



Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats

F Picard¹, Y Deshaies¹, J Lalonde¹, P Samson¹, C Labrie², A Bélanger², F Labrie² and D Richard^{1*}

¹Center for Research on Energy Metabolism, Department of Anatomy and Physiology, School of Medicine, Laval University, Québec, Canada G1K 7P4; and ²Laval University Hospital Research Center, Department of Anatomy & Physiology, School of Medicine, Laval University, Québec, Canada G1K 7P4

OBJECTIVE: The estrogen antagonist EM-652.HCl behaves as a highly potent and pure antiestrogen in human breast and uterine cancer cells. Because of its pure antiestrogenic activity in these cells, and because its prodrug, EM-800, reduces bone loss and decreases serum cholesterol and triglycerides in the rat, EM-652.HCl can be classified as a pure selective estrogen receptor modulator (SERM). This study was conducted to assess the ability of EM-652.HCl to prevent obesity and abnormalities of lipid metabolism induced by ovariectomy in a rat model.

DESIGN: Female rats were left intact or ovariectomized (OVX), and OVX rats were treated with placebo, estradiol (E₂), or EM-652.HCl for 20 days. At the end of the treatment period, parameters of energy balance and determinants of lipid metabolism were assessed.

RESULTS: As expected, OVX increased energy intake, which in turn was accompanied by an increased energy, fat and protein gain and higher food efficiency. OVX also increased the triglyceride content of the liver and produced hypercholesterolemia and hyperinsulinemia. The weight of representative white adipose depots was higher in OVX than in intact rats. Lipoprotein lipase activity was higher in white adipose tissues of OVX rats than in those of intact animals, whereas its activity was lower in oxidative tissues (brown adipose and soleus muscle). Replacement therapy with a physiological dose of E₂ prevented most of the abnormalities in energy and lipid metabolism brought about by OVX, although its orexigenic effect was only partially corrected. In contrast, treatment of OVX rats with EM-652.HCl completely abolished OVX-induced obesity and its related abnormalities in lipid metabolism and glucose/insulin homeostasis.

CONCLUSION: These findings demonstrate that EM-652.HCl can be considered as an effective agent to prevent OVX-induced obesity. The present study also shows that EM-652.HCl reduces cardiovascular risk factors associated with obesity such as hyperlipidemia and insulin resistance.

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Introduction

Obesity results from an imbalance between energy intake and expenditure. Human obesity, particularly its visceral (or android) form, is typically associated with a cluster of metabolic abnormalities termed the insulin resistance syndrome, or syndrome X, which comprises insulin resistance of glucose metabolism, hyperinsulinemia, dyslipidemia, hypertension and endothelial dysfunction.^{1–3} Among the several endocrine factors that are liable to affect the development of obesity, female gonadal steroids have been shown to play a prominent role. Indeed, ovariectomy (OVX) leads to a marked increase in body energy stores of the rat.^{4,5} Estrogen removal appears to be responsible for

this effect, as estrogen replacement after OVX prevents the extra energy gain.^{4–6} The accelerated rate at which rats store energy after OVX is mainly due to an increased energetic efficiency,⁷ with some contribution from a decreased thermogenesis under some conditions.^{5,8} The increase in energy flux brought by OVX is accompanied by concomitant adaptations of peripheral lipid metabolism that include increased hepatic lipid production, elevated levels of circulating lipoproteins, and the induction of pathways involved in fat accumulation.⁶

The estrogen antagonist EM-652.HCl has been developed as a drug for the prevention and treatment of estrogen-sensitive cancers (reviewed in Ref. 9). The compound behaves as a highly potent and pure antiestrogen in human breast and uterine cancer cells *in vitro* as well as *in vivo* in nude mice.⁹ EM-652.HCl binds selectively to both the alpha and beta types of estrogen receptors.¹⁰ Despite its pure antiestrogenic activity in the mammary gland and endometrium, EM-652.HCl can be classified as a selective estrogen receptor modulator, based on some 'estrogen-like'

*Correspondence to: D Richard, Director, Center for Research on Energy Metabolism, Department of Anatomy & Physiology, School of Medicine, Laval University, Québec, QC, Canada G1K 7P4.

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properties of EM-800, the prodrug of EM-652, such as the prevention of bone loss and the lowering of plasma cholesterol and triglycerides.^{11,12} In the course of studies on the action of EM-800, the prodrug of EM-652, collateral observations have suggested that these compounds may exert effects upon metabolic pathways associated with energy balance, glucose and lipid metabolism.^{11,12} These observations, which suggested that the estrogen antagonist EM-652.HCl may exert effects that mimic, rather than antagonize, those of estrogen on energy and lipid metabolism, prompted us to undertake the present study to assess in detail such actions in a rat model. The experimental strategy consisted of evaluating the potential of EM-652.HCl to antagonize the effects of OVX on energy balance and lipid metabolism, and to compare its action with that of estrogen replacement.

Materials and methods

Animals and treatments

Forty-eight female Sprague–Dawley rats initially weighing 175–200 g were purchased from Charles River Laboratories (St-Constant, Québec, Canada) and housed individually in stainless steel cages in a room kept at $23 \pm 1^\circ\text{C}$ with a 10:14 h light–dark cycle (lights on at 06:00). 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 one week and had *ad libitum* access to tap water and a nonpurified rodent diet. They were then fed *ad libitum* a purified diet which contained, in g/100 g diet: corn starch, 21.2; dextrose, 31.2; corn oil, 6.4; casein, 20.0; DL-methionine, 0.3; vitamin mix (Teklad no. 40060, Teklad Test Diets, Madison, WI), 1.0; mineral mix (AIN-76, ICN Biochemicals, Montréal, Québec, Canada), 4.9%; and fiber (Alphacel, ICN Biochemicals), 5.0. Rats were divided into the four following groups of 10 rats each: (1) intact control; (2) ovariectomized (OVX) control; (3) OVX + estradiol (E_2); and (4) OVX + EM-652.HCl. The remaining eight animals were used as the baseline group (see below). At the onset of the study (average body weight of the cohort: 197 ± 1 g), the last three experimental groups of rats were bilaterally ovariectomized 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. A Silastic implant containing E_2 was then inserted subcutaneously in the dorsal area of the OVX + E_2 animals. The animals of the intact

