Do fermented dairy products offer any protection against colorectal cancer ?

26

DANONE

NUTRITOPICS

edito

Editorial
By Prof. Peter R. Holt
(Institute for Cancer Prevention, New York N.Y. ; Professor of Medicine (Emeritus), Columbia University, USA)

Colorectal cancer is the second commonest cause of cancer deaths in the Western world. Treatment of established disease still has little impact on mortality so that prevention is the objective for the 21st century. Colorectal cancer prevention presently consists of detection of pre-neoplastic lesions and their removal – an approach which has been shown to reduce colorectal incidence and mortality by up to 70%. Now, we are heading for primary prevention of the disease in the at risk population as a whole.

This review covers much of the current information about colorectal carcinogenesis and its prevention particularly by dairy foods and probiotic-containing yogurt preparations. Why should prevention work? The process of colon carcinogenesis takes many years and is associated with several pre-cancerous stages. Environmental factors, which can be altered, are believed responsible for at least 70% of the risk of colorectal cancer. Thus, there is time for intervention, the possibility of detection and the likelihood of success.

Non steroidal anti-inflammatory drugs such as Sulindac have been shown to lower the incidence of pre-cancerous polyps in a genetic form of cancer, epidemiologic data points to the benefits of aspirin and very recently the administration of aspirin has been shown to lower polyp recurrence (*NEJM*, March 2003). The population at risk as a whole is much more likely to accept taking “natural” or dietary components rather than medicinals, which have greater side effects. Thus, the potential advantages of calcium which has been shown to reduce the recurrence of pre-neoplastic polyps, especially high-risk polyps, perhaps combined with vitamin D, and the probiotic components of dairy based yogurt preparations may be much more acceptable.

The future is exciting for the possibilities of success in this field. We need to detect those individuals who are particularly at risk for the development of colon cancer. This will need finding biological markers in blood or colorectal biopsies that would indicate a greatly increased risk over that of the general population. We will need to develop simple screening methods to detect the presence of adenomatous polyps and early cancer. We should also recognize that preventive approaches may well require the administration of multiple agents that work on different intracellular signaling pathways leading to cancer. In this way, preventive approaches for colorectal cancer will mimic the approach that oncologists use for chemotherapeutic agents in the treatment of established disease.

Report

What is colorectal cancer?

(Do fermented dairy products offer any protection against colorectal cancer)

summary introduction proliferation

Summary

Colorectal cancer (CRC) is the second commonest cause of cancer deaths in many industrialized countries. It develops very slowly, in the mucosa of the colon or the rectum. During this multi-step process, cell multiplication gradually gets out of control, leading to the accumulation of cells in the form of polyps, which then become malignant tumors.

Currently, prevention depends on colonoscopy, an invasive procedure that is only offered to high-risk patients. Detection of the influence of dietary factors on the risk of CRC makes it possible to envisage preventative measures accessible to entire populations. In Western-type diets, the excessive intake of energy and the consumption of grilled red meat increase the risk of CRC. In contrast, a diet containing large amounts of fruit and vegetables reduces this risk.

Numerous epidemiological and experimental data converge to support the beneficial effects of the dairy products with the lowest energy density, such as fermented dairy products (FDPs). This effect is particularly well-documented for calcium and vitamin D, and for the probiotics, specific to the FDPs, which may act by different mechanisms.

Introduction

During the year 2000, 363,000 new cases of colorectal cancer (CRC) were identified in Europe. It is the fourth most common form of cancer in the world, and the second commonest cause of cancer deaths in many industrialized countries. In these countries, it accounts for 12.6% of cancer cases in men and 14.1% in women (1), and is increasing steadily.

Diet plays a decisive role in the risk of CRC. It has been clearly shown that the diet in Western countries increases this risk, whereas diets based on vegetable-derived products reduce it.

The role of dairy products, which are present in large quantities in Western diets, has been investigated in many studies. In particular, several constituents of fermented dairy products (FDPs) have been shown to have beneficial effects.

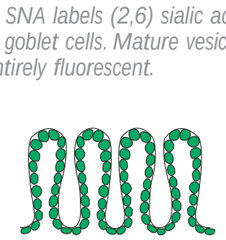
What is the epidemiological and experimental evidence for this effect? How do main constituents of the FDPs act? What human studies are in progress to confirm the promising results reported in animals? What are the prospects in terms of guidelines?
Sufficient data has been accumulated over recent years to answer these questions.

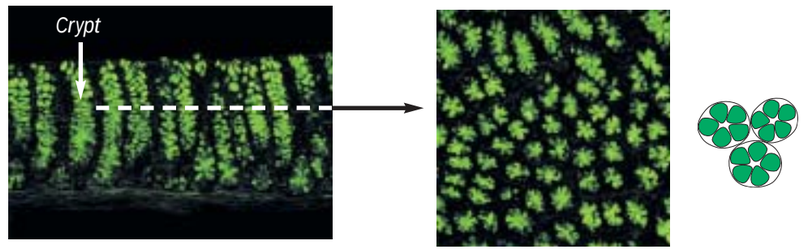
[*Diet plays a decisive role in the risk of CRC.*]

From the normal crypt to neoplastic lesions: a multi-step process

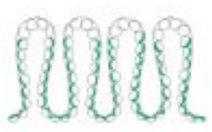
CRC results from the abnormal proliferation of the epithelial cells of the colon or rectum. This loss of control, which develops very gradually, leads to the production of too many cells, which accumulate in the form of polyps. These polyps then develop into carcinomas, according to the adenoma-carcinoma model described by Hill (2).
It is in the **crypts**, which are a sort of invagination formed by the epithelium of the colon and of the rectum, that this process takes place. Lipkin (3) has described the main steps in the process (Figure 2).
In a healthy state the epithelium of the colon or rectum is replaced every 7 to 10 days. **Cell multiplication** demands that this turnover should be strictly **controlled**. It occurs only in the lower part of the crypt known as the "proliferative zone" (Figure 2A). Once the new cells have been formed, they migrate into the upper part of the crypt, where they differentiate and lose their proliferative potential, and then enter the intestinal lumen.
In its earliest stage, CRC is characterized by the **extension of the proliferative zone** to include the upper crypt (Figure 2B), and the acquisition by the cells of an increased ability to proliferate. However, their turnover remains under control, and the equilibrium between new cells and degenerating cells is maintained.
The second stage is marked by a further increase in the ability of the cells to proliferate. This leads to their being produced in excessive numbers and they accumulate in the intestinal lumen, to form **tumors**, which are still benign, and which are known as adenomatous polyps (Figure 2C). During the third stage, further transformations occur, the cells become **pathological**, and the polyps turn into **cancerous tumors** (Figure 2D).
The whole process takes about twenty years, and so far there are no early markers enabling us to detect it before it is too late. Prevention is currently based on the colonoscopic detection of polyps. This invasive procedure is only proposed for subjects at high risk or over 50 years of age. It is therefore important to develop other preventative methods that are more accessible to the whole population.

