

Dehydroepiandrosterone inhibits the amplification of glucocorticoid action in adipose tissue

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Apostolova, Galina, Roberto A. S. Schweizer, Zoltan Balazs, Radina M. Kostadinova, and Alex Odermatt. Dehydroepiandrosterone inhibits the amplification of glucocorticoid action in adipose tissue. *Am J Physiol Endocrinol Metab* 288: E957–E964, 2005. First published December 21, 2004; doi:10.1152/ajpendo.00442.2004.—Dehydroepiandrosterone (DHEA) exerts beneficial effects on blood glucose levels and insulin sensitivity in obese rodents and humans, resembling the effects of peroxisome proliferator-activated receptor- γ (PPAR γ) ligands and opposing those of glucocorticoids; however, the underlying mechanisms remain unclear. Glucocorticoids are reactivated locally by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which is currently considered as a promising target for the treatment of obesity and diabetes. Using differentiated 3T3-L1 adipocytes, we show that DHEA causes downregulation of 11 β -HSD1 and dose-dependent reduction of its oxoreductase activity. The effects of DHEA were comparable with those of the PPAR γ agonist rosiglitazone but not additive. Furthermore, DHEA reduced the expression of hexose-6-phosphate dehydrogenase, which stimulates the oxoreductase activity of 11 β -HSD1. These findings were confirmed in white adipose tissue and in liver from DHEA-treated C57BL/6J mice. Analysis of the transcription factors involved in the DHEA-dependent regulation of 11 β -HSD1 expression revealed a switch in CCAAT/enhancer-binding protein (C/EBP) expression. C/EBP α , a potent activator of 11 β -HSD1 gene transcription, was downregulated in 3T3-L1 adipocytes and in liver and adipose tissue of DHEA-treated mice, whereas C/EBP β and C/EBP δ , attenuating the effect of C/EBP α , were unchanged or elevated. Our results further suggest a protective effect of DHEA on adipose tissue by upregulating PPAR α and downregulating leptin, thereby contributing to the reduced expression of 11 β -HSD1. In summary, we provide evidence that some of the anti-diabetic effects of DHEA may be caused through inhibition of the local amplification of glucocorticoids by 11 β -HSD1 in adipose tissue.

11 β -hydroxysteroid dehydrogenase; adipocyte; obesity; diabetes

THE ELEVATED PRODUCTION OF GLUCOCORTICOIDS and activation of glucocorticoid receptor (GR) lead to increased gluconeogenesis and antagonize the metabolic actions of insulin. In visceral adipose tissue, excessive glucocorticoids further enhance lipolysis, leading to high levels of free fatty acids, ultimately resulting in the development of insulin resistance and type 2 diabetes (21). 11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD1) plays a crucial role in reactivating inactive glucocorticoids (11-dehydrocorticosterone in rodents, cortisone in humans) to their active forms (corticosterone in rodents, cortisol in humans) (46, 51).

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The importance of the local activation of glucocorticoids in adipose tissue has been demonstrated recently in transgenic mice overexpressing 11 β -HSD1 under the adipose-specific AP2 promoter/enhancer (30). These mice expressed approximately threefold higher 11 β -HSD1 levels than normal mice. They developed the full metabolic syndrome characterized by visceral obesity, insulin-resistant diabetes, hyperlipidemia, and high arterial blood pressure due to an increased sensitivity to dietary salt and increased plasma levels of angiotensinogen, angiotensin II, and aldosterone (30, 31). Circulating corticosterone concentrations were normal but adipose corticosterone concentrations elevated. The enhanced adipose glucocorticoid production resulted in an increased corticosterone delivery to the liver. In contrast, mice deficient in 11 β -HSD1 exhibited improved insulin sensitivity and glucose tolerance, had a protective lipid and lipoprotein profile, and showed reduced visceral fat accumulation on high-fat feeding (22, 34). Adipocytes from these mice exhibited elevated basal and insulin-stimulated glucose uptake (35). The important role of the local activation of glucocorticoids by 11 β -HSD1 in adipose tissue is supported by the increased expression of 11 β -HSD1 found in subcutaneous adipose tissue from individuals with acquired obesity and by its positive correlation with the accumulation of subcutaneous and intra-abdominal fat, body mass index, percentage of body fat, waist circumference, as well as fasting glucose, insulin levels, and insulin resistance (17, 26, 42, 45, 56).

The role of 11 β -HSD1 in the liver was investigated by transgenic overexpression using the apoE promoter, yielding mice with two- and fivefold increased hepatic expression levels compared with normal mice (41). These mice represent a model of metabolic syndrome but without obesity and lacking glucose intolerance. Increased hepatic lipid production and impaired lipid clearance result in dyslipidemia and fatty liver in these animals. In addition, a mild insulin resistance and a transgene dose-associated hypertension with elevated hepatic angiotensinogen expression were observed.

