Physiological levels and action of dehydroepiandrosterone in Yucatan miniature swine

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Tagliaferro, A. R., and A. M. Ronan. Physiological levels and action of dehydroepiandrosterone in Yucatan miniature swine. Am J Physiol Regulatory Integrative Comp Physiol 281: R1–R9, 2001.—The biological role of dehydroepiandrosterone (DHEA) and its less active sulphated conjugate DHEAS was investigated in two experiments using Yucatan miniature swine. In experiment 1, plasma levels of both DHEA(S) among males were greater than female pigs that ranged in age from 0.3 to 84 mo old (P < 0.0001). In males, DHEA(S) were related inversely to serum triglycerides; DHEA was positively related to triglycerides in females (P < 0.01). In experiment 2, four 2-yr old male pigs, used as their own control, showed a 5% decrease in body weight, 11% increase in energy expenditure, 88% increase in lipid, and 100% decrease in glucose utilization (P < 0.0001) in response to DHEA vs. placebo treatments when adjusted for body weight. Plasma DHEA(S) were not different between treatment conditions. Glucose tolerance and plasma insulin levels were not different from controls. In vivo response to norepinephrine indicated β-adrenergic sensitivity was altered by DHEA. Present findings suggest DHEA and/or its hormone products are important in modulating energy expenditure and lipid utilization for energy in male animals. The role of DHEA in energy metabolism and the difference between sexes warrant further investigation.

Adiposity has varied with age (34, 53) and body weight (1, 13, 47) and coronary heart disease (4, 33). In human experiments, the effect of DHEA treatment on adiposity has varied with age (34, 53) and body weight status (48). The inconsistency in action of DHEA between body phenotype and gender in humans may be related to oral route of administration and the fact that there is considerable variability in the transforming enzymes present and steroid products synthesized in different peripheral tissues including the liver (5, 19, 20).

The antiobesity effects of DHEA treatment have been found to be more consistent in animal studies. They have included both male and female and obese and nonobese models (7, 10, 24). The reduction in adiposity of laboratory animals has been associated with an elevation of resting metabolism (44) that may involve several less efficient energetic cellular processes (6, 21, 23, 27, 32). Also, there is considerable experimental evidence that would indicate DHEA may affect fat tissue metabolism directly via mechanisms regulating fat synthesis (25), fat mobilization of steroid triglycerides (46), and fatty acid oxidation for energy (9). In related studies, McIntosh and colleagues (22, 28, 29) have shown in vivo and in vitro that DHEA treatment can also decrease adipose cellularity and preadipocyte growth of rodent and human adipose tissue.

One limitation of using the rodent to investigate the biological role of DHEA in energy metabolism is that normal endogenous levels of steroid production are too low for detection (5). Consequently, the observed effects of DHEA on body weight and metabolism have been in response to pharmacological treatment of the steroid, which has made interpretation of their biological relevance less clear.

The Yucatan miniature swine is a natural breed of pig, indigenous to the Yucatan Peninsula of Mexico. It is closely related to the European wild pig Sus scrofa that was bred to the East Indies pig Sus villoatus to produce the modern domestic swine in the United States (31). It has been bred in the U. S. from a primary gene pool and has become a popular biomedical model to study cardiovascular disease (36). The pig is a good animal model to study human obesity because it has a digestive physiology, endocrine system, and intermediary metabolism similar to the human (8). In addition, DHEAS production in the pig is higher in nonobese than obese genotypes (51).
The purpose of the present study was twofold: 1) to determine the circulating levels of DHEA and DHEAS in male and female miniature pigs of different ages and their relationship to serum triglycerides and 2) to determine the effects of DHEA treatment at physiological levels on energy production and substrate utilization in mature adult male swine.

The findings reported herein indicate that circulating levels of DHEA and DHEAS correlate inversely with serum triglycerides in males and positively in females. Also, effects of DHEA treatment, at physiological levels, suggest that the steroid may be involved in the modulation of energy expenditure and lipid utilization for energy in male swine.

