MINIREVIEW

The Antiobesity Effect of Dehydroepiandrosterone in Rats (43158B)

MARGOT P. CLEARY
The Hormel Institute, University of Minnesota, Austin, Minnesota 55912

Abstract. **Initial studies showed that dehydroepiandrosterone (DHEA) treatment in mice resulted in lower body weight gain. Subsequent studies have shown that DHEA treatment in rats has a similar effect. In adult rodents, weight loss is a consequence of DHEA treatment. In general, these effects are independent of changes in food intake and are accompanied by lower body fat. DHEA treatment has been shown in some circumstances to alter a number of serum factors including glucose, insulin, cholesterol, and triacylglycerol. Recent studies have focused on the effects of DHEA on liver metabolism. Studies have been undertaken to determine whether the antiobesity effect of DHEA is mediated by the previously described inhibition of glucose-6-phosphate dehydrogenase by this steroid. It appears that inhibition of glucose-6-phosphate dehydrogenase in liver is not the initial metabolic response to DHEA but may play a contributing role. Inhibition of glucose-6-phosphate dehydrogenase in adipose tissue may affect differentiation of fat cells. A number of other enzymes involved in lipid and carbohydrate metabolism have also been shown to be altered by DHEA treatment, and several futile cycles involving some of these enzymes have been proposed to play a role in DHEA's antiobesity action. In addition, mitochondrial protein content is elevated by DHEA treatment. There appear to be time-dependent changes due to DHEA treatment on hepatic mitochondrial state three rates of respiration. Studies continue to evaluate the role of alterations in mitochondrial metabolism in DHEA's antiobesity action.**

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Obesity is a condition characterized by excessive body fat stored in adipose tissue depots by either increased fat cell size and/or number (1, 2). Metabolic abnormalities such as hypertension, insulin resistance, diabetes, hyperlipidemia, and heart disease are frequently associated with obesity (3–6). The presence of one or more of these factors may lead to accelerated disease and higher death rates in obese subjects. Recently, it has been suggested that the morbidity and mortality attributed to obesity may be better characterized by the location of the excess fat rather than its absolute amount (7, 8).

Methods of treatment for obesity have primarily involved altering food intake. However, the long-term success rates for treatment or prevention of obesity by dietary intervention have been poor (9–13). In fact, intervention by means of lowered food intake does not necessarily result in the expected response. For example, it has been found that, despite being on a calorically restricted diet with exercise for 18 months, obese girls increased fat cell number and had less increase in stature than did normal weight girls (14). During and after weight reduction, resting or basal metabolic rates have been reported to be lower than initial rates, indicating energy conservation (15–18). Furthermore, following weight reduction, some subjects report that they must continue to maintain lowered caloric intake in order to prevent weight regain (19), and such individuals may have elevated adipose tissue lipoprotein lipase activity (19, 20). In addition, weight-reduced individuals recycle adipose tissue fatty acids in a manner suggesting an energy-deficient state (21).

Surgical treatments such as jejunal-ileal and gastric bypasses or gastroplasty have also been used as treatments for obesity. The primary objective of these inter-

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ventions is to lower food intake and/or cause malabsorption. Although some success has been reported, there are numerous metabolic and nutritional problems found upon follow-up in addition to the risks associated with the surgical procedures (22, 23).

There has been increasing realization that other approaches, particularly those that might alter energy requirements or utilization, may provide more effective ways to treat obesity. Repartitioning and/or thermogenic agents have been gaining increasing interest for use in obesity treatment (24–26). One such compound, dehydroepiandrosterone (DHEA), has recently been under investigation as an antiobesity and anticancer agent (27). Despite the high circulating levels of DHEA or its sulfated form, it has no established physiologic role. However, when administered at pharmacologic doses to rodents, it has been shown to lower body weight independently of changes in caloric intake.

DHEA Treatment in Mice

Yen *et al.* (28) reported in 1977 that treatment of lean and obese ($A^{vy/a}$) mice with DHEA (500 mg/kg body wt by intubation three times weekly) resulted in lowered rates of weight gain in comparison to nontreated mice. A much greater response was found in the obese mice whose body weights approached those of lean nontreated mice. Both male and female mice responded to treatment. The intriguing aspect of this investigation was that this change in body weight was not mediated through lowered food intake. The authors also reported that DHEA was effective when administered by injection or included in the food. The ability of DHEA to alter body weight gain in this and other mice strains has been confirmed by a number of investigators (29–35).