Lectin SNA labels (2,6) sialic acid in the goblet cells. Mature vesicles are entirely fluorescent.





Lectin GSI labels α-galactose in the Golgi apparatus. Only the immature vesicles are fluorescent.



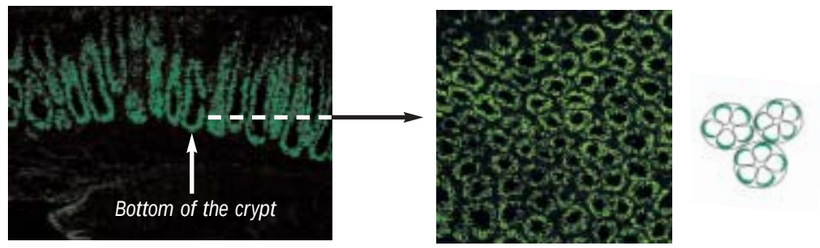


Figure 1. Slices of healthy colon of conventional mice, labeled by fluorescent lectins (Source: Danone Vitapole, M. Freitas)

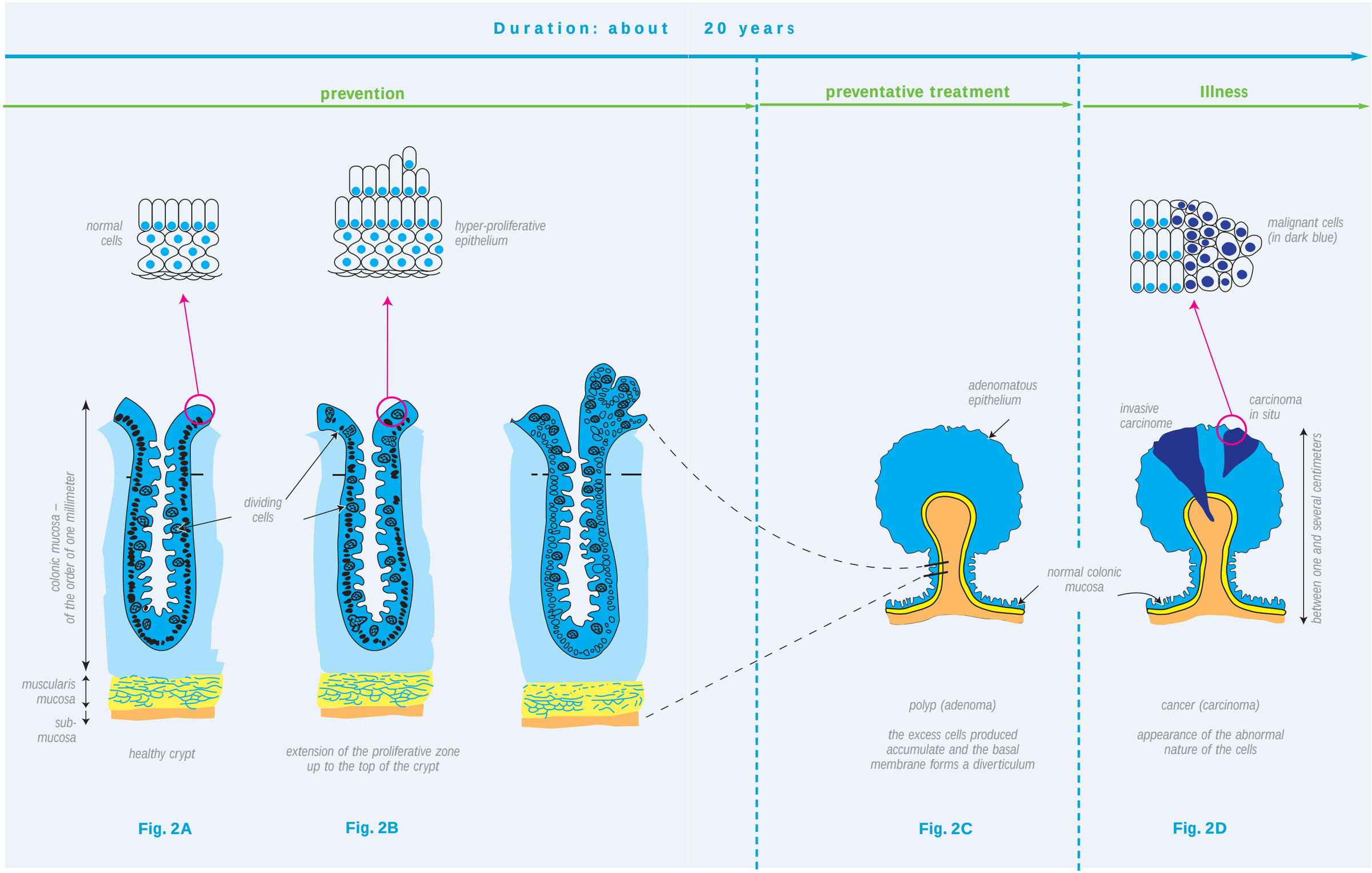


Figure 2. Stages in the development of colorectal cancer, adapted from Lipkin (3), with permission

genes

The accumulation of genetic mutations

The conversion of healthy cells into malignant cells results from an accumulation of genetic mutations (4). Mutations constantly occur in our cells, they are the "copying errors" that occur every time the DNA is copied during cell division. In most cases, these mutations are corrected by enzymes specializing in DNA repair (5). However, they sometimes evade the repair system, and then they are transmitted in subsequent divisions; in this case, all the cells descended from the mutated cell will carry the mutation.

For CRC to develop, several mutations must have accumulated in very specific genes. Some of these have been identified. They include the genes that promote (K-*ras*) or suppress (*APC*, *DCC*, *p53*) tumors, and DNA-repair genes (*hMSH2*, *hMLH1*, etc.).
The more often the cells divide, the more likely mutations are to occur. Thus, increased multiplication of the epithelial cells, in response to different types of aggression of the colorectal mucosa (e.g. by an infection), makes them more likely to occur.

Some mutations can also be induced by **mutagenic agents**. These can be derived directly from the diet (e.g. heterocyclic amines). Some mutagens neutralized by the liver may be released again in the colon as a result of the action of the intestinal flora (e.g. nitrosamines) (6). In animal species, non-dietary mutagenic agents can be used; for instance benzo(a)pyrene is used to induce colon cancer in the rat.

In Man, in 70 to 80% of cases of CRC, the accumulation of mutations occurs entirely randomly (sporadic cases). In the other cases, it involves a hereditary weakness of the repair system (hereditary non-polypous colon cancer), and this allows mutations to accumulate more easily.

