Effect of Dehydroepiandrosterone on Glucose Uptake in Cultured Human Fibroblasts

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Dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEA-S) reportedly have antidiabetic and antiobesity effects. The effect of DHEA on glucose uptake in cultured human fibroblasts was examined. Incubation of cells with supraphysiologic concentrations of DHEA (10^{-5} mol/L) for ≥10 hours enhanced 2-deoxyglucose (2-DG) uptake significantly ($P < .05$). Supraphysiologic concentrations of insulin (10^{-7} mol/L) increased the sensitivity of glucose uptake to DHEA. Conversely, the sensitivity of glucose uptake to insulin was increased by incubating cells with 10^{-6} mol/L DHEA. Both the abundance of transcripts encoding glucose transporter-1 (Glut-1) and the maximal velocity (Vmax) of 2-DG transport were increased in cultured fibroblasts incubated with DHEA. Cultured fibroblasts expressed a specific binding factor with low affinity for [^{3}H]DHEA (maximal number of binding sites, 18,496 sites per cell; K_d, 298 nmol/L). Other androgen hormones exerted a less-marked effect on glucose uptake; DHEA-S had no effect. These results suggested that DHEA increases Glut-1 mRNA through binding to a specific factor in cultured human fibroblasts and thereby stimulates glucose uptake in these cells.

THE ADRENAL ANDROGEN dehydroepiandrosterone ([DHEA] 3β-hydroxy-5-androsten-17-one) and its sulfate derivative (DHEA-S) are produced in abundance in the human body,[1] although their physiologic roles are unknown. The concentrations in serum reach a peak between the ages of 25 and 30 years and thereafter decline steadily, so that by age 60 serum concentrations are only 5% to 10% of the corresponding values in young adults.[1] This decrease in serum concentrations of DHEA and DHEA-S occurs as the incidence of atherosclerosis, obesity, and diabetes increases, suggesting that higher concentrations of DHEA or DHEA-S may protect against the development of these conditions.[2,3]

Studies on the effects of DHEA in animals show that it reduces body mass without affecting appetite or food intake,[4,5] prevents the development of diabetes in genetically diabetic (-db/db) mice[6] or obese (-ob/ob) rats,[7] increases tissue sensitivity to insulin in aged normal mice,[8] and improves the serum lipid profile and retards the development of atherosclerosis.[5,9-11] The effects of DHEA administration in humans remain controversial; thus, DHEA has been shown to have an antiobesity effect in vivo,[2] to increase insulin binding to T lymphocytes from obese women in vitro,[12] to have no effect on glucose disposal in lean men,[13] and to decrease insulin sensitivity after oral administration in postmenopausal women.[14]

We investigated the effects of DHEA and DHEA-S on glucose uptake in cultured human skin fibroblasts, and also characterized the specific DHEA binding sites in these cells in an attempt to determine how this agent exerts its biological effects.

MATERIALS AND METHODS

Cell Culture

Human diploid fibroblasts were obtained from the forearm skin of healthy non-obese volunteers (three men and three women aged 20 to 40 years) as described previously.[15] Cells were cultured in minimal essential medium (MEM) containing 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in an atmosphere of 95% room air and 5% carbon dioxide. The medium was replaced twice weekly. At confluence, cells were exposed to 0.05% trypsin in 0.02% EDTA and then seeded in plastic dishes (Falcon 3001, Becton Dickinson, Lincoln Park, NJ). Cells from the 10th to 20th passages were used in the experiments, and fibroblasts from at least three volunteers were represented in each experiment.

2-Deoxyglucose Uptake Assay

Cultured fibroblasts were incubated in FBS-free MEM for 24 hours, at which time the cells had just achieved confluence, and the medium was then changed to MEM containing 5% dextran-charcoal–treated FBS and DHEA, DHEA-S, testosterone, 17β-estradiol, δ^4-androstenedione, or dexamethasone (all steroids were from Sigma, St Louis, MO). After incubation for 3 to 48 hours, cells were washed and then incubated for 2 hours in glucose-free Dulbecco's phosphate-buffered saline ([DPBS] pH 7.4) containing 0.1% bovine serum albumin (Sigma) and various concentrations (0 to 10^{-7} mol/L) of human insulin (Sigma). Cells were finally incubated for an additional 5 to 30 minutes with 0.1 mmol/L 2-deoxyglucose (2-DG) containing 2-deoxy-D-[1,2-^{3}H(*N*)]glucose (0.2 μCi/mL, 26.2 Ci/mmol, New England Nuclear, Boston, MA). The cells were then quickly washed three times with ice-cold DPBS and solubilized with 1 mol/L NaOH. Portions of cell lysate were assayed for protein and for radioactivity with an LSC-700 liquid scintillation counter (Aloca, Tokyo, Japan). All cell incubations were performed at 37°C. Cell number and morphology were not affected even after the longest incubations with the maximal concentration of DHEA and insulin.

