# Dietary calcium, phosphorus, vitamin D, dairy products and the risk of colorectal adenoma and cancer among French women of the E3N-EPIC prospective study.

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**Key words:** colorectal cancer; colorectal adenoma; calcium; vitamin D; phosphorus; dairy product

A protective effect of calcium and/or dairy products on colorectal cancer has been reported in epidemiological studies but the findings are considered inconsistent. In particular, it is unclear whether they act at a particular step of the adenoma-carcinoma sequence. To investigate the effect of dairy product consumption and dietary calcium, vitamin D and phosphorus intake on the adenoma-carcinoma sequence in the French E3N-EPIC prospective study. The population for the study of risk factors for adenomas was composed of 516 adenoma cases, including 175 high-risk adenomas, and of 4,804 polyp-free subjects confirmed by colonoscopy. The population for the colorectal cancer study was composed of 172 cases and 67,312 cancer-free subjects. Diet was assessed using a self-administered questionnaire completed at baseline. There was a trend of decreasing risk of both adenoma trend5 0.04) and cancer (ptrend50.08) with increasing calcium intake, with RRs for adenoma and cancer of 0.80 (IC 95%50.62– 1.03) and 0.72 (95% CI50.47–1.10), respectively, in the fourth quartile compared to the first. A protective effect of dairy products on adenoma (RRQ4 vs.Q15 0.80, 95% CI50.62-1.05, ptrend5 0.04) was observed and of milk consumption on colorectal cancer Q4vs.Q15 0.54, 95% CI50.33–0.89, ptrend5 0.09), although the latter did not reach significance. Phosphorus intake also decreased the risk of adenoma (RRQ4 vs.Q150.70, 95% CI50.54–0.90, trend5 0.005). No vitamin D effect was identified. Our data support the hypothesis that calcium, dairy products and phosphorus exert a protective effect at certain steps of the adenoma-carcinoma sequence.

The idea that an intake of calcium and vitamin D might protect against colorectal cancer was first suggested by Newmark *et al*.[1] In experimental studies, low calcium diets have consistently been associated with increased cell proliferation and low cell differentiation.[2] It has therefore been suggested that calcium acts directly on epithelial cell proliferation and differentiation, as well as indirectly by forming insoluble soaps with secondary bile acids, although this point has never been formally demonstrated.[2,3] Vitamin D plays a major role in the homeostasis of calcium and 2 studies have also suggested that it reduces epithelial cell proliferation and promotes differentiation.[2,4] It has been further suggested that polymorphisms of the vitamin D receptor (VDR) might modulate this effect.[5]

However, findings from epidemiological studies on the relationship between calcium or dairy product intake and risk of colorectal adenoma or/and of cancer were judged inconsistent in 2 reviews,[6,7] except for a steady protective effect of dairy product reported in cohort studies.[6] Although a meta-analysis[8] concluded that calcium had no protective effect on colorectal tumours, prospective studies and large case-control studies subsequently found a modestly beneficial effect.[9-11] Recently the Pooling

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Project, a pooled analysis of 10 prospective studies,[12] showed a significant protective effect of calcium and milk on colorectal cancer risk.

Additional information has become available from recent intervention trials,[13-15] which suggested a protective effect of calcium supplementation on the risk of colorectal adenoma recurrence, but the level of calcium required for adenoma chemoprevention has yet to be defined. We prospectively studied the association between the intake of dietary calcium, phosphorus, vitamin D and/ or dairy products and the risk of adenomatous polyps and colorectal cancer among women participating in the E3N-EPIC study.

## Material and methods

### *Cohort study*

E3N (Etude Epidémiologique de femmes de la Mutuelle Générale de l'Education Nationale) is a prospective cohort study conducted in France to study the risk factors for the most common cancer sites in women.[16] The cohort was established in 1990 and consisted of 100,000 women living in France, aged 40 to 65 years at baseline and insured with the Mutuelle Generale de l'Education Nationale, a national health insurance scheme for teachers. E3N is also the French part of the EPIC study.[17]

Dietary habits, reproductive factors, use of hormonal treatments, tobacco consumption, anthropometric measurements, personal history of disease, family history of cancer and other factors were recorded in self-administered questionnaires completed approximately every 24 months. Each questionnaire inquired about the occurrence of personal medical events, in particular of colorectal polyps or cancer, since the previous follow-up questionnaire.

### *Dietary data*

Dietary data were collected between June 1993 and July 1995, using a 2-part questionnaire. The first part contained questions on frequency of consumption of food groups and the amounts usually consumed, while the second included qualitative questions allowing to detail individual foods within the food groups. The questionnaire was used to assess the consumption of 208 food items, beverages and recipes. It was accompanied by a booklet of photographs illustrating portion sizes.

Both the questionnaire and the booklet were validated.[18,19] The validity of the dietary questionnaire was assessed using a sample of 115 women, taking as the reference the average of 12 24-hr recalls obtained at monthly intervals over a 1-year period. The reproducibility of the 24-hr recall questionnaires was also tested. The Spearman's correlation coefficient for dairy products consumption was 0.67 for validity and 0.73 for reproducibility. A high proportion of subjects (76% for foods and 72% for nutrients) were classified in the same or adjacent quintile for the dietary questionnaire and the 24-hr recalls. The Pearson's correlation coefficient for calcium intake was 0.38 for validity and 0.75 for reproducibility.

