Effect of Dehydroepiandrosterone on Growth in Lean and Obese Zucker Rats[1]

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ABSTRACT Several studies were undertaken to determine the effect of dehydroepiandrosterone (DHEA) on growth in Zucker rats. In experiment 1, 3 weeks of DHEA treatment in lean rats resulted in decreased body weight gain in comparison to control rats. In experiment 2, both lean and obese rats were treated with DHEA from 6 to 21 weeks of age. Significant decreases in body weight were found for both lean and obese DHEA-treated rats. The food efficiency ratio (FER) was significantly decreased in both DHEA-treated groups. Significant decreases in parametrial and retroperitoneal fat pads were found in both lean and obese DHEA-treated rats. This was primarily attributed to a decrease in fat cell number in lean rats and to decreases in both number and size of fat cells in obese rats. In experiment 3 obese female rats were treated with DHEA from 6 to 21 weeks of age followed by 15 weeks with DHEA removed from the diet. Significantly more weight was gained by the rats previously treated than by the control rats, but body weight remained significantly lower than in the control groups. These data indicate DHEA has an effect on altering body weight and body fat in lean and obese Zucker rats. J. Nutr. *114*: 1242–1251, 1984.

INDEXING KEY WORDS adipose tissue cellularity • food efficiency ratio • obesity • muscle growth • body composition

The obesity of the Zucker rat is characterized by a number of metabolic alterations. These include hyperphagia (1), hyperinsulinemia (2, 3) and hypertriglyceridemia (1, 4, 5). The obesity is of early onset and precedes the abnormalities indicated above. The first detectable differences to be found between lean and obese rats occur during the first to second week of life. These include increased fat cell size and increased adipose tissue lipoprotein lipase activity (6, 7). As the obesity develops, marked hypertrophy of fat cells is followed by increases in fat cell number (8). The hyperplastic adipose tissue appears to be due to increased preadipocyte proliferation during suckling, as well as an extended period of postweaning proliferation, documented by measurement of thymidine kinase activity (9).

A number of techniques have been employed to prevent or treat the obesity of the Zucker rat. Intestinal bypass surgery has been shown to decrease body weight. This is mediated primarily through decreases in food intake; both lean body mass and organ growth are compromised (10, 11). Studies have been undertaken to restrict food intake during preweaning (12), postweaning (4, 13, 14), and both preweaning and postweaning (15) periods. However, the development of hyperplastic-hypertrophic obesity has not been prevented, indicating that hyperphagia

 Received for publication 31 October 1984.

[1]This investigation was supported in part by the Weight Watchers Foundation, The Hormel Foundation and Research Grant AM 27760 from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases of the National Institutes of Health.

is not critical to this obesity syndrome. Adrenalectomy has been shown to reduce food intake and weight gain of obese rats to levels similar to lean rats (16). This intervention was accompanied by decreased fat cell size but with no effect on fat cell number.

Both in vitro and in vivo studies have demonstrated that (−)-hydroxycitrate inhibits lipogenesis (17, 18). Its effectiveness as an antiobesity agent in obese Zucker rats was investigated by Greenwood et al. (19), but as with other forms of intervention, its primary effect appeared to be mediated through decreased food intake.

The 17-ketosteroid, dehydroepiandrosterone (DHEA), has been shown to decrease body weight gain in lean mice as well as in yellow obese mice that have hypertrophic adipose tissue (20–22). The change in body weight occurred without affecting food intake (20–22). Recently, DHEA has been shown to decrease body weight gain independently of changes in food intake in Sprague-Dawley rats (23). No data have been presented on the effects of DHEA in hyperplastic-hypertrophic obesity. In the present study the effect of DHEA on growth and development of Zucker lean and obese female rats was investigated. Body composition, adipose tissue cellularity and liver and muscle cellular growth data are presented.

