Opposing Actions of Dehydroepiandrosterone and Corticosterone in Rats (44405)

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Abstract. **The purpose of this study was to determine the impact of dehydroepiandrosterone (DHEA) and corticosterone (CORT) treatment, using implants as a route of administration, on specific hormones, metabolites, and enzymes involved in energy metabolism. Sixty male Sprague-Dawley rats, 325 g initial weight, were implanted subcutaneously for 3 weeks with time-release pellets containing either DHEA or CORT at doses of 0, 10, 25, 50, or 100 mg in this 2 × 5 factorial experiment. In general, body weights and food intakes decreased as the level of steroid hormones increased. In contrast to DHEA treatment, rats receiving the 50- and 100-mg doses of CORT had lighter thymus glands and spleens and heavier epididymal and retroperitoneal fat pads than their controls. Rats treated with 100 mg of DHEA had lowered serum levels of triglycerides and lipid hydroperoxides whereas rats treated with 100 mg of CORT had higher levels of these blood lipids compared to their respective controls. In contrast to DHEA treatment, there was a dose-dependent increase in liver lipid content and the specific activities of the hepatic lipogenic enzymes glucose-6-phosphate dehydrogenase, malic enzyme, and fatty acid synthase in response to CORT treatment. Rats treated with 100 mg of DHEA had higher serum levels of IGF-1 than control rats. Conversely, rats treated with 100 mg of CORT had lower serum levels of IGF-1 and higher serum levels of testosterone, progesterone, and insulin than their controls. These data demonstrate the lipogenic actions of corticosterone in rats. Conversely, DHEA treatment reduced serum and hepatic lipids. Furthermore, these data suggest that using implants instead of bolus injections of steroids may be a more physiological approach for studying the influence of these steroids on lipid metabolism.**

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Glucocorticoid excess in humans (1) and experimental animals (2) is associated with obesity. For example, patients with Cushing's disease present upper body obesity, insulin resistance (e.g., NIDDM), and hyperlipidemia (3). Glucocorticoids are also essential for the hyperlipogenesis observed in starved-refed rats (4). Moreover, hydrocortisone is a major fetal adipogenic hormone and regulates adipogenesis in fetal pigs (5). Furthermore, glucocorticoids such as dexamethasone are required for optimal differentiation by 3T3-L1 preadipocytes and primary cultures of human and rodent preadipocytes (6). For example, dexamethasone induces the transcription factors C/EBP β and δ during early stages of preadipocyte differentiation (5, 6). C/EBP β and δ synergistically activate C/EBP α, which is known to induce the transcription of adipocyte-specfic genes such as glucose-6-phosphate dehydrogenase, lipoprotein lipase, and aP2 (6). Therefore, chronic elevation of serum glucocorticoids leads to hyperlipidemia, obesity, and NIDDM based on their ability to activate genes regulating lipogenesis.

In contrast to the glucocorticoids, plasma levels of dehydroepiandrosterone (DHEA) and DHEA-sulfate

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(DHEAS), the most abundant steroids in human blood (7), have been positively correlated with lower levels of body fat in both women (8, 9) and men (10). Interestingly, plasma levels of DHEA(S) decrease as adults age (7) whereas aging is associated with an increased risk of adiposity. Indeed, many studies have shown that administration of large bolus doses of DHEA, the intracellular form of the steroid, to rodents blocks or retards fat accretion (11–13, reviews). Moreover, several clinical trials have demonstrated that bolus DHEA treatment reduces body fat (14, 15) and increases lean body mass (15) and strength (16, 17) in mature adults.

Recent data support the hypothesis that the natural decline in both plasma DHEA(S) and maintenance of plasma glucocorticoid levels with age (i.e., decreased DHEA(S): glucocorticoid ratio) contributes to the excess body fat accumulation, hyperglycemia, hyperinsulinemia, hyperlipidemia, cancer, and immunologic anergy associated with aging (11–13, 18–20, reviews). Metabolically, DHEA antagonizes the actions of insulin and glucocorticoids by partitioning energy away from fat synthesis and toward oxidation (11). These thermogenic properties of DHEA may contribute to its therapeutic potential for the treatment of cardiovascular disease, cancer, obesity, lipemia, and diabetes (NIDDM).

Whether supplied in the diet (0.4%–0.6%) or administered as a bolus either orally (30–300 mg/kg body weight) or intraperitoneally (30–100 mg/kg body weight), large doses of DHEA reduce fat synthesis in genetically obese rodents as well as in rodents in which lipogenesis has been induced (21–28). Moreover, DHEA treatment at high levels reduces body weight gain and/or promotes weight loss, depending on the degree of excess body fat and genetic background of the animals (21–32). As an antiobesity agent, pharmacological levels of DHEA reduce adipose tissue depot weight and cellularity and reduce the percentage of body fat when administered to animals (21–32). As an antidiabetic agent, DHEA treatment at high levels decreases serum insulin levels (33–35), reduces pancreatic islet hypertrophy (20), and lowers serum glucose levels (20). As an antihyperlipidemic agent, DHEA treatment at high levels lowers serum levels of triglycerides (25, 28) and total and LDL cholesterol (25, 31). However, the antilipogenic and antidiabetic actions of DHEA when given at these high doses, especially as a bolus, may represent pharmacological rather than physiological effects (11–13).

Based on these reported antagonistic actions of glucocorticoids and DHEA on lipogenesis, the first specific aim of this study was to determine if altering the ratio of DHEA: corticosterone in young rats would influence specific hormones, metabolites, and enzymes involved in energy metabolism. The second aim of this study was to administer DHEA and corticosterone at low levels in a form that would provide a continuous supply of each steroid (i.e., slow-release implants) since earlier studies demonstrated that large bolus treatments of DHEA cause cytotoxicity (27, 28), hepatic carcinogenicity (35), hepatomegaly (23–29, 36), androgenicity (37, 38) and insulin resistance (38).

