DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet

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Hansen, Polly A., Dong Ho Han, Lorraine A. Nolte, May Chen, and John O. Holloszy. DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1704–R1708, 1997.—Visceral obesity is frequently associated with muscle insulin resistance. Rats fed a high-fat diet rapidly develop obesity and insulin resistance. Dehydroepiandrosterone (DHEA) has been reported to protect against the development of obesity. This study tested the hypothesis that DHEA protects against the increase in visceral fat and the development of muscle insulin resistance induced by a high-fat diet in rats. Feeding rats a diet providing 50% of the energy as fat for 4 wk resulted in a twofold greater visceral fat mass and a 50% lower rate of maximally insulin-stimulated muscle 2-deoxyglucose (2-DG) uptake compared with controls. Rats fed the high-fat diet plus 0.3% DHEA were largely protected against the increase in visceral fat (+11.3 g in high fat vs. +2.9 g in high fat plus DHEA, compared with controls) and against the decrease in insulin-stimulated muscle 2-DG uptake (0.94 ± 0.15 µmol·min⁻¹·20 min⁻¹, controls; 0.46 ± 0.06 µmol·min⁻¹·20 min⁻¹, high-fat diet; 0.78 ± 0.07 µmol·min⁻¹·20 min⁻¹, high fat + DHEA). DHEA did not affect food intake. These results show that DHEA has a protective effect against accumulation of visceral fat and development of muscle insulin resistance in rats fed a high-fat diet.

dehydroepiandrosterone; glucose transport

SKELETAL MUSCLE IS quantitatively the most important site of insulin-stimulated glucose disposal (8, 20). Obesity is associated with a diminished ability of insulin to stimulate glucose uptake into skeletal muscle. An animal model that can be used to examine the relationship between obesity and skeletal muscle insulin action is the high-fat diet-fed rat. Numerous studies have shown that rats fed a high-fat diet develop marked skeletal muscle and whole body insulin resistance (11, 21, 24, 30). Impaired insulin action is apparent after only 3–4 wk on the high-fat diet (21, 30). We have found that after 4 wk on the high-fat diet, visceral fat is already significantly increased (unpublished results).

It has been reported that the 17-ketosteroid dehydroepiandrosterone (DHEA) reduces the accumulation of fat in various rodent models of obesity (3, 4, 7, 26, 32). Because of the poor success of programs of diet and exercise in the long-term reversal of obesity and insulin resistance, it appeared of interest to determine whether DHEA would counter these effects of a high-fat diet. Although DHEA is not normally produced by the adrenal in rats, a variety of studies have demonstrated that rat tissues are able to respond to DHEA when it is administered exogenously (1, 3–6, 22, 23, 25–27, 31).

In this context, we tested the hypotheses that 1) DHEA would protect against the increase in visceral fat induced by a high-fat diet and 2) prevention of the increase in visceral fat would protect against the development of skeletal muscle insulin resistance induced by high-fat feeding.

METHODS AND MATERIALS

All experimental procedures were approved by the Washington University Animal Studies Committee. The semipurified high-fat diet was prepared using lard, corn oil, sucrose, and casein (32, 18, 27, and 23%, respectively, of total calories), supplemented with vitamins, 22 g/kg Teklad vitamin mix no. 40077, minerals, 51 g/kg Teklad mineral mix no. 170915, and methionine, 4.4 g/kg. Energy content of this diet was calculated to be 5.1 kcal/g. Control animals were fed constant-formula Purina rodent chow (Purina no. 5001; energy content 3.3 kcal/g). DHEA was added to the diets at 0.3% (wt/wt).

Treatment of animals. At the time of weaning, colony-bred male Wistar rats were assigned to one of the following diet treatment groups: chow, chow + 0.3% DHEA, high fat, or high fat + 0.3% DHEA. Animals were housed individually. To determine food consumption, animals were given preweighed amounts of food each day (~10 g above their ad libitum intake), and the amount remaining in the food dish at the end of each 24-h period was weighed. This value was corrected for food spillage, which was collected on sheets of aluminum foil placed under their food cups.

Muscle preparation. Food was removed at 5:00 PM the day before the experiment. The following morning, rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt) and the epimysial muscles were removed. After dissection of the epimysial muscles, the soleus, plantaris, and gastrocnemius muscles were also removed, washed in saline, cleaned of visible blood and connective tissue, blotted, and weighed.

Fat pad weight. After the muscle dissection was completed, the abdominal cavity was opened, and the visceral fat was removed and weighed. For the purpose of this study, visceral fat weight was calculated as the sum of the retroperitoneal, mesenteric, and epididymal fat pad weights.