Whole-Cell [^{3}H]DHEA Binding Assay

[^{3}H]DHEA (114.7 Ci/mmol, New England Nuclear) binding was measured by a modified version of a whole-cell assay described previously.[16] Fibroblasts were grown to confluence in Falcon 3001 dishes. One day before performing the assay, the medium was replaced with FBS-free MEM. Triplicate dishes were incubated with 10 nmol/L [^{3}H]DHEA in the presence of 0 to 10 μmol/L unlabeled DHEA for 3 hours at 25°C with gentle shaking. Cells were harvested by incubation with 0.05% trypsin and 0.02% EDTA at 37°C, washed three times with 2 mL DPBS, and then suspended

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in 0.2 mL DPBS. One milliliter of absolute ethanol was added to the cell suspension, and portions were then assayed for radioactivity with the scintillation counter. Results were analyzed using the method reported by Scatchard.[17]

Northern Blotting of Glut-1 mRNA

After incubation with or without 10^{-4} mol/L DHEA in MEM containing 5% dextran-charcoal–treated FBS for 24 hours at 37°C, cells were scraped off the dish with a rubber policeman and harvested quickly for determination of Glut-1 mRNA. Total cellular RNA was extracted according to the manufacturer's instructions (Wako Chemical, Tokyo, Japan). Namely, after extraction with chloroform, precipitation in isopropanol, and successive washes with 75% ethanol, the pellet was dissolved in water treated with diethylpyrocarbonate (Sigma). Thirty micrograms of total cellular RNA were denatured with 1 mol/L glyoxal and 50% (vol/vol) dimethyl sulfoxide, subjected to electrophoresis on a 1.0% agarose gel, and transferred to a nitrocellulose filter. The transferred RNA was hybridized with a rabbit Glut-1 cDNA probe labeled with [α-^{32}P]cytidine triphosphate (2,000 Ci/mmol, New England Nuclear) with the use of a random multipriming kit. The filters were washed and exposed to Konika x-ray film with an intensifying screen.

Statistical Analysis

Data are expressed as the mean ± SE. Statistical analyses were performed by ANOVA followed by analysis using Scheffe's method. P less than .05 was considered statistically significant.

RESULTS

Fibroblasts that had been incubated in either the absence or presence of 10^{-5} mol/L DHEA for 24 hours incorporated 2-DG steadily between 5 and 30 minutes after addition of the sugar (Fig 1). However, cells that had been incubated with DHEA showed a significantly enhanced rate of 2-DG uptake at 20 and 30 minutes. Whereas incubation of cells with 10^{-4} mol/L DHEA for 3 hours had no effect on 2-DG uptake by fibroblasts, cells incubated with DHEA for ≥10 hours maintained significantly higher rates of 2-DG uptake than cells without DHEA (Fig 2). Thus, subsequent 2-DG uptake assays were performed with DHEA and 2-DG incubation periods of 24 hours and 20 minutes, respectively.

DHEA enhanced 2-DG uptake in a dose-dependent manner in cells incubated in either the absence or presence of 10^{-7} mol/L insulin (Fig 3). Insulin significantly increased (P < .01) 2-DG uptake in the absence of DHEA. Cells

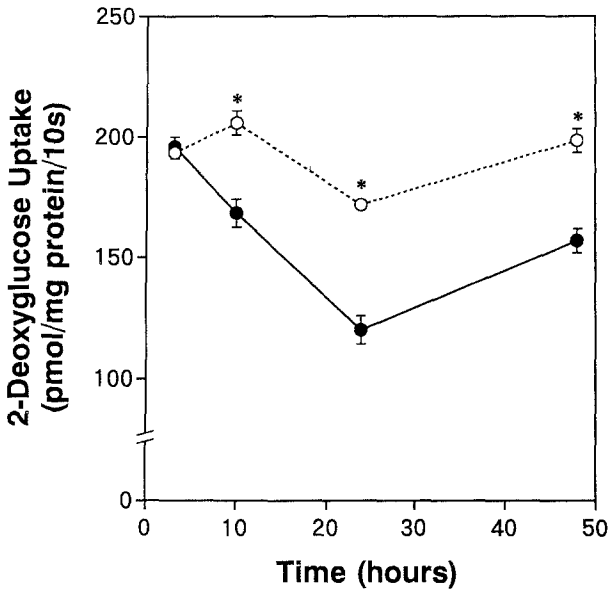

Fig 2. Effect of time of incubation with (○) or without (●) 10^{-4} mol/L DHEA on 2-DG uptake in cultured human fibroblasts. Data are the mean ± SE (n = 4). *P < .001 v without DHEA.

