

# The Estrogen Antagonist EM-652 and Dehydroepiandrosterone Prevent Diet- and Ovariectomy-Induced Obesity

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## Abstract

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**Objective:** EM-652 is a pure antiestrogen in human breast and uterine cancer cells that also reduces bone loss and plasma lipid levels in the rat. This study aimed to assess the ability of EM-652, alone or with dehydroepiandrosterone (DHEA), to prevent obesity and related metabolic abnormalities induced by an obesity-promoting diet and ovariectomy.

**Research Methods and Procedures:** Female rats were fed a high-sucrose, high-fat (HSHF) diet, were left intact or ovariectomized (OVX), and were treated with EM-652, DHEA, or both for 20 days. Variables of energy balance and determinants of lipid metabolism and insulin sensitivity were assessed.

**Results:** The HSHF diet (vs. chow) and OVX both increased energy intake and gain, as well as energetic efficiency. Both EM-652 and DHEA prevented diet- and OVX-induced energy gain mainly by decreasing fat deposition, without being additive. The modest EM-652-induced increase in liver triglycerides of intact rats was prevented by its combination with DHEA. EM-652, but not DHEA, decreased cholesterolemia. The HSHF diet and OVX reduced insulin sensitivity, an effect that was attenuated by EM-652 and abrogated by DHEA and EM-652+DHEA. Treatment with EM-652, DHEA, or their combination abolished the

diet- and OVX-induced increase in adipose lipoprotein lipase activity that accompanied fat gain.

**Discussion:** EM-652 is an effective agent to prevent diet- and OVX-induced obesity and its associated cardiovascular risk factors such as insulin resistance. The addition of DHEA prevents hepatic lipid accumulation and further ameliorates insulin sensitivity. The beneficial metabolic effects of such combined steroid therapy may, therefore, eventually prove to be clinically relevant.

**Key words:** antiestrogen, energy balance, cholesterol, triglycerides, insulin sensitivity

## Introduction

The estrogen antagonist EM-652 has been developed as a drug for the prevention and treatment of estrogen-sensitive cancers (for review, see Ref. 1). The compound behaves as a highly potent and pure antiestrogen in human breast and uterine cancer cells in vitro and in vivo in nude mice (1). EM-652 binds selectively to both the alpha and beta types of estrogen receptors (2). Despite its pure antiestrogenic activity in the mammary gland and endometrium, EM-652 can be classified as a selective estrogen receptor modulator, based on some “estrogen-like” properties such as prevention of bone loss and lowering of plasma cholesterol and triglycerides (3,4), and its antiobesity effect. Indeed, we showed that chronic treatment of female rats with EM-652 was at least as potent as estrogen in preventing ovariectomy (OVX)<sup>1</sup>-induced obesity and its concomitant metabolic abnormalities (5). Therefore, EM-652 shares actions on energy balance with other steroids, such as various anti-carcinogenic agents (6–10) and dehydroepiandrosterone (DHEA) (11–14).

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<sup>1</sup> Nonstandard abbreviations: OVX, ovariectomy; DHEA, dehydroepiandrosterone; SERM, selective estrogen receptor modulator; HSHF, high-sucrose, high-fat; DE, digestible energy; WAT, white adipose tissue; LPL, lipoprotein lipase; HOMA-IR, homeostasis model assessment of insulin resistance.

OVX leads to a marked increase in body energy stores of the rat (15,16) due to estrogen removal (15–17), which leads to increased energetic efficiency (18). Therefore, in humans, the gradual loss of estrogen may contribute to weight gain, especially as visceral fat, that has been reported in women during and after menopause (19,20). The consequences of estrogen loss on energy balance may become particularly relevant in affluent societies characterized by the consumption of high-energy diets. In animal models, obesity induced by either OVX (5,17) or high-energy diets (21–23) leads to abnormalities in glucose and lipid metabolism that are reminiscent of those typical of the human insulin resistance syndrome, or syndrome X, which comprise insulin resistance of glucose metabolism, hyperinsulinemia, dyslipidemia, hypertension, a pro-inflammatory setting, and endothelial dysfunction (24–26).

In view of the recent controversies regarding the health risks/benefits of hormone replacement therapy in postmenopausal women (27,28), it is important to evaluate the metabolic properties of other steroids and selective estrogen receptor modulators (SERMs) to improve the quality of life of this population. In this context, we wished to extend the previous studies mentioned above by assessing the effects of EM-652 on energy balance, lipidemia, and the glucose/insulin status in intact and OVX rats. The ovarian status was altered to test the hypothesis that estrogen removal would amplify the interaction of the compound with estrogen receptors and, hence, its metabolic actions, or alternatively that the potency of EM-652 would be sufficient to counteract the OVX-induced stimulation of obesity-promoting pathways. The study was also designed to assess the full antiobesity potency of EM-652 by combining OVX with diet-induced [high-sucrose, high-fat (HSHF)] obesity. Finally, the effects of EM-652 on energy and lipid metabolism were also investigated in association with DHEA. Such combination may be clinically relevant because DHEA prevents some of the consequences of the absence of estrogens and androgens (29,30) that are not overcome by SERMs, and because DHEA itself exerts beneficial metabolic actions (11,12,14,31–33) that may be distinct from, and, therefore, additive to, those of EM-652.

## Research Methods and Procedures

### *Animals and Treatments*

Eighty-eight female Sprague-Dawley rats initially weighing 200 to 225 g were purchased from Charles River Laboratories (St-Constant, Quebec, Canada) and housed individually in stainless steel cages in a room kept at  $23 \pm 1^\circ\text{C}$  with a 10-hour-light:14-hour-dark cycle (lights on at 6 AM). The animals were cared for and handled in conformity with the Canadian Guide for the Care and Use of Laboratory Animals, and the protocols were approved by our institutional animal care committee. The animals were acclimated

