Food Restriction-Like Effects of Dehydroepiandrosterone: Decreased Lymphocyte Numbers and Functions with Increased Apoptosis (44415)

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Abstract. **Both dietary dehydroepiandrosterone (DHEA) and food restriction can prevent or modulate the initiation or progression of a number of diseases in rodents and prolong life span. We sought to determine if these interventions have common mechanisms of action in regulating lymphocyte functions and cell numbers. We observed that male C57BL/6 mice receiving DHEA in the diet (0.45%, w/w) ate approximately 50% as much food as mice on the DHEA-free diet, and this was reflected in decreased body weights throughout a 10-week period. Mice either fed the DHEA-containing diet or pair-fed to the DHEA-treated mice had decreased spleen and thymus weights and lymphocyte cell numbers compared to mice having free access to the control diet. Mice were fed these diets for 2 weeks before and 2 weeks after exposure to sublethal irradiation (500 cGy). In mice fed DHEA or pair-fed, there was a decrease in spleen cell numbers, and B cells were the most severely affected. The frequency of apoptosis in peripheral blood cells increased from <5% in nonirradiated controls to >50% within 4 days after starting DHEA or pair-feeding. Shortly after irradiation, >87% of blood lymphocytes were hypodiploid (apoptotic) in all groups. By 9 days only 27% of lymphocytes in mice on the control diet were hypodiploid compared to 62% in DHEA and 74% in pair-fed mice. In addition, both DHEA and pair-fed mice had significant reductions in T-cell function (contact hypersensitivity to dinitrofluorobenzene), B cell function (antibody response to trinitrophenolated-lipopolysaccharide), and NK cell function (lung clearance of radiolabeled YAC-1 tumor cells) 2 weeks after irradiation. In a complementary study, peripheral blood lymphocytes from naïve mice were treated overnight with various concentrations of either DHEA or hydrocortisone 21-acetate. Only glucocorticoid-treated cells underwent apoptosis. Thus, DHEA induces apoptosis *in vivo* but not *in vitro.* We conclude that dietary DHEA induces apoptosis and decreased lymphocyte production and function in C57BL/6 mice largely by reducing food intake.** [P.S.E.B.M. 1999, Vol 221]

Dehydroepiandrosterone (DHEA) is a steroid prohormone present in relatively high concentrations in blood of young human adults; these levels gradually decrease to approximately 10% by the eighth decade of life (1). Clinical studies revealed decreased serum and/or urinary concentrations of DHEA significantly lower in patients with Alzheimer's disease (2), HIV infection (3), systemic lupus erythematosus (4), coronary atherosclerosis (5), breast cancer (6, 7), and obesity (8). In studies with animal models of diseases, dietary DHEA (0.2%–1.0% w/w of food) was effective in preventing or inhibiting neoplasia (9), diabetes (10), lupus erythematosus (11), immune decline (12), hemolytic anemia (13), nephrosis (14), atherosclerosis (15), and obesity (16).

Despite the association between decreased serum DHEA levels and disease states in humans, little is known about the mode(s) of DHEA action. Many of the diseases in

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which DHEA is effective have an immune component (4, 11–13). Low doses of DHEA or its sulfate enhances the effectiveness of vaccines against various pathogens, particularly in aged rodents (17–29). At a higher dietary DHEA dose of 0.45% (w/w), there is a decrease in lymphopoiesis (30), but little effect on various lymphocyte functions in mice (31, 32). In long-lived (C3H.SW X B10.RIII)F1 mice, dietary DHEA increased T-cell and NK-cell function (12).

DHEA administration to mice and rats can significantly decrease food intake (12, 14, 33, 34); thus, one of the possible mechanisms by which dietary DHEA may exert its beneficial effects is through the induction of food restriction. Whereas dietary DHEA (0.2%–1.0%) may reduce food intake (10), lower levels of DHEA applied subcutaneously or topically to stimulate the immune system do not affect food consumption or body weights. Limiting an animal's food intake has been found to be therapeutic in many of the same disease models in which dietary DHEA administration is also beneficial (9, 12, 13, 35–41). On the basis of these observations we conducted pair-feeding experiments to determine the possible involvement of food restriction on the observed effects of dietary DHEA on cells of the immune system. Because restriction of food intake has been reported to induce apoptosis in both normal and neoplastic cells (40, 42), we analyzed the incidence of apoptosis in peripheral blood lymphocytes of both DHEA-treated and pair-fed animals before and after irradiation (500 cGy). We also compared the effects of DHEA and hydrocortisone 21-acetate on the rates of apoptosis on lymphocytes maintained in overnight cultures.

Materials and Methods

Animals. Conventionally housed male C57BL/6 mice (10–12 weeks old) were obtained from the Department of Microbiology Colony at this University. Initially all studies were conducted with mice housed individually (1/cage); later, mice were housed in groups of four to six per cage. No differences were observed in body weight, food intake, or immune parameters whether housed singly or in groups. Mice were caged singly for experiments described in Figures 1 and 5. Animals were housed at 22 ± 2°C and maintained on a 12-hr light/dark cycle with lights out at 19:00 hr. The use of mice has been approved by the Institution's Animal Care and Use Committee (IACRAC).