MATERIALS AND METHODS

Rats. All rats were purchased from the Vassar College Biology Department, Poughkeepsie, NY. During the experiments the rats were individually housed in a temperature-controlled room (22–23°C) with a 12-hour light-dark cycle. Water and food were provided ad libitum. Control rats received powdered Purina Rodent Chow (#5001, Ralston Purina Co., St. Louis, MO) and DHEA-treated rats received the powdered Chow with DHEA (Searle Chemicals, Inc., Chicago, IL) mixed in at a level of 0.6% as previously described (23). Food intake and body weight were determined weekly. At the termination of the individual experiments, rats were killed by decapitation in the morning between 0830 and 1030 hours. Tissues were processed as decribed in the individual experiments.

Experiment 1. To ascertain whether DHEA was effective in the Zucker rat strain a preliminary study was undertaken using only lean rats. Lean female rats (Fa/Fa or Fa/?) were randomly assigned to either a control (n = 6) or DHEA (n = 6) group. Initial body weights in the two groups were not significantly different. The experimental period lasted for 3 weeks from 6 to 9 weeks of age. When the rats were killed, livers were removed, weighed and frozen for DNA (24), RNA (25) and protein (26) determinations. Parametrial and retroperitoneal fat pads were removed and weighed.

Experiment 2. Lean and obese rats were divided into control and DHEA groups to give a total of four experimental groups [control-lean (n = 11), DHEA-lean (n = 11), control-obese (n = 7) and DHEA-obese (n = 7)]. Initial body weights of the two lean and two obese groups were not significantly different. Rats were treated for 15 weeks from 6 to 21 weeks of age. When the rats were killed, livers and both right and left gastrocnemius muscles were removed, weighed and frozen for DNA (24), RNA (25) and protein (26) determinations. Parametrial and retroperitoneal fat depots were removed and weighed, and samples were taken for establishment of fat cell size and number by the method of Hirsch and Gallian (27) with a Coulter counter model ZB (Coulter Electronics, Hialeah, FL). Bodies were frozen before determination of body composition (15).

Experiment 3. Female obese rats were either maintained on the control stock diet (n = 4) or treated with DHEA (n = 4) for 15 weeks as described in experiment 2. From 21 weeks of age, all rats were maintained on stock diet alone for another 15 weeks. Rats were killed at 36 weeks of age, and liver weight and parametrial and retroperitoneal fat pad weights were recorded.

Statistical analysis. Data are presented as means ± the standard deviation. Statistical comparisons were made between control and treated rats by using Student's t-test in experiments 1 and 3. In experiment 2, two-way ANOVA was used followed by F-test for comparison of individual means (28). Statistical significance was at either P- or F-value of < 0.05.

RESULTS

Experiment 1

This experiment was undertaken to determine whether DHEA was effective in the Zucker rat strain. Results from this study are presented in table 1. It was found that 3 weeks of DHEA treatment in lean female Zucker rats resulted in a significant decrease in body weight compared to control rats. Weight gain of the treated rats was decreased by 47% compared to control rats during this time interval. There was no effect on cumulative food intake. When food efficiency ratios (FER) (grams of body weight gained ÷ grams of food consumed) were calculated, a significant decrease in FER was found in the DHEA-treated rats compared to control rats.

Liver weight was significantly increased by DHEA treatment (table 1). DHEA treatment resulted in a 100% increase in total liver protein. There were also significant increases in RNA (22%) and DNA (15%). Although decreases in both parametrial (18%) and retroperitoneal (16%) fat pad weights were found in the DHEA-treated rats in comparison to the control rats, these differences were not significant after 3 weeks of treatment.

TABLE 1

Experiment 1: Effect of dehydroepiandrosterone (DHEA) on growth in lean Zucker female rats following 3 weeks of treatment[1]

Measure[2]	Control-lean	DHEA-lean
Final body wt	131.8 ± 10.0*	115.8 ± 8.6
Wt gain	50.7 ± 5.3*	26.8 ± 10.7
Cumulative food intake	307.8 ± 13.6*	303.5 ± 17.3
Food efficiency ratio[3]	0.165 ± 0.010*	0.088 ± 0.008
Liver wt	7.10 ± 0.44*	9.31 ± 0.35
Liver total DNA, *mg*	7.32 ± 0.63*	8.43 ± 0.65
Liver total RNA, *mg*	28.01 ± 2.62*	34.20 ± 1.68
Liver total protein, *mg*	599.58 ± 48.27*	1084.54 ± 63.70
Parametrial fat pat wt[4]	0.377 ± 0.146	0.312 ± 0.054
Retroperitoneal fat pad wt[4]	0.420 ± 0.211	0.357 ± 0.133