Recently, it was shown that the administration of an arylsulfonamidothiazole compound, selectively inhibiting 11 β -HSD1, reduced blood glucose levels and increased insulin sensitivity in diabetic mice (1, 2, 6). Preliminary evidence suggests that this compound may inhibit 11 β -HSD1 in liver and adipose tissue. Treatment with the nonselective inhibitor carbenoxolone was shown in several *in vivo* studies both in animals and humans to improve hepatic insulin sensitivity with no significant effect on systemic glycemia. In these studies, administration of carbenoxolone resulted in reduced hepatic 11 β -HSD1 expression and/or activity, whereas such an effect

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in adipose tissue was not observed or not evaluated (3, 29, 47). The present findings from studies with transgene animals and inhibitors suggest that a reduced local activation of glucocorticoids in adipose tissue by reducing 11 β -HSD1 activity might have beneficial effects in obese and diabetic individuals.

The steroid precursors dehydroepiandrosterone (DHEA) and its sulfated ester (DHEA-S), the most abundant circulating adrenal steroid hormone in humans, are converted by tissue-specific steroidogenic enzymes into active androgens and estrogens in peripheral target tissues (23). Serum concentrations of DHEA and DHEA-S significantly decline with age, and the deficiency of these steroids has been associated with the increasing prevalence of loss of cognitive memory, obesity, insulin resistance, and cardiovascular disease (54). Several studies demonstrated a significant negative association between DHEA and the accumulation of abdominal fat, suggesting that low circulating DHEA is associated with a more central distribution of adipose tissue (8, 9, 53). However, the mechanisms linking obesity, body fat distribution, and serum DHEA or DHEA-S remained unclear. Moreover, several studies reported beneficial effects of dietary administration of DHEA, including effects on cognitive function, immune system, and anti-diabetic effects (54). Many of the anti-diabetic effects described resemble those after treatment with peroxisome proliferator-activated receptor- γ (PPAR γ) agonists including increased insulin sensitivity, lower serum cholesterol and triglycerides, decreased stress-induced hyperglycemia, and increased cognitive memory.

Recently, it was shown that the PPAR γ -dependent increase in subcutaneous fat and reduction in visceral fat are accompanied by a reduced expression of 11 β -HSD1 in visceral adipose tissue, whereas 11 β -HSD1 mRNA levels were not changed in subcutaneous adipose tissue (24). Thus a decreased glucocorticoid level in visceral adipose tissue may contribute to the insulin-sensitizing effects on activation of PPAR γ . A DHEA-dependent reduction in hepatic 11 β -HSD1 activity has been indicated in spontaneously hypertensive rats after treatment with DHEA-S (15). Very recently, a cDNA array analysis of altered gene expression in livers from rats treated with DHEA revealed, among several other genes, the reduced expression of 11 β -HSD1 (11). Moreover, incubation with 500 nM DHEA, but not with the potent androgens testosterone and androstenedione, attenuated 11 β -HSD1 expression in cultured skeletal myoblasts (58). Despite the opposite effects of glucocorticoids and DHEA on body fat distribution, gluconeogenesis, and insulin sensitivity, and a potential relationship between DHEA treatment and 11 β -HSD1 expression in other tissues, the effect of DHEA on 11 β -HSD1 expression and activity in adipose tissue has not been addressed to date.

Therefore, we investigated the effect of DHEA on 11 β -HSD1 expression in 3T3-L1 adipocytes as well as in white adipose tissue and in liver from C57BL/6J mice. We also compared the effects of DHEA and rosiglitazone in white adipose tissue and in liver and investigated the expression levels of several key regulators of 11 β -HSD1.

MATERIALS AND METHODS

Materials. Cell culture media were purchased from Invitrogen (Carlsbad, CA) and the 3T3-L1 cell line from American Type Culture Collection (Manassas, VA). DHEA, corticosterone, and 11-dehydrocorticosterone were from Steraloids (Wilton, NH). Rosiglitazone was

extracted from Avandia tablets (GlaxoSmithKline) using methanol. [1,2,6,7- 3 H]corticosterone was from Amersham Biosciences (Piscataway, NJ); 11-[1,2,6,7- 3 H]dehydrocorticosterone was prepared from radiolabeled corticosterone as described previously (37). Reagents for real-time RT-PCR were purchased from Applied Biosystems (Foster City, CA).

Animals and treatments. Written approval for the present experiments was obtained from the Ethical Committee for Animal Research of the University of Berne, Switzerland. Male C57BL/6J mice (20–22 g body wt; Charles River, France) were housed in groups of four in a room maintained on a 12:12-h light-dark cycle. Animals were transferred to individual cages for 2 h and allowed access to 5 g of standard rodent chow. Water was ad libitum. After an adaptation period of 5 days, groups of eight animals were fed for 12 days with control chow or with chow containing either 0.2% DHEA, rosiglitazone at a final dose of 10 mg/kg body wt, or both. Body weight and food and water intakes were recorded daily. One animal of the DHEA group died for unknown reasons, and one animal of the DHEA/rosiglitazone group ate only half of the chow and was excluded from the study. All other animals ate all of the food. Their body weight was comparable and no significant changes were observed during the time of experiment. After 12 days of treatment, mice were killed and the liver and epididymal tissue were removed, washed from contaminating blood with phosphate-buffered saline, blotted on filter paper, snap-frozen in liquid nitrogen, and stored at -70°C until analysis. Serum levels of DHEA-S were measured using the chemiluminescence immunoassay kit and the Immulite One apparatus following the manufacturer's instruction (Diagnostics Products, Los Angeles, CA).