Measurement of 2-deoxyglucose transport. Glucose transport activity was measured by a slight modification of a method described previously (13). Immediately after dissection, epimysial muscles were placed in 2 ml of oxygenated Krebs-Henseleit bicarbonate buffer (KHB) containing 2 mM sodium pyruvate, 36 mM mannitol, and 0.1% radioimmunoassay-grade bovine serum albumin (BSA) (in the presence or absence of 2 mM/l purified porcine insulin, for 30 min at 30°C. Muscles were then transferred to 1.0 ml KHB containing 1 mM 2-deoxy-0-[3H]glucose (2-DG; 15 µCi/ml), 39 mM [3H]mannitol (0.2 µCi/ml), 0.1% BSA, and 2 mM/ml insulin, if it was present during the previous incubation. The flasks were incubated at 30°C with shaking for 20 min with a gas phase of 95% O2–5% CO2. The assay was terminated by blotting, then clamp freezing the muscle in tongs cooled in
liquid nitrogen. The frozen muscles were cut in half on dry ice; one portion was used for analysis of GLUT-4 glucose transporter content (described below), and the other portion was used to determine muscle extracellular space and intracellular 2-DG concentration (µmol·ml intracellular water \( \text{min}^{-1} \)) according to previously described procedures (13, 33).

Analysis of muscle GLUT-4 protein content. Epitrochlearis muscle GLUT-4 glucose transporter content was determined by Western blotting as previously described (14), using a rabbit polyclonal antibody directed against the COOH terminus of GLUT-4 (F349) followed by horseradish peroxidase-conjugated anti-rabbit immunoglobulin G. Antibody-bound transporter protein was visualized using enhanced chemiluminescence according to the manufacturer’s specifications. Films were scanned using an imaging densitometer.

Statistics. Data are presented as means ± SE. When comparing differences between two groups, statistical analysis was performed using a Student’s t-test. When multiple comparisons were made, a one-way analysis of variance was performed. Post hoc analysis was performed using Tukey’s highly significant difference method. \( P < 0.05 \) was considered to be significant.

RESULTS

Animals fed the high-fat diet tended to be heavier than those fed standard rodent chow, but this difference was not statistically significant after 4 wk on the diet (Fig. 1A). Rats fed the high-fat diet + 0.3% DHEA weighed ~15% less than those fed the high-fat diet alone (\( P < 0.05 \)). Although body weight was changed only slightly, the high-fat diet had a profound effect on visceral fat mass (Fig. 1B); the combined weight of the abdominal fat depots was twofold greater in the high fat-fed animals compared with the chow controls. However, the visceral fat mass in animals fed the high-fat diet + 0.3% DHEA was only 25% greater than that of the chow-fed controls. Average food consumption for the high fat and the high fat + DHEA groups was similar throughout the course of the study (Fig. 2). Average cumulative caloric intake for the 4 wk of the study was not significantly different between the two groups fed the high-fat diet (2,105 ± 112 kcal for fat vs. 2,087 ± 92 kcal for fat + DHEA). Thus the most likely explanation for the reduced body weight and visceral fat mass in the DHEA-treated, high-fat-fed group is a reduction in feed efficiency, i.e., the energy intake required to achieve an increase in body weight. Feed efficiency, averaged over the course of the study, was ~9 kcal/g body wt gain in the high-fat diet group versus ~12 kcal/g body wt gain in the high fat + DHEA group. Neither the fat feeding nor the DHEA had a significant effect on the weights of the soleus or plantaris muscles (Table 1). The gastrocnemius muscles were slightly but significantly smaller in the DHEA + high-fat diet group than in the group fed the high-fat diet alone; however, this difference disappeared when the muscle weights were corrected for body weight.

The effect of DHEA on basal and insulin-stimulated glucose transport activity in isolated skeletal muscle is shown in Fig. 3. The rate of maximally insulin-stimulated 2-DG transport was reduced by 50% in epitrochlearis muscles from high-fat-fed animals compared with chow-fed controls. This effect of high-fat feeding on muscle insulin responsiveness was largely prevented when DHEA was included in the diet; the rate of insulin-stimulated 2-DG transport in muscles from the high fat + DHEA-fed group was only ~17%
less than that elicited by insulin in the chow-fed controls. To evaluate the possibility that this improved insulin responsiveness was mediated by a direct effect of DHEA on muscle, an experiment was performed comparing insulin action in animals fed standard rodent chow, with or without 0.3% DHEA. After 4 wk, animals fed DHEA weighed less than control animals (242 ± 6 g for chow vs. 276 ± 6 g for chow; data are means ± SE for n = 6 per group; P < 0.05). This difference in body weight occurred despite no difference in food intake (average cumulative caloric intake: 1,936 ± 59 kcal for chow vs. 1,954 ± 65 kcal for chow + DHEA; means ± SE for n = 6 per group; P > 0.05). Basal and insulin-stimulated glucose transport activities were not different between the two groups (Fig. 4).

Epitrochlearis muscle GLUT-4 glucose transporter protein content in the three groups is shown in Fig. 5. High-fat feeding, with or without DHEA, had no effect on epitrochlearis muscle GLUT-4 protein expression.