It was speculated by Yen *et al.* (28) that the mechanism of action of the antiobesity effect of DHEA was through inhibition of the enzyme, glucose-6-phosphate dehydrogenase (G6PD). Earlier studies had shown that addition of DHEA to mammalian tissue homogenates or supernatants resulted in inhibition of this enzyme (36–40). Hypothetically, inhibition of G6PD by DHEA would result in limited NADPH production and subsequently to lowered rates of fatty acid synthesis. This hypothesis was supported by the finding of lowered ^3H_2O incorporation into liver lipids of DHEA-treated mice (28). Granholm *et al.* (32) measured G6PD activity in erythrocytes of DHEA-treated mice but did not interpret their findings to support this inhibition as an explanation for the antiobesity effect of DHEA. However, it is not clear how G6PD activity in erythrocytes would reflect lipogenesis in other tissues. The possible relation of the inhibition of G6PD to DHEA's antiobesity action is addressed in more detail later in this article.

Effects of DHEA Treatment on Body Weight in Rats

Following initial reports of the ability of DHEA treatment to alter body weight in mice, it was clearly of interest to determine whether such an effect could be found in other species. Rats were the logical choice for such an experiment. Preliminary studies (41) with Sprague-Dawley rats indicated that, following either intraperitoneal injection or inclusion of DHEA in the food, weight gain was lowered in treated rats compared with control rats without altering food intake. Subsequent studies have confirmed DHEA's ability to lower body weight gain in Sprague-Dawley rats (34, 42–44) and in other "lean" rats such as BHE (45, 46) and Wistar (47) strains.

Results of DHEA treatment in several models of obesity have also been presented. In the genetically obese Zucker rat, a model of early-onset obesity, DHEA treatment has been found to substantially lower the rate of weight gain (48–50) in comparison to nontreated obese rats. As a result of their lower body weights, cumulative food intakes tended to be lower in obese-treated compared with obese-nontreated rats (48–50). However, it has been shown that DHEA-treated obese rats weigh less than pair-fed obese rats (49, 50). Also, even when obese Zucker rats are weight matched to DHEA-treated obese rats, the DHEA-treated rats have significantly lower fat pad weights than do the weight-matched rats (51). These results further indicate that the effects of DHEA treatment are different from those of food restriction.

DHEA treatment has also been investigated in Sprague-Dawley rats with obesity induced by knife cuts of the medial hypothalamus (52). Body weight gain was less in DHEA-treated rats with knife cuts and in DHEA-treated sham-operated rats compared with the two corresponding groups of nontreated rats. Lower food intake was also reported for the DHEA-treated rats, and this was offered as an explanation for their lower weight gain. However, since body fat may be affected independently of body weight change, as described above, one cannot conclude definitively that there was not an effect of DHEA in this experiment without knowledge of either fat pad weights and/or body composition.

Several studies have shown that in adult rats DHEA treatment resulted in weight loss. Cleary and Zisk (53) reported that treatment of adult obese female Zucker rats from 8 to 14 months of age with either 0.6% or 1.0% DHEA included in their food resulted in substantial loss of body weight. In lean rats, 0.6% DHEA prevented weight gain, while 1.0% DHEA resulted in weight loss. Control obese and lean rats gained weight over this interval. Following 17 weeks of DHEA treatment, adult corpulent rats, another genetically obese rat strain, were also found to have lower final body weights compared with their starting weights while con-

trol rats maintained their initial weights (E. Logan and M. P. Cleary, unpublished data). Caloric intakes were similar in both groups.

Recently, the effects of DHEA treatment in a diet-induced model of obesity were determined (54). DHEA treatment was initiated in Sprague-Dawley rats after 7 weeks of consuming an "obesifying" diet mixture consisting of condensed milk, corn oil, and powdered commercial rodent food (Purina Rat Chow, #5001). DHEA treatment resulted in rapid return of body weight of the diet-induced obese rats to that of rats fed the commercial food without additions (Fig. 1). As in earlier studies, caloric intake was not altered by DHEA treatment.

