

Consumption of Fermented Milk Products and Breast Cancer: A Case-Control Study in the Netherlands¹

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ABSTRACT

In a case-control study in The Netherlands, we observed a significantly lower consumption of fermented milk products (predominantly yogurt and buttermilk) among 133 incident breast cancer cases as compared to 289 population controls (mean \pm SD among users only, 116 ± 100 versus 157 ± 144 g/day; $P < 0.01$). The age-adjusted odds ratio of daily consumption of 1.5 glasses (≥ 225 g) of fermented milk versus none was 0.50 (95% confidence interval, 0.23–1.08). When fermented milk was entered as a continuous variable (per g) in either age-adjusted or multivariate analysis, the odds ratio expressed per 225 g was 0.63 (multivariate-adjusted 95% confidence interval, 0.41–0.96). After multivariate adjustment for intake of fat and other confounders, a statistically significant decrease in breast cancer risk was also observed for increasing intake of Gouda cheese. The multivariate-adjusted odds ratio expressed per 60 g of this fermented product was 0.56 (95% confidence interval, 0.33–0.95). For daily intake of milk, no statistically significant differences were observed between cases and controls. These results support the hypothesis that high consumption of fermented milk products may protect against breast cancer.

INTRODUCTION

Results of cross-cultural and regional correlation studies have shown that breast cancer mortality correlates positively with the consumption of milk products (1, 2). In two recent case-control studies, conducted in Italy (3) and France (4), a positive association was observed between consumption of dairy products and breast cancer. In the latter study, however, Lè *et al.* (4) also observed an inverse relationship with the frequency of consumption of yogurt. This observation is of interest since feeding of fermented milk products diminished the development of Sarcoma 180 and Ehrlich ascites tumors (5–7). Lactic acid bacteria, used in fermentation of milk products, may survive in the digestive tract (8, 9) and may interfere with other gut bacteria (9, 10). Goldin and Gorbach showed that the intestinal bacterial enzyme activity was altered during a period of feeding milk supplemented with *Lactobacillus acidophilus*, in both rats (11) and humans (12). Such changes may influence the formation (13) and withdrawal (14) of estrogenic compounds or may alter bile acid metabolism (15–17) in the enterohepatic cycle. Furthermore, microorganisms provided by cultured dairy products may stimulate the immunological activity in the host (18, 19), possibly mediated by glycopeptides in the bacterial cell wall (6).

We studied the relation between breast cancer and the consumption of dairy products in The Netherlands. This country has a high incidence of breast cancer, the consumption of dairy

products is high, and the consumption of fermented milk products is among the highest in the world (20) and varies considerably within the population.

SUBJECTS AND METHODS

Cases. The study was conducted in 1985–1987 among Dutch Caucasian women, age 25–44 or 55–64 years, residing in the middle and southern part of The Netherlands. In order to address additional hypotheses, case ascertainment required collection of preoperative blood as well as interviews after hospital discharge. Consequently, study procedures were complicated and cooperation was obtained from surgeons in 17 of 35 hospitals in the study area, covering 55% of hospital admissions. From these hospitals 168 newly diagnosed eligible breast cancer cases (96% histologically confirmed) were identified either by the surgeons or by regional cancer registries. Of the cases 80% (134 women) responded positively to an invitation letter from our Institute. Data obtained from one case were judged unreliable and were excluded from the analysis.

Controls. A total of 548 women, reflecting the age distribution of cases, were sampled randomly from municipal population registries in the same area as the hospitals. All registries supplied names and addresses of women, who were subsequently invited by a letter that explained the aim, design, and procedures of the study and asked for participation in the 2-h interview at home. When a woman did not respond within 2 weeks, she was called (up to 3 times) or visited (once). Eight subjects appeared to be ineligible and 11 could not be reached, leaving 529 control subjects. A total of 223 subjects (42%) immediately agreed to cooperate and 66 subjects (12%) agreed after the telephone call, while 89 subjects (17%) were willing to return a shortened mailed questionnaire. The remaining 151 subjects (29%) refused any form of participation. Thus, a total of 289 controls were included in the data analysis.

Data Collection. Cases and controls were interviewed by two well trained, registered dietitians in a 2-h home visit. Cases were interviewed 3–6 months after hospital discharge when they were feeling well again (*e.g.*, not during chemotherapy treatment). To exclude the possibility that altered food habits occurring after diagnosis would influence the results, the interviewing of the cases referred to the 12-month period before diagnosis. Interviewing of the controls started 3–4 months before the case interviews and referred to the 12-month period preceding the interview date, *i.e.*, the interviews with cases and controls covered the same period of time.

