Pergamon

European Journal of Cancer Vol. 31A, Nos. 7/8, pp. 1081–1084, 1995

0959–8049/95 $9.50+0.00

0959-8049(95)00135-2

Calcium and Vitamin D: Possible Protective Agents Against Colorectal Cancer?

J.H. Kleibeuker, R. van der Meer and E.G.E. de Vries

Nutritional factors are important determinants of colorectal cancer risk. Diets high in fat and/or low in fibre are especially recognised to increase risk. Dietary calcium and vitamin D have been suggested to be protective against colorectal cancer. With respect to calcium, its possible effect is thought to be mediated at least in part through intraluminal precipitation of hydrophobic, cytotoxic substances, in particular fatty and bile acids, which can promote colorectal cancer development. Data from studies *in vitro* and in animals support a protective effect of calcium, but studies in humans, both epidemiological and interventional, have given inconclusive results. With respect to vitamin D, data from only a small number of studies are available. Results suggest a protective effect by inhibition of cell proliferation, mediated through specific receptors. It is concluded that there are currently insufficient reasons to supplement subjects at increased colon cancer risk with calcium or vitamin D, especially when dietary intake of these substances is in agreement with general guidelines.

Key words: bile acids, calcium, cell proliferation, colon, colorectal cancer, cytotoxicity, fatty acids, vitamin D
Eur J Cancer, Vol. 31A, Nos 7/8, pp. 1081–1084, 1995

INTRODUCTION

EPIDEMIOLOGICAL STUDIES have shown that the composition of food is of major importance in determining the risk of colorectal cancer. A high intake of animal fat and a low intake of dietary fibre are risk factors in this respect, and this is not only true for cancer, but also for its benign precursors, colorectal adenomas [1].

It is generally assumed that a high fat diet affects colorectal cancer risk mainly through the process of promotion, in particular by increasing epithelial cell proliferative activity. Both in animals and in man a high fat intake has been shown to induce an increase of proliferative activity of the colorectal epithelium [2, 3]. Hyperproliferation makes cells more susceptible to adverse mutations and, in addition, stimulates outgrowth of (pre)malignant cells into tumours. Colonic epithelial hyperproliferation has been found by many investigators to be increased in subjects with an increased risk of colon cancer [4]. One possible mechanism through which a high intake of fat induces an increase of epithelial proliferation is that it causes an increase in concentration of fatty acids and bile acids in the contents of the colon. Both fatty acids and bile acids have been shown experimentally to be cytotoxic to colonic epithelial cells and to stimulate compensatory mitotic activity. Also, direct stimulation of the mitotic process may be involved, through stimulating protein kinase C activity in the epithelial cells by fatty acids and bile acids and especially by diacylglycerol [5].

Correspondence to J.H. Kleibeuker at the Division of Gastroenterology, Department of Internal Medicine, University Hospital, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.
J.H. Kleibeuker is at the Division of Gastroenterology and E.G.E. de Vries is at the Division of Medical Oncology, Department of Internal Medicine, University Hospital, Groningen; and R. van der Meer is at the Netherlands Institute for Dairy Research, Ede, The Netherlands.

CALCIUM AND COLORECTAL CANCER

A hypothesis

In 1984, Newmark and associates [6] published a hypothesis on the interactions of fatty and bile acids, calcium and phosphate in relation to colon cancer. They suggested that calcium might have beneficial effects on colonic epithelium by binding the fatty and bile acids in the colonic lumen, thus forming insoluble soaps that would be harmless to the colonic mucosa. Thus, increased oral calcium intake might be able to exert a proliferation inhibiting effect on colonic epithelium, and might protect against the development of colon cancer. The authors further hypothesised that phosphate would compete for calcium in the colon, to produce insoluble calcium phosphate. The proposed protective effect of calcium might thus be opposed by an excess of phosphate. This hypothesis has brought about a large number of investigations into the possible role of calcium as a protective agent against the development of colorectal neoplasms. A concise review of these studies will be presented in this article.

