PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 171, 276–284 (1982)

The Effect of Dehydroepiandrosterone on Adipose Tissue Cellularity in Mice (41511)

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Abstract. The effect of dehydroepiandrosterone (DHEA) on adipose tissue growth in young adult male mice was investigated. In Experiment 1, mice were individually caged and treated by intraperitoneal injections of DHEA (25 mg/kg body weight, 3× weekly) for 20 weeks. No significant differences in body weight and adipose tissue growth were found. In Experiment 2, DHEA was administered by intubation (450 mg/kg body weight, 3× weekly). In addition, mice were housed either one/cage or five/cage. After 10 weeks of treatment body weight and epididymal and retroperitoneal fat pad weights were significantly decreased in both DHEA groups compared to their respective control groups. In both adipose depots fat cell size was significantly decreased in treated mice while there was no effect on fat cell number. No differences were found in adipose tissue lipoprotein lipase or liver fatty acid synthetase of treated mice.

The 17-ketosteroid, dehydroepiandrosterone (DHEA), has been shown by Yen *et al.* (1) and Schwartz (2) to decrease body weight gain in yellow obese mice. This alteration in body weight occurred without a concomitant change in food intake. It was also shown that DHEA treatment decreased total body lipids and the rate of liver lipogenesis (1). In addition, Yen *et al.* (1) reported that body weight was decreased in lean mice but the effect was not as dramatic as that seen in the obese mice. No additional information on lean mice was reported.

DHEA is known to inhibit the enzyme glucose-6-phosphate dehydrogenase (3–6). Inhibition of glucose-6-phosphate dehydrogenase would theoretically result in a decreased availability of NADPH for either lipogenesis or DNA synthesis. The studies in obese mice suggested that DHEA is primarily affecting lipogenesis. However, DHEA has also been shown to inhibit DNA synthesis in cultured cell lines (7) and in mouse epidermis *in vivo* (8) and to decrease the development of spontaneous breast cancer formation in C3H mice (2), indicating it can also affect cellular proliferation. It has been established that adipose tissue, the major site of stored body lipids, can change by either the size of the fat cells or the number of fat cells (9, 10). Therefore, the specific effects of chronic DHEA administration on adipose tissue growth were investigated in mice. Two modes of administration for DHEA were also studied.

Materials and Methods. *Animals.* Six-week-old male C3H mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Mice were housed individually in wire mesh cages, unless otherwise indicated, and were allowed food and water *ad libitum*. A 12-h light–dark cycle was maintained and the temperature was kept at 74°F. Body weight and food intake were recorded weekly.

Experiment 1. Mice were housed individually as described and were fed Purina powdered rodent chow (5001, 5% fat by weight). One-half of the mice (n = 6/group) received intraperitoneal injections 3× weekly with 25 mg/kg body weight DHEA (Sigma Chemical Co., St. Louis, Mo.) in a homogenized suspension of saline:Emulphor (GAF, New York, N.Y.) (95:5, v/v).

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0037-9727/82/110276-09$01.00/0

Control mice were injected with the vehicle only. After 20 weeks of treatment the mice were killed and tissue processed as described below.

Experiment 2. Mice were either housed individually (50 mice) as described or housed five per cage (30 mice) in plastic cages wtih bedding. Mice were fed Purina mouse chow (5015, 11% fat by weight). The mice individually housed received powdered food while the mice housed five per cage were given food in a pelleted form. One-half of each group were intubated with DHEA 450 mg/kg 3× weekly that was homogenized in sesame oil. Each control group received the vehicle only. Mice were treated for 10 weeks and then eight mice from each of the four groups were randomly selected and were killed and tissue processed as described below. Data presented are for the mice that were killed.