[1]DHEA included in the diet at a level of 0.6% from 6–9 weeks of age. Values are means ± SD for six animals in each group. [2]In grams except as noted. [3]Food efficiency ratio = weight gain ÷ food intake. [4]Weight of both right and left pad. *Control-lean rats significantly different from DHEA-lean rats at $P < 0.05$.

Experiment 2

Body weight and food intake. Body weight of the rats at 3-week intervals over the 15-week experimental period are shown in figure 1. Significant differences in body weight between obese-control and obese-DHEA rats were found after 4 weeks of treatment and after 5 weeks of treatment, between lean-control and lean-DHEA rats. DHEA treatment also significantly decreased weight gain in both lean and obese rats compared to their respective control groups (table 2).

Food intake was not affected in the lean-DHEA rats compared to lean-control rats (table 2). A significant decrease in cumulative food intake was found in obese-DHEA rats compared to obese-control rats. However, when examined on a body weight basis, the obese-DHEA rats did not consume less food than control-obese rats (data not shown). When FER were calculated, lean-DHEA rats had a 35% decrease in FER compared to lean-control rats. Obese-DHEA rats were found to have a 55% decrease in FER compared to the obese-control group and a 35% decrease compared to lean-control rats, who had a food intake similar to the obese-DHEA rats (table 2).

Liver and muscle growth. The effects of 15 weeks of DHEA treatment on liver growth are presented in table 3. As previously reported for Sprague-Dawley rats (23) and as cited above in lean female rats (experiment 1), DHEA treatment resulted in an increased liver weight in lean female rats. In obese rats no increase in liver weight was found, on an absolute basis, between the obese-control and obese-DHEA rats, but when liver weight was expressed as a percent of total body weight, a significant increase was found in the obese-DHEA rats (data not shown). In lean-DHEA rats the increased liver size appeared to be attributed to significant increases in DNA, RNA and protein per total liver in comparison to lean-control rats. This resulted in similar RNA:DNA and protein:DNA ratios in comparison to control-lean rats. No significant differences were found for liver DNA,

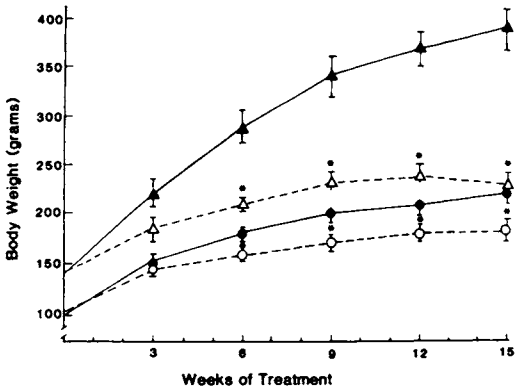

Fig. 1 Growth curves for lean and obese Zucker rats treated with 0.6% DHEA from 6 to 21 weeks of age. Data are shown in 3-week intervals and are presented as means ± SD. ●, Control-lean (n = 11); ○, DHEA-lean (n = 11); ▲, control-obese (n = 7); △, DHEA-obese (n = 7). *Indicates significant difference between control and respective treated group.

RNA and protein measurements in obese-DHEA rats compared to obese-control rats. The RNA:DNA and protein:DNA ratios were similar in both obese groups. Obese-control rats had significant increases in liver weight as well as DNA, RNA and protein when compared to lean-control rats. However, there were no differences in either RNA:DNA or protein:DNA ratios among any of the groups.

Muscle growth was also examined in the rats of experiment 2 (table 3). As previously described in male Zucker obese rats (29, 30), female obese Zucker rats also have compromised muscle growth compared to their lean counterparts. Comparison of respective control and treated animals indicated that DHEA treatment in lean and obese rats had no effect on any aspect of muscle growth. This was in contrast to the effects of food restriction previously reported in Zucker rats (29, 30).

