Cancer Causes Control (2009) 20:601–607
DOI 10.1007/s10552-008-9269-8

ORIGINAL PAPER

Diet and expression of estrogen alpha and progesterone receptors in the normal mammary gland

Pagona Lagiou · Evangelia Samoli · Areti Lagiou · Christina Georgila · Pantelina Zourna · Anastasia Barbouni · George Gkiokas · Dorothy Vassilarou · Annivas Tsikkinis · Constantinos Sfikas · Constantine E. Sekeris · Chung-Cheng Hsieh · Hans-Olov Adami · Dimitrios Trichopoulos

Received: 23 September 2008 / Accepted: 11 November 2008 / Published online: 27 November 2008

Abstract
Objective It has been recently reported that expression of estrogen alpha (ER-α) and progesterone (PR) receptors in the normal mammary gland is inversely associated with breast cancer risk among postmenopausal women. We investigated whether dietary intakes are associated with the expression of ER-α and PR receptors in the apparently *normal*, as opposed to pathological, mammary tissue.
Methods In a study in Greece, we examined associations of dietary intakes with ER-α and PR expression in the adjacent-to-pathological apparently normal mammary tissue of 562 women with either breast cancer (267 women) or BBD (299 women). Diet was assessed through an extensive food frequency questionnaire and results were analyzed using multiple logistic regression.
Results Monounsaturated ($p = 0.03$) and, to a lesser extent, polyunsaturated lipids ($p = 0.08$) were positively associated with ER-α expression. Cereals and starchy roots were inversely associated with ER-α ($p = 0.01$), whereas milk and dairy products were inversely associated with PR expression ($p = 0.02$). Ethanol intake was non-significantly inversely associated with ER-α expression ($p = 0.07$).
Conclusions Our findings suggest that the weak associations of diet with breast cancer risk could be explained, to some extent, by effects of diet on receptor expression in the normal mammary gland.

P. Lagiou · E. Samoli · A. Lagiou · C. Georgila · P. Zourna
Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, 75 M. Asias Street, Goudi 115 27, Greece

P. Lagiou · C.-C. Hsieh · H.-O. Adami · D. Trichopoulos (✉)
Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA
e-mail: dtrichop@hsph.harvard.edu

A. Lagiou
Faculty of Health Professions, Athens Technological Educational Institute, 274 Thivon Avenue, 12241 Athens, Greece

A. Barbouni
Department of Public and Administrative Health, National School of Public Health, 196 Alexandras Avenue, 115 21 Athens, Greece

G. Gkiokas
2nd Department of Surgery, University of Athens Medical School, Aretaieion Hospital, Vas, Sofias Avenue, Athens, Greece

D. Vassilarou
"Prolipsis" Diagnostic Center, 85A Mihalakopoulou St., Athens 11528, Greece

A. Tsikkinis
First Department of Surgery, Elena Venizelou Hospital, 2 Elena Venizelou square, 11521 Athens, Greece

C. Sfikas
Department of Pathology, Elena Venizelou Hospital, 2 Elena Venizelou Square, 11521 Athens, Greece

C. E. Sekeris
National Hellenic Research Foundation, Institute of Biological Research and Biotechnology, 48 Vassileos Constantinou Avenue, 11635 Athens, Greece

C.-C. Hsieh
Division of Biostatistics and Epidemiology, UMass Cancer Center, University of Massachusetts Medical School, 364 Plantation Street, LRB 427, Worcester, MA 01605, USA

Keywords Breast cancer · Estrogen receptor · Progesterone receptor · Mammary tissue

Introduction

There is considerable evidence that mammotropic hormones, particularly estrogens, are central in the etiology of breast cancer in women [1–5]. Given the obligate role of receptors in hormone response [6–9], their expression in normal breast tissue could also be correlated with breast cancer risk. We have recently reported that expression of estrogen alpha (ER-α) and progesterone (PR) receptors in the normal mammary gland is inversely associated with breast cancer risk among postmenopausal women [10].

Although diet has been intensively studied in relation to breast cancer risk, the collective evidence does not indicate an important role of qualitative aspects of nutrition in adult life. There is, however, evidence that alcohol intake may increase this risk [11] and indications that consumption of total fat, particularly saturated fat of animal origin may also increase this risk [11–15]. There have also been reports that intake of olive oil [16, 17], unsaturated lipids [18, 19], and vegetables and fruits [11, 20] could be associated with reduced breast cancer risk, whereas high glycemic load may increase this risk [21].

