Stimulatory effect of voluntary exercise or fat removal (partial lipectomy) on apoptosis in the skin of UVB light-irradiated mice

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Earlier studies indicated that high dietary fat and obesity are associated with an increased risk of cancer at several organ sites in experimental animals and in humans. In a recent study we found that voluntary running wheel exercise decreased body fat and inhibited ultraviolet B light (UVB)-induced carcinogenesis in the epidermis of SKH-1 mice. In the present study we demonstrate that voluntary running wheel exercise stimulated UVB-induced apoptosis in the epidermis by a p53-independent mechanism, and voluntary exercise also stimulated apoptosis in UVB-induced tumors in tumor-bearing mice. Exercise had no effect in non-UVB-treated epidermis or in areas of the epidermis away from tumors in tumor-bearing mice. In addition, we found that removal of the parametrial fat pads (partial lipectomy) 2 weeks before UVB irradiation enhanced UVB-induced apoptosis. The results of our studies suggest that fat cells secrete substances that inhibit apoptosis in cells with DNA damage and possibly also in tumors. Our results help explain why exercise or various dietary regimens that decrease tissue fat inhibit carcinogenesis.

carcinogenesis | sunburn | cancer prevention | sun-induced skin cancer

Nonmelanoma skin cancer is the most prevalent cancer in the United States (>1.0 million cases per year) (1–3), and ultraviolet B light (UVB) in sunlight is a major cause of these cancers (4, 5). Although most skin cancers are squamous cell carcinomas and basal cell carcinomas that are easily cured if detected early, many people still die from these cancers, as well as from the more dangerous sunlight-induced melanomas. Although sunlight plays a causal role for the development of melanomas, other factors are also important. The development of strategies to prevent UVB-induced cancers would have a major impact in decreasing the total load of human cancer.

In earlier studies we found that oral administration of green or black tea but not the decaffeinated teas inhibited UVB-induced skin carcinogenesis in SKH-1 mice (6–8). Adding back caffeine to the decaffeinated teas restored biological activity, and administration of only caffeine strongly inhibited UVB-induced carcinogenesis (7, 8). Interestingly, the degree of inhibition of tumorigenesis (tumors per mouse) in animals given the regular teas or caffeine was associated with decreased tissue fat (9). In other studies we found that oral administration of green tea or caffeine for 2 weeks before irradiation with UVB enhanced UVB-induced apoptosis (10). During the course of these studies we found that oral administration of green tea or caffeine enhanced locomotor activity (11), and we asked whether the increased locomotor activity played a role in the inhibitory effect of administration of green tea and caffeine solutions on UVB-induced carcinogenesis and on the effect of these beverages to decrease tissue fat. We found that enhanced physical activity (voluntary running wheel exercise) increased food intake, decreased body fat, and inhibited UVB-induced carcinogenesis, but body weight was not affected (12). In this earlier study there was a significant relationship between the level of tissue fat and UVB-induced carcinogenesis (tumors per mouse). Animals with more tissue fat had more tumors.

In the present study we found that voluntary running wheel exercise decreased tissue fat, enhanced UVB-induced apoptosis in the epidermis, and enhanced apoptosis in UVB-induced tumors in tumor-bearing SKH-1 mice. In addition, decreasing body fat by removal of the parametrial fat pads also enhanced UVB-induced apoptosis. The results suggest that fat cells secrete antiapoptotic factors that modulate apoptosis after acute DNA damage and in tumors.

Results

Stimulatory Effect of Voluntary Running Wheel Exercise on UVB-Induced Apoptosis in the Epidermis of SKH-1 Mice. Mice in cages with a running wheel for 2 weeks maintained their normal body weight but had a 62% decrease in the weight of their parametrial fat pads and a 42% decrease in the thickness of their dermal fat layer when compared with control mice in cages without a running wheel (mean of two experiments) (Table 1). We estimate that the mice with a running wheel ran ≈2–3 miles/day per mouse. UVB-induced apoptosis in the epidermis of exercising mice, as measured either by apoptotic sunburn cells or by caspase 3 (active form)-positive cells at 6 h after UVB exposure, was increased by 120% when compared with the UVB-induced increase in apoptosis in control mice (mean of two experiments) (Table 2), but exercise did not have a significant effect on the UVB-induced increase in phospho-p53 (Ser-15) (Table 2). In control studies, running wheel exercise had no effect on the small number of apoptotic sunburn cells, caspase 3 (active form)-positive cells, or phospho-p53 (Ser-15)-positive cells present in the epidermis in the absence of UVB irradiation (Table 2).

Stimulatory Effect of Voluntary Running Wheel Exercise on UVB-Induced Apoptosis in the Epidermis of p53 Knockout Mice. $p53^{-/-}$ mice in cages with a running wheel for 2 weeks maintained their normal body weight but had a 35% decrease in the weight of their parametrial fat pads when compared with $p53^{-/-}$ mice in cages without a running wheel. UVB-induced apoptosis in the epidermis of the exercising $p53^{-/-}$ mice as measured by apoptotic sunburn cells was increased by 93% when compared with UVB-induced apoptosis in sedentary $p53^{-/-}$ mice (mean of two experiments) (Table 3), indicating that running wheel exercise

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Abbreviations: UVB, ultraviolet B light; IGF, insulin-like growth factor.