Body composition. Results of the body composition determination are shown in table 4. Data are shown both on an absolute, gram, basis as well as on a percentage basis. On an absolute basis, DHEA treatment decreased body fat by 25% in lean rats and by 57% in obese rats in comparison to their respective control groups. However, this was only significant in the obese group. Grams of body water were significantly decreased in both treated groups. Fat-free dry mass was not significantly decreased in lean-DHEA rats compared to lean-control rats, but was significantly decreased in obese-DHEA rats compared to obese-control rats. The fat-free dry mass in obese-DHEA rats was similar to that found in the two lean groups with the obese-control rats having the greatest absolute fat-free mass of all groups.

On a percentage basis, no differences in body composition were found between the lean-control and lean-DHEA rats. In the two obese groups there was a similar percentage of fat-free dry mass. Obese-DHEA rats had a significant decrease in the percentage of body fat and a significant increase in percentage of body water compared to obese-control rats.

Parametrial and retroperitoneal fat pads. DHEA treatment significantly decreased parametrial and retroperitoneal fat pad weights in both lean and obese treated rats as compared to their respective control groups (fig. 2). Fat pads from obese-DHEA rats still weighed more than fat pads from lean-control rats. This decrease in fat pad weights was due primarily to decreases in

TABLE 2

Experiment 2: Effect of DHEA on weight gain, food intake and food efficiency ratio in lean and obese female Zucker rats[1,2]

Group	Wt gain	Food intake	FER[3]
	g		
Lean-control (n = 11)	122.9 ± 22.8[abcd]	1519.1 ± 88.5[abd]	0.081 ± 0.006[abc]
Lean-DHEA (n = 11)	81.1 ± 16.3	1490.2 ± 65.6	0.053 ± 0.004
Obese-control (n = 7)	253.5 ± 46.8[abc]	2192.0 ± 199.0[abc]	0.116 ± 0.007[abc]
Obese-DHEA (n = 7)	90.5 ± 24.4	1731.5 ± 109.0	0.053 ± 0.008

[1]DHEA administered at a level of 0.6% in the diet from 6 to 21 weeks of age. Values are means ± SD for number of animals in parentheses. [2]Significantly different: [a]two-way ANOVA control groups from DHEA-treated groups; [b]two-way ANOVA lean groups from obese groups; [c]within each genotype control group from respective DHEA group; [d]control-lean group from control-obese group. [3]Food efficiency ratio = weight gain ÷ food intake.

TABLE 3

Experiment 2: Effect of DHEA treatment on liver and muscle weight, and DNA, RNA, and protein content in lean and obese female Zucker rats[1,2]

Tissue and group	Wt	Total DNA	Total RNA	Total protein
	g		*mg*	
A. Liver				
Lean-control (7)	8.01 ± 1.23[abcd]	6.88 ± 1.49[abcd]	32.46 ± 4.91[abcd]	851.77 ± 97.34[abcd]
Lean-DHEA (5)	11.23 ± 2.43	9.11 ± 1.17	44.41 ± 5.88	1374.40 ± 27.38
Obese-control (7)	12.66 ± 1.72[ab]	9.57 ± 1.43[ab]	45.01 ± 6.73[ab]	1371.44 ± 290.87[ab]
Obese-DHEA (7)	13.02 ± 1.21	10.87 ± 1.05	50.55 ± 2.83	1588.43 ± 198.24
B. Muscle				
Lean-control (6)	2.1555 ± 0.2021[bd]	0.52 ± 0.07[bd]	1.18 ± 0.20[bd]	276.00 ± 39.89[bd]
Lean-DHEA (5)	1.9635 ± 0.2254	0.52 ± 0.06	1.13 ± 0.23	260.20 ± 48.16
Obese-control (7)	1.7026 ± 0.2248[b]	0.43 ± 0.08[b]	0.89 ± 0.18[b]	215.06 ± 49.64[b]
Obese-DHEA (6)	1.5576 ± 0.3395	0.39 ± 0.05	0.81 ± 0.19	193.08 ± 47.53