Materials and Methods

Design. Sixty male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), ≈ 325 g initial weight, were randomly allotted to 1 of 10 treatment groups of 6 rats each in this 2 × 5 factorial design. The two main effects tested were steroid type (DHEA or CORT) and steroid dose (0-, 10-, 25-, 50-, or 100-mg implants for 3 weeks). Subcutaneous implants (Innovative Research of America, Toledo, OH) were used in this 3-week-long study to provide a constant amount of exogenous steroid treatment that would be more physiological than administering a large bolus one or more times each day. The 10- and 100-mg doses of each steroid provide ≈ 1.3 and 13 mg/kg body weight/day of steroid, respectively, assuming each pellet released a continuous supply of steroid, and the average weight of the rats during the 21-day study period was 375 g. The actual serum levels of DHEA following 3 weeks of treatment with 10-, 25-, 50-, and 100-mg implants of DHEA were 2.7, 6.2, 11.8 and 17.6 ng/ml, respectively. Each steroid had its own separate control (0 mg DHEA or CORT) based on the vehicle used to solubilize each steroid as supplied by the manufacturer. The serum levels of DHEA in the control and CORT-treated rats were below the lower detectable limit of the RIA kit and therefore could not be determined. Reported physiological levels of DHEA in rodents range between 0.3 and 3.5 ng/ml for males and females, respectively (39).

Rats. Rats were housed individually in hanging wire-mesh cages in a room regulated for temperature (21° ± 2°C), humidity (45%–50%), and light (lights on 0600–1800 hr). Animals were cared for in accordance with guidelines established by the Institute of Animal Resources of the National Research Council, and their use in this experiment was approved by the IACUC Committee of the University of North Carolina at Greensboro. Rats were fed a semipurified diet (AIN 76 A) and provided with drinking water *ad libitum*. Food intakes and body weights were measured every 3 days. Diet ingredients were purchased from U.S. Biochemical (Cleveland, OH). To implant the pellets at the beginning of the study, rats were anesthetized ip with ketamine:xylazine (80:10 mg/kg). A 2-cm incision was made along the midline just behind the front legs, and the implants were inserted with forceps. The incision was sewn together with sutures and cleaned with 70% alcohol. The animals were inspected daily to detect any signs of infection resulting from the surgery.

Sample Collections. After being deprived of food overnight, rats were sacrificed by decapitation, and truncal blood was collected in chilled tubes. Serum was collected following centrifugation at 1600*g* for 20 min at 4°C and stored in 500 μl aliquots at −70°C. Subsamples of the liver were removed and immediately frozen at −20°C for the determination of liver lipid content. The remainder of the liver was diluted 5-fold in a 0.05 *M* Tris, 0.001 *M* EDTA

buffer and homogenized with a tissue homogenizer with Teflon pestles. Following two low-speed centrifugations at 600*g* to remove cell debris and unbroken cells, mitochondrial (3,000 g pellet), peroxisomal plus light mitochondrial (10,000 g pellet), microsomal (105,000 g pellet), and cytosolic (105,000 g supernatant) fractions were isolated by differential centrifugation using JA17 and 50.2 Ti Beckman rotors in Beckman Hi-speed (J2–21) and Ultra-(L7–65) centrifuges (Beckman Instruments, Palo Alto, CA). Epididymal and retroperitoneal fat pads, spleens, and thymus glands were removed and weighed. All chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated.

Levels of Serum Hormones and Metabolites. Serum levels of glucose and triglycerides were determined spectrophotometrically using commercial kits (Sigma Diagnostic Procedures no. 510 and 336, respectively). Serum lipid hydroperoxides were determined using a commercially available kit (Kamiya Biomed Co., Thousand Oaks, CA). Serum insulin, CORT, progesterone, estradiol, and testosterone were determined using commercially available double antibody ^{125}I-Diagnostic RIA kits (ICN Biomedicals, Costa Mesa, CA). Following a petroleum ether extraction of serum, serum DHEA was measured using a ^{3}H-Diagnostic RIA kit from ICN. Following an acid ethanol extraction and subsequent neutralization of serum, serum IGF-1 levels were determined (generously conducted by Dr. Roy Martin, The University of Georgia, Athens, GA) using a double antibody RIA method (40). Serum ACTH levels were determined (generously conducted by Dr. Chuck Eldridge, The Bowman Gray School of Medicine, Winston Salem, NC) using a standard double antibody RIA method (41).

Enzyme Assays. Palmitoyl-CoA oxidase activity was determined in the peroxisomal-rich fraction by measuring the oxidation of leuco-2,7-dichlorofluorescein diacetate (Eastman Kodak, Rochester, NY) at 502 nm catalyzed by exogenous horseradish peroxidase and the production of H_2O_2 in the first step of β-oxidation as previously described (28). Fatty acid synthase activity was determined in the cytosolic fraction by measuring the oxidation of NADPH at 340 nm according to the procedure of Hsu *et al.* (42). Glucose-6-phosphate dehydrogenase (G6 PD) and malic enzyme activities were determined in the cytosolic fraction by measuring the reduction of NADP + at 340 nm as previously described (23). Protein concentration in tissue samples was determined according to the procedure of Bradford using a commercially available assay kit (Bio-Rad Protein Assay, Bio-Rad Labs, Richmond, CA) and bovine serum albumin as a standard.

Statistics. The data were analyzed by the Least Squares ANOVA General Linear Models Procedures (PROC GLM) of SAS (SAS Institute, Cary, NC). The main effects of steroid type ($n = 2$), dose ($n = 5$), and the steroid by dose interactions ($n = 10$) were compared for significance at the $P < 0.05$ level (43). The means ± SE of the treatment interactions and their statistical differences are presented in the tables and figures. In addition, differences between the main effects (i.e., DHEA vs. CORT) are presented at the bottom of each table and at the end of each figure legend.

Results

Animal Performance. Rats treated with the 50- and 100-mg doses of both steroids had significantly lighter final body weights compared to their respective controls (Table I). With the exception of the rats receiving the 100-mg CORT implants, food intake decreased as the level of both steroid hormone treatments increased. The conversion of food to body weight gain, expressed as grams of food consumed per gram of total weight gain (food conversion efficiency), was higher for the rats treated with 100 mg of CORT compared to all other groups. As the level of CORT treatment increased, the weights of the fat pads, spleen and

Table I. Effects of Three Weeks of Treatment with Dehydroepiandrosterone (DHEA) and Corticosterone (CORT) Implants on Food Intake and Body Weight in Young Male Sprague-Dawley Rats*