There have been a few additional studies that have reported on the response of other species to DHEA treatment. Nestler *et al.* (55) reported changes in body composition in young adult human male subjects treated with 1600 mg of DHEA daily for 28 days. Body fat was lower than initial values in the treated subjects while nontreated subjects had no changes. Recently, a study of DHEA treatment in obese dogs has also been published (56). Thirteen of a total of 19 treated obese dogs lost weight over a 3-month period, losing on average 9% of their starting weight. This occurred while the dogs maintained their normal food intake. However, DHEA-treated rabbits had no reported changes in body weight although development of atherosclerosis was ameliorated (57, 58).

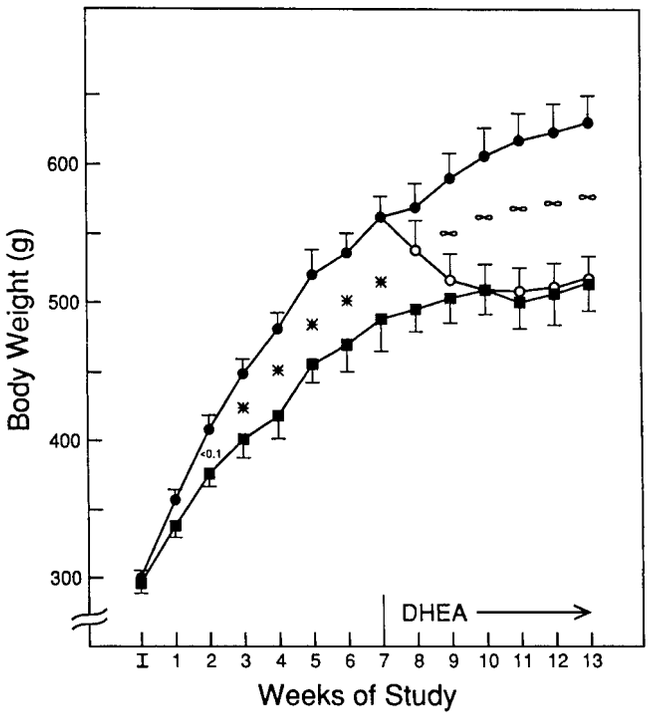

Figure 1. Effects of DHEA treatment on body weight in diet-induced obese rats. ●, Male Sprague-Dawley rats fed a mixture of condensed milk, corn oil, and nonpurified rodent food; ○, male Sprague-Dawley rats fed a mixture of condensed milk, corn oil, and nonpurified diet for 7 weeks, then 0.6% DHEA included in food for 6 weeks; ■, male Sprague-Dawley rats fed nonpurified diet. Data are mean ± SD. *Diet-induced obese rats significantly different from rats fed nonpurified diet. ∞, Diet-induced obese rats significantly different from DHEA-treated diet-induced obese rats and rats fed the nonpurified diet.

Effects of DHEA Treatment on Body Fat

In general, less body fat has been reported in DHEA-treated compared with control rats (46, 48, 51, 53, 54). In several studies, lower body fat has been found even when body weight has not been altered (42, 53). In both obese Zucker rats and diet-induced obese rats, lower fat cell size and number were found in DHEA-treated compared with control rats (48, 54). G6PD, fatty acid synthetase, lipoprotein lipase, and malic enzyme activities were all found to be lower in adipose tissue of obese Zucker rats treated with DHEA for 15 weeks than in nontreated obese rats (59).

It is not clear at present how these effects of DHEA on adipose tissue growth and metabolism are mediated, i.e., whether it is an indirect or direct action. Elevations in resting metabolic rate (42) or oxygen consumption (46) in DHEA-treated rats suggest that ingested nutrients would never reach the adipose tissue for metabolism and storage. Another explanation is that these alterations in adipose tissue metabolism may be mediated through changes in serum insulin. The enzymes mentioned above that were lower in adipose tissue of DHEA-treated obese rats are, in general, regulated by insulin. DHEA treatment has been found to lower serum insulin levels, particularly in obese rats. Thus, lipogenic enzymes and possibly the deposition of body fat in DHEA-treated rats may be regulated by changes in serum insulin levels. Conversely, the smaller adipose depot stores with smaller fat cells in DHEA-treated rats may lower the need for insulin, resulting in less insulin secretion. (See next section for further discussion of insulin and DHEA treatment.)