General procedures. At the end of each experimental period mice were killed by decapitation. Livers were removed for fatty acid synthetase activity determination. The livers were weighed and a (30%, w/v) homogenate was prepared in a 0.15 *M* KCl−4 m*M* $MgCl_2$, pH 7.5, solution. The homogenate was centrifuged at 10,000*g* for 15 min at 4° and the supernatant was removed and recentrifuged at 100,000*g* for 45 min at 4°. The high-speed supernatant was stored at −70°. Fatty acid synthetase activity was determined by the method of Hsu *et al.* (11). Activity was expressed as counts per minute per milligram of supernatant protein. Protein was determined by the method of Lowry *et al.* (12).

Both right and left epididymal and retroperitoneal fat pads were removed. Right and left depots were pooled from each site and weighed. Two small samples from each depot were used to determine fat cell size and number by the method of Hirsch and Gallian (13). One sample was placed in 2:1 chloroform:methanol to extract the lipids and the lipid was quantitated gravimetrically (14). The second sample was placed in a 2% osmium tetroxide mixture and incubated for 72 hr at 37°. Fixed cells were counted electronically using a Coulter Counter (Model ZB). The remainder of the adipose tissue was prepared for lipoprotein lipase activity determinations. Tissue was homogenized (20%, w/v) in 0.25 *M* sucrose−1 m*M* EDTA buffer, pH 7.4. The homogenate was centrifuged at 11,000*g* for 15 min at 4°. The postmitochondrial supernatant was removed and frozen at −70°. Lipoprotein lipase activity was determined by the method of Schotz *et al.* (15) as modified by Hietanen and Greenwood (16).

Statistical analysis. Data are presented as means ± standard deviations. Results from Experiment 1 were analyzed by Student's *t* test. Results from Experiment 2 were analyzed first by two-way analysis of variance followed by *F* test for individual comparisons (17). Level of significance was $P = 0.05$ or less.

Results. *Experiment 1.* Body weight and food intake of treated and nontreated mice are shown in Table I. As can be seen there were no differences in body weights of treated mice compared to nontreated. In addition, food intake was not affected by DHEA treatment. Liver weights and liver fatty acid synthetase activity are also shown in Table I. Neither of these factors was affected by DHEA treatment.

TABLE I. EFFECTS OF INTRAPERITONEAL DHEA TREATMENT[a] ON BODY WEIGHT, FOOD INTAKE, LIVER WEIGHT, AND LIVER FATTY ACID SYNTHETASE ACTIVITY IN MALE C3H MICE (EXPERIMENT 1)

	Body weight (g)	Cumulative food intake (g)	Liver weight (g)	FAS (cpm/mg protein)
Control	32.1 ± 2.3[b]	792.3 ± 38.3	1.7477 ± 0.2020	1.755 ± 0.493
DHEA	31.5 ± 2.6	821.0 ± 47.6	1.7298 ± 0.1977	1.570 ± 0.350

[a] Mice were treated from 6 until 26 weeks of age (25 mg/kg body weight 3× weekly).
[b] Data are means ± standard deviations; $N = 6$, both groups.

TABLE II. EPIDIDYMAL AND RETROPERITONEAL FAT PAD WEIGHTS AND FAT CELL SIZE AND NUMBER IN MALE C3H FOLLOWING INTRAPERITONEAL DHEA TREATMENT[a] (EXPERIMENT 1)

	Epididymal[b]		Retroperitoneal[b]	
	Control	DHEA	Control	DHEA
Pad weight (g)	0.9154 ± 0.2460[c]	0.7115 ± 0.3302	0.2419 ± 0.0809	0.1723 ± 0.0804
Fat cell size (μg lipid/cell	0.4915 ± 0.1852	0.5178 ± 0.2678	0.6637 ± 0.1688	0.4637 ± 0.2443
Fat cell no./ pad ($\times 10^6$)	1.3631 ± 0.4353	0.9308 ± 0.1168*	0.2670 ± 0.0696	0.2398 ± 0.0664
LPL activity[d]	3.3131 ± 3.7065	2.1784 ± 1.3883	3.2115 ± 2.3652	2.5658 ± 1.2319

[a] Mice were treated from 6 until 26 weeks of age, 25 mg/kg body weight intraperitoneally injected three times weekly.
[b] Combined right and left pads.
[c] Data are means ± standard deviations; $n = 6$, both groups.
[d] Lipoprotein lipase activity = μmole free fatty acid released $\times 10^6$ cells.
* DHEA treated significantly different from respective control groups.