Diets. Fourteen days prior to any experimental manipulations, mice were placed on pelleted purified AIN (American Institute of Nutrition)-76A diet (Dyets Inc., Bethlehem, PA) containing either no additives (control diet) or DHEA (0.45%, w/w) (Sigma, St. Louis, MO). All animals except pair-fed mice had free access to food, and all mice received water freely. Pair-fed animals were given the control diet. The amount of control diet fed to pair-fed mice was determined by the mean weight of the food consumed by DHEA-fed mice on the previous day. The diet for pair-feeding was administered once per day between 09:00 hr and 10:00 hr.

Determination of Food Intake and Body Weight. The weight of the food consumed by mice on the DHEA-containing diet, accounting for the weight of spilled food, was determined daily between 09:00 hr and 10:00 hr. When mice were housed four to six animals per cage, only the mean daily intake per mouse could be obtained by dividing the total daily food intake per cage by the number of mice in the group. Mice were weighed weekly during the course of the studies.

Irradiation. Mice were exposed to 500 cGy of gamma radiation in a GammaCell 40 small animal irradiator containing two 137Cs sources (Atomic Energy Ltd., Ottawa, Ontario, Canada) at a rate of 68 cGy/min.

Contact Hypersensitivity. This response is a test of T-cell function and was performed as described (43). Briefly, the abdomen of mice was shaved and painted on 2 consecutive days with 20 μl 0.5% dinitrofluorobenzene (DNFB, Sigma) in acetone/olive oil (4:1, v/v) solution. Five days later, ear swelling was elicited by applying 10 μl of 0.2% DNFB solution to both sides of the pinna of one ear. Response was determined by measuring the difference in ear thickness between pre-elicitation and 24 hr post-elicitation using a Fowler Precision Tools caliper (Lux Scientific Instrument Corp., New York, NY).

T Cell-Independent IgM Antibody Response. The IgM response to trinitrophenolated lipopolysaccharide (TNP-LPS, Sigma), was used to measure B-cell function. Mice were immunized by i.p. injection of 100 μg TNP-LPS; 6 and 10 days later mice were bled *via* the tail vein; sera were diluted in PBS containing 1.0% BSA (Sigma), and IgM concentrations were determined by ELISA (44).

Lung Clearance of Radiolabeled YAC-1 Tumor Cells. This is an assay for natural killer (NK) cell function. YAC-1 tumor cells (31) (5×10^7) were incubated in RPMI containing 5-fluorodeoxyuridine (2.5 μg/ml; Sigma); 20 min later, 30 μCi [^{125}I]5-iodo-2′-deoxyuridine (^{121}IdU, Amersham, Arlington Heights, IL) was added and the cells were incubated for 90 min at 37°C in 5% CO_2/air. The cells were washed three times in RPMI and adjusted to 2×10^6 cells/ml. Cell viabilities were >95% by trypan blue exclusion. 1×10^6 cells (0.5 ml) were injected into mice *via* the lateral tail vein. Four hours later, the mice were sacrificed by CO_2 inhalation, the lungs were removed, and ^{125}I radioactivity was determined. The results are expressed as the geometric mean (95% confidence limits) percentage recovery of the total injected radioactivity (45). The *lack* of retention of radiolabeled YAC-1 cells in the lungs is a measure of NK activity.

Flow Cytometry for Lymphocyte Surface Markers. Animals were bled from tail veins to obtain blood lymphocytes. Other mice were sacrificed CO_2 inhalation. Spleens were removed and gently crushed between the frosted ends of slides in RPMI media (GIBCO BRL, Grand Island, NY). Each spleen was processed individually. Cells were washed twice in RPMI. Erythrocytes were lysed by hypotonic shock; splenocytes were then passed through ny-

lon mesh to make single cell suspensions. Cells were counted using an electronic particle counter (Coulter Electronics Inc., Hialeah, FL). Splenocytes (2×10^6) were suspended in 5% calf serum in PBS, and cells were stained with the following conjugated monoclonal antibodies: $F(ab)'_2$ DTAF-goat anti-mouse IgG + IgM (H + L) (Jackson ImmunoResearch, Avondale, PA), FITC-anti-TCR$\alpha\beta$, PE-anti-NK1.1, (Pharmingen, San Diego, CA), or the irrelevant isotype controls FITC-Rat IgG2a, PE-mouse IgG2a, PE-Hamster IgG (Pharmingen). Collection gates were set on cells of lymphocyte size and internal complexity based on forward and 90 scatter using a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA).

Flow Cytometry for Apoptosis. *In vitro studies.* Peripheral blood was collected from the lateral tail vein of untreated mice and placed in Alsever's solution (GIBCO BRL). Erythrocytes were lysed by hypotonic shock, cells were enumerated using the Coulter electronic particle counter, and lymphocytes (2×10^6 cells/well in 0.2 ml) were plated in 96-well plates containing various concentrations of DHEA or hydrocortisone 21-acetate. DHEA and hydrocortisone 21-acetate stock solutions in ethanol were diluted to the appropriate concentrations with medium. Medium and ethanol-containing medium were used as controls in the flow cytometry studies. Cells were incubated overnight at 37°C in 5% CO_2/air. The next day the cells were placed in Falcon 2054 tubes (Becton-Dickinson) for staining and FACS analysis.