**METHODS**

**Animal Housing**

All animals in experiments 1 and 2 in this study were bred at the University of New Hampshire Burley-Demeritt Miniature Swine Facility that has 1,800 sq. ft. to house animals for logistical purposes. The animals typically were housed in 5-×12-ft. galvanized steel pens. Living area was temperature controlled and well ventilated. Animals were fed a commercial porcine diet low in fat (Agway, Syracuse, NY) in two meals daily; water was available ad libitum.

**Experiment 1**

Blood samples were obtained from 93 healthy male (n = 57) and female (n = 36) swine, age range 10 days to 84 mo. Most of the blood samples in this experiment were obtained from animals that were being culled from the research herd for logistical purposes. With the exception of suckling animals (less than 42 days old), animals were maintained on the commercial diet appropriate for young growing and adult-aged animals. Nonsuckling animals were fasted overnight before blood samples were taken.

Blood assays. Serum triglycerides were measured enzymatically using a commercial kit (Sigma #320, St. Louis, MO). Serum levels of DHEA and DHEAS were measured by radioimmunoassay using commercial kits from Wien Laboratories (Succasunna, NJ). Unless stated otherwise, when reference is made to both forms of the steroid, DHEA and DHEAS, DHEA(S) will be used.

**Experiment 2**

Four male 2 yr-old miniature swine ranging in weight from 72 to 94 kg were tested as their own control in a withinsubject design. The animals were housed at the University of New Hampshire Burley-Demeritt Swine Facility except during testing. Animals were fed the low-fat, commercial swine diet in two meals daily (0800 and 1400); water was available ad libitum. Animals were implanted with two placebo pellets, then two 200-mg DHEA pellets designed for 21-day release (Innovative Research, Sarasota, FL) between back fat and muscle layers in the interscapular region using aseptic surgical techniques. Pellet implantations were separated by 1 wk. Animals were not assigned randomly to treatment conditions because a preliminary experiment showed that animals treated initially with a 21-day DHEA pellet continued to show residual metabolic effects of the steroid 5 wk later.

Energy metabolism. Measurements of energy production and substrate utilization were done by indirect calorimetry using an open-circuit pass-through system. After the afternoon feeding (1500) on 4 successive days during week 2 of placebo and DHEA treatments, the animals were transported to the University of New Hampshire campus and placed in a 2,800-1 thermoregulated, well-ventilated, air-tight chamber until 0800 the next day. Urine was collected during that time. The animal was allowed to acclimate to the chamber for several hours before metabolic measurements were taken. We have found that 2400 to 0800 was an ideal time of day to measure resting metabolism because during that time, the animal was totally relaxed and remained inactive in a recumbent position. Rate of airflow was kept constant by a mass airflow controller (Sierra Instruments, Monterey, CA). Oxygen consumption and production of carbon dioxide were corrected to standard temperature and pressure and analyzed by S-3A O2 (Ametek, Pittsburgh, PA) and LB-2 CO2 analyzers (Beckman, Fullerton, CA) for 5 min every sixth minute or 10 times per hour. For 1 min between gas samples, gas analyzers were recalibrated to ambient air.

Rate of energy production from glucose and lipid following adjustments for protein oxidation was calculated continuously on-line by a computer using a software program based on published equations (11). Because glucose and lipid utilization was not measured directly, utilization of substrates refers to their disappearance.

Glucose tolerance test. The fasted animal (18 h) was suspended in a canvas sling and anesthetized by isoflurane inhalation, and an ear vein was catheterized bilaterally using 22-gauge teflon catheters. In one catheter, the animal was given a bolus injection of 50% dextrose solution at a rate of 0.5 ml/kg body wt. Average glucose dose was 22.25 g. Time to complete an injection varied with volume. On average, glucose injection was completed within 2 min. Blood samples (3 ml) were collected from the contralateral ear vein at 5 min and immediately before administering the dextrose load (time 0) as well as at 2, 5, 10, 20, 30, 40, and 60 min postinjection. Blood flow of the vessel was kept arterialized by applying repeatedly a warm wet towel to the ear. Plasma glucose concentration was measured enzymatically using a commercial kit to assay glucose oxidase (Glucose Kit #510, Sigma); insulin concentration was measured by radioimmunoassay (Linco Research, St. Charles, MO).

