DHEA-PC Slows the Progression of Type 2 Diabetes (NIDM) in the ZDF/Gmi-fa/fa Rat
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Abstract:

The etiology of NIDDM is complex and development is manifested by initial insulin resistance coupled with elevated insulin levels in the early diabetic state with concomitant increases in circulating levels of glucose and triglycerides. This is followed by a decline in insulin levels due to pancreatic exhaustion. Our results show that administration of DHEA-PC, a conjugate of dehydroepiandrosterone (DHEA), delayed the development of NIDDM symptoms and the onset of type 2 diabetes in the ZDF/Gmi-fa/fa rat model. The treatment consisted of weekly implantation of subdermal osmotic infusion pumps in the rats starting at 6 weeks of age (n = 5 animals per group). For the first three weeks the pumps delivered 6 mg/day/rat followed by 12 mg/day/rat for 1 week (control group pumps delivered only carrier vehicle) after which the pumps were removed. Plasma was collected weekly from day 0 through day 58 and glucose, triglycerides, cholesterol, insulin, IGF-1 and IGF-BP3 levels were measured. Data was analyzed by two way ANOVA.

Following 3 weeks of treatment with DHEA-PC, plasma glucose levels in the treated group remained low, 150 +/- 9 mg/dl while the level in the control animals steadily increased to 320 +/- 100 mg/dl (p < 0.05). After the DHEA-PC treatment ended plasma glucose plateaued for 10 days and then took 25 days to reach the level in the control animals (p < 0.05). After 2 weeks of DHEA-PC treatment, plasma triglyceride levels in the treated group remained low, 85 +/- 90 mg/dl while the level in the control rats increased increased to 180 +/- 35 mg/dl (p < 0.05). After the treatment was terminated triglyceride levels in the treated group increased to control levels within two days. Insulin, IGF-1,IGF-1-BP3, cholesterol, body weight and food consumption were not changed by DHEA-PC treatment (p < 0.05). Therefore the delay of increases in plasma glucose and triglycerides, caused by DHEA-PC, was not the result of differences in caloric intake, increased insulin, or increased IGF-1 levels.

The data suggest that DHEA-PC delayed the onset of the two most important parameters of NIDDM, namely hyperglycemia and hypertriglyceridemia.

(ZDF/gmi-fa/fa rats and their care supplied by contract with Genetic Models Inc., Indianapolis, IN).

INTRODUCTION

The etiology of Non Insulin Dependent Diabetes Mellitus (NIDDM) is complex, even though it is the most common form of diabetes. Most people and rodents predisposed to developing NIDDM encounter initial insulin resistance in the early diabetic state. The consequences of advancing NIDDM is the appearance of hyperglycemia and hypertriglyceridemia in circulation. The obese male Zucker diabetic fatty strain of ZDF/Gmi-fa/fa rat are models of type 2 diabetes (1,2). They become hyperglycemic and hypertriglyceridemic as diabetes advances between 7-12 weeks of age and then plateau (3-5). Their diets consist of
high fat (6%) content food (1,5) and their level of physical activity is low. They therefore may adequately reciprocate their human diabetic counterpart. Thus, because of their severe diabetic state of hyperglycemia which is greater than other NIDDM models (2), the ZDF/Gmi-fa/fa rat may be appropriate for addressing the pathophysiology of human NIDDM. The ZDF rat like the human NIDDM condition becomes increasingly unresponsive to insulin secretion as the condition advances (2).