In vivo studies. At different time points after the start of either the DHEA or pair-fed diet, peripheral blood was collected by tail bleeding. Erythrocytes were lysed by hypotonic saline, and lymphocytes were placed in Falcon 2054 tubes for staining and analysis by flow cytometry.

Propidium iodide staining and analysis. Cells were stained as described (46). Briefly, 1×10^5 to 1×10^6 cells were incubated overnight at 4°C in the presence of hypotonic propidium iodide (PI) solution [0.1% Triton X-100 (Baker Chemical Co. Phillipsburg, NJ), 0.1% sodium citrate (Fisher Scientific, Fair Lawn, NJ), 50 μg/ml PI (Sigma)]. The following day the cells were analyzed using a FACScan flow cytometer. Cellular debris was gated out based on low forward scatter. PI fluorescence was monitored in the red fluorescence channels. Apoptotic cells (hypodiploid) appear as a broad peak, staining with considerably lower intensity than the narrow peak of healthy (diploid) cells.

Statistics. Differences between groups were determined using two-way ANOVA followed by Duncan's range test, one-way ANOVA followed by Scheffe's *post hoc* test, or Students *t* test as indicated in the figure legends.

Results

We examined the effect of dietary DHEA (0.45%, w/w) on food intake in C57BL/6 mice for 28 days; 14 days prior to, and 14 days after, sublethal irradiation. Figure 1A presents the mean (± SEM) daily food intake of both control

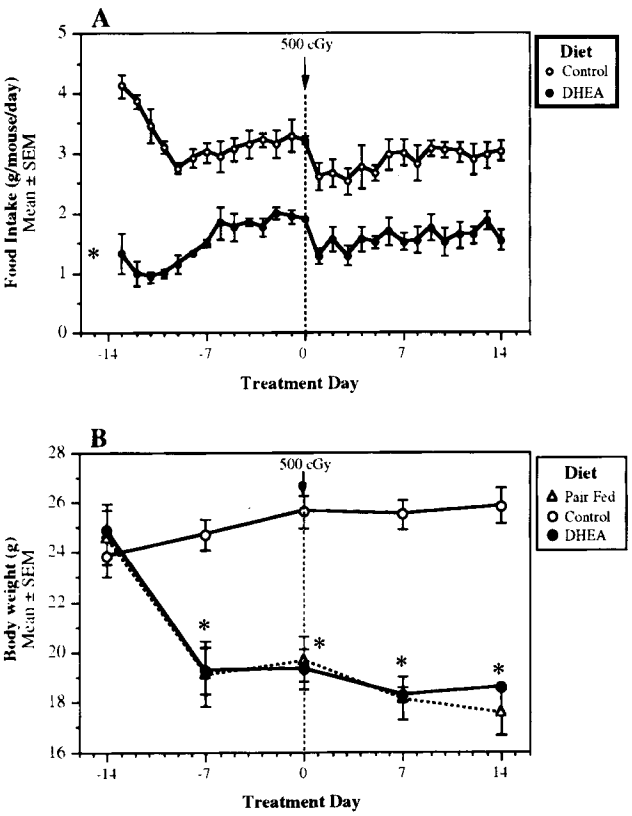

Figure 1. Effect of DHEA treatment and pair-feeding on food intake and body weight of male C57BL/6 mice. Mice received the AIN-76A diet with or without DHEA (0.45%, w/w) freely, or the pair-feeding diet starting 14 days prior to and after sublethal irradiation (500 cGy). Daily food intake and weekly body weights were monitored. Values presented are from six experiments, with four to six mice/group or a minimum of 24 mice per experimental group. *Significantly different from control ($P < 0.01$) by two-way ANOVA with repeated measures followed by Duncan's range test.

and DHEA-treated mice from six independent experiments. The intake of pair-fed mice is not shown because food administered was equal to the intake by the DHEA-fed mice with a 1-day delay. The average intake throughout the course of the studies was 3.04 ± 0.06 g/day (mean ± SEM) for control mice and 1.53 ± 0.05 g/day for DHEA-treated and pair-fed mice ($P < 0.001$). The mean body weights (± SEM) of mice in the same six experiments are indicated in Figure 1B. After the first week, the body weight curves for DHEA and pair-fed mice were significantly different from control, but not from each other, indicating that in C57BL/6 mice, the reduction of food intake accounts for the loss of body weight seen during DHEA treatment. It is interesting to note that DHEA-treated mice consumed significantly less food than control mice even when intake was calculated per gram of body weight (data not shown). In contrast to these findings, in studies conducted with other strains of mice and with obese versus lean Zucker rats receiving either regular chow or palatable food with and without DHEA, variable effects on food intake were observed (10, 12, 47–48).

Pair-feeding experiments were used to determine the role of decreased food intake on the effects of DHEA on lymphoid tissue in unirradiated mice. Male C57BL/6 mice

were fed the control diet or the DHEA-containing diet (0.45%, w/w) freely, or were pair-fed (i.e., given only the amount of control diet that DHEA-fed mice consumed). Mice were sacrificed 10 weeks later, and spleens and thymuses were removed and analyzed. The weights and numbers of cells of spleens of DHEA-treated and pair-fed mice were reduced by approximately 50% (Figs. 2A and 2B). Thymus cell numbers were decreased >80% and weights by >50% in both DHEA and pair-fed animals compared to controls (Figs. 2C and 2D). At 10 weeks of feeding the mean ± SD body weights (g) of control, DHEA fed or pair-fed mice were 30.0 ± 2.5, 20.4 ± 1.8, and 21.3 ± 1.8, respectively ($P < 0.001$ control versus the other two groups). The food intake values (g/day) were 3.2 ± 0.2, 2.1 ± 0.4 and 2.1 ± 0.4, respectively ($P < 0.001$ control versus the other two groups).