The body has several protective systems:
- in the intestinal lumen: rapid transit, which reduces both the time the mutagens have to act, and that available for adsorption, which inactivates them;
- in the epithelial cells: there is a complex detoxification system (for instance cytochrome P450) and a system in the nucleus to repair mutated genes;
- in the liver: chemical neutralization of toxic agents.

epidemio

The environment, a decisive factor

The incidence of CRC varies by a factor of 1 to 20 depending on geographic zone and lifestyle (7). Countries can be divided into high-risk countries, with the highest incidence (North America, Western Europe, Australia), and low-risk countries (Japan, India, Africa). The differences in life expectancy between these regions partly account for these variations. CRC develops slowly and so affects the younger populations. Thus the more populations age, the higher the incidence of CRC.

The environment is a determining risk factor for CRC. Over the past 20 years, the incidence of CRC has been rising steadily in industrialized countries. In Italy, a 12.2% increase was recorded between 1985 and 1997, and similar trends have been reported in other European countries, such as Denmark (8) and Finland (6) (9).
Various environmental factors have been shown to be correlated to the risk of CRC. In the 1980s, a series of epidemiological studies showed that **physical activity** is accompanied by a lower risk of CRC. This relationship has been subsequently confirmed by several prospective and case-control studies, but the protective mechanisms have not been elucidated (10). High **alcohol** intake is another risk factor (11).
In a study carried out amongst Japanese immigrants living in Hawaii and in the United States, Haenszel *et al.* (12) have shown that **diet** plays a major role. Within one or two generations, these migrants had acquired a risk of CRC equal to that of the native populations. These authors attributed this increase in the risk to the fact that these migrants had adopted the diet of the host country. Phillips *et al.* (13) have also shown that the particularly low incidence of CRC in communities of Seventh-Day Adventists living in California is associated with their specific diet, which contains low levels of fat and high levels of fruit and vegetables. It has also been observed that the incidence of CRC was particularly low in Japan, which is characterized by a diet containing low levels of meat and saturated fats, and high levels of fish and cereals, though it is rising rapidly as the mainland Japanese are adopting a more Western lifestyle.

Markers that can be used to investigate colorectal cancer

Animal models and human risk markers are both available to study the factors that influence the development of CRC (24).

The most commonly used animal model is that of the **rat**, in which a dietary carcinogen or a chemical treatment, can be used to induce a cancer of the colon, the development of which follows a pattern very similar to that of human CRC. Another approach in the **mouse** consists of implanting colonic tumor cells and investigating the influence of various treatments on their development. Finally, there are also some genetically modified mouse lines that develop tumors spontaneously.

At the early stage of the development of the cancer, the effect of treatments is investigated on the **morphology of the crypts**, and on the **cell proliferation index**. At more advanced stages, the presence of preneoplastic lesions (**aberrant crypts**) or the presence of tumors is investigated, as well as their morphology and their recurrence. **Fecal markers** can also be used, notably, the concentration of bile acids.

In Man, ethical restrictions as well as practical considerations (cost, duration of the studies, number of subjects) make it difficult to carry out studies in subjects who already have cancer. Intervention studies are carried out in healthy subjects, who have a high risk of CRC because of their genetic predisposition, or diet (6).

Three types of CRC risk marker have been identified in Man:

- **mucosal markers:** in the intestinal mucosa of healthy subjects, the **cell-proliferation index**, the **morphology of the crypts** and the **distribution of proliferative zones** are investigated. These markers are in fact modified in high-risk subjects. However, these markers are not the most reliable ones because anomalies are not always linked to progress towards CRC. An **analysis of the polyps** (morphology, size, recurrence) gives the best prediction of the risk of a development into CRC. Thus, polyps more than 1 cm in diameter are associated with the highest risk. However, polyp analysis cannot be carried out in all the studies because this entails an invasive intervention, and following up the patients over many years.

- **fecal markers:** compounds that irritate the intestinal mucosa, such as the bile acids, are concentrated in the aqueous fraction of the stools (24). The markers about which there is the greatest consensus are the cytotoxicity (toxicity towards cells) and the genotoxicity (mutagenicity) of the aqueous fecal fraction. Several studies have linked high cytotoxicity to an increase in the cell proliferation index and an increased risk of CRC (24).

- **immunological markers:** measuring the activity of the immune system in the colon and in the blood.

Genetic markers have been developed within the past ten years (62). It is now possible to look for mutations of the genes implicated in the development of CRC in the colonic mucosa. These markers, which are still rarely used in Man, have given interesting results in the rat. In the future, they could be used more routinely during colonic biopsies, to identify genetic targets for new therapies. Genomics will also make it possible to go down to a more detailed level in studying mechanisms.

What is the role of diet?

diet
Western diet and higher risk

Many epidemiological studies have been carried out to look for correlations between the consumption of certain foods and the risk of CRC (14). **Overall, these studies suggest that the diet in western countries, which contains high levels of fat and meat and low levels of fruit and vegetables, is associated with a higher risk of CRC** (15) (16) (17) (18).

In these diets, a link has often been made between the excessive consumption of **animal fats** and an increase in the risk of CRC (19). However, this link remains controversial, because this increased risk has been reported by observation studies, but not by intervention studies (14). It is often difficult to distinguish between the contribution of fats and that of energy. **According to some authors, the risk of CRC may be determined by the energy intake rather than by the lipid intake**. According to a case-control study carried out in 4400 adults in the United States, a daily excess of 500 kcal increases this risk by 15% in men and by 11% in women (20). From a meta-analysis of 40 cohort and case-control studies, Giovanucci *et al.* (21) conclude that the total fat intake does not *per se* constitute a risk factor for CRC.

In contrast, some categories of fatty acids are likely to promote the onset of CRC because of their irritant effect.
In animal models, the fat intake has been found to modify some CRC markers (14). The ingestion of fats induces the production of **bile acids**, which are in theory virtually entirely reabsorbed from the small intestine. According to the most generally accepted hypothesis, an excess of fats in the diet induces an excessive amount of bile acids in the small intestine, which cannot be completely reabsorbed, and therefore reach the colon, where they have a detergent effect on the mucosa. This in turn leads to increased proliferation of the epithelial cells (14).
In-vitro, fatty acids stimulate the production by the intestinal flora of **diacylglycerol** (DAG), a substance that induces cell proliferation (14).

It has recently been suggested that **the increase in the level of insulin** in the blood stream (due for example to the consumption of foods with a high glycemic index) **could promote the onset of CRC** (21). It has been shown in animal studies that insulin is an important growth factor for the epithelial cells of the colon (10).

