ABSTRACT

Recently we have shown that supplemental dietary calcium precipitates luminal cytolytic surfactants and thus inhibits colonic epithelial proliferation, which may decrease the risk of colon cancer. In Western diets, milk products are quantitatively the most important source of dietary calcium. However, they also contain large amounts of phosphate, which has been hypothesized to inhibit the antiproliferative effect of calcium. Therefore, we studied in rats the possible differential antiproliferative effects of dairy calcium, calcium carbonate, and calcium phosphate, supplemented to a Western high-risk control diet. We observed that fecal bile acid excretion was similar in the various diet groups, whereas fatty acid excretion was stimulated by the calcium supplements in the order calcium carbonate > calcium phosphate > milk mineral. In fecal water, concentrations of bile acids and fatty acids were drastically decreased in the supplemented groups, resulting in decreased cytolytic activity of fecal water. In vitro incubation of fecal water from the control group with insoluble calcium carbonate also decreased the high concentrations of surfactants and their cytolytic activity. The response of the colonic epithelium to these primary luminal effects of calcium was a decrease in cell damage and cell proliferation. Only minor differences between the supplements were observed. The concentration of serum gastrin, the possible trophic effect of which could counteract the antiproliferative effect of calcium, was increased by the supplements, but no significant correlation was observed between serum gastrin concentration and epithelial proliferation. We conclude that dietary calcium precipitates luminal surfactants and thus inhibits cytolytic activity, epithelial cell damage, and colonic proliferation. The similar efficacy of calcium carbonate, calcium phosphate, and milk mineral indicates that the antiproliferative effect of milk mineral is mediated by its calcium content and is not inhibited by phosphate.

INTRODUCTION

Several epidemiological studies have shown a positive relationship between dietary fat and colon cancer incidence (1, 2) and a negative relationship between dietary calcium and colon cancer (3). These associations have also been observed in experimental tumor induction studies in rodents (see Refs. 4 and 5 for reviews). To explain the promotive effect of dietary fat on colon carcinogenesis, Newmark et al. (6, 7) hypothesized that dietary fat increases the colonic concentrations of soluble bile acids and free fatty acids, which have the potency to damage colonic epithelial cells and consequently stimulate the proliferation of colonic crypt cells (8, 9). Hyperproliferation of colonic epithelium is considered to be the first step in colon carcinogenesis (10). With regard to the protective effect of dietary calcium, Newmark et al. (6, 7) hypothesized that calcium precipitates bile acids and fatty acids in the colonic lumen, thereby decreasing their cytolytic potency. Consequently, colonic epithelial damage may be decreased and epithelial proliferation inhibited. This proposed sequence of calcium-specific effects has been substantiated in in vitro studies (11, 12), as well as in animal (8, 9, 13–15) and clinical (see Ref. 16 for review) studies.

In Western diets, calcium is mainly (about 70%) supplied by milk and dairy products. Therefore, the epidemiological and experimental studies mentioned above may suggest that consumption of these products may contribute to dietary prevention of colon cancer. Indeed, several epidemiological studies have shown a protective effect of milk consumption on the incidence of colon cancer in populations with a high dietary fat intake (17–19). Nevertheless, this extrapolation to milk and dairy products has been questioned because these products also contain a large amount of phosphate (equimolar to calcium), which has been hypothesized to inhibit the antiproliferative effect of calcium (6, 7). However, Stemmermann et al. (20) found a similar trend for dairy and nondairy calcium in decreasing sigmoid cancer risk with increasing calcium. Moreover, our earlier in vitro studies (11, 12) have shown that bile acids are precipitated by insoluble calcium phosphate, and we recently demonstrated that dietary phosphate does not affect the inhibiting effect of calcium on the cytolytic activity of fecal water (15). However, the proposed (6, 7) differential efficacy of dairy calcium, calcium carbonate, and calcium phosphate for colonic epithelial proliferation has never been compared in one study. We therefore investigated in rats fed Western high-risk diets the effects of these different types of dietary calcium on colonic luminal surfactants, on cytolytic activity, and on colonic epithelial proliferation. We also studied the effects of the dietary supplements on the serum concentration of gastrin, because it is known that gastrin release is stimulated by calcium (21, 22) and that gastrin may stimulate colonic epithelial proliferation (23–25) and thus may counteract the protective effect of calcium in the colonic lumen.