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Table 1. Effect of voluntary exercise for 2 weeks on the weight of the parametrial fat pads and the thickness of the dermal fat layer in SKH-1 mice

	Body weight, g		Weight of parametrial fat pads, g per mouse		Thickness of dermal fat layer, μm	
Group	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
No running wheel	26.2 ± 0.61	25.1 ± 0.54	0.81 ± 0.10	0.52 ± 0.07	65 ± 5	57 ± 4
Running wheel	26.4 ± 0.45	24.1 ± 0.63	0.35 ± 0.02*	0.17 ± 0.02*	34 ± 5*	37 ± 3*
% decrease	0	4	57	67	48	35

Female SKH-1 mice (7–8 weeks old, 10 mice per group) were housed in a cage with or without a running wheel for 2 weeks. The two parametrial fat pads were weighed, and the thickness of the dermal fat layer was measured by using an ocular micrometer with an Olympus BHTU light microscope under ×100 magnification at 5–10 representative areas per slide and averaged. Each value represents the mean ± SE. *, $P < 0.01$.

enhanced UVB-induced apoptosis by a p53-independent mechanism.

Stimulatory Effect of Fat Removal (Partial Lipectomy) on UVB-Induced Apoptosis in the Epidermis of SKH-1 Mice. Surgical removal of the two parametrial fat pads (≈0.8 g per mouse) 2 weeks before UVB irradiation enhanced UVB-induced apoptosis in the epidermis by 107% at 6 h after irradiation when compared with the effect of UVB on apoptosis in sham-operated control mice (mean of two experiments) (Table 4). In control studies with mice that did not receive UVB irradiation, partial lipectomy had no effect on the small number of apoptotic cells or phospho-p53 (Ser-15)-positive cells in the epidermis (Table 4).

Stimulatory Effect of Voluntary Running Wheel Exercise on Apoptosis in UVB-Induced Skin Tumors in SKH-1 Mice. Voluntary running wheel exercise during 33 weeks of twice-a-week exposure of the mice to UVB (30 mJ/cm^2) decreased the weight of the parametrial fat pads by 32% and decreased the thickness of the dermal fat layer by 26% (12). The number of keratoacanthomas and squamous cell carcinomas per mouse was decreased by ≈34%, and tumor volume per mouse was decreased by 75% and 69%, respectively (12). Voluntary running wheel exercise increased the percentage of apoptotic cells in keratoacanthomas by 104% and in squamous cell carcinomas by 57%, but there was little or no effect in nontumor areas of the epidermis (Table 5). The percentage of BrdU-positive cells was decreased 17% in keratoacanthomas and by 10% in squamous cell carcinomas (Table 5).

Discussion

In the present study we found that voluntary running wheel exercise (*i*) decreased tissue fat [also observed earlier (12)], (*ii*) stimulated UVB-induced apoptosis in the epidermis, and (*iii*) stimulated apoptosis in UVB-induced tumors in SKH-1 mice. Running wheel exercise, however, did not enhance apoptosis in non-UVB-treated control epidermis or in areas of the epidermis away from tumors in tumor-bearing mice, indicating selectivity for the proapoptotic effect of exercise on DNA-damaged cells. The stimulatory effect of exercise on UVB-induced apoptosis in the epidermis and in UVB-induced tumors in tumor-bearing mice provided a possible mechanism for our recent observation of an inhibitory effect of running wheel exercise on UVB-induced carcinogenesis (12). The lack of an effect of exercise on the UVB-induced increase in phospho-p53 (Ser-15) in SKH-1 mice and the stimulatory effect of voluntary exercise on UVB-induced apoptosis in p53 knockout mice indicated that exercise increased UVB-induced apoptosis by a p53-independent mechanism. The results of our studies with running wheel exercise are similar to the effects of orally administered caffeine or tea, which also decreased tissue fat, stimulated UVB-induced apoptosis, and inhibited UVB-induced carcinogenesis in SKH-1 mice (7, 9, 10).

In an additional study we found that decreasing body fat in mice by surgical removal of the parametrial fat pads enhanced UVB-induced apoptosis, suggesting that the stimulatory effect of exercise on UVB-induced apoptosis in the epidermis (and possibly in precancer cells and in UVB-induced tumors in tumor-bearing animals) may be caused, at least in part, by decreased tissue fat levels. Our results suggest that substances secreted by fat cells have an antiapoptotic effect during tumorigenesis so that lowering the level of tissue fat by exercise, by decreasing dietary fat, by lipectomy, or by administration of caffeine or other substances that decrease cellular fat may inhibit carcinogenesis and tumor growth by selectively enhancing apoptosis in DNA-damaged cells and in tumors but not in normal cells. The mechanisms by which decreased tissue fat levels enhance apoptosis in DNA-damaged cells remain to be explored

Table 2. Effect of voluntary running wheel exercise for 2 weeks on UVB-induced apoptotic sunburn cells, caspase 3 (active form)-positive cells, and phospho-p53 (Ser-15)-positive cells in the epidermis of SKH-1 mice

