Chronic Effects of Dehydroepiandrosterone on Rat Adipose Tissue Metabolism

P. Mauriège, C. Martel, D. Langin, M. Lacaille, J.P. Després, A. Bélanger, F. Labrie, and Y. Deshaies

The goal of the present study was to examine cellular mechanisms that regulate adipose cell metabolism in ovariectomized (OVX) and intact rats that were subjected to long-term (27 weeks) treatment with dehydroepiandrosterone (DHEA). Forty-eight 16-month-old female rats were divided into 4 groups of 9 to 11 animals (intact, intact-DHEA, OVX, OVX-DHEA). Adipose tissue lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), and cyclic adenosine monophosphate (cAMP)-dependent phosphodiesterase (cAMP-PDE) activities were determined, and α_2-, β_1/β_2-, and β_3-adrenoceptors (ARs) were quantified. DHEA did not affect body weight, fat, or muscle mass in intact rats. The similar retroperitoneal fat pad weight of intact-DHEA rats compared to intact animals was in agreement with the lack of difference in the enzyme activities and AR densities. The increased body weight of OVX rat was paralleled by a greater retroperitoneal adipose tissue mass ($P < .01$), which was in turn associated with a marked rise in LPL activity ($P < .005$) and a slight decrease in HSL activity ($P < .05$) compared to intact animals. OVX-DHEA rats, compared to untreated OVX animals, had a smaller retroperitoneal fat depot, which correlated with a decrease in LPL activity ($P < .005$) and moderate increase in both HSL activity and β_3-AR density ($P < .05$). DHEA-treatment lowered fasting insulin and triglyceride levels in both intact and OVX rats ($P < .05$). Plasma testosterone, androsterone, androstenedione, and androstenediol levels were also significantly increased in both intact-DHEA and OVX-DHEA rats compared to untreated animals ($P < .0001$). These findings suggest that the antiobesity action of DHEA may be related in part to changes in lipase activities and in β_3-AR density, and that it is dependent on the ovarian status of the animal.

RENEWED INTEREST has recently been devoted to the antiobesity action of adrenal steroid dehydroepiandrosterone (DHEA), an intermediate in the pathway for the synthesis of androgens and estrogens, and its sulfate conjugate (DHEAS), the major circulating steroid in human bloodstream.[1-3] Although serum levels of DHEAS and DHEA are much lower in rats than in humans,[1,2,4] exogenous DHEA has been shown to display antiobesity and antidiabetic properties in rodents.[1,2] Indeed, long-term treatment of young obese male Zucker[4,5] or Wistar rats[6,7] with DHEA has been shown to lower weight gain, body fat, and adipose cell size. A study addressing regional variation in adipose tissue mass, cell number, and size has revealed smaller fat pads, fewer epididymal, retroperitoneal, and inguinal adipocytes, as well as smaller epididymal adipose cells in young DHEAS-treated rats.[8,9] It was thus hypothesized that DHEA could play a role in the inhibition of lipogenesis and adipogenesis as well as in the activation of lipolysis. However, prior experiments, which were all conducted in young rats, have focused exclusively on DHEA-induced changes in adipose tissue mass or insulin/glucose metabolism,[1,2,4] and the detailed mechanisms by which DHEA modulates triglyceride (TG) storage and mobilization have not yet been investigated. In this regard, lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) are key enzymes that govern the release and deposition of fatty acids in adipose tissue, as LPL regulates the hydrolysis of circulating TG-rich lipoproteins whereas HSL is the rate-limiting step for lipolysis of TG stored in adipose cells.[10] HSL regulation is under the potent control of cyclic adenosine monophosphate (cAMP), whose intracellular concentration depends on both adenylyl cyclase and cAMP-phosphodiesterase (PDE) activities.[11] Although various adrenergic receptors (ARs) that couple positively (β_1-, β_2-, and β_3-ARs) and negatively (α_2-AR) to adenylyl cyclase are involved in the fine tuning of cAMP levels and thereby the lipolytic response to catecholamines,[12] cAMP-PDE activity, through which insulin mediates its antilipolytic effect, is also of importance in the control of cAMP production and lipolysis.[13] It thus appears relevant to verify whether these key metabolic pathways are modulated by DHEA.

Since DHEA has been identified as a potential hormonal replacement therapy in postmenopausal women because of its beneficial actions on bone density, plasma levels of cholesterol, and/or insulin, as well as on fatness,[14-17] this study was undertaken to investigate the effects of a long-term (27 weeks) treatment with DHEA on adipose tissue and lipid metabolism of 16-month-old female rats. The specific aims of the present study were (1) to clarify whether the antiobesity action of DHEA is mediated by an increased lipid mobilization (through greater HSL activity and/or β-AR density) and/or a decreased fat storage (via lower LPL activity and/or number of α2-ARs), and (2) to verify whether these putative changes in adipose tissue metabolism could be predictive of a reduced adiposity and a more favorable lipid/insulin profile. Because it remains unknown whether DHEA exerts its antiobesity action in its native form or after conversion to sex steroids in peripheral tissues, another goal of this study was to examine whether DHEA modifies adipose tissue metabolism identically in intact and ovariectomized (OVX) animals.

From the Department of Social and Preventive Medicine, and the Department of Anatomy & Physiology, Laval University; Oncology and Molecular Endocrinology Research Center, Laval University Medical Center; Québec Heart Institute, Laval Hospital, Québec, Canada; and INSERM U 317, Faculty of Medicine, Toulouse, France.

Submitted August 4, 2000; accepted September 17, 2002.

Supported by the Natural Sciences and Engineering Research Council of Canada and EndoRecherche.

Address reprint requests to Pascale Mauriège, PhD, Division of Kinesiology, Department of Social and Preventive Medicine, P.E.P.S., Laval University, Ste-Foy, Québec, Canada G1K 7P4.

