Shift from a dairy product–rich to a dairy product–free diet: influence on cytotoxicity and genotoxicity of fecal water—potential risk factors for colon cancer

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ABSTRACT Several epidemiologic studies have suggested that dairy product intake is associated with a decreased incidence of colon cancer. To determine whether the cytotoxicity and genotoxicity of the aqueous portion of human stool (two potential risk markers for the disease) were affected by a change in dairy product intake, 18 healthy male and female volunteers were randomly divided into two groups. In a crossover design, the volunteers shifted from their normal dairy product–rich diet to a dairy product–free diet. Nutritional analysis of the food consumed during the study period showed a significant decrease in energy intake from 9000 to 7866 kJ/d because of a decreased intake of protein and fat. Carbohydrate and fiber intakes remained unchanged during the intervention. Calcium intake decreased significantly from 1488 to 372 mg/d, with similar significant decreases in phosphate and vitamin D intakes. Cytotoxicity of fecal water, analyzed by the HT-29 cytotoxicity assay, indicated a significant decrease in cell survival from 34% to 20% when dairy products were excluded from the participants’ diets. Single-cell gel electrophoresis (COMET assay), used to analyze genotoxicity of fecal waters, indicated no differences brought about by the dietary intervention. In conclusion, our findings indicate that a shift from a dairy product–rich to a dairy product–free diet resulted in a significant effect on an accepted risk marker for colon cancer and may suggest that the mechanism by which dairy products are protective is at the level of tumor promotion rather than initiation. Am J Clin Nutr 1997;66:1277–82.

KEY WORDS Dairy products, cytotoxicity, genotoxicity, bile acids, colon cancer, fecal water, risk factors, COMET assay, humans

INTRODUCTION

Colorectal cancer is one of the most common malignancies in westernized countries (1), with an incidence that can be > 10 times that in Asia, Africa, and South America (2). An increased risk for colon cancer has also been observed in epidemiologic studies for populations migrating to westernized countries (3). In addition, migrants from countries with a high-risk of colorectal cancer to countries with a low-risk have a reduced risk (4). Studies of this type clearly indicate that environmental factors play an important role in the etiology of this disease. In this regard, dietary components have received a great deal of attention, with a high intake of fat and a low intake of fiber, fruit, and vegetables being correlated with an increased risk for colon cancer (5). Epidemiologic studies regarding the effects of milk and dairy products on colorectal cancer incidence provide conflicting results. Some studies report a negative association between consumption of dairy products and disease incidence (6–8), whereas others report no (9, 10) or even a positive association (11). One reason for this discrepancy in epidemiologic observations may be that some dairy products, such as cheese and cream, contain large amounts of animal fat, which is considered a risk factor by some for colorectal cancer (12, 13). In line with this assumption, it appears that the positive correlation between dairy products and colorectal cancer found in one study was due to cheese consumption (11). In addition, an international correlation study observed a negative correlation between consumption of dairy products and colorectal cancer only after adjustment for animal fat intake (6). Most of the studies that reported no association did not adjust for fat intake (9, 10). Hence, epidemiologic studies provide weak evidence that consumption of milk and dairy products can protect against colorectal cancer.

The main hypothesis to explain this effect has been the high calcium content of dairy products, although recently, lactic acid bacteria have received increased attention (14). Thus, many previous controlled, dietary intervention studies focused on calcium and consequently examined the effects of dietary calcium supplements (15, 16) or calcium-deficient milk (17) on risk markers for the disease. Although the results of these studies generally show a protective effect of calcium on the biomarkers (8, 18), this is not always the case.

In studies on the dietary etiology of colon cancer, it is now generally accepted that the contents of the fecal stream play an...
important role. Thus, it has been shown, for example, that by dietary modulations one can increase the bile acid concentration in fecal water (19, 20) and that these surfactants can damage the epithelium and stimulate proliferation of the crypt cells (19, 21, 22). Cytotoxicity of fecal water is now an accepted risk marker for colon cancer and several studies have correlated toxicity of this fecal fraction with a higher colonic cell proliferation and increased colon cancer risk (19, 23, 24). Genotoxicity per se in human feces is also generally accepted as a risk marker for colon cancer. Recently, Pool-Zobel et al (25) showed, using the COMET assay, that colon carcinogens induced genotoxicity in the colon of rats and that this genotoxicity was altered by dietary manipulations.