	% Apoptotic sunburn cells		% Caspase 3-positive cells		% Phospho-p53 (Ser-15)-positive cells	
Group	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
No running wheel	0.24 ± 0.04	0.22 ± 0.04	0.26 ± 0.06	0.22 ± 0.04	3.00 ± 0.14	3.53 ± 0.46
Running wheel	0.51 ± 0.09*	0.50 ± 0.10*	0.55 ± 0.10*	0.50 ± 0.10*	3.44 ± 0.33	4.02 ± 0.68
% increase	113	127	112	127	15	14

Female SKH-1 mice (7–8 weeks old, 10 mice per group) were in cages with a running wheel, and other mice (10 per group) were in cages without a running wheel for 2 weeks. The mice were irradiated once with UVB (30 mJ/cm^2) and killed 6 h later. Additional sedentary and exercising mice (10 per group) were not irradiated with UVB. In the absence of UVB irradiation, the number of apoptotic sunburn cells or phospho-p53 (Ser-15)-positive cells in mice with or without a running wheel was ≤0.02%. Apoptotic sunburn cells were measured morphologically, and caspase 3-positive cells and phospho-p53 (Ser-15)-positive cells were measured immunohistochemically. Each value represents the mean ± SE. *, $P < 0.01$.

Table 3. Effect of voluntary running wheel exercise for 2 weeks on the weight of the parametrial fat pads and UVB-induced apoptotic sunburn cells in the epidermis of p53 knockout mice

Group	Body weight, g per mouse	Weight of fat pads, g per mouse	% Apoptotic sunburn cells
$p53^{-/-}$ mice			
No running wheel	30.6 ± 0.98	0.34 ± 0.03	0.15 ± 0.02
Running wheel	31.5 ± 0.66	0.22 ± 0.02*	0.29 ± 0.03*
% change	3	35 ↓	93 ↑
$p53^{+/+}$ mice			
No running wheel	31.1 ± 0.57	0.35 ± 0.02	0.27 ± 0.03
Running wheel	31.1 ± 0.65	0.21 ± 0.02*	0.52 ± 0.06*
% change	0	40 ↓	93 ↑

Male p53 knockout mice and littermate controls (7–8 weeks old, 10 mice per group) were placed in cages with or without a running wheel for 2 weeks. The mice were irradiated once with UVB (30 mJ/cm^2) and killed 6 h later. The weight of the parametrial fat pads was determined, and apoptotic sunburn cells were measured morphologically. Each value represents the mean ± SE. *, $P < 0.01$.

but may be related to a decreased serum level of inflammatory cytokines such as TNFα and other substances that are secretory products of fat cells or by a decreased level of insulin-like growth factor 1 (IGF-1) (13–23). It is of interest that exercise has an antiinflammatory effect, suppresses the formation of TNFα, and increases the level of IL-6 (24). Our study demonstrates that decreasing tissue fat (by partial lipectomy or exercise) is associated with enhanced apoptosis in a DNA-damaged tissue (UVB irradiation/mouse epidermis) or that decreased tissue fat during exercise is associated with enhanced apoptosis in tumors.

Baumann and Rusch (25), >60 years ago, were the first to demonstrate a stimulatory effect of a high-fat diet on UV-induced skin carcinogenesis. Subsequently, Black and colleagues (26–29) confirmed the stimulatory effect of a high-fat diet on UV-induced formation of skin tumors, and they reported that feeding unsaturated fat stimulated carcinogenesis to a greater extent than saturated fat. The stimulatory effect of a high-fat diet on UV-induced formation of skin tumors was observed when the high-fat diet was fed either during the entire experimental period or only after stopping UV administration (29, 30). These results indicated a postinitiation effect of the high-fat diet. Overall, the results indicated that high dietary levels of fats commonly ingested by humans enhanced UV-induced carcinogenesis in mice, but the kind of dietary lipid ingested influenced its effect on carcinogenesis. Our studies suggest that the stimulatory effect of a high-fat diet on UVB-induced carcinogenesis may be related to an increase in the number or size of fat cells and to antiapoptotic substances secreted by these fat cells that inhibit UVB-induced apoptosis and apoptosis in precancer cells and in tumors.

The results obtained by Baumann and Rusch (25) and by Black and his colleagues (26–29) on the stimulatory effect of certain high-fat diets on UVB-induced carcinogenesis in mouse skin described above are similar to studies on the stimulatory effect of a high-fat diet on azoxymethane-induced colon carcinogenesis by Rao *et al.* (31), who showed that feeding a high-fat Western-style mixed-lipid diet had greater tumor-promoting activity in the colon than a low-fat corn oil diet. These investigators also demonstrated that feeding the high-fat mixed-lipid diet was associated with decreased apoptosis and increased COX-2 expression in colon tumors when compared with the low-fat diet, but there was no effect of the high-fat diet on apoptosis in colonic epithelial cells away from the tumors (31). In another study it was found that the effect of a high-fat diet on colon carcinogenesis and apoptosis depended on the type of fat that was administered. Feeding a diet high in fish oil (high in omega-3 fatty acids) inhibited azoxymethane-induced colon carcinogenesis and enhanced apoptosis when compared with a diet high in corn oil (32).