control group were subjected to the same general surgical procedure as OVX animals except that the ovaries were not excised. The E_2 implant, chosen in preliminary studies to yield physiological levels of the hormone as assessed by treatment effect on uterine weight, had the following steroid concentration and size: E_2 :cholesterol (1:50, w/w), 0.5 cm (length of diluted steroid in silastic tubing), 0.5 mm (outer diameter of silastic tubing), and 0.24 mm (inner diameter of silastic tubing). Estradiol implants were immersed in 0.9% NaCl at 37°C overnight before their subcutaneous insertion in animals. Treatment with EM-652.HCl (0.5 mg/rat, or an average of ~ 2.5 mg/kg, a dose that prevents tumor growth in rats⁹) or vehicle alone (0.4% methylcellulose in water, given to the three groups not receiving EM-652.HCl) 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. Treatments were administered for a total of 20 days. The day before completion of the study, the animals were allowed access to food until 21:00. Food was then removed. Between 09:00 and 11:00 (that is following a 12–14 h fasting period, and 24 h after the last EM-652.HCl administration), rats were anesthetized with an intraperitoneal injection 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. The cumulated digestible energy (DE) intake was calculated by subtracting the energy content of the faeces from the gross accumulated energy intake (energy density of the diet \times cumulated intake). Faeces were collected daily during the experimental period. Fecal and food energy concentrations were determined using adiabatic bomb calorimetry (Parr Instrument, Moline, IL). Energy, protein and fat gains were determined with a comparative slaughter technique.⁴ 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 250–300 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 in order to determine the energy as non-protein matter. As carbohydrate represents a negligible part of the total energy, the energy from non-protein matter was assumed to be essentially that of fat. Values of 23.5 and 39.3 kJ/g were taken¹³ 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 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 energy balance trial, 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 (g of fat/g of body weight), protein (g of protein/g of body weight), and energy (kJ of energy/g 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, gender) to those of the experimental groups.

Blood and tissue collection

At time of sacrifice, blood collected by cardiac puncture was centrifuged at 1500 g, 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), interscapular brown adipose tissue (BAT) and the soleus muscle were excised. BAT was cleaned of adhering white fat and muscle tissue. Tissues were weighed, approximately 50 mg were taken from WAT, BAT and the soleus, and tissue samples were homogenized using all-glass tissue grinders (Kontes, Vineland, NJ). The WAT and BAT samples were homogenized 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 soleus muscle samples were homogenized in 1 ml of a solution containing 1 M ethylene glycol, 50 mM Tris-HCl, 3 mM deoxycholate, 10 IU/ml heparin, and 5% (v/v) aprotinin (Trasylol, Miles Pharmaceuticals, Rexdale, Ontario, Canada), pH 7.4. These homogenizing media were found to yield optimal LPL activities in the individual tissues. Homogenates of muscle tissues were quickly frozen and stored at -70°C until measurement of LPL activity. WAT and BAT homogenates were centrifuged at 12 000 g, 4°C for 20 min. The fraction between the upper fat layer and the bottom sediment was removed after tube slicing, diluted with 4 vols 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 total and high-density lipoprotein (HDL) cholesterol were quantified using a reagent kit from Boehringer Mannheim (Montréal, Québec, Canada). The HDL fraction was isolated by precipitation of apolipoprotein B-containing lipoproteins with sodium phosphotungstate-magnesium chloride. Plasma triglycerides were assayed by an enzymatic method using a reagent kit from Boehringer Mannheim, which allowed correction for free glycerol. Plasma nonesterified fatty acids (NEFA) were determined by an enzymatic colorimetric technique using a reagent kit from Wako Pure Chemical Industries (Richmond, VA). Plasma insulin was determined by radioimmunoassay using a reagent kit from Linco Research (St Charles, MO, USA) with rat insulin as standard. Plasma corticosterone was quantified by a competitive protein-binding assay (sensitivity: 0.058 nmol/l; inter-assay coefficient of variation: 9.0%) using plasma from a dexamethasone-treated female rhesus monkey as a source of transcortin.¹⁴

Tissue measurements

After thawing of the frozen liver samples, total lipids were extracted according to the method of Folch *et al.*¹⁵ and solubilized in isopropanol. Cholesterol and triglycerides were quantitated in these lipid extracts using the above mentioned reagent kits. Lipoprotein lipase activity in WAT, BAT, skeletal muscles and heart was measured as described earlier.¹⁶ Upon thawing, samples of 100 µl of tissue homogenates prepared as described above were incubated for 1 hour at 28°C under gentle agitation with 100 µl of a substrate mixture consisting of 0.2 M Tris HCl buffer, pH 8.6, which contained 10 MBq/l [carboxyl-¹⁴C]-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 M or 2 M NaCl. Free oleate released by LPL was then separated from intact triolein,¹⁷ mixed with Universol (New England Nuclear, Montréal, Canada) and sample radioactivity was determined 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 82–91% of total lipolytic activity in all tissue homogenates. LPL activity was expressed as µUnits (1 µU = 1 µmol non-esterified 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.*¹⁸ Treatment effects were essentially similar whether LPL activity was expressed per unit of protein (specific activity) or per whole tissue (total LPL activity), and only specific activity is reported herein.

Statistical analysis

Data are presented as means \pm s.e.m. Comparisons of group means were performed using two-way, one factor analysis of variance (ANOVA) followed by individual mean comparisons using Fisher's Protected Least Squares Difference test. Differences were considered statistically significant at $P < 0.05$. For clarity, only comparisons between the three OVX versus Intact, and OVX+EM-652.HCl versus OVX+E₂ groups are reported.