Thus, whereas dairy products have received much attention in epidemiologic studies, human intervention studies of dairy products as a whole have not been forthcoming. Therefore, we considered it of interest to examine the effect of a shift from a dairy product–rich to a dairy product–free diet in healthy volunteers on two risk markers for colon cancer: cytotoxicity and genotoxicity of fecal water.

 SUBJECTS AND METHODS

Participant selection

A food-frequency questionnaire designed to assess calcium intake from dairy sources was distributed to 145 employees at ARLA in Stockholm; 107 persons filled out the form, which resulted in 37 persons with a calcium intake > 1000 mg/d from dairy products (milk, cheese, and fermented milk products). These 37 persons were asked to participate in the study and from this group 18 healthy volunteers (10 men and 8 women aged 40 y; range: 25–57 y) were chosen for the study. Their mean (± SD) calcium intake from dairy products, based on the food-frequency questionnaire, was 1372 ± 353 mg/d. All volunteers had not taken antibiotics or other medications that may have had an effect on the gastrointestinal tract for 6 mo before the study began. The use of vitamin and mineral supplements was stopped during the study period. The study protocol was approved by the Medical Ethical Committee of Huddinge University Hospital, Stockholm.

Study protocol

A crossover design was chosen for the study and the volunteers were randomly divided into two groups. The study period lasted 14 d. During the first week (Friday to Thursday), group 1 was instructed to continue consuming their normal dairy product–rich diet, whereas group 2 was instructed to abstain from all dairy products. During the dairy product–free period, the participants received advice regarding what foods they might consume to replace the dairy products. Suggestions included, for example, fruit soups, porridge, jams, stewed apple, liver paté, cured meats, beer, and fruit juice. This advice was general in character and it was emphasized that the subjects should eat as normally as possible, the overall aim of the advice being to assist the participants in excluding dairy products during the dairy product–free period. During the second week (Friday to Thursday), group 1 excluded all dairy products from their diet whereas group 2 shifted back to their normal dairy product–rich diet. All participants maintained their usual lifestyle and work patterns during the study period.

Dietary surveys

Food intake was recorded for 3 d (Sunday, Monday, and Tuesday) during both weeks. All participants were taught individually how to generate food records using estimated registration. Estimation was made by using picture models, normal household measures, and weights printed on packages. Nutrients from the 3-d, food-registration forms were calculated by using a computer program based on the Swedish National Food Administration’s latest database (SLV; Uppsala, Sweden). The mean nutrient intake for the 3-d registration in each week was also calculated.

Stool collection and fecal water preparation and fractionation

At the end of each week, volunteers brought in a 24-h stool sample to their place of work. The samples were carefully sealed in airtight boxes and immediately stored in a freezer at −20 °C. All fecal samples were coded so that the origin of the samples was unknown during processing and analysis. After being thawed overnight at 4 °C, each fecal sample was homogenized (Stomacher Lab Blender 400, model BA 6021; Seward Laboratory, London) for 2 min and fecal water was prepared by centrifuging 50 g feces [models JA-17 (rotor) and J2-HS (centrifuge); Beckman Instruments, Stockholm] for 2 h at 30 000 × g at 10 °C. The supernate was carefully decanted and the volume and pH of the fecal water were measured. Fecal water was stored at −20 °C until analyzed; 1 mL fecal water was diluted with 9 mL phosphate-buffered saline (PBS; 10 mmol/L, pH 7.2) and applied to a Sep-Pak C-18 cartridge (Millipore, Bedford, MA). The cartridge was then washed with 10 mL PBS and lipids eluted with 5 mL methanol (Labasco, Partille, Sweden). The methanol eluate was evaporated at 37 °C, resuspended in 1 mL PBS, and used for the cytotoxicity assay described below.

