Commercial Low-Fat Milk Markedly Stimulates Mammary Carcinogenesis in Rats

Adapted from:
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Low-Fat Milk Promotes the Development of 7,12-Dimethylbenz(a)anthracene (DMBA)-Induced Mammary Tumors in Rats
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Abstract

Commercial cows' milk contains considerable amounts of estrogens. This study assessed the effect of commercial low-fat milk on the development of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors in rats. Eighty six-week-old female Sprague-Dawley rats received a single oral dose of 5 mg DMBA. Twenty-four hours later, the animals were divided into 4 groups of 20 animals each and given one of four test solutions for 20 weeks as their drinking liquid: low-fat (1%) milk (M), artificial milk (A), estrone sulfate solution (0.1 μg/ml, E), or tap water (W). The artificial milk was formulated to supply essentially the same calories as the milk. The low-fat milk contained 378 pg/ml estrone sulfate. Tumor incidence, the cumulative number of tumors, and the sum of tumor diameters were higher in the M and E groups than in the A or W groups. Overall, the development of mammary tumors was in the order: M = E > A = W. Whereas the plasma 17β-estradiol concentration in the M group was the second highest after the E group, the plasma level of insulin-like growth factor (IGF-I) was significantly higher in the M group than in the other three groups. In conclusion, commercially available low-fat milk promotes the development of DMBA-induced mammary tumors in rats. The degree of the promotion is almost comparable to that of 0.1 μg/ml estrone sulfate. The high estrogen content in the milk may be responsible for the promotional effects, acting in concert with other hormones such as IGF-I.

Introduction

Breast cancer is the most common female cancer in the world, and is the number one cause of cancer-related death in non-smoking women (1). International differences in the rates of breast cancer incidence and mortality suggest that environmental factors, perhaps dietary ones, may influence risk for this disease (2,3). An epidemiological analysis revealed that the incidence of breast cancer in 42 countries is highly correlated (r = 0.79) with the consumption

of milk and dairy products (4). However, the association between milk and breast cancer has been inconsistent in case-control studies; positive association (5-7), no association (8,9), and inverse association (7,10) have been observed. Cohort studies also have yielded conflicting findings (11-16). In a meta-analysis, a small increase in breast-cancer risk with high milk consumption was observed (17). However, in a pooled analysis of cohort studies, no significant association was found between dairy products consumption and the risk of breast cancer (18). These contradicting results may indicate that different components in milk play complex roles in the development of breast cancer.

Breast cancer is typically hormone dependent. Known risk factors for this cancer involve endogenous hormone exposure-- early menarche, late first full-term pregnancy, and late menopause, all of which increase exposure to hormones, especially estrogens (19). Exogenous estrogens, derived from oral contraceptives and hormone replacement therapy (HRT), have been positively associated with the risk for breast cancer (20,21). In addition to these sources, estrogens, both natural (*e.g.*, estrone) and synthetic (*e.g.*, ethinyl estradiol), have been found in water (22) and in foods (23,24). Remesar *et al.* (23) calculated estrone intake in a standard human diet, and found that 46.6% of estrone came from dairy products. Hartmann *et al.* (24) estimated that dairy products provided 60-80% of dietary estrogens and progesterone, the other female sex hormone in the Western diet. The concentration of estrone sulfate in cows' milk increases greatly during pregnancy (25). Since commercial cows' milk in developed countries is produced mainly from pregnant cows, it contains considerable quantities of estrogens (26).

As components of milk, estrogens have not been addressed as breast-cancer risk factors in epidemiological studies. It is difficult to evaluate the risk of milk estrogens in such studies, as estrogen levels are affected by both endogenous and exogenous factors in women.

Chemically induced mammary tumor models with laboratory animals have been extensively studied, with 7,12-dimethylbenz(a)anthracene (DMBA) being used most frequently (27-29). Although the tumors are not identical across species, hormone dependency is an important similarity between human mammary tumors and those induced by DMBA in rats (30). The DMBA model is useful for the study of human breast cancer, especially in the postmenopausal period (29).

The present study was performed to assess the effects of commercial low-fat milk on the development of DMBA-induced mammary tumors. In order to confirm the role of estrogens, an aqueous estrone sulfate solution was used as a positive control.

