

DHEA Treatment Reduces Fat Accumulation and Protects Against Insulin Resistance in Male Rats

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The purpose of this study was to determine whether administration of dehydroepiandrosterone (DHEA) protects male rats against the accumulation of body fat and development of insulin resistance with advancing age. We found that supplementation of the diet with 0.3% DHEA between the ages of 5 months and ≈25 months resulted in a significantly lower final body weight (DHEA, 593 ± 18 g vs control, 668 ± 12 g, p < 0.02), despite no decrease in food intake. Lean body mass was unaffected by the DHEA, and the lower body weight was due to a ≈25% reduction in body fat. The rate of glucose disposal during a euglycemic, hyperinsulinemic clamp was 30% higher in the DHEA group than in the sedentary controls due to a greater insulin responsiveness. The DHEA administration was as effective in reducing body fat content and maintaining insulin responsiveness as exercise in the form of voluntary wheel running. The DHEA had no significant effect on muscle GLUT4 content. A preliminary experiment provided evidence suggesting that muscle insulin signaling, as reflected in binding of phosphatidylinositol 3-kinase to the insulin receptor substrate-1, was enhanced in the DHEA-treated and wheel running groups as compared to controls. These results provide evidence that DHEA, like exercise, protects against excess fat accumulation and development of insulin resistance in rats.

INSULIN action, as reflected in whole body glucose disposal and in glucose transport into muscle and fat cells, decreases markedly in freely eating sedentary rats between the ages of ≈2 months and ≈6 months (1–3). It has frequently been stated that this development of insulin resistance is due to aging. However, it actually occurs during the period of rapid growth, with no further decline in insulin action attributable to aging (1–4). The development of insulin resistance in sedentary, freely eating rats, as in humans (5, pp. 654–655), appears to be at least in part due to the development of obesity (4,6).

Reducing fat accumulation by means of exercise or food restriction protects against skeletal muscle and adipocyte insulin resistance in middle aged and old rats (4,6). The adrenal steroid dehydroepiandrosterone (DHEA) has also been shown to protect against obesity in rodents (7–10), primarily due to reduced lipid accumulation or loss of body fat stores. In addition, DHEA treatment has antidiabetic effects in some strains of mice (11,12) and reduces serum insulin levels in hyperinsulinemic, diet-induced, or genetically obese rats (8–10). However, it is not known whether DHEA enhances insulin action or protects against the development of the insulin resistance associated with fat accumulation. In this context, we have examined the effect of DHEA administration between the ages of 5 months and ≈25 months on body weight, body composition, and insulin-mediated glucose disposal in freely eating male rats that were either kept sedentary or given access to voluntary running wheels.

METHODS

Animals and treatment. — Specific pathogen-free male Wistar–Long Evans hybrid rats from our rat colony were

housed in temperature- and light-controlled rooms with their own ventilation system, with 15 air changes per h, 100% intake and 100% exhaust (no recirculation), in a facility in which no other animals are housed. To avoid introducing infections into the rat colony, the people who entered the room to care for the animals did not work with other rats or in areas where they could be exposed to other rats. The animal rooms were lighted between 6:00 AM and 6:00 PM and maintained at a temperature between 18–22 °C.

At age 18 weeks, rats were assigned to either sedentary control or voluntary exercise groups. The sedentary rats were housed individually in stainless steel cages measuring 18 × 3 × 20 cm. The voluntary exercise rats were housed in identical cages and had free access to stainless steel running wheels (1.12-m circumference; Wahmann, Baltimore). A revolution counter was attached to the axle of each running wheel. After 2 weeks, half of the rats in both the exercise and sedentary groups were placed on a diet of standard Purina rodent meal (5001), while the other half were given Purina rodent meal supplemented with 0.3% dehydroepiandrosterone (DHEA; Diosynth Inc., Chicago), and water *ad libitum*.

The experimental protocol (# 91169) was approved by the Washington University Animal Studies Committee.