Cell culture. Murine 3T3-L1 cells were cultured in DMEM supplemented with 10% FCS under nondifferentiating and differentiating conditions in a humidified incubator at 5% CO_2 and 37°C as described previously (49). Briefly, preadipocytes were allowed to achieve 2-day postconfluence and were then subjected to differentiation medium for 2 days (DMEM, 10% FCS, 0.25 mM 3-isobutyl-1-methylxanthine, 0.5 μM dexamethasone, 1 $\mu\text{g}/\text{ml}$ insulin). The medium was then replaced with adipocyte growth medium (DMEM, 10% FCS, 1 $\mu\text{g}/\text{ml}$ insulin) and 3 days later with DMEM and 10% FCS but in the absence of insulin.

RNA isolation and analysis. Total RNA was extracted from adherent cultured 3T3-L1 cells or from animal tissues after pulverization in liquid nitrogen, homogenization, and lysis as described previously (4). The mRNA levels from different genes were analyzed using an ABI7000 Sequence Detection System. Briefly, reactions were performed in 25- μl vol on 96-well plates, in reaction buffer containing TaqMan Universal PCR Master Mix and 100 ng of cDNA. Specific primers and sequence probes for each gene were obtained as assay-on-demand gene expression products [11 β -HSD1, PPAR γ coactivator 1 (PGC-1), PPAR α , PPAR γ , CCAAT enhancer-binding protein (C/EBP α), C/EBP δ , liver X receptor (LXR), leptin, H6PDH] or designed using Primer Express software (C/EBP β : forward, 5'-GCCTGG-GACGCAGCAT-3'; reverse, 5'-CCTTTAGACCCATGGAAGT-GGC-3'; probe, 5'-CCC GCCGCCGCCG-3'). The data from the analysis of the relative expression of each gene vs. S18 rRNA control probe were determined using the $2^{-\Delta\Delta\text{CT}}$ method (28). Critical threshold (C_T) values were determined from at least four independent cell experiments or from six to eight animals, each measured in triplicate. Results comparable with those with S18 rRNA were obtained when GAPDH was used as a second control probe. Reactions in the absence of reverse transcriptase did not yield a PCR product.

Determination of 11 β -HSD1 activity. The oxoreductase activity of 11 β -HSD1 was determined by measuring the conversion of 11-dehydrocorticosterone to corticosterone in the presence of radiolabeled 11-[1,2,6,7- 3 H]dehydrocorticosterone tracer as described earlier (37). Briefly, radiolabeled 11-dehydrocorticosterone was added to intact differentiated 3T3-L1 cells (7 days after initiating differentiation) at a final concentration of 200 nM. Fully differentiated control adipocytes or adipocytes pretreated for 48 h with various concentra-

tions of DHEA, rosiglitazone, or both were incubated between 1 and 3 h at 37°C with radiolabeled 11-dehydrocorticosterone. The amount of converted 11-dehydrocorticosterone was assessed by ethylacetate extraction of steroids from the growth medium, evaporation of the solvent, followed by separation by thin-layer chromatography and scintillation counting. Alternatively, to assess a potential direct inhibitory effect of DHEA on 11 β -HSD1, lysates from human embryonic kidney (HEK)-293 cells stably expressing 11 β -HSD1 were incubated in the presence or absence of various concentrations of DHEA, and the conversion of 11-dehydrocorticosterone was determined as described (49). Results were obtained from at least four independent experiments and are expressed as means \pm SD.

Statistical analysis. Experiments were performed at least in triplicates and results are expressed as a percentage of means \pm SD of control values or as detailed in text. Statistical comparisons between groups were made by ANOVA or unpaired *t*-tests where appropriate. $P < 0.05$ was considered significant; * $P < 0.01$, ** $P < 0.05$.

RESULTS

DHEA-induced downregulation of 11 β -HSD1 mRNA expression in 3T3-L1 adipocytes. It has previously been shown that 3T3-L1 cells express high levels of 11 β -HSD1 on terminal differentiation to adipocytes (4, 7, 36). Because both the loss of DHEA with age and inappropriately increased 11 β -HSD1 expression are associated with altered body fat distribution resulting in accumulation of visceral fat, we hypothesized that DHEA might downregulate 11 β -HSD1 activity in adipocytes. To determine the effect of DHEA on 11 β -HSD1 gene expression, 3T3-L1 adipocytes were incubated for 48 h with medium alone or in the presence of various concentrations of DHEA. DHEA caused a marked reduction in the expression of 11 β -HSD1 mRNA by \sim 40%, comparable to the effect observed for the known PPAR γ agonist rosiglitazone (Fig. 1). Maximal inhibition of 11 β -HSD1 mRNA expression on DHEA treatment was observed after incubation at concentrations of 12.5 μ M or higher for 24 h and stayed constant for up to 72 h (not shown), again resembling the effects of rosiglitazone (7). Although 11 β -HSD1 mRNA levels were most efficiently re-

