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DNA Pattern and Dietary Habits in Patients with Breast Cancer

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An association between dietary fat, micronutrients and breast cancer aetiology and prognosis has been found in studies of experimental animals and in epidemiological studies. The relationship between dietary habits and the nuclear DNA content of breast cancer cells was studied in 82 women aged 50–65 years. A dietary history interview was conducted within 4 months following surgery. Patients having tumours with euploid DNA pattern reported lower mean intake of saturated fatty acids (FA) in absolute terms, lower mean intake of total fat, saturated FA, and monounsaturated FA, in percentage of total energy intake (E%), a higher E% from protein, and a higher intake of vitamin D, and selenium per 10 MJ than did patients having tumours with aneuploid DNA pattern. In the stepwise logistic regression analysis, the multivariate odds ratios (OR) for having a tumour with aneuploid DNA pattern was 1.16 (95% confidence interval, 1.04–1.28) for each 1 g increase in intake of total fat (E%) and 0.95 (95% confidence interval, 0.92–0.99) for each mg increase in selenium intake per 10 MJ. When total fat was substituted with types of fat, the OR for having a tumour with aneuploid DNA pattern was 1.30 (95% confidence interval, 1.07–1.59) for each 1 g increase in intake of saturated FA (E%). These results suggest a correlation between a diet rich in fat and protein and the DNA content of breast cancer cells.
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INTRODUCTION

An association between dietary habits and breast cancer has been found in studies of experimental animals as well as in human epidemiological studies. A high-fat diet resulted in a higher incidence of chemically induced mammary tumours in rodents when the fatty acid requirement was met than a low-fat diet [1]. The relationship between total fat intake and incidence of mammary cancer was almost linear suggesting a dose–response pattern. Prolonged survival for rats with mammary tumours on a low-fat diet has also been reported [2]. The precise mechanisms for tumour promotion by fat has not yet been established, although several possible mechanisms have been described, including effects on prostaglandin synthesis, hormonal mechanisms, cell membrane fluidity and cell-to-cell communication [3, 4].

In a recent study in rats by Welsch *et al.* [5] the difference in mammary carcinogenesis between a high-fat and a low-fat diet was abolished by a 12% caloric restriction.

The international variation in breast cancer incidence has been shown to be partly related to differences in dietary habits with a lower incidence in countries with a lower fat intake as opposed to higher incidence rates in western countries [6–8]. Despite conflicting results most of the epidemiological evidence is consistent with a causal association between dietary fat and breast cancer risk [9, 10]. In attempts to study the causal association between fat intake and breast disease, intervention trials have been introduced in the treatment of human breast cancer and benign breast disease [11, 12].

We recently presented an association between dietary habits in 240 Swedish women with breast cancer and prognostic factors, such as tumour size and oestrogen receptor (ER) status [12]. The multivariate odds ratio (OR) for having a tumour ≥20 mm in diameter was 0.95 (95% confidence interval, 0.91–0.99) for each 1g increase in fibre intake per 10 MJ of energy. OR for having an ER-rich tumour was 1.58 (95% confidence interval, 1.08–2.31) for each 1 mg increase in retinol intake per 10 MJ, and 1.08 (95% confidence interval, 1.02 to 1.16) for each 1% increment in % total energy intake (E%) from carbohydrates. The study suggested that the dietary patterns of the Western world affect certain prognostic factors in breast cancer.

An association has previously been shown between DNA ploidy and prognosis in breast cancer [13]. The nuclear DNA content is associated with the histological grading of ductal carcinomas [14] and also to the ER content of the breast cancer cells [15].

The purpose of this study was to analyse whether there is a correlation between dietary habits of women with breast cancer and the nuclear DNA content of the breast cancer cells.

MATERIALS AND METHODS

The study was based on 240 women who had surgery for breast cancer between 1983 and 1986 in Stockholm, Sweden [16]. Patients included in the study were aged 50–65 years at the time of cancer diagnosis. Patients with a previous history of cancer or with a disorder requiring dietary restrictions were excluded from the study. In 82 of these patients, the DNA content of the breast cancer cells had been measured independently as part of a separate research protocol. The mean age of the 82 women at the time of diagnosis was 58 years. 8 patients were premenopausal, and 71 were postmenopausal. The menopausal status was not given for 3 patients.

