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Chronic Administration of Dehydroepiandrosterone Reduces Pancreatic β-Cell Hyperplasia and Hyperinsulinemia in Genetically Obese Zucker Rats (42158)

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Abstract. The Zucker obese (*fa/fa*) rat is a model of hypertrophic/hyperplastic obesity. These rats develop marked hyperinsulinemia, insulin resistance, and pancreatic β-cell hyperplasia. In the present study, chronic (22 weeks) administration of the 17-ketosteroid, dehydroepiandrosterone (DHEA), to obese Zucker rats significantly decreased body weight, and retroperitoneal and parametrial fat pad weights. In addition, β-cell hyperplasia was reduced as well as pancreatic insulin content. DHEA treatment of lean Zucker rats also reduced body weight, fat depot weight, pancreatic islet diameter, and pancreatic insulin content. These data indicate that DHEA treatment appears to inhibit insulin synthesis and β-cell proliferation. Whether this is due to a direct effect on the pancreas or due to improvement of peripheral insulin sensitivity remains to be elucidated.

The obese Zucker (*fa/fa*) rat is considered a good model of early onset hypertrophic-hyperplastic obesity (1). This obesity is associated with hyperlipidemia and hyperphagia. In addition, the obese rats are hyperinsulinemic from an early age (2) and eventually develop insulin resistance of peripheral tissues (3–7). Examination of the pancreata of obese rats has shown an increased number of pancreatic β cells in comparison with pancreata of lean rats and increased insulin production (8). There are also many more enlarged islets in obese rats than in age-matched lean rats (9). These large islets appear to be responsible for the increased insulin secretion found in the obese rats (9).

Administration of the 17-ketosteroid, dehydroepiandrosterone (DHEA), has been shown to decrease the rate of weight gain of several strains of normal and genetically obese rodents (10–14). Recently, chronic DHEA treatment was found to markedly diminish the degree of hyperinsulinemia usually observed in Zucker obese rats (14, 15). This may be due to decreased body fat leading to an improvement in insulin sensitivity or to decreased sensitivity of β cells to glucose or other substances which signal insulin release and islet growth. The purpose of the present study was to determine whether chronic DHEA treatment could prevent the histopathological and metabolic abnormalities usually seen in pancreata of Zucker obese rats. In addition, since an earlier study had shown that the decreased body weight in obese DHEA-treated Zucker rats was accompanied by a slight decrease in food intake (16), a pair-fed group of obese rats was included here since changes in food intake are known to affect insulin metabolism (17).

Materials and Methods. *Animals.* Lean (*Fa/?*) and obese (*fa/fa*) female Zucker rats were purchased from Vassar College, Poughkeepsie, New York. Powdered Purina Rodent Chow (No. 5001) was provided *ad libitum* to the lean and obese control rats with the exception of those in the pair-fed obese group. The pair-fed obese rats received a daily ration of food equal to that consumed by the DHEA-obese group as a previous study had found a 20% decrease in food intake in obese rats treated with DHEA (16). Beginning at 6 weeks of age the DHEA-lean and DHEA-obese groups received DHEA (Searle Chemicals, Inc., Chicago, Ill.) in their food (0.6% W/W) as previously described (13). The two lean groups had eight rats per group and each of the three obese groups contained four rats. Food intake was recorded daily and body weights weekly. Water was provided *ad libitum* and a 12-hr light/12-hr dark cycle was maintained. Rats were fed in the afternoon shortly before the initiation of the dark cycle.

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Tissue preparation and analysis. After 22 weeks of treatment rats were killed by decapitation between 8:30 and 10:30 AM, following an overnight fast. Weights of parametrial and retroperitoneal fat pads were recorded. Blood samples were collected and serum separated for glucose determination (18), using a Beckman glucose analyzer, and for an insulin radioimmunoassay (19).