Contact hypersensitivity responses to DNFB were used as a measure of T-cell function 14 days after sublethal irradiation. The car swelling response to DNFB after priming of DHEA and pair-fed mice was significantly decreased, $P < 0.05$ (Fig. 3A); in fact, the slight response observed was indistinguishable from that seen in unprimed (naive) animals. Similarly treated mice (control, DHEA, pair-fed) were challenged with TNP-LPS to induce a T-cell–independent antibody response to assess B-cell function (44, 49). Serum was obtained prior to immunization and also 6 days after immunization and assayed by ELISA for IgM antibody concentration. The level of IgM antibodies to LPS was significantly decreased in both DHEA and pair-fed mice at serum dilutions of 1:200, 1:1000, and 1:5000, $P < 0.05$ (Fig. 3B). Ten days after immunization there was still a significant difference at the 1:200, but not at the 1:1000 or 1:5000 dilutions (data not shown). NK-cell activity was assayed 14 days after sublethal irradiation by intravenous challenge with ^{125}IdU-labeled YAC-1 cells. Over a 4-hr span, control mice were able to clear the YAC-1 cells from the lungs as reflected by low (8.4%) lung retention of infused YAC-1 cells. The retention of radioactivity was significantly increased in DHEA-treated (25.7%) and pair-fed (24.3%) mice, indicating decreased NK activity, $P < 0.05$ (Fig. 3C).

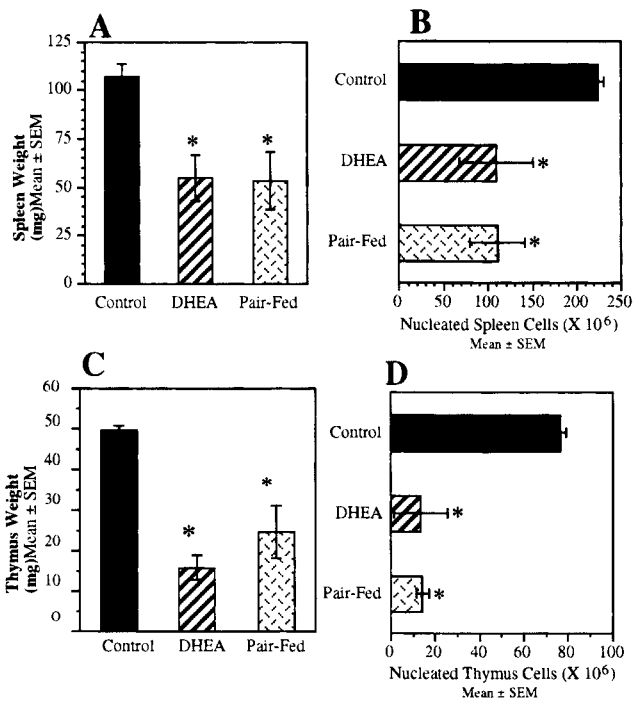

Figure 2. Effect of DHEA treatment and food restriction on spleen and thymus weights and cell numbers. Male C57BL/6 mice were placed on one of the three dietary regimens (Fig. 1) for 10 weeks. (A, B) Spleens and (C, D) thymuses were removed, weighed, and single cell suspensions were prepared. *Significantly different from control ($P < 0.05$) by one-way ANOVA followed by Scheffe's *post hoc* test. This experiment was done twice with four to six mice/group with a minimum of eight mice/group.

Flow cytometric analysis of splenocyte populations 2 weeks after sublethal irradiation demonstrated that the splenic population most affected by dietary DHEA and pair-feeding is the B-cell compartment (i.e., there was a marked decrease in the frequency and numbers of IgG plus IgM positive cells in DHEA and pair-fed animals) (Fig. 4). There was a relative increase in the frequency, but a decrease in total numbers of TCR$\alpha\beta^+$ T cells and NK1.1$^+$ NK cells per spleen.

As internucleosomal DNA cleavage is a hallmark of apoptosis, we monitored the percentage of cells undergoing apoptosis by FACS analysis using PI staining to determine DNA content within nuclei (46). Within 4 days of either DHEA- or pair-feeding, large increases in apoptotic cells were observed (Fig. 5). The numbers of peripheral blood lymphocytes undergoing apoptosis, though variable, remained elevated throughout the study period. As expected, after 500 cGy irradiation 80%–90% of peripheral lymphocytes of all groups (control, DHEA and pair-fed) appeared apoptotic. Over the next 2 weeks the proportion of apoptotic lymphocytes in mice receiving the control diet decreased to 25%, whereas that proportion in DHEA and pair-fed mice remained at approximately 75%–85%.