Fat pad weights are shown in Table II. Both epididymal and retroperitoneal fat pad weights were reduced by DHEA treatment in comparison to nontreated control fat pad weights. However, these differences were not significant. In the epididymal fat pad, DHEA treatment did result in a significant decrease in fat cell number (Table II). No difference in epididymal fat cell size was found. In the retroperitoneal fat pad there was no change in fat cell number. There was a decrease in retroperitoneal fat cell size but this was not significant (Table II). Lipoprotein lipase acitivity in both depots was decreased by DHEA treatment (Table II). However, these differences were not significant.

Experiment 2. Body weight, weight gain, and cumulative food intake data are found in Table III. These values presented for the mice selected for further study were not different from the entire group of mice used. For example body weights of the control one/cage mice were 30.4 ± 2.9 and the body weights for the entire 25 mice in this group was 30.5 ± 2.4. DHEA administered by intubation resulted in decreased body weight in both caging situations (Table III). However, the biggest effect from DHEA treatment on body weight and weight gain was seen in DHEA five/cage mice compared to their control group. In addition, significant differences were found in the body weights of DHEA five/cage

TABLE III. BODY WEIGHT, WEIGHT GAIN, CUMULATIVE FOOD INTAKE AND COMBINED FAT PAD WEIGHTS AS A PERCENTAGE OF TOTAL BODY WEIGHT[a] IN DHEA-TREATED[b] MALE C3H (EXPERIMENT 2)

	Body weight (g)		Weight gain (g)	Cumulative food intake (g)	Combined fat pad weights, percentage total body weight
	Initial	Final			
Control 1/cage	17.8 ± 1.7[c]	30.4 ± 2.9*,†	12.6 ± 1.9*,†	327.3 ± 36.9*	3.92 ± 0.74*,†
DHEA 1/cage	16.6 ± 1.9	27.1 ± 1.5	10.9 ± 1.4	343.3 ± 24.4	2.91 ± 0.70
Control 5/cage	19.9 ± 1.6	35.1 ± 1.7*	15.6 ± 2.1*	225.3‡	4.61 ± 0.60*
DHEA 5/cage	20.1 ± 1.3	28.1 ± 2.2	8.0 ± 1.1	205.7	2.60 ± 0.54

[a] Combined epididymal and retroperitoneal fat pads (both right and left) divided by total body weight ×100.
[b] Treated with DHEA 450 mg/kg body weights from 6–16 weeks of age.
[c] All data are means ± standard deviation; $N = 8$ all groups.
* Control group significantly different from DHEA-treated group.
† Control 1/cage significantly different from DHEA 5/cage.
‡ No statistics done as no individual results were available.

mice compared to control five/cage mice from the second week of treatment while DHEA one/cage mice body weights were significantly different from control one/cage at week 1, 4, 9, and 10 (data not shown). Food intakes were not different between DHEA one/cage and control one/cage mice nor between DHEA five/cage and control five/cage mice (Table III). Both groups of five/cage mice ate less food than one/cage mice. The exact explanation for this finding is unknown. Food given to the one/cage mice was in powdered form and although great care was taken to minimize spillage by placing food in a small glass cup inside a larger cup, it is possible that over 10 weeks some unaccountable loss occurred. In addition, there may be a higher energy cost for mice housed individually as suggested by the decreased body weight in one/cage mice.

Liver weights were not different between any of the groups (Table IV). However, liver weight accounted for a significantly larger percentage of total body weight in both treated groups (Table IV). No significant differences in liver fatty acid synthetase activity were found between control and DHEA-treated groups (Table IV).