However, a direct effect of DHEA on adipose tissue through inhibition of G6PD activity cannot be ruled out. In support of this argument, it has recently been shown (60) that addition of DHEA at Day 7 for 48 hr to 3T3 preadipocytes in a cell culture system prevented the differentiation of these cells to lipid-filled adipocytes. This was assessed by lowered acetate incorporation into lipids and lowered activity of glycerol-3-phosphate dehydrogenase (a marker enzyme of differentiation) and was accompanied by lower cell numbers than those found in samples of untreated cells or in cells treated with DHEA from Days 5 to 7. Inhibition of G6PD activity was also found in the DHEA-treated cells in which there was an interference with differentiation, providing evidence for the involvement of this enzyme in the differentiation process. Further evidence that this metabolic pathway, i.e., pentose shunt, is affected by DHEA has been provided by the fact that lowered cellular 6-phosphogluconate levels were found

in cultures treated with DHEA and reintroduction of 6-phosphogluconate by use of liposomes bypassed this effect of DHEA (61). Other studies have demonstrated antiproliferative effects of DHEA (62, 63). Reintroduction of either deoxyribonucleosides or ribonucleosides has also been shown in some circumstances to overcome the antiproliferative effects of DHEA (62, 64). Thus, it appears that DHEA's effect on adipose tissue may be a result of both direct and indirect actions, but as yet there is not a definitive answer.

Effect of DHEA on Serum Measurements

DHEA treatment has been shown to consistently lower serum insulin levels in obese Zucker rats, and occasionally in lean Zucker rats, without altering serum glucose levels (50, 51, 53, 59, 65). Despite the lower serum insulin and body fat levels in DHEA-treated obese Zucker rats, neither peripheral glucose utilization nor insulin resistance was altered by DHEA treatment. This was assessed by glucose utilization in isolated adipocytes (50, 51) and muscle strips (51) as well as by the oral glucose tolerance test (66). Furthermore, Nestler *et al.* (55) did not find differences in glucose disposal using the euglycemic clamp technique that could be attributed to DHEA treatment when using human subjects. Long-term DHEA treatment lowered pancreatic insulin content and resulted in smaller sized islets in obese Zucker rats (67), but in a short-term study, i.e., 5 weeks, pancreatic insulin content was not affected although serum insulin levels were lower (51).

Recently, serum insulin levels were determined in DHEA-treated diet-induced obese rats (54). In one experiment, diet-induced obese rats treated with DHEA for 6 weeks had lower serum insulin levels compared with levels of rats fed the diet without DHEA or rats fed only the commercial diet. Serum glucose was not altered by treatment. In a second study, older rats were fed the commercial diet or the condensed milk diet. Of the rats fed the condensed milk diet, approximately one-half became obese while the remaining rats weighed a similar amount as rats fed the commercial diet. This resulted in three groups of rats, of which one-half of each group was treated with DHEA for 2 weeks. All DHEA-treated rats had lower serum insulin and glucose levels than did control nontreated rats (Fig. 2). It is interesting to note that the rats fed the condensed milk diet that became obese had higher insulin levels than did those rats fed the condensed milk diet that remained lean. This study indicated that the ability of DHEA treatment to lower serum insulin occurs shortly after initiation of treatment and may be independent of changes in body fat. It is unclear what role lower serum insulin levels may have in enhancing DHEA's antiobesity action, and it is as yet unclear how DHEA treatment lowers serum insulin.