To obtain data on average daily food consumption, a structured dietary history interview was conducted covering the complete dietary pattern. Consumption frequency and amount were recorded for all of the 236 items in the questionnaire, which included all major dairy products used in The Netherlands. In order to obtain an accurate quantification of the usual amounts ingested, portion sizes of the most frequently used household utensils were measured in the kitchen at the end of each interview. This included measurement of the fluid content of cups, glasses, and small dishes used to consume milk and yogurt. Average daily intake of fat and other nutrients was calculated with the standardized Dutch Uniform Encoding system (21) and the 1984 release of the computerized Dutch Nutrient Data Bank (22). The questionnaire also addressed stability of food habits, smoking habits, demographic and socioeconomic characteristics, reproductive history, previous breast diseases, oral contraceptive and other hormone use, and

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prediagnostic weight. In addition, anthropometry was performed, toenail clippings were collected, and a blood sample was drawn (for cases before surgery).

Data Analysis. In this analysis the emphasis was on three groups of dairy products: fermented milk products (*i.e.*, yogurt, buttermilk, curds, and kefir); Gouda cheese; and milk (*i.e.*, skimmed, partly skimmed, and full-cream milk). Consumption of several milk products and fat intake were compared between cases and controls among the women age 25–44 and those age 55–64 years. Since the differences were similar in both age groups, the overall difference of means and their 95% confidence intervals are presented. In further analyses, the intake of dairy products was expressed in g, either in categories of 20 or 75 g (half a glass) or as continuous variables. Odds ratios and their 95% confidence intervals were calculated, with adjustment for age using indicator variables (categories: 25–39, 40–44, 55–59, and 60–64 years). Potential confounders were controlled individually and simultaneously by means of multiple logistic regression. The following variables were considered as potential confounders: age, dietary fat intake, alcohol intake, history of benign breast disease, first- and second-degree familial history, smoking habits, educational level of the woman, use of oral contraceptives, age at menarche, age at first full term pregnancy, parity, body mass index, and geographical area.

RESULTS

Age-adjusted odds ratios and 95% confidence intervals for major risk factors of breast cancer are shown in Table 1. Although none of these variables had a significant impact on the risk of breast cancer in this study, the direction of most associations is as expected.

The consumption of fermented milk products was significantly higher among controls than among cases (Table 2). The differences were in the same direction in both the younger (25–44 years) and older (55–64 years) age group (data not shown). Although cases also consumed slightly less Gouda cheese and

Table 1 Age-adjusted odds ratios and 95% confidence interval for major risk factors of breast cancer, for 133 incident cases and 289 population controls.

| Risk factor | Level | Odds ratio | 95% Confidence interval |
|---------------------------------------|--------------------------|------------|-------------------------|
| Age at menarche (yr) | ≤12 ^a | 1.00 | |
| | 13–14 | 0.79 | 0.48–1.27 |
| | ≥15 | 0.60 | 0.32–1.12 |
| | | | |
| Age at first full-term pregnancy (yr) | Nulliparous ^a | 1.00 | |
| | ≤26 | 0.96 | 0.51–1.80 |
| | >26 | 1.14 | 0.60–2.17 |
| Parity (no. of children) | Nulliparous ^a | 1.00 | |
| | 1–2 | 1.42 | 0.76–2.64 |
| | ≥3 | 0.62 | 0.31–1.22 |
| First-degree familial history | Negative ^a | 1.00 | |
| | Positive | 1.65 | 0.81–3.33 |
| Second-degree familial history | Negative ^a | 1.00 | |
| | Positive | 1.72 | 1.00–2.97 |
| History of benign breast disease | Negative ^a | 1.00 | |
| | Positive | 1.13 | 0.67–1.90 |
| Use of oral contraceptives | Never ^a | 1.00 | |
| | Ever | 1.07 | 0.64–1.78 |
| Body mass index (kg/m ²) | ≤30 ^a | 1.00 | |
| | >30 | 1.18 | 0.63–2.22 |
| Educational level | Low ^a | 1.00 | |
| | Medium | 1.06 | 0.65–1.73 |
| | High | 1.46 | 0.79–2.68 |
| No. of cigarettes smoked daily | 0 ^a | 1.00 | |
| | 1–10 | 0.99 | 0.59–1.65 |
| | ≥11 | 1.41 | 0.81–2.47 |

^a Reference category.