Results

The effects of OVX, with or without treatment with E₂ or EM-652.HCl, on body weight gain are depicted in Figure 1. Untreated OVX rats displayed a greater increase in body weight than intact animals throughout the treatment period (Figure 1A), and the OVX effect was largely prevented by treatment with E₂ and almost completely prevented by treatment with EM-652.HCl. Figure 1B shows that OVX increased final body weight gain fivefold compared with intact rats. Treatment with EM-652.HCl almost completely prevented the OVX-induced increase in weight gain,

whereas E₂ did so slightly less efficiently. As illustrated in Figure 2, the OVX-induced weight gain was contributory to an increase in both fat (Figure 2A) and protein (Figure 2B) gains. In absolute terms, however, fat gain represented approximately 80% of the extra weight gain induced by OVX. E₂ and EM-652.HCl also acted upon both energy components. The greater efficiency of EM-652.HCl to prevent OVX-induced weight gain compared with E₂ was due to its larger effect on fat gain, final fat content being lower in OVX animals treated with EM-652.HCl than in intact controls. As depicted in Figure 3, OVX increased food intake throughout the treatment period, an effect that was dampened by E₂, but completely abolished by EM-652.HCl, the value being inferior to the food intake observed in intact animals.

Treatment effects on variables of whole body energy balance are presented in detail in Table 1. Removal of the ovaries increased digestible energy intake by 44%, an effect that was prevented only partially by E₂, but totally by EM-652.HCl treatment. Treatment effects on energy intake were reflected in body energy gain as well as in apparent energy expenditure, which as expected was proportional to lean mass gain. Changes in energy intake and expenditure brought by OVX translated into an increase in food efficiency that reached 2.5-fold above intact controls. E₂ treatment dampened this increase, but not to a level that was significantly different from that of untreated OVX animals. Treatment with EM-652.HCl, on the other hand, completely prevented the OVX-induced elevation in food efficiency.

Figure 4 illustrates the cholesterol and triglyceride content of the liver. OVX, with or without E₂, did not alter liver cholesterol content, which however was

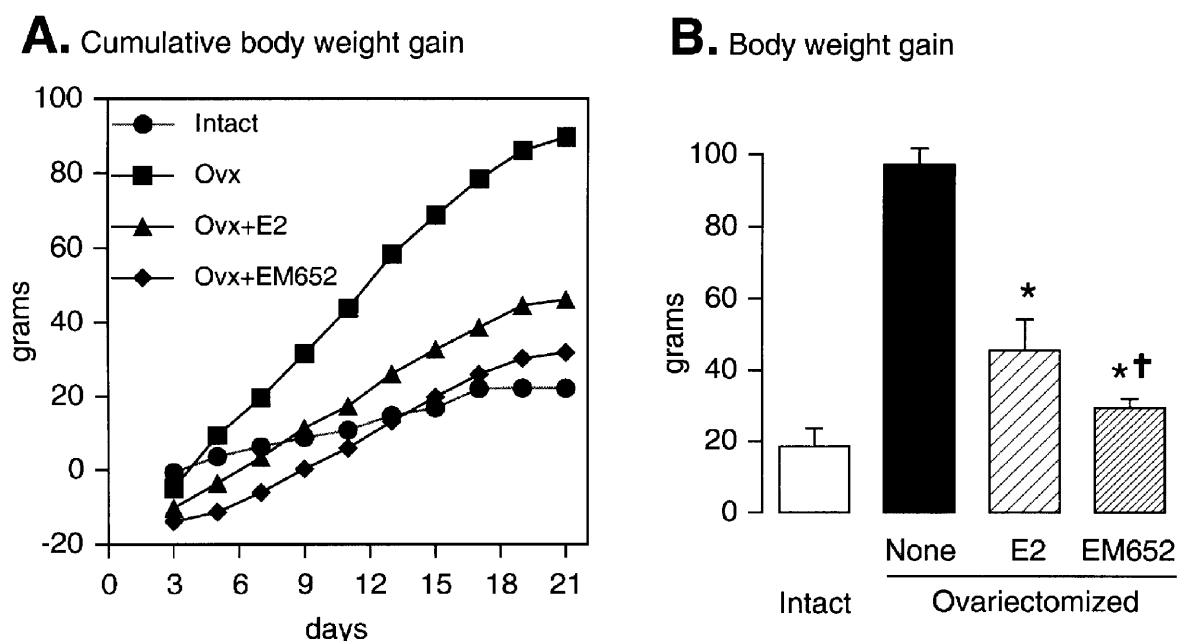


Figure 1 Cumulative (PA) and final (PB) body weight gain of intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Symbols and bars represent means \pm s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$; †different from OVX-E₂, $P < 0.05$.

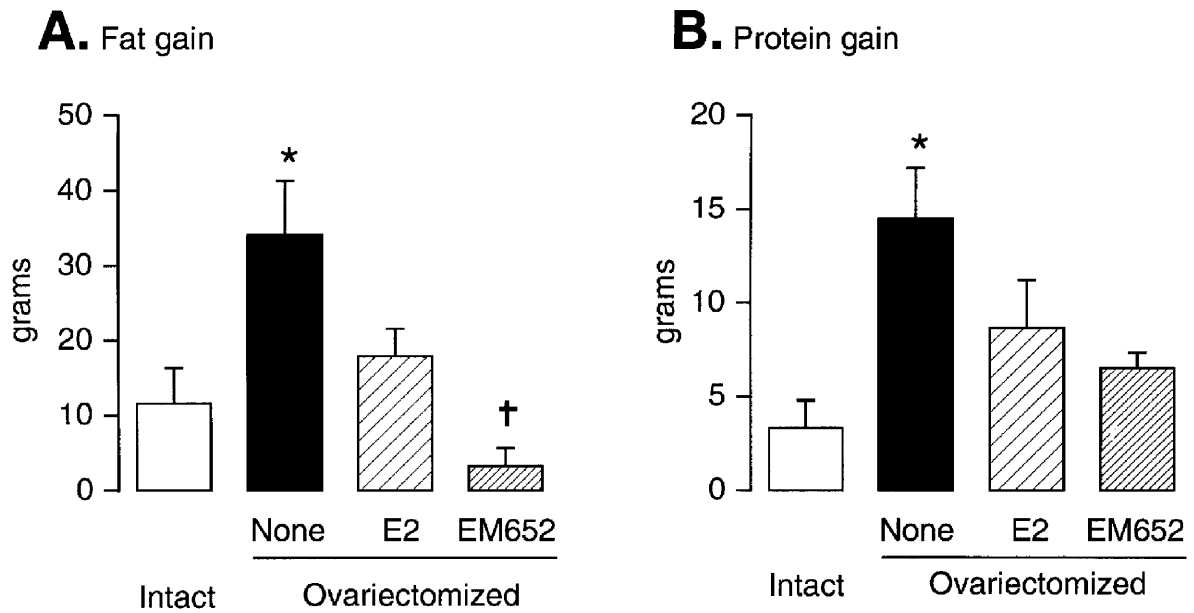


Figure 2 Fat (PA) and protein (PB) gain of intact female rats and of ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, *P* < 0.05; †different from OVX-E₂, *P* < 0.05.

