# Anticancer properties of bovine milk

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Improved means of cancer prevention and treatment remain key goals of global health programmes. This is particularly true in Western society, where the elderly represent a large proportion of the population, and where the likelihood of tumour development is compounded by risk factors such as poor fibre/high fat diets and environmental pollution. Dietary intervention represents an attractive, non-invasive means of providing anticancer preventative and therapeutic benefits to at-risk individuals. This review focuses on the evidence for anticancer properties of bovine milk and milk-derived components. Evidence of a role for whole milk constituents, as well as purified minor components, in combating tumorigenesis is outlined. Shortcomings in current studies are highlighted, and future opportunities for targeted research to characterize important anticancer properties of milk are discussed.

**Milk: Cancer prevention: Whey protein: CLA**

## Introduction

The global incidence of cancer represents a significant challenge to modern medicine and health care. In Western society, disproportionately high incidences of certain forms of cancer indicate a link to life-style as a significant contributor toward an increased risk of tumorigenesis. High dietary fat intake, and low or poor fibre and micronutrient intakes, have been identified as increased risk factors for certain forms of cancer (e.g. colorectal cancer), and the increasing environmental burden imposed by an expanding population may also predispose at-risk individuals to a greater risk of neoplasm development through exposure to pollution (Willett, 1989).

Although improved means of early diagnosis represent a large proportion of research effort in combating cancer, major emphasis is also being placed on non-invasive means of prevention and treatment of disease. In particular, just as poor nutrition can contribute toward an increased risk of cancer, so 'good' nutrition can offer a degree of protection. Nutritional studies, reports and trials to identify anticancer properties of foods have been numerous and extensive, and although there is controversy over which individual foodstuffs may offer the most relative benefit to consumers, it is generally agreed that diets high in grains, green vegetables, fresh fruit and fibre, and low in total and saturated fats, are beneficial to health (Anon, 1997).

Relatively less emphasis has been placed on determining and characterising the beneficial effects of a single food source in detail. In the case of bovine milk, this deficit has arguably been influenced by scientific perceptions that a foodstuff containing saturated fat is unlikely to be beneficial to health. Yet evidence has been accumulating over the last two decades to indicate that the form of dietary fat is very important in determining the relative cancer risk, and overall milk-derived fats may offer significant benefits compared to other common sources of dietary fat (reviewed in detail by Parodi, 1996). Furthermore, this has led to investigation of individual components of bovine milk that have anticancer properties. Several studies have characterised the anticancer properties of synthetic forms of molecules that are known to occur in milk (e.g. CLA), and (by association) have extrapolated these results back to milk. This review will, where possible, focus on the direct evidence for important anticancer properties of milk-derived molecules.

## Evidence for anticancer properties of whole and modified milk: clinical and laboratory studies

In Western societies, environmental factors (including diet) have been suggested to impact significantly on the incidence of cancer in a population (Willett, 1989; Doll, 1992). However, in human clinical research, there have been only a few cross-sectional and cohort studies which have dealt specifically with the potentially beneficial anticancer effects of whole milk or modified milk products. These studies have produced conflicting results, with evidence both for and against a role of milk in tumour prevention. In an epidemiological study, Knekt *et al.* (1996) found that there was a significant inverse correlation between milk intake and the incidence of breast cancer among initially cancer-free women during a 25 year follow-up period. Analysing

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case-controlled and cohort data from several countries, Meera (1998) found no significant association between intake of dairy foods and cancers of the breast, lung, ovary, pancreas or bladder, but a weak positive association between milk intake and the incidence of prostate cancer. Conversely, some case-controlled studies such as those by Ewertz & Gill (1990) and Mettlin *et al.* (1990) have suggested a positive association between milk intake and several cancer types. Overall, however, the issue of whether there is a causal relationship between milk intake and cancer remains unclear, and controlled, properly designed clinical trials are needed to address this question fully.

