Serum Leptin, Lipids, Free Fatty Acids, and Fat Pads in Long-Term Dehydroepiandrosterone-Treated Zucker Rats (44488)

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Abstract. **The obese Zucker rat has a genetically flawed leptin system and is a model of hyperphagia, obesity, hyperlipidemia, and markedly elevated leptin levels. Dehydroepiandrosterone (DHEA) administration reduces hyperphagia, hyperlipidemia, and obesity in Zucker rats. Since serum leptin levels are associated with body fat, we wondered what the effects of fat pad weight reduction from DHEA administration would have on leptin levels. This experiment investigated the effects of DHEA on intra-abdominal fat pads, serum lipids, and peripheral leptin in male lean and obese Zucker rats that were administered DHEA in their food from 4 weeks of age to 20 weeks. Lean and obese rats received plain chow or chow containing DHEA. Additional chow-fed groups of lean and obese weight-matched controls and obese pair-fed rats helped to control for the reduced body weight, food intake, and fat pad weights seen with DHEA administration. DHEA administration to lean Zucker rats reduced body weight and fat pad weights, but leptin levels showed a lower trend. Among obese rats, both DHEA treatment and pair-feeding reduced body weight and fat pad weights, but only DHEA lowered leptin levels. The weight-matched controls had reductions in fat pad weights similar to the DHEA-treated group, but with increased leptin levels. Thus, DHEA may exert a small, independent effect on leptin levels in this animal model, but the reduction is less than what would be expected.** [P.S.E.B.M. 2000, Vol 223]

The obese Zucker rat (*fa/fa*) has a defective leptin receptor due to a single amino acid substitution (1) and subsequently develops markedly increased peripheral levels of leptin (2, 3), presumably due to the loss of the effects of leptin that are mediated by the leptin receptor. It is an animal model of hyperphagia, obesity, and hyperlipidemia (4–6). These rats exhibit greater body fat content than their lean litter mates as early as 1 week of age (7). The obese rats are hyperphagic through age 48 weeks (8), develop larger intra-abdominal fat pads (9), and exhibit elevated serum lipids (5, 10) compared with the lean rats. They develop obesity even in the face of long-term food restriction (11).

There is no question that leptin plays a role in many forms of obesity in animals (12). The realization that a hormone and its receptor can regulate body weight makes obesity an endocrine disorder (13). Other hormones interact with leptin, such as insulin (14–16) and glucocorticoids (17, 18). The adrenal hormone dehydroepiandrosterone (DHEA) has antiglucocorticoid effects (19). Administration of DHEA to Zucker rats has been shown to decrease insulin levels (20–22), decrease intra-abdominal fat pad mass (9, 22–25), and result in less body weight either through weight loss in fully grown rats (9, 21) or less weight gain in growing rats (21–25). Since body fat mass, insulin, and glucocorticoids interact with peripheral leptin levels, we won-

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dered whether DHEA administration would also affect leptin levels in Zucker rats. One would expect lower leptin levels simply because of the smaller fat pads that result from DHEA administration, but the defective leptin receptor may interfere with this expected drop (26, 27). In this study, the effects of DHEA on intra-abdominal fat pad mass, serum lipids, insulin, corticosterone, and peripheral leptin were investigated in young male Zucker rats given DHEA over a prolonged period.

Materials and Methods

Zucker rats were obtained from the breeding colony maintained at the Louisiana State University Medical Center (New Orleans) by the Department of Physiology. The animals are kept in a temperature- and humidity-controlled environment with a daily 12:12-hr light:dark cycle. Heterozygous (*fa/+*) lean ($n = 21$) and homozygous (*fa/fa*) obese ($n = 16$) male Zucker rats were randomly chosen at 4 weeks of age when they could safely be weaned from their mothers. The animals were fed either powdered laboratory rodent diet (chow) #5001 (Purina Mills, Inc., St. Louis, MO) and served as age-matched controls or were fed powdered chow containing DHEA (Sigma Chemical Co., St. Louis, MO) at a concentration of 6 g/kg of chow (0.6% by weight). The physiological fuel value of the rat chow is reported by the manufacturer to be 3.301 kCal/g. The food consumed by each rat was closely monitored through Week 10 and estimated from weekly measurements thereafter. Shortly after reaching 20 weeks of age, the animals were fasted for 12–18 hr and sacrificed by decapitation. Blood was collected on ice and centrifuged under refrigeration to obtain serum that was stored at –70°C. The intra-abdominal fat pads (epididymal, perinephric, and retroperitoneal) were removed and weighed.