The association of decreased tissue fat during exercise with inhibition of carcinogenesis in skin found in our study (12) and the antiinflammatory effect of exercise observed by others (24) differs markedly from enhanced skin and breast carcinogenesis observed in a genetically modified fat-free mouse that is diabetic, has an enlarged fatty liver, has enhanced markers of inflammation, and has increased susceptibility to skin and breast cancer when compared with wild-type mice (33).

It is of considerable interest that serum from exercising men or from exercising men on a low-fat diet inhibited the growth of prostate LNCaP cells and stimulated apoptosis when compared with serum from control subjects (34). This effect was associated with decreased serum IGF-1 and increased IGF binding protein 1 (34). Adding IGF-1 to serum from the low-fat diet/exercising group reversed the effects of the serum on growth and apoptosis in LNCaP cells, and adding IGF binding protein 1 to serum from control subjects enhanced the ability of the serum to reduce the growth of LNCaP cells and to stimulate apoptosis (35). Whether the stimulatory effects of exercise on UVB-induced apoptosis or apoptosis in tumors of tumor-bearing mice is associated with a decreased serum IGF-1 level remains to be investigated.

The effects of dietary fat on sunlight-induced skin cancer in humans is an important issue. Although the animal data described above provide evidence for a stimulatory effect of several

Table 4. Effects of partial lipectomy (surgical removal of the parametrial fat pads) on UVB-induced formation of apoptotic sunburn cells and phospho-p53 (Ser-15)-positive cells in the epidermis of female SKH-1 mice

	% Apoptotic sunburn cells		% Phospho-p53 (Ser-15)-positive cells	
Treatment	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Sham-operated control	0.33 ± 0.05	0.32 ± 0.13	4.41 ± 0.76	3.86 ± 0.51
Partial lipectomy	0.65 ± 0.08*	0.69 ± 0.12*	4.46 ± 0.70	4.29 ± 0.42
% increase	97	116	1	11

Female SKH-1 mice (10 weeks old, 15 per group) underwent surgery to remove their parametrial fat pads or underwent the same surgical procedure, but with their parametrial fat pads left intact (sham-operated controls). The weight of the fat pads removed was 0.79 ± 0.08 g per mouse in Exp. 1 and 0.84 ± 0.07 g per mouse in Exp. 2. At 2 weeks after the surgical procedure, the mice were irradiated with UVB (30 mJ/cm^2), and they were killed 6 h later. Other mice (five per group) underwent surgery as described above and were killed 2 weeks later without receiving irradiation with UVB. In the absence of UVB irradiation, the number of apoptotic sunburn cells or phospho-p53 (Ser-15)-positive cells in sham-operated control mice or in mice with a partial lipectomy was ≤0.01%. Apoptotic sunburn cells were determined morphologically, and phospho-p53 (Ser-15)-positive cells were measured immunohistochemically. Each value represents the mean ± SE. *, $P < 0.01$.

Table 5. Effects of voluntary exercise during UVB irradiation for 33 weeks on the formation of caspase 3 (active form)-positive cells and BrdU incorporation in UVB-induced skin tumors

Treatment	No. of nontumor areas or tumors examined	% Caspase 3-positive cells	% Increase	% BrdU-positive cells	% Decrease
Nontumor areas					
No running wheel	245	0.159 ± 0.011		5.44 ± 0.27	
Running wheel	167	0.192 ± 0.017	21	4.92 ± 0.32	10
Keratoacanthomas					
No running wheel	191	0.239 ± 0.015		14.90 ± 0.64	
Running wheel	125	0.487 ± 0.042*	104	12.39 ± 0.63*	17
Squamous cell carcinomas					
No running wheel	22	0.184 ± 0.023		15.32 ± 1.68	
Running wheel	16	0.288 ± 0.051**	57	13.81 ± 1.84	10

Female SKH-1 mice (7–8 weeks old, 30 mice per group) were treated with UVB (30 mJ/cm^2 twice per week for 33 weeks). One group of mice was put in cages with a running wheel, and another group of mice was put in cages without a running wheel. The mice developed skin tumors gradually, and all the mice were sacrificed at 33 weeks after the start of treatment. All tumors were taken and characterized by histopathology. The caspase 3 (active form)-positive and BrdU-positive cells were determined immunohistochemically. At least 10 microscopic fields per tumor were examined. Each value represents the mean ± SE. *, $P < 0.01$; **, $P < 0.05$.

high-fat diets on UVB-induced skin carcinogenesis, case control and cohort studies on the effects of dietary fat intake on sunlight-induced skin cancer in humans have provided conflicting results (36–46), possibly because of the many confounding variables that are inherent in such studies. These variables include the ingestion of fat with different compositions in different individuals, differences in caloric intake among different individuals, and differences in gene/environment/lifestyle interactions among different individuals. A carefully controlled 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 (precancerous lesions) and nonmelanoma skin cancers (47–49). Although the latter intervention trial indicates that decreasing the level of dietary fat can inhibit the formation of UV-induced skin tumors in humans (possibly by enhancing apoptosis), even this carefully done study may also be affected by confounding variables such as substances that were added to the diet to replace fat.