Implants	Dose (mg)	Final body weight (g)	Total food intake (g)	Food: Gain†
DHEA	0	449 ± 10^{a}	457 ± 15^{a}	3.9 ± 0.6^{b}
	10	417 ± 10^{c,d}	424 ± 15^{a,b,c}	4.5 ± 0.6^{b}
	25	450 ± 9^{a}	418 ± 14^{a,b,c,d}	3.6 ± 0.5^{b}
	50	420 ± 9^{b,c}	408 ± 15^{b,c,d}	4.9 ± 0.6^{b}
	100	417 ± 9^{c,d}	402 ± 14^{c,d}	4.4 ± 0.5^{b}
CORT	0	443 ± 9^{a,b}	442 ± 14^{a,b}	3.8 ± 0.5^{b}
	10	447 ± 10^{a}	437 ± 15^{a,b,c}	3.6 ± 0.6^{b}
	25	419 ± 9^{b,c}	402 ± 14^{c,d}	4.4 ± 0.5^{b}
	50	392 ± 10^{d}	382 ± 15^{d}	5.1 ± 0.6^{b}
	100	397 ± 10^{c,d}	435 ± 15^{a,b,c}	8.6 ± 0.6^{a}
Main Effects: DHEA vs. CORT		NS	NS	DHEA < CORT $P = 0.02$

* Values are least-square means ± SEM, $n = 6$. Values within the same column sharing a common superscript are not significantly different ($P < 0.05$).
† Food: Gain = total food intake (g)/total weight gain (g).

thymus decreased (Table II). In contrast, DHEA treatment did not significantly influence food conversion efficiency, or the weights of the liver, fat pads, spleen, or thymus gland. However, some intradose differences in response to DHEA treatment were observed. For example, liver and fat pad weights of rats treated with the 25-mg DHEA implants were greater than those of rats treated with either 10 or 50 mg DHEA implants.

Hepatic Lipids and Enzymes. In contrast to DHEA treatment, there was a dose-dependent increase in liver lipid content (Fig. 1) and the specific activities of the hepatic lipogenic enzymes glucose-6-phosphate dehydrogenase, malic enzyme, and fatty acid synthase (Table III) in response to CORT treatment. Rats treated with the highest level of DHEA had less liver lipid than the DHEA controls (Fig. 1).

Serum Hormones and Metabolites. As expected, DHEA treatment raised serum DHEA levels, and CORT treatment raised serum CORT levels (Table IV), indicating that the implants were functioning. However, the CORT implants did not raise serum CORT levels to the predicted levels. Rats treated with 100 mg CORT implants had higher serum levels of progesterone, estradiol, and testosterone compared to their respective controls whereas DHEA had no impact on these steroids (Table IV). Rats treated with 100 mg CORT had higher serum insulin and lower IGF-1 levels than their controls whereas rats implanted with 100 mg DHEA had higher IGF-1 levels than their controls (Table V). Serum glucose levels were similar for all groups. Rats treated with DHEA had lower serum triglycerides (Fig. 2; DHEA < CORT; $P < 0.02$) and lipid hydroperoxides (Fig. 3; DHEA < CORT; $P < 0.005$) than CORT-treated rats.

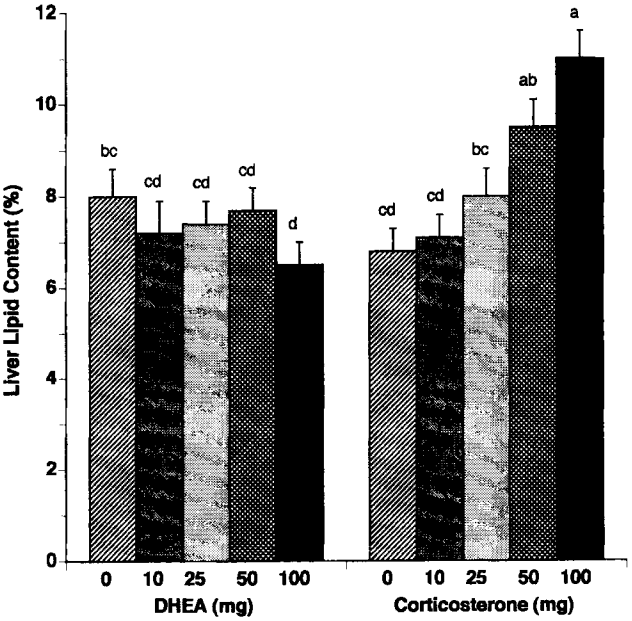

Figure 1. Effects of 3 weeks of treatment with dehydroepiandrosterone (DHEA) and corticosterone (CORT) implants on liver lipid content in young male Sprague-Dawley rats. Values are expressed as means + SEM, $n = 6$. Bars not sharing a common superscript are significantly different, $P < 0.05$. Main effects; DHEA < CORT, $P = 0.003$.

Discussion

These data demonstrate opposing actions of the adrenal hormones DHEA and corticosterone on several hormones, enzymes, and metabolites associated with energy metabolism in male rats. These data are important because: 1) the ratio of DHEA:cortisol decreases with age during adulthood (7); 2) aging is associated with an increased incidence of chronic diseases including heart disease, cancer, obesity and NIDDM; and 3) several antiglucocorticoid properties of DHEA have been demonstrated (11–13), especially on immune function. Therefore, maintaining a "youthful" DHEA: glucocorticoid ratio by manipulating serum levels of DHEA and glucocorticoid may protect against the development of certain chronic diseases whose etiology is glucocorticoid-dependent. In support of this concept, several of our data suggest an antagonism between DHEA and CORT

Table II. Effects of Three Weeks of Treatment with Dehydroepiandrosterone (DHEA) and Corticosterone (CORT) Implants on Tissue Weights in Young Male Sprague-Dawley Rats*