to their environment for 1 week and had ad libitum access to tap water and a nonpurified rodent chow diet (Charles River Rodent Diet no. 5075, Ralston Products, Woodstock, Canada). Eight rats were killed at the beginning of the study period for initial energy balance measurements. Sixteen rats continued to be fed the chow diet throughout the treatment period (dietary reference groups), whereas the remaining 64 rats were switched to an obesity-promoting, HSHF purified diet consisting of 41% energy as carbohydrate, 39% as fat, and 20% as protein. The composition of the diet was as follows (in grams per 100 grams of diet): sucrose, 45.0; corn oil, 10.0; lard, 10.0; casein, 22.5; dl-methionine, 0.3; vitamin mix (Teklad no. 40,060, Teklad Test Diets, Madison, WI), 1.2; mineral mix (AIN-76, ICN Biochemicals, Montreal, Quebec, Canada), 5.5; and fiber (Alphacel, ICN Biochemicals), 5.5. At the onset of feeding the HSHF diet, one-half of the animals (including the chow-fed reference rats) were given an OVX under isoflurane anesthesia. Bilateral removal of the ovaries was achieved through two small lateral skin incisions. The ovaries were pulled out of the body by grasping the periovarian fat, and the junction between the Fallopian tube and the uterine horn was then severed in a single cut. Blood vessels were then ligated, and incisions were appropriately sutured. The animals of the control (Intact) group were subjected to the same general surgical procedure as OVX animals except that the ovaries were not excised. Each of the Intact and OVX, HSHF-fed cohorts were then divided into four groups according to pharmacological treatment. The four groups thus formed within the Intact and OVX cohorts were Placebo (P), EM-652 (E), DHEA (D), and EM-652 + DHEA (ED). Treatment with EM-652 [0.5 mg/rat per day of EM-652.HCl, or an average of  $\sim 2.5$  mg/kg, a dose that prevents tumor growth in rats (1)] or vehicle alone (0.4% methylcellulose in water, given to all groups not receiving EM-652) was initiated on day 2 of the study. The compound or vehicle was given once daily by oral gavage in a total volume of 0.5 mL. DHEA (20 mg/rat per day) was administered percutaneously once daily in 1 mL of vehicle (50% ethanol-50% propylene glycol) applied topically on a shaved dorsal skin area ( $3 \times 3$  cm, shaved once a week). The dose of DHEA was kept constant throughout the treatment according to previous studies in which dosing and conditions of administration of DHEA were established to achieve optimal biological effects while avoiding adverse cutaneous reactions (34,35). Accordingly, the dose of EM-652 was also kept constant throughout the treatment. In groups receiving both drugs, administration was performed simultaneously. The compounds were administered for a total of 20 days. The day before completion of the study, the animals were allowed access to food until 11 PM. Food was then removed. The next day, between 9 and 11 AM (after a 12- to 14-hour fasting period, and  $\sim 24$  hours after the last drug administration), rats were anesthetized with an intraperitoneal in-

jection of 0.4 mL/100 g of body weight of a mixture containing 20 mg/mL of ketamine and 2.5 mg/mL of xylazine. Immediately thereafter, blood and tissues were collected.

#### **Body Weight, Food Intake, and Body Gains in Energy, Fat, and Protein**

Throughout the treatment period, body weight and food intake were monitored every other day. The amount of food spilled on the absorbent paper was collected, allowed to dry, and accounted for in the food intake measurements. At the end of the experimental period, daily food intakes were cumulated. Digestible energy (DE) intake was calculated as being 95% of the gross energy density of the diet, based on previous studies (17,36) in which the energy content of the feces was analyzed. Food energy concentrations were determined using adiabatic bomb calorimetry (Parr Instruments, Moline, IL). Energy, protein, and fat gains were determined by carcass analysis (15). At the time of sacrifice, the gastrointestinal contents were removed from the carcasses, which were then autoclaved at 125 kPa for 20 min to soften hard tissues. The autoclaved carcasses were homogenized in a volume of distilled water corresponding to 1.5 times their weight. Samples of the homogenized carcasses were freeze-dried pending the determination of their energy and nitrogen contents. The energy content of the carcasses was determined in the adiabatic bomb calorimeter calibrated with a dry benzoic acid standard. Carcass nitrogen was determined in 250- to 300-mg samples of dehydrated carcasses using the Kjeldahl procedure, and protein content was computed by multiplying carcass nitrogen content by 6.25. The energy as protein was subtracted from total body energy to determine the energy as nonprotein matter. Because carbohydrates represent a negligible part of the total energy, the energy from nonprotein matter was assumed to be essentially that of fat. Values of 23.5 and 39.3 kJ/g were taken (37) for the energy content of protein and fat, respectively. Initial carcass energy, fat, and protein contents were estimated from live body weights of the rats with reference to the baseline group of eight rats killed at the beginning of the experimental period. Such estimates allow gains in energy, fat, and protein to be determined for the treatment period. The eight rats in the baseline group were killed the day before the beginning of the treatment period, at the end of the adaptation period of 1 week, during which all animals had been fed the nonpurified rodent diet. Their carcass was analyzed for fat, protein, and energy. The body weight densities in fat (grams of fat per grams of body weight), protein (grams of protein per grams of body weight), and energy (kiloJoules of energy per grams of body weight) were computed and averaged. The average densities were then multiplied by the initial body weight of each rat ascribed to the experimental groups. Rats in the initial group

were identical in every respect (strain, age, and gender) to those of the experimental groups.

#### **Blood and Tissue Collection**

At the time of sacrifice, blood collected by cardiac puncture was centrifuged at 1500g, 4 °C for 15 min. Plasma was stored at -70 °C for later biochemical measurements. A sample of liver was immediately frozen and stored at -70 °C until later determination of lipid content. Retroperitoneal and inguinal white adipose tissues (WAT) were excised and tissues were weighed. Approximately fifty milligrams was homogenized, using all-glass tissue grinders (Kontes, Vineland, NJ), in 1 mL of a solution containing 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCL, and 12 mM deoxycholate, pH 7.4. The homogenates were centrifuged at 12,000g, 4 °C for 20 min. The fraction between the upper fat layer and the bottom sediment was removed after tube slicing, diluted with 4 volumes of the homogenization solution without deoxycholate, and stored at -70 °C until later measurement of protein content and lipoprotein lipase (LPL) activity.

#### **Plasma Measurements**

Plasma glucose concentrations were measured with the Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma cholesterol was quantified using a reagent kit from Roche Diagnostics (Montreal, Quebec, Canada). Plasma triglycerides were assayed by an enzymatic method using a reagent kit from Roche Diagnostics that allowed correction for free glycerol. Plasma insulin was determined by radioimmunoassay using a reagent kit from Linco Research (St. Charles, MO) with rat insulin as standard. An index of fasting insulin resistance, consisting of the product of fasting glucose and insulin, was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR) described by Matthews et al. (38).

#### **Tissue Measurements**

After thawing of the frozen liver samples, total lipids were extracted according to the method of Folch et al. (39) and solubilized in isopropanol. Cholesterol and triglycerides were quantitated in these lipid extracts using the above-mentioned reagent kits. LPL activity in WAT was measured as described earlier (40). On thawing, samples of 100  $\mu$ L of tissue homogenates prepared as described above were incubated for 1 hour at 28 °C under gentle agitation with 100  $\mu$ L of a substrate mixture consisting of 0.2 M Tris HCl buffer, pH 8.6, which contained 10 MBq/L [carboxyl-<sup>14</sup>C]-triolein (Amersham, Oakville, Canada) and 2.52 mM unlabeled triolein emulsified in 50 g/L gum arabic, as well as 20 g/L fatty acid free bovine serum albumin, 10% human serum as a source of apolipoprotein C-II, and either 0.2 or 2 M NaCl. Free oleate released by LPL was then separated from intact triolein (41), mixed with Universol (New England Nuclear, Montreal, Canada) and sample radioactivity was determined

**Table 1.** Variables of energy balance in female intact and ovariectomized rats fed rodent chow or a high-sucrose, high-fat diet for 3 weeks

	Chow		High-sucrose, high-fat diet		ANOVA		
	Intact	Ovx	Intact	Ovx	Diet (D)	Surgery (S)	D × S
Final body weight (g)	239 ± 6	294 ± 6	257 ± 10	316 ± 8	0.02	<0.0001	NS
Digestible energy intake (kJ)	5431 ± 189	6241 ± 108	6078 ± 255	7484 ± 354	0.0006	<0.0001	NS
Energy gain (kJ)	341 ± 111	766 ± 129	870 ± 208	2026 ± 253	<0.0001	0.0002	NS
Fat gain (kJ)	299 ± 108	535 ± 117	835 ± 186	1787 ± 248	<0.0001	0.002	<0.05
Protein gain (kJ)	42 ± 15	233 ± 32	37 ± 25	244 ± 20	NS	<0.0001	NS
Apparent energy expenditure (kJ)	5090 ± 187	5475 ± 164	5208 ± 98	5458 ± 186	NS	NS	NS
Energetic efficiency (%)*	6 ± 2	12 ± 2	14 ± 3	27 ± 2	<0.0001	0.0002	NS

Values are means ± SEM of eight animals.