We hypothesized that environmental factors, and diet in particular, might affect hormone receptor expression in the normal breast tissue and, hence, risk of breast cancer. To explore this hypothesis, we conducted a study in Greece with detailed dietary assessment and determination of estrogen alpha (ER-α) and progesterone (PR) receptor expression in the apparently normal, as opposed to pathological, mammary tissue.

Subjects and methods

Recruitment

From March 2001 to May 2005, we asked women who underwent mammary biopsy in two major breast clinics in Athens, Greece to participate in the study. Those who gave their written informed consent filled in a questionnaire with the help of specially trained interviewers, allowed review of their medical records, as well as the use for research purposes of tissue specimens collected in the context of their standard medical care. The study was approved by the Bioethics Committee of the University of Athens.

In breast clinic 1, we recruited women who underwent a breast biopsy during the duration of the study, as well as women who had undergone a biopsy prior to the study initiation (but were interviewed during the indicated study period). In breast clinic 2, all women underwent biopsy during the study period. We estimate that about 75% of eligible women in both clinics agreed to participate in the study. In several instances, women refused to allow any recording of information concerning agreement to participate in the study; thus, the refusal proportion cannot be precisely calculated.

Questionnaire

The interviewer-administered questionnaire covered sociodemographic, lifestyle, as well as gynecological and general medical history variables, and included a semi-quantitative food frequency section. Women were asked to indicate the average frequency of consumption, in the year preceding the recognition of symptoms or signs of their current condition, of about 120 food items or beverage categories per month, per week or per day. For the analysis, the frequency of consumption of different food items was 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 products; milk and dairy products; sugars, sweets, and non-alcoholic beverages.

Energy-generating nutrient intakes—namely protein, saturated, monounsaturated, and polyunsaturated lipids, and carbohydrates—were estimated for individuals by multiplying the nutrient contents of a selected typical portion, for each specified food item, by the frequency that the food item was eaten and adding these estimates for all food items. Estimates were based on a food composition database modified to accommodate the particularities of the Greek diet [22]. The portion size estimation was based on the results from previous validation studies [23], and the nutrient content of complex dishes was calculated on the basis of Greek recipes [22]. Total energy intake in kilocalories was also estimated.

Hormone receptor analyses

We determined receptors in the apparently normal mammary tissue adjacent to the pathological tissue of 267 women with breast cancer and 299 women with benign breast disease (BBD). 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. In all instances, samples from surgical biopsies were examined.

The streptavidine–biotin–superoxidase method [24, 25] was applied on paraffin sections. The sections that were prepared from fresh frozen tissue were fixed in a 10% formol solution of pH 7.4, at 25°C for 24 h, in the automatic immunochemical BioGenex i6000 Consolidated Staining System. The primary specific mouse monoclonal antibodies were obtained from Novocastra Laboratories Ltd., Newcastle upon Tyne, UK. The clone 6F11, specific for estrogen receptor alpha, was applied in a 1:60 dilution, and the clone 1A6, specific for the progesterone receptor, 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 [24, 26]. 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 $[(a_0 \times 0) + (a_1 \times 1) + (a_2 \times 2) + (a_3 \times 3)] \times 100$, 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

For women with BBD or breast cancer, we calculated median values of each nutritional variable by ER-α or PR status. We then used the non-parametric Wilcoxon test for two samples to compare levels of intake of each nutritional variable between receptor positive and receptor negative samples, separately among women with BBD or breast cancer. Subsequently we used multiple logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for ER-α and PR positivity in the normal mammary tissue, among women with BBD or breast cancer, per one standard deviation increment of the major food groups and macronutrients. We stratified for type of PET (stored versus newly processed) and diagnosis (BBD versus cancer), and adjusted for age (categorical in four age groups), parity (parous versus nulliparous), menopausal status (post versus pre), age at menarche (continuously), body mass index (BMI) (continuously), family history (yes versus no), use of hormones (yes versus no, contraceptives for premenopausal women, hormone replacement therapy for postmenopausal) and total energy intake (continuously). Analyses were conducted with the SPSS 16.0 statistical package.

Results

Table 1 presents the distribution of the 299 women with BBD and the 267 women with breast cancer by descriptive characteristics, source of tissue samples and expression of ER-α and PR in the normal mammary tissue. As both clinics were involved in mammographic screening, participating women with breast cancer were somewhat younger than women with breast cancer in general. As expected, women with BBD were younger than breast cancer patients. Expression of ER-α was slightly higher among women with BBD (66%) than among women with breast cancer (62%). Likewise, expression of PR was higher among women with BBD (74%) than among women with breast cancer (62%).