Finally, eating **cooked red meat** also seems to contribute to the higher risk seen with western diets. Thus, Willett *et al.* (19), in a prospective study conducted in the United States in 89,494 women over a period of 6 years, have reported that the risk of CRC is significantly higher in heavy consumers of red meat. Many other studies have reported the same correlation (10). In particular, grilling fatty meat results in the formation of **heterocyclic amines**, which are known to have a mutagenic effect, and indeed they are used in animal studies to induce CRC.

vegetables
The beneficial effect of foods of vegetable origin

The beneficial effect of foods of vegetable origin against cancers in general has been demonstrated in many epidemiological studies. Thus, a review of 200 studies has shown that in people eating a diet containing plenty of fruits and vegetables, the incidence of cancer is only half as high as in the rest of the population (22). In their summary of 37 epidemiological observation studies and 16 case-control studies, Trock *et al.* (23) linked a significantly lower risk of CRC with a diet containing high levels of foods containing plant **fiber**. This study also showed that the reduction in the risk was virtually the same for fruit and for vegetables, but it was not possible to distinguish between the effects of fiber and of the other constituents of vegetables.
More than a dozen putative **protective constituents** have been identified in foods of vegetable origin, in particular in soybean, linseed, tomatoes, garlic, tea, brassicas and citrus fruits (24).
In general, diets that contain more vegetables are also those with **lower energy density**, and this must contribute to their beneficial effects.

yoghurt
The role of fermented dairy products

In North America, Australia and Europe, dairy products provide about 10% of the total energy intake (25). Many studies have been carried out to look for a link between the intake of these products and the risk of CRC (14).
The findings of these studies are discordant, sometimes indicating a reduction of the risk, sometimes no relationship, and sometimes a greater risk (Table 1). According to a very recent meta-analysis conducted by Norat and Riboli (10), the main case-control studies conclude that the consumption of dairy products in general, and of milk in particular, reduces the risk of CRC. However, this reduction is no longer found when cohort studies are taken into account.

The discrepancies between these results can be explained by the diversity of products consumed in the various studies, which makes it difficult to compare them.
Indeed, **some dairy products**, such as cheese or cream, **contain some constituents associated with a reduction of the risk, and others associated with an increase of the risk** of CRC (26). Thus the increased risk observed by Iscovich *et al.* (27) in a case-control study carried out in Argentina seems to result from the high intake of cheese by the population studied. However, cheeses are amongst the dairy products with the highest energy density. It was only after adjusting for the quantity of total fat ingested than an inverse relationship was identified by McKeown-Eyssen (17). However, this adjustment has not been done in most of the studies that have reported no correlation (26).

If we specifically consider **fermented dairy products** (FDPs), which have low energy density, **we observe either a reduction in the risk in people consuming large amounts of FDPs, or no relationship**.
In a study conducted in India, Malhotra (28) attributed the low incidence of CRC to a high intake of yoghurts and other fermented dairy products. Similarly, in two case-control studies, a lower incidence of CRC was found in the people consuming the most yoghurts (29) or fermented milks (30).
In contrast, in two large prospective studies carried out in the United States, Kampman *et al.* (31) did not find this correlation. Similarly, no correlation was reported in another study carried out in the Netherlands in a population of elderly people (32). In these studies, the range of food products consumed was very varied, which made it difficult to interpret the findings.
It should also be noted that overall, these studies were carried out in countries where large amounts of dairy products are consumed. There is therefore little difference between "light consumers" and "high consumers", which does not make it any easier to provide a statistical demonstration of any reduction in the risk.

Study	Characteristics of the study	Products	Findings
Malhotra, India (28)	Retrospective study 37 cases among 360,510 people studied	Yoghurts and other fermented dairy products	Lower risk in highest consumers of FDPs
Peters, United States (29)	Retrospective case-control study 746 cases / 746 controls	Yoghurt	Lower risk in highest consumers of yoghurt
Kampman, United States (31)	Prospective case-cohort studies 681 cases / 17,744 controls	Various products	No correlation
Kampman, Netherlands (32)	Prospective case-cohort study 326 cases / 3,083 controls	Various products	No correlation

Table 1. Results of epidemiological studies that investigated a correlation between the consumption of fermented dairy products (FDPs) and the risk of colorectal cancer (CRC).

What seem to be the protective constituents of FDPs?

Do fermented dairy products offer any protection against colorectal cancer

Animal and *in-vitro* studies suggest that the various constituents of the FDPs could have a beneficial effect in preventing the onset of CRC. In particular, this applies to calcium and vitamin D, which are present in dairy products as a whole, and in the probiotics that are specific to the FDPs.

calcium

Calcium

Many epidemiological and intervention studies in Man, as well as animal studies suggest that calcium has an effect against CRC.

The first large-scale epidemiological study was carried out by Garland *et al.* (33)between 1959 and 1978 in 1954 men employed by "Western Electric" in Chicago. In this prospective study, the authors found that **the risk of CRC was lowest in the men with the highest intake of calcium**. In this study, a daily intake of 1200 mg of calcium was associated with a 75% reduction in the risk of CRC (33). This reduction was reported in most of the epidemiological studies, but not all of them (34).
The hypothesis suggested by Newmark *et al.* in 1984 (35) in fact led to many **intervention studies**. They suggested that an **increased calcium intake** leads to a reduction in the concentration of free fatty acids in the intestinal lumen (36). Overall, these studies revealed a change in the **early and late mucosal markers** of the risk of CRC, after an increase in the dietary intake of calcium.

Lipkin and Newmark (37) studied the effect of calcium supplementation in 10 subjects with a genetic predisposition to CRC, and who had a high **crypt cell proliferation index**. Supplementation led to a reduction in this index, and a reversal of the morphological abnormalities. These effects of calcium supplementation on early markers of CRC were subsequently confirmed in other studies carried out in human subjects and in rats (36) (37).
Holt *et al.* (38) have also shown that a regular intake of calcium over a period of several months restores normal cell proliferation in high-risk subjects. Their results were obtained in a controlled intervention study lasting 1 year in 70 subjects presenting with adenomatous polyps. The subjects treated were following nutritional advice to increase their daily calcium intake to 1200 mg. Six months after the beginning of the study, a significant reduction in the cell proliferation index had been observed in the treated subjects, whereas no change was reported in the control-group. However, this effect was found to be temporary: one year after the beginning of the study, the proliferation index had gone back up again in the supplemented subjects.

Baron *et al.* (39) subsequently demonstrated the beneficial effect of calcium supplementation on the **recurrence of adenomatous polyps**. In this study, 832 subjects with a history of adenomatous polyps received either calcium in the form of a supplement (1200 mg/d), or a placebo. After four years of calcium supplementation, one adenoma or more had been diagnosed in only 31% of the supplemented subjects, versus 38% of the control subjects. A further analysis of these results shows that, interestingly, there was a far greater effect of calcium on more dangerous advanced polyps (63).