\* kJ of energy gained/100 kJ energy ingested. The ANOVA values represent the level of significance of main and interactive treatment effects as determined by factorial ANOVA.

in a scintillation counter. LPL activity was calculated by subtracting lipolytic activity determined in a final NaCl concentration of 1 M (non-LPL activity) from total lipolytic activity measured in a final NaCl concentration of 0.1 M. Under the present conditions, 1 M NaCl inhibited 82% to 91% of total lipolytic activity in all tissue homogenates. LPL activity was expressed as micronunits ( $1 \mu\text{U} = 1 \mu\text{mol}$  nonesterified fatty acids released per hour of incubation at 28 °C). The interassay coefficient of variation was 4.8% and was determined using bovine skim milk as a standard source of LPL. Protein content of the homogenates was measured by the method of Lowry et al. (42). Treatment effects were essentially similar whether LPL activity was expressed per unit of protein or per whole adipose depot (total LPL activity), and only total activity is reported herein.

### Statistical Analysis

Data are presented as means ± SEM and were statistically analyzed by factorial ANOVA. Firstly, to appreciate the effects of the obesity-promoting treatments per se, the respective and interactive effects of diet (chow and HSHF diet) and ovarian status (intact and OVX) on end points were analyzed by a 2 × 2 factorial ANOVA. Secondly, the effects of individual drug treatments in animals fed the obesity-promoting diet were analyzed in intact and OVX animals separately by one-factor ANOVA. Pair-wise comparisons between drug treatments were then made when a significant overall drug effect within a given ovarian status was achieved. Pearson's correlation coefficients were calculated to establish statistical associations between some variables. In all analyses, a  $p$  value ≤ 0.05 was considered statistically significant.

### Results

The metabolic consequences of chronically feeding the obesity-promoting HSHF diet and of OVX on variables of energy balance in rats not treated with drugs are summarized in Tables 1 and 2. Chronic feeding of the HSHF diet resulted in an increase in final body weight (7% over chow-fed), whereas the increase in body weight after OVX reached 23% over intact rats (Table 1). This increase in body weight was associated with a larger energy intake in HSHF-fed (16%) and OVX (19%) rats compared with their respective controls. Total body energy gain over the 3-week treatment period was increased by both HSHF feeding (nearly 3-fold that of chow-fed rats) and OVX (2.5-fold that of intact rats). The HSHF diet and OVX tended to have larger than additive effects on energy gain when combined (diet × surgery interaction:  $p = 0.06$ ). The HSHF-induced increase in body energy gain was entirely explained by fat gain, whereas OVX increased both fat and protein gain compared with intact rats. The HSHF diet and OVX had a synergistic effect on fat gain when combined (significant diet × surgery interaction). Apparent energy expenditure was altered neither by diet nor by surgery. Energetic efficiency was doubled both by the HSHF diet and by OVX and treatment effects were additive. The effects of the HSHF diet and OVX on energy gain were due to increases in DE intake ( $r = 0.89$ ,  $p < 0.0001$ ) and energetic efficiency ( $r = 0.98$ ,  $p < 0.0001$ ); increases in energy gain were not associated with changes in apparent energy expenditure ( $r = 0.17$ , NS). Although energy gain was the result of fat and protein gain, its association was stronger with fat ( $r = 0.99$ ,  $p < 0.0001$ ) than with protein gain ( $r = 0.60$ ,  $p = 0.0003$ ).

**Table 2.** Liver and plasma lipids, plasma glucose and insulin, index of insulin resistance (HOMA-IR), adipose tissue weight, and LPL activity in female intact and ovariectomized rats fed rodent chow or a high-sucrose, high-fat diet for 3 weeks

	Chow		High-sucrose, high-fat diet		ANOVA		
	Intact	Ovx	Intact	Ovx	Diet (D)	Surgery (S)	D × S
Liver cholesterol ( $\mu\text{mol/g}$ )	7.6 ± 0.4	8.2 ± 0.4	8.3 ± 0.2	9.7 ± 0.7	0.04	0.05	NS
Liver triglycerides ( $\mu\text{mol/g}$ )	13 ± 2	17 ± 3	12 ± 1	26 ± 5	NS	0.01	NS
Plasma cholesterol (mM)	1.6 ± 0.2	2.1 ± 0.1	1.6 ± 0.1	1.6 ± 0.3	NS	NS	NS
Plasma triglycerides (mM)	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	NS	NS	NS
Plasma glucose (mM)	7.6 ± 0.2	8.7 ± 0.6	9.1 ± 0.5	10.5 ± 0.7	0.005	0.03	NS
Plasma insulin (nM)	0.07 ± 0.01	0.15 ± 0.03	0.08 ± 0.01	0.12 ± 0.02	NS	0.005	NS
HOMA-IR (arbitrary units)	4.0 ± 0.7	9.9 ± 2.2	5.3 ± 0.9	9.2 ± 1.6	NS	0.003	NS
Retro WAT weight (g)	1.0 ± 0.1	1.5 ± 0.2	1.8 ± 0.3	3.1 ± 0.3	<0.0001	0.0008	NS
Inguinal WAT weight (g)	0.4 ± 0.0	0.9 ± 0.1	0.7 ± 0.1	1.8 ± 0.3	0.0006	<0.0001	NS
Retro WAT LPL ( $\mu\text{U/depot}$ )	17 ± 3	44 ± 7	27 ± 8	64 ± 11	NS	0.0005	NS
Inguinal WAT LPL ( $\mu\text{U/}$ depot)	3 ± 1	10 ± 3	5 ± 2	15 ± 3	0.03	0.0004	NS

Values are means ± SEM of eight animals. Retro, retroperitoneal. The ANOVA values represent the level of significance of main and interactive treatment effects as determined by factorial ANOVA.

Liver cholesterol content was slightly increased by both the HSHF diet and OVX, whereas only OVX increased liver triglyceride content significantly (Table 2). Plasma levels of cholesterol and triglycerides after the 12-hour fast were unaffected by diet or the ovarian status. Fasting glycemia was increased 15% to 20% by both the HSHF diet and OVX, an indication of glucose intolerance, and their effects were additive. After the 12-hour fast, insulinemia did not differ between the two dietary cohorts, but was higher in OVX than in intact animals, such that HOMA-IR, an index of insulin resistance, remained unchanged by the HSHF diet but was doubled by OVX. The gain in body fat caused by the HSHF diet and OVX were reflected in the weight of both the visceral (retroperitoneal) and subcutaneous (inguinal) fat depots. Although diet and OVX did not interact on adipose LPL activity, OVX tended to increase enzyme activity more than did the HSHF diet.