In earlier studies, voluntary running wheel exercise was shown to inhibit chemically induced colon and breast cancer in rodents (50–53), but the effects of exercise on apoptosis during the formation of these cancers were not studied. Case control or prospective epidemiology studies in humans suggest that increased exercise is associated with a decreased risk of melanoma (54), colon cancer (55, 56), and breast cancer (57–59), but more carefully controlled clinical trials are needed.

In conclusion, a recent study from our laboratory showed that voluntary running wheel exercise decreased tissue fat and inhibited UVB-induced skin tumorigenesis in SKH-1 mice (12). In the present study we show that voluntary running wheel exercise enhanced UVB-induced apoptosis in the epidermis as well as apoptosis in UVB-induced tumors in tumor-bearing mice. In addition, we show that decreasing tissue fat by surgical removal of the parametrial fat pads (partial lipectomy) enhanced UVB-induced apoptosis. Our results suggest that substances secreted by fat cells promote tumorigenesis by having an antiapoptotic effect in DNA-damaged cells and in tumors. Although the use of sunscreens and avoiding excessive exposure to sunlight and other sources of UVB radiation continue to be important recommendations, the results of our studies and the available human data by others indicate that dietary and lifestyle changes may also play an important role for the prevention of skin cancer.

Materials and Methods

Animals. ***SKH-1 mice.*** Female SKH-1 hairless mice (6–7 weeks old) were purchased from Charles River Breeding Laboratories (Kingston, NY), and the animals were kept in our animal facility for at least 1 week before use. Mice were housed in a temperature- and humidity-controlled room with free access to water and a Purina Laboratory Chow 5001 diet (Ralston Purina, St. Louis, MO), and they were kept on a 12-h light/12-h dark cycle.

Development of congenic p53 knockout hairless mice. Male $p53^{-/-}$ mice on a C57BL/6J genetic background (The Jackson Laboratory, Bar Harbor, ME) were mated with female hairless p53 wild-type SKH-1 mice to obtain male and female hairless congenic p53-deficient mice. The hairless $p53^{+/-}$ mice were intercrossed for eight generations to obtain heterozygous and homozygous p53-deficient mice and their wild-type littermates (F_8 generation). The mice were housed as described above.

Exposure to UVB. The UV lamps used (FS72T12-UVB-HO; National Biological, Twinsburg, OH) emitted UVB (280–320 nm; 75–80% of total energy) and UVA light (320–375 nm; 20–25% of total energy). The dose of UVB was quantified with a UVB Spectra 305 dosimeter (Daavlin, Byran, OH). The radiation was further calibrated with a model IL-1700 research radiometer/photometer (International Light, Newburyport, MA).

Voluntary Exercise and Preparation of Skin Sections. For studies on the effect of voluntary running wheel exercise on UVB-induced apoptosis in the epidermis of SKH-1 mice, female SKH-1 mice (7–8 weeks old, 10 mice per group) were placed in a cage with a running wheel (13.75-cm diameter, 7-cm width) with free access to the wheel 24 h/day for 2 weeks. Other mice with matched body weights and age were placed in a cage without a running wheel and served as controls. The wheels were attached to a permanent magnetic switch that activated a digital counter to count wheel revolutions. Total wheel revolutions were recorded daily, with total distance run per day determined by multiplying the number of wheel rotations by the circumference of the wheel. A water bottle was attached at the top of the cage unit. After 2 weeks of voluntary running wheel exercise, the exercising mice and their controls were irradiated once with 30 mJ/cm^2 UVB and killed 6 h later. Our previous studies showed that 6–10 h after a single irradiation with UVB is the peak time for the formation of UVB-induced apoptotic sunburn cells (10, 60). Another two groups of mice (five mice per group) with or without a running wheel were not treated with UVB and served as controls. The two parametrial fat pads were surgically removed under anesthesia and weighed. The skin samples (≈2 cm long and 0.5 cm wide) were taken from the middle of the back, stapled flat to a plastic sheet, and placed in 10% phosphate-

buffered formalin at 4°C for 18–24 h. The skin samples were then dehydrated in ascending concentrations of ethanol (80%, 95%, and 100%), cleared in xylene, and embedded in Paraplast (Oxford Labware, St. Louis, MO). Four-micrometer serial sections of skin containing epidermis and dermis were made, deparaffinized, rehydrated with water, and used for regular hematoxylin–eosin or immunohistochemical staining. The thickness of the dermal fat layer was measured by using an ocular micrometer with an Olympus BHTU light microscope (Olympus) under ×100 magnification at 5–10 representative areas per slide and averaged (9). Apoptotic sunburn cells, caspase 3-positive cells, and BrdU labeling were determined as previously described (60).