Implants	Dose (mg)	Liver weight (g)	Fat pad weight† (g)	Spleen weight (g)	Thymus weight (g)
DHEA	0	13.4 ± 0.5^{b}	$15.8 \pm 1.3^{c,d,e}$	$1.20 \pm 0.07^{a,b}$	$0.83 \pm 0.05^{a,b}$
	10	13.2 ± 0.5^{b}	14.1 ± 1.3^{e}	$0.94 \pm 0.08^{b,c}$	$0.77 \pm 0.05^{a,b}$
	25	15.7 ± 0.5^{a}	$17.3 \pm 1.3^{a,b,c}$	$1.10 \pm 0.07^{a,b}$	0.90 ± 0.05^{a}
	50	13.1 ± 0.5^{b}	13.3 ± 1.3^{e}	$1.07 \pm 0.07^{a,b}$	$0.80 \pm 0.05^{a,b}$
	100	13.9 ± 0.5^{b}	$16.8 \pm 1.3^{b,c,d}$	$1.06 \pm 0.07^{a,b}$	$0.86 \pm 0.05^{a,b}$
CORT	0	$14.6 \pm 0.5^{a,b}$	13.9 ± 1.3^{e}	$1.14 \pm 0.07^{a,b}$	$0.88 \pm 0.05^{a,b}$
	10	$14.2 \pm 0.5^{a,b}$	13.3 ± 1.3^{e}	1.21 ± 0.08^{a}	$0.88 \pm 0.06^{a,b}$
	25	13.2 ± 0.5^{b}	$14.4 \pm 1.3^{d,e}$	1.00 ± 0.07^{b}	$0.75 \pm 0.05^{a,b}$
	50	13.2 ± 0.5^{b}	19.5 ± 1.3^{a}	0.78 ± 0.08^{c}	0.54 ± 0.05^{c}
	100	13.9 ± 0.5^{b}	$18.7 \pm 1.3^{a,b}$	0.77 ± 0.08^{c}	0.50 ± 0.06^{c}
Main Effects: DHEA vs. CORT		NS	NS	DHEA > CORT $P = 0.05$	DHEA > CORT $P = 0.001$

* Values are least-square means ± SEM, $n = 6$. Values within the same column sharing a common superscript are not significantly different ($P < 0.05$).
† Combined weight of the retroperitoneal and inguinal fat pads.

Table III. Effects of Three Weeks of Treatment with Dehydroepiandrosterone (DHEA) and Corticosterone (CORT) Implants on the Activities of Hepatic Enzymes Involved in Lipid Metabolism in Young Male Sprague-Dawley Rats*

Implants	Dose (mg)	Glucose-6-phosphate dehydrogenase activity† units/(mg·min)	Malic enzyme activity units/(mg·min)	Fatty acid synthase activity pmol/(mg·min)	Fatty acyl oxidase activity nmol/(mg·min)
DHEA	0	$3.1 \pm 0.6^{b,c,d}$	1.6 ± 0.6^{b}	960 ± 60^{b}	$30 \pm 3^{a,b}$
	10	$2.6 \pm 0.7^{c,d}$	2.0 ± 0.6^{b}	830 ± 70^{b}	33 ± 3^{a}
	25	$3.1 \pm 0.6^{b,c,d}$	2.5 ± 0.6^{b}	830 ± 90^{b}	$27 \pm 3^{a,b}$
	50	2.0 ± 0.7^{d}	2.5 ± 0.5^{b}	810 ± 50^{b}	$29 \pm 3^{a,b}$
	100	$2.7 \pm 0.6^{c,d}$	2.6 ± 0.5^{b}	840 ± 90^{b}	$25 \pm 3^{a,b}$
CORT	0	$3.4 \pm 0.6^{b,c,d}$	1.6 ± 0.6^{b}	950 ± 30^{b}	$29 \pm 3^{a,b}$
	10	$3.7 \pm 0.6^{b,c,d}$	2.0 ± 0.6^{b}	980 ± 100^{b}	$31 \pm 3^{a,b}$
	25	$4.1 \pm 0.6^{b,c}$	2.7 ± 0.6^{b}	980 ± 100^{b}	$24 \pm 3^{a,b}$
	50	4.6 ± 0.6^{b}	$3.0 \pm 0.5^{a,b}$	$1080 \pm 110^{a,b}$	22 ± 3^{b}
	100	8.0 ± 0.7^{a}	4.4 ± 0.5^{a}	1350 ± 130^{a}	$30 \pm 3^{a,b}$
Main Effects: DHEA vs. CORT		DHEA < CORT $P = 0.001$	NS	DHEA < CORT $P = 0.0001$	NS

* Values are least-square means ± SEM, $n = 6$. Values within the same column sharing a common superscript are not significantly different ($P < 0.05$).
† All enzyme activity is expressed per mg of protein per min.

Table IV. Effects of Three Weeks of Treatment with Dehydroepiandrosterone (DHEA) and Corticosterone (CORT) Implants on Serum Hormones in Young Male Sprague-Dawley Rats*

Implants	Dose (mg)	DHEA (ng/ml)	CORT (ng/ml)	ACTH (pg/ml)	Progesterone (pg/ml)	Estradiol (ng/d)	Testosterone (ng/ml)
DHEA	0	ND†	53 ± 14^{c}	76 ± 18	120 ± 40^{c}	82 ± 2^{b}	$1140 \pm 313^{a,b,c}$
	10	2.7 ± 10^{d}	28 ± 13^{c}	118 ± 18	120 ± 50^{c}	84 ± 3^{b}	$1320 \pm 343^{a,b,c}$
	25	6.2 ± 9^{c}	41 ± 16^{c}	99 ± 18	$140 \pm 40^{b,c}$	83 ± 2^{b}	$1610 \pm 314^{a,b}$
	50	11.8 ± 9^{b}	45 ± 16^{c}	85 ± 18	90 ± 40^{c}	88 ± 3^{b}	$1210 \pm 314^{a,b,c}$
	100	17.6 ± 9^{a}	20 ± 14^{c}	88 ± 16	70 ± 40^{c}	84 ± 3^{b}	$930 \pm 314^{a,b,c}$
CORT	0	ND	35 ± 14^{e}	98 ± 18	120 ± 40^{c}	85 ± 2^{b}	650 ± 314^{c}
	10	ND	53 ± 13^{c}	90 ± 18	$250 \pm 50^{a,b}$	84 ± 3^{b}	1770 ± 313^{a}
	25	ND	52 ± 14^{c}	117 ± 16	270 ± 40^{a}	87 ± 2^{b}	$1290 \pm 313^{a,b,c}$
	50	ND	99 ± 13^{b}	95 ± 18	120 ± 40^{c}	85 ± 3^{b}	$800 \pm 343^{b,c}$
	100	ND	144 ± 13^{a}	118 ± 18	$250 \pm 40^{a,b}$	100 ± 3^{a}	1840 ± 384^{a}
Main Effects: DHEA vs. CORT		DHEA > CORT $P = 0.0001$	DHEA < CORT $P = 0.001$	NS	DHEA < CORT $P = 0.001$	NS	NS

* Values are least-square means ± SEM, $n = 6$. Values within the same column sharing a common superscript are not significantly different ($P < 0.05$).
† ND, not detectable.

on lipogenesis. For example, rats treated with DHEA had lower activities of hepatic lipogenic enzymes (e.g., G6 PD and FAS; Table III), less hepatic lipid (Fig. 1), and lower serum triglycerides (Fig. 2) and lipid hydroperoxides (Fig. 3) than CORT-treated rats.

