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# Inhibitory Effects of Orally Administered Green Tea, Black Tea, and Caffeine on Skin Carcinogenesis in Mice Previously Treated with Ultraviolet B Light (High-Risk Mice): Relationship to Decreased Tissue Fat[1]

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## ABSTRACT

**Treatment of SKH-1 hairless mice with ultraviolet B light (UVB; 30 mJ/cm$^2$) twice a week for 22 weeks resulted in tumor-free animals with a high risk of developing malignant and nonmalignant skin tumors during the next several months in the absence of additional UVB treatment (high-risk mice). Oral administration of green tea or black tea (6 mg tea solids/ml) to UVB-pretreated high-risk SKH-1 mice for 23 weeks after stopping UVB treatment decreased the number of tumors/mouse, decreased the size of the parametrial fat pads, and decreased the thickness of the dermal fat layer away from tumors and directly under tumors. Administration of the decaffeinated teas had little or no effect on these parameters, and adding caffeine (equivalent to the amount in the regular teas) to the decaffeinated teas restored their inhibitory effects. Administration of caffeine alone also decreased the number of tumors/mouse, the size of the parametrial fat pads, and the thickness of the dermal fat layer away from tumors and under tumors. Using data from individual mice and linear regression and correlation analysis, we found a highly significant positive correlation between the thickness of the dermal fat layer away from tumors and the number of tumors/mouse ($r = 0.34$; $P = 0.0001$), but the correlation between average tumor size/mouse and the thickness of the dermal fat layer away from tumors was weak ($r = 0.16$; $P = 0.034$). The results suggested that p.o. administered tea or caffeine may have decreased tumor multiplicity in part by decreasing fat levels in the dermis. Additional analysis revealed that oral administration of caffeinated beverages (green tea, black tea, decaffeinated green tea plus caffeine, decaffeinated black tea plus caffeine, or caffeine alone) decreased the thickness of the dermal fat layer under large tumors to a much greater extent than under small tumors. This is the first demonstration of a close association between inhibition of carcinogenesis and the lowering of tissue fat levels by a chemopreventive agent.**

## INTRODUCTION

Sunlight-induced skin cancer is the most prevalent type of human cancer in the United States and in many other temperate parts of the world (1–3). UVB[3] and, to a much lesser extent, UVA are responsible for sunlight-induced cancers (4, 5). The incidence of skin cancer appears to be increasing and is predicted to increase even further because of an increase in recreational exposure to sunlight and also because of the depletion of the stratospheric ozone layer (6, 7).

In an earlier cancer chemoprevention study, we exposed SKH-1 mice to UVB (30 mJ/cm$^2$) twice a week for 22 weeks, and then UVB irradiation was stopped. The mice were tumor-free, but they had hyperplasia and a high risk of developing skin tumors during the next several months in the absence of additional treatment with UVB ("high-risk mice;" Ref. 8). This is a useful animal model that may be comparable with humans previously exposed to moderate/high levels of sunlight who have a high risk of developing skin cancers later in life even in the absence of continued heavy sunlight exposure.

We found that p.o. administration of green tea or black tea to these high-risk mice inhibited the formation and size of malignant and nonmalignant tumors (8). The decaffeinated teas were inactive or less effective inhibitors of tumor formation than the regular teas, and adding caffeine back to the decaffeinated teas restored biological activity (8). Administration of caffeine alone also had a strong inhibitory effect on tumorigenesis in high-risk mice. In the present report, we describe the results of additional studies with tissues from the same mice used in our earlier cancer chemoprevention study. The additional studies indicate a strong relationship between the inhibitory effects of p.o. administered tea, decaffeinated tea plus caffeine, or caffeine alone on skin carcinogenesis and their effects to decrease the size of the parametrial fat pad and to decrease the thickness of the dermal fat layer both away from and directly under tumors. These effects on fat disposition were not observed with the decaffeinated teas.

## MATERIALS AND METHODS

**Chemicals and Tea Solutions.** Lyophilized regular teas and lyophilized decaffeinated teas (prepared by supercritical $CO_2$ extraction) were obtained from the Thomas J. Lipton Company (Englewood Cliffs, NJ), and caffeine was obtained from the Sigma Chemical Co. (St. Louis, MO).

Purified water was prepared by reverse osmosis and used for the preparation of all tea infusions and caffeine solutions. Solutions of caffeine (0.44 mg/ml water; 0.044% solutions) or solutions of lyophilized tea solids (6 mg/ml water; 0.6% solutions) were prepared every 2 days and were used as the sole source of drinking fluid. In these studies, the concentrations of tea solids and caffeine were about 50% higher than normally consumed by humans. The concentration of caffeine (0.44 mg/ml) was the same as that in the 0.6% green tea and black tea solutions.

**Treatment of the Mice.** Female SKH-1 hairless mice (6–7 weeks of age) were obtained from the Charles River Breeding Laboratories (Kingston, NY) and fed Purina Laboratory Chow 5001 diet (Ralston-Purina, St. Louis, MO) *ad libitum*. The mice were treated with UVB (30 mJ/cm$^2$) twice a week for 22 weeks, as described earlier (8–10). For these studies, UV lamps (FS72T12-UVB-HO) that emit UVB (280–320 nm; 75–80% of total energy) and UVA (320–375 nm; 20–25% of total energy) were obtained from the Voltare Co. (Fairfield, CT). When one animal developed a small skin nodule, UVB treatment was stopped and the mouse was discarded. The remaining treated mice without tumors and with a high risk for the development of tumors in the absence of continued UVB exposure were then given various teas or caffeine solutions as their sole source of drinking fluid in the absence of additional UVB treatment. In these studies, the mice were given tea solutions or caffeine that were 25%, 50%, and 75% of full strength (2 days at each concentration), and then full strength solutions were given until the animals were killed after 23 weeks of treatment with the teas or caffeine. None of the treatments had an effect on the food intake or body weight of the mice.

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[3] The abbreviations used are: UVB, ultraviolet B light; UVA, ultraviolet A light; EGCG, (−)-epigallocatechin gallate; CLA, conjugated linoleic acid.

**Tumor Size, Parametrial Fat Pad Size, and Histopathology Studies.** For studies on tumor size, parametrial fat pad size and histopathology studies, the animals were killed, the size of the parametrial fat pads was estimated, and dorsal skins were removed and stapled flat to a plastic sheet before they were placed in 10% buffered formalin phosphate at 4°C for 18–24 h for histological examination. Tumor diameter was determined for each mass by measuring the three-dimensional size (height, length, and width) of each mass before making histological sections and by using the average of the three measurements as the diameter. The formalin-treated skin samples were dehydrated in ascending concentrations (80%, 95%, and 100%) of ethanol, cleared in xylene, and embedded in Paraplast. Four-$\mu$m serial sections were made for regular H&E-phloxine staining. The tumors were characterized by histological criteria, and the thickness of the dermal fat layer was determined directly under tumors and in nontumor areas of the skin (>0.5 cm away from tumors) using a light microscope with an ocular micrometer and ×100 magnification.

