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REDUCTION IN BODY WEIGHT AND CHOLESTEROL IN SPONTANEOUSLY OBESE DOGS BY DEHYDROEPIANDROSTERONE

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We studied the **effect(s)** of **exogenous dehydroepiandrosterone (DHEA) in spontaneously obese dogs. Nineteen euthyroid obese** and six non-obese normal dogs were evaluated. Dogs received reduction in total body weight/month in 68 percent of the obese dogs without reduction in food intake. The normal dogs did not lose weight or reduce food intake. Serum cholesterol in obese dogs went from 226 to 173 mg/dl post-treatment and in normal dogs from 128 to 89 mg/dl. Analysis of lipoproteins in four normal dogs revealed that the marked reduction in cholesterol most significantly affected the LDL-HDL fraction.

Keywords: body weight **reduction,** cholesterol, **dogs,** dehydroepiandrosterone.

Introduction

While the exact pathogenesis of obesity is unknown, most studies suggest that obesity results from an imbalance between energy intake and energy output, and that the balance can be controlled by neurologic, physiologic, metabolic and hormonal factors'. Most attempts to treat obesity involve a reduction in caloric intake. Centrally acting drugs that suppress food intake are the focus of pharmacologic treatment of obesity, but investigators recently have been studying the effects of peripherally acting drugs that modify the metabolic process. One such agent is dehydroepiandrosterone (DHEA).

DHEA is a 17-ketosteroid of adrenal and gonadal origin. DHEA in its sulfate form **(DHEA-S) is the most abundant** steroid circulating in the plasma of humans. More than any other steroid, plasma concentrations of DHEA-S undergo the most marked age-related decline. In man, serum DHEA-S concentration will peak at around the age of 20, progressively decline with age and become constant at 50-80 years of age &. Although DHEA is the main precursor of placental estrogen and may be converted to active androgens and estrogen in peripheral **tissues,** there is no obvious biological role of either DHEA of DHEA-S in normal animals.

The mechanism of action of the antiobesity effect of DHEA is unclear. It has been postulated **that a** decrease in DHEA and DHEA-S concentrations will result in an increase in lipogenesis because DHEA is **a** known inhibitor of the enzyme glucose&-phosphate dehydrogenase.[8] This enzyme is necessary for the hexosemonophosphate shunt, and hence the synthesis of NADPH, which is required for the reductive **synthesis** of fatty acids[8,9]. Lipid synthesis in rat skin has been shown to be inhibited 44-50 percent by DHEA ***in vitro.*** This effect was reduced by addition of NADP. DHEA administered to mice decreased lipogenesis and, thus, prevented weight gain'.

Alteration of the metabolic rate and thus energy expenditure has been suggested as a possible mechanism of action responsible far the antiobesity activity of DHEA. In a study of the metabolic effects of DHEA in rodents, it was shown that rats treated with DHEA had an elevation in resting metabolic rate, leaner body composition, lower serum triglyceride concentrations and lower activity of hepatic glucose-6-phosphate dehydrogenase[2]. The mechanism by which DHEA increases energy expenditure is that it may increase ATP utilization. This might be a result of cycling activity between the enzymes, fatty acyl-CoA hydroxylase and fatty acyl-CoA synthetase, for fatty acid metabolism.

One of the major risk factors for the obese individual is the increased incidence of cardiovascular disease associated with obesity'. One epidemiological study showed that men that have an increase in DHEA-S concentration have a 36 percent

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reduction in mortality from any cause and a 48 percent reduction in mortality from cardiovascular disease". It has also been demonstrated that decreased urinary excretion of DHEA is associated with higher serum cholesterol levels'[6]. In rats made hypothyroid by propylthiouracil, DHEA lowered serum cholesterol levels while cuthyroid rats were unaffected by DHEA In another study, DHEA was found to lower serum cholesterol levels in hypercholesterolemic obese Zucker rats These authors suggested that, since NADPH is a cofactor in cholesterol synthesis, the anticholesterolemic activity of DHEA may be due to its ability to inhibit glucose-6-phosphate dehydrogenase and thus production of NADPH. However, others have concluded that the effect of DHEA on cholesterol synthesis may be by some as yet unknown mechanism other than NADPH-mediated! In a very recent study, Nestler reported that DHEA administration to normal non-obese men reduced total body fat and reduced serum low density lipoprotein cholesterol levels, without weight loss.