The mean self-reported height of the patients was 1.64 m (range, 1.54–1.75 m), and the mean weight was 65 kg (range, 49–98 kg). The mean body mass index (BMI) was 24.1 kg/m^2 (range, 19.1–34.3 kg/m^2).

Measurement of DNA content was carried out on cytological material from the tumour obtained by fine-needle aspiration from the surgical biopsies and smeared onto a glass slide. The aspirated cellular material consisted of single cells or small clusters of cells. Smears of aspirated material were fixed in 10% neutral formalin for a minimum of 15 h, and subsequently stained by the Feulgen method (acid hydrolysis: 5 N HCl,

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60 min, 22°C) [17]. Cytophotometric measurements of the stained cells were performed in a rapid scanning and integrating microspectrophotometer developed by Caspersson and Lomakka [18]. The absorption was measured at 546 nm and used as a measure of the total amount of DNA in the cell nuclei. Generally, 100 morphologically identified tumour cells per tumour were measured randomly. Admixed normal cells, mainly granulocytes, were used as an internal diploid DNA standard.

The modal values of the tumour cell populations were calculated in relation to the DNA content of the internal control cells (2c). In tumours with more than one stemline, the ploidy level was defined by the modal value of the most abnormal peak. Tumours with stemlines <2.5c and/or between 3.5c and 4.5c, and <10% of the cells exceeding 4.5c were defined as euploid. Tumours with stemlines between 2.5c and 3.5c, and >4.5c as well as tumours exhibiting >10% with DNA values above 4.5c were defined as aneuploid. To avoid misinterpretation of G2- and S-phase cells as stemlines, a limit of 20% of the cell population, based on cell-cycle kinetic data, was used as the minimum size of the peaks used for calculations of the modal values.

A dietary history interview was conducted in the patient's home within 4 months after resection of the tumour. The interview assessed the average daily food intake over the year prior to diagnosis. This assessment included usual composition of meals, frequency of consumption of individual foods and various dishes, and portion size as well as demographic characteristics, reproductive history, height, weight, smoking habits and physical activities.

Daily energy and nutrient intakes were calculated using the KOST computer program and the nutrient data base from the Swedish National Food Administration, which includes the energy and nutrient contents of about 1000 foods and 1500 dishes. Intake of the energy-yielding nutrients (protein, fat, carbohydrates and alcohol) was expressed both in absolute values and as percentage of total energy intake (E%). For other nutrients, intake was expressed both in absolute values (g or mg) and in relation to energy intake (g or mg per 10 MJ).

The following dietary variables (in absolute values and in relation to energy) were included in the analyses: energy, total fat, saturated fatty acids (FA), monounsaturated FA, polyunsaturated FA, carbohydrates, protein, alcohol, dietary fiber, retinol, retinol equivalents, carotenoids, selenium and vitamins C, D and E.

Statistics

Analyses of variance and χ^2 analyses with continuity correction were used to compare differences between groups. In the multivariate analyses, a stepwise logistic regression was performed [19]. The multivariate OR was the estimate of the relative risk for the factors that remained statistically significant (<5%) in the multivariate analysis. The OR value for each factor was standardised for all of the other factors. The parameter estimates of beta showed the chances in logarithmic OR of having an euploid tumour when the variables increased by one unit, given that the other variables were unchanged.

RESULTS

The mean values and ranges for the reported intake of dietary variables included in the analyses are shown in Table 1. The number of children, age, BMI, tumour size, stage, ER status or nodal status were similar for patients with different DNA ploidy. Further, no statistically significant differences were found between the two DNA ploidy groups concerning absolute values for the intake of fat, carbohydrates, proteins, alcohol or vitamins.

Table 1. Reported intake of dietary variables in 82 women

	Absolute value			Value in relation to energy		
Variable	Unit	Mean	S.D.	Unit	Mean	S.D.
Total energy	MJ	7.6	1.9	—	—	—
Total fat	g	76.2	26.3	E%	37.1	5.2
Saturated FA	g	34.4	12.9	E%	16.7	3.1
Monounsaturated FA	g	26.2	8.9	E%	12.8	1.9
Polyunsaturated FA	g	10.4	4.5	E%	5.0	1.3
Carbohydrates	g	205.1	51.3	E%	45.3	5.9
Fibre	g	15.8	5.3	g/10 MJ	21.3	6.6
Protein	g	69.6	17.8	E%	15.5	2.5
Alcohol	g	6.6	10.3	E%	2.5	3.3
Retinol	mg	1.2	0.7	mg/10 MJ	1.6	0.9
Retinol equivalents	mg	1.7	0.8	mg/10 MJ	2.3	1.1
Carotenoids	mg	3.2	2.1	mg/10 MJ	4.5	3.1
Vitamin C	mg	73.5	35.4	mg/10 MJ	101.1	54.0
Vitamin D	mg	5.5	2.0	mg/10 MJ	7.3	1.9
Vitamin E	mg	7.8	4.7	mg/10 MJ	10.1	3.8
Selenium	mg	30.4	18.3	mg/10 MJ	40.4	16.3