In contrast to the lack of controlled clinical trials, there have been several studies that have utilised rodent models of tumorigenesis to evaluate potentially protective anti-cancer properties of milk. Klurfeld *et al.* (1983*a*) found that feeding skim milk powder to rats reduced tumour formation in the colon and mammary glands, when compared to rats fed on casein and sucrose. Nelson *et al.* (1987) also found that milk supplementation provided protection against some aspects of carcinogenesis of the colon. Using the chemical carcinogen DMH (dimethylhydrazine) to induce intestinal tumour development, Abdelali *et al.* (1995) found that rats which drank skim milk had approximately half as many aberrant crypts (a neoplastic precursor) per colon as rats which drank an equivalent volume of plain water. However, in contrast to those results, Yanagi *et al.* (1994) showed that DMH-treated mice that were given commercially available whole milk had a higher incidence of mammary tumours than mice given plain water as a liquid source.

## Evidence for anticancer properties of milk fat and milk fat components

Studies have suggested that overall fat intake may play an important role in cancer promotion, however the form of dietary fat is thought to be one of the most important issues in this debate (Carroll, 1991). Experiments using animal models have indicated that saturated animal fats and polyunsaturated fish oils do not promote cancer as effectively as certain polyunsaturated vegetable oils, whereas data gathered from epidemiological studies have shown that total dietary fat correlates with cancer incidence and mortality at least as well as does any particular type of fat (Carroll, 1991). The bulk of scientific evidence now suggests that the risk of cancer can be reduced by a consistently low fat diet (Carroll, 1991).

Dairy fat is highly saturated, and since it is one of the major fat sources in the typical Western diet (Kesteloot *et al.* 1991) it is important to determine the effects on cancer formation and maintenance. Some animal studies have investigated the effects of dairy fats, compared to other dietary fats, in promoting or controlling tumour development. Klurfeld *et al.* (1983*b*) used DMH to induce colon tumour formation in mice, and observed that animals fed butter had a lower tumour incidence than those fed other fat sources. Yanagi *et al.* (1994) further showed that whereas dietary vegetable oil (margarine) caused a dose-dependent increase in the incidence and overall numbers of chemical-induced mammary tumours in rats, butter caused no such effect. Cope & Reeve (1994) and Cope *et al.* (1995) found that UV-induced skin tumours occurred less frequently in butter- and milk fat-fed mice than in mice fed on vegetable oil-based margarine, which is rich in the potential tumour-promoting molecule linoleic acid. Therefore, although total dietary fat is undeniably a risk factor for tumorigenesis, animal studies have clearly shown that the type of dietary fat plays an important role, and that animals fed milk fat are likely to develop fewer tumours than those fed other dietary sources. However, these results could be interpreted in two ways: either milk fat is actively protecting against cancer development, or else it is the least harmful fat in that it does not exacerbate cancer as much as other dietary fats.

Milk fat contains a number of individual components that can be described as having anticarcinogenic properties. In particular, conjugated linoleic acid (CLA) and sphingomyelin have been suggested to have important anticancer properties (Ip *et al.* 1991, 1994, 1996; Dillehay *et al.* 1994). Though much work has been done in this area, few studies have used milk-derived components to investigate anti-cancer properties, and have instead used synthetic analogues that are easier to obtain, purify and standardise.

Conjugated linoleic acid (CLA) is a collective term for one or more isomers of unsaturated linoleic acid. While linoleic acid (which is present in vegetable fats such as margarine) has been shown to induce a higher-than-average cancer rate, CLA has the opposite effect and is a potent anticarcinogen (Ip & Scimeca, 1997). Dairy fat contains the highest natural level of CLA among dietary sources, although this can vary seasonally and regionally depending on the pasture content on which dairy cows are grazed (reviewed by Parodi, 1994).

Synthetic CLA has been shown to be effective *in vitro* in inhibiting the growth of a variety of human tumour cell lines, including melanoma, colorectal, breast and lung cells (Ip *et al.* 1994). In its synthetic form, dietary CLA has also been found to protect against chemically-induced tumour development at a number of sites in animal models (Ip *et al.* 1991, 1994, 1996). Although there is still little evidence that CLA derived from bovine milk is anticarcinogenic, the *cis*-9, *trans*-11-octadecadienoic acid isoform predominant in milk is known to be biologically active (reviewed by Parodi, 1999). Given the important impact of synthetic forms of CLA on tumorigenesis, there is a great need for the efficacy of milk-derived CLA against cancer to be evaluated in human clinical trials.