The diet history questionnaire was sent to 95,644 women, with 2 reminders to nonresponders. In all, 77,613 completed questionnaires (81.1%) were received.

After exclusion of 985 questionnaires because of absence of consent to external health follow-up by the health insurer in case of dropout, 2,050 questionnaires because of miscoded answers, 8 empty questionnaires and 46 duplicate ones, 74,524 questionnaires were available for analysis.

Women with extreme values (in the top or bottom 1%) for the energy intake/energy requirement ratio (calculated taking age, weight and height into account) were excluded. A total of 73,034 women were finally available for analysis. Total dairy products were considered as well as individual products (yoghurt, milk, cottage cheese and cheese). The dietary intake of calcium, vitamin D and phosphorus and the calcium to phosphorus ratio were analysed using a food composition table derived from the French national database[20].

### *Cases and noncases*

No mass-screening for colorectal cancer was achieved in France at the time of our study. Complete colonoscopy was performed mostly for investigating anemia or bowel symptoms, as well as sometimes for screening first degree relatives of subjects with colorectal neoplasia. Therefore adenoma

subjects and adenoma-free controls were selected in the same way. Colorectal cancers were mostly diagnosed when symptomatic.

The risks of adenoma and colorectal cancer were studied separately.

The adenoma study was based on the 1,933 subjects who had reported diagnosis of a colorectal polyp between the return of the dietary questionnaire (1993–1995) and the endpoint of the analysis (December 1997). Pathology reports were obtained for 1,892 (97.9%) of these women; 41 of them had a tumour of unidentified histological type, 388 had no tumour, 108 had a colorectal cancer, 387 had hyperplastic polyps and 968 had adenomatous polyps. After exclusion of subjects with previous cancer (n534), familial adenomatous polyposis (n57), an inflammatory bowel disease (n57), a personal history of previous adenoma (n5390) or an adenoma diagnosed after the end of follow-up (n514), 516 subjects were considered as cases. As adenomas are frequent[21] and are mostly asymptomatic tumours, adenoma cases were compared to a polyp-free population. Noncases were defined as subjects who were polyp-free at endpoint and had never reported a personal history of polyps or cancer. Subjects contributed person-time up to the date of diagnosis of an adenomatous polyp, date of cancer of any site (other than basal cell skin carcinoma), date of last completed questionnaire, date of death or December 1997, whichever occurred first. In order to identify nutritional factors involved in adenoma progression, a high-risk adenoma group was defined. It covered large adenomas (over 1 cm in diameter), adenomas with severe dysplasia, multiple (i.e., 3 or more) adenomas and those with a villous component.[22] The population for the adenoma study was composed of 516 women with adenomatous polyps, including 175 high-risk adenomas and 4,804 polyp-free women.

The colorectal cancer study was based on subjects who had reported diagnosis of a primary colorectal cancer between the return of the dietary questionnaire (1993–1995) and June 2000. We excluded subjects who had reported a cancer diagnosis before the start of follow-up (n54592), nonhistologically confirmed colorectal cancers (n57), a familial polyposis syndrome (n5334), an inflammatory bowel disease (n5556) and subjects lost to followup after dietary assessment (n561). Subjects contributed persontime up to the date of diagnosis of colorectal cancer, date of cancer of any other site (other than basal cell skin carcinoma), date of last completed questionnaire, date of death or June 2000, whichever occurred first. 172 women with colorectal cancer and 67,312 women free of colorectal cancer were finally included in the analysis.

***Statistical analysis***

The association between dairy products, dietary calcium, phosphorus, the calcium to phosphorus ratio, vitamin D and adenomatous polyp or colorectal cancer diagnosis was estimated using Cox proportional hazards models. Age at recruitment was used as the primary time variable. Age at diagnosis of adenomatous polyps (adenoma study), at diagnosis of colorectal cancer (colorectal cancer study) or at censoring date was used as the end-of-study time variable.

Adjustment for total dietary energy intake was made with the energy-adjusted nutrient intake method,[23] using residuals of nutrients intakes on total energy (other than from alcohol). For foods, adjustment for energy was performed by including energy as a continuous variable in the Cox model.

Quartiles for each analysis were calculated on the distribution of noncases. For milk and cottage cheese, a nonconsumer category was defined (as over 25% of subjects were nonconsumers of these products) and consumers were classified in tertiles. Tests for linear trend were performed using the ordinal score. We also tested for potential interaction between calcium and phosphorus.

To control for potential confounding factors, values were adjusted for body mass index at recruitment, total daily alcoholfree energy intake and total daily alcohol intake (all as continuous variables), family history of colorectal cancer (yes/no), physical activity (tertile of weekly energy expenditure), education level ($\leq$11, 12–13, 14–15, $\geq$16 years of schooling) and smoking status at baseline (ever/never). Further adjustment included calcium or vitamin D supplementation as a treatment for osteoporosis (yes/ no), energy-adjusted fibre and folate, consumption of red meat, consumption of processed meat and contribution of saturated fatty acids to dietary energy intake.