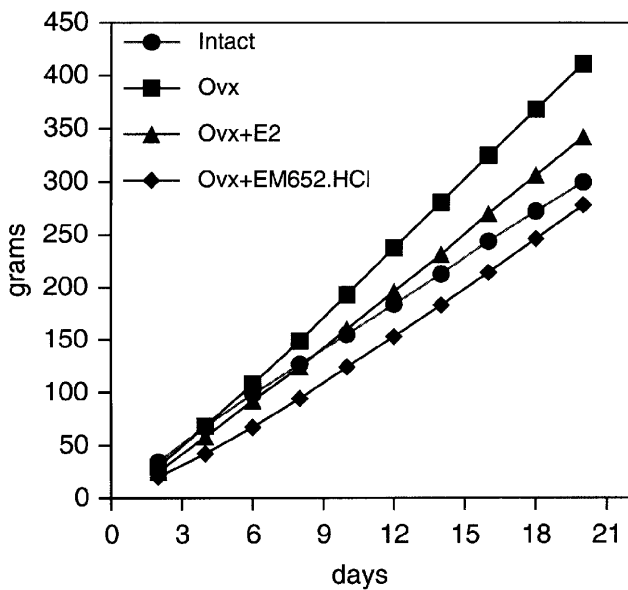


Figure 3 Cumulative food intake of intact female rats and of ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Symbols represent means ± s.e.m. of 8–10 animals.

slightly but significantly increased by EM-652.HCl (Figure 4A). OVX resulted in a two-fold increase in liver triglycerides, an index of long-term hepatic lipid production (Figure 4B). Treatment with EM-652.HCl did not prevent this effect of OVX to any significant extent, whereas administration of E₂ did so. As shown in Figure 5A, plasma total cholesterol was slightly increased by OVX, an effect that was not corrected by replacement therapy with E₂. EM-652.HCl, however, lowered total cholesterol in OVX animals, to levels that were even below those of intact rats. Plasma HDL-cholesterol behaved as did total cholesterol.

Plasma triglyceride concentrations (Figure 5B), on the other hand, were not altered significantly by OVX, with or without EM-652.HCl, but treatment of OVX rats with E₂ increased plasma triglycerides significantly over those of intact, untreated animals.

Plasma levels of energy substrates (glucose and NEFA) are depicted in Figure 6. Removal of the ovaries resulted in a mild degree of fasting hyperglycemia, a condition that was prevented by treatment with either E₂ or EM-652.HCl (Figure 6A). Plasma NEFA levels, an index of adipose tissue lipolysis in the fasted state, were not affected by the ovarian status, but were slightly lowered below intact levels in OVX rats treated with EM-652.HCl (Figure 6B). Plasma levels of insulin and corticosterone, two hormones that are important modulators of lipid metabolism, are presented in Figure 7. OVX brought about fasting hyperinsulinemia, an indication of insulin resistance, which again was prevented by chronic administration of E₂ or EM-652.HCl (Figure 7A). Fasting plasma corticosterone levels were not significantly affected by the status of the ovaries or by the pharmacological treatments under study (Figure 7B).

The consequences of OVX and treatment with E₂ or EM-652.HCl on adipose tissue mass and LPL activity were assessed in the retroperitoneal and inguinal depots, which are representative of visceral and subcutaneous fat, respectively. Figure 8A shows that, in agreement with the total body fat gain described above, retroperitoneal adipose mass was increased two-fold by OVX. Both E₂ and EM-652.HCl prevented this gain in fat depot weight, but the effect of EM-652.HCl was significantly more pronounced than E₂ replacement therapy. The specific activity of LPL, the enzyme responsible for the tissue uptake of fatty acids derived from circulating triglyceride-rich

Table 1 Digestible energy intake, energy gain, apparent energy expenditure, and food efficiency in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days

	Intact	OVX		
		None	E ₂	EM-652.HCl
Digestible energy intake (kJ)	4708 ± 213	6758 ^a ± 231	5511 ^a ± 198	4479 ^b ± 120
Energy gain (kJ)	532 ± 199	1677 ^a ± 264	903 ± 189	278 ^b ± 91
Apparent energy expenditure (kJ)	4176 ± 76	5081 ^a ± 149	4609 ^a ± 143	4201 ^b ± 80
Food efficiency (%)	10.2 ± 3.8	24.1 ^a ± 3.3	15.9 ± 3.0	5.9 ^b ± 1.8

Values are means ± s.e.m. of 8–10 animals. ^aDifferent from Intact, $P < 0.05$; ^bdifferent from OVX + E₂, $P < 0.05$.