Pancreata from two to three rats per group were promptly fixed in Bouin's solution and oriented longitudinally in a paraffin block such that all sections contained portions of pancreatic tail, body, and head. Three sections were stained with hematoxylin and eosin, and one was stained by Scott's aldehyde fuchsin technique (20). Slides were labeled with a numerical code so that we remained unaware of their source until after all data had been compiled. The sections were examined microscopically, taking note of islet shape, cellular composition, inflammation or fibrosis, and cellular granularity.

Using an ocular micrometer, diameters of at least 50 islets from each pancreas were measured. At least two levels from each block were examined quantitatively and only those sections which included portions of head, body, and tail of the pancreas were used. When elliptically shaped islets were encountered, an average of the major and minor diameters was recorded. Since the smallest islets seen in histologic sections may represent sections through the periphery of islets, and since these islets comprise a small proportion of total islet cell mass (21), we recorded data only for those islets exceeding 100 μm in diameter. Median and mean islet diameters, as well as the size distribution of islets, were calculated. It should be noted that this method measures the diameters of random cross sections of islets and produces values lower than methods using squash preparations of pancreatic tissue, which measure the area of each islet at its maximum diameter. Nonetheless, the method we have used produces an index of islet size which is highly correlated with total β-cell mass (22). Also, this method permits simultaneous qualitative microscopic examination of the same tissue, and has been used in a previous study using Zucker rats (8).

Pancreatic insulin was determined as described by Turkenkopf *et al.* (23) on pancreata from an additional series of rats treated in an identical manner as described above. Each group contained six rats. Body and fat pad weights were not significantly different and within 10% of those for the rats presented in this study in Figs. 1A and B. Minced pancreatic tissue was homogenized in a solution of 0.09 *N* HCl in 75% EtOH. Insulin from the acid/ethanol extract was precipitated with ethanol/ether (17/28) and quantitated by radioimmunoassay (19).

Statistics. All data are expressed as the means ± standard error of the mean (SEM). Means from experimental and control groups were compared using a Student's *t* test.

Results. As previously described (14, 16), DHEA treatment in lean and obese Zucker rats resulted in decreased body weight compared to nontreated control rats (Fig. 1A). In addition, the DHEA-obese rats weighed significantly less than the pair-fed obese rats. In the two groups of lean rats there was no difference in cumulative food intake (control-lean, 2314 ± 66 g versus DHEA-lean, 2377 ± 70 g). Although the DHEA-obese rats consumed 16% fewer grams of food than the control-obese rats over the experimental period, this difference was not significant (con-

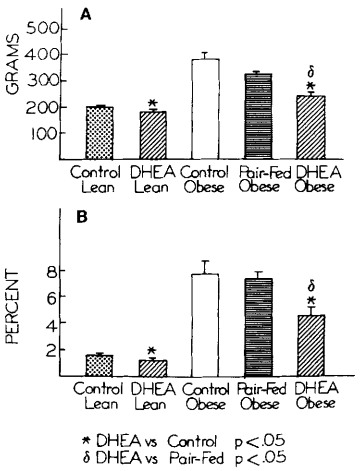

FIG. 1. (A) Body weight in lean and obese Zucker female rats following dehydroepiandrosterone treatment. (B) Combined fat pad weights (left and right parametrial and retroperitoneal pads) contribution to total body weight (fat pad weights/body weight × 100). Data are means ± SEM.

trol-obese, 3262 ± 197 g versus DHEA-obese, 2745 ± 217 g). There was no difference in food intake between the DHEA-obese (2745 ± 217 g) and the pair-fed obese rats (2539 ± 30). These findings demonstrated that the primary effect of DHEA on decreasing body weights is independent of a decrease in food intake.

There was a 40% decrease in the weight that combined (right and left retroperitoneal and parametrial) fat pads contributed to total body weight in the DHEA-obese rats compared to both the control-obese and the pair-fed obese groups (Fig. 1B). In the DHEA-lean group there was a 25% decrease in combined fat pad weight as a percentage of total body weight compared to the control-lean group (Fig. 1B).

