



# Effect of a long-term percutaneous adrenal steroid treatment on rat adipose tissue metabolism

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**OBJECTIVE:** To examine some cellular mechanisms which regulate adipose cell metabolism in ovariectomized (OVX) and intact rats subjected or not to dehydroepiandrosterone (DHEA).

**DESIGN:** Rats were assigned to one of four treatment groups for 27 weeks. The main effects tested were castration (–or+) and DHEA treatment (–or+) which consisted of a single daily percutaneous application of DHEA cream (30 mg/ml in 50% ethanol–50% propyleneglycol).

**SUBJECTS:** Forty female Sprague–Dawley rats (sixteen-month old).

**MEASUREMENTS:** Body weight and fat mass (by dual-energy X-ray absorptiometry), retroperitoneal (RP) fat pad weight; plasma insulin and triglyceride levels, and HDL-cholesterol (C) concentrations; lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activities.

**RESULTS:** No difference in body composition and RP fat pad weight was observed between the intact and intact-DHEA groups. LPL and HSL activities were also similar in both groups. The increased weight of OVX rats was paralleled by a higher adiposity and greater RP adipose tissue mass, which was associated with both a marked rise in LPL activity and a slight diminution in HSL activity in this depot, compared to intact animals. OVX-DHEA rats displayed a reduced adiposity and a lighter RP fat depot, which was associated with a decrease in LPL and an increase in HSL activities, compared to untreated OVX animals. Fasting plasma insulin and TG levels were also decreased whereas plasma HDL-C concentrations were increased in intact-DHEA and OVX-DHEA rats.

**CONCLUSION:** These results show that the antiobesity effects of DHEA are dependent upon the ovarian status of the animal. These effects may involve changes in the lipid storage and the mobilization capacity of adipose tissue.

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**Keywords:** adipose tissue; dehydroepiandrosterone; lipids

## Introduction

A lively interest has recently been offered to the adrenal steroid dehydroepiandrosterone (DHEA), a precursor of testosterone and estrogens.<sup>1,2</sup> In rodents, exogenous DHEA has been shown to display anti-obesity and antidiabetic properties.<sup>1,2</sup> Indeed, obese rats treated with this steroid were characterized by a reduced adiposity, lower plasma triglyceride and cholesterol levels, and a more favorable insulin/glucose profile, compared to untreated obese animals.<sup>1–5</sup> The lighter adipose tissue mass, which has generally been observed in DHEA-treated obese rats, has been mainly attributed to both a decreased fat cell size and number.<sup>3–5</sup> However, an increased basal lipolytic rate has been reported in epididymal adipocytes of DHEA-treated rats,<sup>3</sup> whereas a decreased adipose tissue lipoprotein lipase (LPL) activity has been

observed in genetically obese rats treated with this steroid.<sup>1</sup> With the exception of these two experiments, no study has attempted to elucidate the cellular mechanisms involved in the adipose tissue responses to DHEA treatment. It also remains unclear whether the antiobesity effect of DHEA was due to itself or rather through its conversion to sex steroids in gonads. As castration makes it possible to eliminate the effect of most of the endogenous sex steroids, it was proposed to verify whether DHEA modifies the adipose cell metabolic characteristics in a similar manner, regardless of the hormonal status of the rat, and to examine whether the antiobesity action of DHEA is mediated by an increased lipid mobilization and/or a decreased fat synthesis.

## Methods

### Animals and experimental design

Forty sixteen-month-old female Sprague–Dawley rats were purchased from Charles River (St Constant, Canada). Rats were housed individually, at a tempera-

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ture of 21°C, with a 12:12 h light–dark schedule, and were fed *ad libitum* with a standard laboratory stock diet. One-half of the animals were subjected to a bilateral ovariectomy, while the remaining animals did not undergo any surgery. Two groups of rats (one intact and another castrated) were not subjected to any treatment, whereas two others were treated once daily and percutaneously with 1 ml of a cream preparation (50% ethanol–50% propylene glycol containing 30 mg DHEA) that was applied on the back of the animal, during 27 weeks.

#### Body composition and metabolic characteristics

Body weight, fat mass and muscle mass were measured using dual energy X-ray absorptiometry. At the end of the experimental period, rats were killed by exsanguination, after 12 h fasting. Blood was centrifuged at 1500 g, 4°C for 15 min and plasma was then stored at –70°C until biochemical measurements. Fasting plasma insulin concentrations were determined by radioimmunoassay with an antibody specific against rat insulin (LINCO Research Inc., St Louis, MO, USA) whereas plasma triglyceride and HDL-cholesterol levels were determined enzymatically (Boehringer Mannheim, Montréal, QC).

#### Adipose tissue metabolism

At death, the retroperitoneal adipose tissue was quickly removed and the total fat pad weighed. Samples of approximately 200 mg adipose tissue were immediately frozen in liquid nitrogen and stored at –80°C, for later measurement of heparin releasable LPL activity, using [<sup>14</sup>C]-triolein as substrate.<sup>6</sup> HSL activity was also determined from homogenates of approximately 100 mg of adipose tissue,

using the 1(3)-mono-[<sup>3</sup>H]-oleoyl-2-oleylglycerol as substrate, as previously described.<sup>7</sup>

#### Drugs and chemicals

[<sup>14</sup>C]-triolein was obtained from Mandel Scientific (Canada) whereas 1(3)-mono-[<sup>3</sup>H] oleoyl-2-oleylglycerol was generously provided by Dr P Belfrage (Sweden). All chemicals and organic solvents were of the highest purity grade commercially available.

#### Statistical analysis

The effects of castration (–or+) and of DHEA treatment (–or+) on each variable were tested by a two-way analysis of variance.