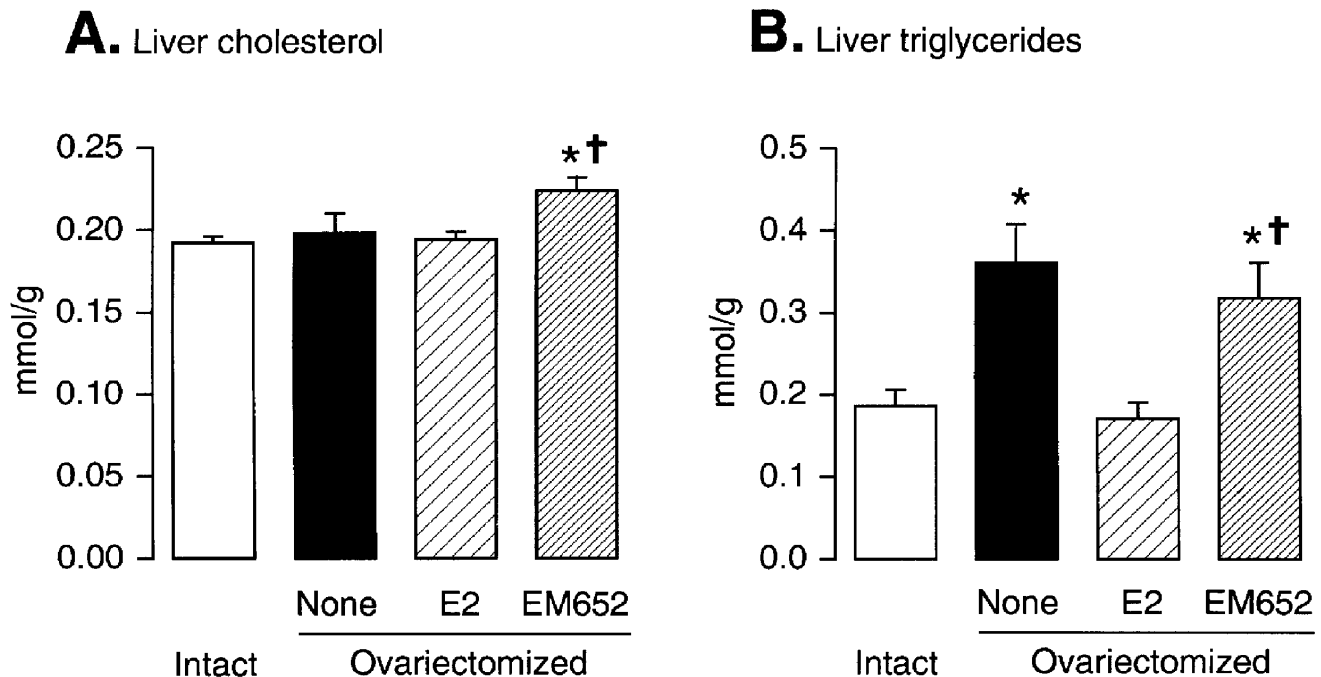


Figure 4 Liver cholesterol (PA) and triglyceride (PB) concentration in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$; †different from OVX-E₂, $P < 0.05$.

lipoproteins, and which is usually associated with fat mass accretion, followed a pattern similar to that of fat depot weight in response to OVX and to pharmacological treatment (Figure 8B). Total LPL activity per depot (data not shown) was four-fold higher in OVX than in intact rats, a difference that was abolished by treatment with E₂ or EM-652.HCl. Identical observations were made in the inguinal adipose depot (data not shown).

The weight of brown adipose tissue (BAT) was also increased by OVX (Figure 9A), mainly because of an increase in the lipid content of the tissue, as total protein remained unaltered (data not shown). Here again, EM-652.HCl was more efficient in preventing the effects of OVX on BAT weight than E₂ replacement therapy. The activity of LPL in BAT, which is frequently associated with BAT thermogenic activity, was slightly lowered by OVX and restored to intact levels by both E₂ and EM-652.HCl (Figure 9B).

In agreement with the total body protein gain presented above, the weight of the soleus muscle

was elevated in OVX, untreated animals compared to intact rats (Figure 10A). Soleus weight of OVX rats was restored to that of intact animals by both E₂ and EM-652.HCl. Soleus LPL activity, which is often positively associated with insulin efficiency upon muscle glucose metabolism as well as with muscle lipid oxidative capacity, was lowered by OVX and restored to values not different from intact controls by treatment with either E₂ or EM-652.HCl (Figure 10B).

Discussion

The present study was prompted by the observation that long-term treatment with EM-800, the prodrug of EM-652, resulted in a reduction in the final body weight and plasma lipid levels of intact female rats.^{11,12} The present study extends these findings by demonstrating that treatment of OVX rats with EM-

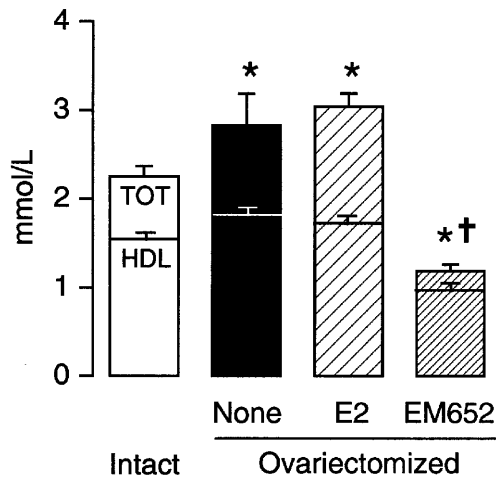
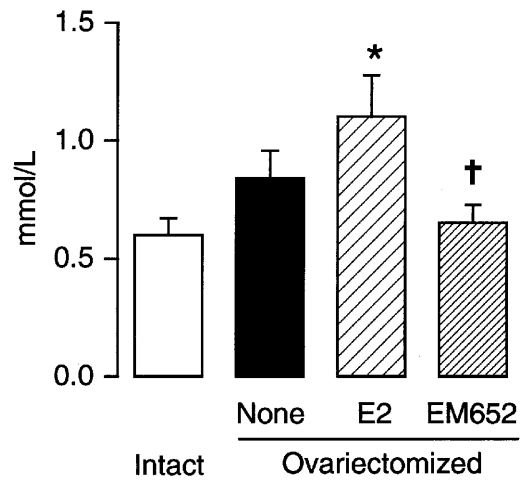
A. Serum cholesterol

B. Serum triglycerides


Figure 5 Serum total and high-density lipoprotein (HDL) cholesterol (PA) and total triglyceride (PB) concentration in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$; †different from OVX-E₂, $P < 0.05$.