Euglycemic hyperinsulinemic clamp. — At ≈25 months of age (range, 24–27 months), animals were anesthetized with an intraperitoneal injection of Brevital, 6 mg/100 g body weight. Catheters were placed in the jugular vein (polyurethane, 0.025" × 0.012") and carotid artery (polyethylene, 0.038" × 0.023") at least 5 days prior to performance of the clamp to allow recovery from the stress of surgery. On the morning of the clamp, following an

overnight fast, ampicillin (5 mg/100 g body weight) was given intravenously to protect against sepsis, and heparin (50 units/100 g body weight) was given intravenously to prevent clotting of the arterial catheter during blood sampling. The animals were allowed to recover for 60 min before the initial blood sample (300 μ l) was obtained. To initiate the clamp, a priming dose of [3 H]-glucose (5 μ Ci; 1Ci = 37 GBq) was administered as an IV bolus, followed by a constant rate (.05 μ Ci/min) IV infusion delivered using a Harvard precision infusion pump. The catheters were attached to a swivel so that the rats were free to move about the cage during the infusions and blood sampling. A continuous IV infusion of insulin was then started at a rate of 4.0 milliunits/kg/min and continued for 2 h. Glucose (20%) was infused through the venous line at a variable rate to maintain blood glucose at 100 mg/dl. Blood samples (40 μ l) for measurement of plasma glucose were taken from the carotid artery catheter at 5 min intervals. Every 30 min an additional .2 ml of blood was withdrawn for determination of plasma insulin concentration and hematocrit. After 2 hr, the insulin infusion rate was increased to 72 milliunits/kg/min, the glucose concentration of the infusate was increased to 50%, and the rate of infusion adjusted to maintain blood glucose concentrations at 100 mg/dl. During the last 30 min of each stage of the clamp, blood samples (150 μ l) were taken at 10 min intervals for measurement of plasma 3 H specific activity. To avoid dilution of the blood samples with blood from the dead space in the catheter, a volume of blood, .5 ml (equivalent to four times the dead space) was drawn into a 1.0 ml syringe prior to each blood sampling. After the blood was drawn, erythrocytes from previous samples were suspended in saline and reinfused with the dead space blood. After completion of the clamp, animals were euthanized with 20 mg/100 g body weight of sodium pentobarbital given intravenously. Whole body glucose appearance and disappearance rates were calculated using Steele's equation for nonsteady state conditions (13). Hepatic glucose output was calculated as the difference between glucose appearance and glucose infusion rate.

Body composition analysis. — Rats aged \approx 25 months were killed by an overdose of pentobarbital sodium (20 mg/100 g body weight) given intraperitoneally. Hair was removed with a mixture consisting of barium sulfide (100 g), detergent (Tide, 50 g), and 10% glycerol (500 ml). Carcass analysis was performed by the method of Mickelsen and Anderson (14) with the following modifications. After homogenizing the autoclaved rat carcass in a Waring blender, a stable suspension was obtained by sonicating the homogenate (10 min). Fat extraction was performed using alcohol/ether, 3:1 (vol/vol). Protein concentration of the defatted sample was determined using the Lowry method (15). For the purpose of this study, the term *lean body mass* is defined as the total body weight minus total body fat.

Determination of IRS-1-bound phosphatidylinositol 3-kinase (PI 3-kinase). — At \approx 25 months of age, rats were anesthetized with an intraperitoneal injection of Nembutal (sodium pentobarbital, 5 mg/100 g body weight), and the

right jugular vein was exposed and catheterized. The catheter was advanced to the right atrium and sutured in place. A bolus of saline (.5 cc) was injected into the catheter, and, after 90 sec, the soleus muscle from one leg was excised and clamp frozen in liquid nitrogen. A second bolus of saline, containing insulin (10 units/kg body weight), was injected into the catheter, and after 90 sec, the other soleus muscle was excised and clamp frozen.

