Dietary Lutein from Marigold Extract Inhibits Mammary Tumor Development in BALB/c Mice

Jean Soon Park, Boon P. Chew, Teri S. Wong
Author Notes

The Journal of Nutrition, Volume 128, Issue 10, October 1998, Pages 1650–1656, https://doi.org/10.1093/jn/128.10.1650

Published:

01 October 1998

Article history

Abstract

High levels of dietary lutein can inhibit mammary tumor growth in mice. However, the antitumor effect of low levels of dietary lutein on mammary tumors is unavailable. Female BALB/c mice and the WAZ-2T (−SA) mammary tumor cell line were used in two experiments. A preliminary tumor cell dose titration study (Experiment 1) was designed to determine the inoculation dose to produce ~65% tumor incidence. Mice (n = 10/dose) were inoculated with 0 to 1 × 10^6 tumor cells in the right inguinal mammary fat pad. A tumor cell load of 2.5 × 10^3 cells/inoculation produced ~65% tumor incidence. This dose was used in a subsequent study (Experiment 2) of the efficacy of dietary lutein against mammary tumor development. Mice (n = 20/treatment) were fed a semisynthetic diet containing 0, 0.002, 0.02, 0.2 or 0.4% lutein from marigold extract. After 14 d, all mice were inoculated with 2.5 × 10^3 tumor cells, and tumor growth was measured daily for 70 d at which time blood, liver, spleen and tumors were obtained. Lutein + zeaxanthin uptake increased dose-dependently ($P < 0.05$) with dietary lutein levels from 0 to 0.02% (spleen) or 0.2% (plasma, liver and tumor). Low levels (0.002 and 0.02%) of dietary lutein lowered ($P < 0.05$) mammary tumor incidence, tumor growth and lipid peroxidation, and increased tumor latency, whereas higher dietary levels (0.2 or 0.4%) were less effective. Therefore, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice.

lutein, mammary tumor, antioxidant, mice

Topic:

- diet
- calendula
- lutein
- animal mammary neoplasms
- mice, inbred balb c
- mice

- neoplasms

Issue Section:

Biochemical and Molecular Roles of Nutrients

Epidemiological studies have suggested that carotenoids may decrease the incidence of major clinical diseases such as cancer (Ziegler 1989), cardiovascular disease (Burri 1997) and age-related macular degeneration (Snodderly 1995). Consumption of carotenoid-rich diets from fruits and vegetables have been associated with a decreased risk of lung, stomach and prostate cancers (Flagg et al. 1995, Poppel and Goldbohm 1995). Optimism concerning the anticancer activity of β-carotene was dampened by recent studies that showed an increased incidence of lung cancer in high-risk population of smokers and/or asbestos workers (Albanes et al. 1995, Omenn et al. 1996) and no effect on cancer and heart disease (Hennekens et al. 1996). Two other studies (Kushi et al. 1996, Verhoeven et al. 1997) reported that β-carotene supplementation had no significant effect on the incidence of breast cancer. In recent years, studies on the anticancer activities of other carotenoids began to emerge. Canthaxanthin (Krinsky 1989), lycopene (Levy et al. 1995) and neoxanthin (Chang et al. 1995) have decreased cancer development or growth of cancerous cells. Lutein is a major carotenoid found in human blood (Parker 1989). Lutein and zeaxanthin have specific biological functions in decreasing cancer development (Chew et al. 1996, Rock et al. 1996), enhancing immune function (Chew et al. 1996) and protecting against age-related macular degeneration (Snodderly 1995). In cultured cells, lutein is more effective than β-carotene in inhibiting the auto-oxidation of cellular lipids (Zhang et al. 1991) and protecting against oxidant-induced cell damage (Martin et al. 1996). In humans, high plasma lutein is correlated with greater expression of estrogen receptors in breast cancer cells and, consequently, with greater survival rates and better response to hormone therapy (Rock et al. 1996). Mice fed dietary lutein had reduced growth of a transplantable mammary tumor and enhanced lymphocyte proliferation (Chew et al. 1996). The latter study raised two questions: *i*) Can dietary lutein prevent breast cancer development or does it merely delay tumor latency? *ii*) Will much lower amounts of dietary lutein be effective against mammary cancer? We hypothesized that low levels of dietary lutein can inhibit the initiation and growth of mammary cancer cells. Therefore our objective is to study the effect of low dietary lutein on mammary tumor development in mice.

MATERIALS AND METHODS

Animals and diet.

Two experiments were conducted to test our hypothesis that feeding low amounts of lutein can inhibit mammary tumor development in mice. In both experiments, female 8-wk-old BALB/c mice were used. Animals were fed a semisynthetic diet (Park et al. 1998) and had free access to water and food. All animals (three mice/cage) were housed in a light- (12 h) and temperature- (23°C) controlled room. All procedures were approved by the Washington State University Animal Care and Use Committee (Pullman, WA).

Tumor cell culture.

The tumor cells used in all studies were from a murine mammary tumor cell line (WAZ-2T; −SA) developed from a spontaneously arising mammary outgrowth in a BALB/c mouse (Ram et al. 1993). This cell line has been characterized (Danielson et al. 1980) and was previously used in similar studies with carotenoids (Chew et al. 1996). To assure consistency with the mammary tumor cells used throughout the experiment, only cell passages 34 to 36 were used.

On the day of mammary tumor challenge, tumor cells were grown to confluence, trypsinized, washed and cell number enumerated using a hemacytometer. Cell viability was assessed by trypan blue exclusion. Tumor cells were resuspended in Dulbecco's Modified Eagle's medium containing (per L) 3.7 g of sodium bicarbonate, 2.38 g of Hepes, 0.1 g of penicillin-G, 0.1 g of streptomycin sulfate, 5 mg of insulin and 100 mL of newborn bovine serum (pH 7.4; Sigma Chemical Co., St. Louis, MO). The cell suspension (50 μL) containing the desired number of tumor

cells was infused into the right inguinal mammary fat pad (Chew et al. 1996). The cells were kept on ice and mixed well prior to each infusion.

Tumor cell infusion.

Animals were anesthetized with an i.p. injection of xylazine hydrochloride (Xyla-ject, Phoenix Pharmaceutical Inc., St. Joseph, MO) and ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, NJ) (dosage: 10 and 100 mg/kg body weight, respectively) in 0.2 mL of sterile phosphate buffered saline (PBS)$_4$. An incision was made along the midline previously sterilized with 70% of alcohol, and the skin was gently teased to expose the right inguinal mammary fat pad. Tumor cell infusion was accomplished using a 100-µL Hamilton glass syringe equipped with a 26-g hypodermic needle. Incisions were closed with 11-mm surgery clips. The length and width of the mammary tumors were measured daily with a pair of calipers (Mitutoya, Tokyo, Japan). To correct for skin thickness, 0.1 cm subtracted these measurements (Chew et al. 1996). Palpable tumors too small to be measured were given a diameter value of 0.2 cm. Tumor volume was calculated by using the volume of a sphere. Mice were killed when tumor diameter exceeded 1.7 cm.

Experiment 1 was designed to determine the tumor cell load that would produce ~65% of tumor incidence, an incidence level we wished to use in Experiment 2 to study the efficacy of dietary lutein in preventing mammary tumor development. Mice (n = 10/treatment) were fed nonpurified rodent food (Teklad F6 Rodent Diet, Madison, WI) and challenged with 0, 5×10^2, 1×10^3, 5×10^3, 1×10^4, 5×10^4, 1×10^5, 5×10^5 and 1×10^6 −SA mammary tumor cells. Mice were killed at 120 d postinoculation or earlier when tumor diameter exceeded 1.7 cm. Tumor incidence and latency were recorded, and mice with no palpable tumors by 120 d postinfusion were given a tumor latency of 130 d.