All analyses were performed using the SAS software, version 8.2.

## Results

### *Characteristics of the studied populations*

The median follow-up time was 3.7 years in the adenoma study and 6.9 years in the cancer study. Baseline characteristics of all groups are presented in Table I. Adenoma cases were older than polyp-free subjects (p<0.001): mean 6 SD was 54.4 6 6.7 years for the total adenoma group, 54.9 6 6.7 years for the high-risk adenoma group and 52.8 6 6.4 years for the polyp-free group. Adenoma cases were also significantly less educated (p50.008), consumed more alcohol (p50.005) and had a higher BMI (p50.002) than polyp-free subjects. Total energy intake, smoking status and calcium and /or vitamin D supplementation did not differ significantly between adenoma cases and polyp-free subjects. Colorectal cancer cases were significantly older (p<0.001) and had a higher BMI (p50.03) than cancer-free subjects. They more frequently had a history of colorectal cancer in their first-degree relatives than the reference population (p<0.001).

Table I – **Baseline characteristics of cases and noncases included in the colorectal adenoma and cancer studies: E3N-EPIC study (1993)**

| | Adenoma study | | | Colorectal cancer study | |
|---|---|---|---|---|---|
| | Polyp-free | All adenoma | High-risk adenoma | Colorectal cancer-free | Colorectal cancer |
| N participants | 4,804 | 516 | 175 | 67,312 | 172 |
| N person-years | 16,489 | | | 425,392 | |
| Age[1] mean years (sd) | 52.8 (6.4) | 54.4[3](6.7) | 54.9[3] (6.7) | 52.7 (6.6) | 57.2[3] (6.4) |
| BMI[1] mean kg/m$^2$ (sd) | 22.74 (3.14) | 23.21[3] (3.35) | 23.16 (3.43) | 23.01 (3.33) | 23.64[3] (3.70) |
| Low level of energy expenditure[1] (%) | 33.14 | 35.27 | 33.71 | 33.34 | 31.40 |
| Familial history of colorectal cancer[1,2] (%) | 30.18 | 31.20 | 33.71 | 12.14 | 22.09[4] |
| Number of years schooling < 12 years (%) | 10.41 | 15.75[4] | 15.34 | 11.58 | 15.57 |
| Current smokers[1] (%) | 12.65 | 14.60 | 16.37 | 14.25 | 13.69 |
| Ex-smokers[1] (%) | 22.49 | 23.86 | 23.98 | 23.03 | 17.26 |
| Calcium and/or vitamin D[1] treatment for osteoporosis (%) | 3.83 | 4.26 | 3.43 | 2.93 | 4.65 |
| Ethanol[1] mean g/day (sd) | 10.84 (13.92) | 12.90[3] (15.93) | 13.96[3] (18.97) | 11.14 (14.10) | 13.04 (20.82) |
| Energy intake[1]: mean Kcal/day (sd) | 2,048.47 (528.24) | 2,026.05 (521.05) | 1,986.60 (519.67) | 2,028.98 (537.55) | 2,055.92 (497.45) |

[1] At time of dietary record.–[2] In first-degree relatives (parents and sibling).–[3] p <0.05 for t-test comparing cases (adenomas or cancers) to their reference population (polyp-free and cancer-free subjects, respectively).–[4] p <0.05 for v -test, testing independence of distribution between cases and their reference population.

Descriptive statistics of the nutrient intake and dairy products consumption of both reference groups are presented in Table II. Intakes of the nutrients studied were higher among polyp-free subjects than among cancer-free subjects.

Table II – **Descriptive statistics of nutrients intakes and food consumption of the two non case populations**

| | Polyp-free subjects (n=4804) | | | | | | | Cancer-free subjects (n=67312) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Standard deviation | 10th percentile | 1st quartile | Median | 3rd quartile | 90th percentile | Mean | Standard deviation | 10th percentile | 1st quartile | Median | 3rd quartile | 90th percentile |
| **Energy-adjusted nutrient intakes (/day)** | | | | | | | | | | | | | | |
| Calcium (mg) | 1,034.71 | 363.65 | 640.08 | 785.62 | 981.67 | 1226.16 | 1,499.42 | 1,014.15 | 359.55 | 617.55 | 766.22 | 962.63 | 1,201.81 | 1,469.18 |
| Dairy calcium (mg) | 594.32 | 323.85 | 258.73 | 370.72 | 538.25 | 754.86 | 1,015.24 | 578.75 | 319.31 | 240.74 | 359.24 | 523.03 | 736.00 | 979.28 |
| Phosphorus (mg) | 1,413.77 | 387.23 | 957.07 | 1,141.86 | 1,374.86 | 1,633.83 | 1,923.29 | 1,391.40 | 388.12 | 934.31 | 1121.23 | 1,352.25 | 1,613.57 | 1,898.93 |
| Vitamin D (μg) | 2.63 | 1.29 | 1.29 | 1.73 | 2.37 | 3.26 | 4.34 | 2.61 | 1.28 | 1.26 | 1.72 | 2.37 | 3.23 | 4.25 |
| Food item (g/day) | | | | | | | | | | | | | | |
| Total dairy products | 324.44 | 200.60 | 120.48 | 184.83 | 280.96 | 424.29 | 578.10 | 316.84 | 201.30 | 114.19 | 179.47 | 274.72 | 409.21 | 564.89 |
| Milk[1] | 102.81 | 156.04 | - | - | 26.27 | 165.47 | 300.00 | 100.74 | 157.90 | - | - | 23.57 | 151.34 | 300.00 |
| Yogurt | 91.06 | 79.65 | - | 32.29 | 80.71 | 129.14 | 193.71 | 88.42 | 78.76 | - | 32.29 | 80.71 | 120.42 | 193.71 |
| Cottage cheese[2] | 39.06 | 52.85 | - | - | 20.85 | 57.14 | 107.14 | 37.44 | 51.31 | - | - | 19.70 | 54.93 | 100.49 |
| Cheese | 55.89 | 42.07 | 11.90 | 27.26 | 47.76 | 71.29 | 115.71 | 54.61 | 40.96 | 11.18 | 26.67 | 46.95 | 70.13 | 109.03 |

