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Effect of Estrogen on Lipoprotein Lipase Activity and Cytoplasmic Progestin Binding Sites in Lean and Obese Zucker Rats (41809)

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Abstract. Injections of 5 μg estradiol benzoate (EB) for 5 days resulted in decreases in the rate of body weight gain in both lean (Fafa) and obese (fafa) Zucker rats. EB administration also resulted in significant induction of cytoplasmic progestin binding sites in both hypothalamic–preoptic area (H–POA) and adipose tissues from rats of both genotypes. However, EB treatment significantly decreased lipoprotein lipase (LPL) activity in adipose tissue from lean, but not obese, Zucker rats and the same treatment increased LPL activity in the uteri from lean, but not obese, Zucker rats. The data are discussed in terms of the metabolic and reproductive dysfunctions observed in the genetically obese rat.

Ovarian hormones affect both reproductive and metabolic systems in a variety of species, and the interactive relations between the two major systems are currently being studied in several laboratories. This paper explores some of the effects of estrogen administration on body weight and on cellular functions in adipose, brain and uterine tissues from genetically obese Zucker (fafa) rats, a strain of rats with apparent deficiencies in the regulation of both metabolic and reproductive systems (1).

In nonobese rats of several strains, ovariectomy (OVX) leads to a transient increase in food intake and rate of body weight gain, and a long-lasting increase in adipose tissue lipoprotein lipase (LPL) activity relative to gonadally intact controls (2–4). Estrogen administration to OVX rats leads to a transient decrease in food intake and rate of body weight gain, accompanied by a rapid and long-lasting decrease in adipose tissue LPL activity (2–6). In addition, the presence of estrogen-sensitive LPL activity has recently been demonstrated in uteri from OVX rats (7). The presence of receptor sites for estrogens and the inducibility of binding sites for progestins have been demonstrated in both adipose (8, 9) and uterine (10, 11) tissues, suggesting that the enzymatic changes which are observed in these tissues following hormone administration may be receptor mediated.

Lean Zucker rats are sensitive to both the anorexic and weight-reducing effects of exogenous estrogen administration (12, 13). Following OVX, obese Zucker rats increase their food intake and gain more weight than do gonadally intact controls. Estrogen administration to OVX obese Zucker rats are effective in inducing changes in food intake ((12), but cf. (13)), but not body weight (12, 13) although similar injections depress both feeding and body weight gain in lean controls.

Early and sustained increases in lipoprotein lipase (LPL) activity are found in the adipose tissues of genetically obese (fafa) Zucker rats, and it has been suggested that deficits in the regulation of LPL activity might be of etiological importance to the development of obesity in the Zucker rat (14–16). There is only one published report of the effects of exogenous estrogen treatment on LPL activity in the obese Zucker rat. Upton *et al.* (17) have demonstrated that very high (33 μg/kg) doses of estradiol benzoate (EB) administration will decrease adipose tissue LPL activity in parametrial tissues of gonadally intact, obese Zucker rats. There are no reports in the literature regarding the effects of estrogen on LPL activity in uterine tissues in the Zucker rat.

Obese Zucker female rats have been reported to be reproductively unsuccessful (18), with reports suggesting either that they are completely sterile (19) or that their reproductive capacities are severely limited due to behavioral irregularities secondary to their obesity (20). In an exploration of possible mechanisms underlying these reproductive deficits, Saiduddin and Zassenhaus (21, 22) have examined the quantitative and qualitative properties of estrogen binding sites in uterus, hypothalamus–preoptic area (H–POA), and pi-

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tuitary, and the properties of estrogen-induced progestin binding sites in uterus of lean and obese Zucker rats. Following OVX, obese rats had only one-half the number of cytoplasmic estrogen binding sites in these tissues, suggesting a decrease in the pool of available sites by which estrogens might exert their influence on cellular events. Although those sites which were measured had similar physicochemical properties, abnormalities were observed in the translocation of the hormone–receptor complex to the nucleus, as well as in [^{3}H]uridine incorporation into RNA in tissues from obese rats (21).

Despite these differences in estrogen–receptor dynamics, induction of progestin receptors in H–POA and uterus following estrogen pretreatment to the rats resulted in similar amounts of induction of progestin binding sites in the two genotypes (22). Uteri from obese rats also took up as much labeled progestin as did uteri from lean controls, and there was similar feedback by progesterone on the amount of estrogen binding sites in uteri of the two genotypes (22). However, reports in the literature examining estrogen binding sites, or the induction of progestin binding sites, in adipose tissues of genetically obese rats are lacking.

In the current study, we further investigated the interactions between the reproductive and metabolic systems of the lean and obese, OVX Zucker rat. We measured the effects of administration of estradiol benzoate (EB) on changes in body weight and adipose and uterine LPL activities, and on the induction of progestin binding sites in brain and adipose tissues.