MATERIALS AND METHODS

Animals and Diets. Nine-week-old male outbred Wistar rats (mean body weight, approximately 300 g; Small Animal Research Center of the Wageningen Agricultural University, Wageningen, The Netherlands) were housed individually. For 2 weeks, four groups of seven rats each were fed semipurified experimental diets, differing only in mineral content. The diets were designed to mimic a Western high-risk diet: 41% of energy as fat, 14% as protein, and 45% as carbohydrate, with 20 g/kg fiber. The milk mineral diet was composed of 650 g/kg diet lactose-treated whole-milk powder (Lactalac; Friesland Frico Domo, Leeuwarden, The Netherlands), containing 150 mmol calcium/kg diet. The composition of the other diets mimicked the macronutrient composition of the whole-milk powder (per kg diet): 133 g casein (acid casein; DMV, Veghel, The Netherlands), 33 g whey protein isolate (Bipro; Le Seur, Northfield, Minnesota), 182 g milk fat, 10 g lactose (BDH Chemicals Ltd., Poole, UK), 132 g glucose monohydrate, and 120 g galactose (both from C.N. Schmidt BV, Amsterdam, The Netherlands). In addition, all diets contained (per kg) 20 g corn oil, 20 g cellulose, 35 g mineral premix, and 10 g vitamin premix. The composition of mineral and vitamin premixes was according to the American Institute of Nutrition 1976 recommendations (26), except for CaHPO4, which was lower and provided 30 mmol calcium/kg diet. In two groups, CaCO3 (150 mmol/kg diet) or CaHPO4 (150 mmol/kg diet) was added. Diets were completed with dextrose monohydrate (AVEBE, Foxhol, The Netherlands). After preparation, diets were analyzed for fat by gas chromatography, and amino acid content (18) was determined. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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of Fiske and Subbarow (27). The dietary concentrations of these minerals are given in Table 1. Other dietary components did not differ between groups. Extrapolating to human diets with a daily intake of approximately 0.5 kg dry matter, the calcium concentrations used mimic a daily calcium intake of 15 and 90 mmol (600 and 3600 mg, respectively). Animals had free access to diets and water. Feed consumption was recorded every 3 days and animal body weights weekly. Feces were collected quantitatively during days 11–14 of the experiment. Serum Gastrin Concentration. Nonfasted blood samples were collected by orbital puncture (after anesthetization with CO2) on day 8 of the experiment, between 9:00 and 11:00 a.m. After 1 h at room temperature, serum was obtained from whole blood by low speed centrifugation (10 min at 1500 g) and was stored at –20°C until analysis. The serum gastrin concentration was determined using a radioimmunoassay (28).

In Vivo Colonic Proliferation. After the experimental feeding period of 14 days, nonfasted rats were given i.p. injections of [methyl-3H]thymidine (specific activity, 25 Ci/mmol; dose, 100 µCi/kg body weight; Amersham International, Buckinghamshire, UK) in 154 mM NaCl. Two h later they were killed by decapitation after anesthetization with CO2, and the colon was excised and opened. Intestinal contents were removed by rinsing with ice-cold 154 mM KCl. The mucosa of the colon was scraped using a spatula and, after homogenization in 154 mM KCl, the scrapings were analyzed exactly as described previously (29). Proliferation is expressed as dpm 3H/µg DNA. For a reliable estimate of effects on proliferation, the amount of DNA per scraping must be constant. In this study no significant difference in the amount of DNA per scraping was observed between groups (mean, 524 µg DNA/scraping).