Other hormones that have been measured in

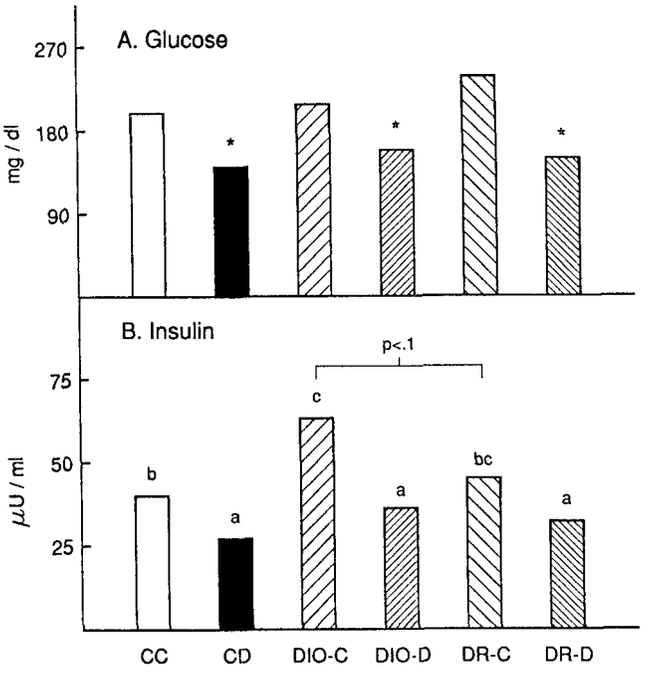

Figure 2. Effects of 2 weeks of DHEA treatment on serum glucose and insulin in rats fed the nonpurified diet and in diet-obese and diet-resistant rats. CC, commercial rodent food-control; CD, commercial rodent food-DHEA; DIO-C, diet induced obese control; DIO-D, diet-induced obese-DHEA; DR-C, diet-resistant control; and DR-D, diet-hesitant + DHEA. *Indicates DHEA values significantly lower than control values by two-way analysis of variance. Different superscripts indicate significant difference among groups with different letters by two-way analysis of variance followed by F test to test for differences between specific groups. From data presented in (54).

DHEA-treated rats include thyroxine and triiodothyronine. Thyroxine levels were not affected, but triiodothyronine was lower after 2 weeks in the DHEA-treated rats in the above described diet-induced obesity study (54). However, these findings are the opposite of those of Kurzman *et al.* (56) in DHEA-treated dogs, indicating that more detailed studies are needed to determine whether modulation of thyroid hormones is associated or involved in DHEA's antiobesity action.

DHEA treatment has also been reported to result in changes in serum lipids. An elevation of serum triacylglycerol and a moderate increase of cholesterol were found in rats made obese by feeding the condensed milk diet when compared with serum lipids of rats fed commercial rodent food (54). Treatment with DHEA lowered triacylglycerol levels regardless of whether it was elevated by diet or not. On the other hand, serum cholesterol levels were lowered by DHEA only when higher than normal due to feeding the condensed milk diet. Several other investigators have measured the effects of DHEA treatment on serum lipids in rats with varied results. For example, DHEA treatment resulted in lower serum cholesterol in male "Sabra" strain rats that had 0.1% propylthiouracil in their water and were fed a 5% cholesterol diet than in nontreated rats with

similar dietary intervention (68). Eighteen-month-old obese DHEA-treated female Zucker rats had lower cholesterol but not triacylglycerol levels than did control rats (65). Tagliaferro *et al.* (42) reported significantly lower serum triacylglycerol levels in both male and female Sprague-Dawley rats following 11 weeks of DHEA feeding. Serum cholesterol levels were not presented in this study. In another investigation, DHEA treatment when included in a high sucrose diet containing 0.5% cholesterol was reported to significantly elevate serum cholesterol but did not alter triacylglycerol (47). Recently, DHEA treatment has been found to lower serum cholesterol in both lean and obese dogs (56). Also, several studies using human subjects have shown decreases in low density lipoprotein cholesterol levels following DHEA treatment (55, 69). These findings suggest that there are species, genetic, and nutritional factors that interact to determine whether DHEA has a hypolipidemic effect.

Effects of DHEA Treatment on Liver Composition

A number of studies have shown that DHEA treatment in rats results in greater liver weights on either an absolute or relative to body weight basis (41, 42, 44, 47–49, 70). Lower liver lipid (47, 71, 42) and glycogen (44) contents have been reported for DHEA-treated compared with control rats. In contrast, hepatic DNA, RNA, and protein content have been reported to be higher (48, 49). Mitochondrial protein in particular appears to be elevated by DHEA treatment (54, 66, 73).