In Table 2, median values and Wilcoxon-derived *p* values are presented for the indicated nutritional variables among women with either BBD or breast cancer, according to ER-α and PR positivity in the normal

Table 1 Distribution of women with benign breast disease (BBD) or breast cancer by descriptive characteristics, source of tissue samples and expression of estrogen alpha and progesterone receptors in the normal mammary tissue adjacent to the pathological tissue

Diagnosis	BBD ($n = 299$)	Breast cancer ($n = 267$)
Age (years)		
−39	105	12
40–49	109	69
50–59	56	91
60+	29	95
Menopausal status		
Pre- and perimenopausal	227	99
Postmenopausal	72	167
Family history of breast cancer		
Yes	28	33
No	271	234
Hormone use (oral contraceptives for pre- and hormone replacement treatment for postmenopausal)		
Yes	47	25
No	252	242
Paraffin Embedded Tissue (PET)		
Stored PETs	79	41
Newly processed PETs	220	226
Expression of receptors in the normal tissue	295[a]	267
Estrogen receptors alpha	196 (66%)	165 (62%)
Progesterone receptors	219 (74%)	165 (62%)

[a] For four women only estrogen receptor alpha expression was determined, whereas for another four, only progesterone receptor expression was determined. For the remaining 291 women expression of both types of receptors was assessed

Table 2 Median values and Wilcoxon-derived *p* values for the indicated nutritional variables among 299 women with benign breast disease (BBD) and 267 women with breast cancer according to estrogen alpha and progesterone receptor positivity in the normal mammary tissue

	Estrogen receptors				Progesterone receptors			
	BBD		Breast cancer		BBD		Breast cancer	
		p value		*p* value		*p* value		*p* value
Energy intake (kcal)		0.48		0.29		0.34		0.60
Positive	1717.5		1692.7		1755.0		1702.3	
Negative	1766.1		1757.8		1681.3		1797.6	
Fruits, vegetables and legumes (times/day)		0.30		0.58		0.94		0.47
Positive	6.12		6.05		6.27		6.05	
Negative	6.72		6.04		6.43		6.04	
Cereals and starchy roots (times/day)		0.35		0.28		0.66		0.04
Positive	3.35		3.34		3.39		3.14	
Negative	3.47		3.63		3.37		3.94	
Meat (times/day)		0.31		0.50		0.26		0.67
Positive	0.63		0.56		0.64		0.57	
Negative	0.66		0.58		0.57		0.57	
Milk and dairy products (times/day)		0.51		0.78		0.63		0.47
Positive	2.26		2.34		2.24		2.29	
Negative	2.47		2.22		2.44		2.29	
Sugars, sweets and non-alcoholic beverages (times/day)		0.93		0.01		0.23		0.65
Positive	2.71		2.07		2.98		2.28	
Negative	2.78		2.94		2.56		2.24	
Saturated lipids (g/day)		0.69		0.48		0.49		0.89
Positive	26.33		26.75		27.29		26.75	
Negative	27.81		26.93		26.17		26.93	
Monounsaturated lipids (g/day)		0.37		0.68		0.45		0.78
Positive	42.81		41.64		42.25		41.64	
Negative	40.30		41.65		40.42		41.60	
Polyunsaturated lipids (g/day)		0.49		0.60		0.15		0.81
Positive	9.64		9.25		9.62		9.26	
Negative	9.18		9.48		9.13		9.45	
Protein (g/day)		0.48		0.81		0.20		0.38
Positive	64.42		62.34		65.72		61.68	
Negative	66.44		62.25		62.38		64.57	
Carbohydrates (g/day)		0.17		0.11		0.56		0.27
Positive	178.07		167.85		185.88		166.76	
Negative	189.95		187.45		180.04		186.64	
Ethanol (g/day)		0.20		0.75		0.48		0.27
Positive	0.32		0.00		0.32		0.0	
Negative	0.63		0.00		0.42		0.0	

mammary tissue. The dietary intake patterns are compatible with what is known about the Greek population. Thus, fruits, vegetables, and legumes are very frequently consumed and consumption of monounsaturated lipids, mostly in the form of olive oil, exceeds 20% of total energy intake. We observed no striking differences with respect to dietary intakes between receptor positive and negative women, either among BBD or among breast cancer patients. The two significant results among women with breast cancer (with respect to consumption of cereals and starchy roots and expression of progesterone receptors, and consumption of sugar and sweets and expression of ER-α) are no more than that would have been expected by chance after undertaking 48 comparisons. However, the data in Table 2 mostly serve descriptive purposes and are not adjusted for possible confounding variables.