Materials and Methods

Animals and Diets

Eighty female Sprague-Dawley rats were obtained at five weeks of age from Shizuoka Laboratory Animal Center (Shizuoka, Japan). They were housed individually in stainless-steel wire-bottomed cages in an air-conditioned room (22 ± 2 ºC with 55 ± 10%

relative humidity) with 12/12 hours light/dark cycle. The care and use of laboratory animals followed the Guidelines for Animal Experiments of the Medical University of Yamanashi.

After one week of acclimation to commercial powder chow (Clea CE-2, Nippon Clea, Tokyo, Japan) and water, all animals were given a single dose of 5 mg DMBA (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.5 ml corn oil by intragastric intubation between 14:00 and 16:00 hours. Twenty-four hours after DMBA administration, the rats were assigned at random to one of four groups, each with a different dietary regimen.

Each rat was maintained on the powder chow (dispensed in a glass container) throughout the experiment. Each group of rats was given low-fat milk (M), artificial milk (A), estrone sulfate solution (E), or tap water (W) as a drinking liquid in a separate glass container of the same kind as used for the powder chow. Commercial, nationally available low-fat milk, the fat content of which was 1%, was purchased locally; the number of the production lot was recorded. The artificial milk was formulated to balance the major nutrients contained in the low-fat milk, and consisted of gluten fortified with lysine, DL-methionine, threonine, and valine as protein, coconut oil as fat, and dextrin-maltose as carbohydrate, supplemented with calcium carbonate (**Table 1**). Estrone sulfate (Sigma-Aldrich) was dissolved in tap water at a concentration of 0.1 μg/ml. The food and drinking solutions were renewed daily at 16:00 hours.

Table 1. The compositions of low-fat milk and artificial milk

Low-fat milk (g)*		Artificial milk (g)	
Protein	38	Gluten	34.7
		Lysine	1.80
		Valine	0.62
		Threonine	0.32
		DL-Methionine	0.56
Fat	10	Coconut oil	10
Carbohydrate	55	Dextrin-Maltose	55
Calcium	1.30	$CaCO_3$	2.58
Sodium	0.60	$NaHCO_3$	1.68
Water	888	Water	892.7
Total	1000	Total	1000

*Kagawa, Y. Standard Tables of Food Composition in Japan. Kagawa Education Institute of Nutrition, 2002.

Chow and liquid consumption was monitored daily by subtracting from what was provided anything not consumed by 10:00 hours the following day. Calculated net intake of energy and body weight was recorded weekly throughout the experiment.

Tumorigenesis assessment.

Rats were palpated weekly to monitor tumor development. The two largest perpendicular diameters of each tumor were measured with calipers (Mitsutoyo CD-15 CP, Kanagawa, Japan) and the mean of the two measures was used to estimate the tumor size. The location of each tumor was also recorded at every palpation.

Autopsy and histopathological examinations

During the 20th week after DMBA administration, rats were decapitated between 14:00 and 16:00, during proestrus, as determined by vaginal smears (31). Trunk blood from the neck was immediately centrifuged at –4 ºC, and the plasma stored at –80 ºC until analysis.

All organs were examined for gross abnormalities, and the location and weight of each tumor was recorded. The visible mammary tumors, ovaries and uteri were rapidly excised and weighed, as was the peritoneal adipose tissue (excepting mesenteric). Mammary tumors were fixed and stored in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 3 μm and stained with hematoxylin and eosin for histopathological examinations. Histological diagnosis was performed according to the classification as described by Konitowski *et al.* (32)

Measurement of plasma hormone concentrations

Because estrogen peaks before ovulation, the period of proestrus was selected to observe the trend of plasma estradiol levels during the experiment. Before DMBA administration, the blood of 20 randomly selected rats was sampled from tail veins between 14:00 and 16:00 hours, using heparinized capillary tubes, to measure the basal 17β-estradiol concentration. Every four weeks, blood from each rat was also collected from the tail between 14:00 and 16:00 hours at proestrus. Plasma 17β-estradiol concentration was measured using a commercial ELISA kit (Immuno-Biological Laboratories, Hamburg, Germany), after extraction with diethyl ether.

