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Review Letter

# Whey proteins in cancer prevention

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**Summary**

*Epidemiological and experimental studies suggest that dietary milk products may exert an inhibitory effect on the development of several types of tumors. Some recent experiments in rodents indicate that the antitumor activity of the dairy products is in the protein fraction and more specifically in the whey protein component of milk. We and others have demonstrated that whey protein diets result in increased glutathione (GSH) concentration in a number of tissues, and that some of the beneficial effects of whey protein intake are abrogated by inhibition of GSH synthesis. Whey protein is particularly rich in substrates for GSH synthesis. We suggest that whey protein may be exerting its effect on carcinogenesis by enhancing GSH concentration.*

**Keywords:** cancer; prevention; diet; whey proteins

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Experimental and epidemiological studies on the effect of food on cancer development, have focused mostly on the role of dietary fibre and fat, while little is known about the influence of protein intake on carcinogenesis. Studies have focused on the quantity of protein and its amino acid supply rather than its source [32]. Only a few data are available on the effect of protein type in nutritionally adequate and similar diets on the development of tumors. A retrospective analysis of several studies on dairy products and tumor development and recent data on milk protein and cancer, suggest an interesting role for dietary milk proteins in cancer prevention.

Jacquet et al. [20] reported that feeding milk, retarded on average by a factor of 0.4, tumor growth in rats implanted with epithelioma T8. This is consistent with some epidemiological studies showing that consumption of milk or dairy products may reduce the risk of cancer [18,19]. In mice inoculated with Ehrlich ascites tumor cells, feeding with yogurt reduced the number of tumor cells by a factor of 0.2—0.28 [29]. It was also reported that mice fed a milk protein formula diet, exhibited inhibition of tumor volume by a factor of 0.2—0.7, following s.c. injection of DMH-induced colon tumor cells in comparison to mice fed other types of protein [24]. A comparable degree of tumor inhibition was noted in milk protein fed mice

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injected s.c. with herpes virus transformed cells [16]. Various types of cheeses and yogurt were recently found to suppress the growth of several experimental tumors in mice in proportion to the duration of feeding. The tumor size was reduced by a factor of 0.17—0.70 depending on the type of tumor [31]. In spite of variations in the type of tumor and in the control diets used in all these studies it is apparent that the level of tumor inhibition reported with dairy product feeding is comparable to that which we obtained with a formula diet containing casein as protein source [25]. Our findings show that in mice fed a casein diet the number and size of dimethylhydrazine (DMH) induced colon carcinomas were reduced by a factor of 0.3 and 0.4 respectively in comparison to Purina fed controls. However, in mice fed a comparable formula diet with similar nutritional efficiency but containing whey protein concentrate prepared under careful and appropriate condition, the number and size of DMH-induced colon carcinomas were reduced four fold in comparison to the Purina fed controls [25]. DMH-induced colon tumors appear to be similar to those found in humans as far as type of lesions and chemotherapeutic response characteristics are concerned [12,14]. The superiority of the anticancer effect of whey protein in comparison to casein has been reported in our previous study [10]. About 80% of the proteins in bovine milk are caseins and the remaining 20% are whey proteins [30,33]. In addition, using the traditional process of preparing casein, the amount of whey protein co-precipitated along with the casein varies from about 40 to 60% of the total amount of whey protein present in the milk [21]. Therefore it is conceivable that the minor anticancer effect seen with casein could be due to the relatively (to caseins) small amount of whey protein co-precipitated with it. It is apparent from the above described studies that the antitumor activity of the dairy products is in the protein fraction and more specifically, in the whey protein component of milk. This conclusion is substantiated by the studies of Birt et al. on the longevity of Syrian hamsters. Whereas carcass nitrogen and serum protein were not influenced by the diets, hamsters of both sexes, fed a whey protein diet since the age of 4 weeks lived longer irrespective of the whey protein levels in the diet. In males, survival increased by 50% when comparing the commercial ration with the 20% whey protein diet [3]. In other experiments, the same authors compared the survival of hamsters fed either a whey protein diet or a corresponding cysteine-enriched casein diet. After 20 weeks of feeding, survival was best in female and male hamsters fed the whey protein diets in comparison to those fed the nutritionally equivalent cysteine enriched casein diets [2]. This type of hamster from the Eppley Institute colony develops spontaneous tumors in different organs, particularly malignant lymphomas [26], cancer of the urogenital system [27], endocrine system [27] and digestive system [28]. This is probably an anti-carcinogenic effect.