Cytotoxicity assay

The HT-29 cell lysis assay, used to determine the cytotoxicity of the fecal water samples, was performed basically as described by Van Munster et al (26). A human tumor adenocarcinoma cell line (HT-29) was cultured in Dulbecco’s modified eagle’s medium (Gibco, Life Technologies Inc, Paisley, United Kingdom) with 10% fetal bovine serum, 2 mmol L-glutamine/L, 1 × 10^5 U penicillin/L, and 100 mg streptomycin/L. The cells were trypsinized, resuspended, and counted in a Burker chamber; 15 000 cells were placed in each well of a 96-multiwell plate (Nunc, Denmark). The cells were cultured for 48 h at 37 °C in a humidified atmosphere of 95% air and 5% CO2. The cultured cells were then incubated for 3 h with the fecal water fractions (100 μL). On each plate, PBS was used as a negative control and deoxycholic acid (DCA; 250 and 500 μmol/L in PBS; Sigma Chemical Co, St Louis) as a positive control. Every experiment was performed in octuple. After incubation, wells were washed and the surviving cells cultured for another 48 h under the same conditions as before. Dye solution (15 μL containing 3–4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Promega, Madison, WI) was placed in each well and incubated for 4 h. The dye and medium in each well were carefully removed and solubilization solution (100 μL; Promega) was placed in each well for 1 h. The plates were read at 540 nm in a spectrophotometer (Titertec).
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Genotoxicity (COMET) assay

The methodology described by Singh et al (27) and Pool-Zobel et al (28) was followed. Briefly, fully frosted and warm microscopic slides (KEBO, Stockholm) were covered with 85 μL normal-melting-point agarose (Gibco BRL, Life Technologies Inc, Gaithersburg, MD). Sixty-five microliters normal-melting-point agarose, together with a coverslip, was applied over the first layer and the agarose was allowed to set for 10 min on an ice tray. All slides were stored in a humidified box at 4 °C and used within 24 h. Caco-2 cells (European collection of animal cell cultures; Salisbury, United Kingdom) from human colon adenocarcinoma were cultured as monolayers in modified eagle's medium containing fetal bovine serum (20%) for 7 d at 37 °C. Cell suspensions were then prepared in RPMI medium (Gibco) containing 20 mmol L-glutamine/L at a concentration of 1 × 10⁶ cells/L. The cell suspension (450 μL) was incubated at 37 °C for 30 min with 50 μL unfractionated fecal water, 0.9% NaCl (negative control), and 25, 50, or 100 μmol H₂O₂/L (positive controls). All experiments were carried out in triplicate and cell viability was assessed by tripyn blue exclusion before and after incubation. After incubation, the cells were centrifuged (100 × g, 3 min) at room temperature and the supernate removed. The cells were resuspended in 75 μL warm, low-melting-point agarose and layered onto the prepared slides. The agarose was allowed to set on an ice tray and 65 μL low-melting-point agarose was finally added on top of the cells and covered with a coverslip.

The cells on the slides were lysed for 1 h at 4 °C in lysing solution [2.5 mol NaCl/L, 100 mmol Na₂EDTA/L, and 10 mmol tris/L, containing 1% Triton-X100 (Sigma Chemical Co) and 10% dimethylsulfoxide]. The slides were then transferred to an electrophoresis tank and placed in cold electrophoresis buffer (1 mmol Na₂EDTA/L and 300 mmol NaOH/L) at 4 °C for 40 min to allow the DNA to unwind before applying a current of 300 mA at 26 V for 20 min. The slides were removed and rinsed twice with neutralization buffer (0.4 mol tris/L, pH 7.5). Ethidium bromide (2 mg/L, 85 μL) was added to each slide and covered with a coverslip. The slides were placed in a humidified airtight box and analyzed within 24 h. Slides were examined at 600× magnification with a fluorescence microscope (Zeiss-photomicroscope II; Zeiss, Jena, Germany). Increased extension of the DNA from the nucleus toward the anode is observed in cells with DNA damage. Each damaged cell has the appearance of a comet with a brightly fluorescent head and a tail whose length and fluorescence intensity are related to the number of DNA-strand breaks induced by the test agent. Undamaged cells appear as intact nuclei (comet heads) without tails. The "tail moment," which is based on the intensity of the fluorescence in the tail and its length, was measured. One hundred randomly selected cells were counted on each slide using KOMET 3.0 for Windows (Kinetic Imaging Ltd, Liverpool, United Kingdom) as the image analysis software. The results are expressed as mean tail moment ± SD.

Statistical analysis

To analyze the change in dietary and fecal indexes brought about by the diet shift, Student's pair-wise t test was used after validation for normal distribution by the Shapiro-Wilk test. Because a crossover design was used, the method proposed by Pocock (29) was used to test whether there was a carryover effect, an interaction between period and diet, or both. None of these effects were present in the data. In addition, descriptive statistics and graphic methods were used to characterize the data. All analyses were carried out with the SAS system (Statistical Analysis System Institute Inc, Cary, NC). A P value < 0.05 indicated a significant difference.