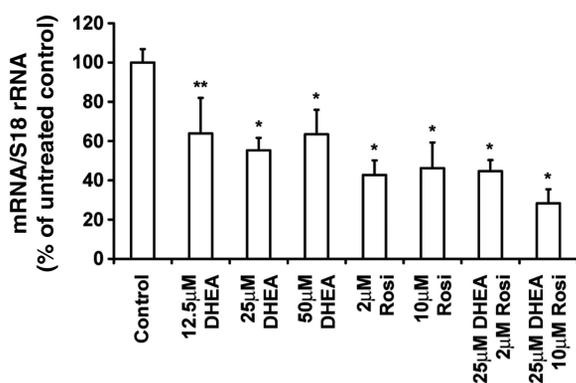


Fig. 1. Inhibition of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) mRNA expression by dehydroepiandrosterone (DHEA) and rosiglitazone (Rosi) in 3T3-L1 adipocytes. 3T3-L1 cells were grown to confluence followed by differentiation into adipocytes as given under MATERIALS AND METHODS. At 7 days postconfluence, the cells were incubated for a further 48 h in media alone or containing DHEA, Rosi, or both at the concentration indicated. The levels of 11 β -HSD1 mRNA expression were quantified by quantitative real-time RT-PCR and are expressed relative to the amount of mRNA found in untreated adipocytes and normalized to the levels of S18 rRNA. The data are shown as means \pm SD from at least 4 independent experiments (* $P < 0.01$, ** $P < 0.05$).

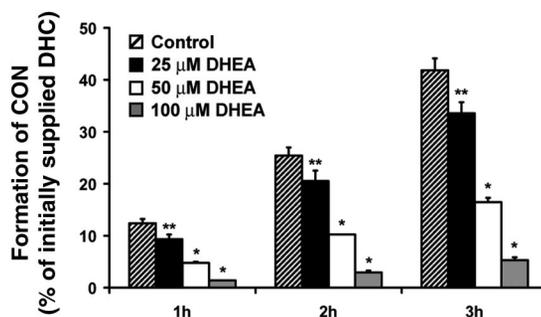


Fig. 2. Effect of DHEA on 11 β -HSD1 activity in 3T3-L1 adipocytes. 3T3-L1 cells were differentiated as given in MATERIALS AND METHODS. At 7 days postconfluence, adipocytes were incubated for 48 h with medium alone or medium containing DHEA at the concentrations indicated. 11 β -HSD1 activity was expressed as the percent conversion of 200 nM 11- 3 H]dehydrocorticosterone (DHC) to 3 H]corticosterone (CON) in medium from intact cells in absence of DHEA after 1, 2, and 3 h, respectively. Data represent means \pm SD and were obtained from 4 independent experiments (* $P < 0.01$, ** $P < 0.05$).

duced when a combination of 25 μ M DHEA and 10 μ M rosiglitazone was used, the effects of DHEA and rosiglitazone were not clearly additive.

DHEA-induced inhibition of 11 β -HSD1-dependent oxoreduction of 11-dehydrocorticosterone in 3T3-L1 adipocytes. We next examined the effect of DHEA on 11 β -HSD1 enzyme activity in intact 3T3-L1 adipocytes. Cells were treated with DHEA or vehicle for 48 h, followed by a medium change and determination of the conversion of radiolabeled 11-dehydrocorticosterone to corticosterone in the absence of DHEA. As shown in Fig. 2, 11 β -HSD1 oxoreductase activity was significantly and dose dependently decreased on treatment of cells for 48 h with DHEA relative to control cells. Treatment with 100 μ M DHEA almost completely inhibited 11 β -HSD1 activity. Cytotoxic effects were observed at concentrations higher than 100 μ M after incubation for 48 h (not shown). Treatment of differentiated control adipocytes with 100 or 200 μ M DHEA for 30 min did not significantly reduce 11 β -HSD1 activity, suggesting that the observed inhibition is not due to a general toxic effect. Furthermore, DHEA did not exert a direct inhibitory effect on 11 β -HSD1 activity, indicated by the fact that incubation of lysates from HEK-293 cells stably expressing 11 β -HSD1 with DHEA did not diminish enzyme activity ($IC_{50} > 50$ μ M, not shown). Together, these results indicate that DHEA decreases 11 β -HSD1 activity by inhibition of gene expression.

Effect of DHEA on the expression of genes associated with the regulation of 11 β -HSD1 in 3T3-L1 adipocytes. By having demonstrated that DHEA reduces the expression of 11 β -HSD1 in adipocytes, we used quantitative real-time RT-PCR to measure the expression of genes that have been associated with the regulation of 11 β -HSD1. PPAR α , PPAR γ , and LXR were shown previously to downregulate the expression of 11 β -HSD1 on activation (7, 13, 52). Treatment of 3T3-L1 adipocytes with 25 μ M DHEA for 48 h differentially regulated the expression of PPAR γ ($68 \pm 15\%$ of untreated control) and PPAR α ($234 \pm 34\%$) but did not alter the expression of LXR (Fig. 3). Interestingly, PGC-1, known to be a coactivator of several receptors including PPAR α , PPAR γ , and LXR, was upregulated 2.1-fold. We next measured the effect of DHEA on C/EBP α , which was shown previously to bind directly to the promoter of *HSD11B1* and exert a potent stimulatory effect on