[1]Non-consumers: 42.99% in the adenoma study; 43.7% in the cancer study. [2]Non-consumers: 30.6% in the adenoma study; 31.02% in the cancer study.

Table III - **RRs of adenoma, high-risk adenoma and colorectal cancer by quartile of energy-adjusted calcium, total calcium, dairy, phosphorus and vitamin D intake.**

| | All adenomas | | | | | High-risk adenomas | | | | | Colorectal cancer | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calcium (mg)** | **<785.62** | **785.62-981.67** | **981.67-1226.16** | **>1226.16** | ***P for trend*** | **<785.62** | **785.62-981.67** | **981.67-1226.16** | **>1226.16** | ***P for trend*** | **<766.22** | **766.22-962.63** | **962.63-1,201.81** | **>1,201.81** | ***P for trend*** |
| Cases (n) | 140 | 141 | 121 | 114 | | 46 | 45 | 46 | 38 | | 49 | 47 | 39 | 37 | |
| RR (Age-adjusted) | 1.00 | 0.96 | 0.83 | 0.79 | *0.03* | 1.00 | 0.92 | 0.92 | 0.77 | *0.27* | 1.00 | 0.94 | 0.77 | 0.70 | *0.07* |
| (IC 95%) | | (0.76-1.22) | (0.65-1.07) | (0.62-1.01) | | | (0.61-1.39) | (0.61-1.40) | (0.50-1.19) | | | (0.63-1.41) | (0.51-1.18) | (0.46-1.08) | |
| RR (Fully[1] adjusted) | 1.00 | 0.97 | 0.83 | 0.80 | *0.04* | 1.00 | 0.93 | 0.94 | 0.80 | *0.34* | 1.00 | 0.94 | 0.78 | 0.72 | *0.08* |
| (IC 95%) | | (0.76-1.22) | (0.65-1.07) | (0.62-1.03) | | | (0.62-1.41) | (0.62-1.42) | (0.52-1.23) | | | (0.63-1.41) | (0.51-1.19) | (0.47-1.10) | |
| **Dairy calcium (mg)** | **<370.72** | **370.72-538.25** | **538.25-754.86** | **>754.86** | ***P for trend*** | **<370.72** | **370.72-538.25** | **538.25-754.86** | **>754.86** | ***P for trend*** | **<359.24** | **359.24-523.03** | **523.03-736.00** | **>736.00** | ***P for trend*** |
| Cases (n) | 132 | 162 | 108 | 114 | | 39 | 61 | 35 | 40 | | 45 | 51 | 36 | 40 | |
| RR (Age-adjusted) | 1.00 | 1.20 | 0.80 | 0.85 | *0.02* | 1.00 | 1.50 | 0.84 | 0.98 | *0.30* | 1.00 | 1.12 | 0.78 | 0.84 | *0.20* |
| (IC 95%) | | (0.95-1.51) | (0.62-1.03) | (0.66-1.09) | | | (1.00-2.25) | (0.53-1.33) | (0.63-1.52) | | | (0.75-1.69) | (0.50-1.22) | (0.55-1.28) | |
| RR (Fully[1] adjusted) | 1.00 | 1.20 | 0.82 | 0.86 | *0.04* | 1.00 | 1.52 | 0.89 | 1.02 | *0.43* | 1.00 | 1.13 | 0.80 | 0.86 | *0.25* |
| (IC 95%) | | (0.95-1.52) | (0.64-1.07) | (0.67-1.11) | | | (1.00-2.28) | (0.56-1.41) | (0.65-1.59) | | | (0.76-1.70) | (0.51-1.25) | (0.56-1.32) | |
| **Phosphorus (mg)** | **<1,141.86** | **1,141.86-1,374.86** | **1,374.86-1,633.83** | **>1,633.83** | ***P for trend*** | **<1,141.86** | **1,141.86-1,374.86** | **1,374.86-1,633.83** | **>1,633.83** | ***P for trend*** | **<1121.23** | **1121.23-1,352.25** | **1,352.25-1,613.57** | **>1,613.57** | ***P for trend*** |
| Cases (n) | 143 | 135 | 129 | 109 | | 45 | 46 | 44 | 40 | | 52 | 41 | 38 | 41 | |
| RR (Age-adjusted) | 1.00 | 0.92 | 0.88 | 0.74 | *0.02* | 1.00 | 0.97 | 0.92 | 0.84 | *0.41* | 1.00 | 0.79 | 0.73 | 0.77 | *0.18* |
| (IC 95%) | | (0.73-1.17) | (0.69-1.11) | (0.58-0.96) | | | (0.64-1.47) | (0.61-1.40) | (0.55-1.29) | | | (0.52-1.20) | (0.48-1.12) | (0.51-1.15) | |
| RR (Fully[1] adjusted) | 1.00 | 0.89 | 0.83 | 0.70 | *0.005* | 1.00 | 0.93 | 0.87 | 0.77 | *0.23* | 1.00 | 0.77 | 0.70 | 0.73 | *0.11* |
| (IC 95%) | | (0.70-1.13) | (0.65-1.06) | (0.54-0.90) | | | (0.61-1.41) | (0.57-1.32) | (0.50-1.19) | | | (0.51-1.17) | (0.46-1.08) | (0.48-1.10) | |
| **Vitamin D (μg)** | **<1.73** | **1.73-2.37** | **2.37-3.26** | **>3.26** | ***P for trend*** | **<1.73** | **1.73-2.37** | **2.37-3.26** | **>3.26** | ***P for trend*** | **<1.72** | **1.72-2.37** | **2.37-3.23** | **>3.23** | ***P for trend*** |
| Cases (n) | 105 | 158 | 129 | 124 | | 38 | 50 | 47 | 40 | | 46 | 48 | 37 | 41 | |
| RR (Age-adjusted) | 1.00 | 1.51 | 1.27 | 1.21 | *0.43* | 1.00 | 1.32 | 1.28 | 1.07 | *0.83* | 1.00 | 1.13 | 0.88 | 0.94 | *0.51* |
| (IC 95%) | | (1.18-1.93) | (0.98-1.64) | (0.93-1.57) | | | (0.87-2.02) | (0.83-1.97) | (0.69-1.67) | | | (0.75-1.70) | (0.57-1.35) | (0.61-1.43) | |
| RR (Fully[1] adjusted) | 1.00 | 1.49 | 1.25 | 1.15 | *0.72* | 1.00 | 1.29 | 1.24 | 1.00 | *0.92* | 1.00 | 1.11 | 0.85 | 0.89 | *0.37* |
| (IC 95%) | | (1.16-1.91) | (0.96-1.62) | (0.88-1.49) | | | (0.85-1.98) | (0.81-1.91) | (0.64-1.57) | | | (0.74-1.67) | (0.55-1.31) | (0.58-1.36) | |