In Experiment 2, mice (n = 30/treatment) were fed a semisynthetic diet (Chew et al. 1996) containing 0, 0.002, 0.02, 0.2 and 0.4% of lutein from marigold extract (INEXA C. A., Quito, Ecuador). Details on the composition of the lutein from marigold extract and on the preparation of the diets are as described in a companion paper (Park et al. 1998). Food intake was measured daily and body weight recorded weekly.

Tumor cell challenge.

Two weeks after the initiation of dietary treatments, mice were inoculated with 2.5×10^3 tumor cells in the right mammary fat pad as described in Experiment 1. This tumor load was selected because Experiment 1 showed that this challenge dose would produce ~65% of tumor incidence on d 50 postinoculation. Mammary glands were palpated daily and tumor size measured with a pair of calipers when mammary tumors became palpable (approximately on d 25 after inoculation). All mice were killed on d 70 after inoculation. Mice with no palpable tumor on d 70 postinoculation were given a latency period of 80 d.

Sampling.

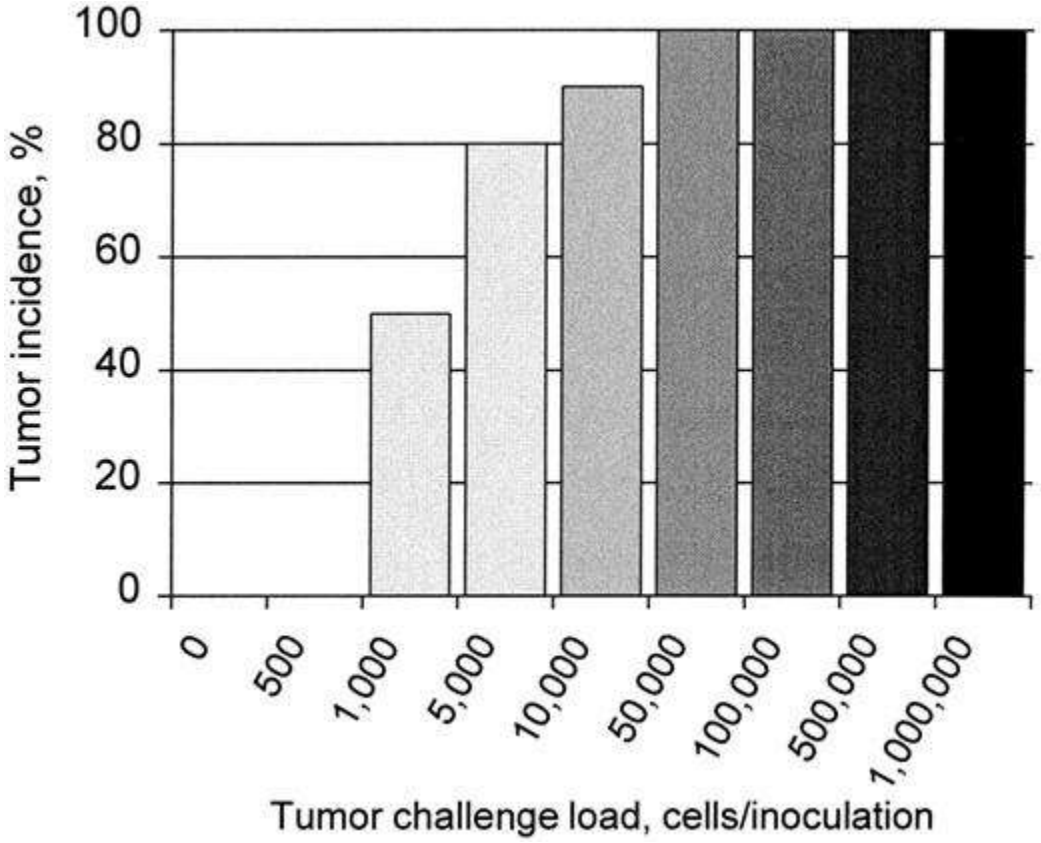
On d 70 postinoculation, blood was collected from anesthetized animals into heparinized tubes by heart puncture. Liver, spleen and tumors were excised, weighed, snap-frozen and stored at −80°C under a layer of nitrogen. All tumors were solid tumors.

Thiobarbituric acid (TBA) assay.

Lipid peroxidation activity in tumors was measured by the TBA procedure (Uchiyama and Mihara 1978). The solid tumors were rinsed with PBS, minced and about 1 g was homogenized in 9 mL of cold KCl (11.5 g/L). Duplicate aliquots of 0.5 mL of homogenate were pipetted, and 3 mL of phosphoric acid (10 g/L) plus 1 mL of TBA solution (6 g/L) were added. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 mL of n-butanol was added and vortexed vigorously. The mixture was centrifuged at 1,000 × g for 10 min, and absorbance of the aqueous phase was read at 532 nm. Tetramethoxypropane was used as the external standard. The thiobarbituric acid reactive substance (TBARS) values were expressed as µmol of malondialdehyde (MDA)/g tumor tissue and also as the difference in optical density between 532 and 520 nm. Because identical

trends were observed with both methods, only values expressed as μmol of MDA/g tumor tissue are presented.

Figure 1



Open in new tabDownload slide

Tumor incidence on d 120 postinoculation in mice challenged with 0 to 1 × 10^6 mammary tumor cells/inoculation. Tumor incidence (%) was calculated by expressing the number of mice with measurable tumors as a percentage of the total number of mice (n = 10/challenge dose). The correlation coefficient (r^2) between tumor incidence and challenge dose is 0.896.

Lutein extraction and high performance liquid chromatography (HPLC).

The extraction and HPLC quantification of lutein and zeaxanthin in plasma, liver, spleen and tumors are described in a companion paper (Park et al. 1998). Again, because lutein and zeaxanthin could not be clearly resolved, results are presented as lutein + zeaxanthin.

Statistical analysis.

Changes in food intake, body weight and tumor volume were analyzed by repeated measures analysis of variance using the General Linear Models Procedure of SAS (SAS 1991). The statistical model was: $Y_{ij} = \mu + \text{treatment}_i + \text{mice(treatment}_i\text{)(error A)} + \text{period}_j + \text{treatment} * \text{period}_{ij} + e_{ij}$ (error B). Treatment differences in lutein + zeaxanthin concentrations in plasma and tissues were compared using the student's t test. Tumor incidence was calculated by expressing the number of mice with measurable tumors as a percentage of total number of mice and differences were compared using Chi square (Steel and Torrie 1980).

