND METHODS

Recruitment

In a 4-yr period from 2001 to 2005, we contacted women who underwent mammary biopsies in two major breast clinics in Athens, Greece and asked them to participate in the study. Following written informed consent, women filled in a questionnaire with assistance from specially trained interviewers and allowed review of their medical records, as well as use of tissue specimens collected in the context of their standard medical care for research purposes. The study was approved by the Bioethics Committee of the University of Athens.

In Breast Clinic 1, recruited women underwent a breast biopsy during the duration of the study, or had undergone a biopsy prior to the study initiation but were interviewed during the indicated study period. In Breast Clinic 2, all women underwent biopsies during the study period. Because in several instances women refused to allow any recording of information concerning agreement to participate in the study, the refusal proportion cannot be precisely calculated, but we estimate that about 75% of eligible women in both clinics agreed to participate in the study.

Although originally both women with benign breast diseases and women with breast cancer were recruited (12), for the purposes of the present study, only women with biopsy-confirmed breast cancer were included.

Questionnaire

The questionnaire was interviewer-administered and covered sociodemographic, lifestyle, medical, and gynecological variables. It also included an extensive semiquantitative food frequency section. Women were asked to indicate the average frequency of consumption of about 120 food items or beverage categories in the year preceding the recognition of symptoms or signs of their current condition. Frequencies were recorded per month, per week, or per day, as appropriate, and, for the analysis, they were quantified approximately in terms of the number of times per day the food was consumed. In the main analysis, food items were considered in groups. The food groups studied were fruits, vegetables and legumes, cereals and starchy roots, meat and meat products, milk and dairy products, sugars, sweets, and non-alcoholic beverages.

Energy-generating nutrient intakes—namely protein, lipids, and carbohydrates—as well as ethanol were estimated for individuals by multiplying the nutrient contents of a selected typical portion for each specified food item or beverage by the frequency it was consumed and adding these estimates for all contributing items. Estimates were based on a food composition database modified to accommodate the particularities of the Greek diet (13). The portion size estimation was based on the results from previous validation studies (14), and the nutrient content of complex dishes was calculated on the basis of Greek recipes (13). Total energy intake in kilocalories was also estimated.

Hormone Receptor Analyses

We determined ER-alpha and PRs in malignant tissue from biopsies of the 421 women diagnosed with breast cancer. In Breast Clinic 1, histological samples were made available in the form of paraffin-embedded tissue (PET) blocks, whereas in Breast Clinic 2, samples obtained during biopsy were frozen in liquid nitrogen before being fixed in 10% neutral buffer formalin at 25°C for 24 h and processed to PETs.

The streptavidine-biotin-superoxidase method (15,16) was applied on paraffin sections using the automatic immunochemical BioGenex i6000 Consolidated Staining System. As indicated, the sections that were prepared from fresh frozen tissue in Breast Clinic 1 were previously fixed in a 10% formol solution of pH 7.4, at 25°C for 24 h, so that the same method was applied to paraffin sections for all samples from both clinics. The primary specific mouse monoclonal antibodies were obtained from Novocastra Laboratories, Ltd. (Newcastle-upon-Tyne, UK). The clone 6F11, specific for ER-alpha, was applied

in a 1:60 dilution, and the clone 1A6, specific for the PR, was applied in a 1:40 dilution.

We scored the immunocytochemical results in a semiquantitative way using the H-score. This score incorporates both the number of cells with positive staining for hormone receptor and the intensity of staining (15,17). Intensity of staining was evaluated on the basis of percentages of stained cells under four categories, denoted as 0 (no staining), 1 (weak but detectably above control), 2 (distinct), and 3 (strong). The H-score was calculated from the formula $[(a_0{*}0)+(a_1{*}1)+(a_2{*}2)+(a_3{*}3)]{*}100$, in which a_0 = percent (expressed as a fraction of 1) of cells with intensity of staining 0, a_1 = percent of cells with intensity staining 1, a_2 = percent of cells with intensity of staining 2 and a_3 = percent of cells with intensity of staining 3. The H-score, therefore, ranges from 0 to 300. We considered scores from 0 to 9 (inclusive) as indicative of ER-α or PR negative tissues and scores from 10 or more as indicative of ER-α or PR positive tissues.