[1] Adjusted for the following variables at the time of dietary record: educational level, current smoking status, family history of colon cancer, body mass index, physical activity level and energy and alcohol intake.

***Nutrient intake***

The RR of adenomas and colorectal cancer associated with nutrient intake are presented in Table III.

*Calcium.* The RR of adenoma decreased significantly with increasing intake of total dietary calcium (p for trend 5 0.04). The fully adjusted RR in the highest quartile of consumption compared to the lowest was 0.80 (95% CI 5 0.62–1.03). The effect was similar although nonsignificant in the high-risk adenoma group. Although significance was not reached, the RR of colorectal cancer also decreased with increasing calcium intake (p for trend 5 0.08), with a RR of 0.72 (95% CI 5 0.47–1.10) in the highest quartile compared to the lowest.

Results for dairy calcium (57.1% of total calcium) were similar but less pronounced.

*Phosphorus.* Increasing intake of phosphorus was associated with a significant decrease in the RR of adenoma (p for trend 5 0.005), with a RR of 0.70 (95% CI 5 0.54–0.90) in the highest quartile compared to the lowest. Phosphorus was observed to have a similar effect on high-risk adenomas and cancer, although it did not reach statistical significance.

A nonsignificant decrease in the risk of colorectal cancer was also observed with increasing intake of phosphorus (p for trend 5 0.11), with a RR of 0.73 (95% CI 5 0.48–1.10) in the highest quartile of intake compared to the lowest. The test for interaction between phosphorus and calcium intake was not significant. No association was found between the calcium to phosphorus ratio and colorectal tumour risk.

***Vitamin D***

No association was found between dietary vitamin D intake and risk of colorectal tumour, with or without adjustment for calcium and/or phosphorus intakes.

***Dairy products***

The age-adjusted, energy-adjusted and fully adjusted RRs of colorectal tumour according to dairy products consumption are shown in Table IV.

Increasing consumption of total dairy products decreased the RR of adenoma (p for trend < 0.05). The fully adjusted RR among subjects in the highest quartile compared to the lowest was 0.80 (95% CI 5 0.62–1.05). The decrease in risk of both high-risk adenoma and cancer was similar to that observed in the analysis of all adenomas but the tests for trend were not statistically significant.

No specific effect of any dairy product (i.e., milk, yoghurt, cottage cheese or cheese) was observed on colorectal tumour risk, except for a protective effect of high milk consumption on colorectal cancer (RR50.54, 95% CI 5 0.33–0.89 in the highest tertile compared to nonconsumers).