The effects of chronic treatment of intact and OVX rats fed the obesity-promoting HSHF diet with EM-652, DHEA, or their combination on cumulative and final weight gains are depicted in Figure 1 (A and B, respectively). In intact animals, chronic treatment with EM-652 decreased weight gain throughout the treatment period. DHEA alone did not affect weight gain and prevented the EM-652-induced decrease in weight gain when both drugs were combined. In OVX animals, EM-652 reduced weight gain to one-half that

of untreated animals. Contrary to its lack of effect in intact animals, DHEA reduced weight gain in OVX rats. The individual effects of the compounds on weight gain of OVX rats were not additive when the drugs were combined. Overall drug effects on weight gain reached a higher level of significance in the OVX ( $p < 0.0001$ ) than in the intact ( $p < 0.02$ ) cohort, indicating more robust drug effects in the absence of ovarian hormones.

The effects of steroid treatment of intact and OVX, HSHF-fed animals on variables of energy balance are presented in Table 3. Overall drug treatment effects on energy intake and energy gain were more marked in the OVX ( $p \leq 0.0001$ ) than in the intact ( $0.05 = p < 0.02$ ) cohort. Chronic treatment of intact rats with EM-652 decreased energy intake by 13%. DHEA alone did not affect energy intake, but somewhat attenuated the anorectic effect of EM-652. Whereas EM-652 did not impact apparent energy expenditure, DHEA appeared to exert its effect on energy gain of intact rats through a tendency to increase energy expenditure (7%). Energetic efficiency strongly tended to be reduced by all drug treatments, but the global drug effect did not reach statistical significance ( $p = 0.08$ ). In OVX animals, drug effects on energy balance were in the same direction as in intact rats, but generally more marked in absolute terms. EM-652 reduced energy intake as it did in intact rats, whereas DHEA, in contrast with its lack of effect

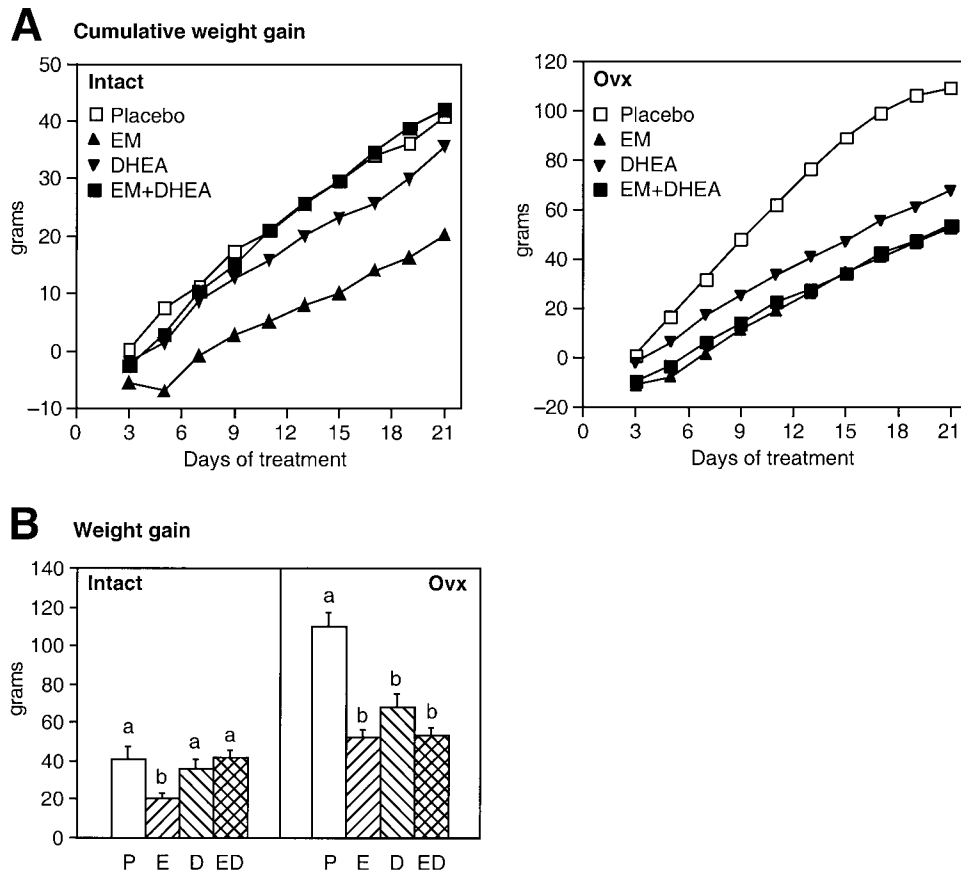


Figure 1: Cumulative (A) and final body weight gain (B) of intact and OVX rats fed an obesity-promoting diet and treated for 20 days with placebo, EM-652, DHEA, or both drugs. Each point and bar represents the mean  $\pm$  SEM of eight animals. Statistical analysis was performed only on final weight gain. Bars not sharing a common superscript are different from each other within the intact or OVX cohort at  $p < 0.05$ .

in intact rats, did reduce energy intake in OVX animals. EM-652 was more potent than DHEA in reducing energy intake, and their combined effect was intermediate. Both drugs reduced body energy gain by more than one-half without exerting additive effects. Apparent energy expenditure remained unaltered by treatments, whereas energetic efficiency was reduced by more than one-half by EM-652, DHEA, and their combination. Drug effects on food intake of intact and OVX animals were present during the whole duration of the treatment period, as illustrated in Figure 2. When the HSHF-fed cohort was analyzed globally, the effects of OVX and drug treatments on energy gain were strongly associated with increases in DE intake ( $r = 0.86$ ,  $p < 0.0001$ ) and food efficiency ( $r = 0.98$ ,  $p < 0.0001$ ), but not with changes in apparent energy expenditure ( $r = 0.20$ , NS).

Fat and protein gains as determined by carcass analysis are depicted in Figure 3. In intact rats, EM-652, DHEA, and the combined drugs all reduced fat gain by approximately one-half that of untreated rats. Similar drug-induced reductions in fat gain were noted in OVX animals, but were

quantitatively larger (60% to 80%) as indicated by a higher level of significance of drug effects ( $p < 0.0001$  in OVX vs. 0.03 in intact). In intact rats, neither drug influenced protein gain significantly, whereas the combination of EM-652 + DHEA slightly increased protein gain above that observed in placebo and EM-652-treated rats. Drug treatments were without significant effect on protein gain of OVX animals. Within the whole cohort of rats fed the HSHF diet, although energy gain was the result of fat and protein gain, its association was stronger with fat ( $r = 0.99$ ,  $p < 0.0001$ ) than with protein gain ( $r = 0.54$ ,  $p = 0.0001$ ).