Sphingomyelin is a milk fat phospholipid, a normal component of the outer membrane of mammalian cells. It has been found to have anticancer properties primarily through its biologically active metabolites: ceramide and sphingosine (Hannun, 1994). Sphingomyelin and its metabolites mediate important transmembrane signalling mechanisms that can control cell proliferation and differentiation. In addition they can regulate the apoptotic signals generated by endogenous cytotoxic factors, such as tumour necrosis factor and interferons. Sphingomyelin thus has the potential to affect important signals in tumour biology, such as cell division and apoptosis (Merrill, 1991).

In milk, sphingomyelin can account for approximately one-third of total milk phospholipids, and during processes such as homogenisation and skimming, these can re-locate

from milk fat globules to enter the aqueous phase, thus increasing bioavailability to consumers (Parodi, 1997). Dillehay *et al.* (1994) used sphingomyelin isolated from low-fat milk to test anticancer properties against chemical-induced carcinoma in mice, and found that mice fed on milk-derived sphingomyelin had half the tumour incidence of control mice. Schmelz *et al.* (1997) also supplemented the diet of mice with sphingomyelin isolated from milk and found a reduction in the number of aberrant crypt foci (an early indicator of the development of colonic tumours) as well as the appearance of colonic adenocarcinoma induced by DMH. In addition, synthetically prepared sphingomyelin has also been found to suppress aberrant crypt foci, establishing that it is indeed sphingomyelin causing tumour suppression and not merely a possible contaminant of the naturally occurring sphingomyelin (Schmelz *et al.* 1997).

## Evidence for anticancer properties of milk protein and protein components

Although total intake of dietary protein may be a risk factor for cancer, the type of protein may be the biggest factor in determining any potential anticancer properties of milk (Parodi, 1998). Investigations have been made into the effects of different dietary proteins on tumour incidence in rodents. McIntosh *et al.* (1995) demonstrated a protective role for dietary dairy proteins against tumour development, showing that dietary whey protein and casein were more protective against the development of intestinal cancers in rats than was red meat or soy bean protein. They concluded that dietary proteins differ in their ability to protect against cancer development and that the proteins in dairy foods, particularly the whey proteins, appear to play a significant role in cancer prevention (McIntosh *et al.* 1995).

Casein is the major protein in skim milk powder and can display comparative anticancer activity. McIntosh *et al.* (1995) found that rats fed on casein had a reduced incidence of DMH-induced colorectal cancer in comparison to rats fed on other dietary sources of protein, although the reduction in DMH-induced colon tumours in comparison to rats fed on red meat or soybean protein was not significant. In comparison, Pence *et al.* (1995) found that rats fed on casein as a major dietary protein did have a significantly lower incidence of DMH-induced colon cancer compared to rats fed on cooked lean beef.

Whole whey protein has a demonstrated ability to protect against chemically induced carcinogenesis in animal models. Papenburg *et al.* (1990) induced colon carcinomas in mice with DMH and found that the incidence of tumours in whey protein-fed mice was significantly less compared to casein- and mouse chow-fed groups. In a 1995 report, McIntosh *et al.* investigated the effects of dietary whey protein on the incidence of DMH-induced tumours in rats, and the effect of different protein diets on antioxidant status and faecal fat. They found that rats fed whey protein had the highest antioxidant status and the lowest content of faecal fat. Low antioxidant status and high levels of fat in the faeces are thought to represent risk factors for colon tumorigenesis (Samelson *et al.* 1985; Potter, 1996).