RESULTS

Effects of a shift from a dairy product–rich to a dairy product–free diet on nutrient intake

Results from the 3-d, food-registration form clearly showed that the participants excluded all dairy products from their diet during the dairy product–free week (Table 1). Mean energy intake decreased significantly by 10%, from 9000 to 7866 kJ/d when dairy products were excluded from the diet. This was mainly due to a 30% decrease in protein intake (from 91 to 60 g/d) but also to a decreased fat intake (from 75 to 65 g/d). Although the fat intake in relation to energy intake (% of energy) remained unchanged, the composition of fatty acids changed: saturated fat decreased from 35 to 25 g/d, monounsaturated fat did not change, and polyunsaturated fat increased from 9 to 11 g/d. Cholesterol also decreased significantly after the dietary shift, from 280 to 240 mg/d. Consumption of carbohydrates did not change significantly when the volunteers excluded dairy products from their diet, nor were there any significant differences in intakes of alcohol and fiber. However, calcium intake decreased significantly, by 75%, from

| TABLE 1 | Dietary intake of the participants during the dairy product–rich and dairy product–free diet
<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Dairy product–rich diet</td>
<td>Dairy product–free diet</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>9000 ± 2105</td>
<td>7866 ± 1569²</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>91 ± 20 (18 ± 2)</td>
<td>60 ± 13 (13 ± 3)³</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>75 ± 29 (31 ± 8)</td>
<td>65 ± 26 (30 ± 7)³</td>
</tr>
<tr>
<td>Saturated (g/d)</td>
<td>35 ± 14 (14 ± 4)</td>
<td>25 ± 11 (12 ± 4)³</td>
</tr>
<tr>
<td>Monounsaturated (g/d)</td>
<td>25 ± 10 (10 ± 5)</td>
<td>25 ± 11 (12 ± 5)²</td>
</tr>
<tr>
<td>Polyunsaturated (g/d)</td>
<td>9 ± 4 (4 ± 1)</td>
<td>11 ± 5 (5 ± 2)²</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>264 ± 72 (51 ± 7)</td>
<td>252 ± 53 (55 ± 10)</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>3 ± 6 (1 ± 2)</td>
<td>5 ± 6 (2 ± 3)</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>19 ± 8</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>280 ± 130</td>
<td>240 ± 130³</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>86 ± 50</td>
<td>141 ± 89³</td>
</tr>
<tr>
<td>Vitamin B-12 (µg/d)</td>
<td>7 ± 3</td>
<td>6 ± 7</td>
</tr>
<tr>
<td>Vitamin D (µg/d)</td>
<td>8 ± 5</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>10 ± 3</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>1488 ± 383</td>
<td>372 ± 70³</td>
</tr>
<tr>
<td>Phosphate (mg/d)</td>
<td>1915 ± 527</td>
<td>936 ± 200³</td>
</tr>
</tbody>
</table>

¹ ± SD; percentage of total energy in parentheses; n = 18.
²-³ Significantly different from dairy product–rich diet (two-tailed paired t test); ² P = 0.004, ³ P < 0.001, ⁴ P = 0.046, ⁵ P = 0.026, ⁶ P = 0.020.
1488 to 372 mg/d when all dairy products were excluded from the diet. Phosphate and vitamin D intakes also decreased significantly, from 1915 to 936 mg/d and from 8 to 4 μg/d, respectively. Intake of vitamin C increased significantly, from 86 to 141 mg/d, whereas iron and vitamin B-12 intakes did not change significantly.

Effects of a shift from a dairy product–rich to a dairy product–free diet on fecal indexes

A summary of the results of the feces and fecal water analyses during the dairy product–rich and dairy product–free diet periods is given in Table 2. Fecal wet weight and fecal water volume did not change significantly as a result of the dietary shift. However, fecal water pH decreased significantly from 7.0 to 6.7 when dairy products were excluded from the diet.