Because DHEA (this paper) and glucocorticoids (49) can induce apoptosis *in vivo,* we examined whether or not DHEA could induce apoptosis *in vitro.* Peripheral lymphocytes in culture were treated with DHEA and hydrocortisone 21-acetate at concentrations from 1×10^{-11} to $1 \times 10^{-5} M$. Although the glucocorticoid induced apoptosis in a dose-dependent manner, DHEA was totally inactive (Fig. 6).

Discussion

The effect of dietary DHEA on thymic and splenic cell numbers and lymphoid cell functional recovery after sublethal irradiation of male C57BL/6 mice appears to be due in large part to a decrease in food intake, as based on similar effects produced by pair-feeding. The dramatic effect of DHEA on food intake by C57BL/6 mice is greater than that reported by other investigators. Dietary DHEA (0.2%–1.0% w/w) exerts dose-dependent effects on food intake in a

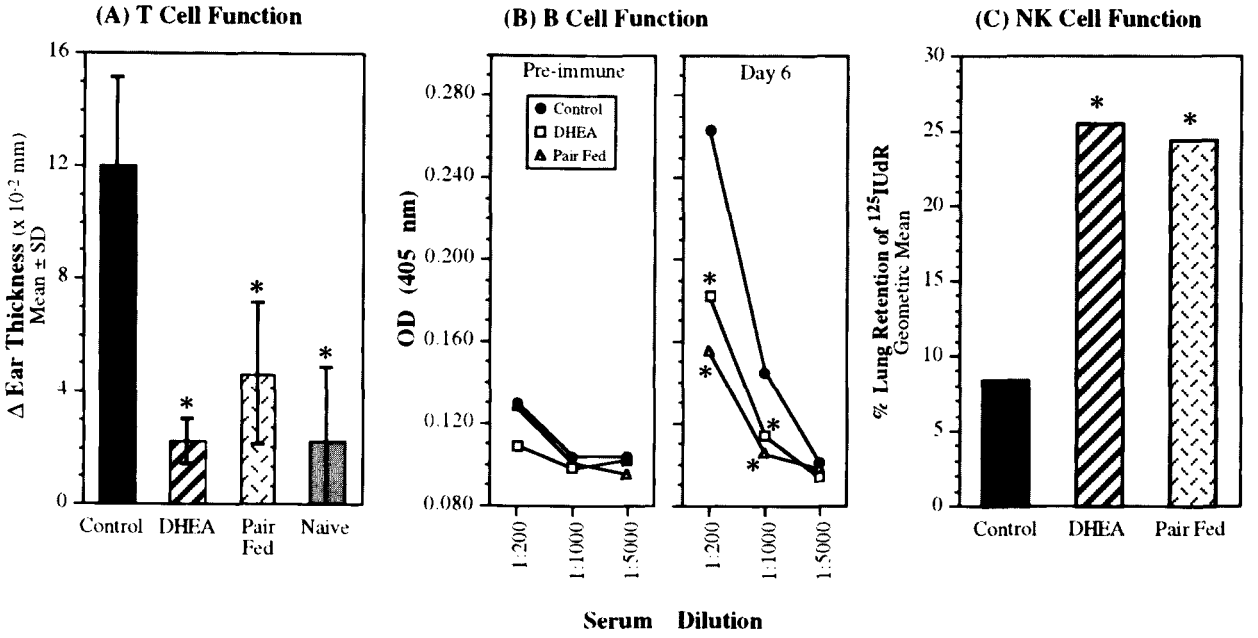

Figure 3. Effect of DHEA treatment and food restriction on recovery of T, B, and NK lymphocyte functions after sublethal irradiation. Male C57BL/6 mice were placed on one of the three dietary regimens (Fig. 1). Two weeks after starting the diets, the mice were exposed to sublethal irradiation (500 cGy). Two weeks after irradiation mice were examined for one of the following parameters: (A) contact hypersensitivity response to DNFB (T cell response); (B) IgM response to TNP-LPS (B cell response); and (C) clearance of radiolabeled YAC-1 tumor cells (NK cell response). *Significantly different from control ($P < 0.05$) by one-way ANOVA followed by Scheffe's *post hoc* test in A and C, and two-way ANOVA followed by Duncan's range test in B. This experiment was done twice with four mice/group or a total of eight mice/group.

given strain of mice, but the effects vary in different animal species and/or strains. For example, Coleman *et al.* (10) observed that food intake was decreased in only one (C57BL/6J) of two strains tested in the narrow ancestral C57BL mouse lineage. Most investigators have found no or only transient effects of DHEA (9, 50–53), whereas others have observed a significant reduction in food intake. A diet we and others have used may contribute to more dramatic effects on food intake due to better absorption (10, 30–32). The suppression of food intake by DHEA in Zucker rats was associated with alterations of hypothalamic serotonin content (47), raising the possibility that DHEA may have a neuromodulatory effect on appetite regulation.