If one assumes that DHEA treatment increased and CORT treatment decreased the DHEA:glucocorticoid ratio, then these data would support the hypothesis that a higher DHEA:CORT ratio results in lower hepatic and serum lipid levels. Unfortunately, actual ratios of serum DHEA and CORT cannot be calculated since DHEA levels could not be detected in control or CORT-treated rats. Interestingly, as the level of corticosterone treatment increased, the weight of the fat pads increased whereas fat pad weights were not affected by DHEA treatment. In addition, CORT treatment increased serum levels of insulin, testosterone, and progesterone; these hormones were not significantly affected by DHEA treatment. Thymus and spleen weights were reduced by corticosterone, but not DHEA treatment. These observations parallel the known immunosuppressive actions of glucocorticoids.

A simple explanation for the reduction in serum and hepatic lipids by DHEA treatment could be due to a reduction in food intake. Several groups have shown a dose-dependent decrease in food intake following DHEA treatment at high levels (11, 12). Rats treated with the 100-mg pellets of DHEA consumed about 7% less food over the 3-week treatment period compared to the DHEA control group. However, their levels of liver lipids and serum levels of triglycerides were 20% and 43%, respectively, lower than

Table V. Effects of Three Weeks of Treatment with Dehydroepiandrosterone (DHEA) and Corticosterone (CORT) Implants on Serum Insulin, IGF-I, and Glucose In Young Male Sprague-Dawley Rats*

Implants	Dose (mg)	Insulin (μU/ml)	IGF-I (ng/ml)	Glucose (mg/dl)
DHEA	0	$36 \pm 3^{b,c}$	$478 \pm 16^{b,c}$	119 ± 4
	10	34 ± 4^{c}	$521 \pm 16^{a,b}$	124 ± 5
	25	$43 \pm 3^{a,b,c}$	527 ± 15^{a}	122 ± 4
	50	$39 \pm 3^{b,c}$	$492 \pm 16^{a,b}$	118 ± 4
	100	$43 \pm 3^{a,b,c}$	531 ± 15^{a}	122 ± 4
CORT	0	$37 \pm 3^{b,c}$	$484 \pm 16^{b,c}$	123 ± 5
	10	$35 \pm 3^{b,c}$	$477 \pm 15^{b,c}$	117 ± 4
	25	$44 \pm 3^{a,b}$	$442 \pm 16^{c,d}$	118 ± 4
	50	$44 \pm 4^{a,b}$	$446 \pm 15^{c,d}$	117 ± 5
	100	49 ± 3^{a}	415 ± 15^{d}	126 ± 5
Main Effects: DHEA vs. CORT		NS	DHEA > CORT $P = 0.0001$	NS

* Values are least-square means ± SEM, $n = 6$. Values within the same column sharing a common superscript are not significantly different ($P < 0.05$).

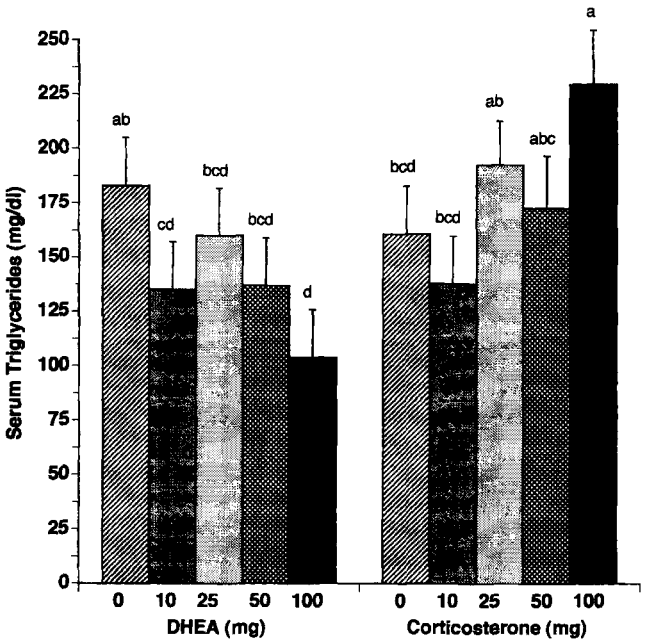

Figure 2. Effects of 3 weeks of treatment with dehydroepiandrosterone (DHEA) and corticosterone (CORT) implants on serum triglyceride levels in young male Sprague-Dawley rats. Values are expressed as means + SEM, $n = 6$. Bars not sharing a common superscript are significantly different, $P < 0.05$. Main effects; DHEA < CORT, $P = 0.02$.

the DHEA control animals. Therefore, it would appear that DHEA treatment reduces liver and serum lipids by a mechanism independent of food intake.

Another mechanism by which DHEA may have reduced liver and serum lipid levels is by reducing fatty acid synthesis and esterification in the liver. This hypothesis is

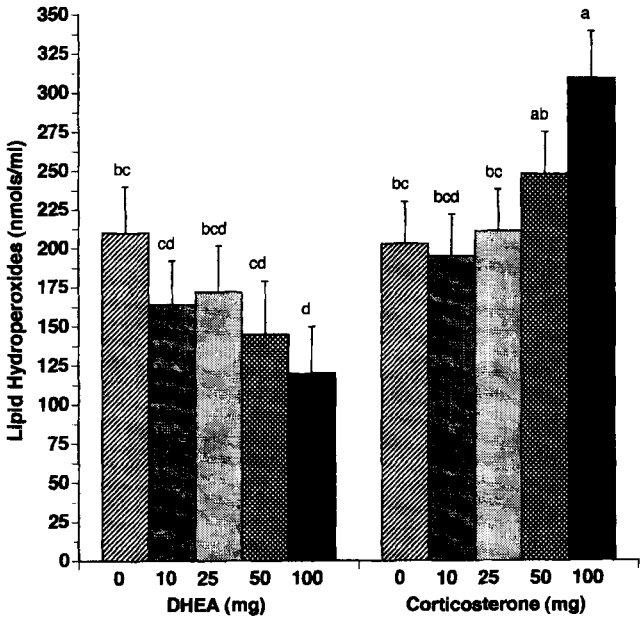

Figure 3. Effects of 3 weeks of treatment with dehydroepiandrosterone (DHEA) and corticosterone (CORT) implants on serum lipid hydroperoxides in young male Sprague-Dawley rats. Values are expressed as means + SEM, $n = 6$. Bars not sharing a common superscript are significantly different, $P < 0.05$. Main effects; DHEA < CORT, $P = 0.0005$.