Epidemiology

Several epidemiological studies have provided support for the beneficial effects of calcium, especially studies from the U.S.A. and Australia [1]. In addition, Sörensen and associates [7], found an inverse association between calcium consumption and age-adjusted colon cancer incidence rates using data from both the U.S.A. and Scandinavia. In contrast, a recent prospective study of more than 100 000 American male health care professionals and female nurses failed to show a relation between calcium intake and the risk of colorectal adenomas [8]. Several European studies have also been unable to show an association between calcium intake and colorectal cancer [1, 8]. Differences in dietary sources of calcium and other confounding factors may account for these discrepancies.

In vitro *studies*

Calcium is an important mediator of many cellular, biological processes, including cell proliferation. An increased intake of calcium might thus affect cell proliferative activity through a direct effect of calcium. Contradictory results have been obtained in this respect, when incubating human colorectal biopsies with increasing concentrations of calcium: one study showing a decrease of cell proliferation [9], and another showing no effect [10]. Other *in vitro* studies have focused on the interactions of calcium with fatty acids and especially bile acids. The binding of calcium to fatty and bile acids to form insoluble soaps is a well known phenomenon. However, the proposed role of phosphate in this, as formulated by Newmark and associates [6], was purely hypothetical. In a series of *in vitro* studies, it has now been shown that bile acids are not precipitated by soluble Ca^{2+}, but that phosphate is a requisite for this [11–13]. In fact, this precipitation is an almost unique property of freshly formed, amorphous calcium phosphate, and is hardly observed with aged, more crystalline calcium phosphates and with other insoluble calcium salts. The mechanism of this precipitation is by hydrophobic aggregation of bile acid monomers at the surface of the amorphous calcium phosphate [13]. As fatty acids and bile acids are thought to promote colon carcinogenesis, at least in part, through their cytotoxic effects on colorectal epithelial cells, whether calcium could counteract these effects was studied. Soluble Ca^{2+} did not reduce the cytotoxic effect of bile acids *in vitro*, whereas calcium phosphate almost abolished it, as measured in a haemolysis assay [14]. Calcium also potently reduced the cytolytic effects of fatty acids and of the highly lytic mixed micelles, formed by the addition of small amounts of bile acids to fatty acids [15].

Studies in animals

Studies in rodents have repeatedly shown that a high calcium intake causes a reduction of epithelial cell proliferation in the colon, whether induced by increased colonic concentrations of fatty and/or bile acids or not [4]. Several studies have also indicated that tumorigenesis is inhibited by calcium supplementation [4]. With regard to the mechanism of the effect of calcium, studies by our group in rats have shown the following. First we determined whether insoluble calcium phosphate is formed *in vivo* in the intestine of rats. Intestinal and faecal samples of rats, fed low and high calcium phosphate purified diets, were analysed [16]. It was found that calcium and phosphate already precipitated in the small intestine, and were almost completely precipitated in colon and faeces. In addition, it was observed that the solubility of faecal calcium and phosphate is determined by the pH of its solubility product, analogous to that observed for amorphous calcium phosphate *in vitro* [17]. Thus, the amount of soluble Ca^{2+} is low, whereas that of amorphous calcium phosphate is high in the colon. The colonic interactions between calcium, phosphate and bile acids, and their effects on colonic epithelial parameters were studied in rats fed a Western high risk control diet, containing a low amount of calcium (20 μmol/g) [18]. This control diet simulates a human calcium intake of approximately 400 mg/day (10 mmol/day). The amount of calcium phosphate in this diet was increased to 180 μmol/g by supplementing the diet with milk mineral. Faecal water was isolated to quantitate the physiologically relevant cytolytic surfactants, as only soluble surfactants are lytic. Supplementation of the low calcium control diet with calcium phosphate drastically decreased the concentration of bile acids and fatty acids in faecal water and inhibited its cytolytic activity, measured with the same haemolysis bioassay mentioned above. Milk mineral also inhibited epitheliolysis, measured as the release of the epithelial cell marker alkaline phosphatase, as well as colonic epithelial proliferation. In other experiments the proposed inhibitory effects of dietary phosphate [16], and the effects of different types of dietary calcium [18] were studied. Taken together, these experiments confirm earlier studies which showed that dietary calcium inhibits colonic epithelial proliferation. They extend these studies by showing that this effect is not inhibited by phosphate, and that milk calcium has at least a similar protective effect. In addition, they show that the protective effect of calcium is mediated by a decrease in solubility of colonic surfactants, such as bile acids and fatty acids, and an inhibition of luminal cytolytic activity, epithelial cell damage and proliferation. It was found that cytolytic activity of faecal water, as well as epitheliolysis were significantly correlated with colonic epithelial proliferation ($r = 0.97$ and 0.88, respectively), suggesting cause–effect relationships.