**Statistical Methods.** Student's *t* test was used for simple comparisons of two groups. The ANOVA models with Dunnett's adjustments (11, 12) were used for the comparisons of multiple treatment groups with the common control (water) group. The Fisher's exact test was used for the comparison of the number of mice with tumors in the different groups.

The random coefficient (hierarchical linear) model was used to study the treatment effect on the relationship between the thickness of the dermal fat layer under tumors and the tumor diameter (13, 14), where the response variable was the logarithm of thickness of the dermal fat layer under tumors and the covariate was the tumor diameter. The mixed (repeated measurement) models (15, 16) were also used to study the relationship between tumor diameter and the thickness of dermal fat layer under tumors. The linear regression models and the Pearson correlation coefficients (17) were also used to study the relation between variables.

## RESULTS

**Effects of Tea, Decaffeinated Tea, and Caffeine on Skin Carcinogenesis, the Size of the Parametrial Fat Pads, and the Thickness of the Dermal Fat Layer in High Risk Mice.** p.o. administration of green tea or black tea (6 mg tea solids/ml) for 23 weeks to high-risk mice decreased the number of tumors/mouse by 66–68%, the size of the parametrial fat pads by 32–54%, the thickness of the dermal fat layer away from tumors by 39–46%, and the thickness of the dermal fat layer directly under tumors by 49–53% (Tables 1 and 2). Administration of the decaffeinated teas had little or no effect on any of these parameters, and adding caffeine (equivalent to the amount in the regular teas) to the decaffeinated teas restored their inhibitory effects (Table 2). Administration of caffeine alone (0.44 mg/ml) decreased the number of tumors/mouse by 61%, the size of the parametrial fat pads by 56%, the thickness of the dermal fat layer away from tumors by 40%, and the thickness of the dermal fat layer directly under tumors by 49% (Table 2). A representative illustration of the effect of p.o. administered green tea on the thickness of the dermal fat layer away from tumors and under tumors is shown in Fig. 1. Although only the regular teas, caffeine, or the decaffeinated teas plus caffeine decreased tumor multiplicity in high-risk mice, tumor size (per tumor) was decreased by the administration of the regular teas, the decaffeinated teas, and caffeine (Table 1). The number of mice with tumors was decreased significantly ($P < 0.05$) in mice treated with green tea, caffeine, decaffeinated green tea plus caffeine, or decaffeinated black tea plus caffeine (Table 1).

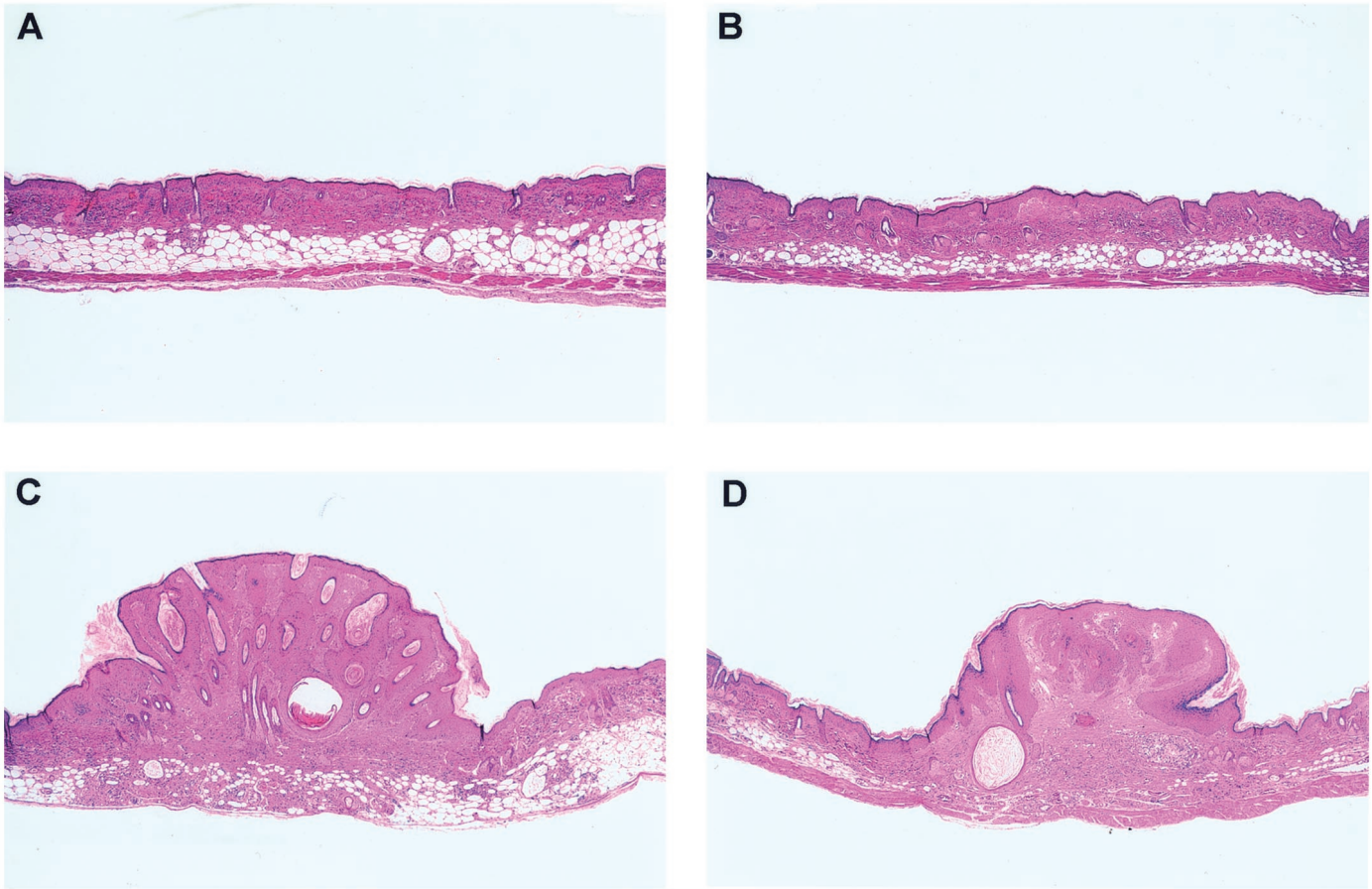


Fig. 1. Effect of p.o. administration of green tea to high-risk mice for 23 weeks on the dermal fat layer (histological evaluation in representative mice). High-risk SKH-1 mice received water (*A* and *C*) or 0.6% green tea (*B* and *D*) as their sole source of drinking fluid for 23 weeks. The dermal fat layer was examined histologically (100-fold magnification) in areas of the skin away from tumors (*A* and *B*) and in areas under keratoacanthomas (*C* and *D*).