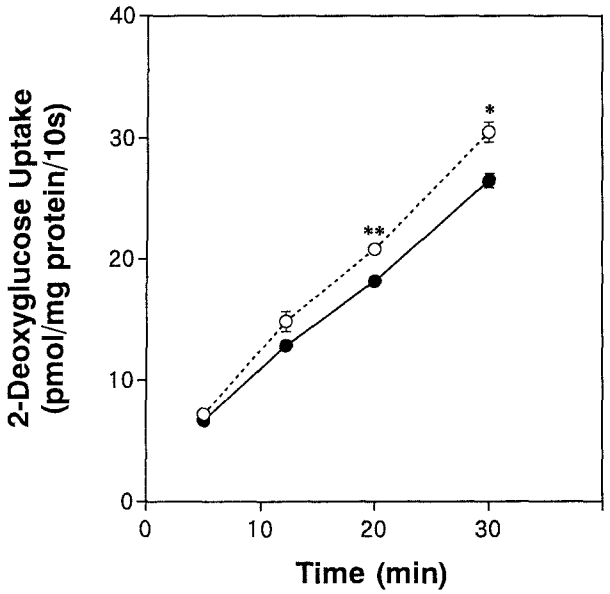

Fig 1. Effect of DHEA on the time course of 2-DG uptake in cultured human fibroblasts. Cells were incubated with (○) or without (●) 10^{-5} mol/L DHEA for 24 hours before measurement of 2-DG uptake. Data are the mean ± SE (n = 3). *P < .05, **P < .01 v without DHEA.

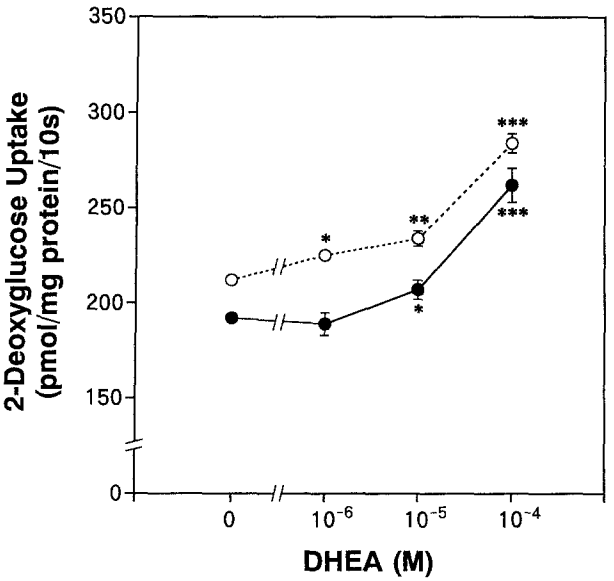

Fig 3. Effect of DHEA concentration on 2-DG uptake in cultured human fibroblasts incubated in the absence (●) or presence (○) of 10^{-7} mol/L human insulin. Data are the mean ± SE (n = 3). *P < .05, **P < .01, ***P < .001 v without DHEA.

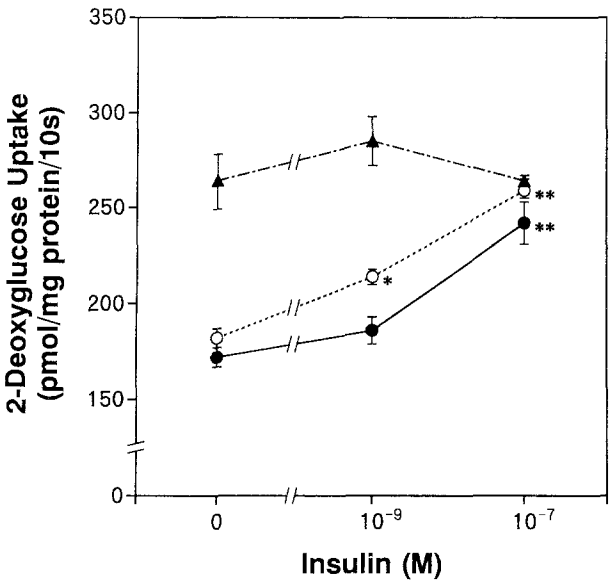

Fig 4. Effect of insulin concentration on 2-DG uptake in cultured fibroblasts incubated either in the absence (●) or in the presence of 10^{-6} mol/L (○) or 10^{-4} mol/L (▲) DHEA. Data are the mean ± SE (n = 4). *$P < .05$, **$P < .001$ *v* without insulin.