These experimental data appear to be consistent with a recent epidemiological study in which reduced risks for frequent consumption of 2% milk relative to not drinking milk were observed for cancer of the oral cavity, stomach, rectum, lung and cervix [23].

The mechanism of cancer prevention by whey protein feeding may be related to some recent findings on the biological activity of dietary whey proteins. Our studies have demonstrated that whey protein feeding specifically enhances mouse immune response to sheep red blood cells [4—9] and resistance to pneumoccocal infection [7].

Our studies have shown that the observed enhancement of the immune response is associated with greater production of splenic glutathione in immunized mice fed whey protein concentrate in comparison to mice fed casein or cysteine-enriched casein in similar dietary concentration [4].

It was then theorized that this might reflect the ability of the lymphocytes of whey protein diet-fed mice to offset potential oxidative damage, thus responding more fully to the antigenic challenge [15]. In fact our studies showed that administration of L-buthionine-sulfoximine, which reduces splenic glutathione in half, significantly reduced the humoral immune

response of whey protein-fed mice. This was taken as further evidence for the important role of glutathione in the immunoenhancing effect of dietary whey protein [4]. Glutathione was found at higher levels in the heart and liver of whey protein fed to old mice in comparison with mice fed the corresponding casein diet, or Purina Mouse Chow [5].

The factors and mechanisms of the observed effect of some whey proteins on glutathione formation is a matter of interest. Tissue glutathione (L-gamma-glutamyl-L-cysteinylglycine) was found to increase following administration of gamma-glutamylcysteine [1]. Indeed, whey protein concentrate from bovine milk has a very high cysteine concentration and contains substantial amounts of glutamylcysteine groups, unlike casein, which does not increase tissue glutathione when fed to mice [4—9]. The glutamylcysteine groups, extremely rare in animal and plant edible proteins, are located primarily in the serum albumin fraction [13]. It is our hypothesis that the glutathione promoting activity of dietary whey protein concentrate is dependent on the glutamylcysteine groups contained in serum albumin fractions, in the $\beta$-lactoglobulin and possibly in the immunoglobulin G fraction. Our studies showed that in order to maintain its glutathione enhancing property, whey protein concentrate must be appropriately prepared to allow the release of the glutamylcysteine groups, during the digestive process [6].

In discussing the effects of milk proteins on tumors it is important to distinguish between anti-tumor effects such as those cited in experiments with implanted tumors [16,20,29,31] and the anti-carcinogenesis effects cited elsewhere [2,10,25]. Our hypothesis is that:

(i) Whey protein may be important in both these effects.

(ii) It does this via its effect on increasing GSH concentration, in relevant tissues, probably by providing high levels of substrates for GSH synthesis.

(iii) That it may have an anti-tumor effect on low volumes of tumor via stimulation of immunity through the GSH pathway.

(iv) That it may have an anti-carcinogenic effect by increasing GSH levels that could detoxify potential carcinogens — in some cases by being conjugated to a known chemical like DMH. In 'spontaneous' carcinogenesis models like that cited here, where the carcinogenesis mechanism is not known, GSH may also be playing a role. Since it is considered that oxygen radical generation is frequently a critical step in carcinogenesis [11] the effect of GSH on free radical detoxification [22] could be important in inhibiting carcinogenesis induced by a number of different mechanisms. GSH conjugation is generally a principal detoxification pathway for many xenobiotics with carcinogenic potential. There are however rare but important examples of GSH conjugation enhancing the toxicity of a chemical. Dihaloalkanes, including ethylene dibromide and ethylene dichloride, are used as pesticides and gasoline additives and so represent environmental hazards. They are known to be carcinogenic in animal models. Recently it was shown that a reaction involving GSH conjugation may result in a reactive metabolite than can form a DNA adduct [17]. Whether this occurs in vivo is not yet certain but remains a serious issue in the present context.

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