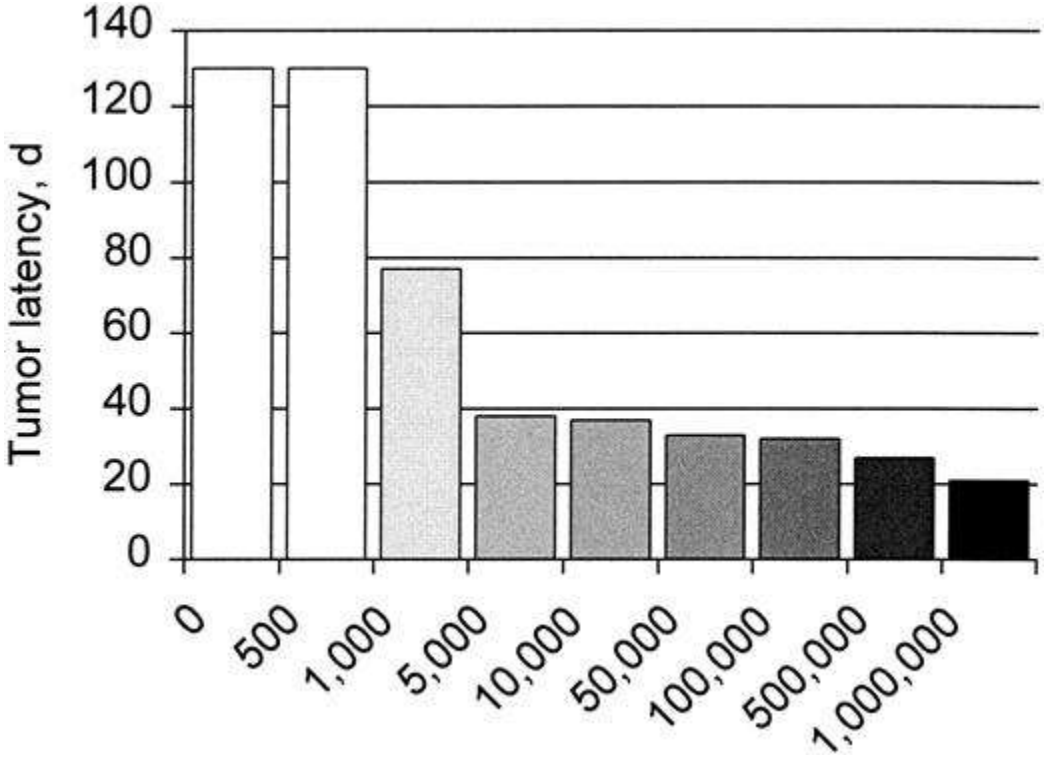
RESULTS

Experiment 1

The incidence of mammary tumor on d 120 postinoculation increased in a dose-related manner (r^2 = 0.896) with inoculation doses from 0 to 5 × 10^4 mammary tumor cells (Fig. 1). With a tumor challenge dose of 0 or 5 × 10^2 cells, none of the mice had palpable tumors. On the other hand,

with challenge doses of greater than 5 × 10^4 cells, all mice developed mammary tumors. Through interpolation of values of Figure 1, we determined that a tumor challenge dose of 2.5 × 10^3 cells is required to produce a tumor incidence of ~65%. This challenge dose was therefore used in Experiment 2.

Figure 2



Open in new tabDownload slide

Tumor latency in mice 120 d after being inoculated with 0 to 1 × 10^6 mammary tumor cells (n = 10/challenge dose). Tumor latency represents the number of days postinoculation when mammary tumors were first palpable. Mice with no palpable tumor on d 120 postinoculation were given a latency of 130 d. The correlation coefficient (r^2) between tumor incidence and challenge dose is 0.887.

Table 1.

Daily food intake and final organ weights in mice fed diets containing 0, 0.002, 0.02, 0.2 and 0.4% of lutein[1]

Treatment (% lutein in diet)				
0	**0.002**	**0.02**	**0.2**	**0.4**
Food intake, g/d[1-2]				
4.65 ± 0.3	4.95 ± 0.3	4.65 ± 0.2	4.72 ± 0.2	4.83 ± 0.3
Lutein intake, mg/d[1-2]				
0a	0.05 ± 0001b	0.46 ± 0.02c	4.95 ± 0.10d	10.11 ± 0.43e
Body weight, g[1-2]				
22.4 ± 0.5	22.5 ± 0.4	22.5 ± 0.4	22.5 ± 0.4	22.6 ± 0.3
Liver weight, g[1-3]				
1.37 ± 0.09a	1.16 ± 0.06b	1.21 ± 0.06b	1.34 ± 0.34ab	1.28 ± 0.07ab
Spleen weight, g[1-3]				
0.19 ± 0.04a	0.10 ± 0.01b	0.19 ± 0.07a	0.16 ± 0.02a	0.14 ± 0.03ab

F1-1

Values are means ± SEM (n = 20/diet group). Means in a row with different superscripts differ significantly ($P < 0.05$).

F1-2

Values calculated from daily food intake measures and represent average values from d 0 to 84 of feeding.

F1-3

Values represent final organ weight taken when animals were killed after 84 d of consuming the diets.

Open in new tab

Tumor latency period decreased linearly with increasing tumor load (**Fig. 2**). None of the mice inoculated with 5×10^2 cells developed tumors by d 120 postinoculation when the study was terminated. Even though tumor incidence was maximal with tumor cell challenge doses greater than 5×10^4 cells, tumor latency period continued to decrease with higher inoculation doses through the highest dose used (1×10^6 cells).

Experiment 2

Body weight and feed intake.

Body weight was not significantly different among treatment groups during the study period (**Table 1**). However, there were significant treatment differences in the weights of livers and spleen, with mice fed the control diet having heavier organs than those fed 0.002% of lutein ($P < 0.05$, Table 1). These differences in organ weight reflected tumor development rather than the level of lutein supplementation. For instance, disregarding dietary treatments, mice with tumors had liver weights 40% heavier ($P < 0.05$) than mice without tumors (**Table 2**). Similarly, spleen weights averaged 26-fold in mice that developed tumors compared to those without tumors. No differences in liver or spleen weights were observed when comparing dietary treatments in mice with tumors, and likewise in mice without tumors.

Table 2.

Differences in spleen and liver weight on d 70 postinoculatoin in mice bearing or not bearing mammary tumors[1]

	n	Liver weight, g	Spleen weight, g
Non tumor-bearing	49	1.06 ± 0.06[b]	0.067 ± 0.01[b]
Tumor-bearing	51	1.48 ± 0.12[a]	0.244 ± 0.03[a]

F2-1

Values are means ± SEM. Means in a column with different superscripts differ significantly ($P < 0.05$).