Serum insulin and glucose levels were unaffected by DHEA treatment in lean rats (Table I). In the obese rats, DHEA treatment decreased both serum insulin levels (80%; $P < 0.05$) and glucose levels (23%; $0.1 > P > 0.05$) in comparison to the control-obese rats. The pair-fed obese rats had lowered serum insulin levels compared to the control-obese group but significantly higher levels than that found for the DHEA-obese group. There was no effect of pair feeding on serum glucose.

Pancreatic insulin content was found to be lower in both DHEA-treated groups when compared to their respective control groups (Table I). There was no effect of pair-feeding on the amount of insulin in the pancreata when compared to the control-obese rats. Both the median and mean pancreatic islet diameters were determined (Table I). Values for the control-obese and the pair-fed obese rats were similar and were higher than those for the remaining three groups.

Microscopic examination of pancreatic sections from lean rats stained with hemotoxylin and eosin revealed islets which were either round or oval in shape. No inflammation or fibrosis of the islets or surrounding exocrine pancreas was noted. Pancreatic sections from lean rats stained by Scott's aldehyde fuchsin method showed islets comprised of well-granulated β cells in a typical trabecular pattern, and aldehyde fuchsin-negative, peripherally located, α cells. Small and medium-sized islets from obese rats were identical in appearance to those of lean rats. However, some of the larger (diameter >400 μm) islets of the obese-control rats showed irregular, angulated contours, disorganization of the trabecular pattern of islet cells, and depletion of β-cell granules (Fig. 2A). These pathologic changes were also seen in the pair-

TABLE I. EFFECT OF DEHYDROEPIANDROSTERONE[a] ON SERUM GLUCOSE, SERUM INSULIN, PANCREATIC INSULIN, AND PANCREATIC ISLET DIAMETERS (x ± SEM)

	Serum glucose (mg/dl)	Serum insulin (ng/ml)	Pancreatic insulin (ng/pancreas)	Median islet diameter (μm)	Mean islet diameter (μm)
Control-lean	101.9 ± 4.5 (8)[b]	0.54 ± 0.06 (8)	324.6 ± 73.0 (3)	161.5 ± 8.5 (2)	180.2 ± 7.1 (2)
DHEA-lean	103.3 ± 6.1 (7)	0.52 ± 0.06 (7)	210.0 ± 38.2** (3)	136.0 ± 17.0 (2)	167.3 ± 15.8 (2)
Control-obese	123.8 ± 10.8 (4)	9.80 ± 1.90 (4)	874.4 ± 127.6 (3)	187.0 ± 9.8 (3)	209.6 ± 11.0 (3)
Pair-fed obese[c]	121.3 ± 8.2 (4)	4.30 ± 1.10** (4)	856.0 ± 269.0 (3)	187.0 ± 9.8 (3)	211.1 ± 12.8 (3)
DHEA-obese	95.3 ± 12.8* (3)	1.95 ± 0.05** (3)	556.6 ± 127.4* (3)	164.3 ± 5.7* (3)	181.4 ± 1.3** (3)

[a] DHEA included in the diet at a level of 0.6%.
[b] Number in parentheses is the number of rats used.
[c] Pair-fed to DHEA-obese rats.
* $P < 0.1$; ** $P < 0.05$. Compared to either control-lean (if DHEA-lean) or control-obese (if DHEA-obese or pair-fed obese).