A group of male obese Zucker rats ($n = 6$) were also randomly chosen from the colony at 4 weeks of age and handled in the same manner as described above with the exception of their diet. Briefly, these calorie-restricted, pair-fed animals were fed an amount of powdered rat chow matching the weekly historical mean amount of food consumed by the DHEA-treated obese rats.

All of these rats were housed individually in group-sized, hanging wire-mesh cages and had unlimited access to water. Except for the pair-fed rats, they had unlimited access to food as well. When the earlier studies had been completed, additional control groups, referred to as the weight-matched controls, were selected from the colony based on body weight. The heterozygous lean rats in the colony were weighed, and six animals were chosen randomly from among those that had body weights that were similar to the final body weights of the lean rats treated with DHEA. In like manner, six obese rats were also chosen randomly from among those having a body weight similar to the final weight of the obese rats treated with DHEA. These weight-matched rats were necessarily younger than the lean and obese controls (lean 9.6 ± 0.0, obese 11.1 ± 0.5 weeks of age) and were also sacrificed by decapitation after being fasted. Prior to being chosen for this protocol, these additional weight-matched controls had shared group-sized, hanging wire-mesh cages with unlimited access to food and water. Their food consumption was not monitored.

Determination of serum triglycerides and total cholesterol was performed by standard automated laboratory techniques using the Paramax 720ZX Automated Chemistry Analyzer (Baxter Healthcare Corp., Paramax Systems Division, Irvine, CA). Serum leptin and insulin were measured with commercially available radioimmunoassay kits specific for rat leptin and rat insulin, respectively (Linco Research, Inc., St. Charles, MO). Serum DHEA-S and corticosterone were measured with commercially available radioimmunoassay kits (Diagnostic Laboratories, Inc., Webster, TX). Serum content of nonesterified (free) fatty acids (FFA) was determined using a commercially available enzymatic colorimetric kit (Wako Chemicals USA, Inc., Richmond, VA). Because the younger, weight-matched animals were added at the end of the experiment after the corticosterone assay was completed, serum corticosterone levels were not determined in the weight-matched animals. In addition, only two specimens from the obese pair-fed group were available for the corticosterone assay due to a laboratory error.

All animals were treated humanely under the guidelines of the National Institutes of Health and the Animal Welfare Act. The protocol for this experiment was approved by the LSU Medical Center Institutional Animal Care and Use Committee (IACUC).

All results are listed as mean ± SEM where appropriate. Results were analyzed with JMP version 3.1.6.2 statistical software (SAS Corp., Cary, NC). The continuous data were analyzed by two-way ANOVA using the "Fit Model" routine with phenotype, diet, and phenotype × diet as independent variables. A P-value < 0.05 was considered significant.

Results

Body Weights, Caloric Intakes, and Fat Pads. The results of this experiment are listed in Table I. The lean and obese rats that were given DHEA weighed less at the time of sacrifice than the lean and obese age-matched controls fed standard rodent chow. The calorie restriction of the obese pair-fed group also resulted in less body weight at the time of sacrifice, but the reduction was not as great as that from DHEA administration. By design, the weight of the weight-matched controls was similar to the weight of the DHEA-treated rats of the same phenotype.

The untreated obese rats consumed more calories than their lean counterparts. DHEA administration reduced caloric intake in both the lean and obese rats. By design, the caloric intake of the obese pair-fed rats was similar to that of the DHEA-treated obese rats.

Compared with the age-matched controls, the total intra-abdominal fat pad mass was decreased in the DHEA-

Table I. Body Weight, Food Intake, Intra-Abdominal Fat Pads, and Serum Measurements (mean ± SEM)