Studies in man

In 1985, Lipkin and Newmark [19] published their study on the effect of calcium supplementation on rectal epithelial proliferation in subjects at increased risk of colorectal cancer. They showed that increasing oral calcium intake by approximately 1200 mg was associated with a marked decrease of epithelial proliferation. This study prompted a series of studies by other investigators on the effect of calcium in high risk subjects. Most of the early studies were not placebo-controlled. Almost invariably they showed a decrease of rectal cell proliferation after a supplementation period of 1 or more months [4]. Only in one study were biopsies for determination of proliferation taken proximal to the rectum, namely from the sigmoid colon. Surprisingly, in that study, proliferative activity increased during calcium therapy [20]. Recently, the results of several placebo-controlled studies have been reported. Two placebo-controlled crossover studies [21, 22] showed a reduction of rectal epithelial proliferation during calcium supplementation, whereas one placebo-controlled parallel groups study showed no appreciable effect [23]. We performed a placebo-controlled parallel groups study in subjects at 50% lifetime risk of hereditary nonpolyposis colorectal cancer, measuring proliferation in rectum, sigmoid and descending colon [24]. Only in the rectum was a slight beneficial effect of calcium found, whereas in the sigmoid and descending colon no effect of calcium was observed. Nevertheless, there is still great interest in the possible beneficial effect of calcium, and currently some large multicentre studies are being performed to evaluate the potential role of calcium in the prevention of colorectal cancer and adenomas.

With regard to the mechanisms through which calcium might affect colorectal carcinogenesis, our group has performed a number of studies. To ascertain the relevance of the results of our biochemical and animal studies for human physiology, we first studied the intestinal association of calcium, phosphate and bile acids [12]. Because, in human diets, phosphate is far in excess of calcium, supplemental dietary calcium (without phosphate) may stimulate complexation with phosphate and/or bile acids. This increased complexation can only be measured as an increase in faecal excretion of phosphate and bile acids, provided that the intake of phosphate is kept constant. In healthy subjects, supplementary calcium carbonate increased the faecal excretion of both phosphate and bile acids, which indicates the intestinal formation of an insoluble complex of calcium,

phosphate and bile acids. Also, in humans, the solubility of faecal calcium and phosphate is determined by the solubility product of amorphous calcium phosphate [25]. Using the calcium chelator, EDTA, we found that resolubilisation of calcium resulted in an increase of soluble phosphate and of soluble bile acids. This shows that calcium, phosphate and bile acids are present in faeces as an insoluble complex. We also studied the effects of calcium on duodenal bile acid composition, and showed that calcium decreased the hydrophobic and cytolytic dihydroxy bile acids, chenodeoxycholate and deoxycholate, and increased the hydrophilic, less cytolytic trihydroxy bile acid, cholate. These results suggest that calcium lowers the cytolytic activity of the soluble bile acids in the intestinal lumen. In line with this, we found that calcium decreased the concentration of hydrophobic surfactants in faecal water, and significantly inhibited the cytolytic activity of faecal water [25]. Similar effects of supplementary calcium on faecal bile acid excretion, duodenal bile acid composition and cytolytic activity of faecal water were obtained in patients with colonic adenomas [26].