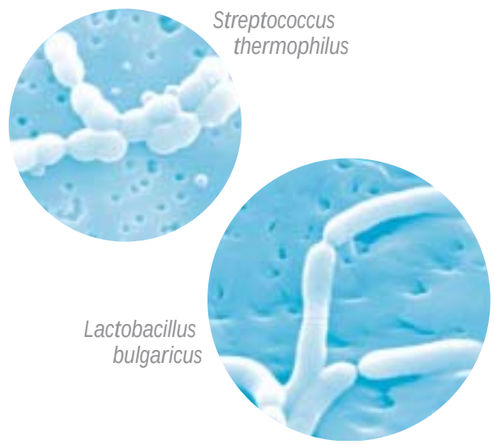
Animal models have made it possible to achieve a better understanding of the **mechanism** of action of calcium. The most likely hypothesis is that the **calcium may form molecular complexes with free fatty acids and bile acids**, thus facilitating their excretion. The concentration of the free forms of these acids in the feces falls when the dietary intake of calcium increases. Calcium may also act by **reducing the toxicity of these acids** towards the intestinal mucosa (14). Finally, these *in-vitro* studies suggest that once it has entered the cell, calcium may be involved in the **control of cell proliferation** (14).

probiotics

Lactic acid bacteria

Several studies in the rat and the mouse, and some studies in Man, suggest that lactic acid bacteria (LAB) may have a beneficial effect, at various levels, on the reduction of the risk of CRC.

Changes in various **mucosal and fecal markers** have been observed with *Lactobacillus, Streptococcus* and *Bifidobacterium* bacteria (40) (41). Several studies in the rat have shown that the dietary intake of LAB affects the development of CRC at various levels. Abdelali *et al.* (42) have reported a significant reduction in the incidence of chemically-induced **aberrant crypts** in the rat, after supplementation with milk fermented by *Bifidobacterium.*
Tavan *et al.* (43) have also reported a reduction of between 93% and 96% in the incidence of aberrant crypts induced by heterocyclic amines, in rats receiving supplements of milk fermented by *B.animalis* or *S.thermophilus*. In comparison, a reduction by only 66% was found in the rats in the same study, supplemented with non-fermented dairy products.
In Man, Biasco *et al.* (44) observed a reduction in **proliferative activity** in the upper part of the crypts, after administering *L. acidophilus* and *B. bifidum* to patients who initially displayed an activity profile which gave them a high risk of CRC.



At least eight different mechanisms have been suggested to explain how the LAB act (Table 2).

- In Man and in animal models, the ingestion of LAB **lowers the concentration of some of the enzymes responsible for the release of mutagenic agents** within the colon. In a study of supplementation with *L.acidophilus* in 21 healthy volunteers, Goldin and Gorbach (45) found lower fecal concentrations of three of these enzymes (β-glucuronidase, nitroreductase and azoreductase). This effect is seen after 10 days of treatment. However, it is reversible and is no longer found 10 to 30 days after the end of the study. It therefore appears that a continuous intake of LAB is required to maintain it (41). Other human and rat studies with *L.acidophilus* have reported a reduction of these enzymes.

- LAB may also act by reducing the **concentration of bile acids**. Lidbeck and Nord have reported a reduction in the fecal concentration of bile acids in patients suffering from colon cancer, after supplementation with fermented milks containing *L. acidophilus* (46).

- *In vitro*, LAB are able to **adsorb some of the mutagens present in Western-style diets**, in particular in grilled meat, and carry them with them when they are excreted. Morotomi and Mutai (47) have shown that several strains of LAB bound to the heterocyclic amines Trp-P1 and Trp-P2. In the rat, the ingestion of *L.acidophilus* reduces the fecal concentration of heterocyclic amines that results from eating meat (45). Abdelali *et al.* (48) have reported a dose-dependent effect of *Bifidobacterium* against the action of benzo[a]pyrene. Tavan *et al.* (49) have also found that several LAB produced a reduction in the mutagenic effect of the three most common heterocyclic amines in the human diet. In the rat, strains of the species *L.acidophilus, L.gasseri, L.confusus, S.thermophilus* and *B.breve* reduce the changes in DNA induced by the action of chemical mutagens on the colon (50). In Man, the ingestion of LAB, in particular of *L.acidophilus*, has been linked to a reduction in the mutagenicity of the urine and feces (64).

- LAB may also have a beneficial effect on the **composition of the intestinal flora**. In Man, the consumption of milk fermented by *L.acidophilus* has been linked to an increase in the fecal concentration of LAB and a reduction in that of **putrefactive bacteria**, which are probably responsible for the release of carcinogenic substances (51).

- **An anti-tumoral effect** of LAB has also been reported on tumor cells implanted in the mouse. This effect has also been reported for ingested LAB as well as after the injection of *Bifidobacterium* into implanted tumors (41). It has also been reported *in vitro* against human cancer cells (52). This latter observation suggests that the soluble compounds released during lactic fermentation have an anti-tumor effect. It is also possible that this effect results from the stimulation by the LAB of the constituents of the **immune system** involved in the antitumor response (24). In a recent study, Perdigon *et al.* (53) found that yoghurt had an anti-tumor effect against CRC induced in the Mouse. They showed in this study that ingesting yoghurt for 10 days stimulates the production of mucosal antibodies (IgA) effective against cancer cells, the production of soluble mediators, and the apoptosis (cell "suicide") of the cancer cells.

- LAB may act on the **cell repair systems**. LAB stimulate glutathione-peroxidase and cytochrome P450, and therefore increase the metabolism of xenobiotics (54) (55).

- Finally, it has also been found that the LAB **modulate the production of butyric acid by the flora of the colon**. *In-vitro* and *in-vivo* studies suggest that butyric acid prevents the proliferation of cancer or precancerous cells from the mouse colon, and inhibit the development of tumors of the colon.

Taken as a whole, these findings are sufficiently encouraging for the European Community to be funding human intervention studies. These are expected to confirm the beneficial effect of LAB, and to identify the specific mechanisms of action of each strain. Some of these studies are particularly interested in the synergistic action of probiotics and prebiotics in the form of synbiotics (56).

vitamin

Vitamin D

Epidemiological data suggest that there may be a link between vitamin D and the reduction in the risk of CRC.
In the United States, the incidence of CRC is 40% higher in the regions at northern latitudes than in the sunny regions of the Southeast and West. On the basis of this observation, Garland and Garland (57) have suggested that **vitamin D, the production of which is stimulated by exposure to the sun, may be associated with a reduction in the risk of CRC**. In the study carried out amongst "Western Electric" employees (33), they also found a lower risk in high vitamin-D consumers. This correlation has also been found in 7th Day Adventists (13), and in various studies carried out in Finland (34).
Other studies have subsequently shown a correlation between the blood level of **25-hydroxyvitamin D** (one of the active forms of vitamin D) and the risk of CRC. The risk is greater for levels of between 10 and 30 ng/mL, levels that are above the vitamin-D deficiency threshold (5 ng/mL) (65).
Vitamin-D supplementation reduces the **development of chemically-induced CRC** in the rat (34). In contrast **no change was found in the early markers** (cell-proliferation index, differentiation) or late markers (polyp formation) of CRC in supplementation studies in Man (65).
Vitamin D may promote the action of calcium by interfering with its distribution around the epithelial cells, and by promoting its entry into the cells (14). It has been shown in a study in the mouse that a dietary intake of vitamin D is required to maintain a high concentration of calcium near the colonic crypts (58).
Vitamin D also modulates the **expression** of many of the **genes** involved in controlling the cell cycle at various points. The colon cells have vitamin D receptors, and so it is possible that this vitamin may act by regulating their **proliferation** (34).
As a conclusion, if vitamin D has a direct effect, it has still to be documented (59) (60) (61).