In addition to research employing whole whey proteins, some studies have looked at individual whey proteins for their potential anticancer properties. Lactoferrin is an iron-binding minor glycoprotein present in bovine milk. A number of physiological roles have been suggested for lactoferrin (Lonnerdal & Iyer, 1995), but it is likely to be the iron-binding properties that contribute to anticancer properties of this whey protein, since free iron may act as a mutagenic promoter by inducing oxidative damage to nucleic acid structure (Weinberg, 1996). It is thought that lactoferrin may bind iron locally in tissues, therefore reducing the risk of oxidant-induced carcinogenesis. Sekine *et al.*(1997) found that rats fed a basal diet supplemented with 0·2 or 2 % of bovine lactoferrin had incidences of carcinogen-induced colon adenocarcinoma of 25 % and 15 %, respectively, in contrast to 57 % for animals fed a basal (non-supplemented) diet. In contrast, iron-saturated lactoferrin was non-protective. Similarly, Tsuda *et al.* (1998) looked at the influence of bovine lactoferrin on colon carcinogenesis in rats exposed to carcinogens. The incidences of colon adenocarcinomas in rats receiving lactoferrin were significantly lower than in control groups, and there were also fewer aberrant crypt foci. Smithers *et al.* (1998) showed that rats fed a soyabean-based diet supplemented with lactoferrin or with β-lactoglobulin (another whey protein) had significantly fewer aberrant colonic crypt cells (cancer precursors) than animals which consumed the parent (non-supplemented) diet. In addition to its effect in dietary inclusion, there is some evidence that lactoferrin administered by a parenteral route may have important anticancer properties. Yoo *et al.* (1997) demonstrated that subcutaneous administration of lactoferrin in its iron-free form could inhibit the metastasis of primary tumours in cancer-bearing mice.

Bovine serum albumin (BSA) is another whey protein which may have anticancer properties. BSA has been shown to inhibit growth of the human breast cancer cell line MCF-7 cell line, when included in *in vitro* cell culture with tumour cells (Laursen *et al.* 1990). BSA has also been reported to exhibit strong antiproliferative effects against a Chinese hamster epithelial cell line *in vitro*, although the mechanisms for this inhibition remain unclear (Bosselaers *et al.* 1994).

Mammary-derived growth inhibitor (MDGI) is a fatty acid-binding protein present in bovine whey in trace levels. MDGI has been shown to inhibit the proliferation of bovine and murine epithelial cell lines *in vitro* (Zavizion *et al.* 1993; Yang *et al.* 1994), and may therefore play a role in limiting early formation of neoplasms in the intestinal epithelium. However, this remains to be determined experimentally, since MDGI and other low-molecular-weight components are notoriously difficult to isolate and purify from bovine milk.

## Milk calcium

Experimental studies suggest that a high dietary calcium intake is associated with a decreased risk of colorectal cancer (Wargovich *et al.* 1991). Although the mechanisms by which dietary calcium might reduce the risk of carcinogenesis are unknown, one proposed mechanism is that calcium might bind and solubilise potentially oxidising fatty acids and bile acids in the intestinal lumen, thus

preventing their direct contact with epithelial cells (Parodi, 1998). In rats, bile acid-induced damage to colonocytes can be reduced by milk calcium phosphate, whereas human milk calcium has been shown to effectively precipitate cytotoxic colonic surfactants and thus inhibit colonic cytotoxicity (van der Meer *et al.* 1998).

Pence *et al.* (1996) investigated the efficacy of two sources of dietary calcium in inhibiting carcinogen-induced colon tumorigenesis in rats. They looked at elemental calcium (in the form of $CaCO_3$) and dairy calcium (as non-fat dried milk), both fed to rats as supplements to either high or low levels of total dietary fat. No difference in tumour incidence was found between the two calcium sources, but a significant interaction was found between dietary fat and calcium, with the lowest tumour incidence seen in the dried milk/high fat diet. It is thus suggested that the source of calcium is not critical, but that calcium may have a significant impact on tumorigenesis among those with a high total dietary fat intake (Pence *et al.* 1996).