Total Feces Analyses. After dry ashing of the freeze-dried feces, calcium and total phosphate were measured as described for the diets. Inorganic phosphate was extracted from the feces with 5% (v/v) trichloroacetic acid and measured as described (27). Organic phosphate was calculated by difference. Total bile acids were extracted from freeze-dried feces with a t-butanol:water mixture (1:1, v/v) as described previously (30). Extracts were assayed for bile acids using a fluorimetric enzymatic assay (31). Total free fatty acids were extracted from freeze-dried feces with diethyl ether, as described previously (32). Extracts were assayed for bile acids using an enzymatic assay (NPEA-C; Wako Chemicals, Neuss, Germany).

Fecal Water Analyses. Fecal water was prepared by reconstituting freeze-dried feces with doubly distilled water to 35% dry weight, which reflects the wet weight to dry weight condition in the distal rat colon. After homogenization, the samples were incubated for 1 h at 37°C in a shaking water bath, followed by centrifugation for 10 min at 15,000 × g. The supernatant was carefully aspirated, pH was measured at 37°C, and fecal water was stored at –20°C until analysis. Control experiments showed that fecal water from freeze-dried feces prepared using this procedure does not differ significantly from fecal water prepared from fresh feces for the parameters studied, except for pH, which is slightly lower in the freeze-dried samples (13, 29). For mineral analyses, fecal water was acidified with trichloroacetic acid (5%, v/v, final concentration) and subsequently centrifuged (2 min at 10,000 × g). Calcium and inorganic phosphate were measured in the supernatant as described above. Organic phosphate was measured in the pellet, after treatment with 12 M HClO4 at 180°C. After acidification of fecal water with 1 M HCl, bile acids and free fatty acids were extracted twice with diethyl ether and then twice with a diethyl ether-heptane mixture (1:1, v/v). After evaporation of diethyl ether and heptane, bile acids and fatty acids were resolubilized in ethanol and quantitated using the enzymatic assays described above. In control experiments, this extraction procedure resulted in >90% recovery of added bile acids and fatty acids. Intestinal alkaline phosphatase (EC 3.1.3.1) activity in fecal water was determined as described previously (14) and was defined as the difference between total alkaline phosphatase activity and the activity remaining after inhibition with L-phenylalanine, a specific competitive inhibitor of the intestinal isozyme.

Cytolytic Activity Assay. Cytolytic activity was determined as described previously (15). Briefly, increasing volumes of fecal water (0–160 µl) were incubated with 40 µl RBC suspension (final hematocrit, 5%) for 2 h at 37°C. Hemolysis was determined by iron release. Cytolytic activity was quantitated as the area under the hemolytic curve and expressed as a percentage of the maximal area, which implies 100% lysis of RBC at each dilution of fecal water.

In Vitro Precipitation Experiment. Increasing amounts of CaCl2 and Na2HPO4 solutions (final amount, 0–80 µmol) were added to Eppendorf tubes containing 50 µl 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (final pH 8.0), analogously to our earlier in vitro experiments (11, 12). NaCl was added to maintain a constant physiological ionic strength of 0.15 M. During incubation for 10 min at 37°C in a shaking water bath, an amorphous calcium phosphate precipitate was formed. After centrifugation of the tubes (2 min at 10,000 × g), the supernatant was removed and 200 µl of pooled fecal water from the control group (pH 8.0), together with 200 µl 154 mM NaCl, were added to the precipitate. After thorough mixing to detach the precipitate from the tube wall, the fecal water (diluted 1:1) was incubated with the precipitate for 20 min at 37°C. Subsequently, tubes were centrifuged (2 min at 10,000 × g) and supernatants were analyzed for bile acids, fatty acids, and organic phosphate as described above. Cytolytic activity was determined by incubating 160 µl supernatant with 40 µl RBC suspension (final hematocrit, 5%) for 6 h at 37°C. Hemolysis was determined by iron release. Because of shortage of fecal water, only two series of incubations were performed, but the results in both series were similar.

Statistics. Results are given as means ± standard errors of seven rats per group. First, differences between the control group and the three calcium-supplemented groups together were tested for their significance, using analysis of variance. If a significant difference was present, the high-calcium groups were subsequently, after a second analysis of variance, subjected to the least significant difference test, to compare the three calcium supplements among each other. The level of significance was preset at P < 0.05.