To test the hypothesis that DHEA administration will decrease body weight and serum cholesterol levels, we undertook a study to evaluate the influence of DHEA in spontaneously obese dogs, In addition, we analyzed plasma lipoprotein concentrations in four non-obese normal dogs prior to and after one month of DHEA administration.

Methods

Twenty-three dogs of various breeds were referred to the University of Wisconsin's School of Veterinary Medicine Teaching Hospital for an obesity problem. Of these 23, four were diagnosed as hypothyroid and were subsequently eliminated from further evaluation. The criteria for entry into this study were that the dog be at least 25 percent overweight and free of concurrent disease. The 19 euthyroid dogs entered were evaluated prior to and after three months of DHEA therapy by the following: a complete physical examination, total body weight, complete blood count (CBC), serum chemistry profile (including cholesterol and triglyceride levels), basal T3, T4, cortisol and insulin levels, adrenal and thyroid stimulation tests, and an intravenous glucose tolerance test (IV-GTT) including the total insulin secreted during the IV-GTT and the fractional clearance rate for glucose (K value). To determine the effects of DHEA on non-obese dogs, six normal dogs of various breeds and sex were evaluated in the same way as the obese dogs.

The adrenal stimulation test was performed using 2.2 units/kg body weight of adrenocorticotropic hormone, injected intramuscularly, and a zero and two-hour plasma sample were collected. The thyroid stimulation test was performed using five units of dermathycin, injected intravenously, and a zero and four-hour serum sample were collected. Assays for baseline and stimulated cortisol, total T3 and total T4 levels were done by the Animal Health Diagnostic Laboratory, Lansing, Michigan.

The IV-GTT was performed after an overnight fast using a glucose dose of 500 mg/kg body weight, infused as a 50 percent solution in 30 seconds. Blood samples for plasma glucose and serum immunoreactive insulin were collected at -20, -10, 0 ,3, 5, 7, 10, 15, 30, 45 and 60 minutes. Plasma glucose was determined by the hexokinase method. The serum immunoreactive insulin was determined by a radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA). The fractional clearance rate for glucose (K value) was calculated by the method of the two variable linear regression from the plasma levels between 15 and 45 minutes[20]. The total insulin secreted was calculated as the total incremental insulin area above the basal resting insulin during the 60-minute IV-GTT.

A dietary recall history questionnaire was Wed. out by the owners of the obese dogs and of 40 non-obese normal dogs. The owners of the obese dogs were also asked to fill out a weekly diet diary to confirm the information obtained in the dietary recall questionnaire. From this information, a total daily caloric intake for each dog was determined and expressed as kilocalories per kilogram body weight per day. The diagnosis of obesity for each dog was determined by gross observation (palpitation) and comparison to ideal body weights for the particular breed and stature[21]. Ideal body weights for mixed breeds were independently estimated by two investigators (I.D.K. and E.G.M.).

DHEA was orally administered in capsules to the obese and non-obese dogs for three months at an initial dose of 30 mg/kg daily, The dose was escalated on a monthly basis to a maximum of 75 mg/kg p.o. daily in an attempt to increase the rate of weight loss. Food intake remained constant and monthly body weights were recorded.

Statistical analysis was performed using the paired Student's t-test for comparing differences between pre-and post-treatment assessments. A *P* value of less than 0.05 was considered significant.

Lipoprotein study

An additional four non-obese normal dogs were maintained on 75 mg/kg of DHEA for 30 days. Prior to and after 30 days of DHEA administration, plasma was collected on each dog for analysis of lipoproteins. Fractionation of the plasma lipoproteins into various density (given throughout as g/ml) classes was accomplished by sequential ultracentrifugation at progressively increasing densities using sodium bromide [L23]. Very low density lipoproteins (VLDL) were isolated at a plasma density of 1,006. Other lipoprotein fractions were isolated by sequentially raising the plasma density to 1 ,019 for intermediate density lipoprotein (LDL), 1,063 for the low density lipoprotein-high density lipoprotein-l faction (LDL-HDL) and 12 1 for HDL, The 1,006, 1 ,019 and 1,063 fractions were centrifuged for 2.34 x 10^{b} gh, and the 1.2 1 fraction for 5 x 1 0^{b} gh. After separation, cholesterol distribution was determined by measuring the amount of cholesterol in each fraction.