Norepinephrine challenge. During the third week of placebo and DHEA treatments, fasted animals were anesthetized by isoflurane inhalation and suspended in canvas slings. A vein in each ear was catheterized with a 22-gauge teflon catheter. In one ear, norepinephrine bitartrate/saline solution (1 μg/ml) was delivered continuously at an infusion rate of ~0.15 ml/min for 1 h by a Beckman syringe pump (Beckman). Baseline blood measures were taken at 6 min and immediately before norepinephrine infusion and at 6-min intervals during catecholamine infusion. A warm wet towel was applied repeatedly to the ear to arterialize blood flow. Plasma-free glycerol and fatty acids were assayed by enzymatic (Kit #320A, Sigma) and colorimetric methods (15), respectively.

Plasma DHEA and DHEAS. Concentrations of the two steroid forms were measured weekly by radioimmunoassay using a commercial kit with tritium as the label (Wien Laboratories).

Fat cell anthropometry and metabolism. During the third week of each treatment condition, a subcutaneous sample of back fat, weighing 1–2 g, was taken from the neck area using aseptic surgical technique. Procedures for isolating and sizing adipocytes and measuring fat cell lipolysis have been...
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described in greater detail previously (46). Briefly, tissue was minced into small cubes and digested enzymatically for 120 min with 0.1% collagenase in Krebs Ringer Buffer (KRB) and 4% BSA. A 0.5-ml aliquot of isolated cells (≈10^6 cells) in KRB and BSA was incubated for 90 min at 37°C in a reciprocating water bath at 90–100 cycles/min. Glycerol release was measured in the presence and absence of the β-agonist isoproterenol (10^-6 mol) to determine rate of basal and stimulated lipolysis, respectively.

Cell size was determined using the mean of three random samples (4 μl) of cells fixed in osmium tetroxide. Cells were visualized on a television monitor and digitized using a Color Space II Videodigitizer (Masa Microsystems, Sunnyvale, CA). Digitized images of cells were sized on a Macintosh II computer (Apple Computers, Cupertino, CA) using Image 123 software. To determine cell size, cell surface area (SA) was calculated as follows: area (μm^2) = 4πr^2. Lipolysis, measured in vitro, was expressed as glycerol release (μM) relative to SA (μm^2).

Statistical Analyses

Experiment 1. Developmental stages of miniature swine were categorized according to scheme defined by Bhathena et al. (8): 0–6 mo of age, prepuberty; 6–24 mo, young adult; 2–10 yr, mature adult; and >10 yr, old age. There were no animals available that could be used in the old age category in this study. Therefore, to examine if there was a relationship between circulating DHEA(S) and age, age categories were subdivided into six age groups: prepuberty 1 (0.3–3 mo, n = 11) and 2 (4–6 mo, n = 36); young adult 3 (7–9 mo, n = 17), 4 (10–12 mo, n = 8), and 5 (13–24 mo, n = 9); and mature adult (≥24 mo, n = 12). Blood values of DHEA(S) were transformed to natural logarithms because of the range of variation in DHEA (pg) and DHEAS (pg) concentrations between animals and the variability between sexes and ages. Differences due to gender and age were analyzed by ANOVA using the general linear model. A multiple regression model using gender, age, and plasma levels of DHEA(S) as independent variables and serum triglycerides, as the dependent factor, was used to determine whether plasma DHEA(S) and serum triglycerides were related.

Experiment 2. Treatment effects were tested by ANOVA with the animal as a blocking factor because order of treatment was not randomized. Body weight was used as covariate in the analysis of energy and substrate metabolism. Pairwise comparisons between means were carried out by Tukey’s honestly significant difference test. Log transformation of data and all analyses were performed using Systat for Windows: Data; Statistics (version 5, 1992; Systat, Evanston, IL; Ref. 43). All values reported were means ± SE. Values were considered statistically significant if the probability of error was 5% or less.