incubated in the presence of insulin showed greater 2-DG uptake at lower concentrations of DHEA than cells incubated in the absence of insulin.

Insulin also increased 2-DG uptake in a dose-dependent manner in fibroblasts not previously exposed to DHEA (Fig 4). Whereas the effect of 10^{-9} mol/L insulin on 2-DG uptake was not significant in cells not exposed to DHEA, the increase achieved statistical significance ($P < .05$) in cells that had been incubated with 10^{-6} mol/L DHEA; that is, DHEA increased the sensitivity of cells to insulin with regard to 2-DG uptake. Insulin, even at 10^{-7} mol/L, did not stimulate 2-DG uptake in cells incubated with 10^{-4} mol/L DHEA, probably because uptake was already maximal.

Uptake of 2-DG was dose-dependent in fibroblasts that had been incubated with or without 10^{-4} mol/L DHEA in the absence of insulin (data not shown). In reciprocal plot analysis, although the K_m for 2-DG uptake was not decreased by incubation of cells with DHEA (K_m: 2.48 mmol/L without DHEA, 4.27 with 10^{-4} mol/L DHEA), the maximal velocity (Vmax) of uptake was increased from 2.35 pmol/mg protein/10 s without DHEA to 4.78 with 10^{-4} mol/L DHEA, suggesting an increased number of glucose transporters in the plasma membrane. The same experiment was performed on a separate occasion with similar results. The similarity of the Vmax and K_m values between this study and a previous study of glucose uptake in cultured human fibroblasts[18] suggests that glucose uptake in our study was glucose transporter–dependent, although we did not use cytochalasin B to demonstrate this. We next evaluated the influence of DHEA on Glut-1 mRNA. Northern blot analysis of Glut-1 mRNA from cultured human fibroblasts showed a single band of 2.9 kb (Fig 5), as previously described.[19] Incubation of cells with 10^{-4} mol/L DHEA for 24 hours increased Glut-1 mRNA 1.5-fold, although expression of β-actin mRNA was not altered.

To determine whether stimulation of 2-DG uptake by DHEA was an effect shared by other androgen hormones, we measured 2-DG uptake in fibroblasts exposed to various steroid hormones at 10^{-4} mol/L (Fig 6). 2-DG uptake was 30.3% greater in cells incubated with DHEA (202.0 ± 9.4 pmol/mg protein/10 s, mean ± SE, n = 3, $P < .001$) than in cells incubated without steroid hormone (155.0 ± 6.5 pmol/mg protein/10 s). δ^4-Androstenedione and testosterone enhanced 2-DG uptake by 20.1% (186.1 ± 6.1 pmol/mg protein/10 s) and 16.3% (180.3 ± 4.2), respectively. In contrast, DHEA-S and 17β-estradiol had no effect on 2-DG uptake, and dexamethasone inhibited this parameter by 12.7% (135.3 ± 2.8).

Finally, we measured [^{3}H]DHEA binding in cultured fibroblasts. The maximal number of specific [^{3}H]DHEA binding sites (Bmax) was 18,496 sites per cell (307 fmol/10^5 cells); however, these sites were of relatively low affinity (K_d, 298 nmol/L) (Fig 7).

DISCUSSION

Hyperandrogenism in women is clearly associated with insulin resistance, as observed in non-obese women with the polycystic ovarian syndrome.[12,20] In addition, administration of anabolic steroids to women is followed by signs of insulin resistance.[21] Women with abdominal obesity also