For studies on the effect of voluntary running wheel exercise on apoptosis in UVB-induced skin tumors of SKH-1 mice, we used tissue samples obtained from our previous tumor experiment (12). Briefly, 60 female SKH-1 hairless mice were divided into two groups (30 mice per group and 10 mice per cage). One group of animals was placed in cages with running wheels whereas the other group had no running wheels. Mice were treated with UVB (30 mJ/cm^2) twice each week for 33 weeks. At the completion of the experiment, the animals were killed, and dorsal skin was removed and stapled to a plastic sheet before being placed in 10% buffered formalin phosphate for histological examination. All tumors were collected and characterized histologically as described earlier (12). Caspase 3- or BrdU-positive cells were determined in serial sections from 316 skin keratoacanthomas (randomly picked but with matched tumor size from both groups) and in all 38 carcinomas (61). The entire areas of the tumor sections were examined. In non-tumor areas, measurements were performed at least 0.5 cm away from tumors.

Effect of Surgical Removal of Parametrial Fat Pads (Partial Lipectomy) on UVB-Induced Apoptosis in Epidermis of SKH-1 Mice. Female SKH-1 mice (10–12 weeks old, 15 mice per group) were injected i.p. with ketamine·HCl (120 mg/kg) and xylazine (10 mg/kg). The abdominal skin of the anesthetized mice was sterilized with 70% alcohol. A 1-cm incision along the midline of the abdominal skin was made. After finding the uterus, the parametrial fat pads together with uterus were pulled out of the peritoneal cavity, and both parametrial fat pads were removed. The uterus was returned to the peritoneal cavity, and the incision was sutured. Sham surgery (15 mice per group) was the same as the lipectomy procedure except that the parametrial fat pads were left intact and placed back inside the peritoneal cavity. The animals were returned to their cages, and recovery from anesthesia was monitored and was uneventful. The mice recovered from the anesthesia within 30–60 min. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Rutgers.

Two weeks after surgery, the mice (with or without fat removal) were irradiated with 30 mJ/cm^2 UVB and killed 6 h later. Another two groups of control mice (five mice per group) with or without fat removal were not treated with UVB. Skin samples (≈2 cm long and 0.5 cm wide) were taken, stapled, and placed in 10% phosphate-buffered formalin. Four-micrometer serial sections of skin were made, deparaffinized, rehydrated, and used for hematoxylin–eosin or immunohistochemical staining. The thickness of the dermal fat layer was measured under ×100 magnification at 5–10 representative areas per slide and averaged.

Measurement of Apoptotic Sunburn Cells. Identification of apoptotic sunburn cells was based morphologically on cell shrinkage and nuclear condensation as we have done previously (60). Apoptotic sunburn cells were identified by their intensely eosinophilic cytoplasm and small, dense nuclei, which was observed in hematoxylin–eosin-stained histological sections of the skin by using light microscopy. The percentage of apoptotic sunburn cells in the epidermis (basal plus suprabasal layers) was calculated from the number of these cells per 100 cells counted from the entire 20-mm length of epidermis for each skin section.

Phospho-p53 (Ser-15) Staining. Polyclonal rabbit anti-phospho-p53 (Ser-15) antibody was purchased from Cell Signaling Technology (Beverly, MA). Skin sections were stained by using the StrAviGen Super Sensitive Universal Immunostaining Kit (Biogenex, San Ramon, CA). Paraffin sections were first treated with 0.01 M sodium citrate buffer (pH 6.0) and then incubated with a protein block (normal goat serum). The sections were incubated with phospho-p53 (Ser-15) antibody (1:100 dilution). The samples were then incubated with a biotinylated anti-rabbit secondary antibody, followed by incubation with conjugated streptavidin solution. Color development was achieved by incubation with 0.02% 3,3′-diaminobenzidine tetrahydrochloride containing 0.02% hydrogen peroxide for 10 min at room temperature. The slides were counterstained, and coverslips were added for permanent mounting. A positive reaction was shown as a brown to dark brown precipitate in the nuclei of the cells. The percentage of phospho-p53 (Ser-15)-positive cells in the epidermis was calculated from the number of stained phospho-p53 (Ser-15)-positive cells per 100 cells counted from the entire 20-mm length of epidermis for each skin section. Similar results were obtained by using polyclonal rabbit NCL-p53 CM5p antibody (1:500 dilution) purchased from Novocastra Laboratories (Newcastle upon Tyne, U.K.).

Caspase 3 Immunostaining. Affinity-purified polyclonal rabbit antibody that reacts with the mouse p20 subunit of caspase 3 but does not react with the precursor form was purchased from R & D Systems (Minneapolis, MN). Skin sections used for the measurement of caspase 3 were stained by the horseradish peroxidase-conjugated avidin method as described previously (60). Endogenous peroxidase was blocked by incubating the tissue sections in 3% hydrogen peroxide in methanol for 30 min at room temperature. Sections were then treated with 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven at a high temperature for 10 min. The sections were incubated with a protein block (normal goat serum) for 10 min, followed by avidin D for 15 min and biotin blocking solution for 15 min (Avidin–Biotin blocking kit; Vector Laboratories, Burlingame, CA) at room temperature. The sections were incubated with caspase 3 primary antibody (1:2,000 dilution) for 30 min at room temperature followed by incubation with a biotinylated anti-rabbit secondary antibody for 30 min and incubation with conjugated avidin solution (ABC Elite kit; Vector Laboratories) for 30 min. Color development was achieved by incubation with 0.02% 3,3′-diaminobenzidine tetrahydrochloride containing 0.02% hydrogen peroxide for 10 min at room temperature. The slides were then counterstained with hematoxylin and dehydrated, and coverslips were added for permanent mounting. A positive reaction was shown as a light brown to dark brown precipitate in the cytoplasm and/or perinuclei of the cells.