The effects of steroid treatments on visceral (retroperitoneal) and subcutaneous (inguinal) adipose tissue depots are depicted in Figure 4. The depots were highly representative of whole-body fat gain as witnessed by their strong association with the latter ( $r = 0.92$ ,  $p < 0.0001$ , and  $r = 0.87$ ,  $p < 0.0001$  for the retroperitoneal and inguinal depots, respectively). Treatment with EM-652, DHEA, and their combination all decreased retroperitoneal depot weight in both intact and OVX animals, with a stronger drug effect in OVX ( $p < 0.0001$ ) than in intact ( $p < 0.02$ ) animals. In the

**Table 3.** Digestible energy intake, energy gain, apparent (app.) energy expenditure, and food efficiency in intact and ovariectomized female rats fed a high-sucrose, high-fat diet and treated with EM-652, DHEA, or the two drugs combined for 3 weeks

	Control	EM-652	DHEA	EM + DHEA
<b>Intact</b>				
Digestible energy intake (kJ)	6078 ± 255 <sup>a</sup>	5261 ± 201 <sup>b</sup>	6010 ± 162 <sup>a</sup>	5631 ± 125 <sup>ab</sup>
Energy gain (kJ)	870 ± 208 <sup>a</sup>	354 ± 95 <sup>b</sup>	416 ± 118 <sup>b</sup>	461 ± 91 <sup>b</sup>
App. energy expenditure (kJ)	5208 ± 98 <sup>ab</sup>	4907 ± 201 <sup>a</sup>	5594 ± 164 <sup>b</sup>	5170 ± 122 <sup>ab</sup>
Energetic efficiency (%*)	14 ± 3 <sup>a</sup>	7 ± 2 <sup>a</sup>	7 ± 2 <sup>a</sup>	8 ± 2 <sup>a</sup>
<b>Ovariectomized</b>				
Digestible energy intake (kJ)	7484 ± 354 <sup>a</sup>	5655 ± 170 <sup>b</sup>	6383 ± 275 <sup>c</sup>	5982 ± 149 <sup>bc</sup>
Energy gain (kJ)	2026 ± 253 <sup>a</sup>	574 ± 73 <sup>b</sup>	924 ± 186 <sup>b</sup>	700 ± 117 <sup>b</sup>
App. energy expenditure (kJ)	5458 ± 186 <sup>a</sup>	5081 ± 161 <sup>a</sup>	5459 ± 115 <sup>a</sup>	5282 ± 117 <sup>a</sup>
Energetic efficiency (%)	27 ± 2 <sup>a</sup>	10 ± 1 <sup>b</sup>	14 ± 2 <sup>b</sup>	12 ± 2 <sup>b</sup>

Values are means ± SEM of eight animals.

\* kJ of energy gained/100 kJ energy ingested. Within a given variable, means not sharing the same superscript are different from each other at  $p < 0.05$ .

inguinal depot (Figure 4B), drug effects on depot weight in intact rats were of lesser magnitude compared with the retroperitoneal depot, but identical to the latter in OVX rats. The activity of LPL (Figure 4, C and D) essentially paralleled the changes in the weight of the two adipose depots, because all drug treatments reduced LPL activity or tended to do so, the effect being particularly strong in OVX rats. In the inguinal depot, EM-652 reduced LPL activity slightly more than DHEA.

The consequences of steroid treatment on liver and plasma lipids of intact and OVX rats fed the obesity-promoting diet are depicted in Figure 5. In intact rats,

only DHEA given alone slightly decreased liver cholesterol content, whereas the drugs had no overall effect in OVX animals (Figure 5A). In intact animals, EM-652 increased liver triglyceride content, an effect that was abrogated by its combination with DHEA, which decreased liver triglyceride content when given alone (Figure 5B). Drug effects on the raised triglycerides found in the liver of OVX animals were modest, and only DHEA reduced liver triglyceride content. In both intact and OVX animals, treatment with EM-652 decreased plasma cholesterol significantly (−30%, Figure 5C). DHEA did not alter plasma cholesterol, nor did it affect the hypo-

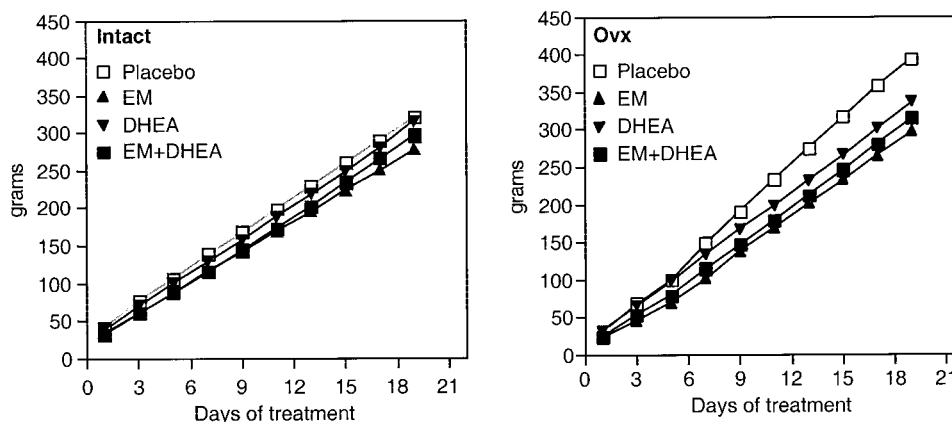


Figure 2: Cumulative food intake of intact and OVX rats fed an obesity-promoting diet and treated for 20 days with placebo, EM-652, DHEA, or both drugs. Each point represents the mean ± SEM of eight animals.

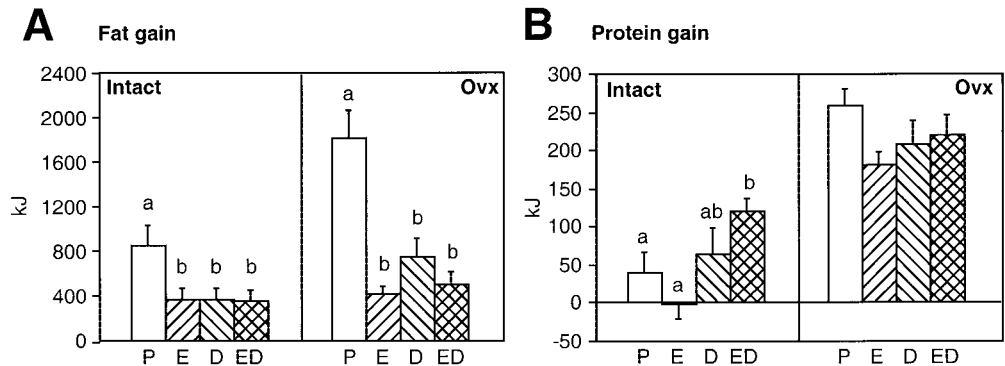


Figure 3: Fat (A) and protein gain (B) of intact and OVX rats fed an obesity-promoting diet and treated for 20 days with placebo (P), EM-652 (E), DHEA (D), or both drugs (ED). Each bar represents the mean  $\pm$  SEM of eight animals. Bars not sharing a common superscript are different from each other within the intact or OVX cohort at  $p < 0.05$ . The absence of superscripts indicates that drug treatments had no overall effect under the corresponding ovarian status, such that groups were not compared pair-wise.

cholesterolemic effect of EM-652. As shown in Figure 5D, treatments did not modify fasting plasma triglycerides.