Frozen muscles (weighing about 200 mg) were pulverized with a mortar and pestle. The resulting powder was homogenized in 1.0 ml ice cold buffer containing 50 mM Hepes (pH 7.4), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1.0 mM EDTA, 10 mM Na₂P₂O₇, 100 mM NaF, 2.0 mM Na₃VO₄, aprotinin (10 μ g/ml), leupeptin (10 μ g/ml), pepstatin (.5 μ g/ml), and phenylmethylsulfonyl fluoride (2 mM), using Kontes glass tissue grinders. Homogenates were incubated at 4 °C with end-over-end rotation for 60 min, then centrifuged (200,000 \times g; 50 min; 4 °C). Samples (1 mg protein) were incubated overnight at 4 °C with a polyclonal anti-rat IRS-1 antibody (catalog no. 06-248; Upstate Biotechnology, Inc., Lake Placid, NY), followed by adsorption with protein A-agarose for 60 min at 4 °C. The immune pellet was washed four times with ice-cold 50 mM Hepes (pH 7.4), 150 mM NaCl, 0.5% Triton X-100, and 200 μ M Na₃VO₄. After the final wash, the immunoprecipitated proteins were solubilized in Laemmli sample buffer, subjected to SDS/PAGE (8% resolving gel), then transferred to nitrocellulose (90 min at 200 mA). PI 3-kinase protein was detected using a mAb directed against the N-terminal SH2 region of the 85-kDa regulatory subunit of the kinase (Upstate Biotechnology Inc.), followed by horseradish peroxidase-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Antibody-bound protein was visualized using enhanced chemiluminescence (ECL; Amersham Corp., Arlington Heights, IL) according to the manufacturer's specifications. Densitometry of the p85 band was carried out using a Bio-Rad GS-670 imaging densitometer and analyzed using Molecular Analyst software (Bio-Rad Laboratories, Inc., Hercules, CA).

Determination of muscle GLUT4 protein. — Plantaris muscle GLUT4 glucose transporter content was determined by Western blot analysis as described (16) using a rabbit polyclonal antibody directed against the C terminus of GLUT4 (F349; the generous gift of Mike Mueckler, Washington University, St. Louis) followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc.). Antibody-bound transporter protein was visualized using ECL according to the manufacturer's specifications. Films were analyzed by densitometry.

Analytical determinations. — Plasma glucose concentration was determined by the glucose oxidase method, using a Beckman Glucose Analyzer II (Beckman Instruments Inc., Fullerton, CA). Plasma insulin concentrations were measured by specific radioimmunoassay (17). For determination of [3 H]-glucose specific activities, 40 μ l of plasma was treated with 60 μ l of 0.3 M Ba(OH)₂ and 60 μ l 0.3 M

ZnSO₄ and centrifuged at 2,500 × g for 10 min. Duplicate aliquots of the protein-free supernatant were dried to eliminate tritiated water, and the specific activity of plasma tritiated glucose was determined by liquid scintillation counting. Muscle glycogen concentration was determined using the amyloglucosidase method (18).

Statistics. — The results are expressed as means ± SE. Comparisons were made using either a two-way analysis of variance (ANOVA) (DHEA × Exercise), or, for the euglycemic clamp study, a three-way ANOVA (DHEA × Exercise × Insulin), and Tukey's HSD (honestly significant difference) test, where appropriate. When interactions were not statistically significant, main effects were explored.

RESULTS

Body weight and wheel running performance. — As shown in Figure 1, DHEA administration resulted in a significantly lower final body weight compared to the freely eating sedentary controls. This reduction of weight gain did not become evident until after the rats had been on the DHEA supplemented diet for ≈5 months (data not shown). The magnitude of the effect of DHEA on final body weight was similar to that observed in the wheel runners (Figure 1). Interestingly, the DHEA feeding and wheel running in combination did not have an appreciably greater effect than the wheel running alone. This finding is explained by the fact that the DHEA-fed runners did considerably less voluntary running than the chow-fed runners (Figure 2). Thus, the increased energy expenditure induced by the DHEA appeared to be balanced almost exactly by a reduction in the amount of running.

Body composition. — As shown in Figure 3, both the DHEA administration and the exercise resulted in significant reductions in percent body fat. Total lean body mass