In short-term studies, the percentage of caspase 3-positive cells in the epidermis was calculated from the number of stained caspase 3-positive cells per 100 epidermal cells counted from the entire 20-mm length of epidermis.

For long-term studies, the percentage of caspase 3-positive cells was determined in 316 skin keratoacanthomas randomly picked but matched for tumor size from exercising and control mice and from all of the carcinomas (total of 38 carcinomas). The entire areas of the tumor sections were examined. In nontumor areas, measurements were performed at least 0.5 cm away from tumors.

BrdU Incorporation into DNA. The methodology was as described previously (60). BrdU was detected by a staining kit obtained from Oncogene Research Products (Cambridge, MA). All animals were injected with BrdU (50 mg/kg) i.p. and killed 1 h later. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The tissue sections were then incubated with 0.125% trypsin, followed by incubation with denaturing solution. The sections were incubated with biotinylated mouse monoclonal anti-BrdU antibody, followed by streptavidin–peroxidase. Color development was achieved with 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.02% hydrogen peroxide.

The BrdU-positive cells were expressed as a percentage of positive cells. The percentage of BrdU-positive cells was determined in serial sections from 316 randomly picked skin keratoacanthomas and from all carcinomas (total of 38 carcinomas) as described above. The entire area of each tumor section was examined. In nontumor areas, measurements were performed at least 0.5 cm away from tumors.

Statistical Analysis. Student's t test was used for comparison of two groups, and the ANOVA model with Dunnett's adjustment was used for comparisons of multiple treatment groups with a common control group. The χ^2 or Fisher's exact test was used for comparing differences between different groups.