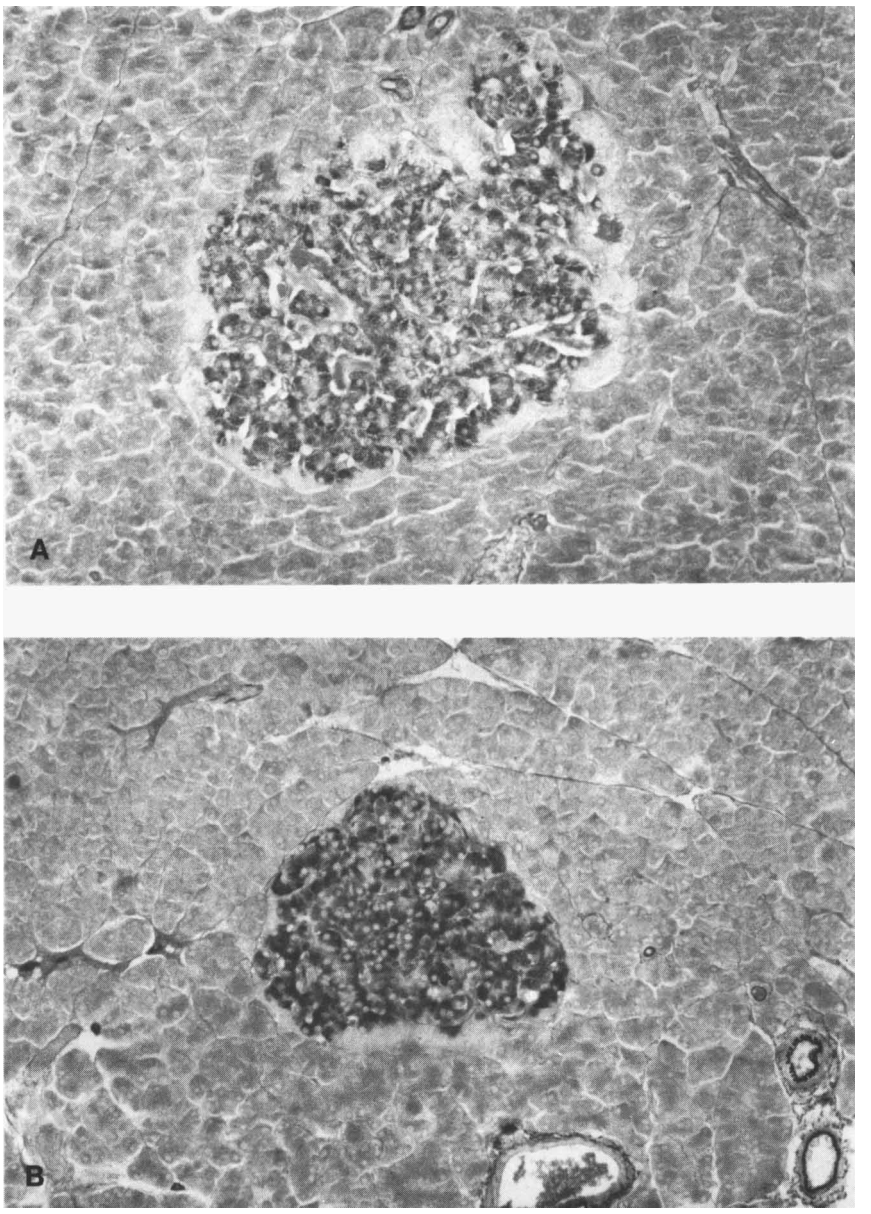

FIG. 2. (A) Typical pancreatic islet from a control-obese rat, illustrating β-cell hyperplasia and hypogranularity. Islets from restricted-obese rats were identical in appearance. (B) Typical islet from a DHEA-obese rat. Note reduced islet diameter and more prominent staining of granules than in (A). (Scott's aldehyde fuchsin, original magnification 200×.)

fed obese rats (not shown) but were less prominent in the obese rats treated with DHEA (Fig. 2B). A summary of the pancreatic morphologic findings is presented in Table II.

Pancreatic sections from all rats showed a predominance of small islets. However, since large and medium-sized islets comprise most of the total islet volume (21), subsequent calculations included only those islets with

TABLE II. SUMMARY OF PANCREATIC MORPHOLOGY IN LEAN AND OBESE ZUCKER RATS

	Islet hypertrophy	Islet contour	Trabecular iselt architecture	Islet inflammation	β-Cell degranulation
Control-lean	Absent	Smooth	Intact	Absent	Absent
DHEA-lean[a]	Absent	Smooth	Intact	Absent	Absent
Control-obese	Severe	Irregular	Frequently distorted	Absent	Moderate
Pair-fed obese[b]	Severe	Irregular	Frequently distorted	Absent	Moderate
DHEA-obese[a]	Absent	Occasionally irregular	Occasionally distorted	Absent	Mild