In the hierarchy of circulating hormones critical to the up- or down-regulation of glucose there are redundancies, down-regulation by insulin is countered by up-regulation of glucagon, epinephrine, growth hormone, IGFs and glucocorticoids. Other factors including adrenal androgens are becoming recognized as regulators of circulating glucose. dehydroepiandrosterone (DHEA) and its 3-sulfate ester (DHEAS) are both implicated in down-regulation of glucose. Furthermore, DHEA has a protective effect against development of insulin resistance in muscle of rats on high fat diets or accumulated visceral fat (6,7). In men free DHEAS or testosterone (T) were inversely correlated with glucose and insulin concentrations (8), but DHEA in men did not alter insulin sensitivity (9). In hyperandrogenic women insulin sensitivity seems to depend more on the ratio of DHEA to T (10). The inverse relationship of DHEA or DHEAS to insulin under physiological conditions occurs over a broad age range in men (30-80 yr.) (11). DHEA and DHEAS have been implicated in lipid metabolism. Pharmacological doses administered to animals or in cell culture have reduced the size of fat cells (12,13) and impaired weight gain or lipogenesis (13). Furthermore, DHEA and DHEAS increased protein content of the body mass (14). In adipocytes they inhibited differentiation from fibroblasts (15). DHEA has a protective effect against accumulation of visceral fat and development of muscle insulin resistance in rats fed a high fat diet (16); furthermore, DHEA does not alter food intake. Feeding DHEA to Zucker rats shows reduced hyperphagia and concomitant hypothalamic reduction (17). This dichotomy may indicate that obese animals eat less or that taste aversion may be a factor.

DHEA-PC (androst-5-ene-3-phosphocholine-17-one) is a congener of DHEA whose identification and synthesis we have recently described (18). Also its biactivity has been demonstrated in its immune stimulation of Balb C mice sensitized concomitantly with dinitrochlorobenzene (19). This effect was not suppressed by the potent glucocorticoid, dexamethasone.

The aim of this study was to investigate the effects of this newly described hormone, DHEA-PC, on the most significant indicators of Type 2 diabetes in an animal model known for its hyperglycemia and hypertriglyceridemia, namely the ZDF/Gmi-fa/fa obese rat.

MATERIAL and METHODS

Synthesis: Dehydroepiandrosterone was reacted with chlorophosphoketal to yield the phosphoketal ester of DHEA as per patent # 5,703,063. Treatment with hypochlorite yields the pentavalent phophodioxolane ester. This was then reacted with trimethylamine in pyridine to yield the desired phosphocholine ester of DHEA.
Animals: Spontaneously diabetic obese male ZDF/Gmi-fa/fa rats from Genetic Models Inc (Indianapolis, IN) were started out on this study at 6 weeks of age, 80 +/- 8 g and just weaned. They were housed in individual ages and randomly assigned to treatment groups and maintained on Purina Mills lab diet 5008 and water ad-lib, with a 12 hr light-dark cycle at 25 C. Blood was collected by tail incision on days 0, 3 and at least weekly throughout the study. Food consumption and body weight were determined weekly through out the study. Animal care was given under IACUC oversight and guidelines at the Genetic Models Inc facilities (Indianapolis, IN).

Treatment: The animals were individually surgically implanted with (2ML1) Alzet osmotic mini-pumps (Alza corp., Palo Alto, CA) delivering 10 µl/h. Animals were assigned to two groups (n = 5 each) with one receiving pumps filled with 25 mg/ml of DHEA-PC calibrated to deliver a dose of 6 mg per day or when two pumps were implanted 12 mg/day, while controls were implanted with pumps only containing water. The pumps were replaced weekly. Anesthesia during the surgical procedure consisted of inhalation isoflurane; USP (Solvay Animal Health Inc.) delivered from the 2-5 minute procedure by an inhalation vaporization system, Isotec III (Matrix Medical Inc., Orchard Park, NY). Animals were allowed to rest for 2 days post surgery before any blood was collected from the day. During this 54 day study, rats were first treated for 3 weeks at the lower dose of 6 mg/cay, then s the animals became more obese by week 4 they received 12 mg/day, On day 28, treatment was stopped, the pumps were removed, and the animals followed until the end of the study period. The controls were treated in exactly the same way except that the pumps contained no drug.

Oral glucose tolerance test
A 60% glucose solution at 3 g/kg was administered in the morning by gavage. Blood samples (100 µl) were taken at 0, 30, 60, and 120 minutes. The samples were analyzed for glucose as described below.

Assays: Plasma glucose, triglycerides, and cholesterol were measured enzymatically using the SychronCX systems, Pechman Instruments, Inc. (Brea, CA) (20-23). RIA of plasma insulin (24) used reagents from Linco Research Inc (St. Louis, MO). the RIAs for IGF-1 (24) and plasma IGF-BP3 (26) used reagents from diagnostic Systems lab (Webster TX). Each assay was done on unextracted plasma and used 125I tracer. The within and between assay percent coefficient of variation were respectively: insulin, 4% and 9%; IGF-1, 5% and 20%; IGF-BP3, 3.2% and 11.5%.