supported by the lower activities of glucose-6-phosphate dehydrogenase (G6 PD) and fatty acid synthase in the livers of DHEA-treated rats compared to rats treated with corticosterone. The less fatty acid and triglyceride synthesized in the liver, the less triglyceride available for storage and/or VLDL transport in the circulation. In support of this speculation, other studies have demonstrated that treatment with high levels of DHEA decreased the rate of fatty acid synthesis (24, 25) and reduced hepatic lipid levels in starved-refed rats (23, 24, 36). Furthermore, DHEA treatment at high levels also decreased G6 PD activity (23, 25, 36), a major supplier of reducing equivalents (NADPH) for lipid synthesis, while stimulating malic enzyme activity (27, 36, 44, 45). In contrast, glucocorticoids increased *de novo* synthesis of hepatic G6 PD and fatty acid synthesis (36) similar to what we found in the present study.

DHEA treatment using low-level implants did not produce any obvious signs of toxicity (i.e., hepatomegaly, lethargy), androgenicity (i.e., increased testosterone levels, aggressiveness, hair loss), or insulin resistance previously observed in studies using higher levels of DHEA *via* bolus administration. However, in contrast to pharmacological levels of DHEA (i.e., 100 mg/kg ip) used in other studies (23–28), fat pad weights were not significantly reduced by the highest level of DHEA implant (i.e., 13 mg/kg/d) used in the present study. In support of our findings, Tagliaferro *et al.* (30) also found that feeding low levels of DHEA (i.e., < 0.1% in the diet) did not reduce body fat levels in rats. Therefore, it appears that higher doses of DHEA are nec-

essary to alter body composition in rodents. In support of this concept, a recent review by Svec and Porter (13) concluded that low doses of DHEA influence neurological and immunologic properties whereas much higher doses of DHEA were required to alter metabolism in rodents and humans. These data suggest that DHEA influences lipogenesis and obesity *via* pharmacological rather than physiological mechanisms. In contrast, our low-level CORT implants significantly increased hepatic (50 and 100 mg CORT) and serum lipids (100 mg CORT) and fat pad weights (50 and 100 mg CORT) compared to the controls.

Treatment variations within doses (i.e., nonlinear effect of steroid dose) were observed for several of the parameters measured. These nonlinear responses to steroid dose may reflect the consistency of the amount and duration of each steroid released from the pellets and/or the physiological responses to different doses of each steroid. For example, serum levels of CORT for rats implanted with 10 and 25 mg implants were identical following 3 weeks of treatment (Table IV). In contrast, it appeared that the DHEA implants were functioning properly based on serum levels of DHEA. It should also be noted that for several of the parameters measured (i.e., serum CORT and testosterone), there were significant differences between the DHEA and CORT placebos. These differences may reflect the different vehicles used to solubilize the steroid within the implants based on the manufacturer's formulations.

A second mechanism by which DHEA may have reduced hepatic and serum lipids is by increasing serum levels of IGF-1. A correlation between obesity and low levels of serum DHEA(S) and IGF-1 has been observed in humans (8, 46). These authors proposed that the lower levels of IGF-1 in severely obese women may be responsible, in part, for the lower levels of DHEA(S) in these patients. In support of this concept, IGF-1 has been shown to stimulate steroidogenesis in adrenocortical cells (47). Furthermore, DHEA enhances the expression of IGF-1 in differentiating rat granulosa cells (48). DHEA(S) treatment of both men and women increases serum IGF-1 levels (16). Moreover, we have demonstrated that rats treated with low levels of DHEAS (0.7 mg/kg body weight) in the drinking water (10 μg/ml) have higher serum IGF-1 levels than control animals (49). For example, acute treatment of patients with NIDDM has demonstrated that IGF-1 increases insulin sensitivity and has beneficial effects on blood lipids (50). Elevated levels of IGF-1 are associated with decreases in body fat and serum lipids. Therefore, it is tempting to speculate that DHEA may reduce tissue and serum lipids by increasing serum levels of IGF-1.

DHEA has been reported to have a number of antiglucocorticoid actions including blocking the glucocorticoid induction of hepatic lipogenesis in starved-refed rats (36) and attenuating the immunosuppressive actions of glucocorticoids (51). For example, DHEA-treated animals (23, 25, 36) and cultures of (pre)adipocytes (52) have lower activities of G6PD, a glucocorticoid-inducible enzyme required for NADPH production for fatty acid synthesis. Inhibition of G6PD activity by DHEA in 3T3-L1 preadipocytes could be partially reversed by adding the hexose monophosphate shunt intermediate 6-phosphogluconate to the cultures (52). DHEA may therefore antagonize the ability of glucocorticoids to activate glucocorticoid-dependent genes involved in lipid metabolism such as G6PD. Possible antiglucocorticoid mechanisms of action of DHEA are: 1) reducing glucocorticoid secretion *via* feedback inhibition (i.e., decreased ACTH) of adrenal glucocorticoid synthesis; 2) interfering with glucocorticoid binding to its cytoplasmic receptor; or 3) altering glucocorticoid receptor binding to nuclear response elements on specific genes. Our research (53) has demonstrated that DHEA does not reduce glucocorticoid binding to its receptor *in vitro*; therefore, we have hypothesized that DHEA may decrease adrenal synthesis and secretion of corticosterone. This hypothesized reduction in plasma corticosterone would also limit glucocorticoid-mediated increases in hepatic lipogenesis, gluconeogenesis, and insulin resistance. However, neither serum corticosterone nor ACTH levels were affected by DHEA in this study. DHEA may therefore interfere with glucocorticoid action at the receptor-DNA binding level in the nucleus.