## Anticancer mechanisms of milk components (both direct and indirect)

As previously outlined, several mineral- and pro-oxidant-binding components in milk are thought to provide important protection to the gastrointestinal tract epithelium against potentially mutagenic oxidising agents (Weinberg, 1996; van der Meer *et al.* 1998). In addition, it has been suggested that antioxidant vitamins and provitamins in milk, such as β-carotene and vitamins A and D, may perform a role in limiting the action of oxidising agents in the gut (Newmark & Lipkin, 1992). Since tumour growth is dependent on accelerated or uncontrolled cellular proliferation, growth inhibitors and antiproliferative molecules in milk may play an important protective role against cancer. Examples of this include MDGI (Yang *et al.* 1994), as well as antiproliferative cytokines such as the transforming growth factor-beta (TGFβ) analogue known as milk-derived growth factor, which is present in bovine milk in trace levels but which displays potent biological activity in the nanomolar range (Stoeck *et al.* 1989). These and other endogenous hormones, present in trace levels in milk and milk fat globule membranes, may perform other mediating protective roles against tumour development, for example binding potentially tumour-promoting oxidised fatty acids, and mediating transmembrane cellular signals such as apoptosis and cellular differentiation (Gorewit & Spitsberg, 1998).

Possibly the most well-documented anticancer mechanism has been outlined in a model proposed by Bounous and colleagues. Bounous *et al.* (1991) demonstrated that dietary whey protein concentrate (WPC) has potent anticancer properties against colon cancer induced by DMH in mice, and there is also some evidence from controlled clinical studies of the effectiveness of WPC in limiting metastasis during anticancer therapy (Kennedy *et al.* 1995). The authors have suggested that the protective efficacy of dietary WPC could be due to whey proteins enhancing tissue glutathione concentration, since whey protein is known to be rich in substrates for glutathione synthesis (Parodi, 1998). The presence of high levels of glutathione in tissues has been suggested to suppress tumour development at various sites in the body, possibly by reducing free radical- and oxidant-induced damage to chromosomal DNA (Cole & Ketterer, 1990). Furthermore, glutathione transferase enzymes catalyse the conjugation of potentially damaging chemical mutagens and carcinogens, which can then be eliminated from the body (reviewed by Parodi, 1998). Bounous *et al.* (1989) reported that mice fed on diets containing WPC as a protein source had higher liver and heart tissue levels of glutathione than mice fed on diets containing casein or mouse chow. Furthermore, McIntosh *et al.* (1995) measured tissue glutathione levels in their study on the inhibitory effect of WPC on colon tumorigenesis. In Sprague–Dawley rats, liver glutathione concentration was highest in WPC- and casein-fed rats and lowest in soybean protein-fed rats. These studies support, indirectly, a role for whey proteins in enhancing tissue glutathione levels and thus providing a degree of protection against tumour development.

## Some concerns over the demonstration of anticancer properties of milk, and future prospects

There is now a substantial body of evidence to suggest that bovine milk contains major and minor components which have anticancer properties (reviewed by Parodi, 1994, 1996, 1998, 1999). The majority of reports which have characterised milk-derived anticancer activity have come from *in vitro* studies using tumour cell lines, or *in vivo* studies using animal models of tumorigenesis. Although both approaches provide valuable evidence as to the potential anticancer actions of milk-derived molecules, caution should be taken when extrapolating results from such studies to statements on disease protection in humans. In the case of *in vitro* studies, the demonstration of an anticancer effect should be taken to imply that the component under test has the *potential* to regress tumour development (not initiation), and moreover any given biological effect of a component *in vitro* must be assessed in light of its perceived *in vivo* performance in the gastrointestinal tract. This is particularly important with respect to human intestinal physiology: many potentially beneficial molecules in milk may be rendered inactive and/or remain unabsorbed in the human digestive tract, following gastric processing. The same cautions apply to *in vivo* studies of tumorigenesis in animal models, where the rodent gastrointestinal system may well respond to anticancer factors in a different way to the human digestive system.

The majority of *in vivo* studies that have utilised rodent models of tumorigenesis have studied the anticancer effects of milk or milk components against potent chemical carcinogens, or in some cases radiation. It must be borne in mind that neoplasms have many different aetiologies, both environmentally induced and genetically predetermined, and it is probable that the benefits of any given anticancer effect will be limited to the particular type of cancer under study, and to cancers initiated by the particular type of causative agent being used, unless otherwise proven. In addition, care should be taken when interpreting the ability of milk-derived components to exert

beneficial effects on secondary anticancer mechanisms. For example, the ability of milk-derived proteins and peptides to affect the functioning of the cellular immune system is well-documented (see articles by Cross & Gill, and Gill *et al.* this supplement), and may imply an enhancement of effective antitumour immunity; however, an enhancement of cellular immunity by milk-derived components which then exerts anticancer benefits remains an unproven theory.