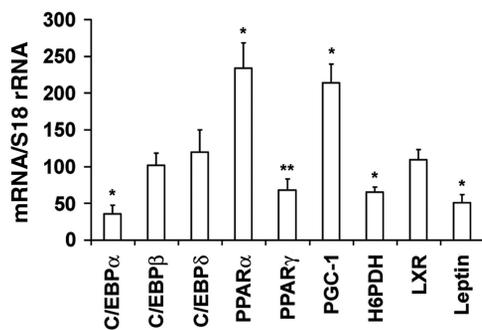


Fig. 3. Effect of DHEA on the expression of genes involved in the regulation of 11 β -HSD1 in 3T3-L1 adipocytes. 3T3-L1 cells were differentiated to adipocytes, treated with 25 μ M DHEA for 48 h as described in Fig. 1. The mRNA levels of the corresponding gene were determined by quantitative real-time RT-PCR and are expressed relative to the amount of mRNA found in untreated adipocytes and normalized to the levels of S18 rRNA. The data are shown as means \pm SD from at least 4 independent experiments (* P < 0.01, ** P < 0.05). C/EBP, CCAAT enhancer-binding protein; PPAR, peroxisome proliferator-activated receptor; PGC-1, PPAR γ coactivator 1; LXR, liver X receptor.

gene transcription (59), and on C/EBP β , a relatively weak activator of *HSD11B1* transcription. DHEA caused a significant reduction in C/EBP α mRNA expression (35 \pm 11%), whereas C/EBP β was not affected and C/EBP δ tended to increase slightly (120 \pm 30%). Recently, evidence was provided that leptin induces 11 β -HSD1 expression via the leptin receptor (27). In our experiment, we observed a downregulation of leptin mRNA (51 \pm 11%). Furthermore, we measured the effect of DHEA on the mRNA of H6PDH, an enzyme required for full oxoreductase activity of 11 β -HSD1 by providing the cofactor NADPH (4, 5), and found a significantly reduced expression (65 \pm 7%).

Inhibition of 11 β -HSD1 mRNA expression by DHEA and rosiglitazone in mice. To examine the effect of DHEA and rosiglitazone on 11 β -HSD1 expression in vivo, we treated C57BL/6J mice with DHEA (0.2% in standard rodent chow), rosiglitazone (10 mg/kg), or both compounds for 12 days. Serum DHEA-S levels were below the detection limit of 0.15 mg/l (400 nM) in three control mice and were 0.16 \pm 0.02 mg/ml in the four other control animals, which revealed the limitations of the chemiluminescence immunoassay used. However, significantly increased DHEA-S levels of 0.21 \pm 0.02 mg/ml (570 \pm 43 nM) were detected in treated mice. Treatment with DHEA and rosiglitazone both caused a significant reduction in 11 β -HSD1 mRNA levels to 56 \pm 5 and 35 \pm 15% of untreated control in white adipose tissue (Fig. 4A) and 65 \pm 10 and 75 \pm 15% in liver (Fig. 4B), respectively, whereby the effects were not additive. These results demonstrate that DHEA and rosiglitazone inhibit 11 β -HSD1 expression both in white adipose tissue and in liver to a comparable extent.

Effect of DHEA on the expression of genes associated with the regulation of 11 β -HSD1 in white adipose tissue and in liver of C57BL/6J mice. To address the possible molecular mechanisms of DHEA-induced downregulation of 11 β -HSD1, mRNAs encoding several potential regulators of 11 β -HSD1 were quantified by real-time RT-PCR (Fig. 5). DHEA treatment caused a downregulation of C/EBP α mRNA to 60% of untreated control both in white adipose tissue and in liver. C/EBP β was upregulated in white adipose tissue (153 \pm 13%)

but unchanged in liver (108 \pm 30%), whereas C/EBP δ was not affected in white adipose tissue (114 \pm 25%) but significantly elevated in liver (195 \pm 24%). DHEA treatment upregulated PPAR α 2.9- and 3.7-fold in white adipose tissue and liver, respectively. In contrast, PPAR γ was differentially regulated in white adipose tissue, where it decreased to 68 \pm 18%, and liver, where it increased to 294 \pm 30%. Interestingly, the effect on PGC-1 mRNA levels was opposite of that on PPAR γ with an upregulation to 150 \pm 9% in white adipose tissue and 77 \pm 18% in liver. LXR did not change in white adipose tissue but increased to 152 \pm 8% in liver. In line with the results obtained in 3T3-L1 adipocytes, leptin was significantly downregulated to 27 \pm 25% with large interindividual differences. H6PDH mRNA levels were also significantly reduced in white adipose tissue (69 \pm 14%) and even more pronounced in liver (39 \pm 17%), an effect also observed in animals treated with rosiglitazone (not shown).