Table 2: Effects of the constituents of fermented dairy products on the development of colorectal cancer

Constituent	Epidemiological and experimental data	Proposed Mécanisms of action
Calcium	**In Man** - High consumption of calcium associated with a lower risk of CRC in epidemiological observation studies - Modification of the early and late mucosal markers of the risk of CRC, and of the fecal markers, in intervention studies **In animal models** - Modification of the mucosal and fecal markers - Inhibition of chemically-induced CRC	- Promotes the excretion of bile acids by trapping them in the form of complexes - Control of cell proliferation
Vitamin D	**In Man** - Differences in the incidence of the CRC depending on exposure to the sun - High consumption of vitamin D is associated with a reduction of the risk - Correlation between the blood level of 25-hydroxyvitamin D and risk of CRC - Vitamin D supplementation has no effect **In animal models** - Reduced development of chemically-induced CRC	- Facilitates the entry of calcium into the cells - Increases the concentration of calcium around the crypts of the colon - Modulation of the expression of many of the genes involved in controlling the cell cycle
Lactic acid bacteria	**In Man** - Reduced proliferation index in the crypts **In animal models** - Lower incidence of chemically-induced tumors - Lower incidence of aberrant crypts	- Reduced concentration of certain enzymes that release carcinogenic substances - Modification of the physico-chemical conditions of the colon - Adsorption of mutagens - Modification of the composition of the intestinal flora - Anti-tumor effect - Stimulation of the DNA repair systems - Increased production of butyric acid

minors

Other constituents of milk

It has also been suggested that other constituents of milk may have a protective effect against CRC (14). Most of the experimental evidence for this comes from animal or *in-vitro* studies.

Conjugated linoleic acids

The effect of the **conjugated linoleic acids (CLA)** has been reported in animals and *in vitro*. These consist of *9 trans* fatty acids, isomers of linoleic acid, of which dairy products (milk, butter, yoghurt, cheese) are the main source. In animals, the ingestion of CLA in concentrations equivalent to that found in dairy products reduces the onset of various chemically-induced cancers. *In vitro*, CLA reduces the proliferation of cancer cells derived from CRC. According to the current hypothesis, it may act indirectly by making the membranes more fluid, reducing the synthesis of prostaglandins, and stimulating the immune response (14).

Sphingolipids

Dairy products also contain high levels of sphingolipids, fatty acids that are required for the activity of the cells of the body. The most common sphingolipid, **sphingomyelin**, is present on the surface of the fatty globules of milk.
The anti-tumor effect of the sphingolipids has been demonstrated *in vitro* and *in vivo* (14). The incorporation of sphingomyelin into the feed of mice reduces the incidence of chemically-induced tumors of the colon by more than one half. This effect has been confirmed by other studies in the mouse. **Sphingosin**, which is produced during the digestion of sphingomyelin, also reduces the metastatic potential and growth of cancer cells *in vitro*.
The mechanism of action of the sphingolipids has not yet been elucidated, but it is known that they are involved in the flexibility of cell membranes, and thus facilitate the vitamin-D mediated entry of calcium into the cells.

Milk proteins

In-vitro and *in-vivo* studies suggest that some of the proteins in milk, in particular lactoferrin, casein and some serum proteins, have the effect of reducing the risk of CRC.

In the rat, administration of bovine **lactoferrin** in the diet inhibits the effect of carcinogenic substances that induce CRC, and anti-metastatic effects have been reported in the mouse (66). This effect seems to be linked to the stimulation of an immune response directed against tumors (T cytotoxic lymphocytes, NK cells, cytokines), and also against certain bacteria that produce toxic substances. Human studies have been carried out to determine the potential benefit of bovine lactoferrin versus CRC and other cancers.
However, it should be noted that milk contains only small amounts of lactoferrin, and that it is inactivated by the heat treatments to which many dairy products are subjected during their manufacture (10).
It has also been reported that milk **casein** inhibits β-glucuronidase (14).
Finally, **serum proteins** contain high levels of various sulfur-containing amino acids, precursors of glutathione, which is the substrate of two enzymes recognized as having anti-cancer activity: glutathione-peroxidase and glutathione-transferase.

conclusion

Conclusion

The dairy products with the lowest energy density, such as **fermented dairy products** (FDPs), seem to be associated with a **reduction of the risk of colorectal cancer** (CRC). Many of the constituents of milk and FDPs act at various stages of the development of CRC.
The many data obtained in Man and in animals using calcium show that it reduces the risk of CRC, and that it inhibits the recurrence of adenomatous polyps. Lactic acid bacteria, which are specific to FDPs, have also been shown to have the effect of reducing the risk, at various stages of the development of CRC. Human intervention studies currently in progress should clarify their effects.
However, it is important to maintain the consumption of dairy products, giving pride of place to FDPs, which have low energy density, and in which the lactic acid bacteria produce their effect synergistically with that of the constituents of milk. Their consumption seems to provide a method of prevention that should be developed, alongside other preventative measures against CRC.