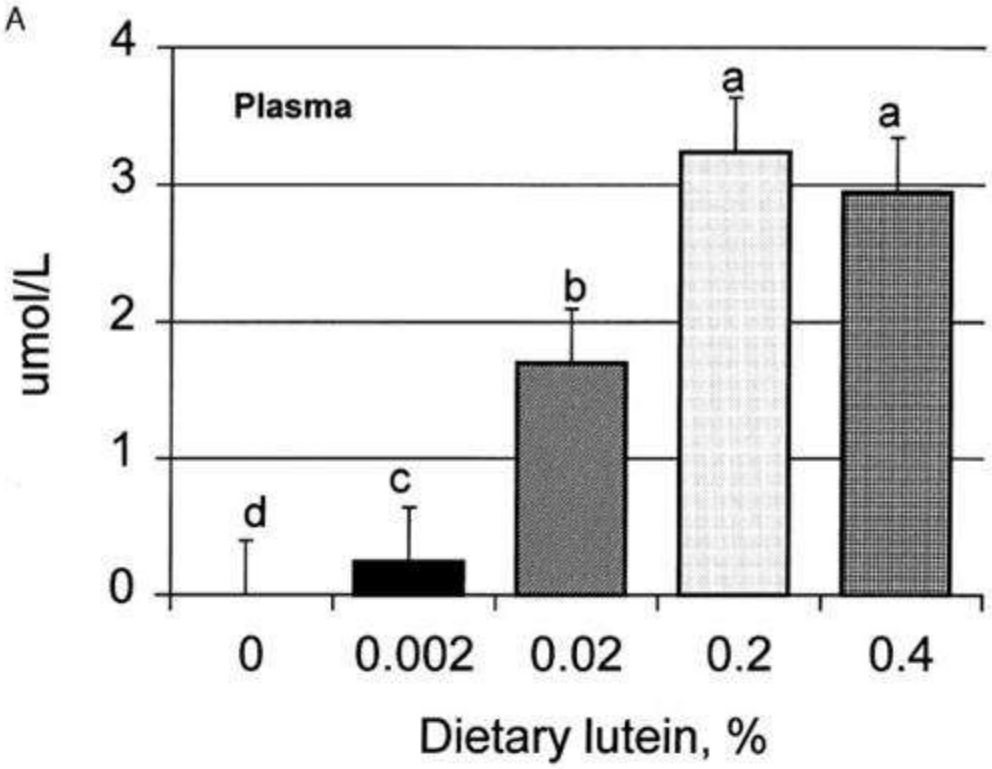
Open in new tab

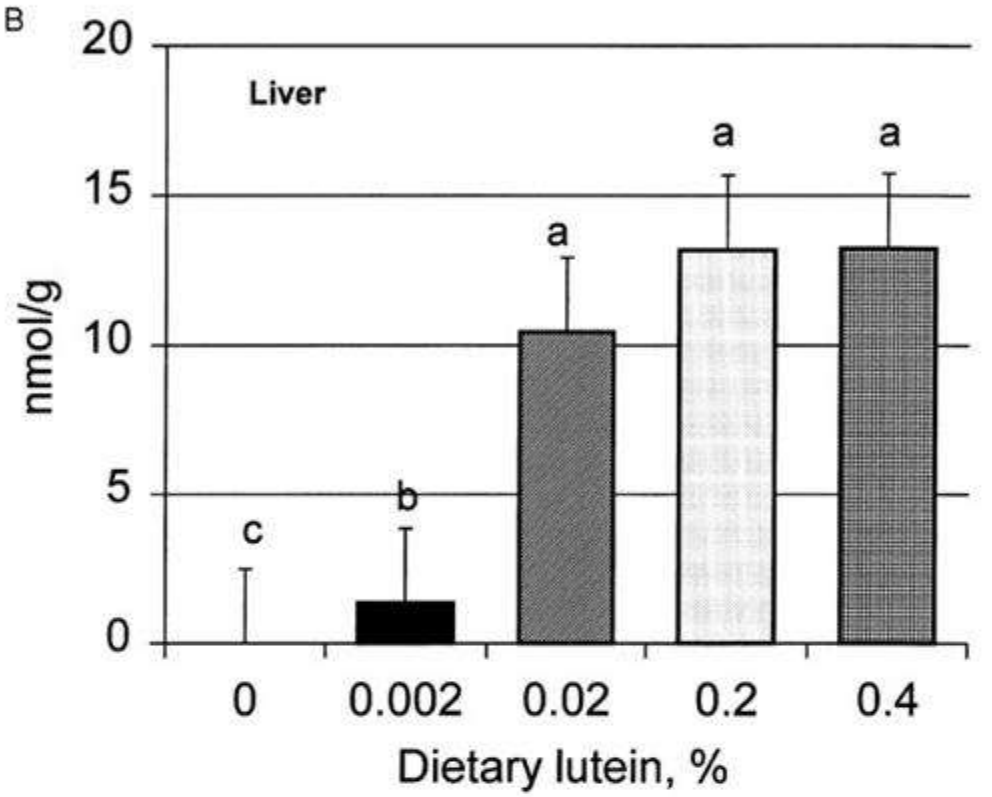
There were no significant treatment differences in total food intake throughout the experimental period (Table 1). Total lutein intake among the treatment groups reflected the level of lutein supplementation ($P < 0.05$, Table 1). Therefore, the level of dietary lutein did not significantly affect body weight, food intake, or liver and spleen weight.

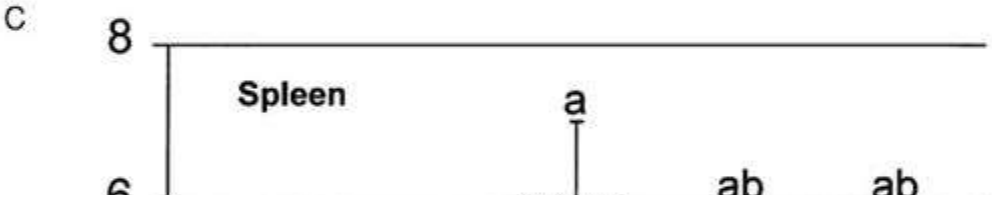
Lutein uptake.

Uptake of lutein + zeaxanthin by plasma, liver, spleen and tumor at the termination of the study (d 70 after inoculation) is illustrated. Lutein + zeaxanthin was not detectable in plasma and tissues of unsupplemented mice (Fig. 3, Table 3). Concentrations of plasma lutein + zeaxanthin increased ($P < 0.05$) in a dose-dependent manner through dietary lutein supplementation levels of 0.2% (Fig. 3). Dietary lutein supplementation of 0.4% did not increase plasma lutein + zeaxanthin concentrations further.

Figure 3


A
Plasma
umol/L
4
3
2
1
0
a
a
b
c
d
0
0.002
0.02
0.2
0.4
Dietary lutein, %


B
Liver
nmol/g
20
15
10
5
0
a
a
a
b
c
0
0.002
0.02
0.2
0.4
Dietary lutein, %


C
Spleen
8
a
ab
ab

Open in new tabDownload slide

Lutein + zeaxanthin concentrations in (A) plasma (B) liver, (C) spleen and (D) tumors of mice fed diets containing 0, 0.002, 0.02, 0.2 and 0.4% of lutein for 84 d. Values are means ± pooled SEM (n = 20/group). Lutein was not detectable in unsupplemented mice and a value of 0.06 μmol/L (plasma) or 0.001 nmol/g (tissues) was given, which is the detection limit. Different letters associated with each bar denote significant difference ($P < 0.05$).

Table 3.

Total lutein uptake by the liver, spleen and tumor of mice fed diets containing 0, 0.002, 0.02, 0.2 and 0.4% of lutein for 84 d[1]

	Treatment (% lutein)				
	0	**0.002**	**0.02**	**0.2**	**0.4**
Liver, *μmol*	0[c]	1.6 ± 0.7[b]	11.9 ± 4.0[a]	17.2 ± 4.0[a]	16.5 ± 6.0[a]
Spleen, μmol	0[c]	0.25 ± 0.07[b]	0.40 ± 0.10[ab]	0.59 ± 0.15[a]	0.44 ± 0.17[ab]
Tumor, *nmol*	0[d]	0.30 ± 0.10[c]	1.42 ± 0.46[b]	4.53 ± 0.70[a]	0.35 ± 0.10[c]

F3-1

Values are means ± SEM (n = 20/group for liver and spleen, n = 8–14 for the tumor). Means in a row with different superscripts differ significantly ($P < 0.05$).

Open in new tab

The concentration (Fig. 3) and total uptake (Table 3) of lutein + zeaxanthin by the liver increased dose-dependently in mice fed 0 to 0.2% of dietary lutein. Like plasma, there was no further increase in liver lutein + zeaxanthin concentrations in mice fed 0.4% of lutein.

In the spleen, the concentration (Fig. 3) and total content (Table 3) of lutein + zeaxanthin increased dose-dependently ($P < 0.05$) in mice fed up to 0.02% of lutein, with no further increase observed with dietary lutein above 0.02%.

The uptake of lutein + zeaxanthin by tumors was similar to that observed with liver although the concentration of lutein + zeaxanthin in liver was about five-fold higher than that of tumors. There was a dose-related ($P < 0.05$) increase in tumor lutein + zeaxanthin concentrations (Fig. 3) and in total lutein + zeaxanthin uptake (Table 3) with dietary supplementation of up to 0.2% of lutein.

Mammary tumor growth.