RESULTS

Experiment 1

Serum triglyceride concentrations decreased with age among male and female animals (P < 0.0001). There were no differences between sexes (Fig. 1). There was a significant gender difference in log DHEA(S) levels (P < 0.0001). Both forms of the circulating steroid were higher in male than female animals (Fig. 2). There was a significant effect of age (P < 0.01) and age × sex interaction (P < 0.01) on log DHEAS (but not on log DHEA) that indicated the circulating levels of DHEAS differed between sexes across age groups. When analyzed separately by ANOVA, it was found that age effects on log DHEAS concentration were higher among some of the older than younger female animals (P < 0.01); there was no effect of age on log DHEAS among the male pigs.

Multiple regression analyses indicated that log DHEAS and age correlated negatively with serum triglyceride levels in male animals (r = −0.43, T = −2.4, P < 0.02; r = −0.36, T = −2.7, P < 0.01, respectively). Also, in males, the relationship between log DHEA and triglycerides was marginally significant (r = −0.34, T = −2.0, P = 0.05). Overall, the model had an R = 0.44 and an R^2 = 0.19 (P < 0.02). In contrast, among female animals, log DHEAS correlated positively to serum triglycerides (r = 0.49, T = 3.19, P < 0.01). The relationship between log DHEA and serum triglycerides was not significant. Age was negatively related to serum triglycerides (r = −0.71, T = −4.9, P < 0.0001). Overall, the model had an R = 0.72 and an R^2 = 0.52 (P < 0.0001).

Experiment 2

Body weights of the animals during DHEA treatment were 5% lower than during placebo treatment (P < 0.001; Fig. 3). After 1 wk of DHEA treatment, plasma levels of DHEA and DHEAS were elevated 6 and 33%, respectively, above baseline. After 2 wk of steroid treatment, plasma DHEAS levels were similar to baseline values, but plasma DHEA remained 82% higher than week 1 (Table 1). Compared with placebo treatment, plasma levels of DHEA and DHEAS during steroid treatment were not different, even when adjusted for differences in body weight. The average plasma levels of DHEA during placebo vs. steroid treatments were 525.56 ± 47.9 vs. 328.89 ± 47.9 pg/ml and for DHEAS, it was 9.49 ± 0.9 vs. 10.75 ± 0.9 ng/ml, respectively.

Effects of placebo and DHEA treatment on energy metabolism and substrate disappearance are pre-
DISCUSSION

In the present investigation, both serum log DHEA(S) were higher in male than female animals in all age groups. Hormone concentration varied inversely to serum triglycerides for male swine and positively for female swine. These effects were independent of age. The variation in hormone levels between males and females and their relationship to serum triglycerides are consistent with the dimorphic differences that have been observed between men and women. That is, plasma DHEAS levels are greater among men vs. women, ages 17–65 yr (19, 35), and have been found to correlate negatively with central adiposity in men (16) and positively in women (14, 50). We believe that the age and age × sex interaction effects on log DHEAS in experiment 1 were not specific to gender but related more to an imbalance in distribution of male and female animals in different age groups. That is, 63% of the male pigs were of prepuberty: group 1 0.3–3 mo, n = 12 m, 3 f; group 2 4–6 mo, n = 24 m, 8 f. Young adult: group 3 7–9 mo, n = 12 m, 5 f; group 4 10–12 mo, n = 3 m, 5 f; and group 5 13–24 mo, n = 2 m, 7 f. Mature adult: group 6 >24 mo, n = 4 m, 8 f. Log DHEA and DHEAS show m > f. GLM ANOVA P < 0.0001. Values are means ± SE.

Plasma glucose and insulin concentrations during the intravenous glucose tolerance test are shown in Fig. 5. Plasma glucose concentration peaked within 2 min following bolus intravenous injection. There were no differences in plasma glucose levels between treatments at any time point. For both treatment conditions, plasma levels of glucose at the end of 1 h remained higher than the preload levels (P < 0.0001). Plasma insulin concentration increased slowly following the glucose load. Insulin concentration was highest at the end of the 1-h test. There were no differences in plasma insulin levels between treatments or in relation to baseline at any time point measured.

Plasma concentrations of glycerol and free fatty acids during norepinephrine peaked ~30 min following the start of the catecholamine challenge. Circulating levels of both glycerol and free fatty acids were found to be lower in response to norepinephrine during DHEA vs. placebo conditions (P < 0.01; Fig. 6). Ratio of glycerol to free fatty acid concentration was also analyzed and found to be similar between DHEA and placebo conditions (3.5 ± 0.3 vs. 3.4 ± 0.3, respectively).