Effects of DHEA Treatment on Hepatic Glucose-6-Phosphate Dehydrogenase Activity

As indicated earlier in this article, DHEA has been reported to inhibit the mammalian form of G6PD. These studies were primarily done *in vitro* with DHEA added to tissue homogenates and supernatants. Several studies in animals showed that in fasted/refed rats administration of DHEA prevented the elevation of hepatic G6PD that is characteristic of this form of dietary intervention (74, 75). Due to the short-term nature of these experiments, no effects of DHEA on either body weight or fat were presented. A number of investigators have subsequently measured hepatic G6PD activity in rats treated with DHEA for several weeks or more. In some cases, G6PD activity was lower, but this was not always the case (41, 42, 44, 45, 53, 54, 59, 64).

In general, it appears that hepatic G6PD activity is "lowered" by DHEA treatment when it is higher than "normal" due to nutritional manipulations such as fasting/refeeding (74–76) and sucrose feeding (45) or when it is higher due to genetics such as in the obese Zucker rat (52, 54). It also appears that when DHEA has been administered for 2 weeks or longer, lower hepatic G6PD activity is usually accompanied by lower body and/or lower fat pad weight. A summary of this relationship is presented in Table I.

Table I. Summary of Hepatic G6PD Activity and Body Weight Changes in DHEA-Treated Rats

Strain	Duration of treatment	G6PD activity	Body weight or fat	Reference
Sprague-Dawley (M)[a]	3–14 weeks	—[b]	—	(41)
Sprague-Dawley (F)	15–26 weeks	↓	↓	(42)
Sprague-Dawley (M)	15–26 weeks	↓	↓	(42)
Sprague-Dawley[c] (M)	35–37 weeks	↓	↓	(54)
Sprague-Dawley[d] (M)	16 days	—	—	(68)
BHE (M)	8–28 weeks	↓	↓	(45)
Zucker lean (F)	6–21 weeks	—	↓	(57)
Zucker obese (F)	6–21 weeks	↓	↓	(57)
Zucker lean (F)	32–56 weeks	↑	↓	(53)
Zucker obese (F)	32–56 weeks	↓	↓	(53)

[a] M, male; F, female.
[b] —, Not significantly different from control group; ↓, significantly lower than control group; ↑, significantly greater than control group.
[c] Includes rats fed commercial rodent food and diet-induced obese and diet-resistant rats.
[d] Control rats in this experiment were meal-fed and pair-fed to DHEA-treated rats. Age of rats not specified; therefore, only the duration of the study is included.

It has been consistently found that DHEA treatment results in an elevation of hepatic malic enzyme activity (41, 45, 53, 59, 65). Since this mechanism could conceivably provide an alternative source of NADPH, the question of the importance of G6PD inhibition in the action of DHEA has been raised. Thus, several other experimental approaches have been undertaken to determine the role of inhibition of G6PD in response to DHEA treatment. Several investigators have used isolated hepatocytes to determine specific effects of DHEA on the pentose pathway. The fate of glucose labeled in different positions has been determined, and contribution of the pentose pathway to overall glucose utilization has been calculated using equations described by Katz *et al.* (77). By using this approach, we found that neither short- nor long-term DHEA treatment was shown to alter this pathway (78, 79). However, when only [1-^{14}C]glucose was used, Garcea *et al.* (64) reported that after a 6-hr incubation there was less CO_2 production in hepatocytes from DHEA-treated rats compared with hepatocytes from control rats, suggesting that there was an effect on this pathway. Others have shown no changes in metabolites of the hepatic pentose pathway or calculated ratios of [NAD^+]/

[NADH] and [$NADP^+$]/[NADPH] in DHEA-treated rats compared with control rats (70). Garcea *et al.* (64) also found no change in [$NADP^+$]/[NADPH] in hepatocytes. In general, it does not appear that inhibition of hepatic G6PD is a primary mediator of DHEA's antiobesity effect, although it may play a contributing role in long-term studies.