0026-0495/03/5203-0001$30.00/0

doi:10.1053/meta.2003.50043

MATERIALS AND METHODS

Animals and Treatment

Forty-eight 12-month-old female Sprague-Dawley rats were purchased from Charles River Laboratory (St Constant, Québec, Canada). The animals were cared for and handled in conformity with the *Canadian Guideline for the Care and Use of Laboratory Animals*, and the protocol was approved by the institution's animal care committee. Upon their arrival, each animal was subjected to a general physical examination by a qualified technician. Any rat deemed abnormal was excluded from the study. Rats were housed individually in plastic cages of conventional design located in a vivarium maintained at an ambient temperature of 23 ± 2°C (humidity: 30% to 50%), under a regimen of 12 hours of light per day (lights on at 7 AM). Upon their arrival, half of the animals were bilaterally ovariectomized (OVX) whereas the other half did not undergo surgery and were used as intact controls. During the subsequent 4-month stabilization period, the animals were given constant access to tap water and were fed a pelleted commercial diet (Certified Rodent Diet no. 5002, PMI Feeds, Richmond, IN) consisting of 55% energy as carbohydrate, 20% as protein, and 4.5% as fat. The amount of food was restricted to 5 pellets, or approximately 20 g/d, which corresponds to the ad libitum intake of intact female rats. By preventing OVX-induced hyperphagia, the procedure allowed maintenance of identical body weights in intact and OVX rats until the onset of the DHEA study period.

At the beginning of the DHEA study period, rats were distributed into the following 4 groups of 12 animals as follows: intact, intact-DHEA, OVX, OVX-DHEA. From then on, food was not restricted and was consistantly available to allow the expression of treatment effects (surgery and DHEA) on energy balance. The intact and OVX groups were treated for a period of 27 weeks once daily with 1 mL of vehicle (50% ethanol–50% propylene glycol) applied topically on a shaved dorsal skin area (3 cm × 3 cm). The intact-DHEA and OVX-DHEA groups were treated percutaneously once daily for 27 weeks with 1 mL of 30 mg DHEA (solubilized in 50% ethanol–50% propylene glycol), which was applied on the back of each animal, as describedt above. Each rat was observed at least once daily for changes in general appearance and behavior, shaved every week, and weighed every 2 weeks. The bioavailability of DHEA administered percutaneously is high.[18,19] This route of administration was therefore selected to avoid first pass DHEA inactivation in the liver and to reduced its potential impact on liver metabolizing enzymes.

Body Composition and Plasma Determinations

Body weight, fat mass and muscle mass were measured using dual energy X-ray absorptiometry (DEXA; QDR 4500A, Hologic Inc., Waltham, MA, USA) using Rat Whole Body Scan software for body lean and fat mass determination.[19,20] At the end of the experimental period, rats were killed by exsanguination under isoflurane anesthesia after a 12-hour fast. Blood was collected from the abdominal aorta and centrifuged at 1,500 × *g*, at 4°C for 15 minutes. Plasma was then stored at −80°C until biochemical measurements. The retroperitoneal adipose tissue, which is highly representative of total body fat, was quickly removed, weighed, frozen in liquid nitrogen, and stored at −80°C until assayed.

Plasma glucose levels were measured enzymatically (Sigma Chemicals Co, St Louis, MO), whereas insulin concentrations were determined by radioimmunoassay with an antibody specific for rat insulin (LINCO Research, St Charles, MO). Plasma TG were assayed by an enzymatic method using a reagent kit from Boehringer Mannheim (Montréal, Québec, Canada), which allowed correction for free glycerol. Plasma total and high-density lipoprotein (HDL) cholesterol levels were quantified using a reagent kit from Boehringer Mannheim. The HDL fraction was isolated by precipitation of apolipoprotein B–containing lipoproteins with sodium phosphotungstate–magnesium chloride.

Plasma steroid levels were determined by gas chromatography–mass spectometry (GCMS).[21] Briefly, steroids were extracted from 200-μL aliquots of plasma with chlorobutane and after centrifugation, the organic solvent was purified on a 4C-Silica cartridge (Supelco, Ontario, Canada). The fraction containing the steroids was evaporated under nitrogen and the dry extract was derivatized, using either heptafluorobutyric anhydride for the measurement of testosterone, DHEA, androsterone, androstenediol, and androstenedione levels, or pentafluorobenzoyl-chloride for the determination of estradiol levels. After evaporation of the reaction mixture, the residue was dissolved with isooctane, with the exception of estradiol for which the residue was cleaned with a diluted $NaHCO_3$ solution before reconstituting the sample with isooctane. Steroids were analyzed using either a GC/electron impact/MS method for testosterone, DHEA, androsterone, androstenediol, and androstenedione assays or a GC/negative chemical ionization/MS method for estradiol. Intra-assay and interassay coefficients of variation for the steroid measurements never exceeded 15%.[21]