Overall, there have been remarkably few well-designed clinical trials to determine conclusively the effects of milk-derived anticancer agents. Although such studies are notoriously difficult to conduct and interpret, the proven efficacy of some milk-derived components (e.g. CLA and lactoferrin) in animal models should merit their trialling under controlled clinical conditions. In a similar fashion, several other minor constituents of milk, especially growth and inhibitory factors, have shown promising action in *in vitro* or laboratory animal studies, but remain untested in humans. Improvements in milk fractionation technology should facilitate the identification of further minor constituents of milk, particularly low-molecular-weight proteins and peptides, which may have important anti-cancer properties. Such technology has been successfully employed in identifying potent immunomodulatory molecules from milk (Stoeck *et al.* 1989), and there is no reason to suppose that the same technology cannot be applied to the identification and purification of small molecular weight molecules for the appraisal of their anticancer properties.

## References


Abdelali H, Cassand P, Soussotte V, Daubeze M, Bouley C & Narbonne JF (1995) Effect of dairy products on initiation of precursor lesions of colon cancer in rats. *Nutrition and Cancer* **24**, 121–132.

Anon. (1997) *Food, Nutrition and Cancer: A Global Perspective.* World Cancer Research Fund publication. Washington DC: American Institute for Cancer Research.

Bosselaers IEM, Caessens PWJ, van Bokel MAJ & Alink GM (1994) Differential effects of milk proteins, BSA and soy protein on 4NQQ- or MNNG-induced SCEs in V79 cells. *Food and Chemical Toxicology* **32**, 905–909.

Bounous G, Batist G & Gold P (1989) Immunoenhancing property of dietary whey protein in mice: role of glutathione. *Clinical and Investigative Medicine* **12**, 154–161.

Bounous G, Batist G & Gold P (1991) Whey proteins in cancer prevention. *Cancer Letters* **57**, 91–94.

Carroll KK (1991) Dietary fats and cancer. *American Journal of Clinical Nutrition* **53**, 1064S–1067S.

Cole B & Ketterer B (1990) The role of glutathione and glutathione transferases in chemical carcinogenesis. *Critical Reviews in Biochemistry and Molecular Biology* **25**, 47–70.

Cope RB & Reeve VE (1994) Modification of 7,12-dimethylbenzanthracene (DMBA) ultraviolet radiation (UVR) co-carcinogenesis, UVR carcinogensis and immune suppression due to UVR anc *cis*-urocanic acid by dietary fats. *Photochemistry and Photobiology* **59**, 24S.

Cope RB, Reeve VE, Bosnic M & Boehm-Wilcox C (1995) Protection from photocarcinogenesis by dietary fats and involvement of eicosanoids in their modulating effect on photoimmunosuppression. *Photochemistry and Photobiology* **61**, 103S.

Dillehay DL, Webb SK, Schmelz E & Merrill AH Jr (1994) Dietary sphingomyelin inhibits 1,2-dimethylhydrazine-induced colon cancer in CF1 mice. *Journal of Nutrition* **124**, 615–620.

Doll R (1992) The lessons of life: keynote address to the nutrition and cancer conference. *Cancer Research* **53**, 2024S–2029S.

Ewertz M & Gill C (1990) Dietary factors and breast-cancer risk in Denmark. *International Journal of Cancer* **46**, 770–784.

Gorewit RC & Spitsberg VL (1998) Anti-cancer properties of proteins in the milk fat globule membranes in whey. In *Proceedings of the Second International Whey Conference*. International Dairy Federation Publication S.I. 9804, pp. 315–325. Brussels, Belgium: IDF Publications

Hannun YA (1994) The sphingomyelin cycle and the second message function of ceramide. *Journal of Biological Chemistry* **269**, 3125–3128.

Ip C, Briggs SP, Haegele AD, Thompson HJ, Storkson J & Scimeca JA (1996) The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. *Carcinogenesis* **17**, 1045–1050.