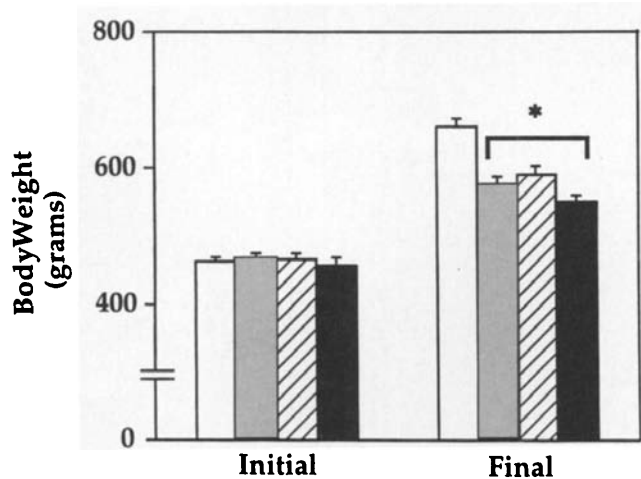


Figure 1. Effect of DHEA feeding and exercise on body weight. Values are mean ± SE for 10–16 animals per group. Initial, body weight at 4 months, prior to initiation of diet and/or wheel running; final, body weight at ≈25 months of age, at time of sacrifice. □ = chow-fed sedentary; ■ = DHEA-fed sedentary; ▨ = chow-fed runners; ■ = DHEA-fed runners. **p* < .001 for both Exercise and DHEA main effects. The DHEA × Exercise interaction was not statistically significant.

was not significantly different in the four groups. The DHEA feeding in combination with wheel running resulted in a somewhat lower total percent body fat (14.7 ± 2.2%) than that found in the chow-fed runners (17.2 ± 1.6%) and the DHEA-fed sedentary controls (18.1 ± 2.5%). However, this difference did not attain statistical significance.

Food intake. — In keeping with the results of previous studies (7,19,20), the diet containing DHEA did not result in a decrease in food intake. Cumulative food intake measured over a period of 10 weeks averaged 1,522 ± 36 g per rat in the chow-fed group and 1,584 ± 51 g per rat in the DHEA-fed group.

Euglycemic clamp. — Fasting blood glucose levels averaged ≈100 mg/dl, and were not significantly different in the four groups. Blood glucose was maintained at this level, i.e., ≈100 mg/dl, throughout the euglycemic clamp. As shown in Figure 4, during the first stage of the euglycemic

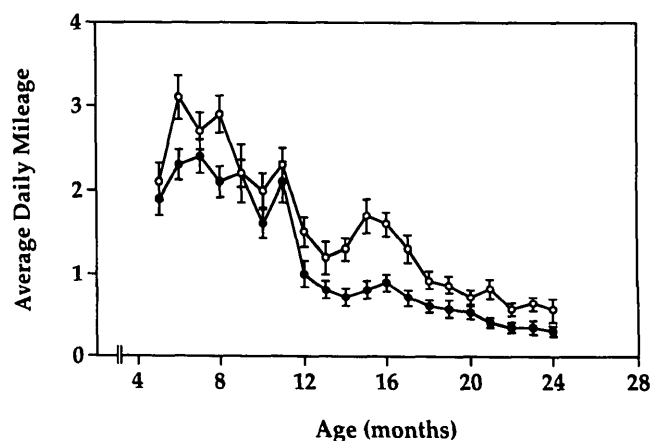


Figure 2. Effect of DHEA feeding on daily wheel running performance. Values are mean ± SE for 10 (chow) or 14 (DHEA) runners/group. ○ = chow-fed runners; ● = DHEA-fed runners. Both DHEA and Age main effects are statistically significant, *p* < .001. The DHEA × Age interaction was not statistically significant.

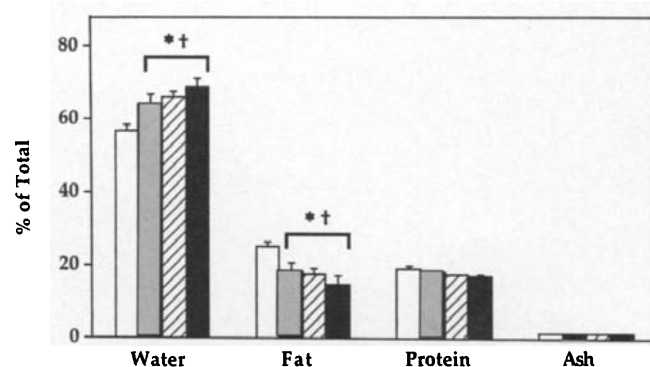


Figure 3. Effect of DHEA feeding and wheel running on body composition. Values are mean ± SE for nine rats in the chow sedentary group, and six animals in each of the three treatment groups. □ = chow-fed sedentary; ■ = DHEA-fed sedentary; ▨ = chow-fed runners; ■ = DHEA-fed runners. **p* < .05 for Exercise main effect; †*p* < .01 for DHEA main effect. The DHEA × Exercise interaction was not statistically significant.

clamp in which plasma insulin was maintained at ≈ 100 microunits/ml, the rate of glucose disposal in all three experimental groups was significantly higher ($\approx 30\%$) than in the freely eating sedentary controls. Hepatic glucose production was nearly completely shut down, averaging ≈ 1 mg/kg/min in all groups.