	Lean controls	Lean DHEA	Lean weight-matched	Obese controls	Obese DHEA	Obese weight-matched	Obese pair-fed
n	10	10	6	8	8	6	6
Body weight (g)	343.0 ± 11.3^{a,d,f}	206.7 ± 6.6^{b}	185.3 ± 2.5^{b}	486.5 ± 15.1^{c}	316.5 ± 17.1^{d,e}	297.9 ± 20.8^{e}	378.1 ± 8.4^{f}
Total food intake (kCal)	7524 ± 115^{a}	5641 ± 118^{b}		8944 ± 238^{c}	6905 ± 423^{a,d}		6723 ± 60^{d}
Fat pads (g):							
Epididymal	3.48 ± 0.25^{a}	1.33 ± 0.07^{b}	0.99 ± 0.05^{b}	13.73 ± 0.80^{c}	6.86 ± 0.62^{d}	6.95 ± 0.85^{d}	8.87 ± 0.55^{e}
Perinephric	0.71 ± 0.07^{a}	0.35 ± 0.04^{a,b}	0.11 ± 0.01^{b}	4.09 ± 0.30^{c}	1.84 ± 0.31^{d}	1.67 ± 0.30^{d}	2.02 ± 0.08^{d}
Retroperitoneal	2.14 ± 0.21^{a}	1.10 ± 0.15^{b}	0.23 ± 0.02^{b}	12.98 ± 0.46^{c}	6.09 ± 0.36^{d}	5.82 ± 0.93^{d}	9.62 ± 0.35^{e}
Total fat pads	6.33 ± 0.49^{a}	2.78 ± 0.23^{b}	1.32 ± 0.07^{b}	30.80 ± 1.33^{c}	14.79 ± 1.20^{d}	14.44 ± 2.01^{d}	20.51 ± 0.44^{e}
Fat pads (% body weight):							
Epididymal	1.01 ± 0.05^{a}	0.64 ± 0.03^{b}	0.53 ± 0.03^{b}	2.82 ± 0.12^{c}	2.14 ± 0.09^{d}	2.29 ± 0.18^{d}	2.35 ± 0.15^{d}
Perinephric	0.21 ± 0.02^{a}	0.17 ± 0.02^{a,b}	0.06 ± 0.01^{b}	0.84 ± 0.04^{c}	0.56 ± 0.06^{d}	0.54 ± 0.06^{d}	0.53 ± 0.02^{d}
Retroperitoneal	0.62 ± 0.06^{a}	0.54 ± 0.08^{a}	0.12 ± 0.01^{b}	2.67 ± 0.08^{c}	1.93 ± 0.08^{d}	1.89 ± 0.18^{d}	2.55 ± 0.13^{c}
Total fat pads	1.84 ± 0.12^{a}	1.35 ± 0.12^{b}	0.71 ± 0.04^{c}	6.33 ± 0.17^{d}	4.64 ± 0.16^{e}	4.72 ± 0.39^{e}	5.44 ± 0.16^{f}
Glucose (mg/dl)	104.8 ± 2.1^{a}	118.3 ± 3.7^{b}	93.8 ± 2.6^{a}	141.5 ± 5.8^{c}	142.1 ± 5.6^{c}	123.0 ± 5.3^{b}	159.5 ± 7.6^{d}
Insulin (ng/ml)	0.77 ± 0.09^{a}	0.49 ± 0.04^{a}	0.15 ± 0.01^{a}	7.03 ± 0.61^{b}	5.93 ± 0.38^{c}	4.92 ± 0.56^{d}	6.78 ± 0.25^{b,c}
Corticosterone (ng/ml)	139.5 ± 38.0^{a}	319.0 ± 29.8^{b}		226.6 ± 39.3^{a,b}	279.5 ± 48.4^{b}		166.6 ± 2.1^{a,b}
Total cholesterol (mg/dl)	78.3 ± 3.1^{a,d}	74.2 ± 1.8^{a,d}	59.5 ± 2.0^{a}	146.8 ± 14.1^{b}	99.1 ± 5.4^{c,e}	82.0 ± 2.7^{c,d}	106.8 ± 11.7^{e}
Triglycerides (mg/dl)	44.3 ± 2.2^{a,d}	37.3 ± 1.5^{a}	31.8 ± 1.4^{a,d}	480.0 ± 101.2^{b}	332.6 ± 51.9^{c}	170.5 ± 27.0^{d}	379.0 ± 61.9^{b,c}
FFA (mEq/liter)	0.69 ± 0.04^{a,c}	0.53 ± 0.05^{a}	0.72 ± 0.05^{b,c}	1.33 ± 0.09^{d}	0.96 ± 0.07^{e}	0.98 ± 0.07^{e}	1.40 ± 0.06^{d}
Leptin (ng/ml)	1.35 ± 0.30^{a}	0.64 ± 0.12^{a}	0.42 ± 0.04^{a}	31.72 ± 0.37^{b}	28.96 ± 0.46^{c}	34.95 ± 1.44^{d}	31.08 ± 0.46^{b}
Leptin to total fat pad ratio	0.21 ± 0.04^{a}	0.22 ± 0.03^{a}	0.32 ± 0.02^{a}	1.06 ± 0.05^{b}	2.08 ± 0.15^{c}	2.65 ± 0.34^{d}	1.52 ± 0.04^{e}