Treatment with supraphysiological levels of DHEA increased whole body (22, 24, 26, 53) and hepatic (24, 53) oxygen consumption. The increase in hepatic oxygen utilization has been attributed to an increase in both mitochondrial (22, 24, 53) and peroxisomal (27, 28, 54) fatty acid oxidation. In contrast to mitochondrial β-oxidation, peroxisomal fatty acid oxidation is less efficient in the transfer of electrons from energy substrates to reducing equivalents and their subsequent use for ATP synthesis in mitochondrial electron transfer chain. We previously hypothesized that high levels of DHEA increase peroxisomal fatty acid oxidation, thereby lowering energy efficiency. This DHEA-mediated reduction in energy efficiency in liver reduces the availability of energy substrates for hepatic lipid synthesis. However, the activity of fatty acyl oxidase, an indicator of peroxisomal fatty acid oxidation, was not increased by low levels of DHEA in this study. Therefore, it does not appear that peroxisomal β-oxidation contributes to the lower levels of hepatic and serum lipids in rats treated with low levels of DHEA implants.

In summary, treatment of male rats with 100-mg implants of DHEA for 3 weeks reduced serum triglycerides and lipid hydroperoxides and hepatic lipid content whereas rats treated with 100-mg CORT implants had significantly higher levels of these lipids. However, 100-mg implants of DHEA (i.e., 13 mg/kg/d) did not reduce body fat levels as previously demonstrated in rats treated with 100 mg DHEA/kg/d ip. Future studies will examine the potential antago-

nism between DHEA and corticosterone on the growth and metabolism of preadipocytes.

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1. Ross E, Marshall-Jones P, Friedman M. Cushing's Syndrome: Diagnostic criteria. Q J Med **35:**149–192, 1966.
2. Edwardson J, Hough C. The pituitary-adrenal system of the genetically obese (*ob/ob*) mouse. J Endocrinol **65:**99–107, 1975.
3. Orth D, Kovacs W, Debold C. The adrenal cortex. In: Wilson J, Foster V, Eds. Williams Textbook of Endocrinology (8th ed). Philadelphia: W.B. Saunders, pp489–1188, 1992.
4. Boullion D, Berdanier C. Role of glucocorticoid in adaptive hyperlipogenesis in the rat. J Nutr **110:**286–297, 1980.
5. Martin R, Hausman G, Hausman D. Regulation of adipose cell development *in utero*. Proc Soc Exp Biol Med **219:**200–210, 1998.
6. MacDougal O, Lane D. Transcriptional regulation of gene expression during adipocyte differentiation. Annu Rev Biochem **64:**345–373, 1995.
7. Orentreich N, Brind J, Rizer R, Vogelman J. Age changes and sex differences in serum DHEA-sulfate concentrations throughout adulthood. J Clin Endocrinol Metab **59:**551–555, 1985.
8. DePergola G, Giagulli V, Garruti G, Cospite M, Giorgino F, Cignarelli M, Giorgino R. Low DHEA levels in premenopausal obese women with very high body mass index. Metabolism **40:**187–190, 1991.
9. Williams D, Boyden T, Pamenter R, Lohman T, Going S. Relationship of body fat percentage and fat distribution with dehydroepiandrosterone sulfate in premenopausal women. J Clin Endocrinol Metab **77:**80–85, 1993.
10. Haffner SM, Valdez RA, Mykkanen L, Stern MP, Katz MS. Decreased testosterone and DHEAS concentrations are associated with increased insulin and glucose concentrations in nondiabetic men. Metabolism **43:**599–603, 1994.
11. Berdanier C, Parente J, McIntosh M. Is DHEA an antiobesity agent? FASEB J **7:**414– 419, 1993.
12. Cleary M. Role of DHEA in obesity. In: Kalimi M, Regelson W, Eds. The Biological Role of Dehydroepiandrosterone. Berlin: Walter de Gruyter, pp356–361, 1990.
13. Svec F, Porter J. The actions of exogenous dehydroepiandrosterone in experimental animals and humans. Proc Soc Exp Biol Med **218:**174–191, 1998.
14. Nestler J, Barlascini C, Clore J, Blackard W. DHEA reduces serum LDL levels and body fat but does not alter insulin sensitivity in older men. J Clin Endocrinol Metab **66:**57–61, 1988.
15. Jakubowicz D, Beer N, Rengifo R. Effect of DHEA on c-GMP in men of advancing age. Ann N Y Acad Sci **774:**312–315, 1995.
16. Morales A, Nolan J, Nelson J, Yen S. Effects of replacement dose of DHEA in men and women of advancing age. J Clin Endocrinol Metab **78:**1360–1367, 1994.
17. Yen S, Morales A, Khorram O. Replacement of DHEA in aging men and women: Potential remedial effects. Ann N Y Acad Sci **774:**128–142, 1995.
18. Gordon G, Helzlouer K, Comstock G. Serum levels of DHEA and its sulfate and the risk of developing bladder cancer. Cancer Res **51:**1366–1369, 1991.
19. Schwartz A, Whitcomb J, Fairman D, Hastings L, Lewbart M. Inhibition of tumorigenesis by DHEA and structural analogues. In: Lardy H, Stratman F, Eds. Hormones, Thermogenesis, and Obesity. New York: Elsevier, pp441–452, 1989.
20. Coleman DL. Therapeutic effects of DHEA and its metabolites in diabetes-obesity mutants. In: Lardy H, Stratman F, Eds. Hormones, Thermogenesis, and Obesity. New York: Elsevier, pp377–384, 1989.
21. Cleary M. The antiobesity effects of DHEA in rats. Proc Soc Exp Biol Med **196:**8–16, 1991.
22. Mohan P, Ihnen J, Levin B, Cleary M. Effect of DHEA treatments with diet-induced obesity. J Nutr **120:**1103–1114, 1990.
23. McIntosh M, Berdanier C. Strain differences in the dose-response curves of adrenalectomized, starved-refed rats to DHEA. Proc Soc Exp Biol Med **187:**216–222, 1988.
24. Berdanier C, McIntosh M. Further studies on the effects of DHEA on hepatic metabolism in BHE rats. Proc Soc Exp Biol Med **192:**242–247, 1989.
25. McIntosh M, Berdanier C. Antiobesity effects of DHEA are mediated by futile substrate cycling in the hepatocytes of BHE/cdb rats. J Nutr **121:**2037–2043, 1991.
26. McIntosh M, Berdanier C. Influence of DHEA on the thyroid hormone status of BHE/cdb rats. J Nutr Biochem **3:**194–199, 1992.
27. McIntosh M, Goldfarb A, Cote P, Griffin K. Vitamin E reduces peroxisomal fatty acid oxidation and indicators of oxidative stress in exercised rats treated with DHEA. J Nutr Biochem **4:**298–303, 1993.
28. McIntosh M, Goldfarb A, Curtis L, Cote P. Vitamin E alters hepatic antioxidant enzymes in rats treated with DHEA. J Nutr **123:**216–224, 1993.
29. Tagliaferro AR, Davis JR, Truchon S, Van Hamont N. Effects of DHEA acetate on metabolism, body weight, and composition of male and female rats. J Nutr **116:**1977–1983, 1986.
30. Tagliaferro A, Ronan A, Payne J, Meeker L, Tse S. Increased lipolysis to β-adrenergic stimulation after DHEA treatment in rats. Am J Physiol **268:**R1374–R1380, 1995.
31. Kurzman I, Panceira D, Miller J, MacEwen E. The effect of dehydroepiandrosterone combined with a low-fat diet in spontaneously obese dogs: A clinical trial. Obes Res **6:**20–28, 1998.
32. Hansen PA, Han DH, Nolte LA, Chen M, Holloszy JO. DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. Am J Physiol **273:**R1704–R1708, 1997.
33. Cleary M, Zabel T, Sartin J. Effects of short-term DHEA treatment on serum and pancreatic insulin in Zucker rats. J Nutr **118:**382–387, 1988.
34. Nestler J, Barlascini C, Clore J, Blackard W. DHEA: Effects on insulin sensitivity, serum lipid levels, and body composition in normal men. In: Lardy H, Stratman F, Eds. Hormones, Thermogenesis, and Obesity. New York: Elsevier, pp405–414, 1989.
35. Rao M, Subbarau V, Yeldandi A, Reddy J. Hepatocarcinogenicity of dehydroepiandrosterone in the rat. Cancer Res **52:**2977–2979, 1992.
36. McIntosh M, Berdanier C. Differential effects of adrenalectomy and starvation-refeeding on hepatic lipogenic responses to DHEA and glucocorticoid in BHE and Sprague-Dawley rats. J Nutr **118:**1011–1017, 1988.
37. Mortola J, Yen S. The effects of oral DHEA on endocrine and metabolic parameters in post-menopausal women. J Clin Endocrinol Metab **71:**696–709, 1990.
38. Pashko L, Schwartz A. Antihyperglycemic effect of dehydroepiandrosterone analogue 16-fluoro-5-androsten-17-one in diabetic mice. Diabetes **42:**1105–1108, 1995.
39. Leiter E, Beamer W, Coleman D, Longcope C. Androgenic and estrogenic metabolites in serum of mice fed dehydroepiandrosterone: Relationship to antihyperglycemic effects. Metabolism **36:**863–869, 1987.
40. Latimer A, Hausman G, McCusker R, Buonomo F. The effects of thyroxine on serum and tissue concentrations of insulin-like growth factors (IGF-I and -II) and IGF-binding proteins in the fetal pig. Endocrinology **133:**1312–1319, 1993.
41. Fink G, Dow R, Casley D, Johnston C, Bennie J, Carroll S, Dick H. Atrial natriuretic peptide is involved in the ACTH response to stress and glucocorticoid negative feedback in the rat. J Endocrinol **135:**37–43, 1992.
42. Hsu R, Butterworth P, Porter J. Pigeon liver fatty acid synthase. Methods Enzymol **71:**33–39, 1981.