During the second stage of the hyperinsulinemic-euglycemic clamp, the steady state insulin levels were above 7,000 microunits/ml in all four groups; this concentration is well above that (2,000 microunits/ml) required to produce a maximal effect on glucose disposal (Han & Holloszy). Hepatic glucose production was completely shut down at this high insulin concentration. Insulin responsiveness, as reflected in the glucose disposal rates at this high insulin concentration, was also significantly higher ($\approx 30\%$) in all three experimental groups than in the sedentary chow fed controls (Figure 4). There were no significant differences in the maximally insulin stimulated rates of glucose disposal between the chow-fed runners, the DHEA-fed sedentary controls, and the DHEA-fed runners. However, although DHEA-fed sedentary animals were significantly different from chow-fed sedentary animals, DHEA-fed runners and chow-fed runners were not significantly different

from each other at either insulin concentration (DHEA \times Exercise interaction significant at $p < .01$). The finding that DHEA and exercise in combination did not have a greater effect than DHEA alone can probably be attributed to the reduced voluntary running performed by the DHEA-fed runners compared to the chow-fed runners (see Figure 2).

In view of the fact that the differences in body weights between the sedentary chow-fed controls and the three experimental groups were due to differences in body fat content, we have also expressed the glucose disposal rates per kg of lean body mass (Table 1). Expressed in this way, the rates of glucose disposal during both the first and second stages of the euglycemic clamp were $\approx 20\%$ higher in each of the three experimental groups than in the controls.

Muscle GLUT4 expression. — One possible mechanism by which the interventions might have increased insulin-stimulated glucose uptake is by an increase in skeletal muscle content of the GLUT4 isoform of the glucose transporter protein (21). However, a two-way ANOVA revealed no significant effects of either DHEA ($p = .17$) or exercise ($p = .97$) on skeletal muscle GLUT4 protein content (Figure 5).

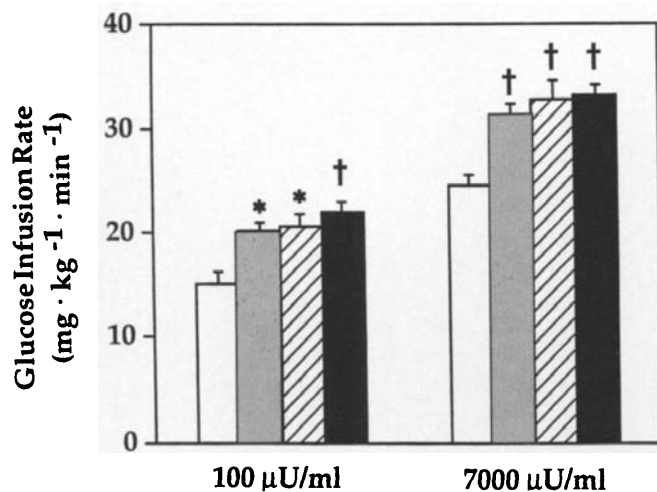


Figure 4. Rate of glucose disposal during euglycemic submaximal (≈ 100 $\mu\text{U/ml}$) and maximal (≈ 7000 $\mu\text{U/ml}$) euglycemic hyperinsulinemic clamp. Values are mean \pm SE for four to seven rats per group. \square = chow-fed sedentary; \blacksquare = DHEA-fed sedentary; \square (hatched) = chow-fed runners; \blacksquare (solid) = DHEA-fed runners. * $p < .05$; † $p < .01$ vs. chow-fed sedentary at each insulin level. DHEA \times Exercise interaction significant at $p < .01$; all other interactions were not statistically significant.