These effects of calcium supplementation of the diet prompted us to study whether calcium in the habitual diet has similar protective effects on luminal metabolic risk factors. In a typical Western diet, approximately 70% of dietary calcium is derived from milk and dairy products. Therefore, we studied the effects of habitual dietary calcium in a double-blind crossover experiment using specially prepared milk products [27]. During the experimental period of 2 weeks, the male volunteers consumed a constant habitual diet in which all liquid dairy products were replaced by either placebo milk/yoghurt or regular, high calcium containing, milk/yoghurt. These products differed only in calcium content and provided 3 and 30 mmol of Ca^{2+}/day for the placebo and calcium period, respectively. At the end of each period, faeces were quantitatively collected for 3 days and urine for 1 day. Minerals were measured in faeces and urine to determine whether the total daily output was in accordance with the designed intake of nutrients. Faecal water was prepared by centrifugation of homogenised faeces and its composition determined by standard procedures. Cytolytic activity was measured as lysis of erythrocytes by faecal water. The measured total excretion of calcium differed by 27 mmol/day which is exactly the difference in calcium content of the supplied placebo and calcium milk products. Milk calcium significantly increased faecal pH and the faecal excretion of phosphate, bile acids, and fatty acids, indicating intestinal calcium phosphate formation and precipitation of bile acids and fatty acids. Calcium approximately halved the concentration of bile acids and fatty acids in faecal water which indicates that it precipitates these lipids. Neutral steroids and phospholipids are probably solubilised by hydrophobic bile acids and thus also precipitated by calcium. To quantitate the effects on hydrophobicity of bile acids, we also determined their composition in faecal water by capillary gas chromatography. On placebo milk, faecal water bile acids mainly consist of the secondary bile acids, deoxycholate and lithocholate (approximately 80%). Only the concentration of these bile acids was significantly decreased by milk calcium. Our *in vitro* work shows that these hydrophobic secondary bile acids particularly have very severe cytolytic effects [14]. In line with these *in vitro* results, we found that milk calcium drastically inhibited the cytolytic activity of faecal water.

As mentioned previously, a high fat intake might also stimulate proliferation by increasing the activity of protein kinase C by fat-derived substances such as diacylglycerol. Studies in patients with an intestinal bypass for morbid obesity, have shown that these subjects have an increased rectal epithelial cell proliferative activity [28], and have very high diacylglycerol concentrations in their faeces [29], both of which are reduced by calcium supplementation, suggesting an association between the two. The precipitation of fatty acids and bile acids by calcium might also be involved in the reduction of cell proliferation in these cases.

Taken together, the studies in humans indicate that calcium supplementation strongly reduces several metabolic risk factors in the colonic lumen. However, to what extent this reduces epithelial cell proliferation, and whether this may affect colorectal carcinogenesis, is still the subject of study.

VITAMIN D AND COLORECTAL CANCER

Because of the role of vitamin D in calcium homeostatis, possible effects of calcium and of vitamin D on colorectal carcinogenesis have been evaluated together, particularly in epidemiological studies. Studies by Garland and associates [30] have indicated that vitamin D consumption is inversely correlated with colon cancer incidence. Studies *in vitro* have subsequently shown that vitamin D_3 has antiproliferative effects on human colon cancer cells *in vitro*, and the same was found for analogues of vitamin D_3, which were devoid of any hypercalcaemic effect [31]. Similarly, *in vitro* incubation of human rectal biopsies with the vitamin D analogue, calcipotriol, reduced cell proliferative activity compared with control values [32]. Also, in rats, vitamin D_3 significantly affects cellular kinetic indices, especially a decrease of the proliferative zone [33]. The effects of vitamin D are probably due to direct binding of the substance to specific receptors on the epithelial cells, and are independent of intraluminal factors, including calcium.