Methods. Young adult (10- to 12-week-old) female genetically obese (fafa) and lean (Fafa) Zucker rats, derived from a crossing of Fafa females with fafa males from our own laboratory stock, were used in this study. Lean and obese rats were identified following visual inspection and examination of growth curves. Lean rats ranged in weight from 180 to 210 g at the beginning of the experiment, while age-matched obese rats weighed between 275 and 325 g. All animals were maintained on a 12 hr:12 hr light:dark cycle, and were fed laboratory rat pellets (Charles River) and tap water *ad libitum*.

Rats were ovariectomized via bilateral, dorsolateral incisions while the animals were under ether anesthesia. Care was taken to minimize disturbance to the uterus and surrounding parametrial adipose depot.

Starting 3 days following surgery, daily measurements of body weight (to the nearest 0.1 g) were taken. On postoperative Day 10, two subgroups within each genotype were formed, matched with respect to average body weight. One subgroup received daily subcutaneous injections of sesame oil vehicle (0.1 cc), while the other received injections of estradiol benzoate (EB: 5 μg/day). Daily body weight measurements were taken throughout the experiment.

In the first experiment, 16 lean and 16 obese rats were split into two treatment subgroups, matched for average body weight ($N = 8$ per group). One control obese rat died during the experiment, and all data from it were dropped from the analyses. Rats were killed by decapitation following 5 days of oil or hormone treatment. Parametrial adipose tissues were dissected out and homogenized in medium containing 0.25 *M* sucrose and 1 m*M* EDTA, pH 7.4. Uteri were dissected and incubated in 1 ml Krebs–Ringer phosphate containing one-third the usual concentration of calcium and with 10 units heparin (KRP-hep; (6)). Lipoprotein lipase (LPL) activity was measured in the high-speed supernatants from parametrial tissue (23) and the KRP-hep incubates from uteri (7) according to a modification (6) of the methods of Schotz *et al.* (24).

In the second experiment, rats were killed by overdose injections of sodium pentobarbital (Nembutal) following 5 days of oil or EB treatment. Animals were perfused with cold saline, and parametrial adipose tissues and hypothalamic–preoptic areas of the brain were rapidly dissected. Tissues were homogenized in cold Tris (10 m*M*) buffer, containing 1.5 m*M* EDTA, 12 m*M* monothioglycerol, and 10% (v/v) glycerol, pH 7.4. Homogenates were centrifuged at 48,000*g* for 1 hr. Cytoplasmic progestin binding sites were measured using 3H-R5020 (^{3}H-promegestone; S.A. 87 Ci/mmole; New England Nuclear) as a ligand (9, 25).

All data were analyzed using Student's *t* between EB-treated and oil-treated rats of the same genotype. Data were considered to be statistically significant if $P \leq 0.05$.

Results. EB administration led to a significant decrease in the change in body weight

over the 5-day treatment period (final weight − initial weight) in both lean and obese Zucker rats (Table I). Uterine weight and total uterine protein significantly increased in both lean and obese rats following EB administration (Table I). EB treatment had no effect on either total parametrial pad weight or parametrial pad protein, whether protein levels are expressed as total amounts per pad or on a per gram tissue weight basis (Table I).

LPL activity was several-fold higher in parametrial adipose tissues from obese, as compared with lean Zucker rats (Figs. 1A, B). LPL activity was significantly lower in parametrial adipose pads taken from EB-treated lean rats as compared with oil-treated lean controls. This was true whether data were expressed as units (FFA released per hour) per milligram protein or as units per whole parametrial pad (Figs. 1A, B). Parametrial adipose tissue LPL activity was not altered by EB treatment in obese Zucker rats, whether data were expressed relative to protein levels or per pad.

Total uterine LPL activity was significantly increased in EB-treated lean rats, although there was no effect on total uterine LPL activity in uteri from obese animals (Fig. 1C). When uterine LPL activity was expressed in units per milligram protein (Fig. 1D), apparent, although not statistically significant ($P = 0.06$), decreases in enzyme activity were found for lean animals and significant decreases in activity were found for obese rats.

Cytoplasmic progestin (3H-R5020) binding was measured in both brain (H–POA) and parametrial adipose tissues from lean and obese OVX Zucker rats which had been treated with either EB or oil. Significantly higher levels of progestin binding were found in both H–POA and adipose tissues in EB-treated rats of both the lean and obese genotypes (Table II). EB treatment resulted in a twofold greater induction of progestin binding sites in both H–POA and parametrial adipose tissue of lean, as compared with obese, rats (Table II).