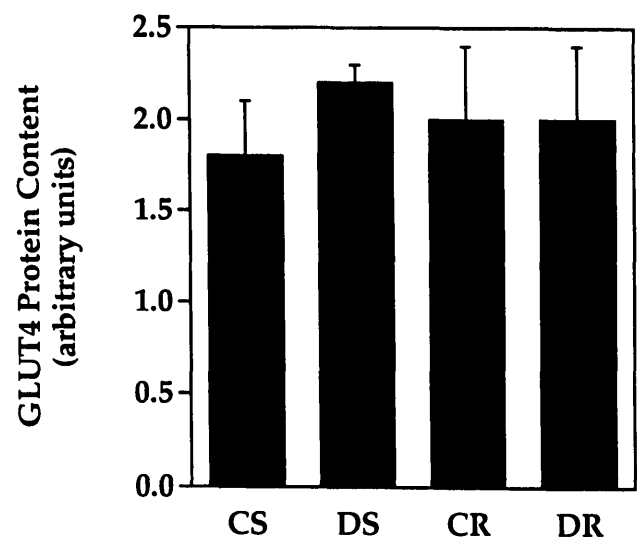


Figure 5. Effect of DHEA feeding and wheel running on skeletal muscle GLUT4 protein content. Total plantaris muscle GLUT4 protein was assessed by Western blot analysis as described under *Methods*. Values are means \pm SE for three to six muscles per group. CS, chow-fed sedentary; DS, DHEA-fed sedentary; CR, chow-fed runner; DR, DHEA-fed runner.

Table 1. Rates of Glucose Disposal During the Hyperinsulinemic, Euglycemic Clamp

Glucose Disposal Rate, mg/kg LBM (Lean Body Mass)/min	Control Sedentary	DHEA-fed Sedentary	Control-fed Runner	DHEA-fed Runner
First stage — Plasma insulin (≈ 100 microunits/ml)	21.3 \pm 1.4	25.8 \pm 1.4*	26.3 \pm 1.1*	26.8 \pm 1.2*
Second stage — Plasma insulin (≈ 7000 microunits/ml)	32.2 \pm 2.1	38.2 \pm 2.2*	38.4 \pm 1.4*	38.5 \pm 1.2*

Note: Values are means \pm SE for six or seven rats in the sedentary groups and four animals in the runner groups.

*Significantly different from control sedentary at same insulin level, $p < .05$. DHEA-fed sedentary animals are significantly different from control sedentary animals, but DHEA-fed runners and control runners are not different (DHEA \times Exercise interaction significant at $p < .01$).

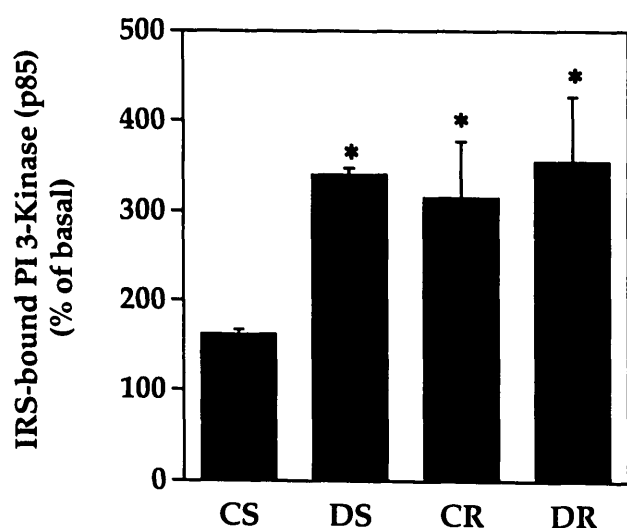


Figure 6. Effect of DHEA feeding and wheel running on insulin stimulated association of PI 3-kinase with IRS-1. IRS-1 protein was immunoprecipitated from the soluble fraction of soleus muscle homogenates prepared following in situ exposure to saline (basal) or insulin (10 U/kg body weight). The amount of p85 bound to IRS-1 was determined by Western blot analysis as described under *Methods*. Values are means \pm SE for two or three animals per group. CS, chow-fed sedentary; DS, DHEA-fed sedentary; CR, chow-fed runner; DR, DHEA-fed runner. Treatment groups were compared to controls using unpaired Student's *t* tests. * $p < .05$ vs. chow-fed sedentary.