After adjustment for calcium or vitamin D supplementation as a treatment for osteoporosis, intake of dietary fibre, folate and saturated fatty acids (as percent of total energy intake), consumption of red meat and consumption of processed meat, the results were essentially unchanged and are not tabulated.

Table IV - **RRs of adenoma, high-risk adenoma and colorectal cancer by quartiles or tertiles (versus non-consumers) of consumption of total dairy product, milk, yogurt, cottage cheese and cheese consumption.**

| | All adenomas | | | | | High-risk adenomas | | | | | Colorectal cancer | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total dairy products (g)** | **<184.83** | **184.83-280.96** | **280.96-424.29** | **>424.29** | ***P for trend*** | **<184.83** | **184.83-280.96** | **280.96-424.29** | **>424.29** | ***P for trend*** | **<179.47** | **179.47-274.72** | **274.72-409.21** | **>409.21** | ***P for trend*** |
| Cases (n) | 146 | 145 | 114 | 111 | | 50 | 51 | 37 | 37 | | 47 | 44 | 43 | 38 | |
| RR (Age-adjusted) (IC 95%) | 1.00 | 0.99 (0.78-1.24) | 0.79 (0.61-1.01) | 0.77 (0.60-1.00) | *0.02* | | 1.05 (0.71-1.55) | 0.76 (0.50-1.18) | 0.80 (0.51-1.25) | *0.17* | | 0.93 (0.62-1.41) | 0.88 (0.58-1.34) | 0.74 (0.48-1.16) | *0.19* |
| RR (Fully[1] adjusted) (IC 95%) | 1.00 | 1.01 (0.80-1.27) | 0.81 (0.63-1.05) | 0.80 (0.62-1.05) | *0.04* | 1.00 | 1.07 (0.72-1.60) | 0.81 (0.52-1.25) | 0.85 (0.54-1.33) | *0.28* | 1.00 | 0.94 (0.62-1.43) | 0.90 (0.59-1.38) | 0.78 (0.49-1.22) | *0.28* |
| **Milk (g)** | **NC[2]** | **<80** | **80-210** | **>210** | ***P for trend*** | **NC[2]** | **<80** | **80-210** | **>210** | ***P for trend*** | **NC[2]** | **<79.72** | **79.72-210** | **>210** | ***P for trend*** |
| Cases (n) | 229 | 104 | 90 | 93 | | 81 | 35 | 32 | 27 | | 82 | 29 | 42 | 19 | |
| RR (Age-adjusted) (IC 95%) | 1.00 | 0.99 (0.78-1.25) | 0.87 (0.68-1.11) | 0.93 (0.73-1.18) | *0.33* | 1.00 | 0.95 (0.64-1.41) | 0.88 (0.58-1.32) | 0.77 (0.50-1.20) | *0.24* | 1.00 | 0.80 (0.52-1.22) | 1.08 (0.74-1.56) | 0.53 (0.32-0.87) | *0.07* |
| RR (Fully[1] adjusted) (IC 95%) | 1.00 | 1.01 (0.80-1.28) | 0.87 (0.68-1.11) | 0.93 (0.73-1.19) | *0.36* | 1.00 | 0.98 (0.66-1.46) | 0.91 (0.60-1.37) | 0.80 (0.51-1.23) | *0.31* | 1.00 | 0.81 (0.53-1.23) | 1.08 (0.75-1.57) | 0.54 (0.33-0.89) | *0.09* |
| **Yogurt (g)** | **<32.29** | **32.29-80.71** | **80.71-129.14** | **>129.14** | ***P for trend*** | **<32.29** | **32.29-80.71** | **80.71-129.14** | **>129.14** | ***P for trend*** | **<32.29** | **32.29-80.71** | **80.71-120.42** | **>120.42** | ***P for trend*** |
| Cases (n) | 163 | 110 | 144 | 99 | | 61 | 37 | 42 | 35 | | 58 | 37 | 40 | 37 | |
| RR (Age-adjusted) (IC 95%) | 1.00 | 0.89 (0.69-1.13) | 0.97 (0.78-1.22) | 0.84 (0.65-1.07) | *0.28* | 1.00 | 0.82 (0.55-1.24) | 0.77 (0.52-1.15) | 0.81 (0.53-1.23) | *0.24* | 1.00 | 0.88 (0.58-1.33) | 0.91 (0.61-1.37) | 0.80 (0.53-1.21) | *0.34* |
| RR (Fully[1] adjusted) (IC 95%) | 1.00 | 0.91 (0.72-1.17) | 1.03 (0.82-1.29) | 0.87 (0.68-1.13) | *0.52* | 1.00 | 0.85 (0.56-1.29) | 0.82 (0.55-1.22) | 0.85 (0.56-1.30) | *0.38* | 1.00 | 0.90 (0.60-1.36) | 0.95 (0.63-1.42) | 0.82 (0.54-1.25) | *0.42* |
| **Cottage cheese (g)** | **NC†** | **<22.47** | **22.47-59.71** | **>59.71** | ***P for trend*** | **NC†** | **<22.47** | **22.47-59.71** | **>59.71** | ***P for trend*** | **NC†** | **<21.43** | **21.43-57.14** | **>57.14** | ***P for trend*** |
| Cases (n) | 160 | 125 | 111 | 120 | | 52 | 36 | 40 | 47 | | 63 | 40 | 36 | 33 | |
| RR (Age-adjusted) (IC 95%) | 1.00 | 1.07 (0.85-1.36) | 0.93 (0.73-1.19) | 0.98 (0.77-1.24) | *0.63* | 1.00 | 0.98 (0.64-1.50) | 1.06 (0.70-1.61) | 1.20 (0.81-1.79) | *0.35* | 1.00 | 0.88 (0.59-1.31) | 0.79 (0.52-1.19) | 0.69 (0.45-1.06) | *0.07* |
| RR (Fully[1] adjusted) (IC 95%) | 1.00 | 1.09 (0.86-1.38) | 0.95 (0.74-1.21) | 1.01 (0.80-1.29) | *0.85* | 1.00 | 1.00 (0.65-1.54) | 1.11 (0.73-1.68) | 1.27 (0.85-1.90) | *0.23* | 1.00 | 0.88 (0.59-1.32) | 0.80 (0.53-1.21) | 0.71 (0.46-1.08) | *0.09* |
| **Cheese (g)** | **<27.26** | **27.26-47.76** | **47.76-71.29** | **>71.29** | ***P for trend*** | **<27.26** | **27.26-47.76** | **47.76-71.29** | **>71.29** | ***P for trend*** | **<26.67** | **26.67-46.95** | **46.95-70.13** | **>70.13** | ***P for trend*** |
| Cases (n) | 140 | 115 | 136 | 125 | | 47 | 41 | 45 | 42 | | 41 | 37 | 52 | 42 | |
| RR (Age-adjusted) (IC 95%) | 1.00 | 0.83 (0.65-1.07) | 0.98 (0.77-1.25) | 0.92 (0.71-1.20) | *0.85* | 1.00 | 0.91 (0.60-1.39) | 1.01 (0.67-1.54) | 1.01 (0.64-1.59) | *0.87* | 1.00 | 0.90 (0.57-1.40) | 1.23 (0.81-1.87) | 0.99 (0.62-1.57) | *0.68* |
| RR (Fully[1] adjusted) (IC 95%) | 1.00 | 0.82 (0.64-1.05) | 0.97 (0.76-1.24) | 0.90 (0.69-1.17) | *0.72* | 1.00 | 0.89 (0.58-1.36) | 0.99 (0.65-1.50) | 0.98 (0.62-1.55) | *0.96* | 1.00 | 0.89 (0.57-1.40) | 1.22 (0.80-1.85) | 0.97 (0.61-1.54) | *0.74* |