Dietary lutein inhibited the growth and establishment of mammary tumors in BALB/c mice inoculated with 2.5 × 10[3] −SA mammary tumor cells. The incidence and latency of mammary tumors in unsupplemented mice (Table 4) were similar to that observed in Experiment 1. Mice not supplemented with lutein reached a maximal tumor incidence of 70% by d 50 postinoculation whereas only 20 to 37% of mice fed 0.02 to 0.4% of lutein produced tumors ($P < 0.05$, Fig. 4). Interestingly, none of the mice fed 0.002% of lutein had palpable tumors prior to d 50 postinoculation. Tumor incidence in mice fed lutein gradually increased until the end of the study period. However, final tumor incidence on d 70 (termination of the experiment) was higher ($P <$ 0.05) in unsupplemented mice than in mice fed 0.002 or 0.4% of lutein (Table 4).

Table 4.

Mammary tumor growth on d 70 postinoculation in mice fed diets containing 0, 0.002, 0.02, 0.2 and 0.4% of lutein[1]

	Treatment (% lutein)				
Measure	**0**	**0.002**	**0.02**	**0.2**	**0.4**
Tumor incidence,[4-2]*%*	70[a]	40[b]	45[ab]	55[ab]	40[b]
Tumor latency, *d*	52 ± 4[c]	71 ± 2[a]	64 ± 4[b]	60 ± 4[bc]	67 ± 4[b]
Tumor weight, *g*	2.90 ± 0.8[a]	0.39 ± 0.2[c]	0.64 ± 0.3[c]	1.47 ± 0.3[b]	0.90 ± 0.4[bc]
Tumor volume, *cm3*	1.75 ± 0.3[a]	0.57 ± 0.2[d]	0.68 ± 0.2[d]	1.24 ± 0.2[b]	0.72 ± 0.3[c]

F4-1

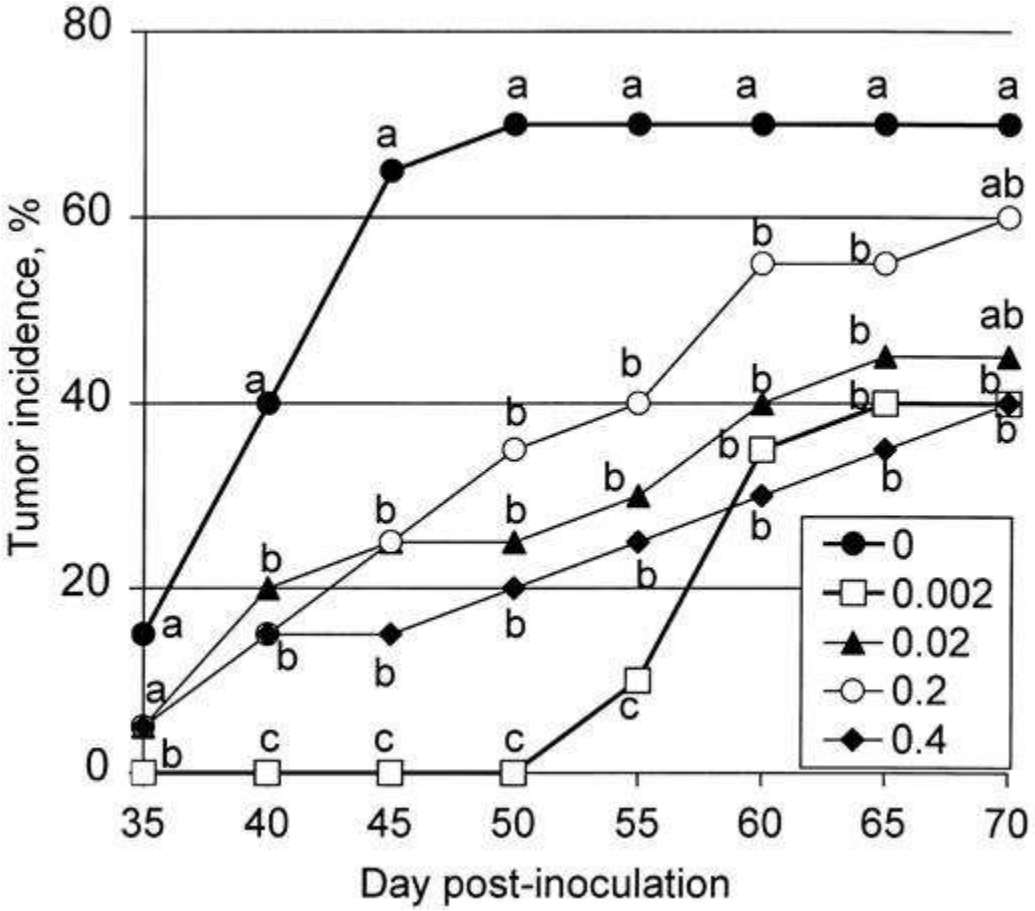
Values are means ± SEM (n = 20/group). Means in a row with different superscripts differ significantly ($P < 0.05$).

F4-2

Calculated by expressing the number of mice with measurable tumors as a percentage of total number of mice. Each tumor-bearing mouse had one tumor. The total number of mice/treatment = 20 for all groups. Treatment differences were compared using Chi-square.

Open in new tab

Figure 4

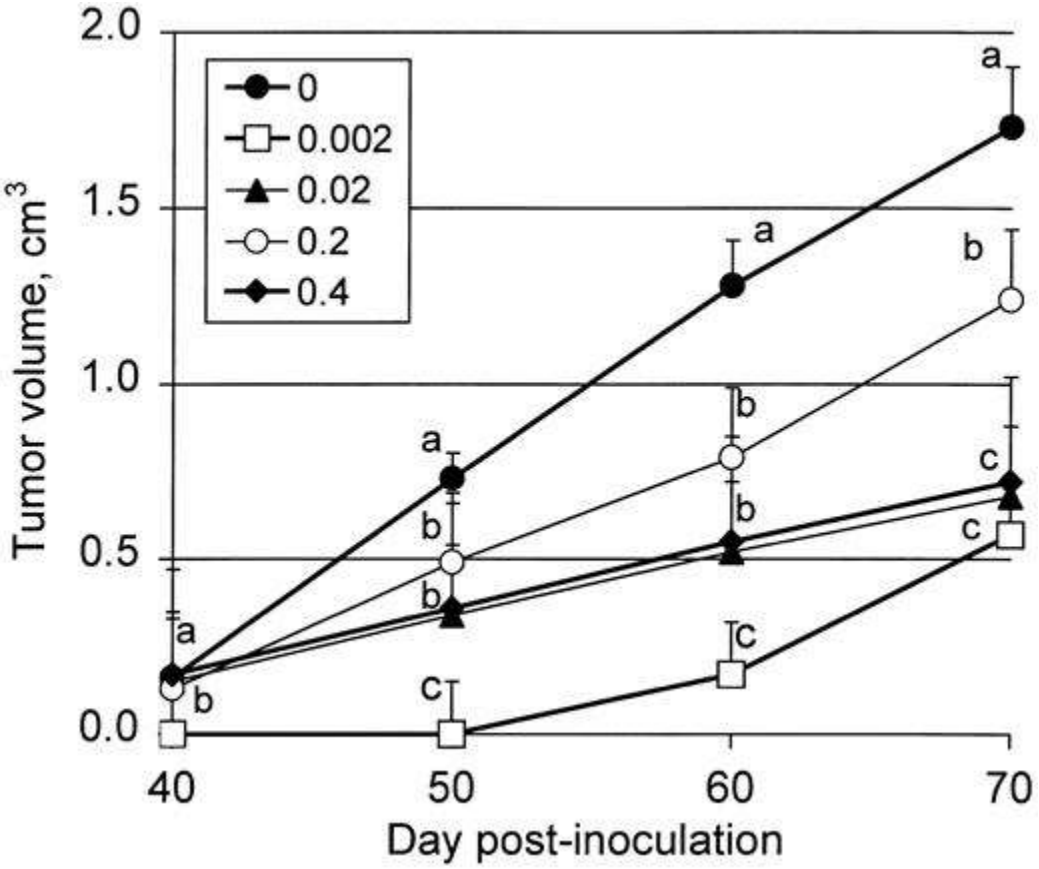


Open in new tabDownload slide

Tumor incidence in mice fed 0, 0.002, 0.02, 0.2 and 0.4% of lutein and challenged with 2.5 × 10^3 mammary tumor cells/inoculation. Tumor incidence (%) was calculated by expressing the number of mice with measurable tumors as a percentage of the total number of mice (n = 20/treatment). Treatment differences, indicated by no letters in common at a time point, were identified using Chi-square.