The blood plasma collected at autopsy was used to measure the concentrations of 17β-estradiol, estrone, insulin-like growth factor (IGF)-I, and prolactin. Plasma estrone and IGF-I concentrations were measured using an estrone RIA kit (Diagnostic Systems Laboratories, Webster, TX, USA) and a rat IGF-I EIA kit (Diagnostic Systems Laboratories), respectively. Plasma prolactin concentration was measured using a rat prolactin EIA kit (SPI-BIO, Massy Cedex, France).

Measurement of estrogens in low-fat milk

A sample of the low-fat milk was stored at -80º C every four weeks, and was later assayed for estrone, 17β-estradiol and estriol concentrations. For the analysis of each free estrogen, 8 ml of diethyl ether was added to each 2-ml milk sample (prepared in duplicate), and shaken vigorously. After centrifugation at 3,000 rpm for 10 min, 7 ml of the ether layer was recovered and evaporated to dryness in a vacuum centrifuge. The residue was dissolved in 200 μl of methanol, and 10 μl of this solution was injected into an HPLC system with a coulometric electrode array detector (eight channels, ESA, Chelmsford, MA, USA) (33,34).

For determination of total estrogen (free + conjugated), 2-ml milk samples (prepared in duplicate) were mixed with 200 μl of buffer containing 10 mg sulphatase

(Sigma-Aldrich) and incubated at 37 ºC for 3 hours before extraction with diethyl ether (33). The concentration of conjugated estrogen was calculated by subtracting the free from the total estrogen concentration.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using StatView 4.0 (Abacus Concepts, Berkeley, CA, USA), followed by a *t* test when significant differences existed among groups. Tumor incidence was analyzed by Fisher's exact probability test. The number, size and weight of tumors (at autopsy) were analyzed by a Mann-Whitney U-test. *P*-values less than 0.05 were considered significant.

Results

Body weight and food intake

No significant difference was observed in body weight between the M and A groups (**Fig. 1**). Rats in the E group occasionally weighed less than rats in the W group. In general, rats in the M and A groups were heavier than rats in the E and W groups.

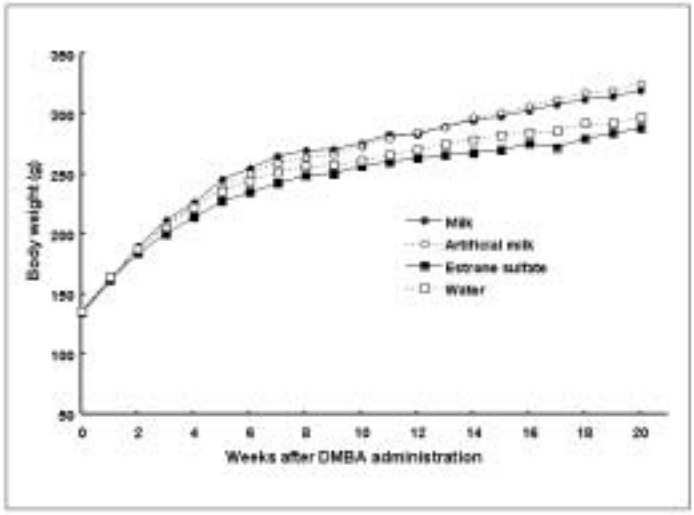
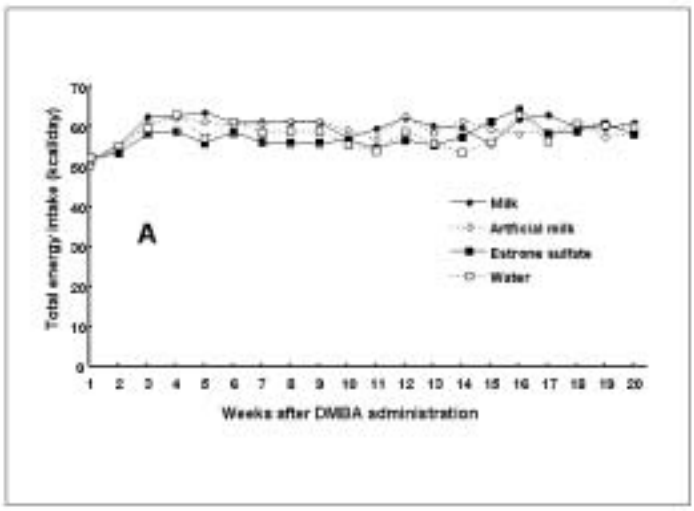
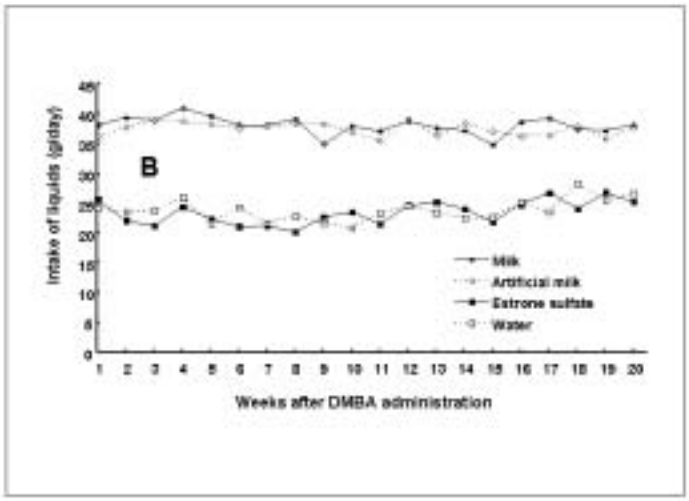