Table 2 Consumption of milk products and fat intake of 133 breast cancer cases and 289 control subjects

| Milk product | % nonusers | | Intake (g/day) among users ^a | | Difference (95% confidence interval) ^b |
|--------------------|----------------|----------|---|-----------|---|
| | Cases | Controls | Cases | Controls | |
| Fermented milk | | | | | |
| Yogurt | 32 | 33 | 64 ± 55 | 74 ± 70 | 10 (–7, 26) |
| Buttermilk | 59 | 54 | 106 ± 92 | 150 ± 130 | 44 (6, 82) |
| Curds | 77 | 80 | 18 ± 18 | 30 ± 66 | 12 (–12, 36) |
| Kefir | 99 | 100 | 84 ± 4 | 86 ± 0 | 2 |
| Total ^c | 21 | 21 | 116 ± 100 | 157 ± 144 | 41 (9, 72) |
| Gouda cheese | 5 | 3 | 39 ± 27 | 43 ± 29 | 4 (–2, 10) |
| Milk | 28 | 24 | 189 ± 154 | 203 ± 80 | 14 (–27, 56) |
| Total fat intake | — ^d | — | 100 ± 36 | 92 ± 30 | 8 (1, 14) |

^a Mean ± SD among users only.

^b Mean difference (95% confidence interval) among users only.

^c Sum of fermented milk products: yogurt, buttermilk, curds, and kefir.

^d Not applicable.

Table 3 Relation between consumption of fermented milk products (sum of yogurt, buttermilk, curds, and kefir), Gouda cheese, and milk and breast cancer for 133 cases and 289 population controls, assessed by age-adjusted and multivariate logistic regression

| Amount consumed | % | | Odds ratio (95% confidence interval) | |
|-------------------------|-------|----------|--------------------------------------|---------------------------|
| | Cases | Controls | Age-adjusted | Multivariate ^a |
| Fermented milk products | | | | |
| Nonusers | 21 | 21 | 1.00 | 1.00 |
| 0–75 g | 35 | 28 | 1.25 (0.70–2.24) | 1.29 (0.69–2.42) |
| 75–150 g | 22 | 20 | 1.10 (0.58–2.09) | 1.12 (0.56–2.25) |
| 150–225 g | 14 | 13 | 1.14 (0.55–2.37) | 1.29 (0.59–2.83) |
| >225 g | 9 | 18 | 0.50 (0.23–1.08) | 0.55 (0.24–1.27) |
| Per 225 g ^b | | | 0.63 (0.42–0.93) | 0.63 (0.41–0.96) |
| Gouda cheese | | | | |
| Nonusers | 5 | 3 | 1.00 | 1.00 |
| 0–20 g | 25 | 23 | 0.76 (0.24–2.42) | 0.53 (0.15–1.89) |
| 20–40 g | 32 | 30 | 0.81 (0.26–2.53) | 0.49 (0.14–1.76) |
| 40–60 g | 23 | 23 | 0.75 (0.23–2.44) | 0.41 (0.11–1.52) |
| >60 g | 16 | 21 | 0.63 (0.19–2.11) | 0.26 (0.07–1.05) |
| Per 60 g | | | 0.83 (0.52–1.31) | 0.56 (0.33–0.95) |
| Milk | | | | |
| Nonusers | 28 | 24 | 1.00 | 1.00 |
| 0–75 g | 22 | 21 | 0.95 (0.52–1.74) | 1.08 (0.56–2.10) |
| 75–150 g | 11 | 14 | 0.68 (0.33–1.39) | 0.69 (0.31–1.51) |
| 150–225 g | 15 | 17 | 0.74 (0.38–1.43) | 0.86 (0.41–1.81) |
| >225 g | 24 | 23 | 0.91 (0.51–1.63) | 0.82 (0.43–1.57) |
| Per 225 g ^b | | | 0.86 (0.65–1.15) | 0.81 (0.59–1.12) |

^a Adjusted for age, fat and alcohol intake, history of benign breast disease, first- and second-degree familial history, number of cigarettes smoked daily, woman's educational level, ever use of oral contraceptives, age at menarche, age at first full-term pregnancy, parity, body mass index, and geographic area.

^b Derived from the logistic regression model, with intake specified in g. An average cup of milk contains about 150 g.

milk than did controls, these differences were not statistically significant.