Table 1 *Effect of p.o. administration of tea, decaffeinated tea, and caffeine to SKH-1 mice previously treated with UVB (high-risk mice) on the number of mice with tumors, the number of tumors, and the size of tumors*

Female SKH-1 mice (7–8 weeks of age) were treated with UVB (30 mJ/cm$^2$) twice weekly for 22 weeks, and UVB was stopped. The mice were then treated for 23 weeks with 0.6% lyophilized green tea (6 mg tea solids/ml), black tea (6 mg tea solids/ml) or decaffeinated green or black tea (6 mg tea solids/ml) as their sole source of drinking fluid. Other mice received 0.044% caffeine (0.44 mg/ml) or 0.6% lyophilized decaffeinated teas plus 0.044% caffeine. The number of mice alive at the end of the experiment, the number of mice with skin nodules (used for histopathology examination), the number of mice with histologically identified tumors, the number of tumors, and the size of tumors in each group are indicated. The values for body weight and tumor diameter are expressed as the mean ± SE. Statistical evaluation of differences from the water-positive control group was done by the Dunnett's *t* test or Fisher's exact test.

| Treatment[a] | No. of mice | Body weight (g/mouse) | No. of mice with skin nodules | No. of mice with histologically identified tumors | No. of tumors | Tumor diameter (mm/tumor) | % decrease in tumor diameter |
|---|---|---|---|---|---|---|---|
| Water | 28 | 32.0 ± 0.7 | 28 | 25 | 173 | 1.90 ± 0.11 | |
| GT | 28 | 32.1 ± 0.6 | 21 | 17[c] | 56 | 1.37 ± 0.18[d] | 28 |
| BT | 27 | 32.5 ± 0.6 | 24 | 20 | 57 | 1.23 ± 0.18[b] | 35 |
| dGT | 27 | 32.9 ± 0.4 | 22 | 21 | 134 | 1.36 ± 0.13[b] | 29 |
| dBT | 26 | 32.1 ± 0.4 | 22 | 21 | 120 | 1.38 ± 0.13[c] | 28 |
| CF | 27 | 32.0 ± 0.4 | 22 | 17[c] | 65 | 1.08 ± 0.17[b] | 44 |
| dGT + CF | 25 | 32.3 ± 0.5 | 21 | 16[c] | 50 | 1.26 ± 0.19[c] | 34 |
| dBT + CF | 29 | 31.5 ± 0.4 | 19 | 15[b] | 34 | 1.44 ± 0.22 | 24 |
| Total | 217 | | 179 | 152 | 689 | | |

[a] GT, green tea; BT, black tea; dGT, decaffeinated GT; dBT, decaffeinated BT; CF, caffeine.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P < 0.1$.

An additional conclusion that can be made from the information in Table 2 is that the dermal fat layer is much thinner under tumors than away from tumors in all experimental groups. For instance, in high-risk mice given only water as their drinking fluid for 23 weeks, the thickness of the dermal fat layer away from tumors was 162 μm, but it was only 60 μm directly under tumors (Table 2). In high-risk mice given 0.6% green tea for 23 weeks, the thickness of the dermal fat layer away from tumors was 100 μm, but it was only 28 μm directly under tumors (Table 2).

**Relationship between the Thickness of the Dermal Fat Layer away from Tumors and Tumor Multiplicity.** The 179 mice with skin nodules (Table 1) were analyzed histologically for tumors; 152 of these mice had a total of 689 tumors, and 29 mice had no tumors. The relationship between the thickness of the dermal fat layer away from tumors (possible surrogate for total body fat levels) in individual mice and the number of tumors/mouse in all 179 mice is shown in Table 3. Fourteen mice with a very thin dermal fat layer (≤50 μm) away from tumors had an average of only 1.6 ± 0.7 tumors/mouse, whereas seven mice with a thick dermal fat layer (>250 μm) away from tumors had 7.4 ± 1.8 tumors/mouse. Regression analysis was performed with data from all 179 mice to assess the relationship between the thickness of the dermal fat layer away from tumors for each mouse and the number of tumors/mouse. There was a highly significant positive linear association between the number of tumors/mouse and the thickness of the dermal fat layer away from the tumors ($P = 0.0001$). The regression equation was: tumors/mouse = 0.83 + 0.025 × (thickness of the dermal fat layer away from tumors in μm).

The relationship between the thickness of the dermal fat layer away from tumors and the number of tumors/mouse in animals that received only noncaffeinated beverages (water, decaffeinated green tea, and decaffeinated black tea; 72 mice) is shown in Table 4. For mice ingesting only noncaffeinated beverages, there was still a highly

Table 2 *Effect of p.o. administration of tea, decaffeinated tea, and caffeine on tumorigenesis, the size of the parametrial fat pads, and the thickness of the dermal fat layer in SKH-1 mice previously treated with UVB (high-risk mice)*

| Treatment | No. of mice | Keratoacanthomas (tumors per mouse)[a] | Squamous cell carcinomas (tumors per mouse) | Total tumors (tumors per mouse) | Size of fat pads (relative units) | Thickness of dermal fat layer away from tumors (μm) | Thickness of dermal fat layer under tumors(μm) |
|---|---|---|---|---|---|---|---|
| Water | 28 | 4.00 ± 0.47 | 1.82 ± 0.30 | 6.18 ± 0.71 | 1.41 ± 0.11 | 162 ± 9 | 60 ± 4 |
| 0.6% GT | 28 | 1.25 ± 0.47[a] | 0.68 ± 0.30[b] | 2.00 ± 0.71[a] | 0.64 ± 0.11[a] | 100 ± 11[a] | 28 ± 6[a] |
| | | (69) | (63) | (68) | (54) | (39) | (53) |
| 0.6% BT | 27 | 1.30 ± 0.48[a] | 0.70 ± 0.31[c] | 2.11 ± 0.72[a] | 0.95 ± 0.11[b] | 88 ± 10[a] | 31 ± 6[a] |
| | | (68) | (61) | (66) | (32) | (46) | (49) |
| 0.6% dGT | 27 | 2.93 ± 0.48 | 1.85 ± 0.31 | 4.96 ± 0.72 | 1.62 ± 0.11 | 152 ± 10 | 55 ± 4 |
| | | (27) | (0) | (20) | (0) | (6) | (8) |
| 0.6% dBT | 26 | 2.88 ± 0.49 | 1.58 ± 0.31 | 4.62 ± 0.74 | 1.23 ± 0.12 | 150 ± 10 | 51 ± 4 |
| | | (28) | (13) | (25) | (13) | (8) | (14) |
| 0.044% CF | 27 | 1.70 ± 0.48[a] | 0.63 ± 0.31[b] | 2.41 ± 0.72[a] | 0.62 ± 0.11[a] | 98 ± 10[a] | 31 ± 6[a] |
| | | (57) | (65) | (61) | (56) | (40) | (49) |
| dGT + CF | 25 | 1.04 ± 0.50[a] | 0.92 ± 0.32 | 2.00 ± 0.75[a] | 0.61 ± 0.12[a] | 105 ± 11[a] | 29 ± 6[a] |
| | | (74) | (50) | (68) | (57) | (35) | (51) |
| dBT + CF | 29 | 0.72 ± 0.46[a] | 0.38 ± 0.30[a] | 1.17 ± 0.70[a] | 0.89 ± 0.11[a] | 87 ± 11[a] | 24 ± 7[a] |
| | | (82) | (79) | (81) | (37) | (47) | (61) |