Tumor latency averaged 15 d longer ($P < 0.05$) in lutein-supplemented mice than in control mice (Table 4). Mice fed 0.002% of lutein had a longer tumor latency period than all other groups. Although mammary tumor incidence in control mice reached a maximal level of 70% by d 50 (Fig. 4), tumor volume continued to increase until animals were terminated on d 70 (Fig. 5). Final tumor volume and tumor weight were greater ($P < 0.05$) in control mice than in mice fed lutein (Table 4). Tumor growth throughout the study period was slowest in mice fed 0.002% of lutein.

Figure 5



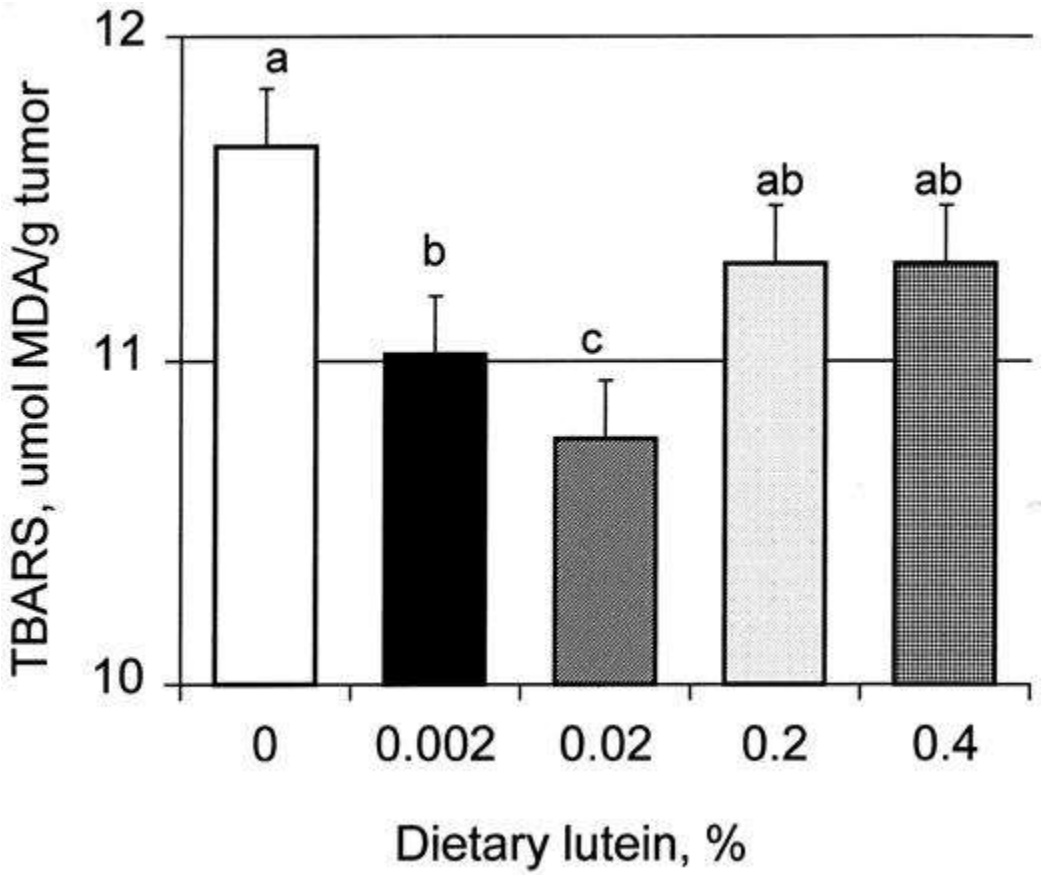
Open in new tabDownload slide

Tumor volume (calculated by using the volume of a sphere) in mice fed 0, 0.002, 0.02, 0.2 and 0.4% of lutein and challenged with 2.5 × 10$_3$ mammary tumor cells/inoculation. All mice (n = 20/group) were included in the calculation, with mice not bearing tumors given a tumor volume value of zero. Values are means ± pooled SEM (repeated measures analysis of variance). Means at a time point with no letters in common differ significantly, $P < 0.05$.

TBARS.

Lipid peroxidation activity in mammary tumors as measured by the TBARS assay decreased ($P <$ 0.05) in a linear fashion in mice fed from 0 to 0.02% of lutein (Fig. 6). However, mice fed higher levels of lutein (0.2 or 0.4%) had TBARS values that did not differ from that of the control group. The changes in TBARS activity (lowest in mice fed 0.02% of lutein) did not reflect the uptake of lutein by tumors which increased in a dose-dependent manner through 0.2% of lutein. Lipid peroxidation activity, expressed as the difference in optical density between 532 and 520 nm, showed similar trends (data not shown).

Figure 6



Open in new tabDownload slide

Lipid peroxidation as measured using thiobarbituric acid reactive substance (TBARS) in tumors from mice fed 0, 0.002, 0.02, 0.2 and 0.4% of lutein. Values are means ± SEM (n = 8–14/group). Means with no letters in common differ significantly, $P < 0.05$.

DISCUSSION

Chew et al. (1996) reported that dietary lutein inhibited the growth of mammary tumors in mice. Their study used very high amounts of supplemental lutein (0.1 and 0.4%) and a high mammary tumor challenge dose (1×10^6 −SA tumor cells). In fact, 100% of the mice in all treatment groups developed tumors and consequently did not permit the assessment of the possible effects of lutein in preventing the establishment of mammary tumor cells inoculated into the mammary fat pad. The present study was designed to refine these findings by *i*) selecting a more optimal tumor challenge dose to assess the inhibitory action of lutein against the establishment of mammary tumor cells prior to the promotional stage and *ii*) using lower amounts of dietary lutein. A dose-titration study (Experiment 1) showed that 65% of mice developed tumors when challenged with 2.5×10^3 −SA mammary tumor cells. Therefore, a challenge dose of 2.5×10^3 cells was used in Experiment 2 to study the activity of dietary lutein in inhibiting the development and growth of mammary tumors.

In this study, dietary lutein did not significantly influence body weight and feed intake. Liver and spleen weights were generally higher in control mice than in those fed lutein. However, differences in liver and spleen weights reflected mammary tumor development rather than dietary treatment. Within a treatment group, mice bearing tumors had larger liver and spleen than those not bearing tumors. This is expected because hepatomegaly and splenomegaly are conditions that result from cancer development. These observations suggest a lack of adverse effects of dietary lutein from marigold extract and is in agreement with other reports (CARIG 1996, Chew et al. 1996, Gartner et al. 1996).