Statistical Analysis: The data are preseted as mean +/- SEM and statistical analysis were performed using two way ANOVA. post-hoc tests comparing individual differences between means used Scheffe F statistic.

RESULTS:

Plasma glucose suppression by DHEA-PC
We hypothesized that ZDF-Gmi-fa/fu obese type 2 diabetic rats when started on DHEA-PC treatment when immature (6 weeks old) would have the progression of the diabetic trait blocked. Disease symptoms begin to emerge in
this strain at about 6 weeks and progress rapidly to maturity at about 10 weeks. While in our control groups the symptoms of NIDDM began to develop rapidly, our DHEA-PC treated animals were spared from developing hyperglycemia (Fig 1). Clearly DHEA-PC suppressed plasma glucose levels by 100 mg/dl or 50% for 16 days from day 23 to day 39 compared to the untreated control animals. Even after the treatment was terminated and the pumps removed, glucose remained low for an additional 10 days. However, by day 58 of the study, 4 weeks post treatment, plasma glucose in the treated animals had risen to the level in the control animals. Plasma glucose in the control animals was not static but continued to increase from 141 +/ - 2 to 475 +/ - 21 mg/dl. Treatment with DHEA-PC at 6 mg/dl failed to alter the rise in plasma glucose which only responded by day 23 when the dose was doubled to 12 mg/day when a significant decrease was observed. It was not clear whether the ultimate suppression was a result of the increased dose or a cumulative response to the drug over time.

Plasma triglycerides suppression by DHEA-PC:

Plasma triglycerides were significantly (p < 0.05) suppressed during DHEA-PC treatment by 100 mg/dl, which is 50% below the level in the control animals (Figure 2). This response occurred on day 16 of the treatment (6 mg/day) while the animals were still on the lower dose of the drug, showing that triglycerides were more responsive to the treatment than glucose levels. By day 9 into the treatment triglycerides were still at the baseline level, but two weeks later on day 223 they had decreased below the baseline level. This nadir on day 23 of 83 +/ - 7 mg/dl in plasma triglycerides occurred during the week when the dose was doubled and the exposure to the drug was longest. Immediately after the treatment ended and the pumps were removed, plasma triglycerides increased rapidly to those in the control animals and thereafter the triglyceride levels in the two groups of animals increased in parallel for the rest of the study. Contrasting the immediate response in triglycerides when the treatment was terminated with 10 day delay in an increase in plasma glucose, suggests that different physiological mechanisms are involved.

Plasma cholesterol was not altered by the treatment and levels ranged between 95 and 83 mg/dl on day 0 in the control and treatment groups (figure 3). Post treatment cholesterol levels were elevated (p < 0.05) above the baseline levels by 10 days after the pumps were removed.

Oral glucose tolerance: The treated animals showed a consistent decrease in the glucose curve over time (figure 4) (P < 0.05) when the treatment was started before the OGT was carried out.

Body weight was not affected by DHEA-PC treatment (Figure 5) (P < 0.05). The day 0 body weights were 188 +/ - 8 g for the control group and 193 +/ - 8 g for the treated group. By day 58, weights had increased to 437 +/ - 21 and 422 +/ - 19 g respectively. These are normal weight gains for this animal model. Similarly food consumption was the same in the two food groups ranging from 28-30 g/rat. These results make it clear that the responses observed in glucose and triglyceride levels were not the result of increased caloric intake in the controls or
starvation in the treatment group but reflect physiological changes. The other parameters which were measured, insulin, IGF-1, and IGF-BP3 were similarly unaltered (P < 0.05) showing that the changes in glucose and triglyceride levels were the result of cellular events which do not reflect changes in these hormone levels.