RESULTS

No differences between groups were observed in feed intake (mean, 19.0 g/day) or in final body weight (mean, 350 g). The daily output of feces was lower with the control diet (0.96 ± 0.06 g dry matter/day, n = 7) than with the supplemented diets (1.42 ± 0.03 g dry matter/day, n = 21). No differences between the calcium supplements were observed.

The daily fecal output of calcium and phosphate was strongly related to dietary intake (Fig. 1). The increased excretion of inorganic phosphate with the CaCO3 supplement indicates the formation of an insoluble calcium phosphate complex in the intestine, induced by

Table 1. Composition (mmol/kg diet)

<table>
<thead>
<tr>
<th></th>
<th>CaCO3</th>
<th>CaP</th>
<th>Milk mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>188</td>
<td>183</td>
<td>178</td>
</tr>
<tr>
<td>P</td>
<td>55</td>
<td>198</td>
<td>175</td>
</tr>
<tr>
<td>Mg</td>
<td>17</td>
<td>19</td>
<td>39</td>
</tr>
<tr>
<td>Na</td>
<td>81</td>
<td>90</td>
<td>178</td>
</tr>
<tr>
<td>K</td>
<td>89</td>
<td>91</td>
<td>255</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of calcium supplements on the daily fecal excretion of calcium (Ca), inorganic phosphate (P), and organic phosphate (P). The control diet contains 30 mmol calcium/kg, whereas the other diets contain 180 mmol calcium/kg diet. Excreta amounts of calcium, inorganic phosphate, and organic phosphate not sharing the same superscript are significantly different (P < 0.05).

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The control diet contains 30 mmol calcium/kg, whereas the other diets contain 180 mmol calcium/kg diet. Values are mean ± SE (n = 7).

Table 2. Effects of diets on mineral concentrations and pH in fecal water

The control diet contains 30 mmol calcium/kg, whereas the other diets contain 180 mmol calcium/kg diet. Values are mean ± SE (n = 7).

<table>
<thead>
<tr>
<th>Concentration (mmol)</th>
<th>Control</th>
<th>CaCO₃</th>
<th>CaP</th>
<th>Milk mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>24.4 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>8.8 ± 1.3</td>
<td>1.6 ± 0.2</td>
<td>9.3 ± 2.2</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>P&lt;sub&gt;f&lt;/sub&gt;</td>
<td>6.1 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.8 ± 0.2</td>
<td>0.0 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>8.1 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.0 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 0.1</td>
<td>7.7 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in the same row not sharing the same superscript are significantly different (P < 0.05).

<sup>b</sup> P<sub>i</sub>, inorganic phosphate; P<sub>f</sub>, organic phosphate.

The daily fecal excretion of bile acids was not affected by the diets, whereas the excretion of free fatty acids was drastically stimulated by the calcium supplements (Fig. 2). The extent of stimulation was apparently dependent on the mineral composition; CaCO₃ supplementation of the diet stimulated the fecal fatty acid excretion significantly more than did milk mineral supplementation.

Because the cytolytic potency of surfactants is dependent not on their total but on their soluble concentrations (13–15), we quantitated the concentrations of bile acids and fatty acids in fecal water. In the control group, fecal water isolation resulted in a turbid viscous suspension, whereas the fecal waters of the supplemented diets were homogeneous clear solutions. In contrast to the absence of effect on total bile acids and the calcium-induced increase in total fatty acids, the soluble concentrations of these surfactants in fecal water were decreased with the supplemented diets (Fig. 3). These changes in luminal cytolytic surfactant concentrations resulted in a drastically decreased cytolytic activity of fecal water (Fig. 3). No differences between the calcium supplements were observed.