IRS-1-bound PI 3-kinase. — One of the last known steps in the insulin signaling pathway that leads to activation of glucose transport is the binding of PI 3-kinase to phosphorylated tyrosine residues on the insulin receptor substrate-1 protein, IRS-1. The few animals that were not needed for the euglycemic clamp study were used for a preliminary evaluation of the effects of DHEA feeding and exercise on the insulin signal as reflected in the amount of PI 3-kinase associated with IRS-1. As shown in Figure 6, despite the small number of animals in each group, the three experimental groups all had significantly greater amounts of PI 3-kinase bound to IRS-1 in maximally insulin stimulated muscles.

DISCUSSION

The purpose of this study was to test the hypothesis that long-term administration of DHEA would, by protecting against body fat accumulation, maintain the insulin-responsiveness of glucose disposal at a high level even in old age. Our results, obtained on male rats treated with DHEA up to the age of ≈ 25 months, provide evidence in support of this hypothesis. In a previous study (4) it was found that regularly performed exercise that protects against development of obesity also maintains insulin action on glucose disposal at a high level in old rats. This finding was confirmed in the present study in which voluntary wheel running and DHEA had very similar beneficial effects on body fat content and insulin responsiveness.

Previous studies, most of which were relatively short-term (≤ 24 weeks), have provided evidence that DHEA administration reduces weight gain in young rodents by inhibiting fat accumulation (7,8,19,20). In addition, a loss of body weight, primarily body fat, occurs when adult rats are treated with

DHEA (9,10). This effect of DHEA is not mediated by a reduction in food intake (7,19,20, and the present study). The mechanism by which DHEA exerts its antiobesity effect is still unclear. It was originally postulated that DHEA impaired the synthesis of lipids by inhibiting glucose-6-phosphate dehydrogenase activity, thereby limiting the availability of reduced nicotinamide-adenine dinucleotide phosphate (7). However, unless enzyme activity is elevated above normal levels (e.g. in the obese Zucker rat), DHEA seems to have no consistent inhibitory effect on this enzyme (8,9,10,19). An increase in substrate (futile) cycling (22) and/or an increased flux of fatty acids through the peroxisomal β -oxidation pathway (23) have been proposed as mechanisms by which DHEA increases metabolic inefficiency, and thereby prevents accumulation/storage of energy as body fat. In support of these possibilities, DHEA-treated rats have been reported to have a higher resting metabolic rate and heat production compared to untreated controls (20).

It has frequently been stated that insulin resistance develops in rats (2,24,25) and humans (26) as a result of the aging process. However, the decrease in insulin action in sedentary freely eating rats occurs during the period of growth and development, which is also a period of fat cell hypertrophy and fat accumulation, and no further increase in insulin resistance occurs as a result of aging between the ages of ≈ 6 months and 24 months (1–4,27). In humans, the development of insulin resistance also appears to be associated with the development of obesity, particularly of the central-visceral type, rather than due to aging per se (5). In both rats and humans, avoidance of obesity, either by limiting food intake or by means of regularly performed exercise protects against the development of insulin resistance (4,5,27–30).

In very preliminary experiments to obtain information regarding the mechanisms by which reduction in body fat accumulation protects against insulin resistance, we found that there was no significant effect of DHEA on muscle GLUT4 glucose transporter content. Although it is well established that exercise training induces an increase in muscle GLUT4 (21), no such increase was seen in the current study, probably because the rats markedly reduced the amount of running they performed as they aged. We also looked at the effect of the interventions on the signal produced by a maximal insulin stimulus in skeletal muscle by measuring the amount of PI 3-kinase bound to IRS-1. Association of PI 3-kinase with tyrosyl phosphorylated IRS-1 results in activation of the kinase (31); PI 3-kinase activity is necessary for insulin stimulation of glucose transport (32). Despite the small number of animals per group, there was a significantly greater effect of insulin on the binding of PI 3-kinase to IRS-1 in muscles of the three experimental groups, suggesting that enhanced insulin signaling is involved in the greater insulin responsiveness of the DHEA-treated and wheel running groups.

The results of the present study provide evidence that administration of DHEA is as effective as exercise or food restriction in protecting against excess fat accumulation and development of insulin resistance in male rats. There is currently not sufficient information available regarding the effects of long-term DHEA treatment in humans to speculate

regarding whether or not this is a feasible approach to preventing obesity and insulin resistance in patients. However, the possibility appears worthy of investigation.

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