**DISCUSSION**

The results of this study show that DHEA administration to a large extent protects against the development of visceral obesity associated with high-fat feeding. This finding is consistent with previous studies showing a potent antiobesity effect of DHEA in several rodent models of obesity (3, 4, 6, 7, 26, 32). DHEA administration reduces weight gain in young rodents by inhibiting fat accumulation (3, 4, 31, 32), whereas in adult rats, treatment with DHEA causes decreases in body fat and body weight (6, 26). This effect of DHEA is not mediated by a reduction in caloric intake (3, 31, 32). Although the exact mechanism is still unclear, it appears that, at least in rodents, DHEA prevents accumulation and/or storage of energy as body fat by increasing metabolic inefficiency, possibly via an increase in futile cycling.
(22, 25) and/or an increased flux of fatty acids through the peroxisomal β-oxidation pathway (23). In support of these possibilities, DHEA-treated rats have been reported to have a higher resting metabolic rate and heat production compared with untreated controls (1, 31).

The current findings demonstrate that DHEA can markedly ameliorate the muscle insulin resistance induced by high-fat feeding in rodents. It seems unlikely that DHEA exerts a direct effect on skeletal muscle insulin action, as feeding DHEA to animals on the standard chow diet had no effect on the insulin responsiveness of glucose transport. Instead, the present results support the interpretation that the marked attenuation by DHEA administration of the insulin resistance in the high-fat-fed rats was mediated by reduced accumulation of visceral fat. It is well documented that visceral obesity is associated with insulin resistance in humans (9, 19), and the high-fat-fed rat appears to be a useful model for the visceral obesity syndrome. There has recently been considerable interest in the possibility that hypertriglyceridemia and hyperinsulinemia can produce an "insulin resistance factor," e.g., tumor necrosis factor-α, that inhibits insulin action in peripheral tissues (15, 16). If this hypothesis is correct, it could explain why protection against the development of obesity by DHEA protects against insulin resistance. The lack of change of muscle GLUT-4 protein expression with high-fat feeding in this study is consistent with the findings of Rosholt et al. (29) and Okamoto et al. (28) showing that the decreased insulin action in skeletal muscle of Zucker rats fed a high-fat diet is independent of changes in the muscle content of the insulin-sensitive glucose transporter.

Although the effectiveness of DHEA in reducing body fat accumulation has been demonstrated in a number of animal models of obesity, the antiobesity effects of this compound have been best characterized in the genetically obese Zucker (fa/fa) rat. The fa/fa rat is a widely studied model of insulin resistance characterized by hyperinsulinemia, obesity, hyperlipidemia, and glucose intolerance (2, 17). DHEA administration reduces weight gain in young fa/fa rats by inhibiting fat accumulation (3, 4) and induces weight loss, primarily body fat, in adult fa/fa rats (6). In addition, fasting serum insulin levels in the fa/fa rats are reduced by DHEA treatment (6, 27). Despite the marked reduction in body fat, DHEA has been reported to have no effect on insulin-stimulated glucose metabolism in either adipocytes or skeletal muscle isolated from DHEA-treated fa/fa rats (5, 27). This is in marked contrast to the findings in the present study, in which attenuation of the high-fat feeding-induced increase in body fat resulted in a clear improvement in skeletal muscle insulin responsiveness of the glucose transport process. The difference between the animals on the high-fat diet and the obese Zucker rat provides evidence that the mechanisms responsible for the development of insulin resistance in these two models are different. Further evidence for this conclusion has been provided by studies showing that high-fat feeding causes resistance to activation of glucose transport by exercise as well as insulin (12, 29), whereas in the fatty Zucker, only the insulin-stimulated pathway is impaired (10, 18).

In conclusion, our results show that DHEA administration largely protects against both the accumulation of visceral fat and the development of skeletal muscle insulin resistance in rats fed a high-fat diet. Because DHEA had no effect on insulin action in muscles of Chow-fed rats, it appears likely that the reduction in visceral fat accumulation mediates the protective effect of DHEA against development of muscle insulin resistance in rats on a high-fat diet.

**Perspectives**

Visceral, i.e., abdominal, obesity and the insulin resistance associated with it are uncommon in young people and generally develop in middle age, even though caloric intake is normally lower in middle age than in youth. Plasma DHEA levels decrease markedly with advancing age and are much lower in middle-aged than in young people. In contrast to primates, rats do not produce DHEA in significant amounts. Nevertheless, as shown in numerous studies, rat tissues are responsive to DHEA administration, and the rat has been used extensively to study the effects of DHEA. It has been hypothesized that the decline in DHEA levels with advancing age in humans may play a role in the development of visceral obesity and insulin resistance. The results of the present and previous studies on rodents showing that DHEA has an energy-wasting effect that protects against development of obesity provide a rationale for conducting similar studies in older humans with visceral obesity and low DHEA levels.

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