Adipose Tissue Measurements

LPL and cAMP-PDE determinations. Samples of approximately 150 mg adipose tissue were immediately frozen in liquid nitrogen and stored at −80°C for later measurement of heparin-releasable LPL activity, using [^{14}C]-triolein as substrate.[22] cAMP-PDE activity was assayed in homogenates obtained from samples of 50 mg of adipose tissue, according to the original method of Solomon,[23] as slightly modified by DeMazancourt and Giudicelli.[24] Briefly, adipose tissue was homogenized in a Potter apparatus in 0.5 mL of Tris-HCl (75 mmol/L), $MgCl_2$ (10 mmol/L) buffer (pH 7.4), and centrifuged for 5 minutes at 795 × *g*, at 4°C. The resulting suspension was immediately used for cAMP-PDE assays. cAMP-PDE activity was determined in the liquid phase; 50 μg of protein was incubated under vigorous shaking at 37°C for 10 minutes in a final volume of 250 μL containing 0.62 μmol/L cAMP, 20 nmol/L [^{3}H]-cAMP, 5 mmol/L $MgCl_2$, 0.04% bovine serum albumin (BSA), and 30 mmol/L Tris-HCl (pH 7.4). A warm denaturized homogenate (2 minutes at 95°C) was used as blank. The reaction was stopped by adding a mixture of 200 μL $Ba(OH)_2$ (0.3 mol/L) and 200 μL $ZnSO_4$ (0.3 mol/L). After centrifugation for 10 minutes at 5,000 × *g*, 200 μL of the supernatant were removed and counted in 4 mL of scintillation solution. Blank values obtained from tubes incubated with boiled membranes were subtracted (0.1% of the total radioactivity). Under these conditions, less than 15% of the substrate was hydrolyzed and the reaction was linear for up to 10 minutes with 50 μg protein. LPL and PDE activities were expressed per milligram of protein and the protein content was determined according to the method of Lowry et al[25] using BSA as standard. Treatment effects were similar whether enzyme activities were expressed per unit of total protein (specific activity) or per whole tissue (total activity), and only specific activity is reported herein.

HSL assay. This assay was performed according to the original method of Fredriksson et al[26] with some modifications for the handling of small samples,[27] as previously described in detail by our group.[28] Briefly, small pieces of adipose tissue (100 mg) were homogenized at 4°C in 0.8 mL of a buffer containing 0.25 mol/L sucrose, 1 mmol/L EDTA, 1 mmol/L dithiothreitol, and the protease inhibitors leupeptin and antipain, both at 20 μg/mL (pH 7.4). Samples were then centrifuged at 100,000 × *g* for 45 minutes at 4°C in a Beckman ultracentrifuge (Beckman Instruments, Palo Alto, CA), and the fat cake was removed. Pellets containing crude adipose tissue membranes obtained after centrifugation were used for radioligand binding studies. The fat-free infranatant was recovered for analysis of maximal enzyme

activity, using 1(3)-mono-[^{3}H]oleoyl-2-*O*-oleylglycerol as substrate.[26] All samples were incubated for 30 minutes at 37°C and were analyzed together. Sensitivity of the assay is enhanced by the use of a diacylglycerol analog as substrate, since HSL has a 10-fold higher activity towards diacylglycerol than triacylglycerol.[26,27] All measurements were run in triplicate and HSL activity was expressed per milligram of protein. Treatment effects were comparable whether HSL activity was expressed per unit of protein or per whole tissue, and only specific activity is reported herein.

Radioligand binding studies. α_2- and β-ARs were quantified with radioligands selective for each AR subtype, ie, [^{3}H]-RX821002 (RX, a more selective α_2-AR antagonist than the widely used [^{3}H]-yohimbine),[29,30] as well as [^{3}H]-CGP12177 (CGP), a β_1/β_2-AR antagonist[31,32] and a partial β_3-AR agonist in rat fat cells.[30,33] Pellets previously obtained were rehomogenized with 1 mL of Tris-HCl (50 mmol/L), $MgCl_2$ (0.5 mmol/L), pH 7.5 (Tris-$MgCl_2$ buffer), recentrifuged at 40,000 $\times$ *g* for 15 minutes at 4°C, filtered through a nylon mesh, and then adjusted to a final protein concentration of approximately 0.5 mg/mL.

Adipose tissue membranes were incubated with 5 nmol/L of either [^{3}H]-RX or [^{3}H]-CGP for α_2- or β_1/β_2-AR tracer experiments, respectively. Both radioligands displayed saturable specific binding to crude membranes prepared from the different tissues, and nonspecific binding did not exceed 20% to 30% of total binding (P. Mauriège, unpublished observations). Specific binding was directly proportional to the protein content at the concentrations of both ligand and membrane used. The radioligand concentrations corresponding to almost twice the affinity of each radioligand (ie, 5 nmol/L) label the totality of α_2- and β_1/β_2-ARs.[28,32] For the identification of β-ARs, the incubation medium consisted of 50 μL of [^{3}H]-CGP and 50 μL of membrane suspension made up to a final volume of 200 μL with Tris-$MgCl_2$ buffer. This radioligand was used either at a concentration of 5 nmol/L to label the high-affinity binding sites that exhibited exclusively the pharmacological characteristics of β_1/β_2-ARs,[29-31] or at a concentration of 50 nmol/L to recruit both the high (β_1/β_2-ARs) and low-affinity binding sites, the latter being ascribed to β_3-ARs.[12] Specific binding was defined as the difference between total binding and binding in the presence of 100 μmol/L unlabeled (-) bupranolol (nonselective β-adrenergic antagonist).[33] A similar radioligand binding technique was used to quantify α_2-ARs. Total binding was determined by incubating 50-μL aliquots of the resuspended membrane preparation with a fixed concentration of [^{3}H]-RX (5 nmol/L) in a final volume of 200 μL Tris-$MgCl_2$ buffer (50 mmol/LTris-HCl, 10 mmol/L $MgCl_2$, 0.1 mmol/L ascorbic acid, pH 7.5). Specific binding was defined as the difference between total and nonspecific binding, which was evaluated in the presence of 10 μmol/l unlabeled (-) phentolamine (nonselective α-adrenergic antagonist).[28]

For both types of binding assays, incubations were performed in a water bath for 25 to 30 minutes at 37°C, under constant shaking at approximately 120 cycles/min and the reaction was stopped by the addition of 4 mL of ice-cold binding buffer, followed by rapid filtration under reduced pressure through Whatmann GF/C glass-fiber filters (Fisher, Montreal, Canada) placed on a Millipore manifold sampling unit (Millipore, St-Laurent, Canada). The tubes and filters were then washed twice with 10-mL portions of ice-cold binding buffer. The radioactivity retained on the filters was counted in minivials containing 2 mL of liquid scintillation cocktail, using an LKB scintillation spectrometer (Wallac, Turku, Finland) (at an efficiency of 35%).