$^{a-f}$ Sharing the same letter denotes similarity between groups ($P < 0.05$).

treated groups and in the weight-matched control groups of both phenotypes whereas the total intra-abdominal fat pad mass of the lean and obese weight-matched controls was similar to that of the lean and obese DHEA-treated rats, respectively. The group of obese pair-fed rats had a total intra-abdominal fat pad mass that was smaller than that of the obese age-matched controls, but larger than that of both the DHEA-treated rats and the weight-matched controls. With only a few exceptions, the same patterns were evident when examining the individual epididymal, perinephric, and retroperitoneal intra-abdominal fat pads. The intra-abdominal fat pad weights expressed as a percentage of total body weight also exhibited similar relationships as the absolute fat pad weights.

Glucose, Insulin, and Corticosterone. In the lean rats, serum glucose was increased with administration of DHEA compared with the age-matched controls. The obese weight-matched controls had lower serum concentrations of glucose than did any other group of obese rats. The obese pair-fed group had a higher glucose level than any of the other obese groups. All of the lean groups had lower glucose levels than their obese counterparts.

Insulin levels were markedly elevated in all of the obese groups compared with their lean counterparts. DHEA treatment reduced insulin levels in the obese rats, but not in the lean rats. Pair feeding the obese rats did not change the insulin levels compared with either the age-matched controls or the DHEA-treated obese rats. The obese weight-matched controls had lower insulin levels than the other obese groups. Among the lean rats, the insulin level was unchanged in either the DHEA-treated or weight-matched controls.

Corticosterone levels were increased by DHEA administration to the lean rats. The obese rats showed no effect from DHEA.

Lipids and FFA. Total cholesterol and triglycerides were lower in each lean group than in the corresponding obese group except for triglycerides that were nearly different ($P < 0.06$) in the weight-matched control groups. The obese weight-matched controls had lower levels of total cholesterol and triglycerides than their respective age-matched controls. DHEA treatment in the obese rats reduced the total cholesterol and triglyceride levels compared with their age-matched controls. In the lean rats, DHEA treatment had no effect on either triglycerides or total cholesterol. Pair-feeding the obese rats lowered total cholesterol but not triglycerides, when compared with the obese age-matched controls.

Compared with the lean age-matched controls, FFA levels were unaffected by DHEA administration to lean rats and unchanged in the lean weight-matched controls. The FFA levels of each lean group were less than those of their obese counterparts. Among the obese rats, FFA levels were lowered similarly in both the DHEA-treated and weight-matched control groups compared with both the age-matched controls and the pair-fed groups that also exhibited similar levels.

Leptin. Leptin levels were markedly increased in all obese groups compared with any lean group whereas the lean groups all had similar leptin levels. Among the obese rats, DHEA treatment lowered leptin levels compared with those of the obese age-matched controls. The obese weight-matched controls had higher leptin levels than any other group. The pair-fed group had leptin levels that were similar to those of the age-matched controls.

Discussion

The obese Zucker rat is a well-known animal model of hyperphagia, obesity, and hyperlipidemia (5, 6). The adre-

nal hormone DHEA has a beneficial impact upon each of these derangements (9, 20–25). As expected, the obese rats given DHEA gained less weight and had smaller intra-abdominal fat pads than the obese age-matched controls, whether expressed in grams or as a percentage of total body weight, but DHEA administration resulted in less caloric intake, and this would be expected to result in less body weight and smaller fat pads. However, the obese DHEA-treated group weighed less than the obese pair-fed group indicating that DHEA may have more of an effect on body weight than simply reducing caloric intake (20–24). This effect of DHEA on body weight necessitated the inclusion of additional groups of weight-matched controls having similar weights as the DHEA-treated lean and obese rats, respectively. Because the DHEA-treated rats gained less weight than the controls, these weight-matched rats were necessarily younger than the other rats in the study (see Materials and Methods). The body weight and intra-abdominal fat pads were similar in the obese weight-matched control and obese DHEA-treated groups indicating that their body compositions were grossly comparable, whereas the lean DHEA-treated group exhibited similar body weights as the lean weight-matched controls, but with greater total fat pad weights. With less body weight and smaller fat pads, one would expect the DHEA-treated and weight-matched control rats to have reduced leptin levels since leptin may be a sensor for fat mass (28, 29). The trend in lean rats was toward a reduction in leptin with lower body weights and fat pads, but the leptin levels were not statistically different. The obese DHEA-treated, pair-fed, and weight-matched control groups all demonstrated marked reductions in body and fat pad weights, but either no or small and variable changes in leptin levels.