43. Steele R, Torrie J. Principles, Procedures of Statistics. New York: McGraw-Hill, pp336–376, 1980.
44. Marrero M, Prough R, Frenkel R, Milewich L. Dehydroepiandrosterone feeding and protein phosphorylation, phosphatases, and lipogenic enzymes in mouse liver. Proc Soc Exp Biol Med **193:**110–117, 1990.
45. Song MK, Grieco D, Rall JE, Nikodem VM. Thyroid hormone-mediated transcriptional activation of the rat liver malic enzyme gene by dehydroepiandrosterone. J Biol Chem **264:**18981–18985, 1989.
46. DePergola G, Cospite M, Giagulli V, Giorgina F, Garruti G. Insulin-like growth factor-1 (IGF-1) and dehydroepiandrosterone sulfate in obese women. Int J Obesity **17:**481–483, 1991.
47. Pham-Huu M, Binoux M. Insulin-like growth factor-1 (IGF-1) induces cortisol production in bovine adrenocortical cells in primary culture. J Steroid Biochem **36:**583–588, 1990.
48. Yan Z, Lee G, Anderson E. Influence of dehydroepiandrosterone on the expression of insulin-like growth factor-1 during cystogenesis in polycystic rat ovaries and in cultured granulosa cells. Biol Reprod **57:**1509–1516, 1997.
49. Lea-Currie R, Wu S, McIntosh M. Effects of acute administration of dehydroepiandrosterone sulfate on adipose tissue mass and cellularity in male rats. Int J Obes Relat Metab Disord **21:**147–154, 1997.
50. Moses A, Young S, Morrow L, O'Brian M, Clemmons D. Recombinant human IGF-1 increases insulin sensitivity and improves glycemic control in patients with NIDDM. Diabetes **45:**91–100, 1996.
51. Araeno B, Daynes R. Dehydroepiandrosterone functions as more than an antiglucocorticoid in preserving immunocompetence after thermal injury. Endocrinology **136:**393–401, 1995.
52. Gordon G, Newitt J, Shantz L, Weng D, Talalay P. Inhibition of the conversion of 3T3-L1 fibroblast clones to adipocytes by DHEA and related anticarcinogenic steroids. Cancer Res **46:**3389–3395, 1986.
53. McIntosh M, Pan J, Berdanier C. *In vitro* studies on the effects of dehydroepiandrosterone and corticosterone on hepatic steroid receptor binding and mitochondrial respiration. Comp Biochem Physiol **104:**147–153, 1993.
54. Frenkel R, Slaughter C, Orth K, Moomaw C, Hicks S. Peroxisome proliferation and induction of peroxisomal enzymes in mouse and rat liver by dehydroepiandrosterone feeding. J Steroid Biochem **35:**333–342, 1990.