Statistical Analyses

We have used logistic regression to calculate the odds ratio for expression, alternatively, of ER-alpha and PRs according to categories of non-dietary variables, with and without mutual adjustment. We have used the non-parametric Wilcoxon test to compare intake of dietary variables between breast cancer patients with expression or not of ER-alpha and PRs. Then, we used multiple logistic regression to calculate the odds ratio for expression, alternatively, of ER-alpha and PRs per one standard deviation increase in the intake of the examined dietary variables, stratifying for source of tissue sample and controlling for age (categorical in 3 age groups), parity (parous vs. nulliparous), menopausal status (post vs. pre), age at menarche (categorical in 4 age groups), body mass index (categorical in 3 groups), family history of breast cancer (yes vs. no), use of exogenous hormones (yes vs. no; oral contraceptives for premenopausal women, hormone replacement treatment for postmenopausal women) and total energy intake (continuously). Analyses were conducted with the SPSS 16.0 statistical package.

RESULTS

Table 1 presents the distribution of breast cancer patients by source of tissue samples, characteristics of the patients, and expression of ER-alpha and PRs in the malignant tissue. This table serves descriptive purposes. Most women are postmenopausal and overweight, in accordance with what is known about the high prevalence of excess body weight among postmenopausal women in Greece and the association of postmenopausal obesity with breast cancer. Only a small minority of women have been using oral contraceptives or hormone replacement therapy at the time of diagnosis, again in line with the known practices among Greek women. More than 80% of women are positive for ER-alpha and PRs, and the proportions are slightly higher in the newly processed compared to the stored PETs.

Table 2 shows logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CI) for ER-alpha and PR expression by non-nutritional variables, univariate and after mutual control of the indicated variables. Expression of ER-alpha is higher among older ($P < 0.05$) and among postmenopausal women ($P < 0.05$) and mutual adjustment suggests that

TABLE 1
Distribution of 421 women with breast cancer by source of tissue samples, characteristics of the women, and expression of estrogen alpha and progesterone receptors in the malignant tissue

Characteristic	Stored PET $N = 259$ (100%)	Newly processed PETs $N = 162$ (100%)
Age (years)		
−49	86 (33)	44 (27)
50–59	62 (24)	55 (34)
60+	111 (43)	63 (39)
Age at menarche (years)		
−11	45 (18)	24 (15)
−12	56 (22)	48 (30)
−13	78 (31)	49 (30)
13+	72 (29)	40 (25)
Menopausal status		
Pre- and perimenopausal	80 (31)	58 (36)
Postmenopausal	178 (69)	103 (64)
Parity		
Nulliparous	54 (21)	20 (12)
Parous	205 (79)	142 (88)
Body mass index (kg/m^2)		
−25	94 (41)	58 (39)
25–29,99	80 (35)	49 (33)
30+	56 (24)	41 (28)
Family history of breast cancer		
Yes	32 (12)	26 (16)
No	227 (88)	136 (84)
Hormone use (OC for pre-meno and HRT for post)		
Yes	27 (10)	14 (9)
No	232 (90)	148 (91)
Expression of estrogen alpha receptors		
Yes	203 (79)	140 (86)
No	55 (21)	22 (14)
Expression of progesterone receptors		
Yes	199 (77)	150 (93)
No	60 (23)	12 (7)

Notes. There are some missing values with respect to the risk factors; for one stored PET sample estrogen receptor alpha determination was missing. PET = paraffin embedded tissue, OC = oral contraceptives, HRT = hormone replacement treatment.