We have previously reported that expression of ER-α and PR receptors in the normal mammary gland is inversely associated with breast cancer risk among postmenopausal women [10]. The multivariate associations of dietary variables with expression of ER-α and PR receptors should be evaluated in this context. In Table 3 we present logistic regression-derived ORs and 95% CIs for ER-α and PR positivity in the normal mammary tissue, per one standard deviation increment of the indicated food groups and macronutrients, stratifying for diagnosis and type of PET (stored versus newly processed). Expression is examined with respect to ER-α and PR, separately, with an additional comparison between women with expression of both receptors to those with expression of none. We found no striking results, but some suggestive findings. Cereals and starchy roots are inversely associated with ER-α expression ($p = 0.01$), whereas milk and dairy products are inversely associated with PR expression ($p = 0.02$). There is also some evidence that ethanol intake is inversely associated with ER-α expression ($p = 0.07$). Monounsaturated lipids ($p = 0.03$) and, to a lesser extent, polyunsaturated lipids ($p = 0.08$) are positively associated with ER-α expression. Because expression of these two types of receptors is generally concordant, positive or inverse associations are broadly consistent across the three contrasts shown in Table 3. There was no evidence for interaction by diagnosis (BBD versus cancer) and type of PET (stored versus newly processed) for any of the examined associations.

Discussion

This is the first study to evaluate the association of diet with ER-α and PR expression in the normal mammary gland adjacent to pathological tissue from biopsies of women diagnosed with benign breast diseases or breast cancer. Using the same study base, we have recently reported that expression of ER-α and PR receptors in the normal mammary gland is inversely associated with breast cancer risk among postmenopausal women [10]. In the present analysis, we examined the association of diet with expression of these receptors in the normal mammary tissue. We found evidence that receptor expression is inversely associated with intake of cereals and starchy roots (for ER-α $p = 0.01$; for PR $p = 0.06$; for expression of both receptors versus none $p = 0.02$), as well as suggestive results indicating that it may also be inversely associated with intake of milk and dairy products (for ER-α $p = 0.21$; for PR $p = 0.02$; for expression of both receptors versus none $p = 0.06$). We also found weak evidence for an inverse association of receptor expression with alcohol intake (for ER-α $p = 0.07$; for PR $p = 0.38$; for expression of both receptors versus none $p = 0.14$), but no

Table 3 Logistic regression-derived odds ratios (ORs) and 95% confidence intervals (CIs)[a] for estrogen alpha (ER) and progesterone (PR) receptor positivity in the normal mammary tissue, among women with benign breast disease (BBD) or breast cancer, per one standard deviation increment of the major food groups and macronutrients

	ER positivity ($n = 562$)			PR positivity ($n = 562$)			Both ER and PR positivity versus both ER and PR negativity ($n = 455$)		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Energy intake (per 558.9 kcal/day)	0.98	0.81–1.17	0.80	1.09	0.89–1.33	0.40	1.07	0.85–1.33	0.59
Fruits, vegetables and legumes (per 2.8 times/day)	0.94	0.75–1.18	0.62	0.97	0.76–1.23	0.79	0.92	0.71–1.19	0.52
Cereals and starchy roots (per 2.1 times/day)	0.74	0.60–0.92	0.01	0.82	0.66–1.01	0.06	0.75	0.59–0.95	0.02
Meat (per 0.5 times/day)	0.96	0.77–1.21	0.74	1.07	0.83–1.37	0.62	1.03	0.78–1.36	0.85
Milk and dairy products (per 2.0 times/day)	0.88	0.72–1.07	0.21	0.78	0.63–0.96	0.02	0.80	0.63–1.01	0.06
Sugars, sweets and non-alcoholic beverages (per 3.1 times/day)	0.93	0.76–1.14	0.50	1.07	0.84–1.35	0.60	1.00	0.78–1.29	>0.99
Saturated lipids (per 13.4 g/day)	1.10	0.78–1.55	0.59	1.03	0.70–1.52	0.89	1.08	0.71–1.63	0.72
Monounsaturated lipids (per 13.3 g/day)	1.45	1.03–2.05	0.03	1.14	0.81–1.60	0.46	1.33	0.90–1.98	0.16
Polyunsaturated lipids (per 3.80 g/day)	1.31	0.97–1.77	0.08	1.31	0.94–1.83	0.11	1.50	1.01–2.22	0.04
Protein (per 23.4 g/day)	1.07	0.73–1.56	0.73	0.93	0.61–1.41	0.73	1.00	0.64–1.56	0.99
Carbohydrates (per 69.0 g/day)	0.78	0.55–1.13	0.19	0.95	0.64–1.41	0.80	0.81	0.53–1.26	0.36
Ethanol (per 6.1 g/day)	0.82	0.66–1.01	0.07	0.92	0.77–1.11	0.38	0.82	0.63–1.07	0.14