DISCUSSION

DHEA and glucocorticoids exert opposite effects on cognitive function, blood glucose levels, insulin sensitivity, and body fat distribution; however, a link between the actions of these two steroids remains unclear. Using 3T3-L1 adipocytes and C57BL/6J mice treated with DHEA, we observed a reduced expression and activity of 11 β -HSD1, resulting in diminished local amplification of glucocorticoid action. DHEA downregulated 11 β -HSD1 in vitro in 3T3-L1 adipocytes and in vivo in liver and white adipose tissue from mice to an extent

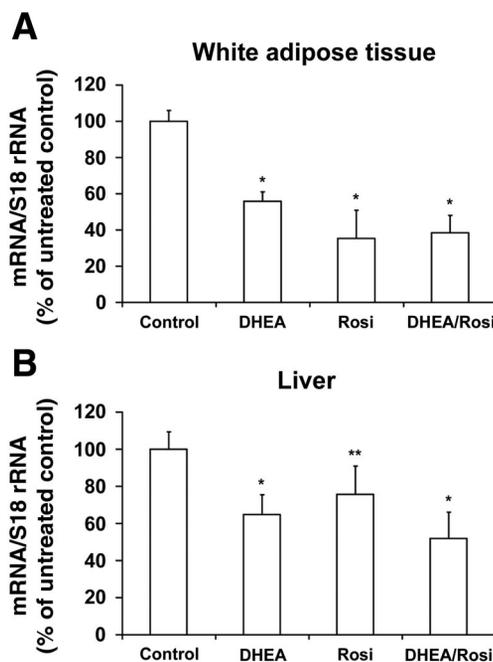


Fig. 4. Inhibition of 11 β -HSD1 mRNA expression by DHEA and Rosi in mice. C57BL/6J mice were fed for 12 days with standard chow (control) or chow containing either 0.2% DHEA (DHEA), Rosi at a final dose of 10 mg/kg body wt (Rosi), or both (DHEA/Rosi). After 12 days, animals were killed, RNA was isolated from liver and epididymal tissue, and the levels of 11 β -HSD1 mRNA were quantified by real-time RT-PCR as described under MATERIALS AND METHODS. 11 β -HSD1 mRNA levels in white adipose tissue (epididymal fat; A) and in liver (B) were normalized to S18 rRNA control probe, and data (means \pm SD from 6–8 animals) are expressed relative to the amount of mRNA found in untreated animals (* P < 0.01, ** P < 0.05).

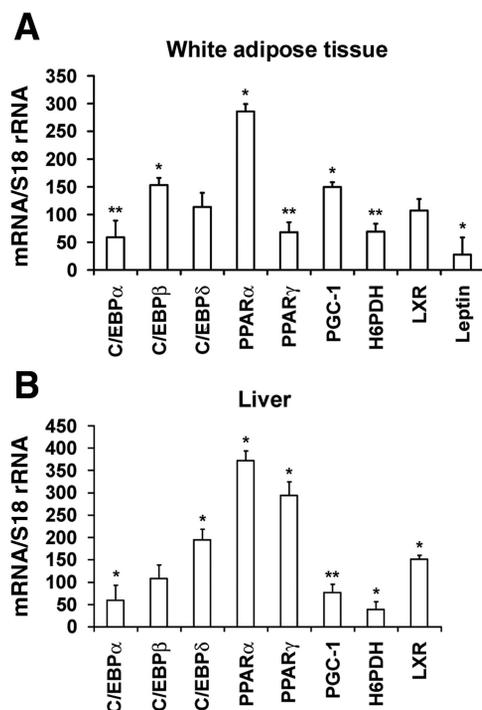


Fig. 5. Effect of DHEA on the expression of genes involved in the regulation of 11 β -HSD1 in mice. Animals were treated with 0.2% DHEA for 12 days as described in Fig. 4. RNA was isolated from liver and epididymal tissue and the mRNA levels from genes associated with the regulation of 11 β -HSD1 expression and activity were measured by quantitative real-time RT-PCR as described under MATERIALS AND METHODS. The mRNA levels in white adipose tissue (epididymal fat; A) and in liver (B) were normalized to S18 rRNA control probe and data are expressed relative to the amount of mRNA found in untreated animals. The data represent means \pm SD from 6–8 animals (* P < 0.01, ** P < 0.05).

comparable with that of the known PPAR γ agonist and anti-diabetes drug rosiglitazone (7). Therefore, some of the anti-diabetic effects of these two compounds may be explained by inhibition of 11 β -HSD1.

Moreover, we found that DHEA, which acts as a potent uncompetitive inhibitor of the cytoplasmic glucose-6-phosphate dehydrogenase but does not directly inhibit H6PDH (10, 38), causes a significant decrease in H6PDH expression. This effect was more pronounced in the liver than in white adipose tissue. We recently showed that H6PDH determines the reaction direction of 11 β -HSD1 as an oxoreductase by regenerating the cofactor NADPH in the lumen of endoplasmic reticulum (4). Thus a reduced expression of H6PDH further decreases 11 β -HSD1 oxoreductase activity and may explain the continuous decline in 11 β -HSD1 activity observed at increasing DHEA concentrations despite comparable mRNA levels be-

tween 12.5 and 50 μ M (Figs. 1 and 2). In addition, DHEA-mediated inhibition of the pentose phosphate pathway via downregulation of H6PDH in the endoplasmic reticulum and by direct inhibition of glucose-6-phosphate dehydrogenase in the cytoplasm results in reduced cellular NADPH levels. This may diminish the generation of oxygen-free radicals, which have been shown to contribute to various disease states such as diabetes, atherosclerosis, and cancer (48).