Female SKH-1 mice were treated with UVB (30 mJ/cm$^2$) twice a week for 22 weeks, and UVB administration was stopped. These tumor-free mice had a high risk of developing skin tumors during the next several months, and they were treated for 23 weeks with 0.6% lyophilized green tea (6 mg tea solids/ml), black tea (6 mg tea solids/ml), or decaffeinated green or black tea (6 mg tea solids/ml) as their sole source of drinking fluid. Other mice received 0.044% caffeine (0.44 mg/ml) or 0.6% lyophilized decaffeinated teas plus 0.044% caffeine. Tumors were classified by histological evaluation of skin samples from the 179 mice with skin nodules, and calculations of tumors/mouse included data from all 217 mice alive at the end of the experiment.

The combined length of the two parametrial fat pads from all 217 mice alive when the experiment was terminated was measured and graded as follows: 1 (<1 cm), 2 (1–2 cm) or 3 (>2 cm). The thickness of the dermal fat layer was measured in 5–10 representative nontumor areas of the skin (>0.5 cm away from tumors) from 19–28 mice/group (total of 179 mice; see Table 1) using a light microscope with an ocular micrometer and × 100 magnification. The 152 tumor-bearing mice were used for calculations of the thickness of the dermal fat layer under tumors.

[a] The values represent the mean ± S.E., and the values in parentheses represent percentage decrease compared with the drinking water positive control group. Statistical evaluation of differences from the water positive control group ([a] $p < 0.01$, [b] $p < 0.05$, [c] $p < 0.1$) was done by the Dunnett's T test (Taken from ref. 1 except for fat pad and dermal fat layer data which have not been published). The data for total tumors includes a small number of squamous cell papillomas.

Table 3 *Relationship between the thickness of the dermal fat layer away from tumors, the number of tumors/mouse, and the tumor size/mouse in mice receiving noncaffeinated or caffeinated beverages*

UVB-pretreated high risk SKH-1 mice were given water, green tea (GT), black tea (BT), decaffeinated green tea (dGT), decaffeinated black tea (dBT), caffeine (CF), dGT + CF or dBT + CF for 23 weeks as described in Tables 1 and 2. The thickness of the dermal fat layer in areas away from tumors or in mice with no tumors was determined. The data were obtained from 28 mice in the water group, 21 mice from the GT group, 24 mice from the BT group, 22 mice from the dGT group, 22 mice from the dBT group, 22 mice from the CF group, 21 mice from the dGT + CF group and 19 mice from the dBT + CF group (total, 179 mice). Each value represents the mean ± SE.[a]

| Thickness of dermal fat layer away from tumors ($\mu$m) | No. of mice | No. of tumors/mouse[a] | Tumor diameter/mouse[b] (mm) |
|---|---|---|---|
| ≤50 | 14 | 1.6 ± 0.7 | 0.70 ± 0.26 |
| 50–100 | 63 | 2.9 ± 0.4 | 1.18 ± 0.13 |
| 100–150 | 68 | 3.8 ± 0.6 | 1.26 ± 0.15 |
| 150–200 | 17 | 5.5 ± 1.0 | 1.51 ± 0.26 |
| 200–250 | 10 | 7.8 ± 1.4 | 1.56 ± 0.18 |
| >250 | 7 | 7.4 ± 1.8 | 1.24 ± 0.15 |

[a] $P = 0.0001$ (from the Pearson correlation coefficient) for the thickness of the dermal fat layer away from tumors *versus* the number of tumors/mouse for all 179 mice.

[b] $P = 0.034$ (from the Pearson correlation coefficient) for the thickness of the dermal fat layer away from tumors *versus* the average tumor diameter/mouse for all 179 mice.

significant positive linear association between the number of tumors/mouse and the dermal fat thickness away from tumors ($P = 0.009$). The regression equation was: tumors/mouse = 2.84 + 0.02 × (thickness of the dermal fat layer away from tumors in $\mu$m).

**Weak Relationship between the Thickness of the Dermal Fat Layer away from Tumors and Tumor Size.** Although regression analysis of data from all 179 mice examined histologically indicated a statistically significant relationship between the thickness of the dermal fat layer/mouse (away from tumors) and average tumor size/mouse ($P = 0.034$), this relationship was not very strong (Table 3).

**Effects of Caffeinated Beverages on the Thickness of the Dermal Fat Layer under Tumors of Different Sizes.** For the 152 tumor-bearing mice ingesting noncaffeinated or caffeinated beverages, the thickness of the dermal fat layer under the 689 tumors described in Table 1 was related to tumor size, and it can be seen that larger tumors had a smaller dermal fat layer under the tumors than did smaller tumors (Table 5; $P < 0.0001$). For tumors ≤0.5 mm diameter, the thickness of the dermal fat layer directly under these tumors was 58 ± 3 $\mu$m (202 tumors), and for tumors >3 mm, the thickness of the dermal fat layer under the tumors was 24 ± 5 $\mu$m (50 tumors). Analysis of the data using the random coefficient model showed that there was a significant negative linear association between tumor size and the log thickness of the dermal fat layer directly under tumors ($P = 0.0001$).

Studies on the relationship between the log thickness of the dermal fat layer under 427 tumors from mice ingesting noncaffeinated beverages and tumor diameter revealed a highly significant negative linear association ($P = 0.0001$). Similarly, the relationship between the log thickness of the dermal fat layer under 262 tumors and the tumor diameter from mice ingesting caffeinated beverages also revealed a highly significant negative linear association ($P = 0.0001$). Moreover, the slope of the regression line for the caffeinated beverage group was significantly more negative than the slope for the noncaffeinated beverage group ($P = 0.0001$). This implies that the rate of decrease in the thickness of the dermal fat layer under tumors as tumor size increased for the caffeinated beverage group was significantly greater than that for the noncaffeinated beverage group.