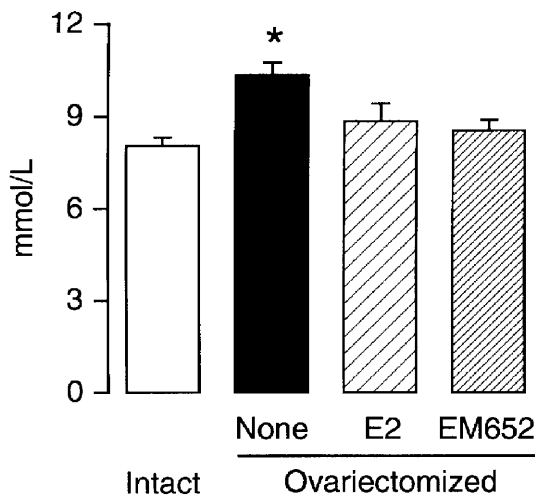
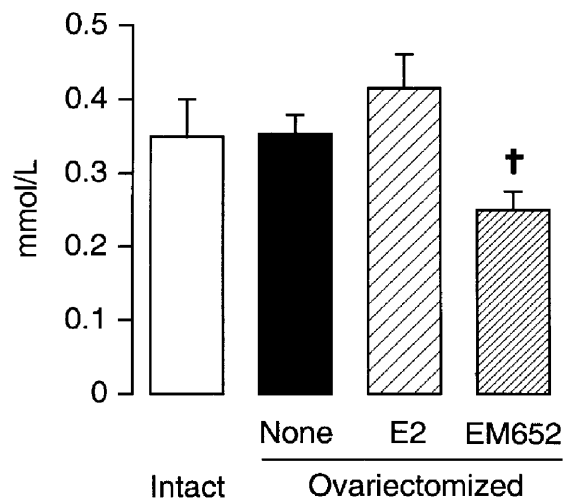
A. Serum glucose

B. Serum nonesterified fatty acids


Figure 6 Serum glucose (PA) and nonesterified fatty acid (PB) concentration in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$; †different from OVX-E₂, $P < 0.05$.

652.HCl is at least as effective as estrogen replacement therapy in reverting obesity and its related lipid and glucose/insulin abnormalities induced by OVX.

Estrogen removal through OVX profoundly altered energy balance and lipid metabolism, as previously described by us and others^{4–7,19,20} In brief, OVX increased energy intake and food efficiency, which resulted in a larger deposition of body energy, mainly in the form of fat. Determinants of lipid metabolism such as liver triglyceride content, an index of long-term hepatic lipid production, and adipose tissue lipoprotein lipase activity, the enzyme which hydro-

lyzes lipoprotein-bound triglycerides and favors tissue uptake of fatty acids thus liberated, were altered in congruence with the increased energy flux, as observed earlier.⁶ 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 pre-diabetic state of insulin resistance.

Ovariectomy induces obesity by removing the catabolic action of estrogen, which acts upon as yet poorly defined central neuropeptidergic pathways that regu-

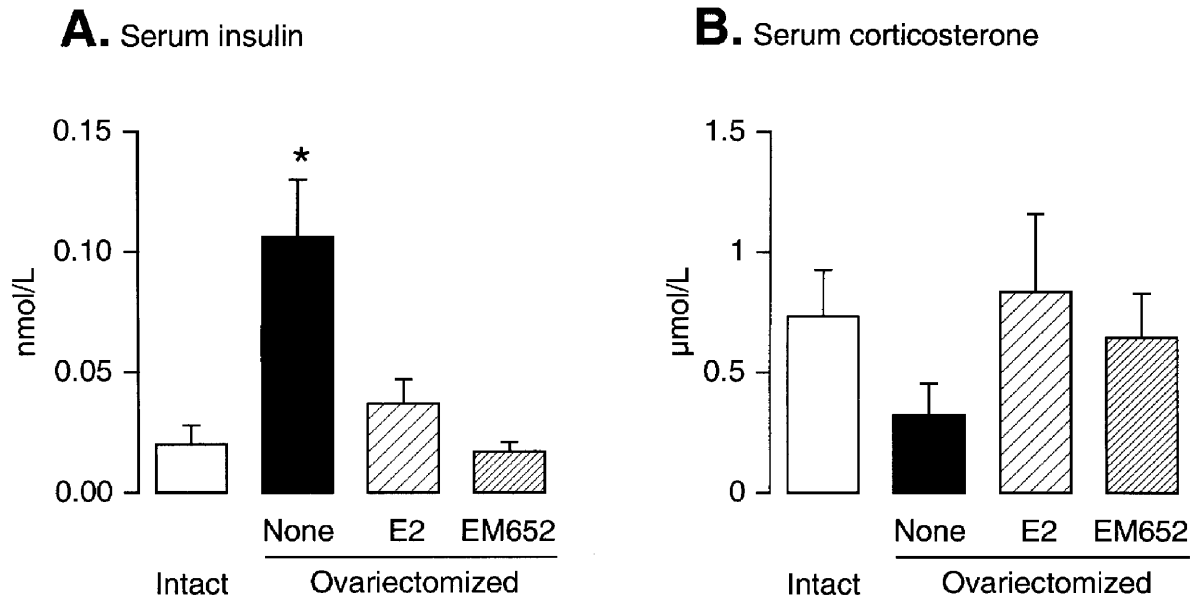


Figure 7 Serum insulin (PA) and corticosterone (PB) concentration in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$.