Whereas drug treatments did not affect fasting glycemia in intact rats, EM-652, DHEA, and their combination all decreased plasma glucose levels of OVX rats (Figure 6A).

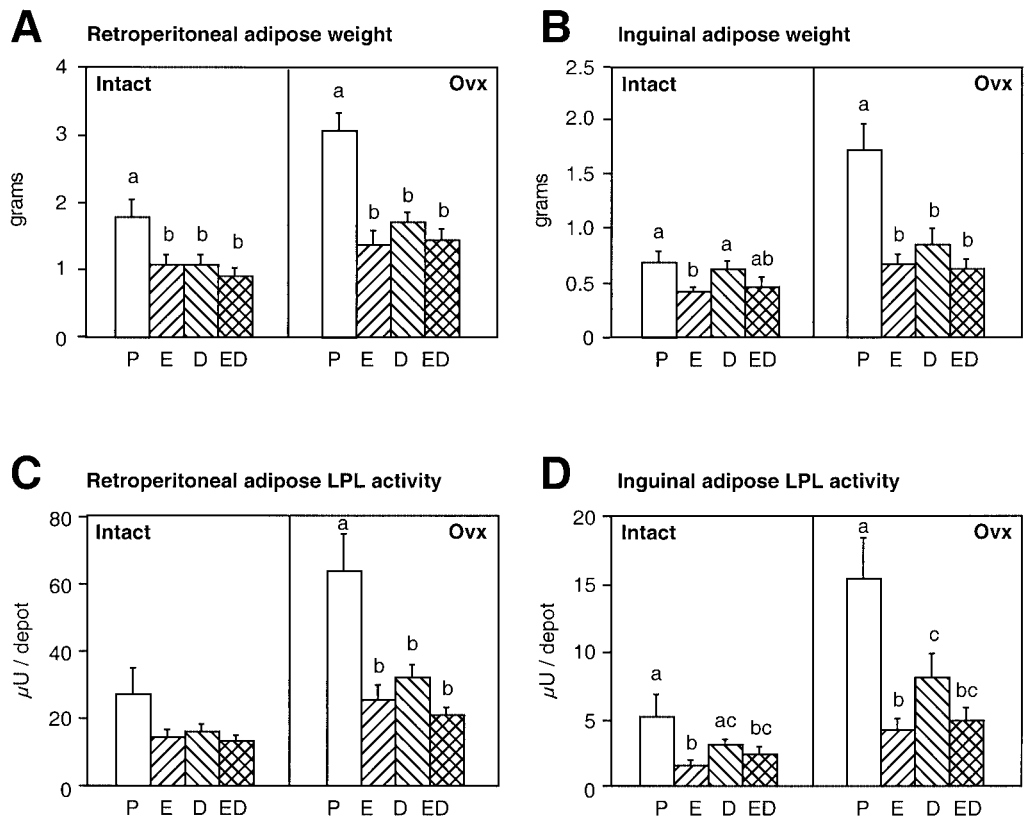


Figure 4: Retroperitoneal (A) and inguinal adipose depot weight (B) and retroperitoneal (C) and inguinal adipose depot LPL activity (D) of intact and OVX rats fed an obesity-promoting diet and treated for 20 days with placebo (P), EM-652 (E), DHEA (D), or both drugs (ED). Each bar represents the mean  $\pm$  SEM of eight animals. Bars not sharing a common superscript are different from each other within the intact or OVX cohort at  $p < 0.05$ . The absence of superscripts indicates that drug treatments had no overall effect under the corresponding ovarian status, such that groups were not compared pair-wise.

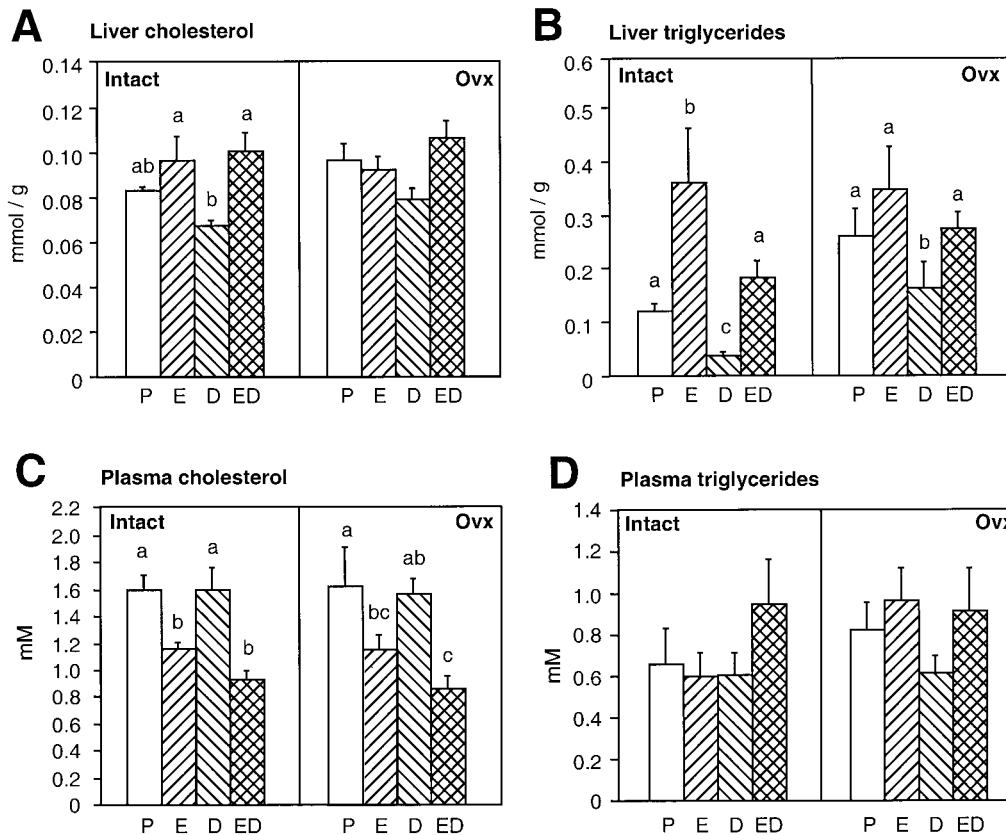


Figure 5: Liver cholesterol (A) and triglyceride content (B) and plasma levels of cholesterol (C) and triglycerides (D) of intact and OVX rats fed an obesity-promoting diet and treated for 20 days with placebo (P), EM-652 (E), DHEA (D), or both drugs (ED). Each bar represents the mean  $\pm$  SEM of eight animals. Bars not sharing a common superscript are different from each other within the intact or OVX cohort at  $p < 0.05$ . The absence of superscripts indicates that drug treatments had no overall effect under the corresponding ovarian status, such that groups were not compared pair-wise.