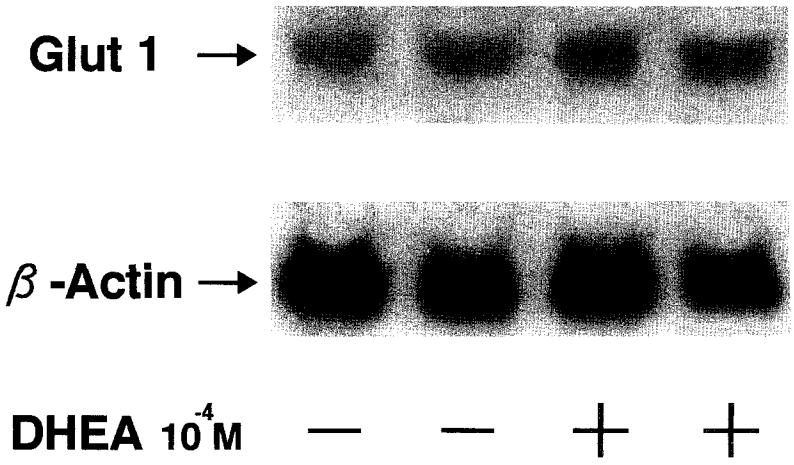

Fig 5. Northern blot analysis of total cellular RNA from cultured human fibroblasts. Total RNA (30 γ) from cells incubated in the absence (control) or presence of 10^{-4} mol/L DHEA for 24 hours was resolved on a 1% agarose gel and hybridized with ^{32}P-labeled rabbit Glut-1 cDNA or ^{32}P-labeled β-actin cDNA.

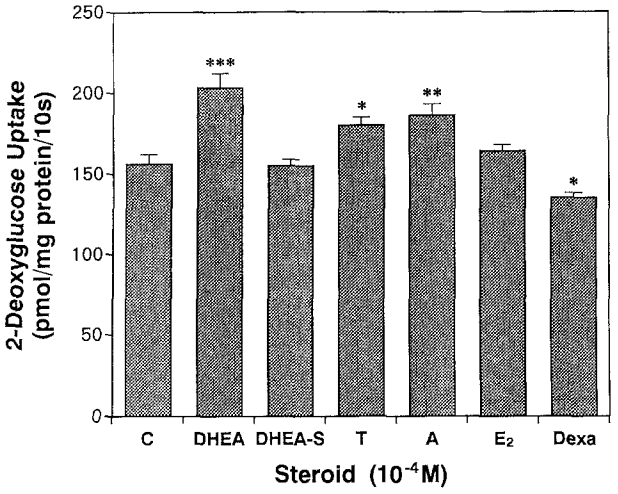

Fig 6. Effects of various steroid hormones (10^{-4} mol/L) on 2-DG uptake in cultured human fibroblasts. C, control; T, testosterone; A, δ^4-androstenedione; E_2, 17β-estradiol; and Dexa, dexamethasone. Data are the mean ± SE (n = 3). *$P < .05$, ** $P < .01$, ***$P < .001$ v control.

have increased serum concentrations of free testosterone and are insulin-resistant.[22] However, serum concentrations of DHEA and DHEA-S, which are adrenal androgens, show an opposite trend, being negatively correlated with serum insulin concentration and positively correlated with insulin binding in women.[23] In individuals with adrenal hyperplasia whose serum DHEA concentration was increased, insulin sensitivity was enhanced[12] or reduced.[24] Thus, these adrenal androgens seem to have certain salutary effects on glucose metabolism; however, the mechanisms are unknown, and the effect of DHEA on insulin action remains controversial.

We now describe a stimulatory effect of DHEA on glucose uptake in cultured human fibroblasts. This effect

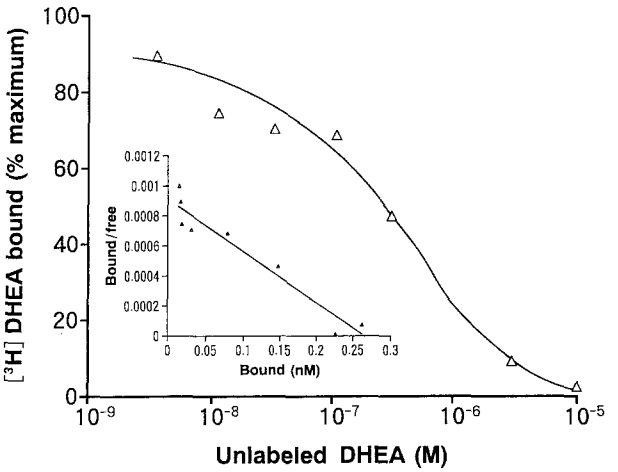

Fig 7. Inhibition of [^{3}H]DHEA binding by unlabeled DHEA in cultured human fibroblasts. Insert shows Scatchard analysis of DHEA binding, with a K_d of 298 nmol/L and a Bmax of 18,496 sites per cell (307 fmol/10^5 cells).