Drugs and Chemicals

Leupeptin, antipain, cold triolein, and cAMP were purchased from Sigma Chemical, whereas phentolamine mesylate came from Ciba Geigy (Mississauga, Canada). 1(3)-mono-[^{3}H]oleoyl-2-*O*-oleylglycerol was provided by Sevicon AB (Lund, Sweden). (-)[^{3}H]-RX821002 (RX) (1,4-[6,7(n)-^{3}H]benzodioxan-2-methoxy-2-yl)-2-imidazoline hydrochloride) (specific activity, 53 Ci/mmol), (-)[^{3}H]-CGP12177 (CGP) ((-)-4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H] benzimidazol-2-1)) (specific activity, 44 Ci/mmol) and cyclic [^{3}H]-AMP (specific activity, 49 Ci/mmol) were obtained from Amersham International (Oakville, Canada), whereas [^{14}C]-triolein was purchased from Mandel Scientific (St-Laurent, Canada). (-) Bupranolol was kindly provided by American Cyanamid Co (Lederle Laboratories, Pearl River, NY). All other chemicals and organic solvents were of the highest purity grade commercially available. The same batches of protease inhibitors, radioligands, and pharmacological agents were used in all experiments.

Statistical Analysis

Values presented in figures are means $\pm$ SE. The effects of the ovarian status (intact or OVX) and of DHEA treatment (- or +) on each variable were assessed by a 2 $\times$ 2 factorial analysis of variance (ANOVA) to determine main and interactive effects. When treatment interactions were detected, individual between-group comparisons were performed using Fisher's protected least squares difference post hoc test. Differences were considered significant at $P < .05$.

RESULTS

The physical characteristics of the four groups of rats studied are presented in Fig 1. Neither body composition nor retroperitoneal fat depot weight was significantly modified by DHEA treatment in intact animals. OVX rats were characterized by an increased adiposity and a heavier retroperitoneal fat pad compared to their intact counterparts ($P < .01$). Although body weight, total adipose tissue, and retroperitoneal fat depot mass were reduced in OVX-DHEA rats compared to untreated OVX animals ($P < .01$), muscle mass was not affected by ovariectomy or by chronic administration of DHEA. DHEA treatment lowered both fasting insulin and TG levels ($P < .01$), although it did not modify CHOL and HDL cholesterol concentrations, regardless of the ovarian status (Fig 2). Fasting glucose levels were also comparable as mean values averaged 8.5 $\pm$ 0.5, 9.0 $\pm$ 0.5, 8.7 $\pm$ 0.7, and 8.9 $\pm$ 0.6 mmol/L (mean $\pm$ SEM) in intact, intact-DHEA, OVX, OVX-DHEA rats, respectively (not shown).

Plasma sex steroid concentrations in the 4 groups of rats are given in Fig 3.

DHEA levels considerably increased in response to treatment with this sex steroid in both intact and OVX animals (15- to 20-fold, respectively) ($P < .0001$). DHEA treatment also caused marked rises in plasma testosterone (4- to 6-fold), androsterone (15- to 20-fold), androstenedione (3.5- to 4-fold), and androstenediol (10- to 15-fold) levels ($P < .0001$), although it did not modify estradiol concentrations, irrespective of the ovarian status.

Since changes in lipid storage and/or mobilization may lead to either a decrease or an increase in body fatness, adipose tissue–LPL and HSL activities were therefore investigated. As shown in Fig 4, the 2 lipase activities were similar in both intact and intact-DHEA groups. In contrast, the greater retroperitoneal adipose tissue mass observed in OVX rats was accompanied by a marked rise in LPL activity (7.4-fold increase, $P <$