[a] Stratified for type of paraffin-embedded tissue (stored versus newly processed) and diagnosis (BBD versus cancer); adjusted for age (categorical in 4 age groups), parity (parous versus nulliparous), menopausal status (post versus pre), age at menarche (continuously), body mass index (BMI) (continuously), family history (yes versus no), use of hormones (yes versus no, contraceptives for premenopausal women, hormone replacement therapy for postmenopausal) and total energy intake (continuously)

association with saturated lipid intake. Intake of both mono- and polyunsaturated lipids, however, appeared to be positively associated with this expression, notably with respect to ER-α and monounsaturated lipids ($p = 0.03$) and with respect to expression of both versus none receptors with polyunsaturated lipids ($p = 0.04$). These findings suggest that consumption of foods imparting high glycemic load and of dairy products, as well as alcohol intake, may reduce expression of receptors, whereas consumption of unsaturated lipids may increase this expression.

Expression of ER-α and PR in the normal mammary gland is likely to play a role in the pathogenesis of breast cancer. These receptors' intimate involvement in the mammotropic action of the corresponding hormones would suggest a positive association between receptor expression and breast cancer risk. However, mammary cells expressing these receptors are considered to be distinct and more differentiated than the actively replicating cells [27, 28], which would be compatible with an inverse association between receptor expression and breast cancer risk.

Two studies, both of the case–control design, have examined ER-α receptor expression in the normal mammary gland in relation to breast cancer risk [10, 29]; one of them [10], also examined expression of progesterone receptors. The study by Khan and colleagues [29]—including data from an earlier study [30]—reported a statistically non-significant positive association of ER-α expression with breast cancer risk among premenopausal women and a significant positive association among postmenopausal women. In contrast, the study by Lagiou and colleagues [10], which relied in the same database as the present investigation, found a non-significant inverse association of ER-α expression with breast cancer risk among premenopausal women and a significant inverse association among postmenopausal women, as well as a significant inverse association of PR expression with breast cancer risk among postmenopausal women. The reason for the discrepant results is not apparent and additional studies are needed to clarify the issue.

Breast cancer risk has been positively associated with alcohol intake [11] and, perhaps, with glycemic load [21], and inversely associated with intake of unsaturated lipids [18, 19]. Thus, on the basis of the existing literature on diet and breast cancer, the results of this study are more compatible with an inverse, rather than positive, association of ER-α and PR expression with breast cancer risk, in line with the results reported by Lagiou and colleagues [10].

Strengths of this investigation are the fairly large sample size, the determination of ER-α and PR expression with a standard protocol by the same investigators (CS and CES) and the use of a dietary questionnaire which has been used in several Greek case–contol studies, that have generated results compatible with those of several major investigations [31, 32]. An inherent limitation imposed by ethical constrains, is the investigation of normal mammary gland in patients with a diagnosis of either BBD or cancer rather than in women with no breast disease. Therefore, we had to rely on apparently normal tissue which was adjacent to pathological and may or may not have the properties of normal tissue in women with no breast pathology.

ER-α and PR expression in the normal mammary gland is influenced by levels of the corresponding hormones, which have been linked to breast cancer risk. Thus, a role of the expression of these receptors in breast cancer etiology is plausible, and the identification of their possible dietary determinants important. Although the evidence at this time is limited, our findings suggest that the weak associations of diet with breast cancer risk could be mediated, to some extent, by the effects of diet on receptor expression in the normal mammary gland.

Acknowledgments This study was partially supported by: the National Institutes of Health/National Cancer Institute, USA (CA89823); the European Union—European Social Fund and Greek Ministry of Development—General Secretariat for Research and Technology (03ED44 PENED project); the Academy of Athens and the University of Athens, Greece (Kapodistrias Program). The authors wish to thank Ms. P. Drogari for administrative assistance.

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