TABLE 2
Logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CI) for estrogen alpha and progesterone receptor expression in women with breast cancer, stratified by source of tissue samples—univariate and after mutual control of the indicated variables

	Estrogen alpha receptors				Progesterone receptors			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age (yr)								
<49		Baseline				Baseline		
50–59	1.81	0.95–3.43	1.01	0.42–2.41	0.95	0.48–1.89	0.80	0.30–2.13
60+	2.09	1.17–3.73	1.32	0.48–3.63	1.01	0.55–1.85	1.07	0.37–3.11
Parity (parous vs. nulliparous)	1.20	0.65–2.24	1.19	0.58–2.43	0.89	0.46–1.75	0.98	0.44–2.16
Menopausal status (post vs. pre)	2.05	1.23–3.41	1.68	0.69–4.09	0.95	0.54–1.67	1.11	0.42–2.95
Age at menarche (yr)								
−11		Baseline				Baseline		
−12	0.61	0.28–1.32	0.82	0.35–1.92	1.17	0.54–2.57	1.30	0.54–3.12
−13	0.98	0.45–2.14	1.30	0.56–3.06	1.15	0.55–2.41	1.23	0.54–2.79
13+	1.09	0.49–2.44	1.31	0.55–3.15	2.15	0.94–4.91	2.61	1.02–6.68
Body mass index (kg/m^2)								
−25		Baseline				Baseline		
25–29,99	1.09	0.59–2.00	0.92	0.48–1.75	0.82	0.43–1.56	0.76	0.38–1.50
30+	1.27	0.64–2.51	1.03	0.49–2.16	1.01	0.49–2.10	0.94	0.43–2.07
Family history (yes vs. no)	1.42	0.64–3.14	1.62	0.68–3.85	1.48	0.64–3.46	1.75	0.69–4.42
Exogenous hormones (yes vs. no)	1.74	0.66–4.59	2.42	0.81–7.21	1.65	0.62–4.42	1.76	0.63–4.86

menopausal status may be a more important determinant than age per se. In contrast, expression of PRs does not vary with age at diagnosis or menopausal status, but it is significantly higher among women with late menarche.

Table 3 shows daily frequency of consumption of standard portions of 8 major food group categories, as well as daily intakes in grams of energy-generating nutrients, according to ER-alpha and PR expression. There is no consistent evidence across columns that any food group or any energy-generating nutrient is strongly or significantly associated with expression of ER-alpha and/or PRs. However, the results in this table are not controlled for non-dietary variables or mutually, which limits their interpretability.

Table 4 presents logistic regression-derived ORs and 95% CIs for ER-alpha and PR per one standard deviation increment of the indicated nutritional variables, controlling for non-nutritional variables and energy intake. There are no statistically significant associations for any of the examined nutritional variables, although there is a suggestion that high consumption of carbohydrates reduces the expression of both ER-alpha and PRs ($P\sim0.08$ in both instances), and high consumption of total lipids increases the expression of PRs ($P\sim0.051$). We have mutually adjusted the 3 energy-generating nutrients as well as ethanol (but not energy intake to avoid colinearity), also controlling for non-nutritional variables, and the results are shown in Table 5. Only with respect to carbohydrate intake are the results fairly consistent for ER-alpha ($P = 0.04$) and PRs ($P = 0.10$), the ORs for expression of receptors being around 0.70 per one standard deviation increment of intake.

DISCUSSION

In a study of 421 women with histologically confirmed breast cancer, we have found no evidence for a strong or consistent association of the intake of any particular food group or energy-generating nutrient with the expression of ER-alpha or PRs in breast tumors. A suggestive inverse association of carbohydrate intake with the expression of these receptors was significant only with respect to ER-alpha, after mutual adjustment among energy-generating nutrients.

A possible association of diet with the expression of ER-alpha and/or PRs in breast tumors could be of importance, given the different prognosis of receptor positive and negative tumors. The empirical evidence in this context is weak and inconclusive (10). Olsen and colleagues (18) found an inverse association of fruit and vegetable intake with ER negative breast tumors among postmenopausal women. Linseisen and colleagues (19) have reported an inverse association of phytoestrogen intake with

TABLE 3
Median values and Wilcoxon-derived *P* values for the indicated nutritional variables among 421 women with breast cancer according to estrogen alpha and progesterone receptor expression in the malignant mammary tissue by source of tissue samples