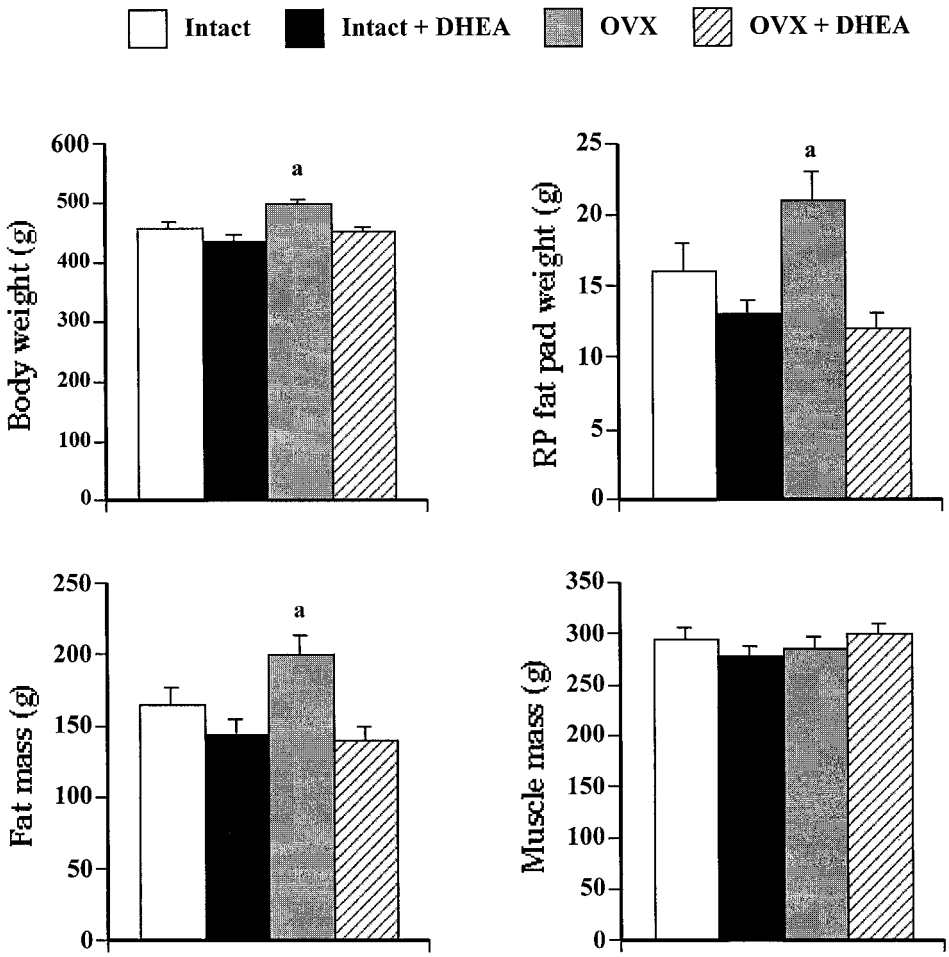

Fig 1. Physical characteristics of intact and OVX rats treated or not with DHEA. Values are means ± SEM of 9-11 animals in each group. RP, retroperitoneal. [a]Between-group difference at $P < .01$.

.005) and a slight reduction in HSL activity (1.7-fold decrease, $P < .05$). The smaller retroperitoneal fat depot of OVX-DHEA animals was associated with a 3.6-fold decrease in LPL activity ($P < .005$) and a 2.2-fold increase in HSL activity ($P < .01$), indicating that DHEA was able to restore levels of enzyme activities close to values found in intact animals. DHEA treatment did not change cAMP-PDE activity, regardless of the ovarian status of the rats (Fig 4).

Finally, as the balanced cross-talk between α_2- and β-AR-dependent pathways seems to be of importance in controlling lipolysis and thereby adipose tissue mass,[12] α_2- (antilipolytic) and $\beta_1/\beta_2/\beta_3$- (lipolytic) ARs were also determined. As illustrated in Fig 5, the α_2-AR density was almost 10-fold lower than that of β-ARs, irrespective of the ovarian status and of hormonal treatment ($P < .001$). On the other hand, β_3-ARs were approximately 6- to 8-fold more numerous than β_1/β_2-ARs, also regardless of treatment conditions ($P < .001$). Although β_3-AR density was significantly reduced by ovariectomy ($P < .01$), that of β_1/β_2-ARs remained unchanged following surgery. However, in OVX animals, DHEA was able to restore β_3-AR levels to those observed in intact and intact-DHEA rats.

DISCUSSION

The present study was conducted to investigate the chronic effects of DHEA administration on adipose tissue metabolism of old female rats, and focused more particularly on the functional balance between extracellular and intracellular TG lipolysis, which modulates the expansion of adipose tissue mass and thereby the degree of obesity.[10-13]

The markedly increased adipose tissue LPL activity of ovariectomized rats (Fig 4), which has already already been described,[34,35] is consistent with the greater fat mass and the heavier retroperitoneal fat pad of the animals (Fig 1). The drastic DHEA-induced reduction in AT-LPL activity, which returned to levels of intact rats (Fig 4), is also in agreement with the lighter fat pad weight and reduced adiposity (Fig 1). The combination of high LPL and low HSL activities (Fig 4) thus probably explains the increased adipose tissue mass observed after ovariectomy (Fig 1). The fact that DHEA seems to counteract the increase in LPL activity and the decrease in HSL activity brought by ovariectomy re-emphasizes the implication of this sex steroid in the inhibition of lipid storage,[4] while it suggests a modest role for DHEA in the activation of lipid

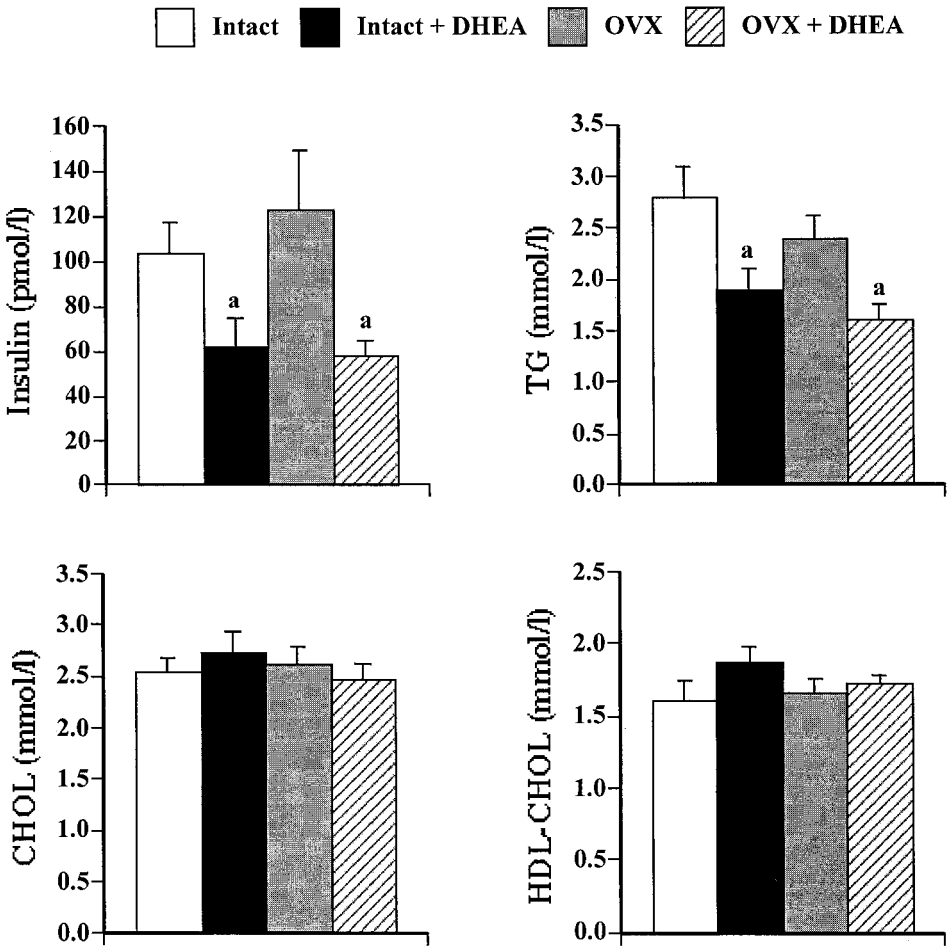

Fig 2. Metabolic profile of intact and OVX rats treated or not with DHEA. Values are means ± SEM of 9-11 experiments run in duplicate, in each group. For cholesterol (CHOL) and HDL-CHOL, values are means of 7-8 experiments run in duplicate, in each group of rats. [a]Between-group difference at $P < .05$.