Isolated adipocytes of animals sampled during DHEA and placebo treatments were found to be similar in size (DHEA: 153 ± 36 μm² vs. placebo: 144 ± 36 μm² SA). There were no statistical differences observed in either the rate of basal lipolysis (DHEA: 3.0 ± 1 nm/μm² SA vs. placebo: 3.0 ± 1 nm/μm² SA) or in response to isoproterenol stimulation (DHEA: 12 ± 3 vs. placebo: 16 ± 3 nm/μm² SA).

Fig. 2. Serum concentration of DHEA (A) and DHEAS (B) among fasted m and f swine of different age groups; values were transformed to natural logarithms. Age categories are as follows. Prepuberty: group 1 0.3–3 mo, n = 12 m, 3 f; group 2 4–6 mo, n = 24 m, 8 f. Young adult: group 3 7–9 mo, n = 12 m, 5 f; group 4 10–12 mo, n = 3 m, 5 f; and group 5 13–24 mo, n = 2 m, 7 f. Mature adult: group 6 >24 mo, n = 4 m, 8 f. Log DHEA and DHEAS show m > f. GLM ANOVA P < 0.0001. Values are means ± SE.

Fig. 3. Weekly body weights during 3-wk placebo (weeks 1–3) and DHEA (weeks 4–6) treatments. Body weights during DHEA condition were significantly lower than placebo (n = 4). ANOVA P < 0.01. Values are means ± SE.
berty age, 30% were young adult, and only 7% were mature adult animals at the time of the study; whereas only 31% of the female animals were of prepuberty age, 47% were young adult, and 22% were mature adult age.

It has been reported that during the first 8 postnatal mo, circulating levels of DHEAS follow a U-shaped pattern in the pig (40). It is noteworthy that in experiment 1, both male and female animals of the same age range (i.e., groups 1-3, Fig. 2) showed a similar U-shaped pattern of serum DHEAS. The biological significance of this cyclic pattern is not clear but may represent an age-dependent modulation of hormone production that is important in growth and development in the pig. Interestingly, a similar type of pattern has been reported to occur between birth and late adolescence in humans (5).

In experiment 2, the plasma DHEAS levels of the male pigs during placebo condition were considerably lower than normal values reported for similar aged Yorkshire swine and crossbred Duroc/Yorkshire swine (51). In contrast, plasma DHEAS levels of the male pigs in experiment 2 were severalfold greater than Hormel miniature swine of similar age (8). These findings would indicate that there is a wide variation in DHEA production among porcine breeds. To our knowledge, the present study is the first to report circulating values of DHEA(S) in female swine.

During steroid treatment, the changes in plasma DHEA levels were not different statistically from control conditions. However, by week 2 of steroid treatment, plasma levels of DHEA were more than two times greater than the average normal range in variation of DHEA levels shown during placebo weeks (Table 1). So, we are confident that the physiological changes observed were related to steroidal treatment.

During DHEA treatment, the animals showed a small but significant decrease in body weight compared with placebo treatment. These differences in body weight were paralleled by an 11% higher rate in energy metabolism. The higher metabolic rate of the DHEA-treated animals observed in this study is consistent

### Table 1. Plasma DHEA and DHEAS

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<th>Placebo</th>
<th>DHEA</th>
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<tr>
<td>DHEAS, ng/ml</td>
<td>9.9 ± 0.7</td>
<td>9.3 ± 0.7</td>
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<td>DHEA, pg/ml</td>
<td>441.3 ± 115</td>
<td>264.1 ± 132</td>
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Values are means ± SE. Weekly plasma concentrations of dehydroepiandrosterone (DHEA) and its less active sulphated conjugate DHEAS of male adult animals (n = 4) in experiment 2 during placebo and DHEA conditions. Week 0 refers to baseline values before implantation of respective treatment. Weeks 1 and 2 are plasma values taken during the first 2 wk after pellet implantation. There were no statistical differences between treatments.