Our data suggest that DHEA mediates its effects on 11 β -HSD1 by a C/EBP-dependent mechanism. As shown in Table 1, DHEA treatment caused a significant downregulation of C/EBP α , a potent transcriptional activator of 11 β -HSD1 expression (59). C/EBP β , which was shown to attenuate the activating effect of C/EBP α , was upregulated in white adipose tissue but not altered in the liver, whereas C/EBP δ was upregulated in liver but unaffected in white adipose tissue, indicating the tissue-specific action of these transcription factors. Importantly, the ratios of C/EBP α :C/EBP β and C/EBP α :C/EBP δ are two to three times lower in liver and adipose tissue from DHEA-treated compared with untreated mice. The DHEA-dependent reduction of C/EBP α mRNA levels found in the present study is in line with findings by McIntosh et al. (32), who also reported a DHEA-induced reduction of C/EBP α levels in 3T3-L1 cells, although at very high DHEA concentrations of up to 250 μ M.

The mechanisms by which DHEA downregulates C/EBP α are unclear. It was recently shown that DHEA, like insulin, leads to the activation of phosphatidylinositol 3-kinase (PI3K) and the formation of phosphatidylinositol 3,4,5-triphosphate (PIP $_3$) (43). The increase in PIP $_3$ stimulates phospholipase C γ , enhances Ca $^{2+}$ flux, and activates protein kinase C β (PKC β), finally resulting in the translocation of GLUT4 and GLUT1 to the plasma membrane and explaining the enhanced glucose uptake into adipocytes. Whether activation of PKC β downregulates C/EBP α in adipose tissue remains to be investigated. However, unlike insulin, DHEA does not activate AKT1 and MEK or ERK kinases (43), which play an essential role in the initial upregulation of C/EBP α during adipogenesis (44). Recently, Sekine et al. (50) demonstrated that the chronic activation of PI3K by overexpression of a membrane-targeted catalytic subunit of PI3K, like treatment with insulin, induced the expression of C/EBP β and C/EBP δ in vascular smooth muscle cells. Thus DHEA may attenuate the transcription of 11 β -HSD1 by inducing a switch in the expression of C/EBPs by the downregulation of the mRNA level of C/EBP α together with a PI3K-dependent increase in C/EBP β and/or C/EBP δ .

PPAR α , PPAR γ , and LXR have been shown to downregulate the expression of 11 β -HSD1 (7, 13, 52). The regulation by these receptors is indirect and involves other yet unidentified transcription factors, possibly including C/EBPs. DHEA (Fig.

Table 1. DHEA alters the expression of C/EBP transcription factors

	C/EBP α	C/EBP β	C/EBP δ	C/EBP α :C/EBP β	C/EBP α :C/EBP δ
3T3-L1 cells	0.35 \pm 0.11	1.02 \pm 0.16	1.20 \pm 0.30	0.34	0.29
White adipose tissue	0.59 \pm 0.20	1.53 \pm 0.13	1.14 \pm 0.25	0.39	0.52
Liver	0.60 \pm 0.30	1.08 \pm 0.30	1.95 \pm 0.23	0.555	0.31

Data represent means \pm SD from at least 4 independent cell experiments or from 6–8 animals. C/EBP, CCAAT enhancer-binding protein. 3T3-L1 adipocytes and C57BL/6J mice were treated with dehydroepiandrosterone (DHEA) followed by the determination of mRNA levels using real-time RT-PCR as described in MATERIALS AND METHODS. The mRNA levels were normalized to S18 rRNA control probe and data are expressed relative to the amount of mRNA found in untreated controls (control set as 1).

5) and rosiglitazone (not shown) stimulated PPAR γ expression in the liver, resembling the effects reported recently for pioglitazone and glucose on PPAR γ expression in HK2 kidney cells (40). An elevated expression of the receptor is expected to elicit a more pronounced response in the presence of an agonist, hence the increased receptor expression could constitute a protective role by preventing inappropriately high levels of 11 β -HSD1. In contrast, both DHEA and rosiglitazone treatment resulted in a slight downregulation of PPAR γ mRNA in adipose tissue. Whether the DHEA-induced upregulation of PGC-1 in adipose tissue results in a net stimulation of PPAR γ receptor response remains to be determined. Decreased PGC-1 expression was found in adipose tissue but not in skeletal muscle from insulin-resistant patients (12). In contrast, PGC-1 is elevated in the liver in fasting and in diabetes and increases the hepatic gluconeogenesis (60). Because PGC-1 is regulated in a tissue-specific manner and interacts with several other transcription factors, including LXR and PPAR α , the potential effect of each receptor on the expression of 11 β -HSD1 is complex.