mobilization. On the other hand, the unchanged basal cAMP-PDE activity (another key enzyme controlling cAMP levels and thus lipolysis) following ovariectomy (Fig 4) is in agreement with the similar enzyme activity previously found in subcutaneous and parametrial adipocytes of ovariectomized rats.[36] That DHEA did not modify cAMP-PDE activity may suggest an insensitivity of this enzyme to steroid hormones. In this regard, estradiol and progesterone were shown to reverse the defective cAMP-dependent activation of PDE in ovariectomized rats.[36] However, these hormones have not been tested on basal cAMP-PDE activity in the latter report, and further studies are therefore warranted to clarify this issue. Another possible explanation for the lack of effect of DHEA on cAMP-PDE is that under our experimental conditions, enzyme activity measured in crude adipose tissue membranes corresponds to the total population of cAMP-PDE[24] and therefore does not discriminate between the different isoenzymes, among which the particulate isoform associated with the endoplasmic reticulum fraction appears to play a key regulatory role in regulating lipolysis.[13]

Although moderate, the decreases in both HSL activity and β_3-ARs in OVX rats are consistent with the reduced adipose cell lipolysis previously reported by others[34,37] and the enlargement of adipose tissue mass observed in the present study (Fig 1). The fact that β_1/α_2-ARs (the high-affinity binding sites) were less numerous than β_3-ARs (which can be ascribed to the low-affinity binding sites) (Fig 5) has commonly been observed in rat adipose tissue.[12,29,30] However, it should be kept in mind that despite the preponderance of β_3-ARs, the classical β_1/β_2-ARs may still play an important role in regulating fat cell lipolysis because of their higher affinity for catecholamines, in rodents.[38] On the other hand, the β_1/β_2-AR density (15 fmol/mg protein) observed here is lower than that reported in previous experiments, in which it averaged 50 to 90 fmol/mg protein.[30,37] A possible explanation for such discrepancy is that our binding assays were performed on crude membrane preparations obtained from adipose tissue homogenates instead of purified adipocyte plasma membranes.[30,37] Also, the nature of the radioligand, [^{125}I]-cyanopindolol[37] versus [^{3}H]-CGP12177 (used in the present study), cannot be ruled out. Finally, the lower α_2- than β-AR density (Fig 5) re-emphasizes the minor role of this component in the modulation of rat adipose cell lipolysis.[12,29,30,37]

The fact that administration of DHEA was able to restore in OVX rats HSL activity and β_3-AR number (Fig 5) suggests an androgenic-like effect of this hormone, since testosterone in-