Measuring the uptake of lutein by the tumor, liver and spleen allowed us to determine the tissue that correlates with the antitumor activity of lutein. The antitumor activity of lutein could indeed be mediated through immune modulation that involves other organs. Uptake of dietary lutein + zeaxanthin increased in a dose-dependent manner in plasma, liver and tumor in mice fed 0 through 0.2% of lutein. In contrast, uptake of lutein + zeaxanthin by the spleen was maximal in mice fed 0.02% lutein. A previous study using similar experimental conditions showed very rapid uptake of lutein + zeaxanthin by plasma, liver and spleen (Park et al. 1998). In that study, plasma lutein + zeaxanthin was saturated by d 7 in mice fed 0.05 to 0.4% of lutein. In contrast, lutein + zeaxanthin concentrations in the liver and particularly in the spleen generally continued to increase through the 28-d study period. In addition, uptake of lutein + zeaxanthin by the spleen was generally dose-dependent through 28 d of feeding. Mice fed the highest dose of lutein (0.4%) had the highest accumulation of lutein + zeaxanthin (0.015 nmol/g spleen) in the spleen (Park et al. 1998). In the present study, mice were fed lutein for 84 d. Uptake of lutein + zeaxanthin by the spleen was dose-dependent with dietary lutein levels of up to 0.02%. Feeding doses higher than 0.02% did not result in greater accumulation of lutein + zeaxanthin in the spleen, thereby indicating maximal lutein + zeaxanthin uptake by the spleen with dietary lutein of 0.02%. It is entirely possible that results on lutein + zeaxanthin uptake by the spleen in this study would be similar to that reported by Park et al. (1998) if the feeding period (28 d) had been the same. Overall, the concentration of lutein + zeaxanthin in the tumor in this study was 50–80% lower than in the spleen and liver, respectively.

Results of the present study showed that dietary lutein reduced mammary tumor growth and development. Tumor incidence, weight and volume generally were lower and tumor latency longer in lutein-fed mice. The highest antitumor activity was observed in mice fed 0.002% of lutein. Chew et al. (1996) similarly showed decreased tumor growth in mice fed lutein. In the latter study, very high amounts of lutein were fed (0.1 and 0.4%) and a very high challenge dose (1×10^6 cells) was inoculated. Results from the present study are in general agreement with Chew et al. (1996) for mice fed similar amounts of lutein. However, as expected, mammary tumor growth and development differed between the two studies due to a much lower challenge dose (1×10^6 vs. 2.5×10^3 tumor cells) used in the present study. Because the lower tumor challenge dose was expected to produce a tumor incidence of about 65% on d 50 postinoculation, it permitted us to study if dietary lutein could prevent the initial establishment of the tumor cells rather than inhibit tumor growth. Indeed, mice fed lutein, especially those fed 0.002% of lutein, had a lower incidence of mammary tumors on d 70 postinoculation. This suggests, for the first time, that lutein is not only capable of inhibiting mammary tumor growth but possibly of preventing tumor initiation. Krinsky (1989) suggested that carotenoids, specifically β-carotene and canthaxanthin, exert their effects by interfering with the promotional phase of carcinogenesis. Therefore, it is likely that the latter carotenoids (β-carotene and canthaxanthin) may exert different biological actions than that of lutein. Alternatively, definitive studies on the possible action of β-carotene and canthaxanthin in inhibiting carcinogenesis prior to the promotional stage of tumor development have not been conducted.

Lutein may exert its antitumor activity through its antioxidant function. In vitro, lutein protects human liver cells against oxidant-induced damage (Martin et al. 1996). Lutein is capable of quenching singlet oxygen even though its activity is lower than that of β-carotene (Di Mascio et al. 1989). While lipid peroxidation may be positively related to tumor growth, Chew et al. (1996) reported that the TBARS activity of tumors from mice fed 0.1 or 0.4% of lutein was not influenced even though dietary lutein inhibited tumor growth. Results from the present study showed lower TBARS levels in tumors from mice fed 0.002 or 0.02% of lutein compared to unsupplemented mice. However, TBARS activity increased in mice fed higher levels of dietary lutein (0.2 and 0.4%) even though total accumulation of lutein in tumors increased dose-dependently. Tumor growth inhibition also was higher in mice fed the lower levels of dietary lutein

(especially those fed 0.002% of lutein) compared to those fed 0.2 or 0.4% of lutein. Therefore, these studies suggest that tumor lipid peroxidation activity may be positively correlated with tumor growth only with lower levels of dietary lutein supplementation, whereas higher dietary lutein levels (0.02%) may result in increased lipid peroxidation activity and lower tumor growth inhibition. Indeed, the lack of positive correlation between lipid peroxidation activity and tumor growth reported by Chew et al. (1996) may be due to the high (0.1 to 0.4%) levels of supplemental lutein. High levels of dietary lutein, and therefore, high concentrations of lutein in tissues may indeed create a pro-oxidant instead of an antioxidant environment (Crabtree and Adler 1997).

These findings may be relevant to two recent studies that reported negative effects of supplemental β-carotene on lung cancer. The ATBC study (Albanes et al. 1995) showed an 18% increase in the incidence of lung cancer among heavy smokers given 20 mg of β-carotene daily for 5 to 8 y. The CARET study (Omenn et al. 1996) reported 28% more lung cancer cases and 17% more deaths among heavy smokers and asbestos-exposed workers given 30 mg of β-carotene plus 25,000 of IU retinyl palmitate daily. The possibility remains that the level of β-carotene supplementation in these human studies may be too high (coupled with a long supplementation period), thereby producing an adverse instead of a beneficial effect. Indeed, the antitumor and antilipid peroxidation activity of dietary lutein observed in the present study was diminished or even abolished in mice fed higher levels of lutein.

The orientation of the various carotenoids in the membrane lipid bilayer may explain their protection against free radical-induced damage. For example, zeaxanthin is more effective than β-carotene because the zeaxanthin molecule is anchored in both polar sides of the membrane bilayer, thereby providing an exceptionally good condition for the rigidifying effect of zeaxanthin in the hydrophobic part of the membrane (Gabrielska & Gruszecki 1996). Aside from its potential antioxidant function, lutein may act through other mechanisms. These include immunomodulation (Chew 1993), cell-to-cell communication (Bertram and Bortkiewicz 1995) and prostaglandin production (ElAttar and Lin 1991).

Previous studies have demonstrated anticancer activities of carotenoids other than β-carotene. For example, canthaxanthin is more effective than β-carotene in delaying skin tumor growth in mice (Krinsky 1989), while lycopene is more active than α- and β-carotene in inhibiting human cancer cell proliferation (Levy et al. 1995). Neoxanthin is effective as a chemopreventative agent in reducing cancer in hamster buccal pouch (Chang et al. 1995). Our studies (this study and Chew et al. 1996) have similarly demonstrated that the oxycarotenoid, lutein, can inhibit the growth of mammary cancer.

In summary, low amounts of dietary lutein from marigold extract can inhibit the development and growth of mammary tumors in mice.

LITERATURE CITED

1
Albanes
,
D.
,
Heinonen
,
O.
,
Huttunen
,
J. K.
,

Taylor
,
P. R.
,
Virtamo
,
J.
,
Edwards
,
B. K.
,
Haapakoski
,
J.
,
Rautalahti
,
M.
,
Hartman
,
A. M.
,
Palmgren
,
J.
&
Greenwald
,
P.
(
1995
)
Effects of α-tocopherol and β-carotene supplements on cancer incidence in the alpha-tocopherol beta-carotene cancer prevention study
.
Am. J. Clin. Nutr.