[1] Adjusted for the following variables at the time of dietary record: educational level, current smoking status, family history of colon cancer, body mass index, physical activity level and energy and alcohol intake. - [2]Non-consumer.

## Discussion

Our findings support the hypothesis that intakes of total dietary calcium and phosphorus reduce the risk of colorectal tumour at all stages of the adenoma-carcinoma sequence, while dietary vitamin D does not appear to be associated with colorectal tumour risk. Our study also revealed a protective effect of dairy products consumption on the risk of colorectal tumours.

Epidemiological data on the relationship between calcium and colorectal tumour risk are conflicting. A meta-analysis of 24 studies did not support a potential protective effect of calcium on colorectal tumours (cancer and/or adenoma) and emphasised the heterogeneity of the findings[8]. A review of over 20 case-control and cohort studies on colorectal cancer risk concluded that calcium had no protective effect.[7]

However, our finding of a decrease in risk of colorectal cancer with a high intake of calcium was in agreement with recent studies. The ATBC trial,[24] using male smokers, observed a strong decrease in colorectal cancer risk in subjects with a high calcium intake (median of 4th quartile51,789 mg/day) compared to subjects with a low calcium intake (median of 1st quartile5856 mg/ day). In the Cancer Prevention Study II Nutrition (CPSN) cohort, a protective effect of total (i.e., supplemental and dietary) calcium was also observed in both men and women.[9] A recent pooled analysis[12] of 10 cohort studies (not including the CPSN cohort) found a significant protective effect of calcium on colorectal cancer risk. Three case-control studies,[10,25,26] but not a fourth,[27] found similar results, but 2 cohort studies[11,28] failed to confirm them. Few studies have reported results on the association between calcium and risk of colorectal adenoma. Most of these[29-31] found that calcium had nonsignificant protective effects or no effect. One found that calcium from a low-fat dairy product had a protective effect on the risk of large adenomas in men.[30]

A recent review[7] and several recently published studies[9,10,24,25] concluded that dietary vitamin D had no protective effect on colorectal cancer, but it has been reported to have a slight protective effect on adenoma risk.[30,31] However, 2 of these studies[9,25] found a protective effect of total (i.e., supplemental and dietary) vitamin D on colorectal cancer. The lack of effect of dietary vitamin D in our population can be explained by the low dietary intake of vitamin D, as most dairy products available in France during the dietary assessment period were not vitamin-enriched. Detailed quantitative data on calcium and vitamin D supplementation are not available for our population.