As compared to nonusers, the age-adjusted odds ratio of consumption of more than 1.5 glasses (>225 g) of fermented milk was 0.50 (95% confidence interval, 0.23–1.08) but slightly exceeded 1.00 for lower levels of consumption (Table 3). This result was not materially affected by multivariate analysis. When fermented milk was included as a continuous variable (in g) in the logistic regression model, the odds ratio of breast cancer expressed per 225 g fermented milk daily (one and a half glasses *versus* none was 0.63 in both the age-adjusted and multivariate model and the corresponding 95% confidence intervals did not include 1.00.

Consumption of Gouda cheese showed a lower odds ratio at higher intake, but this association was not statistically significant. However, since fat intake was associated with both the

consumption of Gouda cheese (Pearson coefficient of correlation = 0.34) and breast cancer (Table 2), it might have obscured a protective effect. Indeed, in multivariate analysis the association became more pronounced, and in the model specifying Gouda cheese as a continuous variable the association became statistically significant (Table 3).

Consumption of milk was not significantly associated with the odds ratio for breast cancer in either the age-adjusted or the multivariate model.

DISCUSSION

Our results suggest that consumption of a high amount of fermented milk products may have a protective effect on the risk of breast cancer. After allowance for fat intake, a similar relationship was observed for Gouda cheese, which is also a cultured milk product. No statistically significant relationship was observed between consumption of milk and breast cancer.

Until now, only one epidemiological study (4) has addressed the consumption of a fermented milk product, *i.e.*, yogurt, in relation to breast cancer. In most studies conducted so far, no distinction has been made between fermented and nonfermented dairy products. In our study, we distinguished different types of milk products, we inquired about consumption frequency and number of glasses or dishes, we actually measured the contents of relevant household utensils, and we conducted analyses using average daily intakes with and without adjustment for total fat intake and other potential confounders.

We considered whether the inverse association with fermented milk products might be explained by confounding factors or other sources of bias. Adjustment for a large number of breast cancer risk factors (see "Subjects and Methods") and the most widely discussed dietary factors with regard to breast cancer, *i.e.*, fat and alcohol intake (23, 24), however, did not appreciably affect the results. Furthermore, it was considered that response could be related to socioeconomic status and food habits. However, when the intake of fermented milk of cases and controls was stratified according to the educational level of the women (who generally buy and prepare the food) or their partners, cases consumed less fermented milk products than did controls in all strata. Results of the multivariate analysis were similar when the educational level of the partners (rather than the women) was included in the model. Finally, no major differences were observed between partial (89 women) and complete responders (289 women; see "Subjects and Methods"), either with regard to age, age at first full-term pregnancy, parity, height, weight, and body mass index or with regard to fat content of milk products and food items used on bread and usual consumption of bread, eggs, and fish.

The inverse association between consumption of Gouda cheese and breast cancer seems to disagree with the results of Lê *et al.* (4), who observed a positive association with the frequency of consumption of French cheese. Their data, however, required extensive classification of many types of cheese, while our analysis could be restricted to Gouda cheese, accounting for more than 90% of total cheese consumption in our study population. Moreover, the inverse association appeared after adjustment for total daily fat intake, which was not taken into account by Lê *et al.* The association, however, can also partially be attributed to the small number of nonusers and was weakened when educational level of the partners (instead of the women) was included in the multivariate model (data not shown). Because microorganisms used in the fermentation of Gouda cheese are similar to those in some other fermented milk products,

this association is in line with the study hypothesis.

Biological evidence supporting the hypothesis that fermented milk products may protect against breast cancer points to lactic bacteria interfering with the enterohepatic circulation (13–17) or stimulating immunological activity (6, 18, 19). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* play an important role in the production of yogurt, while other lactic acid bacteria (*Streptococcus* spp. and *Leuconostoc* spp.) are used in the production of buttermilk and Gouda cheese (25, 26). In our data, the lowered odds ratio at high intake of fermented milk can be attributed, to a large extent to buttermilk (Table 2). For Gouda cheese, with an initially higher concentration of live bacteria, the decreased odds ratio was observed at a lower intake. Since the number of live bacteria in Gouda cheese decreases considerably during storage, this suggests that the protective effect of fermented milk products might not be limited to live lactic acid bacteria from yogurt but could extend to the microorganisms (not necessarily live) in these other fermented products.

In conclusion, our results are in agreement with the observations of Lê *et al.* (4) and support experimental research suggesting favorable effects of lactic acid-forming bacteria on the intestinal microflora and the immune system. The suggested protective effect of a high intake of fermented milk products (including Gouda cheese) on the risk of breast cancer needs to be substantiated by further observational and experimental research.

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