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1. American Cancer Society (2004) *Cancer Facts & Figures 2004* (Am Cancer Soc, Atlanta), p 17.
2. Scotto J, Fears TR, Fraumeni JF, Jr (1996) in *Cancer Epidemiology and Prevention*, eds Schottenfeld D, Fraumeni JF, Jr (Oxford Univ Press, New York), pp 355–372.
3. Parker SL, Tong T, Bolden S, Wingo PA (1997) *CA Cancer J Clin* 47:5–27.
4. Cole CA, Forbes PD, Davies RE (1986) *Photochem Photobiol* 43:275–284.
5. de Gruijl FR, Sterenborg HJ, Forbes PD, Davies RE, Cole C, Kelfkens G, van Weelden H, Slaper H, van der Leun JC (1993) *Cancer Res* 53:53–60.
6. Wang Z-Y, Huang M-T, Lou Y-R, Xie J-G, Reuhl KR, Newmark HL, Ho C-T, Yang CS, Conney AH (1994) *Cancer Res* 54:3428–3435.
7. Huang M-T, Xie J-G, Wang Z-Y, Ho C-T, Lou Y-R, Wang C-X, Hard GC, Conney AH (1997) *Cancer Res* 57:2623–2629.
8. Lou Y-R, Lu Y-P, Xie J-G, Huang M-T, Conney AH (1999) *Nutr Cancer* 33:146–153.
9. Lu Y-P, Lou Y-R, Lin Y, Shih WJ, Huang M-T, Yang CS, Conney AH (2001) *Cancer Res* 61:5002–5009.
10. Lu Y-P, Lou Y-R, Li X-H, Xie J-G, Brash D, Huang M-T, Conney AH (2000) *Cancer Res* 60:4785–4791.
11. Michna L, Lu YP, Lou YR, Wagner GC, Conney AH (2003) *Life Sci* 73:1383–1392.
12. Michna L, Wagner GC, Lou Y-R, Xie J-G, Peng Q-Y, Lin Y, Carlson K, Shih WJ, Conney AH, Lu Y-P (May 13, 2006) *Carcinogenesis*, 10.1093/carcin/bgL057.
13. Ahima RS, Flier JS (2000) *Trends Endocrinol Metab* 11:327–332.
14. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW (1999) *Arterioscler Thromb Vasc Biol* 19:972–978.
15. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) *J Clin Invest* 95:2409–2415.
16. Mohamed-Ali V, Bulmer K, Clarke D, Goodrick S, Coppack SW, Pinkney JH (2000) *Int J Obes* 24(Suppl 2):S154–S155.
17. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW (1997) *J Clin Endocrinol Metab* 82:4196–4200.
18. Coppack SW (2001) *Proc Nutr Soc* 60:349–356.
19. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) *Science* 259:87–91.
20. Fried SK, Bunkin DA, Greenberg AS (1998) *J Clin Endocrinol Metab* 83:847–850.
21. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, Coppack SW (1999) *Am J Physiol* 277:E971–E975.
22. Tsigos C, Kyrou I, Chala E, Tsapogas P, Stavridis JC, Raptis SA, Katsilambros N (1999) *Metabolism* 48:1332–1335.
23. Lyngso D, Simonsen L, Bulow J (2002) *J Physiol (London)* 543:373–378.
24. Petersen AM, Pedersen BK (2005) *J Appl Physiol* 98:1154–1162.
25. Baumann CA, Rusch HP (1939) *Am J Cancer* 35:213–221.
26. Black HS, Lenger W, Phelps AW, Thornby JI (1983) *Nutr Cancer* 5:59–68.
27. Black HS, Lenger WA, Gerguis J, Thornby JI (1985) *Cancer Res* 45:6254–6259.
28. Black HS (1983) in *Diet, Nutrition, and Cancer: From Basic Research to Policy Implications*, ed Roe DA (Liss, New York), pp 49–60.
29. Black HS, Thornby JI, Gerguis J, Lenger W (1992) *Photochem Photobiol* 56:195–199.
30. Black HS (1993) *Cancer Bull* 45:232–237.
31. Rao CV, Hirose Y, Indranie C, Reddy BS (2001) *Cancer Res* 61:1927–1933.
32. Chang WL, Chapkin RS, Lupton JR (1998) *J Nutr* 128:491–497.
33. Nunez NP, Oh W-J, Rozenberg J, Perella C, Anver M, Barrett JC, Perkins SN, Berrigan D, Moitra J, Varticovski L, *et al.* (2006) *Cancer Res* 66:5469–5476.
34. Barnard RJ, Ngo TH, Leung PS, Aronson WJ, Golding LA (2003) *Prostate* 56:201–206.
35. Ngo TH, Barnard RJ, Leung PS, Cohen P, Aronson WJ (2003) *Endocrinology* 144:2319–2324.
36. van Dam RM, Huang Z, Giovannucci E, Rimm EB, Hunter DJ, Colditz GA, Stampfer MJ, Willett WC (2000) *Am J Clin Nutr* 71:135–141.
37. Hunter DJ, Colditz GA, Stampfer MJ, Rosner B, Willett WC, Speizer FE (1992) *Ann Epidemiol* 2:231–239.
38. Davies TW, Treasure FP, Welch AA, Day NE (2002) *Br J Dermatol* 146:1017–1022.
39. Veierod MB, Thelle DS, Laake P (1997) *Int J Cancer* 71:600–604.
40. Bain C, Green A, Siskind V, Alexander J, Harvey P (1993) *Ann Epidemiol* 3:235–238.
41. Hakim IA, Harris RB, Ritenbaugh C (2000) *Nutr Cancer* 36:155–162.
42. Gallagher RP, Elwood JM, Hill GB (1986) *Recent Results Cancer Res* 102:38–55.
43. Graham S (1983) *Cancer Res* 43:2409s–2413s.
44. Millen AE, Tucker MA, Hartge P, Halpern A, Elder DE, Guerry DT, Holly EA, Sagebiel RW, Potischman N (2004) *Cancer Epidemiol Biomarkers Prevent* 13:1042–1051.
45. Osterlind A, Tucker MA, Hou-Jensen K, Stone BJ, Engholm G, Jensen OM (1988) *Int J Cancer* 42:200–206.
46. Granger RH, Blizzard L, Fryer JL, Dwyer T (2006) *BioMed Central* 6:141.
47. Jaax S, Scott LW, Wolf JE, Jr, Thornby JI, Black HS (1997) *Nutr Cancer* 27:150–156.
48. Black HS, Herd JA, Goldberg LH, Wolf JE, Jr, Thornby JI, Rosen T, Bruce S, Tschen JA, Foreyt JP, Scott LW (1994) *N Engl J Med* 330:1272–1275.
49. Black HS (1998) *Mutat Res* 422:185–190.
50. Reddy BS, Sugie S, Lowenfels A (1988) *Cancer Res* 48:7079–7081.
51. Cohen LA, Choi K, Backlund JY, Harris R, Wang CX (1991) *In Vivo* 5:333–344.
52. Thompson HJ (1997) *Breast Cancer Res Treat* 46:135–141.
53. Thompson HJ, Zhu Z, Jiang W (2004) *J Nutr* 134:3407S–3411S.
54. Shors AR, Solomon C, McTiernan A, White E (2001) *Cancer Causes Control* 12:599–606.
55. Chao A, Connell CJ, Jacobs EJ, McCullough ML, Patel AV, Calle EE, Cokkinides VE, Thun MJ (2004) *Cancer Epidemiol Biomarkers Prev* 13:2187–2195.
56. Martinez ME (2005) *Recent Results Cancer Res* 166:177–211.
57. Gammon MD, John EM, Britton JA (1998) *J Natl Cancer Inst* 90:100–117.
58. Verloop J, Rookus MA, van der Kooy K, van Leeuwen FE (2000) *J Natl Cancer Inst* 92:128–135.
59. Rockhill B, Willett WC, Hunter DJ, Manson JE, Hankinson SE, Colditz GA (1999) *Arch Intern Med* 159:2290–2296.
60. Lu Y-P, Lou Y-R, Li X-H, Xie J-G, Lin Y, Weichung JS, Conney AH (2002) *Oncol Res* **13,** 61–70.
61. Lu Y-P, Lou Y-R, Xie J-G, Peng Q-Y, Liao J, Yang CS, Huang M-T, Conney AH (2002) *Proc Natl Acad Sci USA* 99:12455–12460.