Fig. 4. Hourly rates of energy expenditure, lipid and glucose utilization during placebo and DHEA conditions are presented. Metabolic values were adjusted for body weight. Metabolism was measured by indirect calorimetry during week 2 of each treatment period. Values represent hourly changes from 2400 to 0800. A: rate of energy expenditure on the pig was higher during DHEA than placebo (P < 0.0001). B: respiratory quotient during DHEA was lower than placebo (P < 0.0001). Rates of lipid utilization (C) were greater and glucose utilization (D) lower during DHEA than placebo conditions (P < 0.0001). There were no differences in rate of protein oxidation (data not shown). Treatment effects determined by ANOVA. Values are means ± SE.
with findings reported previously in rodents (44). The present effect of DHEA treatment and energy metabolism also agrees with the positive relationship between plasma DHEAS and basal rate of metabolism that has been observed among obese and nonobese women (3). On the other hand, our findings do not agree with those of Welle et al. (49), who reported no significant effects of daily oral treatment (1,600 mg) of DHEA on energy expenditure or protein oxidation of young and middle-aged men, using doubly labeled water and labeled leucine, respectively.

It is possible that the reduction in body weight of the animals during DHEA treatment may have been caused by a restriction in food intake, which was not measured. Typically, the antiobesity effects of DHEA in most animal studies (7, 10), but not all (37, 42), have not been associated with a decrease in appetitive behavior. If weight loss of the pigs did involve a reduction in food intake, it most likely was secondary to the increase in energy metabolism, because a primary effect of food restriction is to lower energy metabolism (12).

Concomitant with an elevation in energy metabolism during DHEA treatment were changes in respiratory quotient and substrate utilization. Respiratory quotient refers to the ratio of oxygen consumed and carbon dioxide produced, and it varies directly with the amount of glucose, relative to protein and fatty acids oxidized for energy. When adjusted for body weight and corrected for protein oxidation, the respiratory quotient of the animals during DHEA treatment was ~12% lower than during placebo treatment. This corresponded to a percentage decrease in glucose and an increase in fatty acid utilization that were similar in magnitude. We believe that this shift toward greater lipid and less glucose disappearance represented an increase in oxidation of lipids and not a disappearance related to an increase in the intracellular recycling of fatty acids. The observed increase in lipid utilization here is also consistent with the reported elevation in biochemical indexes of lipid oxidation that have been reported with DHEA treatment in rodents (9, 23). And, the absence of differences in the ratio of glycerol to free fatty acids released during norepinephrine infusion during placebo and DHEA conditions also would support this interpretation.

The effects of DHEA treatment on energy metabolism in this study do not seem to be unique to adult-aged animals, because we have found that DHEA treatment can normalize a suppression in lipid utilization in juvenile-aged castrated pigs (45). In a related clinical study, it was reported that adolescent boys with delayed pubescence who were treated with testosterone responded with an increase in rates of energy

Fig. 5. Intravenous glucose tolerance test (IVGTT). Results of 1-h IVGTT are presented. Blood glucose values at 60 min were significantly greater than preglucose load for both conditions (ANOVA $P < 0.0001$). There were no differences in either glucose (A) or insulin concentrations (B) at any time point between conditions. Values are means ± SE.

Fig. 6. Norepinephrine challenge. Plasma concentration of glycerol (A) and free fatty acids (B) before and in response to norepinephrine bitartrate (1 μg/ml) at an infusion rate of 0.15 ml/min. Release of glycerol and free fatty acid concentration was significantly lower during DHEA than placebo conditions (ANOVA $P < 0.01$). Values are means ± SE.
metabolism and lipid utilization (2). These latter findings would suggest that the metabolic effects of DHEA may include other products of its metabolic pathway.

The biological action of DHEA on substrate utilization did not appear to involve a change in insulin sensitivity, because neither plasma glucose nor the insulin response to intravenous glucose injection was found to be different between placebo and DHEA treatments. These findings are consistent with negative effects of DHEA treatment on insulin sensitivity reported in human trials (34) but vary with the effects on insulin reported in rodents (11). The differences observed in this latter study could be related to methodological differences. In the rodent study, DHEA was administered orally in pharmacological doses via the animals’ diet. Also, we cannot rule out that DHEA treatment in the present study did not affect insulin sensitivity, because at the end of 60 min, glucose levels remained higher than baseline, and insulin curves between placebo and DHEA conditions were starting to diverge. This effect may have been anesthesia related because isoflurane has been found to suppress glucose clearance and insulin secretion in the pig (18).