## Results

Table 1 shows selected physical and metabolic characteristics, as well as the RP adipose tissue lipase activities, of the 4 groups of rats studied. Neither the body composition, nor the retroperitoneal (RP) adipose tissue mass was modified by DHEA treatment in intact animals. Both the LPL and HSL activities assessed in this depot were similar in intact and intact-DHEA rats. Castration has significantly increased the adiposity of the animals ( $P < 0.01$ ), whereas it was devoid of effect on selected metabolic indices, compared to intact rats. The higher RP adipose tissue mass observed in OVX rats was accompanied by a marked rise in LPL activity (6.5-fold increase,  $P < 0.005$ ) and a slight reduction in HSL activity (1.7-fold decrease,  $P < 0.05$ ). On the other hand, both the body weight and the fat mass were

**Table 1** Physical and metabolic characteristics, as well as adipose tissue lipase activities of intact and ovariectomized (OVX) rats treated or not with dehydroepiandrosterone (DHEA)

	Intact (10)	Intact-DHEA (9)	OVX (11)	OVX-DHEA (10)
<i>Physical characteristics</i>				
Body weight (g)	455 ± 15 <sup>a</sup>	437 ± 10 <sup>a</sup>	500 ± 13 <sup>b</sup>	465 ± 6 <sup>a</sup>
Fat mass (g)	165 ± 12 <sup>a</sup>	144 ± 11 <sup>a</sup>	200 ± 14 <sup>b</sup>	155 ± 9 <sup>a</sup>
RP fat depot (g)	16 ± 2 <sup>a</sup>	13 ± 1 <sup>a</sup>	21 ± 2 <sup>b</sup>	12 ± 1 <sup>a</sup>
<i>Fasting metabolic variables</i>				
Insulin (pmol/l)	107 ± 12 <sup>a</sup>	66 ± 13 <sup>b</sup>	121 ± 26 <sup>a</sup>	63 ± 11 <sup>b</sup>
Triglycerides (mmol/l)	2.8 ± 0.3 <sup>a</sup>	1.9 ± 0.2 <sup>b</sup>	2.1 ± 0.3 <sup>a</sup>	1.5 ± 0.2 <sup>b</sup>
HDL-C (mmol/l)	1.6 ± 0.1 <sup>a</sup>	2.0 ± 0.1 <sup>b</sup>	1.5 ± 0.1 <sup>a</sup>	1.7 ± 0.1 <sup>ab</sup>
<i>Adipose tissue lipase activities</i>				
LPL (μU/fat pad weight)	2.9 ± 0.3 <sup>a</sup>	4.4 ± 0.8 <sup>a</sup>	19.3 ± 3.6 <sup>b</sup>	5.0 ± 1.8 <sup>a</sup>
HSL (mU/fat pad weight)	1315 ± 353 <sup>a</sup>	1016 ± 122 <sup>a</sup>	735 ± 73 <sup>b</sup>	1957 ± 291 <sup>a</sup>

Values are means ± standard error (SE) of (n) experiments. RP: retroperitoneal; C: cholesterol; LPL: lipoprotein lipase; HSL: hormone-sensitive lipase.

Values not sharing a common superscript are significantly different,  $P$  values ranging from 0.005–0.05.

All determinations were run in triplicate and both lipase activities were expressed per retroperitoneal fat pad weight.

For LPL, one μU of enzyme activity is equivalent to 1 pmol of fatty acid released per minute.

For HSL, one mU of enzyme activity equaled 1 nmol of fatty acid produced per minute.

<sup>a,b</sup>Indicate between group-differences.

reduced in OVX-DHEA rats, compared to untreated OVX animals ( $P < 0.01$ ). The lighter RP fat depot observed in these animals was associated with a 4-fold decrease in LPL activity ( $P < 0.005$ ) and a 2.7-fold increase in HSL activity ( $P < 0.01$ ). Treatment with DHEA also resulted in a decrease of both plasma insulin and TG levels, and in an increase in HDL-C concentrations ( $P < 0.05$ ), regardless of the hormonal status of the animal. Finally, muscle mass was neither affected by castration nor by DHEA-treatment (not shown).

## Discussion

HSL and LPL are key enzymes that govern the release and deposition of fatty acids in adipose tissue.<sup>8</sup> Indeed, HSL is the rate-limiting step for lipolysis of intracellular TG in adipocytes, whereas LPL regulates the hydrolysis of extracellular TG in lipoproteins. A change in the activity between these two lipases could result in an alteration of fatty acid metabolism, and may therefore lead to either a decrease or an increase in total body fat. The similar RP fat pad weight (which reflected an unchanged adiposity) which characterized intact and intact-DHEA rats was concordant with the lack of difference in both LPL and HSL activities. In contrast, the lighter RP adipose tissue mass of OVX-DHEA rats, compared to OVX untreated animals, could be mainly attributed to both a reduction in TG storage and an increase in lipid mobilization. On the other hand, the more favorable lipid/insulin profile observed in response to DHEA treatment, regardless of the hormonal status of the rat, was in good accordance with previous observations.<sup>1,2,4,5</sup> Taken together, these results suggest that the antiobesity action of DHEA is dependent upon the ovarian status of the animal, as DHEA is devoid of effect in intact animals. The reduced adiposity observed in castrated-DHEA rats may involve changes in the lipid storage and, to a less extent, in the mobilizing capacity of adipose tissue. It also appears possible that DHEA does not exert its antiobesity effect by itself

but probably through its conversion to sex steroids in ovaries. However, further studies are clearly warranted to confirm this hypothesis. It is also clear that the measurement of plasma steroid levels following treatment would help to clarify the effective agent which acts on adipose cell lipolytic (HSL) and 'lipogenic' (LPL) potencies.

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