[a] DHEA included in the diet at a level of 0.6% from 6 to 29 weeks of age.
[b] Pair-fed obese rats fed same amount of food as DHEA-obese rats from 7 to 29 weeks of age.

diameters of at least 100 μm. The islet diameter histograms in Fig. 3 illustrate the differences between cross-sectional islet diameters among the various groups of rats

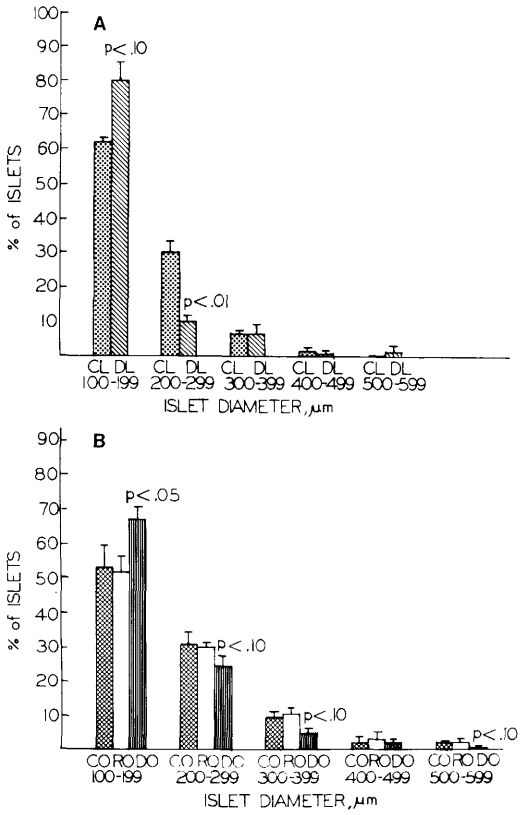

FIG. 3. Islet diameter distribution in lean (A) and obese (B) Zucker rats following dehydroepiandrosterone treatment. See Materials and Methods for description of methodology. CL = control-lean, DL = DHEA-lean, CO = control-obese, PFO = pair-fed obese, DO = DHEA-obese. Data are means ± SEM.

described above. Compared with their lean counterparts, the obese rats have fewer small islets (<200 μm) and more large islets (>300 μm). Caloric restriction had no detectable effect on the islet size distribution of obese rats, while DHEA administration resulted in a significant shift in the size distribution of both lean and obese rats toward an increased proportion of smaller islets. Mean and median islet diameters were decreased in both treated groups but was significant only in the DHEA-obese rats with this group having values similar to those of the control-lean rats.

Discussion. Obese Zucker rats invariably develop severe peripheral insulin resistance (4–7, 24, 25). It has been hypothesized that β-cell hyperplasia and hyperinsulinemia might be a compensatory reaction to peripheral insulin resistance (7). However, it has been shown that adipocytes and hepatocytes from young hyperinsulinemic obese rats are insulin sensitive (2, 3). Increased insulin secretion has been associated with large islets in the obese rats (9). These and other observations suggest that a defect in β-cell metabolism may contribute to the development of obesity in the Zucker rat.

In the present study we have confirmed earlier observations (14, 16) that DHEA treatment reduces body weight and fat depot weights of both lean and obese Zucker rats. Although pair-feeding decreased body weight and serum insulin concentration in obese rats, DHEA treatment decreased these values to a much greater extent relative to control, *ad libitum*-fed obese rats. These results support our earlier hypothesis that DHEA's effects on body weight are due to alterations

in energy metabolism and not simply to a decrease in food intake (16).

Both lean and obese DHEA-treated rats had reductions in pancreatic insulin content compared to their respective nontreated control groups. Pair-feeding had no effect on pancreatic insulin content in the obese rat and did not decrease fasting serum insulin levels to the extent that DHEA treatment did. Only one previous study reported data of pancreatic insulin content in food-restricted obese Zucker rats (26). Following either 142 or 193 days of nondefined food restriction, excessive insulin was still present in the pancreata of obese rats.