The results indicate that p.o. administration of caffeinated beverages decreased the thickness of the dermal fat layer under tumors, and this effect was greatly magnified with large tumors. The administration of caffeinated beverages decreased the thickness of the dermal fat layer directly under the tumors by 36% for small tumors (≤0.5 mm diameter), by 57% for 0.5–1-mm tumors, by 70% for 1–2-mm tumors, by 90% for 2–3-mm tumors, and by 97% for tumors >3 mm (Table 5).

**Effect of Administration of Green Tea, Black Tea, and Caffeine on the Weight of the Parametrial Fat Pads and the Thickness of the Dermal Fat Layer in Normal Non-UVB-treated Mice.** p.o. administration of green tea, black tea, decaffeinated green tea plus caffeine, decaffeinated black tea plus caffeine, or caffeine alone to normal non-UVB-treated SKH-1 mice for 4 or 8 weeks decreased the weight of the parametrial fat pads and the thickness of the dermal fat layer, but the decaffeinated teas had little or no effect (Table 6). None of the treatments had an effect on body weight (Table 6). There was a strong positive correlation between the weight of the parametrial fat pads and the thickness of the dermal fat layer ($P < 0.0001$; Table 6, *experiments 5* and *6*). Administration of caffeine or green tea for only 2 weeks decreased the weight of the parametrial fat pads and the thickness of the dermal fat layer (Table 6, *experiments 1–3*). Oral administration of EGCG (2 mg/ml) for 2 or 4 weeks had little or no effect on the weight of the parametrial fat pad (Table 6, *experiments 3* and *4*) or the thickness of the dermal fat layer (Table 6, *experiment 4*). The concentration of EGCG used was equivalent to the concentration of total catechins in 0.6% green tea.

In an additional experiment, we explored the effect of p.o. administration of the regular teas, the decaffeinated teas, the decaffeinated teas plus caffeine, or caffeine alone for 8 weeks on food consumption and body weight. None of the treatments influenced body weight or food consumption (Table 7).

We explored the possibility that the effect of green tea on lipid disposition was mediated by an effect on the adrenal gland. This was not observed, because p.o. administration of 0.6% green tea to adrenalectomized SKH-1 mice for 4 weeks (13–14 mice/group) decreased the weight of the parametrial fat pads by 72%, and the thickness of the dermal fat layer was decreased by 36%. In a parallel group of intact mice, administration of 0.6% green tea for 4 weeks decreased the weight of the parametrial fat pads by 65%, and the thickness of the dermal fat layer was decreased by 31%. Tea administration did not affect body weight in intact or adrenalectomized mice.

## DISCUSSION

In the present study, we found that the inhibitory effects of p.o. administered green tea, black tea, the decaffeinated teas plus caffeine, or caffeine alone on the formation of skin tumors (tumor multiplicity) in UVB-pretreated, high-risk mice were associated with an effect of these treatments to decrease the size of the parametrial fat pads and to decrease the thickness of the dermal fat layer away from tumors and directly under tumors (Table 2). These effects on tumor multiplicity and fat disposition were not observed in animals treated with the decaffeinated teas, thereby pointing out the importance of caffeine for the effects of the regular caffeinated teas on carcinogenesis and in

Table 4 *Relationship between the thickness of the dermal fat layer away from tumors and the number of tumors per mouse in mice receiving non-caffeinated beverages*

UVB-pretreated, high-risk mice were treated p.o. with water, decaffeinated green tea or decaffeinated black tea for 23 weeks as described in Tables 1 and 2. Each value for the number of tumors or the average tumor diameter/mouse represents the mean ± SE.

| Thickness of dermal fat layer away from tumors ($\mu$m) | No. of mice | No. of tumors/mouse[a] | Tumor diameter/mouse (mm) |
|---|---|---|---|
| ≤50 | 1 | 0 | 0 |
| 50–100 | 11 | 5.2 ± 1.1 | 1.40 ± 0.17 |
| 100–150 | 31 | 5.0 ± 1.1 | 1.51 ± 0.26 |
| 150–200 | 13 | 6.9 ± 1.0 | 1.80 ± 0.27 |
| 200–250 | 10 | 7.8 ± 1.4 | 1.56 ± 0.18 |
| >250 | 6 | 8.2 ± 2.0 | 1.35 ± 0.12 |

[a] $p = 0.009$ (from regression analysis) for the thickness of the dermal fat layer *versus* the number of tumors/mouse for all 72 mice.

Table 5 *Effect of p.o. administration of caffeinated beverages on the thickness of the dermal fat layer under tumors of different sizes in SKH-1 mice previously treated with UVB (high-risk mice)*

UVB-pretreated high-risk SKH-1 mice were treated p.o. with noncaffeinated beverages (water, dGT, dBT) or caffeinated beverages (GT, BT, CF, dGT + CF, and dBT + CF) for 23 weeks as described in Tables 1 and 2. The thickness of the dermal fat layer under tumors of different sizes was measured in all 152 tumor-bearing mice (67 mice from the noncaffeinated beverage groups and 85 mice from the caffeinated beverage groups). The values in parentheses indicate the number of tumors studied. The mean value for the thickness of the dermal fat layer under tumors ± SE is given for tumors of different sizes.[a]

| Tumor diameter (mm) | Thickness of dermal fat layer under tumors ($\mu$m) | | | % decrease |
|---|---|---|---|---|
| | Noncaffeinated plus caffeinated beverages[b] | Noncaffeinated beverages[c] | Caffeinated beverages[c] | |
| ≤0.5 | 58 ± 3 (202) | 70 ± 4 (100) | 45 ± 2 (102) | 36 |
| 0.5–1 | 49 ± 3 (204) | 63 ± 3 (125) | 27 ± 2 (79) | 57 |
| 1–2 | 43 ± 3 (177) | 54 ± 3 (125) | 16 ± 2 (52) | 70 |
| 2–3 | 31 ± 5 (56) | 39 ± 5 (42) | 4 ± 1 (14) | 90 |
| >3 | 24 ± 5 (50) | 34 ± 6 (35) | 1 ± 1 (15) | 97 |
| Mean value | 47 ± 1 (689) | 58 ± 2 (427) | 29 ± 2 (262) | 50 |

[a] GT, green tea; BT, black tea; dGT, decaffeinated GT; dBT, decaffeinated BT; CF, caffeine.
[b] There was a significant negative linear association between tumor diameter and the log thickness of the dermal fat layer under tumors ($P = 0.0001$) using the random coefficient (hierarchical linear) model.
[c] The rate of decrease in thickness of the dermal fat layer under tumors of increasing size in mice treated with caffeinated beverages was significantly greater than that for mice treated with the noncaffeinated beverages ($P = 0.0001$) using the random coefficient (hierarchical linear) model.

lowering tissue fat levels. The importance of caffeine as a major component of tea that is responsible for the inhibition of UV-induced carcinogenesis was pointed out in our earlier studies (8, 10). The effects of caffeinated teas or caffeine to decrease the size of the parametrial fat pads and to decrease the thickness of the dermal fat layer were also observed in normal non-UVB-treated mice (Table 6). The mechanism of the effects of caffeinated teas or caffeine to decrease tumor multiplicity is not known but could result from effects of these agents on cell proliferation, apoptosis, angiogenesis, and/or the immune system. Tea-induced changes in these parameters could be mediated by changes in body fat levels.