Furthermore, DHEA may exert a protective effect in adipose tissue and in liver by increasing PPAR α expression and preventing excessive 11 β -HSD1 levels. In line with a recent study in Wistar rats (20), we observed significantly increased expression of PPAR α both in liver and adipose tissue (Fig. 5). The chronic treatment with a PPAR α agonist decreased hepatic 11 β -HSD1 mRNA (13). Thus some of the anti-diabetic effects of DHEA may be explained by lowering local glucocorticoid action by downregulation of 11 β -HSD1. Elevated cortisol concentrations stimulate gluconeogenesis and increase the susceptibility to diabetes. It was also reported that glucocorticoids stimulate PPAR α mRNA in hepatocytes by a GR-dependent mechanism, thus there is a negative feedback loop between PPAR α and GR. Indeed, transgenic mice overexpressing 11 β -HSD1 in the liver exhibited increased PPAR α mRNA levels (41). These animals showed an impaired response to high-fat diet with altered lipoprotein assembly and metabolism and a reduced lipoprotein clearance from the circulation and decreased LDL receptor levels. In addition, the induction of major transcriptional regulators of cholesterol homeostasis such as SREBP-2, FXR, LXR, and RXR was attenuated under a high-fat diet. On the other side, mice lacking PPAR α accumulated hepatic fat on a normal diet and were prone to obesity (25). They also exhibited increased leptin levels.

DHEA may exert at least part of its effects via leptin. In line with a study in rats (20), we observed a significant decrease in leptin expression in white adipose tissue of DHEA-treated C57BL/6J mice and in murine 3T3-L1 adipocytes after DHEA treatment. Liu et al. (27) reported that the treatment of *ob/ob* and normal mice with leptin increased hepatic 11 β -HSD1 expression and reduced body weight. The leptin-dependent increase in hepatic 11 β -HSD1 was mediated by the leptin receptor. The leptin effect on food intake and on body weight is inhibited by glucocorticoids, which may contribute to the development of leptin resistance in obese humans. By downregulating 11 β -HSD1, DHEA counteracts the effects of glucocorticoids, which stimulate the expression of the leptin receptor and leptin secretion, thereby antagonizing leptin action. Although the molecular mechanisms underlying leptin action remain unclear, there is increasing evidence for a role of the PI3K pathway. Activation of PI3K by the anorexigenic

ciliary neurotrophic factor led to the suppression of leptin expression and secretion (39). Mice deficient in the p85 α regulatory subunit of PI3K showed elevated PIP $_3$ production in adipocytes, had enhanced insulin sensitivity and increased serum leptin levels, and exhibited significantly greater increases in body weight and white adipose tissue mass (55). In addition, glucocorticoids rapidly inhibited leptin-induced JAK/STAT pathway, partly via the MAP kinase pathway (16). These observations suggest that the antidiabetic effect of DHEA may be due, at least in part, by a decrease in leptin levels, possibly by a mechanism involving PI3K.

The DHEA concentrations applied in our study with mice represent pharmacological doses well above physiological concentrations, and species-specific effects have to be considered when trying to extrapolate these results to humans. Whereas a concentration of DHEA-S of 2,400–4,200 ng/ml (6.5–11.4 μ M) is found in young human individuals (57), the serum concentrations of DHEA and DHEA-S reported for rodents are highly controversial. Levels of 1,000 ng/ml (2.7 μ M) (18) and 2,200 ng/ml (6.0 μ M) (33) were measured in C57BL/6 mice, either using radioimmunoassay or liquid-phase kinetics enzyme immunoassay kits. In contrast, we found concentrations of 160 ng/ml (0.43 μ M) or below detection limit in untreated animals using chemiluminescence immunoassay, concentrations that are ~20-fold lower than those found in young humans. Even lower levels of DHEA-S were reported in DBA/1J mice (25 nM, determined by gas chromatography/mass spectrometry) (19) and very low concentrations of 0.62 ng/ml (1.7 nM) were detected in Wistar rats (14), indicating significant methodological differences and species-specific variability.

Nevertheless, a recent randomized controlled trial in elderly women and men demonstrated that administration of 50 mg/day of DHEA for 6 mo significantly reduced abdominal visceral fat as well as abdominal subcutaneous fat (57). DHEA treatment also led to a significant increase in insulin sensitivity. In these individuals serum DHEA-S concentrations increased from 700 ng/ml (1.9 μ M) to 3,600 ng/ml (9.8 μ M), levels found in young humans. Thus supplementation therapy may help to treat the progressive decline of circulating DHEA levels with aging and may prevent elevated intra-adipose cortisol levels (or corticosterone in rodents), which antagonize the effects of insulin and induce hypertrophy and visceral fat accumulation (30, 35).

In conclusion, DHEA downregulates 11 β -HSD1 both in the liver and in adipose tissue, thereby inhibiting the local amplification of glucocorticoids. The reduction of H6PDH expression in both tissues may further contribute to the inhibition of the oxoreductase activity of 11 β -HSD1 by limiting the availability of its cofactor NADPH. Our results suggest that this DHEA-mediated downregulation of 11 β -HSD1 is caused by a switch in the expression from its potent transcriptional activator C/EBP α to the weak activators C/EBP β and/or C/EBP δ . These findings may explain some of the anti-glucocorticoid effects of DHEA.

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