Bibliography

1. Boyle P, Langman JS. ABC of colorectal cancer: Epidemiology. *BMJ* 2000;321:805-8.
2. Hill MJ, Morson BC, Bussey HJ. Aetiology of adenoma-carcinoma sequence in large bowel. *Lancet* 1978;1:245-7.
3. Lipkin M. Phase 1 and phase 2 proliferative lesions of colonic epithelial cells in diseases leading to colonic cancer. *Cancer* 1974 Sep;34(3):suppl:878-88.
4. Le Cancer. In: Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J, eds. Biologie moléculaire de la cellule. Paris: Flammarion 2003:1192-202.
5. Les mécanimes génétiques fondamentaux. In: Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J, eds. Biologie moléculaire de la cellule. Paris: Flammarion 2003:220-7.
6. Gill C, I, Rowland IR. Diet and cancer: assessing the risk. *Br. J. Nutr.* 2002;88:S73-S87.
7. Yoon H, Benamouzig R, Little J, François CM, Tomé D. Systematic review of epidemiological studies on meat, dairy products and egg consumption and risk of colorectal adenomas. *Eur. J. Cancer Prev*. 2000;9:151-64.
8. Johansen C, Mellemgaard A, Skov T, Kjaergaard J, Lynge E. Colorectal cancer in Denmark 1943-1988. *Int. J. Colorectal Dis.* 1993;8:42-7.
9. Kemppainen M, Raiha I, Sourander L. A marked increase in the incidence of colorectal cancer over two decades in southwest Finland. *J. Clin. Epidemiol.* 1997;50:147-51.
10. Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. *Eur. J. Clin. Nutr.* 2003;57:1-17.
11. Wu AH, Paganini-Hill A, Ross RK, Henderson BE. Alcohol, physical activity and other risk factors for colorectal cancer: a prospective study. *Br. J. Cancer* 1987;55:687-94.
12. Haenszel W, Berg JW, Segi M, Kurihara M, Locke FB. Large-bowel cancer in Hawaiian Japanese. *J. Natl. Cancer Inst.* 1973;51:1765-79.
13. Phillips RL, Garfinkel L, Kuzma JW *et al.* Mortality among California Seventh-Day Adventists for selected cancer sites. *J. Natl. Cancer Inst.* 1980;65:1097-107.
14. Dairy foods and the colon cancer. In: Miller GD, Jarvis JK, McBean LD, eds. Handbook of diary foods and nutrition. CRC Press LLC 2000:155-91.
15. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer* 1975;15:617-31.
16. Drasar BS, Irving D. Environmental factors and cancer of the colon and breast. *Br. J. Cancer* 1973;27:167-72.
17. McKeown-Eyssen GE, Bright-See E. Dietary factors in colon cancer: international relationships. *Nutr. Cancer* 1984;6:160-70.
18. Liu K, Stamler J, Moss D, Garside D, Persky V, Soltero I. Dietary cholesterol, fat, and fibre, and colon-cancer mortality. An analysis of international data. *Lancet* 1979;2:782-5.
19. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE, *et al.* Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *N. Engl. J. Med.* 1990;323:1664-72.
20. Kampman E, Slattery ML, Caan B, Potter JD. Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *Cancer Causes Control* 2000;11:459-66.
21. Giovannucci E, Goldin B. The role of fat, fatty acids, and total energy intake in the etiology of human colon cancer. *Am. J. Clin. Nutr.* 1997;66:1564S-71S.
22. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* 1992;18:1-29.
23. Trock B, Lanza E, Greenwald P. Dietary fiber, vegetables, and colon cancer: critical review and meta- analyses of the epidemiologic evidence. *J. Natl. Cancer Inst.* 1990;82:650-61.
24. Rafter JJ. Scientific basis of biomarkers and benefits of functional foods for reduction of disease risk: cancer. *Br. J. Nutr*. 2002;88:S219-S224..
25. http://apps.fao.org
26. Rafter JJ. The role of lactic acid bacteria in colon cancer prevention. *Scand. J. Gastroenterol.* 1995;30:497-502.
27. Iscovich JM, L'Abbe KA, Castelleto R *et al.* Colon cancer in Argentina. I: Risk from intake of dietary items. *Int. J. Cancer* 1992;51:851-7.

28. Malhotra SL. Dietary factors in a study of cancer colon from Cancer Registry, with special reference to the role of saliva, milk and fermented milk products and vegetable fibre. *Med. Hypotheses* 1977;122-6.

29. Peters RK, Pike MC, Garabrant D, Mack TM. Diet and colon cancer in Los Angeles County, California. *Cancer Causes Control* 1992;3:457-73.

30. Young TB, Wolf DA. Case-control study of proximal and distal colon cancer and diet in Wisconsin. *Int. J. Cancer* 1988;42:167-75.

31. Kampman E, Giovannucci E,Veer P et al. Calcium, vitamin D, dairy foods, and the occurrence of colo-rectal adenomas among men and women in two prospective studies. *Am. J. Epidemiol.* 1994;139:16-29.

32. Kampman E, van't Veer P, Hiddink GJ,Van Aken-Schneijder P, Kok FJ, Hermus RJ. Fermented dairy products, dietary calcium and colon cancer: a case- control study in The Netherlands. *Int. J. Cancer* 1994;59:170-6.

33. Garland C, Shekelle RB, Barrett CE, Criqui MH, Rossof AH, Paul O. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* 1985;1:307-9.

34. Holt PR. Dairy foods and prevention of colon cancer: human studies. *J. Am. Coll. Nutr.* 1999;18:379S-91S.

35. Newmark HL, Wargovich MJ, Bruce WR. Colon cancer and dietary fat, phosphate, and calcium: a hypothesis. *J. Natl. Cancer Inst.* 1984;72:1323-5.

36. Mobarhan S. Calcium and the colon: recent findings. *Nutr. Rev.* 1999;57:124-6.

37. 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;313:1381-4.

38. Holt PR, Atillasoy EO, Gilman J *et al.* Modulation of abnormal colonic epithelial cell proliferation and differentiation by low-fat dairy foods: a randomized controlled trial. *JAMA* 1998;280:1074-9.

39. Baron JA, Beach M, Mandel JS *et al.* Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N. Engl. J. Med.* 1999;340:101-7.

40. Hirayama K, Rafter J. The role of probiotic bacteria in cancer prevention. *Microbes Inf.* 2000;2:681-6.

41. Rafter J. Lactic acid bacteria and cancer: mechanistic perspective. *Br. J. Nutr.* 2002;88:S89-S94.

42. Abdelali H, Cassand P, Soussotte V, Daubeze M, Bouley C, Narbonne JF. Effect of dairy products on initiation of precursor lesions of colon cancer in rats. *Nutr. Cancer* 1995;24:121-32.

43. Tavan E, Cayuela C, Antoine JM, Trugnan G, Chaugier C, Cassand P. Effects of dairy products on heterocyclic aromatic amine-induced rat colon carcinogenesis. *Carcinogenesis* 2002;477-83.

44. Biasco G, Paganelli GM, Brandi G *et al.* Effect of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on rectal cell kinetics and fecal pH. *Ital. J. Gastroenterol.* 1991;23:142.

45. Goldin BR, Gorbach SL. Alterations of the intestinal microflora by diet, oral antibiotics, and *Lactobacillus*: decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. *J. Natl. Cancer Inst.* 1984;73:689-95.

46. Lidbeck A, Nord CE. Lactobacilli in relation to human ecology and antimicrobial therapy. *Int. J.Tissue React.* 1991;13:115-22.

47. Morotomi M, Mutai M. *In vitro* binding of potent mutagenic pyrolysates to intestinal bacteria. *J. Natl. Cancer Inst.* 1986;77:195-201.

48. Abdelali H, Cassand P, Soussotte V, Koch-Bocabeille B, Narbonne JF. Antimutagenicity of components of dairy products. *Mutat. Res.* 1995;331:133-41.

49. Tavan E, Cayuela C, Antoine JM, Cassand P. Antimutagenic activities of various lactic acid bacteria against food mutagens: heterocyclic amines. *J. Dairy Res.* 2002;69:335-41.

50. Bolognani F, Rumney CJ, Pool-Zobel BL, Rowland IR. Effect of lactobacilli, bifidobacteria and inulin on the formation of aberrant crypt foci in rats. *Eur. J. Nutr.* 2001;40:293-300.