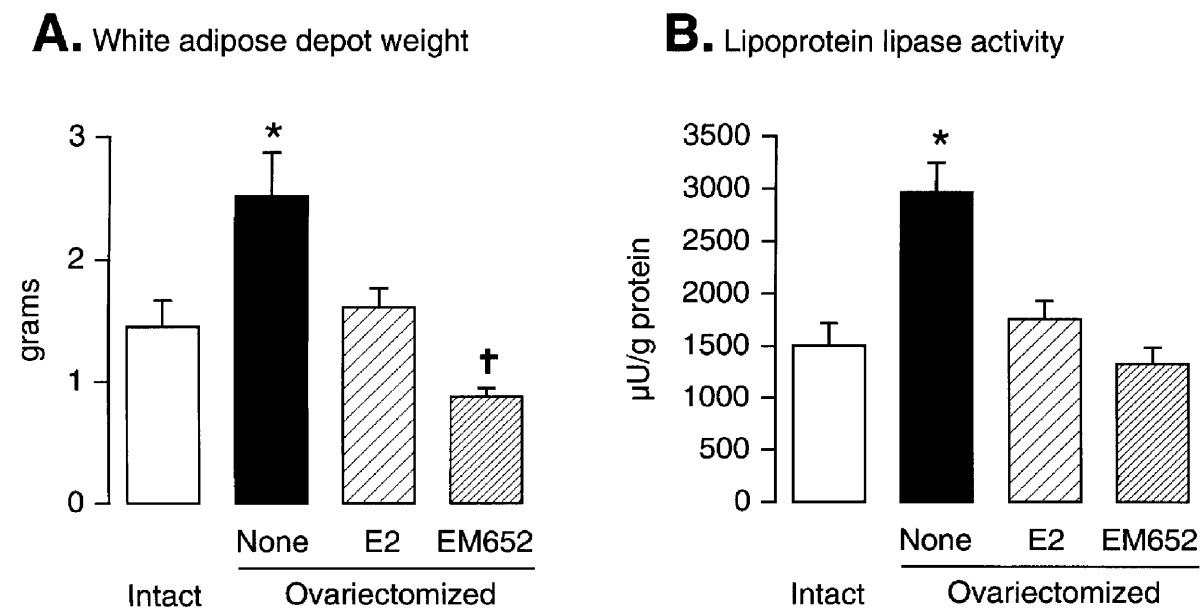


Figure 8 Retroperitoneal white adipose depot weight (PA) and lipoprotein lipase-specific activity (PB) in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from Intact, $P < 0.05$; †different from OVX-E₂, $P < 0.05$.

late energy balance.^{4,5} Previous studies have shown that the hypothalamic-pituitary-adrenal axis is somehow involved in the modulation of these pathways.^{6,21} With regard to peripheral lipid metabolism, it is likely to be affected by gonadal steroids both directly through their action on the liver and adipose tissue and indirectly through their central action on food intake, which in turn affects peripheral lipid metabolism, for instance, via changes in insulin levels and its efficiency of action. The quantitative contribution of central and peripheral steroid actions on lipid metabolism remains to be more fully defined.

Treatment with a physiological amount of E₂ that ensured the maintenance of a normal uterine weight

greatly dampened most of the OVX-induced changes in energy balance parameters, determinants of lipid metabolism, and indexes of insulin sensitivity. However, some of the orexigenic effect of OVX was preserved, leading to the partial maintenance of accumulated energy, fat and protein deposition. It should be mentioned that somewhat larger doses of E₂ have been found to totally prevent the extra energy gain brought by OVX.^{4,5} OVX-induced hypercholesterolemia, however, was not reversed by E₂ treatment either, which also caused hypertriglyceridemia, as shown earlier.²²

Treatment with EM-652.HCl was very effective in preventing most of the effects of OVX on energy

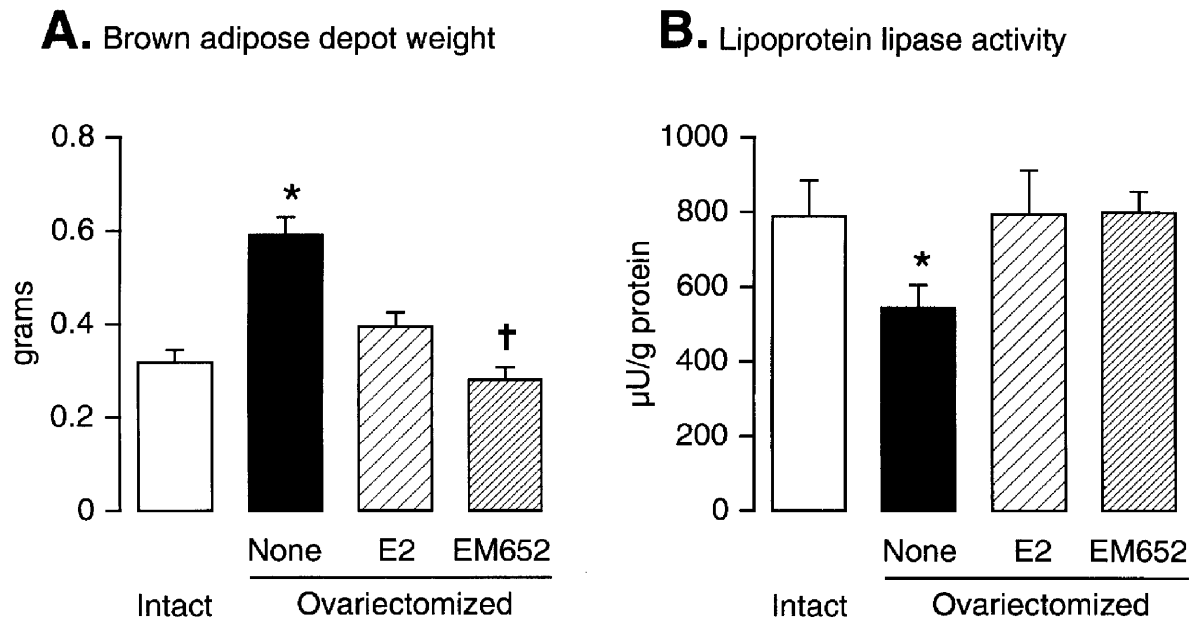


Figure 9 Interscapular brown adipose depot weight (PA) and lipoprotein lipase-specific activity (PB) in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$; †different from OVX-E₂, $P < 0.05$.