Ip C, Chin SF, Scimeca JA & Pariza MW (1991) Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Research* **51**, 6118–6124.

Ip C, Singh M, Thompson HJ & Scimeca JA (1994) Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Research* **54**, 1212–1215.

Ip C & Scimeca JA (1997) Conjugated linoleic acid and linoleic acid are distinctive modulators of mammary carcinogenesis. *Nutrition and Cancer* **27**, 131–135.

Kennedy RS, Konok GP, Bounous G, Baruchel S & Lee TDG (1995) The use of whey protein concentrate in the treatment of patients with metastatic carcinoma: a phase I-II clinical study. *Anticancer Research* **15**, 2643–2649.

Kesteloot H, Lesaffre E & Joossens JV (1991) Dairy fat, saturated animal fat, and cancer risk. *Preventative Medicine* **20**, 226–236.

Klurfeld DM, Weber MM & Kritchevsky D (1983*a*) Comparison of semi-purified and skim milk protein containing diets on DMBA-induced breast cancer in rats. *Kiel Milchwirtschaft Forschun* **35**, 421–422.

Klurfeld DM, Weber MM & Kritchevsky D (1983*b*) Inhibition of chemically induced colon or breast cancer by milk fat and milk solids. *Federation Proceedings* **42**, 802.

Knekt P, Jarvinen R, Pukkala E & Aromaa A (1996) Intake of dairy products and the risk of breast cancer. *British Journal of Cancer* **73**, 687–691.

Laursen I, Briand P & Lykkesfeldt AE (1990) Serum albumin as a modulator of the human breast cancer cell line MCF-7. *Anticancer Research* **10**, 343–352.

Lonnerdal B & Iyer S (1995) Lactoferrin: molecular structure and biological function. *Annual Review of Nutrition* **15**, 93–110.

McIntosh GH, Regester GO, Le Leu RK, Royle PJ & Smithers GW (1995) Dairy proteins protect against dimethylhydrazine-induced intestinal cancers in rats. *Journal of Nutrition* **125**, 809–816.

Meera J (1998) Dairy foods, dairy fats, and cancer, a review of epidemiological evidence. *Nutrition Research* **18**, 905–937.

Merrill AH (1991) Cell regulation by sphingosine and more complex sphingolipids. *Journal of Bioenergetics and Biomembranes* **23**, 386–391.

Mettlin CJ, Schoenfeld ER & Natarajan N (1990) Patterns of milk consumption and risk of cancer. *Nutrition and Cancer* **13**, 89–99.

Nelson RL, Tanure JC & Andrianopoulos G (1987) The effect of dietary milk and calcium on experimental colorectal carcinogenesis. *Diseases of the Colon and Rectum* **30**, 947–949.