Although caffeine is the major component of p.o. administered tea that inhibits skin carcinogenesis in UVB-pretreated, high-risk mice (8) or in mice treated concurrently with tea and UVB in a complete carcinogenesis model (10), noncaffeine components of tea are strong inhibitors of UVB-induced carcinogenesis in 7,12-dimethylbenz[a]-anthracene-pretreated mice, and they also decrease tumor size in UVB-pretreated high risk mice (8, 9). Additional studies have indicated inhibitory effects of decaffeinated teas or EGCG on the formation of several chemically induced cancers (18, 19).

The relationship between the effects of the various teas and caffeine on carcinogenesis and body fat was evaluated in more detail by examining the relationship between tumors/mouse and dermal fat thickness away from tumors (as a surrogate for body fat levels) in

Table 6 *Effect of administration of green tea, black tea, caffeine, or EGCG on the weight of the parametrial fat pads and thickness of the dermal fat layer in normal non-UVB-treated SKH-1 mice*

Normal non-UVB-treated female SKH-1 mice (7–8 weeks of age were treated with green tea (6 mg tea solids/ml), black tea (6 mg tea solids/ml), decaffeinated green tea (6 mg tea solids/ml), decaffeinated black tea (6 mg tea solids/ml), caffeine (0.44 mg/ml) or EGCG (2 mg/ml) as their sole source of drinking fluid for 2–8 weeks as indicated. The two parametrial fat pads were weighed. The thickness of the dermal fat layer was measured with an ocular micrometer and × 100 magnification. Five to 10 representative areas for each mouse were measured and averaged. The values represent the mean ± SE. Statistical evaluation of differences from the water-positive control group were done by the Student's *t* test in experiments 1 and 2 by the Dunnett's *t* test in experiment 3–6. In experiments 5 or 6 or 5 plus 6, the weight of the parametrial fat pads was highly correlated with the thickness of the dermal fat layer ($P < 0.0005$; Pearson correlation coefficient).

| Experiment | Duration of treatment (wk) | Treatment[a] | No. of mice | Body weight (g/mouse) | Weight of parametrial fat pads | | Thickness of dermal fat layer | |
|---|---|---|---|---|---|---|---|---|
| | | | | | (g/mouse) | (% decrease) | ($\mu$m) | (% decrease) |
| 1 | 2 | Water | 35 | 24.5 ± 0.29 | | | 85 ± 4.2 | |
| | | GT | 35 | 24.7 ± 0.29 | | | 67 ± 3.5 | 21 |
| 2 | 2 | Water | 40 | 24.2 ± 0.28 | | | 97 ± 5.4 | |
| | | CF | 40 | 23.3 ± 0.27 | | | 77 ± 5.3[b] | 21 |
| 3 | 2 | Water | 10 | 23.4 ± 0.50 | 0.42 ± 0.05 | | 84 ± 6.6 | |
| | | CF | 10 | 23.2 ± 0.44 | 0.24 ± 0.05[c] | 43 | 56 ± 6.4[b] | 33 |
| | | EGCG | 10 | 23.5 ± 0.58 | 0.37 ± 0.06 | 12 | 73 ± 5.9 | 13 |
| 4 | 4 | Water | 16 | 26.2 ± 0.38 | 0.49 ± 0.05 | | | |
| | | CF | 16 | 25.8 ± 0.28 | 0.21 ± 0.02[b] | 57 | | |
| | | EGCG | 16 | 26.6 ± 0.36 | 0.43 ± 0.04 | 12 | | |
| 5 | 4 | Water | 15 | 26.4 ± 0.48 | 0.35 ± 0.04 | | 107 ± 7.2 | |
| | | 0.6% GT | 15 | 26.6 ± 0.54 | 0.20 ± 0.04[c] | 44 | 75 ± 7.2[c] | 30 |
| | | 0.6% BT | 15 | 26.7 ± 0.45 | 0.22 ± 0.04[c] | 37 | 78 ± 7.2[c] | 27 |
| | | 0.6% dGT | 14 | 26.4 ± 0.28 | 0.37 ± 0.04 | −6 | 93 ± 7.5 | 13 |
| | | 0.6% dBT | 15 | 26.4 ± 0.50 | 0.47 ± 0.04[d] | −35 | 103 ± 7.2 | 3 |
| | | 0.044% CF | 15 | 26.4 ± 0.32 | 0.23 ± 0.04[d] | 34 | 58 ± 7.2[b] | 45 |
| | | dGT + CF | 13 | 26.0 ± 0.26 | 0.15 ± 0.04[b] | 56 | 75 ± 7.8[c] | 30 |
| | | dBT + CF | 15 | 26.2 ± 0.48 | 0.16 ± 0.04[b] | 55 | 63 ± 7.2[b] | 41 |
| 6 | 8 | Water | 15 | 27.1 ± 0.29 | 0.42 ± 0.04 | | 152 ± 8.0 | |
| | | 0.6% GT | 15 | 27.2 ± 0.42 | 0.21 ± 0.04[b] | 50 | 109 ± 8.0[b] | 28 |
| | | 0.6% BT | 15 | 26.7 ± 0.37 | 0.21 ± 0.04[b] | 50 | 111 ± 8.0[b] | 27 |
| | | 0.6% dGT | 15 | 26.7 ± 0.24 | 0.42 ± 0.04 | −1 | 141 ± 8.0 | 7 |
| | | 0.6% dBT | 15 | 26.7 ± 0.66 | 0.49 ± 0.04 | −16 | 140 ± 8.0 | 8 |
| | | 0.044% CF | 15 | 26.9 ± 0.44 | 0.24 ± 0.04[b] | 42 | 100 ± 8.0[b] | 34 |
| | | dGT + CF | 14 | 27.3 ± 0.93 | 0.25 ± 0.04[c] | 42 | 101 ± 8.3[b] | 33 |
| | | dBT + CF | 15 | 26.7 ± 0.40 | 0.21 ± 0.04[b] | 51 | 112 ± 8.0[b] | 27 |

[a] GT, green tea; BT, black tea; dGT, decaffeinated GT; dBT, decaffeinated BT; CF, caffeine.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P \leq 0.1$.