Patients having tumours with euploid DNA pattern reported lower mean intake of saturated FA in absolute terms. A lower mean E% was also reported for total fat, saturated FA, and monounsaturated FA. A higher E% was reported for intake of protein and for reported intake of vitamin D and selenium per 10 MJ (Table 2).

Comparisons with χ^2 analyses were performed for patients in lowest vs. highest quartiles according to the reported intake of different food items. The proportion of patients with aneuploid tumours was significantly larger in the highest quartiles for intake of saturated FA in absolute terms and in relation to energy (all $P < 0.05$). It was lower for reported intake in relation to energy for vitamin D and selenium (all $P < 0.05$).

When the stepwise logistic regression procedure was used to analyse the relationships between ploidy and the dietary variables with absolute values [total fat (but not types of fat) as well as protein, selenium and vitamin D] no statistically significant

Table 2. Results of bivariate analyses of variables in relation to DNA pattern, n = 82

		DNA pattern				
		Euploid (n=31)		Aneuploid (n=51)		
Variable	Unit	Mean	S.D.	Mean	S.D.	P value
Total energy	MJ	7.3	2.1	7.8	1.8	ns
Total fat	g	70.1	29.7	80.0	23.6	ns
	E%	35.3	4.9	38.2	5.2	<0.01
Saturated FA	g	30.7	12.9	36.6	12.5	<0.05
	E%	15.5	2.7	17.4	3.1	<0.01
Monounsaturated FA	E%	12.2	1.8	13.1	1.9	<0.05
Protein	E%	16.2	3.2	15.1	2.0	<0.05
Vitamin D	mg/10 MJ	7.9	2.2	6.9	1.5	<0.01
Selenium	mg/10 MJ	46.5	22.8	36.6	9.0	<0.01

ns, not significant.

Table 3. Multivariate logistic regression analysis for diploid DNA pattern as dependent variable, and statistically significant variables in the bivariate analyses as predictors of relative risk

Predictor	Increment	Beta	S.D.	*P* value	OR	95% CI
Total fat						
Intercept		−2.75	2.03			
Selenium	1 mg/10 MJ	−0.05	0.02	<0.01	0.95	0.92–0.99
Fat	1 g/10 MJ	0.15	0.05	<0.01	1.16	1.04–1.28
Types of fat						
Intercept		−0.56	1.02			
Saturated FA	1 g	0.09	0.03	<0.01	1.09	1.03–1.15
Selenium	1 mg	−0.06	0.03	<0.06	0.94	0.89–1.00
Intercept		−1.88	1.75			
Selenium	1 mg/10 MJ	−0.05	0.02	<0.02	0.95	0.92–0.99
Saturated FA	1 g/10 MJ	0.26	0.10	<0.01	1.30	1.07–1.59

relationships were shown. Substitution of saturated, monosaturated and polyunsaturated FA for total fat showed statistical significance for saturated FA ($P < 0.01$). When the variables were related to energy, significance was shown for selenium ($P < 0.01$) and total fat ($P < 0.01$). When substitution was made for total, fat significance was shown for saturated FA ($P < 0.01$; Table 3). The OR of having an aneuploid tumour was 1.09 for each 1 g increase in intake of saturated FA and 0.94 for each 1 mg increase in selenium intake. When expressed in relation to energy the OR was 0.95 for each 1 mg increase in intake of selenium, 1.16 for each 1 g increase in intake of fat, and 1.30 for each 1 g increase in intake of saturated FA (Table 3).