CONCLUSION

Calcium supplementation would be a very attractive option to prevent colorectal cancer in high risk subjects. It is easy to administer, well tolerated and non-toxic. It may easily be incorporated into the diet by increasing consumption of dairy products. Concerning possible adverse effects, only interference with iron absorption has been well documented [4]. Biochemical studies and metabolic studies in animals and man are all in accordance with each other, and indicate that calcium precipitates luminal cytolytic substances, such as bile acids and fatty acids, thus reducing luminal cytolytic activity. In rats, this reduction correlates well with the reduction of parameters of intestinal epitheliolysis and colonic cell proliferation. In addition, colonic tumorigenesis is generally inhibited by calcium in animal models. Studies on the effects of calcium on human colorectal epithelium are not as uniform in their conclusions, and the same is true for epidemiological studies. Thus, currently, the role of calcium in the prevention of colorectal carcinogenesis is still unclear, and results of current and future studies are awaited before this can be concluded. This is even more true of vitamin D and its analogues, for which so far only very few data are available.

1. Potter JD, Slattery ML, Bostick RM, Gapstur SM. Colon cancer: a review of the epidemiology. *Epidemiol Rev* 1993, **15**, 499–545.
2. Bird RP, Medline A, Furrer R, Bruce WR. Toxicity of orally administered fat to the colonic epithelium of mice. *Carcinogenesis* 1985, **6**, 1063–1066.
3. Stadler J, Stern HS, Yeung KS, *et al.* Effect of high fat consumption on cell proliferation activity of colorectal mucosa and on soluble faecal bile acids. *Gut* 1988, **29**, 1326–1331.