[1]DHEA included in the diet at a level of 0.6% from 6 to 21 weeks of age. Values are means ± SD for number of animals in parentheses. [2]Significantly different: [a]two-way ANOVA control groups from DHEA groups; [b]two-way ANOVA lean groups from obese groups; [c]within each genotype control group from respective DHEA group; [d]control-lean group from control-obese group.

fat cell numbers in both parametrial (fig. 3*A*) and retroperitoneal (fig. 3*B*) fat pads for both lean and obese DHEA-treated groups in comparison to their respective control groups. In lean rats there was no effect of DHEA on fat cell size in either depot (fig. 3*C*, 3*D*). However, a 25% decrease in both parametrial and retroperitoneal fat cell size was found in the obese-DHEA treated rats compared to obese-control rats.

Experiment 3

Body weights of the obese rats used in this experiment are shown in table 5. After 15 weeks of DHEA treatment, these rats responded in a similar manner to the obese rats in experiment 2, i.e., a significant decrease in body weight was found in the treated obese rats. Following 15 weeks without DHEA treatment, the DHEA-obese rats still weighed significantly less than the control-obese rats (table 5). However, during this time interval the DHEA-obese rats had a body weight gain three times greater than that of the control rats. Liver weights were still similar in the two groups. In addition, retroperitoneal and parametrial fat depot weights of DHEA-obese rats were still significantly decreased in comparison to the

TABLE 4

Effect of DHEA on body composition in lean and obese Zucker rats[1,2]

Group	Body fat	Body water	FFDM[3]	Body fat	Body water	FFDM[3]
		g			%	
Lean-control (11)	13.1 ± 4.2[abc]	107.4 ± 15.3[ac]	21.1 ± 6.6[ab]	9.5 ± 3.0[abd]	76.2 ± 5.9[abd]	14.6 ± 4.8
Lean-DHEA (11)	9.7 ± 3.6	79.9 ± 10.0	17.0 ± 3.4	9.1 ± 2.9	75.4 ± 3.0	16.0 ± 2.5
Obese-control (7)	146.3 ± 25.4[abc]	118.9 ± 29.7[ac]	33.4 ± 7.1[abc]	50.0 ± 6.3[abc]	40.4 ± 8.1[abc]	11.5 ± 2.5
Obese-DHEA (7)	63.3 ± 17.0	76.0 ± 16.5	19.4 ± 3.9	40.2 ± 6.3	49.0 ± 6.7	12.9 ± 3.8

[1]DHEA administered at a level of 0.6% in the diet from 6 to 21 weeks of age. Values are means ± SD for number of animals in parentheses. [2]Significantly different: [a]two-way ANOVA control groups from DHEA groups; [b]two-way ANOVA lean groups from obese groups; [c]within each genotype control group from respective DHEA group; [d]control-lean group from control-obese group. [3]FFDM, fat-free dry mass.

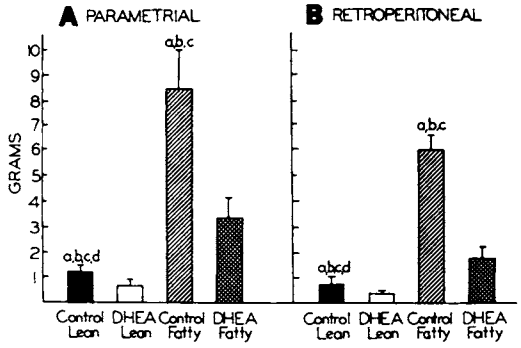

Fig. 2 Parametrial (2*A*) and retroperitoneal (2*B*) fat pad weights of lean and obese Zucker rats treated with 0.6% DHEA from 6 to 21 weeks of age. Data are means ± SD for control-lean (n = 11), DHEA-lean (n = 11), control-obese (n = 7), DHEA-obese (n = 7). [a]Two-way ANOVA control groups significantly different from DHEA groups. [b]Two-way ANOVA lean groups significantly different from obese groups. [c]Within each genotype control group significantly different from respective DHEA group. [d]Control-lean rats significantly different from control-obese rats.

control-obese rats, as was the percentage contributed by the fat pads to total body weight (table 5).