Both food restriction and dietary DHEA can prevent and/or treat many of the same diseases in animal models, as well as inhibit immune decline (1–10, 35–42); this list includes autoimmune disease (35–37), nephrosis (14, 39) and neoplasia (9, 40, 41, 54). Also, both dietary DHEA and food restriction greatly extend the life expectancy of laboratory rodents (14, 55). These observations indicate a possible role for food restriction in the effects of DHEA. For many years little was known about the mechanism by which food restriction mediates its various effects. As early as the 1960s it was noted that food restriction inhibited cellular proliferation, and it was proposed that proliferation was inhibited through suppression of DNA synthesis (56, 57). More recently, food restriction has been shown not only to inhibit proliferation, but also to eliminate pre-neoplastic cells through induction of apoptosis (40). The protective effect of food restriction against cancer was associated with modulation of the rates of apoptosis and proliferation (42). In our studies, DHEA administration and food restriction dramatically increased the number of apoptotic blood lymphocytes in mice within 4 days. The high rates of apoptosis were surprising, because apoptotic cells are usually rapidly engulfed and removed by phagocytic cells. The action of DHEA is likely mediated through food restriction, because pair-fed mice behave in a similar manner. DHEA, unlike glucocorticoids, does not directly induce cells to undergo apoptosis *in vitro* (Fig. 6), indicating that DHEA and food restriction act indirectly. However, it is conceivable that the stress or toxicity of DHEA and/or food restriction stimulated the secretion of corticosterone or other direct mediators of apoptosis, or induced the expression of *Fas* on lymphocytes and *Fas* ligand on other cell types or other lymphocytes. The effects of DHEA observed here may have been mostly indirect and may have obscured any direct effects of DHEA on the immune system.

It is possible that apoptosis detected by flow cytometry as described (46) is a result of the manipulations involved in isolating peripheral blood lymphocytes, and not a result of the treatment. However, the fact that apoptotic cells are not detectable in lymphocytes of mice on the control diet until after the mice are sublethally irradiated indicates that the manipulations performed here do not induce healthy cells to undergo apoptosis. It is still possible that apoptotic cells are not present in the blood of DHEA-treated or pair-fed mice, but that these cells are more susceptible than cells from

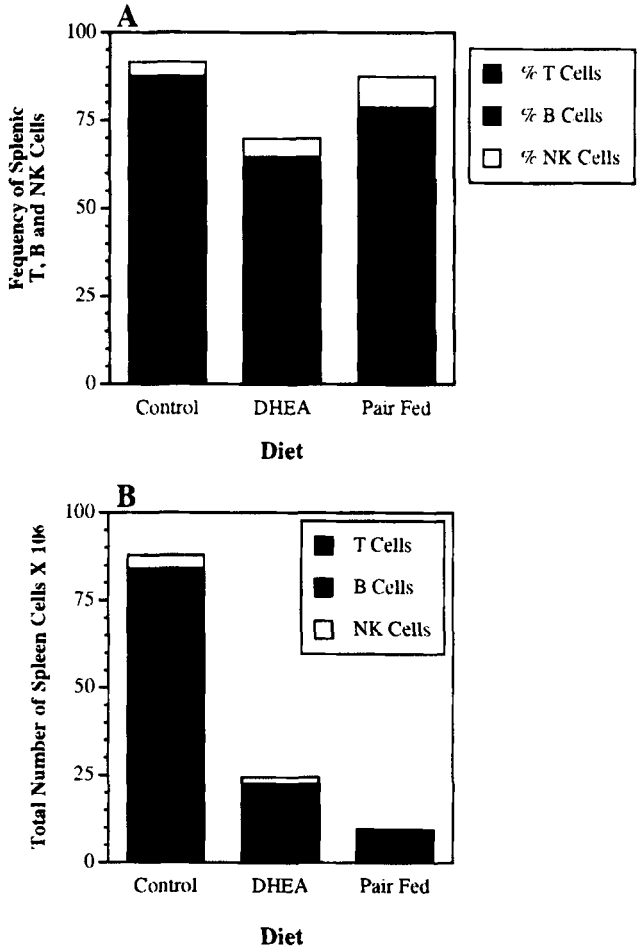

Figure 4. Effect of DHEA treatment and pair-feeding on splenic lymphocyte populations after sublethal irradiation of male C57BL/6 mice. Male C57BL/6 mice were placed on one of the three dietary regimens, as described (Fig. 1) 14 days prior to sublethal irradiation (500 cGy). Two weeks after irradiation, animals were sacrificed, and spleens were removed and pooled for flow cytometry studies. Cell populations were defined by the following criteria: FITC-anti-TCRαβ (T cells), DTAF-goat anti-IgG/IgM (H+L) (B cells), and PE-anti-NK 1.1 (NK cells). (A) Frequencies and (B) absolute numbers of T, B, and NK cells are presented. This experiment was done twice with 5 mice/group or a total of 10 mice/group.

mice on the control diet and, thus, undergo apoptosis more readily when manipulated. Whether or not this is the case, administration of DHEA and restriction of food intake lead to an increased incidence and/or susceptibility to apoptosis.