Epididymal and retroperitoneal fat pad weights of both treated groups were significantly decreased compared to their respective nontreated groups (Tables V, VI). The differences in fat pad weight were much greater than those found for body weights. For example, DHEA five/cage mice weighed 15% less than control five/cage, while epididymal fat pad weight was decreased 55%. The effect of DHEA was greater on fat pad weights in the five/cage mice than in the one/cage mice. In both treated groups the combined epididymal and retroperitoneal weights accounted for significantly less of total body weight than the combined weights of fat pads of the untreated groups (Table III).

Cellularity data for epididymal and retroperitoneal fat pads are shown in Tables V and VI, respectively. In general, the biggest effect of DHEA treatment was on fat cell size in both depots. Both DHEA one/cage and DHEA five/cage mice had significantly decreased fat cell size in epididymal and retroperitoneal fat pads compared to their respective control groups. No effect of treatment was found in epididymal fat cell number. In the retroperitoneal pad a slight but significant decrease was found in fat cell number of DHEA five/cage compared to control five/cage while no difference was found in retroperitoneal fat cell number of DHEA one/cage compared to control one/cage.

Lipoprotein lipase activity results for Experiment 2 are found in Tables V and VI. In the mice housed one/cage DHEA treatment resulted in decreased lipoprotein lipase activity in both epididymal and retroperitoneal fat pads. Slight decreases in lipoprotein lipase activity were found in both pads of DHEA five/cage mice compared to controls but these values were not significant.

Discussion. These data are in agreement with previous studies (1, 2) that have shown an obesity prevention effect of dehydro-

TABLE IV. LIVER WEIGHT, LIVER WEIGHT AS A PERCENTAGE OF BODY WEIGHT,[a] AND LIVER FATTY ACID SYNTHETASE (FAS) ACTIVITY IN DHEA-TREATED[b] MICE (EXPERIMENT 2)

	Liver weight (g)	Liver weight, percentage of body weight	FAS (cpm/mg protein)
Control 1/cage	1.6988 ± 0.2348[c]	5.5 ± 0.4*	3.463 ± 1.639
DHEA 1/cage	1.6458 ± 0.0939	5.9 ± 0.2	2.848 ± 1.111
Control 5/cage	1.6845 ± 0.1296	4.8 ± 0.2*	4.284 ± 1.528
DHEA 5/cage	1.7361 ± 0.1063	5.9 ± 0.4	5.172 ± 1.889

[a] Liver weight divided by body weight × 100.
[b] Treated with DHEA, 450 mg/kg body weight.
[c] Data all means ± standard deviations; $n = 8$ all groups.
* Control group significantly different from DHEA-treated group.

TABLE V. EPIDIDYMAL FAT PAD WEIGHT, CELLULARITY AND LIPOPROTEIN LIPASE ACTIVITY IN DHEA-TREATED[a] MICE (EXPERIMENT 2)

	Pad weight (g)	Fat cell size (μg lipid/cell)	Fat cell no./pad (×10⁶)	LPL activity[b]
Control 1/cage[c]	0.9471*,†	0.1292*,†	4.3498	1.301*
	±0.2819	±0.0432	±0.9174	±0.315
DHEA 1/cage	0.6197	0.0711	4.2530	0.790
	±0.1817	±0.0149	±0.4931	±0.313
Control 5/cage	1.3024*	0.1827*	3.9419	2.035
	±0.2359	±0.0435	±1.2025	±0.595
DHEA 5/cage	0.5903	0.0800	4.0199	1.786
	±0.1491	±0.0213	±0.5937	±0.512

[a] Treated by intubation with 450 mg/kg body weight DHEA from 6 to 16 weeks of age.
[b] LPL activity: μmole free fatty acid released × 10^6 cells per hour.
[c] Data are means ± standard deviations; $N = 8$ for all groups.
* Control group significantly different from DHEA-treated group.
† Control 1/cage significantly different from DHEA 5/cage.