	Estrogen alpha receptors				Progesterone receptors			
Variable	Stored PET	*P* value	Newly processed PETs	*P* value	Stored PET	*P* value	Newly processed PETs	*P* value
Vegetables and legumes (times/day)								
Positive	3.3	0.39	3.5	0.47	3.3	0.50	3.4	0.03
Negative	3.3		3.8		3.2		4.5	
Fruits (times/day)								
Positive	2.9	0.76	2.5	0.37	3.0	0.77	2.5	0.37
Negative	3.3		3.1		2.9		3.1	
Cereals (times/day)								
Positive	3.1	0.30	3.2	0.24	3.1	0.37	3.2	0.50
Negative	3.4		3.3		3.3		3.3	
Starchy roots (times/day)								
Positive	0.2	0.21	0.3	0.43	0.3	0.87	0.3	0.09
Negative	0.3		0.3		0.2		0.2	
Meat (times/day)								
Positive	0.6	0.66	0.6	0.45	0.6	0.61	0.5	0.40
Negative	0.6		0.6		0.6		0.6	
Milk and dairy products (times/day)								
Positive	2.1	0.13	2.3	0.09	2.2	0.91	2.3	0.09
Negative	2.7		1.9		2.5		1.6	
Sugars, sweets, and non-alcoholic beverages (times/day)								
Positive	1.5	0.14	2.1	0.09	1.6	0.12	2.3	0.82
Negative	2.3		3.4		2.1		2.5	
Added lipids (times/day)								
Positive	2.1	0.30	2.2	0.58	2.1	0.72	2.2	0.58
Negative	2.2		2.0		2.1		2.1	
Energy intake (kcal/day)								
Positive	1658.6	0.45	1626.4	0.36	1675.6	0.65	1627.0	0.59
Negative	1759.4		1728.9		1600.9		1794.4	
Total lipids (g/day)								
Positive	74.8	0.80	77.2	0.49	75.3	0.31	77.6	0.49
Negative	76.5		78.1		75.3		76.3	
Protein (g/day)								
Positive	63.9	0.46	60.6	0.81	65.0	0.65	60.9	0.69
Negative	64.5		63.3		63.3		62.0	
Carbohydrates (g/day)								
Positive	167.5	0.15	162.2	0.25	170.7	0.43	164.8	0.50
Negative	188.3		170.4		172.3		173.6	
Ethanol (g/day)								
Positive	0.0	0.26	0.0	0.94	0	0.56	0	0.94
Negative	0.0		0.0		0		0	

Note. PET = paraffin embedded tissue.

TABLE 4
Logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CIs)[a] for estrogen alpha and pregesterone receptor expression in women with breast cancer per one standard deviation increment of the major nutritional variables

	Estrogen alpha receptors			Progesterone receptors		
Dietary variable (per 1 SD increment)	OR	95% CI	*P* value	OR	95% CI	*P* value
Vegetables and legumes (per 1.8 times/day)	0.92	0.67–1.27	0.61	0.89	0.63–1.25	0.48
Fruits (per 2.0 times/day)	0.90	0.66–1.22	0.50	1.24	0.86–1.79	0.25
Cereals (per 2.1 times/day)	0.75	0.55–1.03	0.07	0.76	0.54–1.07	0.12
Starchy roots (per 0.2 times/day)	1.12	0.80–1.57	0.52	1.05	0.73–1.51	0.79
Meat (per 0.6 times/day)	0.95	0.72–1.25	0.71	0.93	0.68–1.27	0.66
Milk and dairy products (per 1.99 times/day)	1.00	0.74–1.33	0.97	1.08	0.74–1.55	0.70
Sugar, sweets, and non-alcoholic beverages (per 2.8 times/day)	0.89	0.68–1.16	0.38	0.84	0.63–1.13	0.25
Added lipids (per 1.0 times/day)	0.91	0.72–1.14	0.41	0.99	0.75–1.30	0.94
Energy intake (per 653.9 kcal/day)	0.87	0.69–1.10	0.25	1.02	0.78–1.33	0.90
Total lipids (per 31.68 g/d)	1.60	0.85–3.01	0.15	2.16	1.00–4.67	0.051
Proteins (per 28.7 g/day)	1.41	0.81–2.45	0.22	1.19	0.66–2.15	0.57
Carbohydrates (per 82.4 g/day)	0.59	0.33–1.06	0.08	0.54	0.27–1.06	0.08
Ethanol (per 5.0 g/day)	1.14	0.79–1.64	0.48	1.03	0.73–1.45	0.87

Note. Results are stratified by source of tissue samples.