Fig. 1. Body weights of rats in the four groups during the experimental period. No significant difference was observed between Milk and Artificial Milk or between Estrone and Water.





During the first three weeks, the energy intake of the rats increased in every group, and remained at a similar level thereafter (**Fig. 2A**). The average energy intake (kcal/day) throughout the experiment was 59.7 ± 3.3, 59.1 ± 2.9, 57.8 ± 2.6, and 57.8 ± 3.3 for the M, A, E, and W groups, respectively (no significant difference between M and A or between E and W).

No significant difference was observed in the consumption of drinking liquid (g/day) between the M and A groups or between the E and W groups (**Fig. 2B**). However, consumption (g/day) was much greater in the M (38.2 ± 1.5) and A (37.6 ± 1.1) groups than in the E (23.4 ± 1.8) or W (23.9 ± 1.9) groups.

Fig.2. Daily intake of food and liquids after DMBA administration. A, Total energy intake; B, Intake of liquid. No significant difference was observed between Milk and Artificial Milk or between Estrone and Water.

The body weights (g) of the M (332.8 ± 27.9)- and A (325.8 ± 44.9)-group rats at autopsy were significantly higher than those of the E (293.2 ± 24.7)- or W (301.8 ± 32.2)-group rats. The weight of peritoneal adipose tissue (g) was significantly higher in both the M (25.3 ± 5.6) and A (26.2 ± 7.1) groups than in the E (15.4 ± 4.7) or W (18.6 ± 5.6) groups. There was no significant difference in the adipose tissue weight between the M and A groups or between the E and W groups. However, the weight of adipose tissue as a fraction of body weight (%) was found to be significantly lower in the E group (5.2 ± 1.3) than in the W (6.1 ± 1.3) group, whereas no significant difference was noted between the M (7.8 ± 1.3) and A (7.9 ± 1.5) groups. The weights of uteri and ovaries were not significantly different among the four groups.