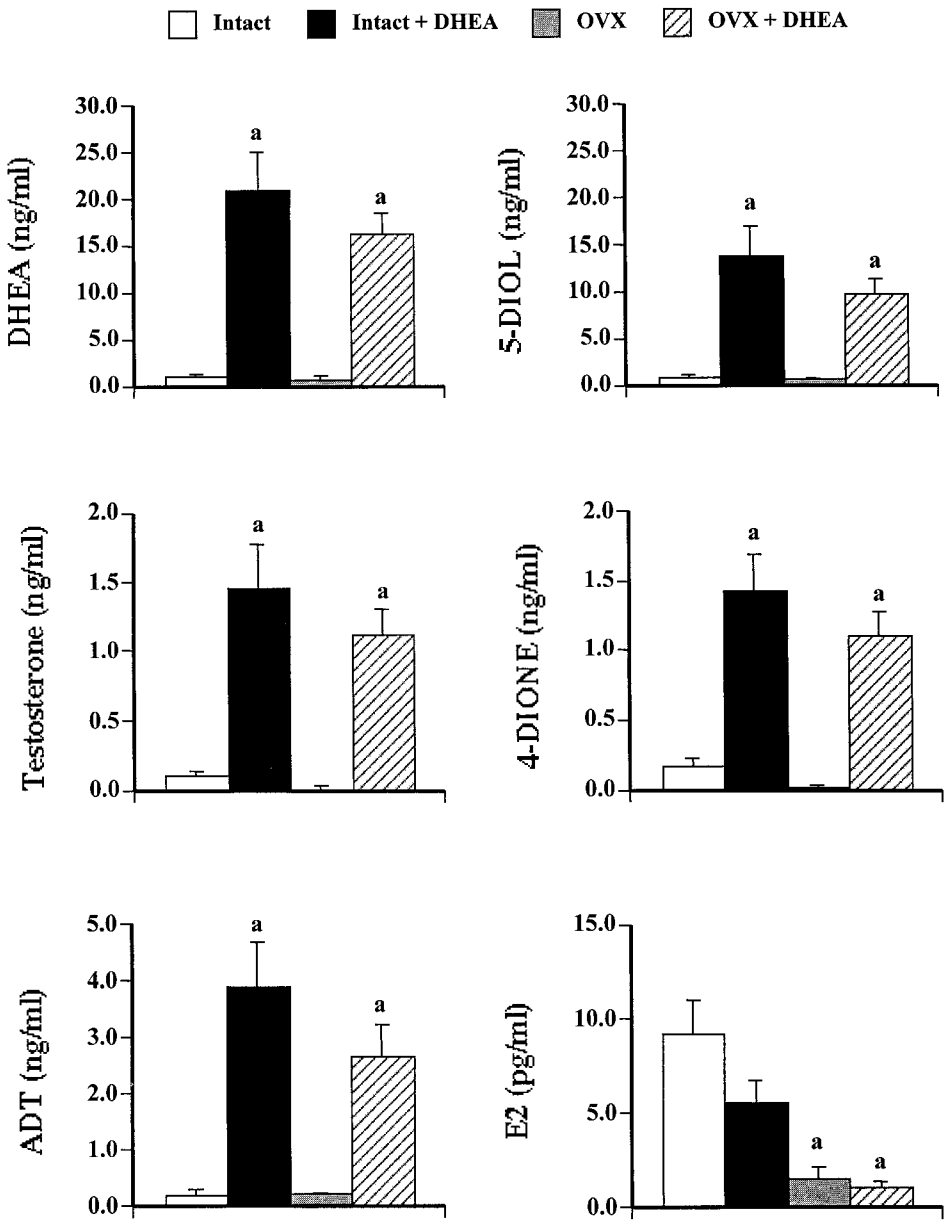

Fig 3. Plasma steroid levels of intact and OVX rats treated or not with DHEA. Values are means ± SEM of 9-11 animals, in each group. ADT, androsterone; 4-dione, androstenedione; 5-diol, androstenediol; E_2, estradiol. [a]Between-group difference at $P < .0001$.

creases β-AR density and promotes activation of the protein kinase A–HSL complex, thereby leading to stimulation of lipolysis.[37,39] This hypothesis is supported by the fact that chronic administration of DHEA promoted a significant rise in plasma testosterone and other androgens without affecting estradiol concentrations (Fig 3). This has previously been observed in postmenopausal women treated with pharmacological doses of DHEA during 28 days,[16] and supports the notion of a preferential conversion of DHEA into androgens, as mentioned above. Indeed, preclinical data have suggested that the effects of DHEA in the bone, mammary gland, and skin were mediated by the intracrine action of androgens synthesized from DHEA in these peripheral tissues.[17] Another finding that argues in favor of an androgenic-like effect of DHEA is that its percutaneous administration results in 10 times more androgenic than estrogenic activity in rats.[18] Surprisingly, muscle mass remained unchanged by DHEA treatment regardless of the ovary status (Fig 1). However, the androgenic rather than estrogenic-like effects of DHEA observed in the present study appear to be tissue-specific, since the androgenic effects of percutaneous DHEA in rats were lower in the dorsal prostate, seminal vesicles, and uterine weight than in the ventral prostate.[18] Different levels of expression of locally acting steroidogenic and steroid-metabolizing enzymes are likely responsible for these important tissue-specific differences.[40]

It is of interest to note that DHEA appeared to improve insulin sensitivity, as indicated by lower insulinemia and normal glycemia, regardless of the ovarian status (Fig 2). There are

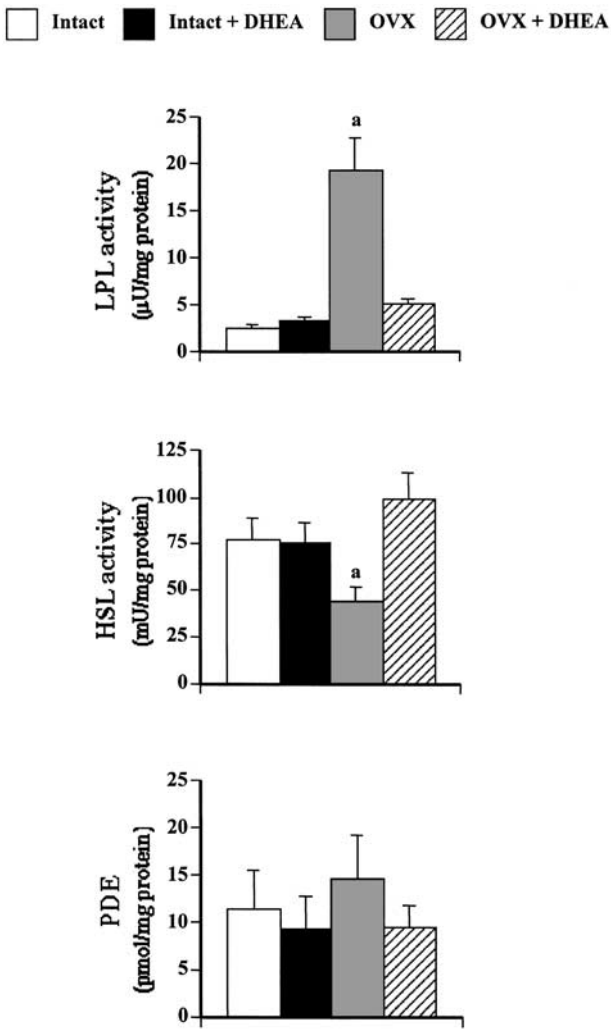

Fig 4. Adipose tissue LPL, HSL, and PDE activities in adipose tissue of intact and OVX rats treated or not with DHEA. Values are means ± SEM of 9-11 experiments run in triplicate, in each group. Protein levels averaged 1.49 ± 0.20, 1.44 ± 0.24, 1.51 ± 0.25, and 1.56 ± 0.31 mg protein/mL in intact, intact-DHEA, OVX, and OVX-DHEA groups, respectively. For LPL and HSL determinations, [a]indicates between-group difference at $P < .005$ and $P < .05$, respectively.