**DISCUSSION:**

The results demonstrate that the hyperglycemia, which develops in the NIDDM model of diabetes ZDF/Gmi-fa/fa rat, was suppressed by DHEA-PC treatment. These results also show that hypertriglyceridemia, which develops during the maturation period as this strain of rats grows in size and obesity was also suppressed by DHEA-PC. Because DHEA-PC is a recently synthesized conjugate of DHEA, no literature exists on its physiological properties; however the physiological and pharmacological effects of DHEA and DHEAS are known and comparisons can be made about the activities of these three related molecules. The fact that plasma glucose (Fig 1) and triglyceride (Fig 2) levels increased during maturation beginning at 6-7 weeks in this rat strain is consistent with the observations of other investigators (2,5). The hyperglycemia and hypertriglyceridemia observed in this strain may result from hyperphagia, which is a common trait (12, 27). Cholesterol levels are not a factor in this study since the treated and control animals had comparable levels.

Similarly body weight which doubled in both groups of animals played no role in the observed responses. The increased food intake, although not necessary for obesity does increase the magnitude of the condition (27). Both control and DHEA-PC treated rats consumed about the same amount of food (30 +/- 2 g/day/rat), while the lean non-obese strains are less (27). To eliminate the problem of taste aversion seen when diets containing DHEA were used (27) osmotic pumps were used to administer the drug. The results suggest that the suppression of hyperglycemia and hypertriglyceridemia were the results of the administered DHEA-PC acting by an alternative pathway not involving decreased food consumption. The problem of taste aversion to DHEA, which is also seen with the standard Zucker rat initially is overcome with time and after 4-5 weeks on an 0.04% DHEA diet Zucker rats begin to eat the same amount as the untreated control animals (unpublished observations from this laboratory).

The time frame for suppression of these two parameters was different for the two effects suggesting that different mechanisms are involved. The decrease in hypertriglyceridemia responded more quickly at the lower doses of DHEA-PC, while the decrease in hyperglycemia only occurred after the dose was doubled from 6 to 12 mg/day. Hypertriglyceridemia also declined more quickly at the end of the treatment period rising to control levels in 3 days while the hyperglycemic response did not reappear until 10 days after the treatment ended. The delayed increase is not due to lingering DHEA-PC since we have established that DHEA-PC is cleared within 24 hours (unpublished data from this laboratory). This is comparable to the clearance of DHEA and DHEAS in rats. While a number of alternative hypotheses may be offered to explain these results, no firm mechanism can be advanced at this time. It should be noted that muscle cells develop increased glucose transport under DHEA stimulation (12). Further as
these obese rats develop, there is no drop-off in the number of skeletal muscle GLUT-4 receptors or insulin receptor mRNA which were reported to be 50% higher than in the lean non-diabetic litter mates (28). Despite many studies, the mechanism of insulin resistance in the muscles of genetically obese rats or people is not clearly understood. The difference in polarity of glucose and triglycerides suggest an explanation for the differences in the post-treatment responses. Triglycerides because of their non-polarity and size require no transport system to enter through cell membranes, thus DHEA-PC has no post-treatment effect on their transport even though membrane passage is enhanced while the compound is being given. This same enhancement may account for the earlier suppression of triglyceride levels which was observed.

No differences (p < 0.05) were seen in insulin, IGF-1 and IGF-BP3 levels between the treated and control animals over the course of the study. These observations are surprising due to the magnitude of the hypoglycemia and hypertriglycerideremia and the extent of suppression by DHEA-PC. Plasma glucose and triglycerides were suppressed without any enhanced disappearance of insulin or IGF-1. This result differs from what might have been anticipated from human studies where Nestler (11) showed an inverse relationship between DHEA and DHEAS levels and insulin levels. Adrenal insufficiency is not likely since Svec has shown that the HPA axis of the Zucker rat does not appear to be altered under chronic DHEA treatment or act as an antiglucocorticoid (28).

This study shows that the initiation of DHEA-PC therapy before the onset of frank diabetes in these ZDF rats prevented hyperglycemia. The ZDF rats become obese and insulin resistant but remain pre-diabetic with regard to glucose levels. Thus the ZDF rat is a good model for NIDDM which resembles that in people. The defect in insulin secretion described by Sturtis is another parameter in which the ZDF rat mimics the human results (29). Abnormal disruptions in the periodicity and pulsatility of insulin secretion occur early on in NIDDM development in both humans and the ZDF rat (29, 30).