In the rodent, isoproterenol-stimulated lipolysis of adipocytes has been found to be increased following several weeks of dietary treatment of DHEA (46). In the present investigation, we did not observe the same effect on lipolysis of isolated adipocytes. However, the lowered glycerol and free fatty acid release in vivo to norepinephrine infusion did indicate that adrenergic sensitivity of lipolysis was affected by DHEA treatment.

Adrenergic action of norepinephrine involves both β-mediated stimulation and α2-mediated inhibition of fat cell lipolysis. β-Adrenergic receptors (i.e., β1, β2, and β3), particularly β1, are the predominant receptors involved in lipolysis in the pig (30). Affinity of β1- adrenergic receptors to the agonist isoproterenol, however, has been found to be attenuated under conditions of chronic sympathetic activation (26). We speculate that the lower serum levels of glycerol and free fatty acid release in response to norepinephrine infusion during week 2 of DHEA vs. placebo treatments may have reflected a desensitization of adrenergic receptors to lipolysis due to chronic β-adrenergic stimulation that was central in origin. That is, electrophysiological (38) and lesion studies (17) have shown that the hypothalamus is a central integrator of neural pathways involved in the regulation of energy balance. In particular, the ventromedial nucleus (VMN) controls sympathoadrenergic outflow to peripheral organs and tissues involved in the control of food intake and adipose tissue metabolism. Neurons of the paraventricular nucleus (PVN) of the hypothalamus are part of the neuronal network with the VMN, which has also been shown to be important in the regulation of energy balance (39).

In the Zucker rat, both oral and systemic administration of DHEA has been found to stimulate the release of serotonin in the lateral nucleus of the hypothalamus and norepinephrine in the PVN in both lean and obese Zucker rats (53). The increase of these brain transmitters in their respective sites is associated with decreased food intake with increase in sympathoadrenergic activation. Hence, a chronic elevation of central sympathoadrenergic outflow induced by DHEA treatment may have caused a desensitization of β-adrenergic receptors in peripheral adipose tissues. This possible mechanistic warrant further investigation.

In summary, the present investigation showed that the Yucatan miniature swine is a useful animal model to study the role of DHEA and related steroid hormone metabolism in obesity. The present findings also support the hypothesis that DHEA has a biological role in energy balance that, in males, protects against excess adiposity. DHEA treatment, at physiological levels, increased energy expenditure and lipid utilization for energy and a possible downregulation in sensitivity to hormone-stimulated lipolysis. In addition, a positive relationship between circulating DHEA levels and triglycerides suggested that DHEA favors adiposity in female animals.

The mechanism(s) by which the steroid may be protective against obesity in males and not in females is not clear but most likely is related to a delicate balance or ratio between androgen and estrogen hormones, of which DHEA is the major hormone precursor.

Perspectives

DHEA is an ubiquitous hormone that is present in high concentration both in peripheral circulation and the central nervous system. By the third decade of life in humans, the steroid declines steadily. Depletion of this hormone has been associated with development of chronic health conditions that include obesity, heart disease, and Alzheimer’s disease. Although experimental evidence strongly suggests that DHEA is closely linked to the maintenance of health, elucidation of its biological mechanism and physiological importance to humans has been slowed by the complexity of DHEA metabolism and the lack of a suitable animal model. Findings of the present investigation indicate that the miniature pig may be a very useful experimental model to assess the action of DHEA action in human metabolism. Production of the steroid is measurable at a young age and varies between sexes. Furthermore, manipulation of DHEA in the physiological range produced reliable effects on energy and lipid metabolism. Because the life span of the miniature pig is ~20 yr, this model would enable the investigator to conduct controlled longitudinal studies of energy metabolism and obesity using invasive procedures to examine simultaneously the effects of variation in DHEA and its steroidal products at both the cellular level and in the whole animal.

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