51. Shahani KM, Ayebo AD. Role of dietary lactobacilli in gastrointestinal microecology. *Am. J. Clin. Nutr.* 1980;33:2448-57.

52. Baricault L, Denariaz G, Houri JJ, Bouley C, Sapin C, Trugnan G. Use of HT-29, a cultured human colon cancer cell line, to study the effect of fermented milks on colon cancer cell growth and differentiation. *Carcinogenesis* 1995;16:245-52.

53. Perdigon G, de Moreno dL, Valdez J, Rachid M. Role of yoghurt in the prevention of colon cancer. *Eur. J. Clin. Nutr.* 2002;56 Suppl 3:S65-S68.

54. Nugon-Baudon L, Rabot S, Flinois JP, Lory S, Beaune P. Effects of the bacterial status of rats on the changes in some liver cytochrome P450 (EC 1.14.14.1) apoproteins consequent to a glucosinolate-rich diet. *Br. J. Nutr.* 1998;80:231-4.

55. Humblot C, Kassie F, Nugon-Baudon L, Knasmuller K, Lhoste EF. Diet modulates the genotoxicity of IQ (2-amino-3-methylimidazo[4,5- f]quinoline) in rats associated with a human faecal flora. *IARC Sci. Publ.* 2002;156:417-8.

56. Van Loo J, Jonkers N. Evaluation in human volunteers of the potential anticarcinogenic activities of novel nutritional concepts: prebiotics, probiotics and synbiotics (the SYNCAN project QLK1-1999-00346). *Nutr. Metab Cardiovasc.Dis.* 2001;11:87-93.

57. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int. J. Epidemiol.* 1980;9:227-31.

58. Brenner BM, Russell N, Albrecht S, Davies RJ. The effect of dietary vitamin D3 on the intracellular calcium gradient in mammalian colonic crypts. *Cancer Letters* 1998;127:43-53.

59. Holt PR, Arber N, Halmos B *et al.* Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D. *Cancer Epidemiol. Biomarkers Prev.* 2002;11:113-9.

60. Levine AJ, Harper JM, Ervin CM *et al.* Serum 25-hydroxyvitamin D, dietary calcium intake, and distal colorectal adenoma risk. *Nutr. Cancer* 2001;39:35-41.

61. Pritchard RS, Baron JA, Gerhardsson de Verdier M. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. *Cancer Epidemiol. Biomarkers Prev.* 1996;5:897-900.

62. Lipkin M, Bhandari M, Hakissian M, Croll W, Wong G. Surrogate endpoint biomarker assays in phase II chemoprevention clinical trials. *J. Cell. Biochem. Suppl.* 1994;19,47-54.

63. Wallace K, Baron JA, Cole BF, Karagas MR, Beach ML. Calcium carbonate chemoprevention in the large bowel: effects on hyperplastic polyps, tubular adenomas, and more advanced lesions. *Proc AARC* 2002 March; 43, abstract n°816.

64. Fric P. (Probiotics in gastroenterology). *Zeitschrift für Gastroenterologie* 2002;40:197-201.

65. Cascinu S, Ligi M, Del Ferro E *et al.* Effets of calcium and vitamin supplementation on colon cell proliferation in colorectal cancer. *Cancer Investigation* 2000;411-6.

66. Tsuda H, Sekine K, Fujita K, Ligo M. Cancer prevention by bovine lactoferrin and underlying mechanisms - a review of experimental and clinical studies. *Biochem. Cell. Biol.* 2002;80(1):131-6.

key words

cancer, colon, rectum, epithelium, crypt, colonic mucosa, polyp, adenoma, diet, fermented dairy product, yoghurt, calcium, vitamin D, lactic acid bacteria, risk reduction, epidemiological study, clinical study, Man.

contacts

Publication Director: Sven THORMAHLEN

Editor in Chief, Subscriptions Manager: Martine PIAIA

Writer: Catherine VERRIER-MARY (Avicenne)

Danone Vitapole Editing Board: Jean-Michel ANTOINE, Anne-Marie CAHART, Miguel FREITAS, Nicolas GAUSSERES, Irene LENOIR-WIJNKOOP, Céline PICARD.

Our warmest thanks to Prof. Peter HOLT (Institute for Cancer Prevention, New York N.Y.; Professor of Medicine (Emeritus), Columbia University, USA) for his contribution to this issue.

Danone Vitapole

NUTRITOPICS
Route Départementale 128
91767 PALAISEAU CEDEX
France
Fax : +33 1 69 35 76 89
E-mail : science-corner@danone.com

profile

Danone Vitapole

A fresh worldwide multidisciplinary R&D dynamism

Name: Danone Vitapole
Date of birth: 28 July 2000
Nationality: international
Address: one Center at Palaiseau (France) and local branches throughout the world
Skills: Fresh dairy products, Biscuits and Cereal Snacks, Bottled water.
Players: 800 people in R&D, including 500 researchers and development scientists, of all nationalities, based at Palaiseau.
Mission: to catalyse innovation for the Groupe DANONE brands, build tomorrow's products, and strive to ensure the health, enjoyment and wellbeing of consumers worldwide.
Distinguishing features: publishes Danone Nutritopics and organizes scientific events (Symposia and Workshops), it has a website at:

www.danonevitapole.com.

Nutritopics (English ed.) ISSN 1634-1678

Print : French: 4500 expl. - English : 5000 expl. - Spanish : 2000 expl.
Photographs : Danone Vitapole, ©Patrick Shendell O'Caroll / PhotoAlto PhotoDisk / Production : ID Source + 33 1 64 80 19 64

Subscription form: to be returned to the above address

You may photocopy this form, and if needed several times, for several persons to subscribe.

☐ I would like to subscribe free of charge to Nutritopics Text in ☐ French ☐ English ☐ Spanish
☐ I would like to correct my address

☐ Mr ☐ Mrs ☐ Miss ☐ Dr ☐ Pr

Name

First Name

Company / University

Service / Department

Address

Zip Code - City

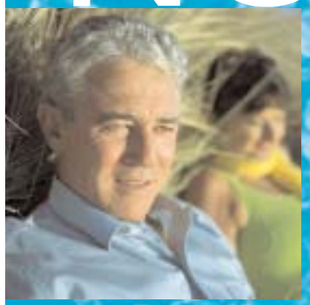
Country E-mail

This information is required to enable Danone Vitapole to send you the "Danone Nutritopics" journal. The e-mail address is optional. This information will under no circumstances be given to any third parties within or outside Groupe DANONE, for reasons other than sending Danone Vitapole scientific publications.
Under the terms of the French Data Protection Act no. 78-17 of 6 January 1978 concerning information technology, data files and freedom, you have the right to access and correction of the information concerning you. To exercise your rights, please contact us at the address above.

MARCH 2003

DANONE

NUTRI



DANONE Danone Vitapole