Cytotoxicity of fecal water

Cytotoxicity of fecal waters was assessed by monitoring survival of HT-29 cells in these fecal fractions before and after the dietary shift. Visual inspection with a microscope during incubation of the fecal water samples with the HT-29 cells indicated cell lysis not only in the positive controls (ie, DCA) but also in the fecal water samples. In fact, all fecal water samples exhibited cytotoxicity in this assay. Percentage cell survival for the positive controls was in the same range in all experiments, ie, 250 μmol DCA/L resulted in 73 ± 29% cell survival (± SD) whereas 500 μmol DCA/L resulted in 16 ± 10% cell survival. As is evident from Table 2, cell survival in fecal water decreased from 34 ± 28% to 20 ± 22% as a result of the shift from a dairy product–rich to a dairy product–free diet. This translated into a 40% decrease in cell survival and reflected a significant increase in fecal water cytotoxicity associated with the dietary shift. There was no significant correlation between cell survival and pH of the fecal water (dairy product–rich period: r = 0.35, P = 0.15; dairy product–free period: r = 0.09, P = 0.72).

Genotoxicity of fecal water

Genotoxicity of fecal water before and after the dietary shift was analyzed by using the COMET assay with Caco-2 cells. In all experiments performed, cell viability exceeded 90% after incubation of the cells with the various test samples. Saline (negative control) gave a mean (± SD) tail moment of 1.9 ± 0.8 in all experiments, whereas hydrogen peroxide (25, 50, and 100 μmol/L; positive control) resulted in mean tail moments of 10.8 ± 4.7, 17.8 ± 7.1, and 23.6 ± 6.0, respectively. DNA damage induced by individual fecal water samples from the participants consuming the dairy product–rich and dairy product–free diets is shown in Figure 1. It is clear that several of the fecal water samples had a genotoxicity well above that of the saline controls. However, mean tail moments of 6.1 ± 5.7 for fecal water samples from the individuals consuming the dairy product–rich diet and 6.5 ± 5.9 for fecal water samples from individuals consuming the dairy product–free diet indicated that the dietary shift had no significant effect on the genotoxic potential of the fecal water fractions (Table 2).

DISCUSSION

Dairy products per se have not been well studied in dietary intervention studies related to colon cancer, although most of the calcium in a normal Western diet originates from dairy sources. To our knowledge this is the first such study in which a group of free-living individuals was asked to shift from their normal diet rich in dairy products to a dairy product–free diet and accurately record their nutrient intake. Thus, the study did not focus on any one component of the dairy products but

<p>| TABLE 2 |</p>
<table>
<thead>
<tr>
<th>Fecal water indexes during the dairy product–rich and dairy product–free diets</th>
<th>Dairy product–rich diet</th>
<th>Dairy product–free diet</th>
</tr>
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<tbody>
<tr>
<td>Wet weight of feces (g)</td>
<td>130 ± 63</td>
<td>115 ± 50</td>
</tr>
<tr>
<td>Volume (mL fecal water/g wet wt)</td>
<td>0.17 ± 0.15</td>
<td>0.13 ± 0.12</td>
</tr>
<tr>
<td>pH of fecal water</td>
<td>7.0 ± 0.5</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>HT-29 cell survival (%)</td>
<td>34 ± 28</td>
<td>20 ± 22</td>
</tr>
<tr>
<td>Caco-2 genotoxicity (tail moments)</td>
<td>6.1 ± 5.7</td>
<td>6.5 ± 5.9</td>
</tr>
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</table>

1, 2, 4 Significantly different from dairy product–rich diet (two-tailed paired t test): 2 P = 0.012, 4 P = 0.025.

4 n = 12; fecal water from the remaining participants was not sufficient to carry out the genotoxicity assay.
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rather attempted to examine the effect of this group of products as a whole on risk markers for colon cancer.

As was evident from our dietary surveys, excluding dairy products had a marked effect on the intake of several nutrient components. Energy intake decreased, presumably because of decreased protein and fat intakes, although the intake of carbohydrates did not change significantly. Intakes of saturated fat, cholesterol, calcium, phosphate, and vitamin D decreased significantly because they are present in high amounts in dairy products. Intakes of polyunsaturated fat and vitamin C increased, presumably reflecting the food sources that replaced dairy products. Intakes of monounsaturated fatty acids, alcohol, fiber, vitamin B-12, and iron did not change significantly during the intervention. However, the dietary intake data obtained during both dietary periods should allow conclusions to be drawn regarding any alterations observed in the analyzed risk markers.