appears to be relatively specific for DHEA among androgen hormones and is abolished by sulfation of DHEA. These results suggest that DHEA acts on fibroblasts directly rather than as a precursor of estrogen or other androgens. The serum concentration of DHEA in healthy humans is generally between 10^{-9} and 10^{-7} mol/L.[1] Although the stimulatory effect of DHEA on glucose uptake in the absence of insulin was observed at pharmacologic concentrations of DHEA ($\geq 10^{-5}$ mol/L), in the presence of insulin, stimulation of glucose uptake was apparent at lower concentrations of DHEA (10^{-6} mol/L). Thus, under physiologic conditions, the sensitivity of glucose uptake to DHEA may be greater than that observed in vitro. Interestingly, 10^{-6} mol/L DHEA increased the sensitivity of glucose uptake to insulin. This synergism of DHEA and insulin is consistent with the observation that insulin binding to T lymphocytes from individuals with polycystic ovarian syndrome and simple obesity was increased by incubation with DHEA.[12] On the other hand, in lean healthy men, Nestler et al[13] observed no change in glucose disposal after DHEA treatment; and in postmenopausal women, Mortola and Yen[14] demonstrated deterioration of insulin sensitivity together with increased serum testosterone and dihydrotestosterone concentrations after oral administration of DHEA. Thus, in contrast to experimental animals, the effects of DHEA on in vivo glucose metabolism and insulin sensitivity in humans are controversial. Changes in other androgens after DHEA administration seemed to influence glucose metabolism and insulin sensitivity in previous studies[8,22,24]; such changes may have greater effects in humans than in animals. DHEA analogs (16α-fluoro-5-androsten-17-one) and metabolites (etiocholanolones) that lack the androgenic action have antidiabetic effects in mice.[25,26] Thus, administration of these agents to humans may clarify the effects of DHEA on glucose metabolism and insulin sensitivity.

We showed that the Vmax of glucose transport was increased by incubation of fibroblasts with DHEA, even though the K_m was not decreased. Thus, the number of glucose transporters in the plasma membrane may be increased by DHEA, leading to enhanced glucose uptake. A major portion of insulin-sensitive glucose transport in peripheral tissues, especially in muscle and adipose tissue, is mediated by the Glut-4 transporter. However, Glut-4 mRNA is present in small amounts in cultured human fibroblasts,[27] and Glut-1 is thought to contribute significantly to glucose transport in the basal state and to translocate from cytosol to plasma membrane in response to insulin stimulation of these cells.[19,28] The amount of Glut-1 mRNA in cultured fibroblasts was increased by incubation of cells with 10^{-4} mol/L DHEA. This increase in Glut-1 mRNA may result in an increased number of glucose transporters in fibroblasts. An increase in glucose uptake was observed in fibroblasts incubated with DHEA for more than 10 hours, but not in cells exposed to DHEA for only 3 hours. We anticipate that an increase in Glut-1 protein subsequent to the DHEA-induced increase in Glut-1 mRNA requires greater than 3 hours. Insulin also

increases Glut-1 mRNA in cultured fibroblasts; however, the response was observed to be maximal within 3 hours, and basal levels were restored by 24 hours.[29]

Although we detected specific binding of DHEA in cultured fibroblasts, the affinity of the binding sites was relatively low. Classic steroid receptors have a high affinity for ligand and often regulate the expression of specific genes by binding to DNA regulatory elements[30]; such a receptor might not exist for DHEA. Although the mechanisms of DHEA action are unknown, DHEA may affect a factor that regulates glucose transporter expression and glucose metabolism.

Fink et al[31] showed that aging in humans is associated with a significant postreceptor defect in in vivo insulin action and in the glucose transport system in isolated adipocytes. Our in vitro study with supraphysiologic concentrations of DHEA suggests that the decrease in serum DHEA concentration with age might induce this postreceptor insulin resistance. Further in vivo studies, including glucose-clamp experiments and DHEA administration to diabetic subjects, are necessary to elucidate the effect of DHEA on glucose metabolism.

In conclusion, DHEA enhanced glucose uptake in cultured human fibroblasts. Insulin increased the sensitivity of glucose uptake to DHEA. Conversely, the sensitivity of glucose uptake to insulin was increased by DHEA. The abundance of Glut-1 mRNA and the Vmax of glucose transport were both increased by DHEA. The stimulatory effect of DHEA on glucose uptake may be mediated by a binding factor that exhibits a low affinity for DHEA yet is relatively specific for this adrenal androgen.

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