epiandrosterone in mice when DHEA is administered by intubation at a level of 450 mg/kg body weight. Although, Yen *et al.* (1) have reported that intraperitoneal injections of DHEA at 10 mg/kg were effective in decreasing weight gain in female yellow obese mice, in the present investigation intraperitoneal injection of DHEA at 25 mg/kg body weight did not affect body weight of lean male mice. This lack of agreement may be due to some combination of sex and strain differences as well as housing conditions, diet composition, and body weight or body fatness level. In general, DHEA seemed to have a larger effect on altering body weight when the mice were growing at a more rapid rate as shown from previous work in obese mice (1) and the present results obtained in Experiment 2 in mice housed five per cage. However, in order to more clearly differentiate the effectiveness of different modes of administration, levels of DHEA present in the treated mice should be determined and related to body weight.

DHEA appears to have no toxic effects in mice as noted by Yen *et al.* (1) and Schwartz *et al.* (18) found that mice that

TABLE VI. RETROPERITONEAL FAT PAD WEIGHT, CELLULARITY AND LIPOPROTEIN LIPASE ACTIVITY IN DHEA-TREATED[a] MICE (EXPERIMENT 2)

	Pad weight (g)	Fat cell size (μg lipid/cell)	Fat cell no./pad (×10⁶)	LPL activity[b]
Control 1/cage[c]	0.2574*,†	0.1494*,†	0.9356	1.484*
	±0.0576	±0.0215	±0.1962	±0.371
DHEA 1/cage	0.1867	0.1050	0.9137	0.567
	±0.0507	±0.0360	±0.1858	±0.203
Control 5/cage	0.3198*	0.1275*	1.1225*	1.729
	±0.0650	±0.0334	±0.1524	±0.553
DHEA 5/cage	0.1817	0.0983	0.9068	1.429
	±0.0529	±0.0249	±0.1851	±0.345

[a] Treated by intubation with 450 mg/kg body weight DHEA from 6 to 16 weeks of age.
[b] LPL activity; μmole free fatty acid released × 10^6 cells per hour.
[c] Data are means ± standard deviations; $N = 8$ for all groups.
* Control group significantly different from DHEA-treated group.
† Control 1/cage significantly different from DHEA 5/cage.

were treated with DHEA for over one year showed no apparent side effects. In fact, DHEA treatment inhibited the development of spontaneous development of breast cancer in female C3H mice and decreased their mortality rate (18).

Although a number of metabolic inhibitors of lipogenesis have been investigated in *in vitro* or acute animal studies, few have been used in chronic studies. (−)-Hydroxycitrate, an inhibitor ATP: citrate lyase (19), is one of the exceptions. Its antilipogenic effects have been studied in both lean (20, 21) and obese rats (22, 23). However, in general its effects on weight gain and body fat seem to be mediated primarily through decreasing food intake as demonstrated from pair-feeding studies (21, 23). Therefore, DHEAs effect on decreasing weight gain without affecting food intake poses an interesting metabolic puzzle as to the disposition of the ingested calories. We are presently investigating this.

In both Experiments 1 and 2, DHEA treatment was found to decrease both epididymal and retroperitoneal fat pad weights. In Experiment 2 where the results are most consistent and significant this effect was primarily due to decreased fat cell size in both fat depots. These results are consistent with the observation that fat cell number is not usually altered in adult lean mice (24). A decrease in fat cell number was found in both the epididymal fat pad of treated DHEA mice in Experiment 1 and in the retroperitoneal fat pad of DHEA five/cage in Experiment 2. These decreases are probably due to a failure to recruit previously made fat cells which are maintained in a precursor pool until some later time (25, 26). Although recent studies have shown the ability to increase fat cell number by overfeeding (27−29), no studies have shown the ability to decrease fat cell number once they have been produced (9). In addition, it should be noted that although lean mice were used in this study, similar results would be expected in adipose depots of the yellow obese mice since this rodent's obesity is due to increased fat cell size only (24).