Apoptosis is believed to play a critical role in the development and regulation of the immune system. *In vivo,* it is believed that immune tolerance, as well as the extent of an immune response, are regulated through induction of apoptosis of responding cells (58). Thus, it is not surprising that lymphocytes can be induced to undergo apoptosis through a variety of signals. After cell contact, binding of the *Fas* antigen of target cells to the *Fas* ligand induces apoptosis in certain cells (59). Cytokines also can play a major role in this process (e.g., cells may undergo apoptosis after withdrawal of such required growth factor as GM-CSF or IL-3) (60, 61). TGFβ, TNFα, and IFNγ can directly induce apoptosis (62–64). We confirmed our earlier observations (31, 32) that the thymus and spleen are both reduced in size and cell numbers. While the normal thymus has a high rate of apoptosis to mediate T-cell selection, apoptosis in the spleen is only prominent in germinal centers during B-cell selection. Splenic B cells, a major target of DHEA or food restriction (Fig. 4), undergo apoptosis unless reduced by agents such as IL-4 or protein kinase C activators (65). The microenvironment in the spleens of DHEA-treated and pair-fed mice may have decreased levels of cytokines necessary for B-cell survival or increased levels of cytokines that induce apoptosis. Deliberate starvation for 2 days resulted in decreased T-cell–mediated immunity, which could be reversed by the administration of leptin to mice (66). Therefore, it is conceivable that food restriction could decrease leptin levels to mediate some of the effects we observed.

Although our data suggest a role for food restriction in some of the effects produced by dietary DHEA, food restriction cannot account for all of the effects of this steroid. For example, a single subcutaneous administration of a low dose of DHEA increased immunity against viral challenge (17). Similar enhancement of immune-related effects of low doses of DHEA given topically or by injection was observed particularly in aged rodents (18–29). It is unlikely that the effect of DHEA administered as a single, small dose is mediated through a decrease in energy intake or lack of some essential component present in food, supporting the concept that DHEA may act through a variety of mechanisms. Although highly effective in aged mice, short-term treatment of aged humans with a relatively small amount of DHEA did not significantly improve the age-related decreased response to immunization against influenza or tetanus (67–69). However, oral dehydroepiandrosterone supplied in physiological replacement doses to postmenopausal women had an immunomodulatory effect (i.e., decreased $CD4^+$ (helper) T cells and increased $CD8^+/CD69^+$ NK cells and cytotoxicity) (70). DHEA is a steroid pro-hormone for estrogens and androgens, and the immune and endocrine systems are considerably intertwined. It is possible that DHEA affects the immune system at many levels; the decreased DHEA levels in aged individuals might contribute to immunosenescence.

Obese women placed on a restricted diet were found to have decreased levels of NK cell function and circulating IgG (71). Also, after 6 months on a 30% caloric reduction diet, the four men and four women of Biosphere 2 demonstrated a 30% decrease in leukocyte count (72). In addition, preliminary findings of studies on life span extension and reduction in age-related diseases by food restriction in rhesus monkeys appear to be very similar to those found in rodents (73). Humans subjected to fasting for 7 or 10 days had decreased numbers of peripheral lymphocytes, but NK-cell killing activity per cell increased significantly (74).

The observation that some of the effects produced by dietary DHEA may be mediated through food restriction raises interesting possibilities for human therapy. Humans given 1.6 g DHEA/day orally did not decrease food intake

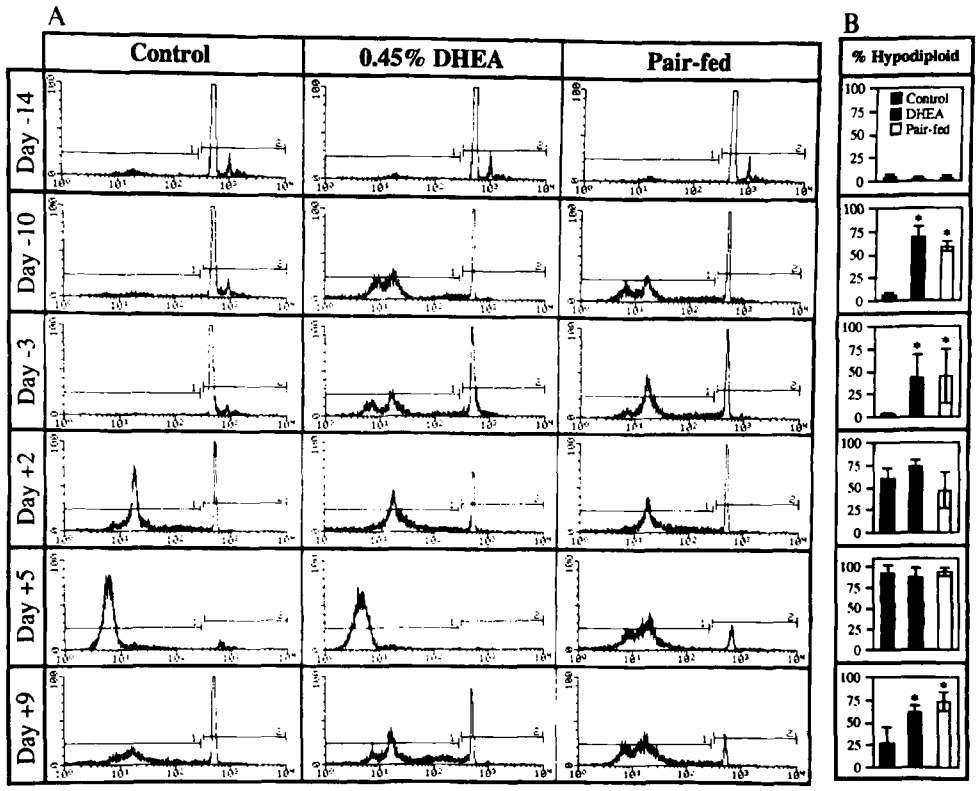

Figure 5. Effect of DHEA treatment and pair-feeding of male C57BL/6 mice on apoptosis rates of peripheral blood lymphocytes *in vivo*. Male C57BL/6 mice were placed on one of the three dietary regimens (as described in Fig. 1) 14 days prior to sublethal irradiation (500 cGy). The feeding regimens were continued for 2 weeks after irradiation. Blood was obtained from the lateral tail vein, erythrocytes were lysed, and cells were stained with propidium iodide for FACS analysis to determine the percentage of hypodiploid cells. *Significantly different from control ($P < 0.05$) by one-way ANOVA followed by Scheffe's *post hoc* test. This experiment was done twice with four mice/group or a total of eight mice/group.