4. Kleibeuker JH, Cats A, van der Meer R, Lapré JA, De Vries EGE. Calcium supplementation as prophylaxis against colon cancer? *Dig Dis* 1994, **12**, 85–97.
5. Morotomi M, Guillem JG, LoGerfo P, *et al.* Production of diacylglycerol, an activator of protein kinase C, by human intestinal microflora. *Cancer Res* 1990, **50**, 3595–3599.
6. Newmark HL, Wargovich MJ, Bruce WR. Colon cancer and dietary fat, phosphate, and calcium: a hypothesis. *J Natl Cancer Inst* 1984, **72**, 1323–1325.
7. Sörenson AW, Slattery ML, Ford MH. Calcium and colon cancer: a review. *Nutr Cancer* 1988, **11**, 135–145.
8. Kampman E. Do fermented dairy products and calcium protect against colorectal cancer? Thesis, Agricultural University, Wageningen, The Netherlands, 1994.
9. Lipkin M, Friedman E, Winawer SJ, *et al.* Colonic epithelial cell proliferation in responders and nonresponders to supplemental dietary calcium. *Cancer Res* 1989, **49**, 248–254.
10. Bartram H-P, Scheppach W, Schmid H, *et al.* Proliferation of human colonic mucosa as an intermediate biomarker of carcinogenesis: effects of butyrate, deoxycholate, calcium, ammonia and pH. *Cancer Res* 1993, **53**, 3283–3288.
11. Van der Meer R, De Vries HT. Differential binding of glycine- and taurine-conjugated bile acids to insoluble calcium phosphate. *Biochem J* 1985, **229**, 265–268.
12. Van der Meer R, Welberg JWM, Kuipers F, *et al.* Effects of supplemental dietary calcium on the intestinal association of calcium, phosphate and bile acids. *Gastroenterology* 1990, **99**, 1653–1659.
13. Govers MJAP, Termont DSML, Van Aken GA, van der Meer R. Characterization of the adsorption of conjugated and unconjugated bile acids to insoluble, amorphous calcium phosphate. *J Lipid Res* 1994, **35**, 741–748.
14. Van der Meer R, Termont DSML, De Vries HT. Differential effects of calcium ions and calcium phosphate on cytotoxicity of bile acids. *Am J Physiol* 1991, **260**, G142–G147.
15. Lapré JA, Termont DSML, Groen AK, van der Meer R. Lytic effects of mixed micelles of fatty acids and bile acids. *Am J Physiol* 1992, **263**, G333–G337.
16. Govers MJAP, van der Meer R. Effects of dietary calcium and phosphate on the intestinal interactions between calcium, phosphate, fatty acids, and bile acids. *Gut* 1993, **34**, 365–370.
17. Lapré JA, De Vries HT, van der Meer R. Dietary calcium phosphate inhibits intestinal cytotoxicity. *Am J Physiol* 1991, **261**, G907–G912.
18. Govers MJAP, Termont DSML, van der Meer R. The mechanism of the antiproliferative effect of milk mineral and other calcium supplements on colonic epithelium. *Cancer Res* 1994, **54**, 95–100.
19. Lipkin M, Newmark H. Effect of added dietary calcium on colonic epithelial cell proliferation in subjects at high risk for familial colonic cancer. *N Engl J Med* 1985, **313**, 1381–1384.
20. Kleibeuker JH, Welberg JWM, Mulder NH, *et al.* Epithelial cell proliferation in the sigmoid colon of patients with adenomatous polyps increases during oral calcium supplementation. *Br J Cancer* 1993, **67**, 500–503.
21. Wargovich MJ, Isbell MJ, Shabot M, *et al.* Calcium supplementation decreases rectal epithelial cell proliferation in subjects with sporadic adenoma. *Gastroenterology* 1992, **103**, 92–97.
22. Barsoum GH, Hendrickse C, Winslet MC, *et al.* Reduction of mucosal crypt cell proliferation in patients with colorectal adenomatous polyps by dietary calcium supplementation. *Br J Surg* 1992, **79**, 581–583.
23. Bostick RM, Potter JD, Fosdick L, *et al.* Calcium and colorectal epithelial cell proliferation: a preliminary randomized, double-blinded, placebo-controlled clinical trial. *J Natl Cancer Inst* 1993, **85**, 132–141.
24. Cats A, Kleibeuker JH, Van der Meer R, *et al.* Randomized, double-blinded, placebo-controlled intervention study with supplemental calcium in families with hereditary nonpolyposis colorectal cancer. *J Natl Cancer Inst* 1995, **87**, 598–603.
25. Lapré JA, De Vries HT, Termont DSML, Kleibeuker JH, De Vries EGE, van der Meer R. Mechanism of the protective effects of supplemental dietary calcium on cytolytic activity of fecal water. *Cancer Res* 1993, **53**, 248–253.
26. Welberg JWM, Kleibeuker JH, van der Meer R, *et al.* Effects of oral calcium supplementation on intestinal bile acids and cytolytic activity of fecal water in patients with adenomatous polyps of the colon. *Eur J Clin Invest* 1993, **23**, 63–68.
27. Govers M, Lapré J, Kleibeuker J, Vonk R, Van der Meer R. Calcium in milk products precipitates intestinal surfactants and inhibits luminal cytolytic activity in healthy subjects. *Gastroenterology* 1994, **106**, A388.
28. Steinbach G, Lupton J, Reddy BS, Kral JG, Holt PR. Effect of calcium supplementation on rectal epithelial hyperproliferation in intestinal bypass subjects. *Gastroenterology* 1994, **106**, 1162–1167.
29. Steinbach G, Morotomi M, Nomoto K, *et al.* Calcium reduces the increased fecal 1,2-*sn*-diacylglycerol content in intestinal bypass patients: a possible mechanism for altering colonic hyperproliferation. *Cancer Res* 1994, **54**, 1216–1219.
30. Garland C, Barrett-Conner E, Rossof AH, *et al.* Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1985, **i**, 307–309.
31. Shabahang M, Buras RR, Davoodi F, *et al.* Growth inhibition of HT-29 human colon cancer cells by analogues of 1,25-dihydroxyvitamin D_3. *Cancer Res* 1994, **54**, 4057–4064.
32. Thomas MG, Brown GR, Alison MR, *et al.* Divergent effects of epidermal growth factor and calcipotriol on human rectal cell proliferation. *Gut* 1994, **35**, 1742–1746.
33. Beaty MM, Lee EY, Glauert HP. Influence of dietary calcium and vitamin D on colon epithelial cell proliferation and 1,2-dimethylhydrazine-induced colon carcinogenesis in rats fed high fat diets. *J Nutr* 1993, **123**, 144–152.

Acknowledgements—Supported by the Dutch Cancer Society (GUKC 89-08, RUG 94-785) and the Netherlands Organization of Scientific Research (NWO 900-562-078).