As in the case of plasma glucose, drug treatments did not modify insulinemia in intact rats (Figure 6B). In OVX animals, however, EM-652 tended to reduce insulinemia, which was significantly reduced by DHEA, either alone or in combination with EM-652. The index of insulin resistance (HOMA-IR) was reduced by both drugs, DHEA tending to be more potent than EM-652, and by their combination in both intact and OVX animals (Figure 6C). Drug effects were more marked in OVX ( $p = 0.0008$ ) than in intact ( $p < 0.04$ ) animals.

### Discussion

The present study aimed to assess the ability of EM-652, an anticarcinogenic SERM, to prevent diet- and OVX-induced obesity and its metabolic complications. An obesity-promoting diet was chronically fed to intact and OVX rats that were treated with EM-652, either alone or in combination with DHEA. It was found that both EM-652 and DHEA prevented obesity induced by diet and OVX, either alone or combined, and most of its concomitant lipid

and glucose/insulin abnormalities. The compounds were effective in both intact and OVX animals, generally with more marked effects in the latter. The compounds did not exert additive effects, but their combination resulted in the DHEA-mediated prevention of EM-652-induced modest liver triglyceride accumulation and in a more marked improvement in fasting insulinemia and insulin sensitivity.

The HSHF diet, which mimics to some extent the high-energy diets frequently consumed by many individuals, has been used previously by us and others to induce obesity in rats, with concomitant dyslipidemia and insulin resistance reminiscent of the human insulin resistance syndrome (syndrome X) (43–45). The sucrose component of the diet potentiates the deleterious effect of high-fat diets on insulin-stimulated glucose uptake in skeletal muscle, WAT, and the liver, and on lipemia (21,45–47). After the relatively long fasting period, insulin resistance in HSHF-fed rats was no longer evident, unlike in a previous study (43), but HSHF-fed rats were clearly glucose intolerant, as indicated by fasting hyperglycemia. Estrogen removal through OVX also

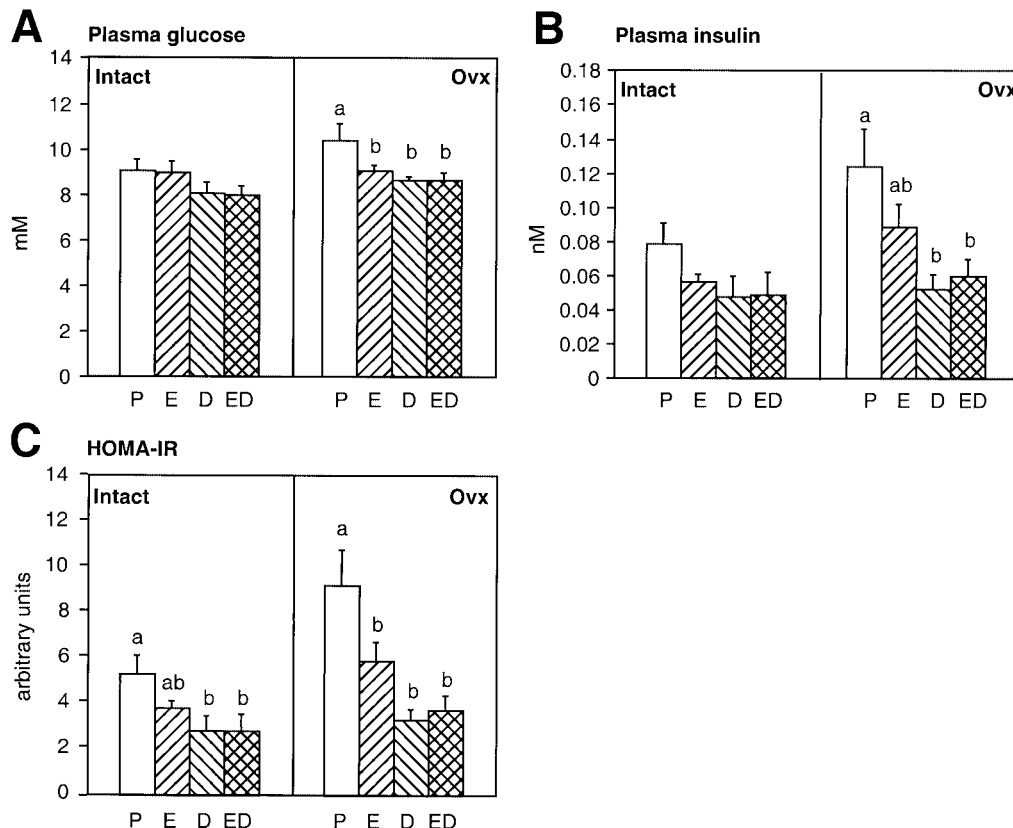


Figure 6: Plasma levels of glucose (A) and insulin (B) and insulin sensitivity index (HOMA-IR) of intact and OVX rats fed an obesity-promoting diet and treated for 20 days with placebo (P), EM-652 (E), DHEA (D), or both drugs (ED). Each bar represents the mean  $\pm$  SEM of eight animals. Bars not sharing a common superscript are different from each other within the intact or OVX cohort at  $p < 0.05$ . The absence of superscripts indicates that drug treatments had no overall effect under the corresponding ovarian status, such that groups were not compared pair-wise.

profoundly altered energy balance and lipid metabolism, as previously described by us and others (5,15–18,48,49). In brief, OVX increased energy intake and food efficiency, which resulted in a larger deposition of body energy, mainly in the form of fat but also as protein. Determinants of lipid metabolism such as liver triglyceride content, a crude index of long-term hepatic lipid production, and adipose tissue LPL activity, the enzyme that hydrolyzes lipoprotein-bound triglycerides and favors tissue uptake of fatty acids, thus liberated, were altered in congruence with the increased energy flux, as observed earlier (17). Plasma levels of insulin, a major positive modulator of peripheral lipid metabolism, were also increased after OVX, and the development of obesity and the increase in global lipid flux were accompanied by the prediabetic state of insulin resistance. The HSHF diet and OVX exerted additive effects on energy intake and deposition. The two treatments differed from one another in that the obesity-promoting diet increased fat deposition, but not that of protein, whereas OVX increased both fat and protein gain—the former being quantitatively more important than the latter—in accordance with the

catabolic action of estrogens (5,15,16). It should be noted that the effects of the HSHF diet and OVX on liver and plasma lipids were of lesser magnitude than in previous studies (17,22,43). The responsiveness of determinants of lipid metabolism tends to vary across studies. Also, the somewhat stressful gavage procedure used similarly in all groups to administer the placebo or EM-652 may conceivably have altered the feeding behavior of the animals, thereby dampening diet and OVX effects on lipid variables.