Mammary tumorigenesis

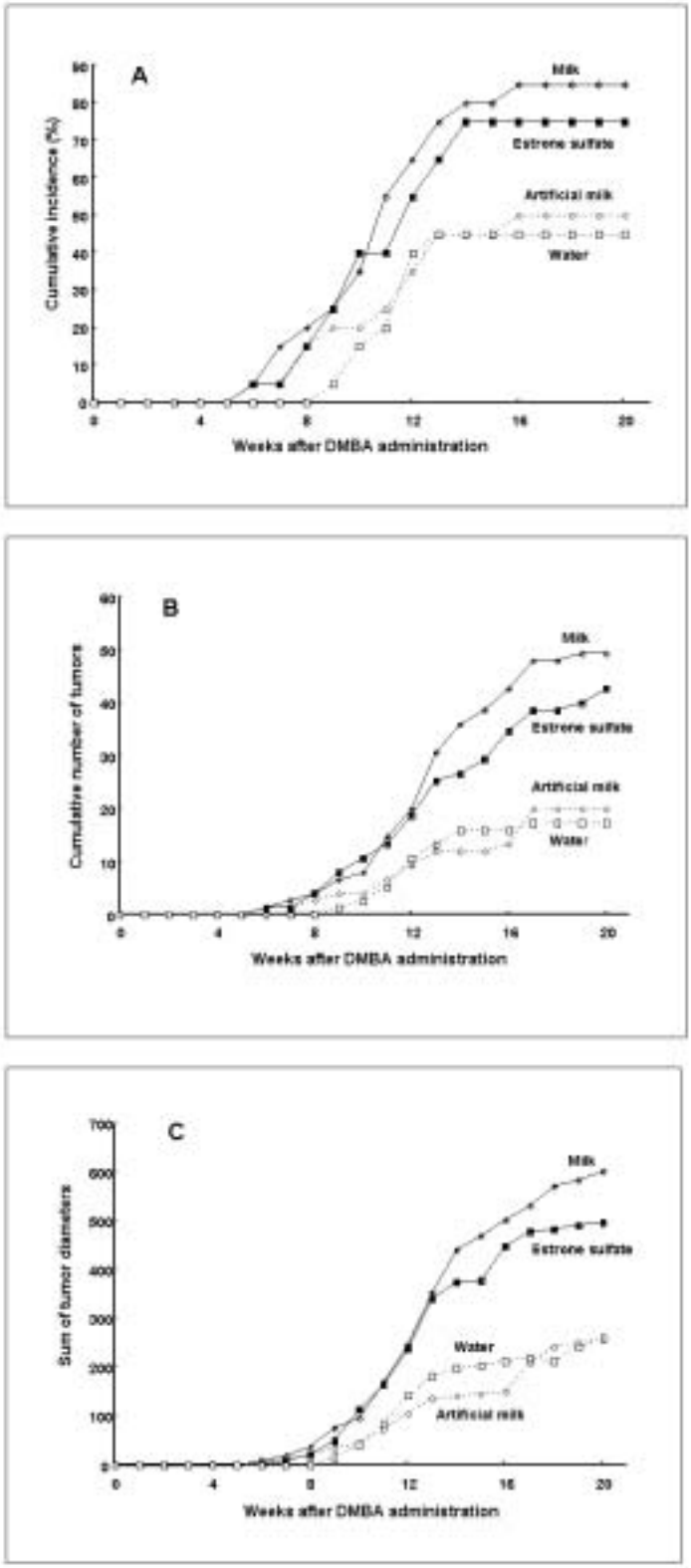


Figure 3 shows the tumor incidence (**A**), the number of tumors (**B**) and the size of tumors (**C**) in the experimental groups, as detected by palpation. With each of the three indices of tumorigenesis, the M and E groups could be grouped together separately from the A and W groups, the former having much higher values than the latter.

At week 6 after DMBA administration, the first tumors were palpated in the M, A, and E groups (**Fig. 3A**). Palpable tumors in the W group did not appear until week 9 after DMBA administration. The incidence increased with time, more rapidly in the M and E groups than in the A and W groups. Incidence reached or surpassed 50% at weeks 11, 12, and 16 for the M, E, and A groups, respectively. The incidence in the W group never reached 50%. A significant difference in incidence between the M and A groups was noted consistently from week 14 onward. The incidence in M-group rats was higher than in W-group rats, also from week 14 onward. The difference between the E and W groups was not statistically significant at the end of the experiment ($P = 0.0528$).

Fig. 3. Time course of palpable mammary tumor appearance. A, Tumor incidence; B, Cumulative number of tumors; C, Sum of tumor diameters.

At the final palpation (week 20), tumor incidence was 85% (17/20), 50% (10/20), 75% (15/20), and 45% (9/20) for the M, A, E, and W groups, respectively.

More than twice as many tumors were seen in the M group as in the A group, as of week 11 after DMBA administration (**Fig. 3B**). Similarly, rats in the E group had almost twice as many tumors as did rats in the W group. No significant difference in number was observed between the M and E groups or between the A and W groups. At the final palpation (week 20), the cumulative number of tumors in each group was 49, 20, 43, and 17 in the M, A, E, and W groups, respectively.