[a]Adjusted for age, parity, menopausal status, age at menarche, body mass index, family history of breast cancer, use of exogenous hormones (oral contraceptives for premenopausal women, hormone replacement treatment for postmenopausal women), and total energy intake.

hormone receptor positive tumors among premenopausal women, whereas Touillaud and colleagues (20) reported a similar association among postmenopausal women. Fung and colleagues (21,22) have found inverse associations between plant food-rich diets and ER negative breast cancer. Suzuki and colleagues (23) focused on alcohol intake and reported a positive association between alcohol drinking and risk of postmenopausal ER positive breast cancer, whereas McCullough and colleagues (24) have focused on dairy products and found an inverse association of the intake of these foods with risk for ER positive postmenopausal breast cancer. A positive association of meat intake with hormone receptor positive breast cancer was reported by Cho and colleagues (25) among premenopausal and by Cui and colleagues (26) among postmenopausal women. The results of these studies do not converge in pointing to a particular direction. Moreover, several other studies examining the association of various dietary aspects with breast cancer risk by hormone receptor expression have been reported as null (27–32).

Carbohydrate intake per se has not been the focus of previous studies concerning the etiology or the prognosis of breast cancer or the expression of receptors, although in a large case-control study in Italy, intake of carbohydrates was positively associated with breast cancer risk (33). Moreover, in a cohort study in the United States, carbohydrate intake was reported to be inversely associated with the simultaneous expression of ERs and PRs (11) and, thus, inferentially, associated with less

TABLE 5
Logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CIs)[a] for estrogen alpha and pregesterone receptor expression in women with breast cancer, per one standard deviation increment of the indicated energy-generating nutrients

	Estrogen alpha receptors			Progesterone receptors		
Nutrient (per 1 SD increment)	OR	95% CI	*P* value	OR	95% CI	*P* value
Total lipids (per 31.68 g/day)	1.05	0.67–1.64	0.84	1.55	0.85–2.83	0.16
Proteins (per 28.7 g/day)	1.23	0.73–2.10	0.44	0.94	0.51–1.74	0.85
Carbohydrates (per 82.4 g/day)	0.69	0.48–0.98	0.04	0.72	0.49–1.07	0.10
Ethanol (per 5.0 g/day)	1.09	0.77–1.54	0.61	0.99	0.71–1.37	0.95

Note. Results stratified by source of tissue samples and mutually adjusted for all energy-generating nutrients.

[a]Adjusted also for age, parity, menopausal status, age at menarche, body mass index, family history of breast cancer, use of exogenous hormones (oral contraceptives for premenopausal women, hormone replacement treatment for postmenopausal women).

favorable survival. In contrast, however, isoflavones, which are present in carbohydrate-rich legumes, have been reported to be beneficial with respect to breast cancer incidence and survival, although short-term intervention studies suggest a worrisome possible stimulatory effect of these compounds on breast tissue (34). Clearly, the issue deserves further investigation.

In our study, we did not compare women with hormone receptor positive or negative breast cancer with women in the underlying population, as reflected in a control series, but we focused instead on a direct comparison among women with breast cancer and contrasted hormone receptor positive breast cancer patients with hormone receptor negative ones. We believe that our approach has three advantages. First, it minimizes selection bias, which is always a concern in case-control studies, particularly those that are not strictly nested in an underlying cohort. Second, it minimizes information bias, as receptor status is unlikely to affect reporting of diet by breast cancer patients. And last, our approach may have power advantages because it generates a single odds ratio for each dietary exposure, rather than relying on comparison of two odds ratios per dietary exposure (one for receptor positive breast cancer patients vs. controls and one for receptor negative breast cancer patients vs. controls). Limitations of our investigation are those inherent in studies relying on dietary ascertainment, which is suboptimal even with interviewer-administered questionnaires, and statistical power concerns, because our study was only of moderate size.

In conclusion, our results are in line with those generated through alternative approaches in indicating no strong association of qualitative aspects of diet with expression of hormone receptors in breast cancer tumors. The weak inverse association of carbohydrate intake with the expression of ER-alpha, and perhaps progesterone, receptors in breast cancer tumors, however, merits further study in future investigations.

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