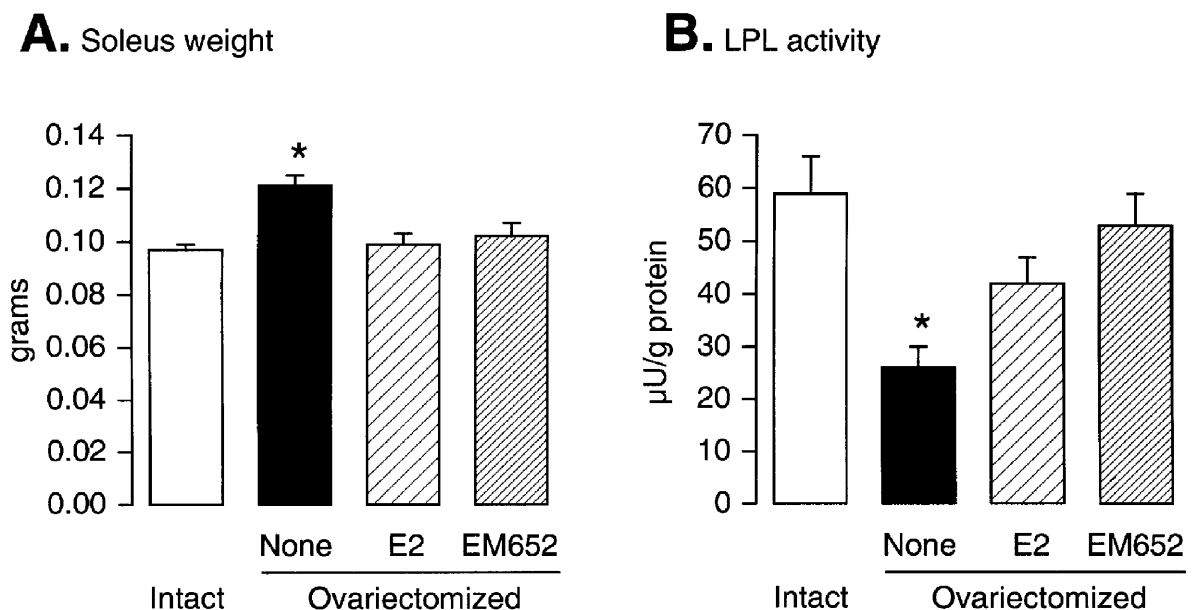


Figure 10 Soleus muscle weight (PA) and lipoprotein lipase specific activity (PB) in intact female rats and in ovariectomized (OVX) rats treated with placebo (none), estrogen (E₂), or EM-652.HCl for 20 days. Bars represent means ± s.e.m. of 8–10 animals. *Different from intact, $P < 0.05$.

balance and lipid metabolism. The drug therefore shares actions on energy and lipid metabolism that have been described previously for other antiestrogens such as nafoxidine, tamoxifen, raloxifene, ICI-182,780 and CP-336,156.^{23–27} EM-652.HCl totally abolished the OVX-induced increase in energy intake, and even led to a somewhat reduced food efficiency and energy deposition in the form of fat compared with intact animals. The compound was also able to prevent the OVX-induced changes in LPL activity, that is the increase in white adipose tissue (an index of a higher potential for lipid storage), the decrease in brown adipose tissue (sometimes asso-

ciated with a lesser thermogenic activity of the tissue), and the decrease in skeletal muscle (sometimes associated with muscle insulin resistance and with a lesser capacity of muscle to oxidize fat). These effects of EM-652.HCl on LPL activity constitute indirect evidence for the normalization of the metabolic activity of these tissues that obviously play key roles in energy and lipid metabolism.

Treatment with EM-652.HCl normalized plasma cholesterol levels in OVX rats, and did not elevate plasma triglycerides as did treatment with E₂. Not surprisingly, HDL-cholesterol concentrations responded identically to total cholesterol, in accordance with

the fact that HDL is the main cholesterol carrier in the rat. Such hypolipidemic actions have been reported recently for the prodrug EM-800.¹² In that study performed in fed animals, EM-800 was shown to be frankly hypotriglyceridemic, contrary to the neutral effect of EM-652.HCl observed here in fasted animals. It is most likely that the hypotriglyceridemic action of EM-652.HCl was masked in the present study by the rather long fasting period imposed on the animals before blood sampling. Estrogen and some antiestrogen compounds lower serum cholesterol levels in the rat and human, but estrogen elevates serum triglyceride levels in both species (reviewed in Ref. 12). This is due to a direct action of estrogen on the secretion of triglyceride-rich very-low density lipoproteins by the liver.²² The suggested ability of EM-652.HCl to reduce both plasma cholesterol and triglyceride levels is therefore of particular interest and appears to be unique to this compound among antiestrogens.¹²

Ovariectomy did not alter liver cholesterol content, but increased liver triglyceride content, an effect that was prevented by treatment with E₂. The observation that EM-652.HCl resulted in a small increase in liver cholesterol and triglycerides was therefore surprising given the expected lowering of liver lipid levels following a reduction in energy intake. This points to the possibility that EM-652.HCl may act directly on hepatic cholesterol synthesis, its excretion in the bile or its secretion into lipoproteins (VLDL and HDL), as well as on hepatic triglyceride synthesis or secretion into lipoproteins. It should however be noted that the increases in liver lipid concentrations brought by EM-652.HCl were quantitatively modest and did not reach levels indicative of significant steatosis. These observations may also be linked to the marked decrease in serum cholesterol and triglycerides brought by the prodrug of EM-652 and observed under fed conditions.¹²

It is of interest to note that, like E₂, EM-652.HCl totally prevented fasting hyperglycemia and hyperinsulinemia that were observed in the untreated OVX rats compared with intact animals. The latter profile is a reliable index of the presence of an impaired insulin action on glucose metabolism (insulin resistance), and is frequently associated with obesity.^{1–3} It is likely that the effect of E₂ and EM-652.HCl on energy balance, particularly on food intake, was involved in such an improvement, 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 variables of lipid metabolism and insulin sensitivity were assessed in the fasted state, that is at a time when lipid flux is minimal. Treatment effects therefore represent robust phenomena that persisted after 12 h of fasting. The consequences of OVX and pharmacological treatments observed in the present study would most likely become much more evident in the postprandial state, during which glucose and lipid metabolisms are fully active. The postprandial state could also unveil treat-

ment effects (eg plasma triglyceride lowering) that may not have been present in the fasted state. Since postprandial lipemia is strongly associated with the risk for cardiovascular disease,^{1–3} the consequences of EM-652.HCl treatment on postprandial glucose and lipid metabolism certainly deserve future attention.

In conclusion, in addition to the beneficial effects of EM-652.HCl on carcinogenesis and bone loss previously reported, the present study demonstrates that the compound can be considered as an effective agent to prevent OVX-induced obesity. The study also shows that EM-652.HCl reduces cardiovascular risk factors associated with obesity such as dyslipidemia and insulin resistance. Therefore, the present study lengthens the list of beneficial 'estrogen-like' actions of the compound. Further research is required to identify the central and peripheral mechanisms by which EM-652.HCl acts upon energy balance and lipid metabolism.

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