The total size of tumors in the M group, estimated by the sum of tumor diameters, was more than twice that of tumors in the A group (**Fig. 3C**). The tumors that developed in the E group were also almost twice as large as those in the W group. No significant difference in tumor size was found between the M and E groups or between the A and W groups. Altogether, the palpation data show that the mammary tumors developed in the order: M = E > A = W.

Autopsy data supported the palpation data described above, except that some small, impalpable tumors were found at autopsy (**Table 2**).

Table 2. DMBA-induced mammary tumors among the groups at autopsy

Group	Number of rats	Number of tumor-bearing rats	Number of tumors	Tumor weight (g)
Milk (M)	20	17[ab]	73[ab]	49.2[ab]
Artificial milk (A)	20	10	33	24.3
Estrone sulfate (E)	20	15	64[b]	47.3[b]
Water (W)	20	9	25	23.1

[a]Significantly different from A. [b]Significantly different from W.

The tumor incidence was 17/20, 10/20, 15/20, and 9/20 in the M, A, E, and W groups, respectively. The respective total numbers of tumors were 73, 33, 64, and 25 in the four groups. The sums of tumor weight were 49.2, 24.3, 47.3, and 23.1 g in the respective groups. It is noteworthy that both the total number of tumors and the total weight of tumors in the M group were more than double the respective values in the A group. The same relationship was seen between tumors of the E and A groups.

Histologically, almost all the tumors that developed in each group were adenocarcinomas. Overall, the four groups could be ranked by the development of mammary tumors as follows: M = E > A = W.

Plasma hormone concentrations

The time courses of plasma 17β-estradiol concentrations in the four groups during the experiment are shown in **Fig. 4**. From week eight onward, the concentration in the E group was significantly higher than that in the other three groups. The concentration in the M group increased slightly over time, with no significant difference from that in the A group throughout the experiment. At weeks 16 and 20, the M group had a significantly higher estradiol concentration in the plasma than did the W group.

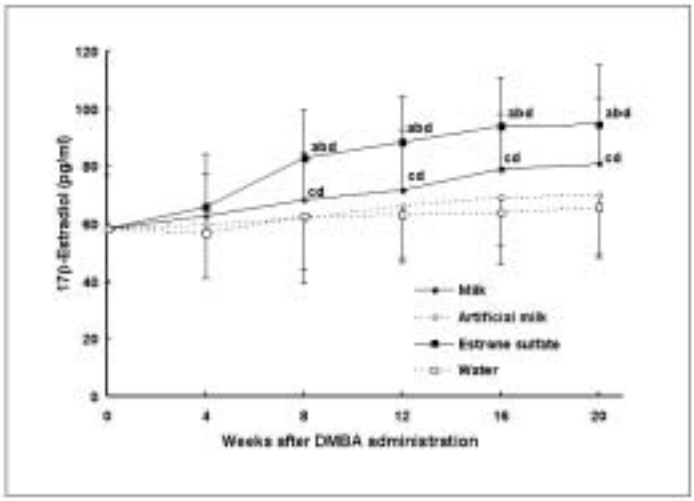


Fig. 4. Time course of 17b-estradiol concentration in the plasma. A, Significantly different from Milk; b, from Artificial Milk; c, from Water.

Plasma concentrations of estrone, 17β-estradiol, IGF-Ị and prolactin, measured at autopsy, are shown in **Table 3**. Although the estrone level in the M group was not significantly different from that of the A group ($p = 0.064$), it was significantly higher than that in the W group. The IGF-I concentration was significantly higher in the M group than those in the other three groups, while no significant differences were found among the A, E and W groups. The plasma prolactin concentrations were significantly higher in the M and A groups than in the E and W groups, although the intra-group variation of the concentrations was considerable. There were no significant differences in the plasma prolactin concentration between the M and A groups or between the E and W groups.