Newmark HL & Lipkin M (1992) Calcium, vitamin D and colon cancer. *Cancer Research* **52**, 2067–2070.
Papenburg R, Bounous G, Fleiszer D & Gold P (1990) Dietary milk proteins inhibit the development of dimethylhydrazine-induced malignancy. *Tumour Biology* **11**, 129–136.
Parodi PW (1994) Conjugated linoleic acid: an anticarcinogenic fatty acid present in milk fat. *Australian Journal of Dairy Technology* **49**, 93–97.
Parodi PW (1996) Milk fat components: possible chemopreventive agents for cancer and other diseases. *Australian Journal of Dairy Technology* **51**, 24–32.
Parodi PW (1997) Cows' milk fat components as potential anticarcinogenic agents. *Journal of Nutrition* **127**, 1055–1060.
Parodi PW (1998) A role for milk proteins in cancer prevention. *Australian Journal of Dairy Technology* **53**, 37–47.
Parodi PW (1999) Conjugated linoleic acid and other anti-carcinogenic agents of bovine milk fat. *Journal of Dairy Science* **82**, 1339–1349.
Pence BC, Butler MJ, Dunn DM, Miller DF, Zhao C & Landers M (1995) Non-promoting effects of lean beef in the rat colon carcinogenesis model. *Carcinogenesis* **16**, 1157–1160.
Pence BC, Dunn DM, Zhao C, Patel V, Hunter S & Landers M (1996) Protective effects of calcium from non-fat dried milk against colon carcinogenesis in rats. *Nutrition and Cancer* **25**, 35–45.
Potter JD (1996) Nutrition and colorectal cancer. *Cancer Causes and Control* **7**, 127–146.
Samelson SL, Nelson RL & Nyhus LM (1985) Protective role of faecal pH in experimental colon carcinogenesis. *Journal of the Royal Society of Medicine* **78**, 230–233.
Schmelz EM, Bushnev AS, Dillehay DL, Liotta DC & Merrill AH Jr (1997) Suppression of aberrant colonic crypt foci by synthetic sphingomyelins with saturated or unsaturated sphingoid base backbones. *Nutrition and Cancer* **28**, 81–85.
Sekine K, Watanabe E, Nakamura J, Takasuka N, Kim DJ, Asamoto M, Krutovskikh V, Bab-Toriyama H, Ota T, Moore M, Masuda M, Sugimoto H, Nishino H, Kakizoe T & Tsuda H (1997) Inhibition of azoxymethane-initiated colon tumor by bovine lactoferrin administration in F344 rats. *Japanese Journal of Cancer Research* **88**, 523–526.
Smithers GW, McIntosh GH, Regester GO, Johnson MA, Royle PJ, Le Leu RK & Jelen, P (1998) Anti-cancer effects of dietary whey proteins. In *Proceedings of the Second International Whey Conference*. International Dairy Federation Publication S.I. 9804, pp. 306–309. Brussels, Belgium: IDF Publications
Stoeck M, Ruegg C, Miescher S, Carrel S, Cox D, Fliedner V, Alkan S & Von-Fliedner V (1989) Comparison of the immunosuppressive properties of milk growth factor and transforming growth factors beta1 and beta2. *Journal of Immunology* **143**, 3258–3265.
Tsuda H, Sekine K, Nakamura J, Ushida Y, Kuhara T, Takasuka N, Kim DM, Asamoto M, Baba-Toriyama H, Moore MA, Nishimo H & Kakizoe T (1998) Inhibition of azoxymethane initiated colon tumour and aberrant crypt foci development by bovine lactoferrin administration in F344 rats. *Advances in Experimental Medicine and Biology* **443**, 273–284.
Van der Meer R, Bovee-Oudenhoven IMJ, Sesink ALA & Kleibeuker JH (1998) Milk products and intestinal health. *International Dairy Journal* **8**, 163–170.
Wargovich MJ, Lynch PM & Levin B (1991) Modulating effects of calcium in animal models of colon carcinogenesis and short term studies in subjects at increased risk of colon cancer. *American Journal of Clinical Nutrition* **54**, 2025–2055.
Weinberg ED (1996) The role of iron in cancer. *European Journal of Cancer Prevention* **5**, 19–36.
Willett WC (1989) The search for the causes of breast and colon cancer. *Nature* **338**, 389–394.
Yanagi S, Yamashita M, Ogoshi K & Imai S (1994) Comparative effects of milk, yogurt, butter and margarine on mammary tumourigenesis induced by 7, 12-dimethylbenz(a)anthracene in rats. *Cancer Detection and Prevention* **18**, 415–420.
Yang Y, Spitzer E, Kenney N, Zschiesche W, Li M, Kromminga A, Muller T, Spener F, Lezius A, Veerkamp JH, Smith GH, Salomon DS & Grosse R (1994) Members of the fatty-acid binding protein family are differentiation factors for the mammary gland. *Journal of Cell Biology* **127**, 1097–1109.
Yoo YC, Watanabe S, Watanabe R, Hata K, Shimazaki KI & Azuma I (1997) Bovine lactoferrin and lactoferricin inhibit tumour metastasis in mice. *Advances in Experimental Medicine and Biology* **443**, 285–291.
Zavizion B, Politis I, Gorewit RC, Turner JD, Spitzer E & Grosse R (1993) Effect of mammary-derived growth inhibitor on proliferation of MAC-T bovine mammary epithelial cells. *Journal of Dairy Science* **76**, 3721–3726.