Table 7 *Effect of p.o. administration of green tea, black tea, and caffeine on food consumption in SKH-1 mice*

Female SKH-1 mice (15 per group; 7–8 weeks of age) were treated with green tea (GT, 6 mg tea solids/ml), black tea (BT, 6 mg tea solids/ml), decaffeinated green tea (dGT, 6 mg tea solids/ml), decaffeinated black tea (dBT, 6 mg tea solids/ml), caffeine (CF, 0.44 mg/ml), dGT + CF or dBT + CF as their sole source of drinking fluid for 8 weeks. Food consumption was determined (3 cages/group; 5 mice/cage) each week for 8 weeks. Each value represents the weekly mean ± SE for food consumption for the three cages (expressed as g/mouse/day). No statistically different effects of the different treatments on body weight or food consumption were observed.

| | | Weeks of treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Group | Body weight (g) | Food consumption (g/mouse/day; mean ± SE) | | | | | | | |
| Water | 27.2 ± 0.64 | 5.6 ± 0.15 | 5.7 ± 0.10 | 5.8 ± 0.08 | 5.5 ± 0.06 | 5.6 ± 0.04 | 5.8 ± 0.08 | 5.8 ± 0.48 | 6.0 ± 0.09 |
| 0.6% GT | 27.5 ± 0.24 | 5.5 ± 0.11 | 6.0 ± 0.17 | 6.1 ± 0.07 | 5.6 ± 0.04 | 5.6 ± 0.06 | 5.6 ± 0.18 | 5.8 ± 0.14 | 5.8 ± 0.08 |
| 0.6% BT | 27.4 ± 0.23 | 5.6 ± 0.05 | 6.1 ± 0.10 | 6.0 ± 0.06 | 5.6 ± 0.02 | 5.8 ± 0.11 | 5.7 ± 0.10 | 5.8 ± 0.09 | 5.7 ± 0.04 |
| 0.6% dGT | 27.5 ± 0.23 | 5.3 ± 0.07 | 5.6 ± 0.20 | 5.7 ± 0.06 | 5.3 ± 0.02 | 5.3 ± 0.06 | 5.6 ± 0.11 | 5.7 ± 0.11 | 5.8 ± 0.05 |
| 0.6% dBT | 26.8 ± 0.82 | 5.0 ± 0.09 | 5.5 ± 0.08 | 5.8 ± 0.06 | 5.3 ± 0.02 | 5.4 ± 0.10 | 5.7 ± 0.09 | 5.7 ± 0.11 | 5.8 ± 0.08 |
| 0.04% CF | 27.1 ± 0.07 | 5.7 ± 0.03 | 6.4 ± 0.07 | 6.0 ± 0.02 | 5.6 ± 0.03 | 5.5 ± 0.06 | 5.6 ± 0.06 | 5.7 ± 0.11 | 5.9 ± 0.03 |
| 0.6% dGT + 0.04% CF | 27.7 ± 0.70 | 5.4 ± 0.07 | 5.9 ± 0.15 | 5.8 ± 0.05 | 5.4 ± 0.03 | 5.4 ± 0.03 | 5.6 ± 0.05 | 6.1 ± 0.29 | 5.9 ± 0.03 |
| 0.6% dBT + 0.04% CF | 27.1 ± 0.07 | 5.4 ± 0.09 | 6.0 ± 0.07 | 5.9 ± 0.01 | 5.4 ± 0.03 | 5.5 ± 0.14 | 5.6 ± 0.06 | 5.7 ± 0.08 | 5.7 ± 0.10 |

individual mice. We observed a highly significant direct correlation between the thickness of the dermal fat layer (measured in areas away from tumors) and the number of tumors/mouse (Table 3; $P = 0.0001$). The relationship between tumor size/mouse and dermal fat thickness (away from tumors), however, was much less robust (Table 3; $P = 0.034$). These results suggest that tumor multiplicity and tumor size may be regulated differently. The use of dermal fat thickness as a surrogate for body fat is supported by a positive correlation between the thickness of the dermal fat layer and the weight of the parametrial fat pads in individual mice (Table 6, *experiments 5*, and *6*).

Although several dietary modifications are known to inhibit carcinogenesis, and some may decrease body fat levels in rodents [*e.g.*, calorie restriction (20–23), low-fat diets (23), and CLA administration (24, 25)], this is the first study to show a close relationship between the effect of a cancer chemopreventive agent to inhibit carcinogenesis and to lower tissue fat levels. The results of the present study suggest that the inhibitory effect of p.o. administration of tea or caffeine on carcinogenesis in high-risk mice may be related—at least in part—to their effects on tissue levels of fat. The linkage between tea-induced decreases in tissue fat levels and decreases in tumor multiplicity is much stronger than the linkage between tea-induced decreases in tissue fat levels and tumor size. These results suggest that changes in dermal fat levels may be more important for modulating tumor formation (multiplicity) than for modulating tumor growth (size).

The thickness of the dermal fat layer directly under tumors was less than that away from tumors (Table 2) suggesting that dermal fat under tumors may be used by the tumors as a source of energy. This concept is also in accord with finding less dermal fat under large tumors than under small tumors (Table 5). Administration of caffeinated beverages decreased the thickness of the dermal fat layer under both small and large tumors, but the effect of caffeinated beverages on fat was greatly magnified under large tumors (Table 5). Administration of caffeinated beverages decreased the thickness of the dermal fat layer by 36% under small tumors (≤0.5 mm diameter) and by 97% under large tumors (>3 mm diameter; Table 5). It will be of interest to determine whether tumors secrete substances that synergize with caffeine to decrease dermal fat thickness under tumors.

It should be noted that the inhibitory effects of the regular caffeinated teas and caffeine on tumorigenesis and body fat levels (as measured by changes in the size of the parametrial fat pads and dermal fat thickness) occurred in the absence of effects on body weight (Tables 1, 6, and 7) or food consumption (Table 7). These results suggest that administration of the regular caffeinated teas or caffeine decreased the proportion of body fat without changing overall energy intake or utilization. Although additional studies are needed to determine the effects of tea and caffeine on total body fat and protein levels, our results indicate that p.o. administration of the caffeinated teas or caffeine increased lipolysis or decreased the absorption or synthesis of fat without changing body weight.