Table 3. Concentrations of estrone, 17β-estradiol, IGF-I and prolactin in the plasma at autopsy

Group	Estrone (pg/ml)	17β-Estradiol (pg/ml)	IGF-I (ng/ml)	Prolactin (ng/ml)
Milk (M)	75.7 ± 35.8[c]	81.1 ± 22.6[bc]	748 ± 125[abc]	1660 ± 1550[bc]
Artificial milk (A)	58.7 ± 17.4[b]	70.1 ± 21.7[b]	629 ± 109	1200 ± 1080[bc]
Estrone sulfate (E)	77.7 ± 36.4[c]	94.8 ± 20.6[ac]	648 ± 152	622 ± 292
Water (W)	40.3 ± 15.0	66.0 ± 16.6[b]	669 ± 65	577 ± 394

Values are mean ± SD.
[a]Significantly different from A. [b]Significntly different from E.
[c]Significantly different from W.

Within each group, there were no significant differences in 17β-estradiol and estrone concentrations between tumor-bearing and non-tumor-bearing rats (data not shown). However, in both the M and E groups, the plasma IGF-I concentration (mean ± SD in μg/ml) was higher in the tumor-bearing rats (0.789 ± 0.100 [n = 17] and 0.698 ± 0.117 [n = 15], respectively) than in the non-tumor-bearing rats (0.586 ± 0.065 [n = 3] and 0.511 ± 0.166 [n = 5], respectively). Although plasma prolactin concentration (ng/ml) in the M group was much higher in rats with tumors (1.94 ± 1.68, n = 17) than in tumor-free rats (0.51 ± 0.19, n = 3), the difference was not statistically significant.

When data from rats in all groups were pooled, no significant difference was observed in 17β-estradiol and estrone concentrations in the plasma (mean ± SD in pg/ml) between tumor-bearing (n = 51; 81.3 ± 25.3 and 68.3 ± 29.6, respectively) and non-tumor bearing rats (n = 29; 72.4 ± 21.7 and 54.0 ± 21.2, respectively). However, both IGF-I (μg/ml) and prolactin (ng/ml) concentrations in the plasma were significantly higher in tumor-bearing rats (0.716 ± 0.113 and 1.324 ± 0.258, respectively) than in non-tumor-bearing rats (0.600 ± 0.104 and 0.505 ± 0.228, respectively).

Estrogen concentrations in milk

The levels of estrogens in the milk used in the present study are shown in **Table 4**.

Table 4. Estrogen concentrations (pg/ml) in the low-fat milk[a] used in this study

Group	Estrone	17β-Estradiol	Estriol	Total
Free	58.1 ± 23.2	54.4 ± 49.3	*	113
Conjugated	378 ± 151	159 ± 114	53.4 ± 34.8	591
Total	436 ± 151	214 ± 91	53.4 ± 34.8	703

Values are mean ± SD.
[a]The milk had been sterilized at 130 C for 2 sec during production.
*Values were under the limit of detection.

Total estrogen content in the commercial low-fat milk was 703 pg/ml, of which estrone conjugates comprised 378 pg/ml. The conjugated form accounted for 87% of total estrone. The 17β-estradiol concentration was approximately half that of estrone.

Discussion

In most of the epidemiological studies that have identified milk consumption as a risk factor for breast cancer, special attention has been given to the fat content of the milk (2,5,7,11,17). Milk fat cannot, however, explain all of the breast cancer risk related to milk consumption. In fact, consumption of whole milk has declined steadily since the 1950s, and been replaced by

fat-reduced milk (35), yet the incidence of breast cancer has increased worldwide over the last 50 years (3).

In the present study, we examined the effect of low-fat milk (with a fat content of 1%) on the development of DMBA-induced mammary tumors, excluding the effect of milk fat. As the development of mammary tumors is influenced by energy intake (27), we included, as a negative control, a group of rats that consumed artificial milk (A group). The protein, fat and carbohydrate contents of the artificial milk were the same as those of the commercial low-fat milk used. We used gluten, fortified with some essential amino acids (**Table 1**) as the source of protein, rather than casein (which is produced from milk), to avoid the potential for carry-over of other milk components. As we expected, rats in the M and A groups consumed almost the same number of calories (**Fig. 2**). Body weights in the two groups were similar throughout the experiment (**Fig. 1**); at autopsy, both body weights and ratios of adipose tissue weight to body weight were similar.