Treatment with EM-652 was very effective in preventing most of the effects of the HSHF diet and OVX on energy balance and lipid metabolism, even when the two obesity-promoting procedures were combined. Therefore, the drug shares actions on energy and lipid metabolism with other antiestrogens such as nafoxidine, tamoxifen, raloxifene, ICI-182.780, and CP-336.156 (6–10), as well as with estradiol metabolites with low estrogenic activity (50). EM-652 abolished the excess gain in energy and fat associated with consumption of the HSHF diet and OVX. Indeed, in intact rats, EM-652 treatment of HSHF-fed animals (Table 3) reduced energy gain to that of untreated, chow-fed rats

(Table 1). Likewise, EM-652 reduced energy gain of OVX rats to that of untreated, intact animals (Table 3). These findings confirm our earlier study in OVX rats (5), and extend the antiobesity action of EM-652 to intact and OVX rats with diet-induced obesity. The major level of action of EM-652 on energy balance appeared to be food intake, which was reduced concomitantly with food efficiency. Total energy expenditure was not affected by EM-652; however, one could have expected lower energy expenditure in the smaller EM-652-treated rats. Therefore, in addition to its anorectic action, the compound may lower energy gain partially by maintaining high levels of energy expenditure. It is worthy to note that the absolute effect of EM-652 on energy intake, body weight, energy, and fat gains was considerably larger in OVX than in intact animals, supporting the initial hypothesis that the drug may have had a higher degree of access to estrogen receptors, and, therefore, a stronger effect in OVX than in intact animals. Alternatively, and independently of the degree of access of EM-652 to estrogen receptors, the findings show that the potency of the antiestrogen was sufficient to counteract the OVX-induced stimulation of obesity-promoting pathways. Although the mechanisms by which EM-652 exerts such pro-estrogenic actions on energy and lipid metabolism remain to be fully established, preliminary studies strongly suggest that the metabolic actions of EM-652 are mostly, if not all, mediated by estrogen receptor alpha agonism (C. Lemieux, D. Richard, F. Labrie, and Y. Deshaies, unpublished data).

As was the case for EM-652, DHEA given alone abrogated diet-induced obesity, because it reduced energy (Table 3) and fat gain (Figure 3) of intact HSF-fed rats to that of untreated chow-fed animals (Table 1). Identical findings were obtained when considering OVX-induced obesity (Table 3). In intact females, DHEA appeared to alter body composition mainly through energy expenditure rather than food intake, in agreement with previous studies in mice (51) and rats (33). The mechanisms by which DHEA affects energy expenditure remain obscure, but the steroid has been reported to activate liver peroxisome proliferator-activated receptor  $\alpha$ , a key modulator of lipid metabolism that favors fatty acid oxidation and increases resting metabolic rate (52,53). In OVX animals, however, food intake was the determinant that was altered by DHEA. These differences in the action of DHEA on energy balance according to the ovarian status may be related to the fact that DHEA is a precursor of both male and female sex steroids (29,30). In the rat, DHEA was shown to be more androgenic than estrogenic in its action on adipose tissue metabolism (14). The present findings suggest that the estrogen-like, anorectic action of DHEA is expressed only in the absence of endogenous estrogen, but the reasons for such an interaction remain to be determined. As in the case of EM-652, apparent energy expenditure in DHEA-treated OVX rats was

identical to that in the heavier untreated rats, suggesting maintenance of the DHEA-mediated increase in energy expenditure per unit weight.

The reasons for the lack of additivity of the drugs on energy metabolism are not evident a priori because their mechanisms of action remain obscure. DHEA tended to attenuate the anorectic effect of EM (Table 3), whereas EM-652 tended to reduce the DHEA-induced increase in energy expenditure in intact rats. Such tendencies may have contributed to prevent additivity, and could reflect the fact that, in addition to putative peripheral actions, the drugs may act on common, saturable central pathways of energy balance regulation. Evidently, studies directly addressing this issue are needed to reach a definitive conclusion.

Diet, OVX, and drug effects on whole-body fat gain were all observed at the level of both the subcutaneous and visceral adipose depots, and globally, no manifest depot specificity was noted. The decrease in retroperitoneal and inguinal depot mass was paralleled by an identical reduction in the activity of LPL, indicating that modulation of LPL is a key adaptation through which diet, ovarian hormones, DHEA, and EM-652 exerted their divergent actions on fat deposition. The overall action of OVX on adipose LPL was probably the result of a combination of direct, i.e., removal of the down-regulating action of estrogens (54,55), as well as indirect mechanisms such as changes in insulin (56,57) associated with altered energy consumption. It remains to be established whether EM-652 and DHEA modulated adipose LPL through direct or indirect pathways.

The HSHF diet tended to increase liver lipid content, particularly in OVX rats, whereas OVX per se did not alter liver cholesterol but increased liver triglyceride content, as shown earlier (5,17,22). The tendency of EM-652 to increase liver lipid content in intact rats and its inability to prevent OVX-induced liver triglyceride accumulation, also noted earlier (5), is at variance with the expected lowering of liver lipids after a reduction in energy intake. This points to the possibility that EM-652 may act directly on hepatic cholesterol and triglyceride metabolism to promote lipid accumulation. However, it should be noted that the increases in liver lipid concentrations brought by EM-652 were quantitatively modest and did not reach levels indicative of significant steatosis. Importantly, in the absence of any marked effect of diet or OVX on plasma cholesterol concentrations, a significant hypocholesterolemic effect of EM-652 was noted in both intact and OVX animals, confirming earlier studies (4,5). Preliminary evidence indicates that EM-652 may in fact promote cholesterol uptake by the liver (C. Lemieux and Y. Deshaies, unpublished data). On the other hand, the drug did not reduce triglyceridemia, contrary to its prodrug EM-800 (4), which, however, was studied under fed conditions. In contrast with EM-652, DHEA slightly decreased liver lipid content and prevented EM-652-induced liver triglyceride accumulation, but did

not affect cholesterolemia. The DHEA-induced reduction in liver triglycerides may be associated with its peroxisome proliferator-activated receptor  $\alpha$  agonist properties discussed above (52,53).

Both EM-652 and DHEA reduced fasting hyperglycemia, hyperinsulinemia, and insulin resistance (HOMA-IR) that were observed in the untreated intact and OVX rats (more so in the latter), DHEA tending to be more potent than EM-652. Insulin resistance is frequently associated with obesity, particularly with its visceral form (24–26). It is likely that the effect of EM-652 and DHEA on energy balance was involved in such an improvement in insulin action, although a direct action of the compounds on the pancreas and on insulin target organs cannot be excluded a priori. It must be noted that indices of lipid metabolism and insulin sensitivity were assessed in the fasted state, i.e., at a time when lipid flux is minimal. Therefore, treatment effects represent robust phenomena that persisted after 12 hours of fasting. The consequences of OVX and pharmacological treatments observed in the present study would conceivably become more evident in the postprandial state, during which the metabolic handling of glucose and lipids becomes fully active.

To summarize, in addition to the previously reported beneficial effects of EM-652 on breast cancer proliferation and bone mineral density, this study demonstrates that the compound can be considered as an effective agent to prevent diet- and OVX-induced obesity and its metabolic complications such as insulin resistance. The adrenal steroid DHEA given alone did not alter body weight of intact rats but reduced their fat gain, and prevented OVX-induced obesity. The combination of EM-652 and DHEA did not exert additive effects on energy and lipid metabolism, but had the advantage of preventing the modest EM-652-induced liver accumulation of triglycerides, and of completely normalizing fasting insulinemia and insulin sensitivity. Therefore, the beneficial metabolic effects of such combined steroid therapy may eventually prove to be clinically relevant.

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