Other investigators have also demonstrated the effects of tea administration on lipid disposition. Kimura *et al.* (26) reported that administration of green tea, black tea, or oolong tea inhibited elevations in serum and liver lipids (total cholesterol and triglycerides) that occurred in rats fed peroxidized oil for 1 week. Additional studies showed a hypolipidemic effect of green tea (lowered serum total cholesterol, triglycerides, and low-density lipoprotein-cholesterol) along with a lowered body weight in rats treated with green tea (27). In another study, administration of oolong tea prevented the obesity and fatty livers that were induced by a high fat diet (28). An antilipidemic constituent of tea was identified as caffeine, which was shown to stimulate norepinepherine-induced lipolysis in fat cells (28). A very recent study by Dulloo *et al.* (29) indicated that oral administration of a green tea extract rich in catechin polyphenols and caffeine during breakfast, lunch, and dinner stimulated 24-h energy expenditure and fat oxidation in humans, but administration of an equivalent amount of caffeine alone was reported to have little or no effect. The amounts of catechin polyphenols administered with each meal were comparable with the amounts commonly ingested by tea drinkers. In other studies, administration of caffeine together with sympathetic stimulation (cold stress or exercise) or with sympathomimetic drugs such as ephedrine stimulated thermogenesis (30, 31). Dulloo *et al.* (29) suggested that caffeine and the tea catechins may have a synergistic stimulatory effect in enhancing energy expenditure and fat oxidation because of an inhibitory effect of tea polyphenols on catechol *O*-methyltransferase activity (32), which would be expected to elevate endogenous norepinepherine levels, and because of an inhibitory effect of caffeine on phosphodiesterase activity, which would be expected to elevate the levels of cyclic AMP and potentiate the action of catecholamines on thermogenesis. The studies and concepts by Dulloo are interesting, and they require confirmation. In a preliminary study in SKH-1 mice, p.o. administration of EGCG (2 mg/ml) together with a low concentration of caffeine (0.011%) as the drinking fluid for 4 weeks did not enhance the fat-lowering effect of caffeine itself (data not presented).

Baumann and Rusch (33)—more than 60 years ago—were the first to demonstrate a stimulatory effect of high levels of dietary fat on UV-induced skin carcinogenesis. Subsequently, Black *et al.* (34–37) confirmed the stimulatory effect of high levels of dietary fat on UV-induced skin carcinogenesis, and they reported that unsaturated fat stimulated carcinogenesis to a greater extent than saturated fat. These investigators also reported that dietary antioxidants inhibited UV-induced carcinogenesis but only when high levels of lipids were administered (35, 36). The stimulatory effect of a high fat diet on UV-induced carcinogenesis was observed when the high fat diet was

fed either during the entire experimental period or only after stopping UV administration (37, 38). These results indicate a postinitiation effect of the high fat diet. Although corn oil and menhaden oil both contain unsaturated lipids, high levels of dietary corn oil (rich in ω-6 unsaturated fatty acids) enhanced UV-induced carcinogenesis, whereas high levels of dietary menhaden oil (rich in unsaturated ω-3 fatty acids) did not (37). Overall, the results indicate that high levels of dietary fat enhance UV-induced carcinogenesis in mice, and the kind of dietary lipid administered can influence the carcinogenic response.

Leyton *et al.* (39) observed that changes in the diet can influence the fatty acid composition of epidermal phospholipids in mice. Increasing the dietary linoleate levels resulted in an increased level of linoleate and a decreased level of arachidonate in phosphatidylcholine (39). Similar diet-induced changes were observed for linoleate and arachidonate in other phospholipids (39). It will be of interest to determine the effects of administration of tea and caffeine on the profile of fatty acids in epidermal phospholipids and in the neutral fat of the parametrial fat pads. Decreased levels of arachidonic acid in the epidermis could result in decreased levels of prostaglandins that have been implicated in carcinogenesis. Pariza *et al.* (40–42) recently identified CLA as an anticarcinogenic substance in grilled ground beef and in dairy products. Administration of this substance inhibited chemically induced tumorigenesis in several animal models (24, 25, 41), and the levels of arachidonic acid and linoleic acid-derived polyunsaturated fatty acids in the mammary gland (a neutral fat-containing tissue) was decreased (43). Total body fat was also decreased and total body protein and water were increased (44, 45). Mechanistic studies suggested a stimulatory effect of CLA administration on the rate-limiting enzyme for fatty acid β-oxidation (increases in carnitine palmitoyltransferase activity in fat and muscle) and enhanced lipolysis (44). Additional studies indicated an inhibitory effect of CLA on stearoyl-CoA desaturase (46, 47) and an effect of CLA administration on monounsaturated fatty acid composition (48). The effects of tea and caffeine on fatty acid synthesis, fatty acid β-oxidation, stearoyl-CoA desaturase, and fatty acid composition have not yet been studied.

Although some case-control and prospective epidemiology studies indicated no relationship between fat intake and risk of nonmelanoma skin cancer in humans (49, 50), a recent large prospective epidemiology study in men suggested an inverse association between dietary intake of monounsaturated fat and basal cell carcinomas (51). An additional case-control study suggested an inverse relationship between a high intake of polyunsaturated fatty acids and melanoma in women (52). A more rigorous randomized intervention trial in human subjects indicated that reducing the percentage of fat calories from 39% to 21% of total calories caused a marked reduction in the number of actinic keratoses and skin cancers (53–55). Although the latter rigorous intervention study indicates that decreasing the level of dietary fat can inhibit the formation of UV-induced nonmelanoma skin tumors in humans, an extension of these intervention trials is needed.

In conclusion, p.o. administration of caffeinated teas or caffeine to UVB-pretreated, high-risk mice decreased tumor multiplicity, the size of the parametrial fat pads, and the thickness of the dermal fat layer. The decaffeinated teas were essentially inactive, and adding back caffeine restored activity. There was a highly significant correlation between dermal fat thickness away from tumors and tumor multiplicity in individual mice. Additional studies are needed to determine whether the inhibitory effect of the caffeinated teas and caffeine on tumor multiplicity are caused by their effects on dermal fat (cause/effect relationship) and whether caffeinated teas or caffeine decrease the arachidonic acid content or modify the levels of other fatty acids in dermal fat or in epidermal phospholipids. Tea- or caffeine-induced decreases in the arachidonic acid level in fat could result in decreased levels of prostaglandins that are believed to play a role in the carcinogenic process. The possibility that administration of the caffeinated teas or caffeine inhibit tumorigenesis by decreasing the amount of fat available as a source of energy under tumors also requires exploration. Additional studies are also needed to determine whether other cancer chemopreventive agents inhibit carcinogenesis by decreasing body fat levels.

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