Rats in the E group consumed almost the same energy as the W group (**Fig. 2**). The volume of drinking solution consumed was also comparable between the two groups. This suggests that estrone sulfate (0.1 μg/ml) had no influence on food and water consumption. However, the adipose tissue weight and the adipose tissue weight/body weight ratio were lower in the E group than in the W group. It is possible that estrone sulfate at a concentration of 0.1 μg/ml inhibited energy storage.

Since fat and energy intake were balanced between the M and A groups, the significantly higher incidence, greater number, and greater growth of mammary tumors in the M group (**Fig. 3**) may be attributed to an undetermined component or components in milk, other than fat or calories.

In the present study, the incidence, number, and size of tumors in the M and E groups were similar. The number and weight of mammary tumors that developed were significantly higher in the E group than in the W group, although the difference in incidence between the two groups (75% vs. 45%) was not statistically significant. Special attention should be paid to the role of estrone sulfate in the development of DMBA-induced mammary tumors.

The findings at autopsy (**Table 2**) and the measurement of palpable tumors (**Fig. 3**) indicate that the potential of estrone sulfate to promote the development of DMBA-induced mammary tumors in rats is comparable to that of commercially available low-fat milk.

The conjugated estrone concentration in the low-fat milk used was about 380 pg/ml (**Table 4**). In addition to the long plasma half-life of estrone sulfate, it is absorbed in the intestinal mucosa virtually unchanged (36,37). Although the oral bioactivity of free estradiol and estrone may be relatively low, estrone sulphate, which is a major estrogen in milk, has high oral bioactivity (26). Once inside the body it can be readily converted to estrone and estradiol. The entero-hepatic circulation also plays a role in the absorption of estrone

conjugates. In the present study, plasma 17β-estradiol concentration increased gradually in the E-group rats, followed by that in the M-group rats (**Fig. 4**).

Some human studies have also demonstrated that milk consumption increased the level of estrogen in circulation. South African black males switched from a vegetarian to a Western diet--with milk, butter and meat--showed an increase in circulating estrogen levels (38). The relationship between milk consumption and plasma estrogen concentrations is also supported by the fact that Asian women, whose consumption of milk and dairy products is low, have lower plasma estrogen concentration than do Caucasian women, whose dairy product consumption is high (39).

Estrogens in Silastic implants have been documented to increase mammary tumor development in rats (28,29,40). Moreover, increasing evidence has implicated 17β-estradiol as a cause of breast cancer in postmenopausal women (41-43). Indirect effects of estrogens may occur through the stimulation of prolactin secretion, which is linked with mammary cancer (19). In the present study, however, the E group, with a high incidence of DMBA mammary tumors, had a prolactin concentration similar to the W group, with a low incidence of tumors (**Table 3**). By contrast, plasma prolactin was about three-fold higher in the M group, and two-fold higher in the A group than in the E and W groups. Calories or fat intake, which were slightly higher in both the M and A groups than in the E or W groups, is likely to have mediated the increase in prolactin level, as suggested by Clinton *et al.* (44)

Another component in cows' milk may play an additive or synergistic role with estrogens in the development of DMBA-induced mammary tumors. IGF-I has recently attracted increasing attention, as studies have demonstrated a link between breast cancer risk and high levels of this growth factor (45,46). IGF-I concentrations in cows' milk have been reported to range from 6 to 162 ng/ml (47). In a human study, plasma IGF-I concentration increased by 10% when healthy subjects consumed cows' milk (48). In the present study, plasma IGF-I concentration was significantly higher in the M group than in the other three groups (**Table 3**). In addition, tumor-bearing rats in the M and E group had a higher IGF-I concentration than did non-tumor-bearing rats. It is possible that IGF-I from cows' milk is active in rats, because IGF-Is in mammals are highly similar in the structure (49). Estrogens and other hormones in milk may have been involved in the promotion of DMBA-induced mammary tumors in the M group.

In conclusion, commercially available low-fat milk promotes the development of DMBA-induced mammary tumors in rats. The degree of the promotion is almost comparable to that of 0.1 mg/ml estrone sulfate. The high estrogen content of the milk may be responsible for the promotional effects, acting in concert with other hormones such as IGF-I. Further epidemiological and mechanistic studies are needed to verify the hypothesis that milk consumption is a factor responsible for the development of breast cancer.

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