62
(suppl):
1427S
–
1430S
.
Google Scholar
Crossref

PubMed

2
Bertram

J. S.

Bortkiewicz

H.
Dietary carotenoids inhibit neoplastic transformation and modulate gene expression in mouse and human cells
.
Am. J. Clin. Nutr.
62
1995
1327S
1336S
Google Scholar
Crossref
PubMed

3
Burri

B. J.
Beta-carotene and human health: a review of current research
.
Nutr. Res.
17
1997
547
580
Google Scholar
Crossref

4
Chang

J.

Chen

W.

Hong

D.

Lin

J.
The inhibition of DMBA-induced carcinogenesis by neoxanthin in hamster buccal pouch
.
Autr. Cancer
24
1995
325
333
Google Scholar

5
Chew

B. P.
Role of carotenoids in the immune response
.
J. Dairy Sci.
76
1993
2804
2811
Google Scholar
Crossref
PubMed

6
Chew

B. P.

Wong

M. W.

Wong

T. S.
Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice
.
Anticancer Res.
16
1996
3689
3694
Google Scholar
PubMed

7
Crabtree

D. V.

Adler

A. J.
Is β-carotene an antioxidant?
Med. Hypoth.
48
1997
183
187
Google Scholar
Crossref

8
Danielson

K. G.

Anderson

L. W.

Hosick

H. L.
Selection and characterization in culture of mammary tumor cells with distinctive growth properties in vivo
.
Cancer Res.
40
1980
1812
1819
Google Scholar
PubMed

9
Di Mascio

P.

Kaiser

S.

Sies

H.
Lycopene as the most efficient biological carotenoid singlet oxygen quencher
.
Arch. Biochem. Biophys.
274
1989
532
583
Google Scholar
Crossref
PubMed

10
El Attar

T. M.

Lin

H. S.
Effect of retinoids and carotenoids on prostaglandins formation by oral squamous carcinoma cells
.
Prostagl. Leukotrienes and Essential Fatty Acids
34
1991
175
178
Google Scholar
Crossref

11
Flagg

E. W.

Coates

R. J.

Greenberg

R. S.
Epidemiological studies of antioxidants and cancer in humans
.
J. Am. Coll. Nutr.

14
1995
419
427
Google Scholar
Crossref
PubMed

12
Gabrielska

J.

Gruszecki

W. I.
Zeaxanthin (dihydroxy-β-carotene) but not β-carotene rigidifies lipid membranes: a 1H-NMR study of carotenoid-egg phosphatidylcholine liposomes
.
Biochim. Biophys. Acta
1285
1996
167
174
Google Scholar
Crossref
PubMed

13
Gartner

C.

Stahl

W.

Sies

H.
Preferential increase in chylomicron levels of the xanthophylls lutein and zeaxanthin compared to β-carotene in the human
.
Internat. J. Vit. Nutr. Res.
66
1996
119
125

Google Scholar

14
Hennekens

C. H.

Buring

J. E.

Manson

J. E.

Stampfer

M.

Rosener

B.

Cook

N. R.

Belanger

C.

Lamotte

F.

Gaziano

J. M.

Ridker

P. M.

Willett

W.

Peto

R.
Lack of effect of long-term supplementation with beta-carotene on the incidence of malignant neoplasm and cardiovascular disease
.
N. Engl. J. Med.
334
1996
1145
1149
Google Scholar
Crossref
PubMed

15
Krinsky

N. I.
Carotenoids and cancer in animal models
.
J. Nutr.
119
1989
123
126
Google Scholar
Crossref
PubMed

16
Kushi

L. H.

Fee

R. M.

Sellers

T. A.

Zheng

W.

Folsom

A. R.

Intake of vitamins A, C, and E and postmenopausal breast cancer
.
Am. J. Epidemiol.
144
1996
165
174
Google Scholar
Crossref
PubMed

17
Levy

J.

Bosin

E.

Feldman

B.
Giat
y.
Miinster

A.

Danilenko

M.

Sharoni

Y.
Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene
.
Nutr. Cancer
24
1995
257
266
Google Scholar
Crossref
PubMed

18

Martin

K. R.

Eailla

M. L.

Smith

J. C.
β-Carotene and lutein protect HepG2 human liver cells against oxidant-induced damage
.
J. Nutr.
126
1996
2098
2106
Google Scholar
PubMed

19
Omenn

G. S.

Goodman

G. E.

Thornqvist

M. D.

Balmes

J.

Cullen

M. R.

Glass

A.

Keogh

P. J.

Meyskens

F. L.

Valanis

B.

Williams

J. H.

Barnhart

S.

Hammar

S.
Effects of a combination of beta-carotene and vitamin A on lung cancer and cardiovascular disease
.
N. Engl. J. Med.
334
1996
1150
1155
Google Scholar
Crossref
PubMed

20
Park
,
J. S.
,
Chew
,
B. C.
&
Wong
,
T. S.
(
1998
)
Dietary lutein absorption from marigold extract is rapid in BALB/c mice

.
J. Nutr.

128
: (in press).
Google Scholar

21
Parker

R. S.
Carotenoids in human blood and tissues
.
J. Nutr.
119
1989
101
104
Google Scholar
Crossref
PubMed

22
Poppel

G.

Goldbohm

A.
Epidemiologic evidence for β-carotene and cancer prevention
.
Am. J. Clin. Nutr.
62
1995
1393S
1402S
Google Scholar
Crossref
PubMed

23
Ram

T. G.

Reeves

R.

Hosick

H. L.
Elevated high mobility group-I(Y) gene expression is associated with progressive transformation of mouse mammary epithelial cells
.
Cancer Res.
53
1993
2655
2659
Google Scholar
PubMed

24
Rock

C. L.

Saxe

G. A.

Ruffin

M. T.

August

D. A.

Schottenfels

D.
Carotenoids, vitamin A, and estrogen receptor status in breast cancer
.
Nutr. Cancer
25
1996
281
296
Google Scholar
Crossref
PubMed

25

SAS

1991
.
Users Guide (Release 6.03).

SAS Inst. Inc.
,
Cary, NC
.
Google Scholar

26
Snodderly
,
D. M.
(
1995
)
Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins
.
Am. J. Clin. Nutr.

62
(suppl):
1448S
–
1461S
.
Google Scholar
Crossref
PubMed

27
Steel
,
R. G. D.
&
Torrie
,
J. H.
(
1980
)
Principles and procedures of statistics. A Biometrical Approach.
pp.
477
–

494
.
McGraw-Hill Book Co.
,
New York, NY
.
Google Scholar

28
The Carotenoid Research Interactive Group (CARIG)
Beta-carotene and the carotenoids: beyond the intervention trials
.
Nutr. Rev.
54
1996
185
188
PubMed

29
Uchiyama

M.

Mihara

M.
Determination of malondialdehyde precursor in tissues by thiobarbituric acid test
.
Anal. Biochem.
86
1978
271
278
Google Scholar
Crossref
PubMed

30
Verhoeven

D. T. H.

Assen

N.

Goldbohm

R. A.

Dorant

E.
van't Veer
P.
Sturmans

F.

Hermus

R. J. J.

van den Brandt

P. A.
Vitamins C and E, retinol, beta-carotene and dietary fiber in relation to breast cancer risk: a prospective cohort study
.
Brit. J. Cancer
75
1997
149
155
Google Scholar
Crossref

31
Zhang

L.-X.

Cooney

R. V.

Bertram

J. S.
Carotenoids enhanced gap junctional communication and inhibit lipid peroxidation in C3H/10T/2 cells: relationship to their cancer chemopreventative action
.
Carcinogenesis
12
1991
109

114
Google Scholar

32
Ziegler

R. G.
A review of epidemiologic evidence that carotenoids reduce the risk of cancer
.
J. Nutr.
119
1989
116
122
Google Scholar
Crossref
PubMed