DISCUSSION

This study indicates that DHEA treatment results in decreases of body weight gain in both lean and obese female Zucker rats. Although Yen et al. (22) and Schwartz (21) have presented data on the antiobesity action of DHEA in yellow obese mice, this is the first description of the effect of DHEA in hyperplastic-hypertrophic obesity. As previously reported for mice (20–22) and Sprague-Dawley rats (23), this effect on body weight was independent of a decrease in food intake in the lean Zucker rats. In the obese-DHEA Zucker rats, a significant decrease in cumulative food intake was found in comparison to obese-control rats. However, a significant difference in food intake between obese-control and obese-DHEA rats was not observed until after the fourth week of treatment. By this time there was a 30% difference in body weight between the two obese groups. This would indicate that the effect of DHEA in the obese rat is not due primarily to a decreased food intake, but rather, that the decrease in food intake is a consequence of the decrease in body weight.

Calculation of the FER resulted in decreased values for both DHEA-treated groups in comparison to their respective controls. This indicated that lean-DHEA and obese-DHEA rats were calorically inefficient, i.e., they gained less weight in relation to caloric intake than either lean-control or obese-control rats. This is in contrast to the FER of either food-restricted male (15) or female (Cleary, M. P., Fox, N. and Lazin, B., unpublished observation) obese Zucker rats, which remains unchanged when compared to ad libitum–fed obese rats. In fact, DHEA-treated obese rats gained 65% less weight and 57% less body fat, while caloric intake was decreased by only 22%. A previous study of male food-restricted rats showed that weight differences paralleled food intake changes.

Other evidence support the notion that the effect of DHEA is different than that of food restriction in the obese rat. For example, obese-DHEA rats did not have compromised muscle growth as has previously been found in obese Zucker rats pair-fed to lean ad libitum–fed rats (29, 30) or in rats following

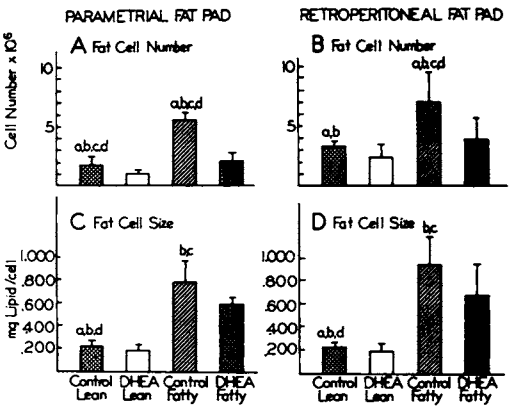

Fig. 3 Effect of 0.6% DHEA on number (3*A*, 3*B*) and fat cell size (3*C*, 3*D*) of parametrial and retroperitoneal fat pads from lean and obese Zucker rats. Data are presented as means ± SD for control-lean (n = 11), DHEA-lean (n = 11), control-obese (n = 7), DHEA-obese (n = 7). [a]Two-way ANOVA control groups significantly different from DHEA groups. [b]Two-way ANOVA lean groups significantly different from obese groups. [c]Within each genotype control group significantly different from respective DHEA group. [d]Control-lean rats significantly different from control-obese rats.

TABLE 5

Experiment 3: Effect of DHEA on growth following cessation of treatment in obese Zucker rats[1]

Measure[2]	Control-obese	DHEA-obese
Body wt (A) after 15 weeks 0.6% DHEA	362.5 ± 32.4*	182.0 ± 37.6
Body wt (B) after 15 weeks no DHEA	410.2 ± 9.3*	322.0 ± 19.5
Wt gain from A – B	47.8 ± 30.8*	140.0 ± 46.7
Liver wt	12.01 ± 0.03*	11.81 ± 3.43
Parametrial fat pad wt[3]	7.399 ± 0.932*	4.980 ± 1.169
Retroperitoneal fat pad wt[3]	8.621 ± 2.101*	3.956 ± 0.492
Fat pads, *% of total body wt*	7.79 ± 0.91*	5.52 ± 0.47

[1]DHEA in diet from 6 to 21 weeks of age followed by no DHEA from 22 to 36 weeks of age. Values are means ± SD for four animals in each group. [2]In grams except as noted. [3]Per pad weight. *Control significantly different from DHEA ($P < 0.05$).

intestinal bypass surgery (10). In addition, percentage of body fat was decreased and percentage of body water was increased by DHEA treatment. These changes are in the direction of a normalization of body composition in the obese rats. Food restriction did not improve body composition in obese rats (15).