conflicting data to support both the presence and the absence of effect of DHEA on insulin and/or glucose levels in humans[14-16] and animal models.[5,8,9,41-43] Indeed, previous studies performed in obese postmenopausal women treated with DHEA have shown either a decrease,[14] an increase,[16] or even a lack of change in insulin sensitivity.[15] Fasting insulin and glucose levels have also been reported to remain unchanged after DHEA administration alone[8] or combined with a high-fat diet[9,41] in different strains of rats. However, the improvement in insulin sensitivity noted here in response to DHEA administration has previously been observed in intact and castrated,[5] fed and fasted Zucker rats,[42] as well as in young BL/6 mice.[43] Treatment with DHEA significantly reduced plasma TG levels without affecting cholesterol concentrations both in intact and OVX rats (Fig 2). Not surprisingly, HDL cholesterol concentrations responded identically to total cholesterol, in accordance with the fact that the HDL subfraction is the main cholesterol carrier in rats. The hypotriglyceridemic action of DHEA has been shown in some studies[8,41] but not in others.[5,9,16] Similarly, strong[14,44] or modest[5,16] cholesterol-lowering effects of DHEA have been detected in both humans and animal models. It is believed that such discrepancies could be attributed to the dose and mode of administration of DHEA, the duration of treat-

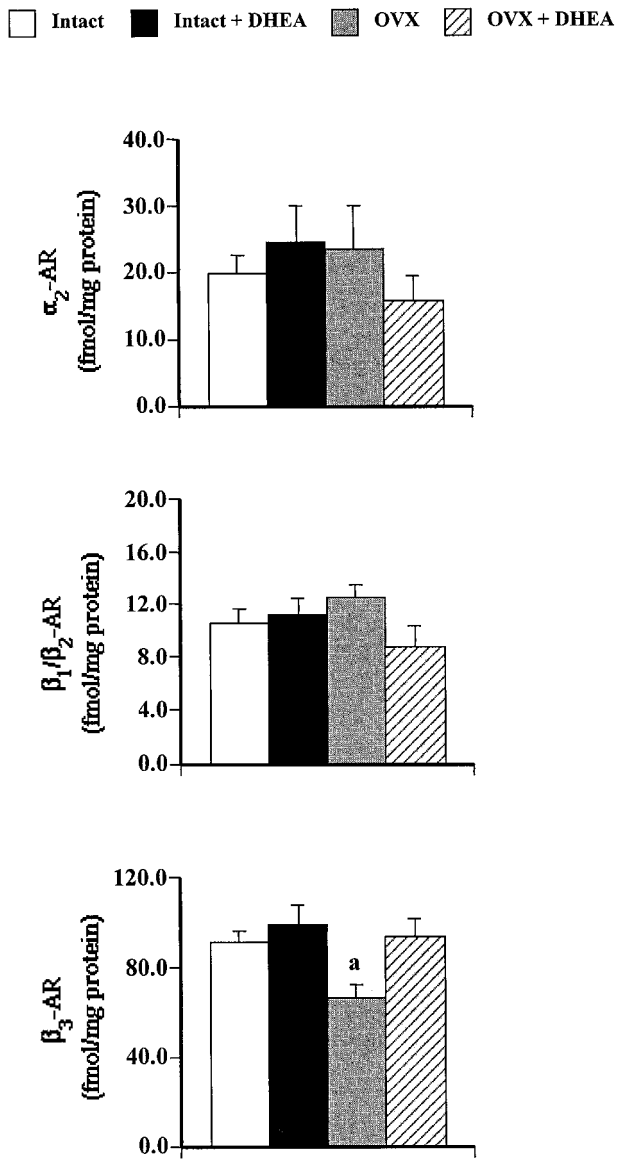

Fig 5. Maximum number of α_2-, β_1/β_2-, and β_3-ARs in adipose tissue membranes of intact and OVX rats treated or not with DHEA. Values are means ± SEM of 9-11 experiments run in triplicate, in each group. Protein levels averaged 0.46 ± 0.08, 0.42 ± 0.07, 0.45 ± 0.05 and 0.43 ± 0.06 mg protein/mL in intact, intact-DHEA, OVX, and OVX-DHEA groups, respectively. For β_3-AR assays, [a]indicates between-group difference at $P < .05$.

ment, species differences, the genetic background, the degree of obesity and age of animals, and/or the composition of the diet. It is also important to note that DHEA may exert its beneficial metabolic effects independently of its anti-obesity activity, as plasma insulin and TG levels were significantly reduced in intact rats whose body weight and fat mass remained unaltered by DHEA treatment (Fig 1). Additional studies are clearly warranted to elucidate the obesity-independent metabolic actions of DHEA. Finally, bearing in mind that lipid metabolism and insulin sensitivity were assessed in the fasted state, that is at a time when lipid flux is minimal, treatment effects represent robust phenomena that persisted after 12 hours of fasting. The consequences of ovariectomy and DHEA treatment observed in the present study would likely become more evident in the postprandial state, during which glucose/ lipid metabolism is fully active. As postprandial lipemia is strongly related to increased risk of cardiovascular disease,[45] the effect of DHEA treatment on postprandial glucose and lipid metabolism deserves further investigation.

The present study showed that (1) chronic administration of DHEA led to a marked decrease in LPL activity and to a moderate increase in HSL activity and β_3-AR density, (2) DHEA administered percutaneously may exert an androgenic effect at the adipose tissue level, (3) the lower fat mass of DHEA-OVX rats compared to untreated OVX animals appeared to be linked to changes in adipose tissue metabolism, and (4) improvements of the fasting lipid/insulin profile of rats by DHEA was independent of adiposity and the ovarian status.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Josée Lalonde (Department of Physiology, Laval University, Québec, Canada) and Michèle Dauzats (INSERM Unit 317, CHU Rangueil, Toulouse, France) for their excellent collaboration. Thanks are also expressed to the staff of the Oncology and Molecular Endocrinology Research Center from the Laval University Medical Center (CHUL) for their excellent work at various stages of the study.

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