DISCUSSION

The results of this study of mainly postmenopausal women with breast cancer suggest an association between dietary habits and nuclear DNA content of breast cancer cells. The limitations in measurement procedures may have led to a random misclassification of dietary habits. It is possible that the true association between dietary parameters and the DNA content of the breast cancer cells are stronger than shown in the present study since misclassification may have weakened the association. None of the interviewers and none of the patients had any knowledge of the nuclear DNA pattern at the time of the interview since this was not available in the records. It is, therefore, unlikely that this would have introduced any bias. The dietary factors associated with DNA euploidy in this study has previously been correlated to breast cancer risk and prognostic factors for breast cancer [9, 16, 20–22]. However, an association between DNA content of cancer cells and dietary habits has to our knowledge not been reported before.

Patients with an euploid cellular DNA pattern, corresponding to a higher degree of cell differentiation, reported a significantly higher intake of selenium related to total energy intake. An evaluation of the nutrient data base that was used in this study has recently been performed showing that the previous values of selenium are adequate [23]. Selenium with its antioxidant effects has been shown to have preventive properties in virally induced mammary carcinomas in mice [24]. Lower than normal levels of selenium has been reported in patients with breast cancer and fibrocystic disease [25, 26]. The significance of these findings remain unclear. The present study supports the hypothesis of a relationship between breast cancer and the dietary selenium.

No correlation between intake of vitamin C, carotenoids or retinol and the DNA content of the breast cancer cells were found in the present study. This is in agreement with the results of Marubini *et al.* who did not find any correlations between the intake of these nutrients and breast cancer [27].

No correlation between tumour size or axillary node status and ploidy was found in the present study. Such relationships have been shown in larger studies [13]. Dietary habits of this group of breast cancer patients were recently associated with ER content and tumour size [16]. An euploid DNA value of breast cancer cells is also related to a high ER content and euploidy is associated with a low ER content [15].

This rather small study of dietary habits of women with breast cancer and the DNA content of the breast cancer cells implies a correlation between the Western dietary patterns and the DNA content of the breast cancer cells. Further studies are desired to corroborate these findings.

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Trends in Lung Cancer Mortality in Three Broad Italian Geographical Areas Between 1969 and 1987

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Trends in death certification rates from lung cancer in broad Italian geographical areas (north/centre/south) were analysed over the period 1969–1987. In northern Italy, lung cancer rates in young and middle-aged males reached a peak between the mid and late 1970s, and tended to decline afterwards; only above age 60 was mortality still rising in the 1980s. A similar pattern of age-specific rates was observed in central areas, while in the South rates tended to level off in the early 1980s only below age 55, but were still upwards in subsequent age groups. Consequently, the north/south ratio for the overall age-standard rate increased slightly between the late 1960s and mid 1970s, from 1.68 (corresponding to a world standardised rate of 47.1/100 000 in the north vs. 28.1 in the south) to 1.73, but declined to 1.55 between 1985 and 1987 (for a rate of 69.1/100 000 males in the north vs. 44.6 in south). In the younger age groups a diverging pattern was observed: at ages of 25–34 rates in 1985 and 1987 were apparently higher in the south (1.0 vs. 0.9/100 000 in the north), and in the 35–44 age group the north/south ratio decreased from 1.7 to 1.2 (with rates of 12.9 and 10.7, respectively, in 1985 and 1987). Among females, lung cancer rates increased in all geographical areas and age groups except the youngest (25–34 years). Under the age of 50, the rises were proportionally similar in various geographical areas, thus widening the north/south difference in absolute terms. Above the age of 50, the north/south difference tended to be wider in relative terms too, reaching a factor of 2 in the 65–74 age group. The overall age-standardised north/south ratio for females increased from 1.51 in 1969–1974 (5.6 vs. 3.7/100 000) to 1.87 in 1985–1987 (8.4 vs. 4.5/100 000). These trends reflect changes in smoking habits in subsequent generations of Italian males and females from different areas of the country, and confirm the central role of cigarette smoking in lung cancer rates in various populations, although this does not exclude some influence by other, mainly occupational, lung carcinogens on the substantial differences in lung cancer rates in various Italian geographical areas.
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INTRODUCTION

THERE ARE substantial differences in mortality from lung cancer in various Italian geographical areas, with a clear north/south gradient. In the early 1970s, overall age-standardised lung cancer rates for males were 67% higher in the north than in the south of the country, and 55% higher in females, with central areas showing generally intermediate rates [1]. In the mid 1970s geographical differences in overall lung cancer rates tended to rise to 79% higher in the north for males and 83% for females [2, 3]. This was essentially attributed to the pattern of change in