The decrease in fat cell number and size found in DHEA-obese rats further supports the interpretation that the action of DHEA is distinct from that of food restriction. Initially food restriction decreased fat cell number in obese rats, but there was an eventual catching up of fat pad weight and fat cell number even while food was restricted (15). In the present experiment, when DHEA was removed from the diet, the significantly reduced fat pad weight was maintained even after 15 weeks without DHEA. Fat cell size in obese Zucker rats has been found to be highly resistant to change (12, 15, 19) but was decreased 25% by DHEA treatment. Adrenalectomy has also been shown to decrease fat cell size but not to decrease fat cell number (16).

Therefore, although there is a decrease in food intake in the Zucker obese rat, there is supportive evidence that the effect of DHEA in obese rats is primarily independent of any effect on food intake just as in lean and obese mice and lean rats. A summary of the comparisons discussed between the DHEA-treated obese rats in this study and food-restricted obese rats (15, 29) is outlined in table 6. It is acknowledged that the rats are of different sexes and slightly different ages at the termination of the experiment. At the present time an experiment incorporating pair-feeding is underway to address this issue directly.

Obese rats appeared to respond to DHEA treatment much more than did the lean rats. Possibly this is due to differences in the metabolism of DHEA by lean and obese rats. DHEA intake was calculated on a milligram/kilogram body weight basis, and similar amounts were ingested by lean and obese treated rats. For example, during week two the mean value for DHEA intake for lean rats was 4453.2 ± 383.9 versus 4854.6 ± 563.4 for obese rats. At that time these two groups weighed similar amounts. This relationship continued throughout the experiment and does not provide conclusive evidence about metabolism of DHEA by either lean or obese rats. One could argue that since no differences in DHEA intake or body weight were found, there were no differences in metabolism. On the other hand, since the expected growth of the obese rat was so much greater than that of the

TABLE 6

A comparison of the effects of food restriction and DHEA treatment in in obese Zucker rats

Measure	Food restriction[1]	DHEA treatment[2]
Body wt, *g*	Decreased (26%)[3]	Decreased (40%)
Food intake, *g*	Decreased (28%)	Decreased (22%)
Food efficiency ratio	No effect[4]	Decreased (55%)
Body fat, %	No effect	Decreased (20%)
Muscle wt, *mg*	Decreased (30%)	No effect
Fat pad wt, *g*		
Gonadal	Decreased (33%)	Decreased (46%)
Retroperitoneal	Decreased (26%)	Decreased (62%)
Fat cell size, *g lipid/cell*	No effect	Decreased (25%)

[1]Data from references (15, 29). [2]Data from Experiment 2. [3]Comparisons made between experimental obese and ad libitum-fed obese-controls. Number in parentheses is the percent difference between values significantly different in the direction indicated. [4]No effect indicates no significant difference between experimental and ad libitum-fed obese controls.

lean rat, one could argue that a given amount of DHEA had a much greater impact on the obese rat. This remains an aspect of the work to be studied in more detail.

The mechanism by which DHEA treatment results in decreases in fat cell number is not known. DHEA is known to inhibit glucose-6-phosphate dehydrogenase (described below), an enzyme important in NADPH production. A limited supply of this cofactor might directly inhibit DNA synthesis and result in the production of fewer fat cells. Both in vitro and in vivo studies have shown that DHEA can inhibit DNA synthesis (31, 32). It is also possible that a decrease in NADPH levels would inhibit lipogenesis. There would then be less lipid to fill fat cells allowing them to remain below detectable limits. If overall energy metabolism is increased by DHEA treatment, as suggested by the decrease in caloric efficiency, there could also be less substrate to be stored resulting in a similar observation. Thus, data available at this time cannot answer whether this decrease in fat cell number is real or apparent. Measurements of thymidine kinase or DNA polymerase activites or actual [^{3}H]thymidine incorporation studies may help clarify this point (9, 33, 34).