Lipoprotein lipase is the enzyme in adipose tissue responsible for clearing triglycerides from either chylomicra or very low density lipoproteins. A number of studies have investigated the role of lipoprotein lipase in the regulation of body weight and obesity. Elevated levels are found in obese mice (30), obese Zucker rats (31−33), and in obese humans (34). In general adipose tissue lipoprotein lipase activity correlates with fat cell size (16, 35). However, in a number of situations this relationship has been found to be uncoupled. For example, during development lipoprotein lipase activity increases prior to an increase in fat cell size (16). Steinsgrimsdottir *et al.* (36) have shown that progesterone injections also resulted in increased lipoprotein lipase activity before fat cell size was found to increase. Chronic food restriction in both obese humans (37) and obese Zucker rats (38) has been found to result in increased adipose lipoprotein lipase activity compared to normally fed controls. It has also been shown that corticosteroid treatment appears to lower lipoprotein lipase activity (39, 40). In the present experiment DHEA treatment did not lead to consistent changes in lipoprotein lipase activity. DHEA one/cage mice did have decreased lipoprotein lipase activity that paralleled the smaller fat cell size. However, in the DHEA five/cage mice there was no statistical difference in lipoprotein lipase activity between the two groups. Whether the time difference in killing these groups of mice led to diurnal variations in the enzyme activity, or hormonal factors associated with the grouped housing altered lipoprotein lipase activity independent of fat cell size changes remains to be investigated.

What role endogenous DHEA may play in the etiology or maintenance of obesity is not known. No animal studies have been done and the results of human studies have been contradictory. Lopez and Krehl (41) found that an increase in body weight in obese subjects paralleled increases in red blood cell glucose-6-phosphate dehydrogenase activity and that less DHEA was excreted in the urine. When the subjects were placed on a low carbohydrate diet a de-

creased glucose-6-phosphate dehydrogenase activity and an increase in urinary DHEA was noted. Results of another calorie-restricted study (42) showed a decrease in DHEA excretion. However it has also been reported that obese subjects have an increased clearance rate for DHEA (43). A recent study in female runners found decreased body weight to be correlated with an increase in DHEA in the blood (44). Thus, although differences in DHEA metabolism can be found in humans, clearly more research is necessary to determine the precise role of DHEA in regulation of body weight. However, clinical and epidemiological studies have shown that women who have subnormal plasma concentrations of DHEA have an increased risk of developing breast cancer (45–47). Interestingly, increased body weight is also a risk factor in breast cancer.

In conclusion the results of this study are consistent with DHEA acting as an inhibitor of glucose-6-phosphate dehydrogenase activity and of the hexose monophosphate shunt (3–6, 48, 49) resulting in a limitation of the NADPH needed for lipid synthesis. No decrease in the activity of the NADPH-dependent enzyme, fatty acid synthetase, was found *in vitro*. However, since NADPH is added to the reaction mixture, this does not exclude the possibility that fatty acid synthetase activity was reduced *in vivo* and Yen *et al.* did report a decrease in *in vivo* lipogenesis in DHEA-treated mice (1). Clearly more work is necessary to determine the effect of long-term DHEA administration on glucose-6-phosphate dehydrogenase activity and possible compensatory mechanism of providing NADPH such as malic enzyme which has been shown to increase following short-term DHEA treatment in rats (48). In addition, one would like to know where the additional calories are going which are not being laid down as lipid. Preliminary studies in rats indicate DHEA treatment may increase oxygen consumption.

The assistance of Sharon Merkin, Stewart Avart, Louise Jackson, and Denise Fairman in the care of the mice is gratefully acknowledged. We thank Brenda Jones for typing the manuscript. This study was supported by Grants CA-14661 and AG-00368 in addition to a grant from the Weight Watchers Foundation and Drexel Research Scholar Award 1980–1981 to Margot P. Cleary.

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Received April 15, 1982. P.S.E.B.M. 1982, Vol. 171.