A slight protective effect of phosphorus on adenoma risk was previously observed in 1 case-control study,[30] although it did not reach statistical significance. However, calcium and phosphorus were strongly correlated in our population, making it difficult to identify the effect of each mineral independently. The observed protective effect of phosphorus might be partially confounded by that of calcium, but the reverse is also possible.

A recent review of epidemiological data[32] concluded that dairy products had a potential protective effect on adenoma occurrence. Another review revealed a consistent protective effect of dairy products on colorectal cancer occurrence in cohort studies but not in case-control studies.[6] The effect was not found in the CPSN cohort.[9] The analysis of the Pooling Project[12] (which did not include the CPSN cohort) revealed a lower risk of colorectal cancer in subjects who consumed over 250 g/day of dairy products than in those consuming under 70 g/day. However, the effect was restricted to cancers of the distal colon and the rectum.

The mechanisms that are claimed to explain a potential protective effect of calcium, vitamin D and/or dairy products have recently been reviewed.[2,3,6] Newmark et al.[1] first suggested in 1984 that ionised calcium might precipitate with secondary bile acids and fatty acids to generate insoluble soap, protecting the mucosa from their deleterious effect. Later, others[33] suggested that phosphorus might also be involved in this mechanism through the formation of calcium phosphate.

Two intervention trials conducted on adenoma recurrence found a beneficial effect[13,15] of calcium supplementation, although it was significant in only 1 trial.[13] A more recent analysis found that a stronger beneficial effect of calcium supplementation on the risk of recurrence with advanced neoplasms than with

tubular adenomas.[14] The effect was greater for subjects with a high dietary calcium intake. As far as the effect of vitamin D is concerned, experimental studies have shown that 1a,25-dihydroxyvitamin D3 might directly inhibit the proliferation of colonic cells and stimulate differentiation of a wide variety of cells, particularly colon cells, through the vitamin-D-receptor.[3] Some of these studies argued that the concentration of vitamin D receptors in cells might be greater than the levels of vitamin D.[3,6] Polymorphism of the vitamin D receptors could modulate the effect of both calcium and vitamin D.[3] Endogenous vitamin D synthesis is dependent on skin colour and sunshine exposure. Our data do not support a protective effect of dietary vitamin D. Further adjustment for sunshine exposure in our predominantly Caucasian population did not modify the findings.

The prospective design of our study made it possible to avoid recall bias, which can lead to a misleading estimation of the effect of diet on colorectal tumour risk. Results of the validation study[18] for the dietary history questionnaire suggested a good quality of dietary data, attributable in large part to the high educational level of our volunteers and to their high degree of health consciousness. Furthermore, the response rate after 14 years of follow-up (85%) indicates the high degree of interest of participants and argues for the reliable quality of the recorded data. The exclusion of subjects in the top and bottom 1% of the energy intake/energy requirement ratio helped to reduce the impact of outliers on the estimation of risks.

All cases of adenoma or cancer were histologically confirmed, making it possible to correctly classify the subjects and to limit any decrease in power induced by false positives. Moreover, the use of polyp-free subjects as controls in the adenoma study limited the number of false negatives, i.e., controls bearing asymptomatic adenomas, the proportion of which exceeds 10% in women over 50 years of age.[34] In addition, women who undergo colonoscopy are a selected group, as demonstrated for example by a higher proportion of family history of colorectal cancer both in the polypfree group and in the adenoma-free group as compared to the whole population. These 2 aspects underline the need for using polyp-free subjects recruited after a colonoscopy as controls for adenoma cases.

Additional analyses were performed, omitting cases diagnosed during the first year of dietary assessment. Findings were not subsequently changed but statistical significance was not reached, because of decreased statistical power.

The moderate associations observed in our study are at least in part attributable to the relatively short follow-up time, resulting in a small number of cases and a lack of statistical power. However, the shortness of the follow-up also limits changes in dietary habits, which are likely to occur in long prospective studies that fail to provide for updating of the dietary record. Another possible limitation derives from the homogeneity of our study population. Its narrow range of consumption levels and healthy dietary habits result in a relatively high intake of calcium and dairy products. Our population had a median calcium intake of approximately 1,000 mg, in agreement with the French guidelines[35] and the cut-off for the 1st quartile was 860 mg/ day). In the Cancer Prevention Study II Nutrition cohort,[9] the protective effect of total calcium was associated with intakes of over 1,255 mg/day compared to those of below 561 mg/day. The analysis of the NHS and HPFS data,[11] both of which had an interquintile range of the same magnitude (<500 mg to >1,250 mg/day), also found a protective effect of calcium on distal cancer risk. In contrast, no calcium effect was found in the Finnish study, where calcium intake was high (bottom quartile <1,178 mg). The authors of the Pooling Project[12] suggested a threshold effect for total calcium intake, with little further decrease in colorectal cancer risk for calcium intakes in excess of 1,000 mg/day, which agrees with our findings. We can therefore hypothesise that only low calcium intake is deleterious.

Our data support an inverse association between calcium, phosphorus and dairy products and colorectal tumour risk. Further investigations should help to determine the optimum intakes for colorectal cancer prevention.

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