Most of the data presented are consistent with the interpretation that the effect of DHEA is mediated through its known ability to inhibit the activity of the enzyme glucose-6-phosphate dehydrogenase. This would theoretically limit NADPH production and result in inhibition of either lipid or DNA synthesis. However, we have been unable to consistently demonstrate a decrease in the activity of this enzyme following DHEA treatment (23, 35, 36). In addition, an increase in another NADPH-producing enzyme, malic enzyme, has been found (23, 35, 36). Furthermore, even if glucose-6-phosphate dehydrogenase activity is decreased, the fate of glucose molecules not metabolized by the pentose-phosphate shunt needs to be determined. Thus, the mechanism of the caloric inefficiency or energy wasting due to DHEA treatment is unknown. Recently, we have described a possible energy-wasting, substrate (futile), cycle of fatty acid metabolism that may at least partially explain DHEA's effect (35). It was found that DHEA treatment in both lean and obese rats resulted in an increase in hepatic long-chain fatty acyl-CoA hydrolase activity. This enzyme removes the CoA group from fatty acids. Prior to further metabolism of the fatty acid, the CoA group must be reattached. The reaction responsible for this is catalyzed by the enzyme fatty acyl-CoA synthetase and requires ATP hydrolysis. Therefore, energy is required to reverse the consequences of the action of the hydrolase.

Some of the observations made here are similar to those described for adrenalectomized obese rats (16, 37, 38). However, the body weight differences found in adrenalectomized rats parallel the 40% decrease in food intake observed (16). In addition, in adrenalectomized rats the main effect on adipose depots was a decreased fat cell size (16), while in DHEA-treated obese rats, decreases in both fat cell size and number were found. These changes induced by adrenalectomy have been attributed to an enhanced response to corticosterone. One cannot rule out the possibility that DHEA levels may be elevated such that they inhibit adrenal function, and this may play some role in the antiobesity action of exogenously administered DHEA. Further studies including serum analyses need to be performed to determine whether the results of these two types of intervention are mediated through a common factor.

Of all steroid hormones, DHEA has the highest circulating level in plasma. Despite this there is no known biological function for DHEA except its ability to contribute slightly to body pools of testosterone and estrogen. It is possible that some of DHEA's effect may be attributable to conversion to these two hormones, but our data do not support this. No effect on gonadal weight of treated rats has been found. The findings that DHEA treatment prevents development of breast cancer in mice (21) and that there is an inverse correlation between DHEA plasma levels and the development of breast cancer in humans (39) indicate that estrogen is not the active compound for the effect of DHEA.

The dose of DHEA being administered is quite high. However, no toxic effects of long-term DHEA treatment have been described (21, 22). Even higher doses of 1%

appear to have no adverse effects in older lean and obese rats (Cleary, M. P. and Zisk, J., unpublished). In fact, as mentioned above, DHEA treatment prevented the development of mammary tumors in mice and increased life expectancy (21). DHEA has also been found to prevent the development of several types of chemically induced tumors in mice (40, 41). The only possible adverse effect noted in this and a previous study with Sprague-Dawley rats (23) is the liver enlargement. However, this is a common finding after steroid treatment (42) as well as treatment with hypolipidemic drugs such as clofibrate (43).

The fact that DHEA decreases body weight and more importantly body fat in mice and rats without being mediated by changes in food intake and without compromising muscle growth is evidence that altered energy metabolism can be induced by DHEA. As examination of the body weight curve shows that this effect is not overcome with time, and when the compound is removed there is a slow catch-up period. The potential for such an agent in obesity treatment is clear since the many varied attempts to prevent or treat obesity in rodents and humans by food restriction alone have not been successful. Although it is unlikely that DHEA itself would be used as a therapeutic agent in humans, delineating its mechanism of action may provide insight for future analog and drug development.

ACKNOWLEDGMENTS

We would like to thank Stuart Avart for his expert technical assistance.

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