Results

There were a total of 19 cuthyroid obese dogs studied. Of 15 females, six were intact and nine were neutered. There were four males, one was intact and the other three were castrated. The median age was 7 years old with a range of 2-13 years. Thirteen of the dogs were of various purebreds and six were of mixed breed. DHEA was well tolerated and no toxic effects were noted.

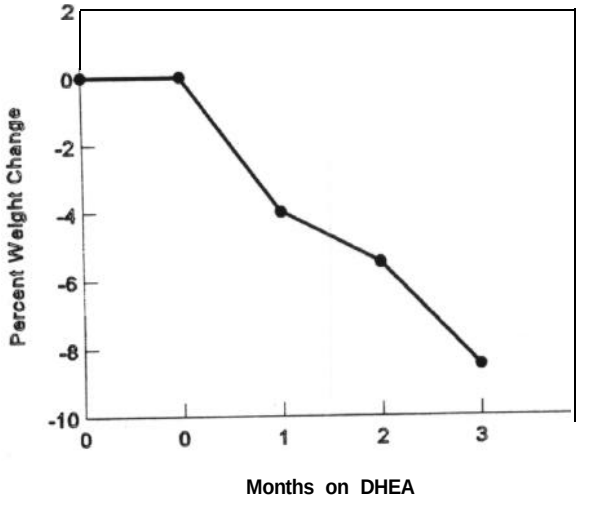

Fig. ***1. Mean percent total body weight (+/- standard error,) lost during DHEA administration in 13 of 19 obese dogs (68 percent).***

The effect of DHEA administration on weight loss is shown in Fig. 1. Thirteen of the 19 obese dogs (68 percent) lose weight while on DHEA. Due to the wide variation in size of the obese dogs in this study (weight ranged from 7.9 to 51 kg), weight loss was recorded for each dog as a percentage of total body weight. The mean weight lost for the 13 dogs was 3 percent of total body weight per month. Monthly escalation of the daily dose of DHEA did not increase the rate of weight loss. Of the six obese dogs that did not lose weight, five were intact females and one was a castrated male. Only one obese dog voluntarily reduced his food intake during the observation period. All other dogs maintained their normal food intake. Body weight and food intake remained stable in the non-obese dogs.

The comparisons of the pre-and post-treatment assessments of blood profiles and the IV-GTT for the obese dogs are shown in Table I, those for the non-obese dogs are shown in Table 2.

The most marked change observed during DHEA therapy was the alteration in serum cholesterol levels. The mean cholesterol for the obese dogs went from 226 mg/dl pre-treatment to 173 mg/dl post-treatment, a reduction of 23 percent ($P < 0.0005$). The non-obese dogs went from a mean of 128 mg/dl to 89 mg/dl, a

Table I. ***Comparisons of the means of the laboratory findings pre-and post-DHEA treatments for 19 euthyriod spontaneously obese dogs.***

	Pre-DHEA	*Post-DHEA*	*P-value*
Resting insulin (uU/ml)	17.7+/-3.2	21.3+/-3	n.s.[b]
TIS (uU/ml/h)	2861+/-349	2615+/-461	
Cholesterol (mg/dl)	226+/-14	173+/-14	P <0.:0005
Triglyceride (mg/dl)	82+/-8	67+/-6	n.s.
T3 (ng/ml)	1.06+/-0.08	101+/-0.05	
T4 (ng/ml)	23.4+/-2.03	13.8+/-1.5	P<0.0001
Cortisol (ng/ml)	38.5+/-5.6	23.8+/-3.9	n.s.
Fasting glucose (mg/dl)	92+/-1.5	99+/-3.8	n.s.
Peak glucose (mg/dl)	416+/-13	398+/-10	n.s.
60 minute glucose (mg/dl)	93+/-4	103+/-8	n.s.
K value	3.26+/-.16	2.9+/-0.16	n.s.

'Standard error of the mean. [b]n.s.= not significant.