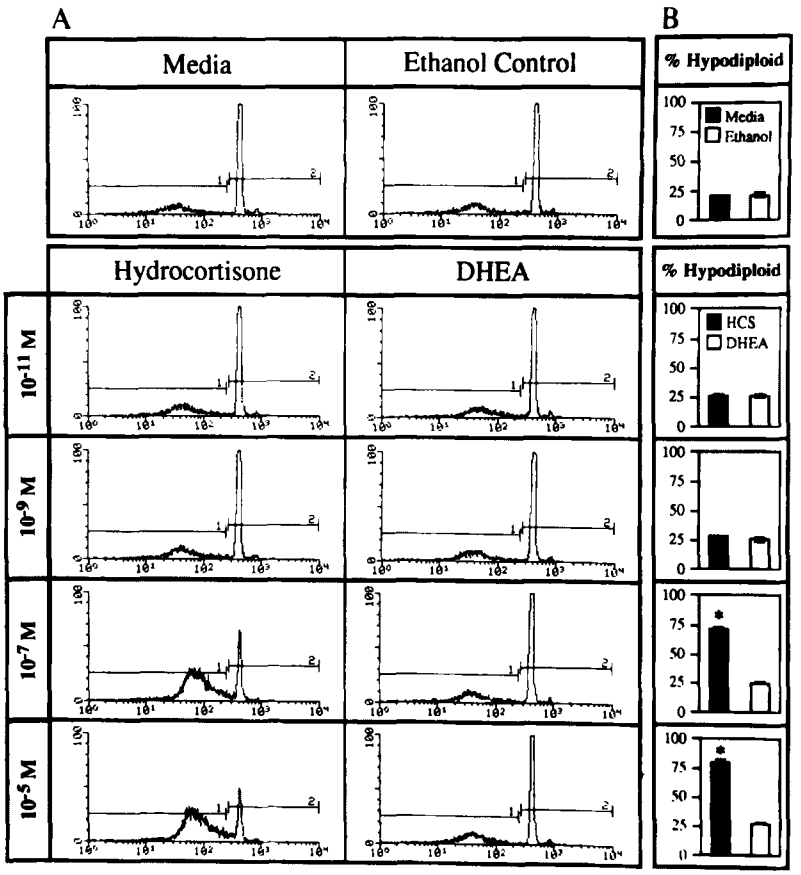

Figure 6. Effect of DHEA and hydrocortisone 21-acetate *in vitro* on apoptosis of cultured peripheral blood lymphocytes. Peripheral blood lymphocytes obtained from untreated male C57BL/6 mice were cultured overnight in 96 well plates (2×10^6 per well) in the presence of either DHEA or hydrocortisone 21 acetate at the concentrations indicated. Cells were harvested and stained with propidium iodide, as described in Materials and Methods, and analyzed by flow cytometry. Gray bars represent hypodiploid cells (%; mean ± SD) observed in cultures with media containing 0.1% ethanol. *Significantly different from either medium or 0.1% ethanol-containing medium and from DHEA-treated cells ($P < 0.001$) by Student's *t* test. This experiment was done twice with very similar results.

(75–77). However, these doses are much lower when expressed in DHEA/kg body weight/day than doses used in animal studies. The dose of DHEA that can be administered safely to human subjects is not precisely known, but it is limited by the possible adverse effects of increasing the levels of the androgenic and estrogenic metabolites of DHEA. Oral doses of 3 g/day were safe in a study of treatment of patients with multiple sclerosis (78).

Whether the benefits of reducing food intake are due to immunological or other mechanisms remains to be determined, yet it is important to note that some immunological effects are observed in both laboratory animals (35–42) and humans (71, 72, 74). Unfortunately, the reality of human compliance to a restricted diet is likely to fall short of what can be imposed on caged laboratory animals.

In conclusion, dietary DHEA decreased food intake in male C57BL/6 mice, and this decrease apparently led to increased apoptosis of lymphocytes, with decreased lymphoid cell numbers and functions. Adrenalectomy reversed the inhibitory effects of food restriction on skin tumor promotion by phorbol esters in mice (79), supporting the potential importance of adrenal steroids (e.g., glucocorticoids) in at least some of the effects observed. Defective apoptosis is thought to contribute to autoimmune disease and immunosenescence (80, 81), whereas increased apoptosis is protective in liver cancer models (40, 42). DHEA, by reducing food intake, may exert some of its beneficial effects through apoptosis. The pleiotropic effects of DHEA on metabolic, hormonal, or brain functions argue that apoptosis is not the only important effect of this abundant human steroid (82).

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