A major finding of the present study was that a shift from a dairy product–rich to a dairy product–free diet resulted in a significant increase in the cytotoxicity of the fecal water samples, as measured by the HT-29 cell assay. Recently, Van Munster et al (26) showed that HT-29 cells are considerably more sensitive to the toxicity of fecal water than are erythrocytes, which were used for this purpose in earlier studies. They also showed that fecal water toxicity correlated with the deoxycholate content of the fecal water. Indeed, several researchers have reported that bile acid concentration is highly correlated with the cytotoxicity of fecal water (19, 20, 26). Our observation that we recovered all of the cytotoxic activity of the fecal water samples in the methanol eluate from Sep-Pak C-18 cartridges (data not shown) is also consistent with speculation that the cytotoxicity observed was due to the content of bile acid, fatty acids, or both in the fecal water samples. The dietary shift did not alter the volume of fecal water per gram feces, ruling out the possibility that the altered cytotoxicity we observed after the dietary shift was simply due to dilution effects. Volume of fecal water has been shown to increase, with a concomitant decrease in cytotoxicity, after a shift from a mixed to a lactovegetarian diet (30), and this was attributed to the increased intake in dietary fiber. However, fiber intake was not altered in the present study. Fecal water pH decreased significantly during the dairy product–free period, which could have been due to a decreased fecal buffer capacity because of lower amounts of calcium and phosphate in the colon, as has been suggested (31).

The observed increase in fecal water cytotoxicity brought about by the shift from the dairy product–rich to the dairy product–free diet could theoretically be explained by any of the dietary components that changed during the intervention. Dietary components that have been reported to have the ability to influence the cytotoxicity of this fecal fraction include fat, calcium, fiber, and resistant starch (8, 20, 32, 33). Newmark et al (34) proposed that 1) calcium ions bind soluble bile and fatty acids in the colon and reduce their cytolytic activity by precipitation, and 2) intake of phosphate would in this regard bind free calcium and prevent calcium ions from reacting with these cytolytic surfactants. Interestingly, the nutritional implications of Newmark’s hypothesis was that protective calcium could not be derived from dairy products. Van der Meer et al (8, 35) proposed an alternative hypothesis: the intake of phosphate together with calcium leads to complexes of insoluble calcium phosphate in the colonic lumen, which bind and precipitate bile and fatty acids and consequently reduce the surface activities of these acidic lipids. Thus, although we cannot rule out an effect of polyunsaturated fat, clearly the most plausible explanation for the dramatic effect on this biomarker for colon cancer was the significant decrease in calcium intake, and possibly in phosphate intake, brought about by the dietary shift. This is supported by the recent report by Govers et al (17), who showed that shifting healthy subjects from normal dairy products to calcium-depleted dairy products caused an increase in the concentration of bile and fatty acids in fecal water, which was associated with an increase in the cytotoxicity of this fecal fraction. In addition, the results of our study may indicate that dairy products can exert a protective effect, which support Van der Meer et al’s hypothesis described above.

Although the cytotoxicity of the fecal water samples increased as a result of the shift from the dairy product–rich to the dairy product–free diet, no such effect was observed in the genotoxicity of these fecal fractions, as measured by the COMET assay. However, it is interesting that several of the fecal water samples studied were significantly genotoxic to the Caco-2 cells. This is the first time that the COMET assay has been used to study human fecal water, and the nature of the genotoxic agents is unknown.

In conclusion, the present findings indicate that shifting from a dairy product–rich to a dairy product–free diet significantly increased the cytotoxicity of human fecal water, an accepted risk marker for colon cancer. In our opinion, this effect was most likely due to the decreased intake of dairy calcium and possibly phosphate. In general terms, if one regards the COMET assay as an assay for tumor initiators and the HT-29 cell assay as an assay for tumor promoters, our findings might suggest that the mechanism for the protective effect of dairy products may be at the level of tumor promotion rather than initiation. This study may indicate that milk and dairy products can contribute significantly to the nutritional modulation of colorectal cancer risk.

We thank Ragne Fondén for valuable discussions during the course of this study, Eva Callmer for useful advice regarding food-registration materials, Agneta Yngve for valuable help with the diet analyses, Kristina Helmberg for valuable technical assistance, Per Nasman for help with the statistical analyses, and Richard Hamby for skillful instructions on the COMET assay.

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