To demonstrate that the decreased bile acid and fatty acid concentrations and cytolytic activity in the supplemented groups are indeed due to increased amounts of intestinal insoluble calcium phosphate, we added increasing amounts of insoluble calcium phosphate to the turbid fecal water of the control group. In the supplemented groups, 1 g of freeze-dried feces, which is used to prepare about 1.9 ml fecal water (35% dry weight), contains about 1500 μmol insoluble calcium phosphate. Therefore, we added up to 80 μmol insoluble calcium phosphate to 200 μl fecal water, which was diluted 1:1. From 40 μmol calcium phosphate, clear supernatants (pH 8.0) were observed after incubation with the turbid fecal water. The solubility of bile acids, fatty acids, and organic phosphate (predominantly phospholipids; see above) was drastically decreased with increasing calcium phosphate (Fig. 4). Consequently, the cytolytic activity of the supernatants was decreased.

To study the effect of the changes in luminal cytolytic potency on the colonic epithelium, we determined the release of the epithelial marker alkaline phosphatase, which might reflect epithelial cell damage (14, 33). The alkaline phosphatase activity in fecal water was lower with the supplemented diets than the control diet, and no dif-
ferences between the calcium supplements were observed (Fig. 5). Finally, we determined the response of the colonic epithelium to this increased epithelial cell damage. Epithelial proliferation was lower with the supplemented diets than the control diet (Fig. 5). The antiproliferative effect of calcium supplementation was most pronounced with the milk mineral diet.

In addition to the dietary calcium-induced luminal effects on colonic proliferation, we studied the possible interfering effect of serum gastrin. The gastrin concentration in nonfasted serum samples was increased by the calcium supplements (Fig. 6). When all four groups were included, a negative correlation \((r = -0.59, n = 28, P < 0.001)\) was observed between serum gastrin concentration and colonic proliferation. To eliminate the interfering effect of dietary calcium on proliferation, this correlation was also calculated for the three supplemented groups only. Then, no significant correlation \((r = -0.38, n = 21)\) was found between serum gastrin concentration and colonic proliferation (Fig. 7).

**DISCUSSION**

The present study shows that dietary calcium decreases epithelial cell damage and thus inhibits colonic epithelial proliferation. The mechanism by which calcium inhibits proliferation is by decreasing the concentrations of bile acids and fatty acids in fecal water and thus inhibiting their cytolytic activities, as was recently reported by Lapré et al. (14). The present results extend the latter study by showing, for the first time, that the mechanisms of the antiproliferative effects of milk mineral and of calcium carbonate and calcium phosphate are similar. Therefore, the inhibiting effect of milk mineral on colonic proliferation is most likely mediated by its calcium content. The extent of the effects on luminal and epithelial parameters is almost equal for all three equimolar calcium supplements, which supports and extends our earlier results showing that phosphate does not interfere with the protective effect of dietary calcium on the cytolytic activity of fecal water (15). Therefore, the present results offer a molecular explanation for the epidemiological observations that consumption of milk products is negatively associated with the incidence of colon cancer (17–19).

To our knowledge, this is the first strictly controlled experimental study on the role of milk mineral in the prevention of colon cancer. Only Nelson et al. (34) previously reported an experimental study concerning the effect of milk on the risk of colon cancer. Studying dimethylhydrazine-induced tumorigenesis in rats, those authors could...
not demonstrate a decrease in the amount of colorectal tumors, either by milk or by calcium carbonate. However, the calcium concentrations of their supplements can be calculated to be <10 mmol/kg diet. Those calcium supplements are definitely too low to exert an effect, especially when supplemented to a regular diet, probably containing normal (130 mmol/kg) calcium concentrations. Moreover, the diets used in their study were not controlled for differences in energy density, which limits the interpretation of their results.

Regardless of the counterion of calcium in the diet, in the small intestine dietary calcium is precipitated by phosphate (15, 35), which is present in large amounts in animal and human diets. We have previously shown that glycine-conjugated and unconjugated, but not taurine-conjugated, bile acids are bound and thus precipitated by calcium phosphate (11, 12). Because in rats bile acids are predominantly conjugated with taurine (36), precipitation of bile acids by calcium phosphate hardly occurs proximal to ileal reabsorption (15). This explains why fecal bile acid excretion was not affected by the diets (Fig. 2), in accordance with the results of Appleton et al. (37). After microbial deconjugation of bile acids in the colonic lumen, precipitation by calcium phosphate can take place. In contrast to bile acids, our results show that free fatty acids are precipitated by calcium before their absorption site is reached, resulting in strongly increased fecal excretion with the calcium-supplemented diets (Fig. 2). The observation that addition of other calcium ligands, like phosphate, to the diet resulted in decreased fecal fatty acid excretion suggests that precipitation of fatty acids in the proximal small intestine occurs via ionic binding to soluble Ca^{2+}.