DHEA treatment was further characterized in both lean and obese rats by a reduced degree of β-cell hyperplasia as judged by the islet diameter distribution, and the mean and median values of islet diameter. The islet size histogram (Fig. 3) illustrates the DHEA-associated shift toward a greater proportion of small islets in both lean and obese rats. Both mean and median values for islet diameter were lowered by DHEA treatment but were significant only in the obese rats. The mean values for all groups were higher than the median values, due to the fact that the median is less influenced by the small number of very large islets. Presentation of morphometric data as islet size distribution rather than as mean or median values appears to allow more sensitive detection of islet hyperplasia. In general, the response to DHEA treatment has been greater in obese than lean rats for a number of parameters determined such as body weight, fat pad weight, insulin, and numerous enzymes (14–16). The present results on changes in the pancreas would thus be in agreement.

Some large islets from control and pair-fed obese rats showed irregular borders, focal loss of the usual trabecular pattern, and depletion of β-cell granules. These pathologic changes have previously been noted in obese Zucker rats (8) and other insulin-resistant rodents (27, 28) and were reduced by DHEA treatment. Histologic examination revealed no evidence of DHEA-associated islet inflammation or hyalinization. Thus, DHEA treatment appeared to reduce serum insulin levels by altering insulin homeostasis and not via a toxic destruction of insulin-producing cells. This appeared to be a specific effect for insulin as there was no significant effect on serum glucagon levels by DHEA treatment in lean or obese rats (data not presented).

Our findings are similar to those of Coleman *et al.* (29, 30). They have found that DHEA treatment of (*db/db*) C57BL/6J mice resulted in decreased body weight, decreased plasma insulin levels, reduced islet area, and increased β-cell granularity. Like the Zucker rat, these mice develop a syndrome of obesity and insulin resistance without severe diabetes. In contrast, (*db/db*) C57BL/KsJ mice usually develop severe life-shortening diabetes. Treatment of these mice with DHEA prevented islet atrophy and severe diabetes, and they instead developed a syndrome of well-compensated mild diabetes with obesity and insulin resistance (30). Thus, DHEA treatment reduces β-cell hyperplasia and hyperinsulinemia in Zucker rats and (*db/db*) C57BL/6J mice, and prevents islet exhaustion and atrophy in (*db/db*) C57BL/KsJ mice.

Precisely how the effect of DHEA on the pancreas is mediated is unknown. One possible explanation is through DHEA's known ability to inhibit glucose-6-phosphate dehydrogenase thus decreasing activity of the pentose phosphate pathway and availability of NADPH (32–34). It has been documented that pancreatic insulin secretion and synthesis are dependent on NADPH and/or the $NADPH/NADP^+$ ratio (35, 36) and it has been reported that the exposure of cultured mouse pancreatic islets to DHEA resulted in a dose-related inhibition of insulin release (31). The relevance of these mechanisms to our *in vivo* observations of Zucker rats remains to be investigated. Alternatively, DHEA could improve the insulin sensitivity of peripheral tissues, resulting in increased glucose uptake or decreased glucose production and thereby inhibiting β-cell metabolism in an indirect manner. The reduction of serum glucose levels in the DHEA-obese rats is consistent with this hypothesis. However, we have previously observed that isolated adipocytes from DHEA-treated obese rats metabolized less glucose into CO_2 and fatty acids than did cells from control-obese rats and therefore did not seem to have improved response to insulin (37). Lean rats, on the other hand, appeared more insulin responsive,

thus providing inconclusive data as to whether changes in peripheral insulin response play a role in the altered insulin metabolism induced by DHEA treatment. Similar studies of other insulin-sensitive tissues are in progress. We cannot completely exclude the possibility that the decreased body weight of the obese rats is partially responsible for the effect of DHEA on the pancreas. However, effects on the pancreas are found in *db/db* mice treated with DHEA that had no alterations in body weight (29, 30) and in both lean and obese Zucker rats. Although the available data suggest that alterations in pancreatic structure and function are important in DHEA's anti-obesity action, the precise mechanism of DHEA's effect on the pancreas remains to be elucidated.

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