Effects of DHEA Treatment on Other Liver Enzymes

In addition to affecting G6PD activity, DHEA treatment has been shown to alter activities of a number of other hepatic enzymes. Activities of glycogen phosphorylase *a*, glycogen phosphorylase *a* + *b*, hexokinase, glucokinase, pyruvate kinase and fructose 1, 6-bisphosphatase were all lower following DHEA treatment (44) as was the activity of pyruvate carboxylase (70). Lower activity of hepatic fatty acid synthetase has been shown to accompany the lower G6PD activity measured in obese Zucker rats, but this was not true for lean rats (53, 59). Attempts to verify DHEA's ability to lower liver lipogenesis using *in vivo* incorporation of tritium from ^3H_2O have recently been reported for rats (46). It was found that *ad libitum*-fed DHEA-treated rats had 6-fold *higher* incorporation of tritium into fatty acids than did *ad libitum*-fed nontreated rats, indicating higher rates of lipogenesis, but starved/refed rats treated with DHEA had 2.5-fold lower incorporation than did the corresponding control group. In addition, in livers of DHEA-treated corpulent rats, no differences in tritium incorporation from ^3H_2O into fatty acids were found when compared with control rats (E. Logan and M. P. Cleary, unpublished data). Thus, it is still not clear what effects DHEA treatment has on liver lipogenesis and whether this, in turn, is related to DHEA's antiobesity effect.

In addition to malic enzyme, activities of other enzymes have been reported to be elevated in response to DHEA treatment. These include glucose-6-phosphatase (44), β-glucosidase (44), mitochondrial and cytosolic glycerol-3-phosphate dehydrogenase (80), lactate dehydrogenase (80), isocitrate dehydrogenase (70, 80), peroxisomal and microsomal carnitine acetyl transferase (54), and peroxisomal fatty acid oxidase (43). Catalase activity has also been found to be higher after DHEA treatment (43, 81). Recently, induction of a microsomal NADPH-cytochrome P-450 reductase has also been reported in response to DHEA treatment (82).

The activity of the enzyme, long-chain fatty acyl-CoA hydrolase, has been found to be elevated in livers of DHEA-treated rats (45, 65, 73). This led to the speculation that DHEA treatment induces a futile or substrate cycle of deacylation/reacylation of fatty acids. Recently, the activity of the other enzyme that would be involved in such a cycle, fatty acyl-CoA synthetase, was also found to be elevated following DHEA treatment (73), supporting that such a pathway may indeed be activated. We are currently attempting to verify the activity of such a pathway by alternative means.

Table II. Summary of Time-Related Effects of DHEA Treatment on Rates of Hepatic Mitochondrial Respiration

	Length of DHEA treatment (days)				
	3[a]	7[a, b]	14[c]	20[d]	35[e]
Respiration rate					
Per mg protein	↑[f]	—	↓	↓	↓
Per liver	↑	↑	↑	↑[g]	↑
Mitochondrial protein					
Per liver	↑	↑	↑	NI	↑
Liver weight	—	↑	—	↑	↑

[a] Male obese Zucker rats, DHEA administered 50 mg/kg intraperitoneally Day 1; 25 mg/kg thereafter (73).
[b] Female obese rats, DHEA included in diet at 0.6% (83).
[c] Male Sprague-Dawley rats, DHEA included in diet at 0.6% (54).
[d] Male BHE rats, DHEA injected 120 mg/kg daily (46).
[e] Female obese Zucker rats, DHEA included in diet at 0.6% (66).
[f] ↑, Significantly greater than control group; ↓, significantly lower than control group; —, not significantly different from control group; NI, not included.
[g] Estimated from data presented in article.

Table III. Summary of Short-Term Effects of DHEA Treatment on Various Liver Measurements

	Time (hr)		
	3	12	24
Liver mitochondrial respiration			
Glutamate-malate			
State 3 rate, per mg protein	↑[a]	↑	↑
State 3 rate, per gram	↑	↑	↑
State 4 rate, per mg protein	—	—	—
State 4 rate, per gram	—	—	↑
Succinate			
State 3 rate, per mg protein	—	—	↑
State 3 rate, per gram	—	↑	↑
State 4 rate, per mg protein	—	—	—
State 4 rate, per gram	—	↑	—
Liver enzyme activities			
G6PD	ND	ND	—
Malic enzyme	—	—	↑
Catalase	ND	—	↑
Long chain fatty acyl-CoA hydrolase	ND	—	↑
β-Oxidation			
Peroxisomal	ND	—	↑
Mitochondrial	ND	ND	—
Liver weight	—	—	—
Mitochondrial protein (mg/g liver)	—	—	—

[a] ↑, Significantly greater than control rats; —, no difference compared with control rats; ND, not determined because later values were not found to be significantly different. From data presented in (83).