Analogously to fatty acids, supplemental dietary calcium stimulated the fecal excretion of organic phosphate (Fig. 1), whereas its soluble concentration was drastically decreased (Table 2). Also, in human volunteers we found an increase in organic phosphate excretion after dietary calcium supplementation (38). These observations suggest that luminal organic phosphate is also precipitated by dietary calcium. The lipid extraction experiment indicated that the organic phosphate is derived mainly from phospholipids. It can be speculated that these phospholipids also contribute to the luminal cytolytic activity and the compensatory epithelial proliferation, because it is well known that lyosphospholipids are extremely cytolytic surfactants (39, 40). Whether precipitation of these phospholipids with the milk mineral diet can explain the lowest proliferation in this group will be investigated in future experiments.

The viscous fecal water of the control group contained relatively high concentrations of calcium (Table 2) and fatty acids (Fig. 3), indicating the presence of soluble calcium soaps which are kept in suspension by the high concentration of bile acids (41). Apparently, these solubilized aggregates of soaps and bile acids have a high cytolytic potential, which is in accordance with our in vitro studies showing that mixed micelles of fatty acids and bile acids are very lytic to RBC and Caco-2 cells (33). Analogously to the in vitro results (Fig. 3), the present in vivo experiment (Fig. 4) demonstrates that these surfactants in fecal water can be precipitated by insoluble calcium phosphate, thereby reducing their cytolytic activity. Therefore, the results of this experiment support our proposed mechanism of the antiproliferative effect of dietary calcium, implying that insoluble calcium phosphate precipitates luminal surfactants, which decreases their cytolytic potential and consequently decreases epithelial cell damage and epithelial proliferation.

Whereas dietary calcium decreased colonic proliferation, it increased serum gastrin concentrations (Fig. 6). Thus, quite artificially, a negative relationship between serum gastrin concentration and colonic proliferation was observed (Fig. 7). Therefore, a possible specific effect of serum gastrin on colonic proliferation can be ascertained only by using solely the data of the calcium-supplemented groups. Although a wide range of serum gastrin concentrations were observed, no stimulating effect of gastrin on in vivo colonic proliferation was found, which was recently also reported for colonic mucosa in humans (42). In fact, the correlation between gastrin and proliferation was, although not significant, slightly negative. Therefore, in the present study serum gastrin does not counteract the strong inhibiting effect of dietary calcium on colonic epithelial proliferation.

In conclusion, dietary calcium decreases the concentrations of bile acids and fatty acids in fecal water. Consequently, the cytolytic activity of fecal water is decreased. As a response to these luminal effects of calcium, epithelial cell damage is decreased and colonic epithelial proliferation is inhibited. The mechanism of milk mineral is similar to that of calcium carbonate and calcium phosphate, indicating that the antiproliferative effect of milk mineral is mediated by its calcium content. Because milk and dairy products are quantitatively the most important source of dietary calcium in Western diets, the present results indicate that further research is warranted on the mechanism and the physiological relevance of the observed protective effects of milk mineral in humans. Recently, we demonstrated the inhibiting effect of calcium carbonate supplementation on the cytolytic activity of fecal water in healthy individuals (43). Therefore, we will now substantiate the results of the present study in a strictly controlled human intervention trial, by studying the effects of calcium in milk on colonic luminal parameters in healthy volunteers.

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REFERENCES

EFFECT OF MILK MINERAL ON COLONIC EPITHELIUM


Mechanism of the Antiproliferative Effect of Milk Mineral and Other Calcium Supplements on Colonic Epithelium

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