DHEA given chronically to lean or obese Zucker rats results in decreased body weights, intra-abdominal fat pad weights, and insulin concentrations (30). The investigation being reported here also showed decreased body weights and intra-abdominal fat pad weights in lean and obese Zucker rats, along with decreased insulin levels in obese Zucker rats. In the DHEA-treated obese rats, the reduction in fat pad weight may be the main reason that leptin levels fell, but the reduction in insulin may also have contributed because insulin can stimulate leptin production in normal rodents (3, 26). However, glucocorticoids can increase leptin production (18), and corticosterone, a glucocorticoid, was increased with DHEA administration in the lean rats. The lean weight-matched controls also had reduced fat pads, but only a trend toward lower insulin and leptin.

Another way of analyzing leptin levels is with the ratio of leptin-to-fat pad weight (31). If leptin expression were solely controlled by adiposity, this ratio should be constant. The ratio was similar among the lean groups whereas each obese group had a different ratio. In fact, a possible age-effect was demonstrated by the fact that the obese weight-matched controls, though younger than the age-matched controls, had lower body weights and less intra-abdominal fat, but greater leptin-to-total fat pad weights. DHEA-treatment and pair-feeding the obese rats also decreased body weights and intra-abdominal fat pad weights while increasing the ratio to intermediate values compared with the age-matched controls. The data presented here do not allow the determination of whether DHEA exerted an independent effect on leptin levels or an indirect effect by leading to lower body weights. However, in contrast to the lean rats, the obese DHEA-treated rats had reduced fat pad weights but with only slightly reduced leptin levels and no change in corticosterone or insulin levels. The unexpectedly small change in leptin levels exhibited by the obese rats associated with marked reductions in intra-abdominal body fat could be due to the impaired leptin receptor because leptin receptors are apparently required for the downregulation of leptin expression (26, 27), but could DHEA also play a role? By controlling for the decreased caloric intake that results from DHEA administration, the obese pair-fed group showed that less food intake could explain some of the reductions in body weight and intra-abdominal fat pad weights of the DHEA-treated obese rats, but the pair-fed rats had no change, compared with age-matched controls, in leptin, triglycerides, insulin, or corticosterone despite their reductions in body and fat pad weights due to caloric restriction, whereas DHEA-treatment reduced leptin, triglycerides, total cholesterol, and insulin, compared with the same age-matched controls. It is interesting that food restriction alone does not prevent the development of obesity in obese Zucker rats (11) and that the pair-fed group weighed more than the DHEA-treated group and had larger fat pads. These results support the view of some researchers that DHEA exerts an independent effect on body weight and fat pad weights beyond that of a reduced caloric intake (20, 24, 32). To help control for this observation, the obese weight-matched controls were added to the study and showed no differences in intra-abdominal fat pad weights compared with the obese DHEA-treated rats, but insulin and triglyceride levels were higher. It is further interesting that these weight-matched obese controls actually had higher leptin levels than any other group and that these higher leptin levels occurred in rats with lower body weight, fat pad weights, and insulin levels than the obese age-matched controls. However, it must be emphasized that the lean and obese weight-matched groups were necessarily younger in age and that this age difference could indicate different levels of sexual maturity that could alter hormone levels.

The purpose of this study was to investigate the effect of DHEA on leptin levels. In the lean Zucker rats, DHEA administration did not exert any significant changes in leptin or insulin levels despite a reduction in caloric intake, body weight, and total intra-abdominal fat pad weight, though the leptin levels did tend to be lower. However, the effects of DHEA in obese Zucker rats were more complicated. Chronic DHEA administration to obese Zucker rats reduced total caloric intake, body weight, and total intra-abdominal fat pad weight to a greater degree than would be

expected from the DHEA-associated reduction in caloric intake based on the pair-fed group, implying an independent effect on body weight and fat pad weights. Despite this very marked reduction in body weight and total fat pad weight with both DHEA treatment and pair-feeding, the leptin levels did not drop to any great degree. Why this is so is not clear at this time. Although leptin is produced by adipocytes and is a marker of adipose tissue mass (28, 29), this investigation shows that serum leptin levels do not depend solely upon adipose tissue mass and insulin levels in the obese Zucker rat. Furthermore, if duplicated in other studies, the observation that the younger, weight-matched obese rats had higher leptin levels than the obese controls, despite less body weight and total intra-abdominal fat pad weight, may indicate an age and/or maturity relationship to leptin levels in obese Zucker rats.

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