Effects of DHEA on Hepatic Mitochondrial Respiration

Several preliminary studies suggested that DHEA treatment resulted in an increase in hepatic mitochondrial protein content and that there were changes in mitochondrial respiration rates. These initial observations have been followed by more detailed experiments. In the first study, it was shown that rats treated with DHEA for either 3 or 7 days, in general, had increases in mitochondrial protein content (73). After 3 days, treated obese rats also had 20% higher mitochondrial state 3 respiration rates expressed on a per milligram of protein basis with both glutamate-malate and succinate as substrates. There were no effects on state 4 rates. After 7 days, state 3 and 4 rates were similar when expressed per milligram of protein in DHEA-treated rats compared with the corresponding control groups. When rates were expressed per gram or per liver, DHEA-treated rats had greater respiration rates than did control rats.

Several other studies have shown that DHEA treatment for longer than 1 week results in the same or lower state 3 respiration rates when expressed per milligram of protein than found in the corresponding control rats (46, 54, 66). However, as indicated above, when calculated per gram or per liver, an elevation of respiration is found. A summary of the time-related changes for 3, 7, 14, 20, and 35 days of DHEA treatment on hepatic mitochondrial respiration per mg protein and per liver is presented in Table II.

The effect of short-term DHEA treatment on mitochondrial respiration has been recently investigated in some detail (83). Obese rats were treated for 3, 12, or 24 hr with DHEA. The earliest metabolic response to DHEA treatment was an elevation of mitochondrial state 3 respiration seen after 3 hr. Metabolic factors shown in other experiments to respond to DHEA administration such as various enzyme activities and peroxisomal β-oxidation were not affected until later time points. These findings are summarized in Table III.

Mitochondria in brown adipose tissue have unique thermogenic capacity (84), and defects in this tissue have been implicated in the development or maintenance of obesity. It was therefore of interest to see if DHEA treatment had similar effects on brown fat mitochondria, particularly with respect to respiration rates. However, neither brown fat weight, mitochondrial protein content, or respiration (expressed per milligram of protein, per gram, or per total tissue weight) was altered by DHEA treatment (54).

In order to determine how these metabolic changes in mitochondria were mediated, DHEA was added directly to mitochondria (85). Addition of DHEA to isolated mitochondria resulted in inhibition of state 3 and 4 respiration rates over a dose of 6–200 μM with a number of substrates except succinate. This was found for mitochondria isolated from liver, heart, kidney, adrenal, brain, and brown fat. Some of these results confirmed an earlier observation by Sonka *et al.* (86) for *in vitro* effects of DHEA on heart mitochondria. These results contrast those for short-term DHEA treatment where an elevation of respiration has been found and therefore provided little help in understanding short-term effects of DHEA treatment. Evaluation of a number of other steroids suggested that this inhibition of state 3 rate was also found with some, but not all, compounds tested (85), suggesting that this phenomenon is a characteristic of some steroids.

Conclusions

In addition to a substrate cycle of deacylation/reacylation of fatty acids, substrate cycles of carbohydrate (44) and protein metabolism (46) and increased peroxisomal oxidation of fatty acids (43) have been hypothesized to play roles in DHEA's antiobesity action. However, the evidence to support the involvement of these pathways has been obtained from long-term studies where there is no way to separate out the effects of lower body weight versus the action of DHEA treatment.

To date, our findings indicate that there are time-related changes of mitochondrial respiration in DHEA-treated rats, particularly in state 3 rates. The effects on state 3 respiration can be documented after only 3 hr of DHEA treatment before changes occur in body weight or fat. How this is mediated is not presently known, but very recent data suggest that mitochondrial protein synthesis may be of importance. Changes in mitochondrial phospholipid fatty acid composition (83) have also been found which might contribute to changes in mitochondrial respiration. Investigations continue to determine what role these effects on mitochondria play in DHEA's antiobesity effect.

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