Discussion. Adipose tissue LPL activity is elevated in the obese Zucker rat (Fig. 1) (14–16) and this altered enzyme activity, reflecting a possible regulatory deficit in the appropriate gating of metabolic fuels, might be important in the development and maintenance of the obese condition (16). In the current experi-

TABLE I. EFFECT OF 5 DAILY INJECTIONS OF 5 μg EB ON BODY WEIGHT, AND UTERINE AND PARAMETRIAL PAD WEIGHTS AND PROTEIN CONTENTS IN OVARIECTOMIZED RATS[a]

	Lean (Fafa)		Obese (fafa)	
	Oil ($N = 8$)	EB ($N = 8$)	Oil ($N = 7$)	EB ($N = 8$)
Body weight (g)				
Initial (I)	192.3 ± 5.7	194.0 ± 7.9	300.0 ± 17.7	287.7 ± 19.0
Final (F)	205.0 ± 5.8	191.2 ± 6.9	322.7 ± 18.3	301.9 ± 18.6
Change (F − I)	12.7 ± 1.2	−2.8 ± 1.5**	22.9 ± 2.7	14.2 ± 2.1*
Uterine weight (g)	0.113 ± 0.008	0.363 ± 0.022**	0.130 ± 0.045	0.254 ± 0.019*
Total uterine protein (mg)	1.06 ± 0.12	3.46 ± 0.37**	1.03 ± 0.43	2.29 ± 0.18*
Uterine protein (mg/g uterus)	9.20 ± 0.54	9.40 ± 0.49	7.48 ± 0.80	9.19 ± 0.68
Parametrial pad weight (g)	0.398 ± 0.050	0.352 ± 0.046	1.696 ± 0.241	1.519 ± 0.171
Total parametrial pad protein (mg)	0.301 ± 0.039	0.326 ± 0.042	0.809 ± 0.121	0.839 ± 0.186

Note. One obese control animal died during experiment; all data from it were dropped.

[a] Data are presented as mean ± SEM values.

* $P < 0.05$, Student's t Oil vs EB within genotype.

** $P < 0.01$, Student's t Oil vs EB within genotype.

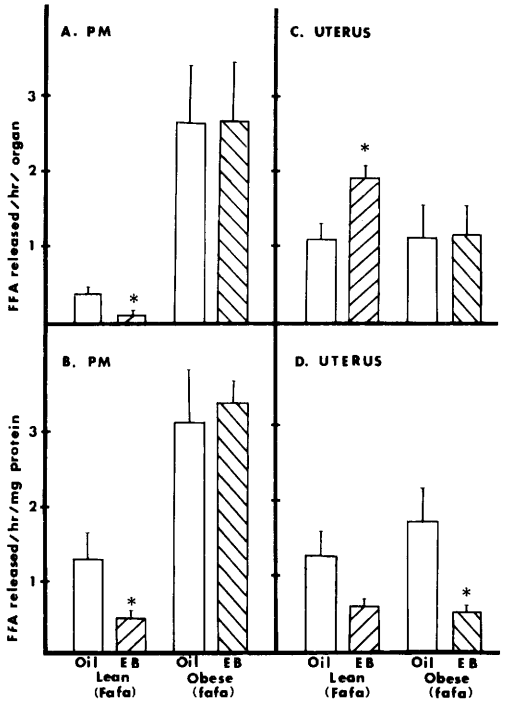

FIG. 1. The effect of administration of 5 μg EB for 5 days on parametrial (PM) (A, B) and uterine (C, D) LPL activity in lean (Fafa) and obese (fafa) OVX Zucker rats. Data are calculated as units of enzyme activity (FFA released per hour) per whole organ (A, C) or per milligram protein (B, D) and are presented as mean values ±SEM. * Denotes significant differences between oil- and EB-treated rats within genotypes.

ments, parametrial adipose tissue LPL activity in obese Zucker rats was insensitive to the regulatory effects of exogenous EB administration (Fig. 1), further supporting the possibility that there is a primary deficit in the regulation of adipose tissue LPL activity in the obese Zucker rat.

Although no changes in adipose tissue LPL activity were observed with the short-term, relatively low dose of EB used in the current study, injection of a higher dose of EB for longer treatment times has been shown to result in estrogen-induced decreases in adipose tissue LPL activity in obese, as well as lean Zucker rats (17). Relative differences in EB dosage for the two genotypes in the current study must be acknowledged, as all rats received a constant dose (5 μg) of EB, rather than a dosage corrected for body weight. These data suggest that the doses of EB given to the obese rats in the current experiment might have been too low to induce the commonly observed decrease in enzyme activity, or that the obese Zucker is relatively insensitive to the estrogen effects on adipose tissue LPL activity.

However, the lack of an EB-induced decrease in adipose tissue LPL activity in the obese animals in the current study cannot be explained solely by an insufficient dose of the hormone or by a relative tissue insensitivity to the administered EB, as EB treatment did result in the induction of progestin binding sites in the same parametrial adipose tissues (as well as other tissues, Table II; 22) taken from the obese animals (Table II). It is unclear whether the lack of hormone effectiveness in altering LPL activity is a reflection of a direct deficit in the endocrine system (i.e., at, or near, the receptor level), or whether this is a reflection of a more general deficit in the regulation of LPL activity in the obese Zucker rat.

In addition, estrogen pretreatment resulted in increases in uterine protein content, without altering uterine LPL activity in tissues from OVX, obese rats (Table I, Fig. 1). These data suggest that, at least at the level of induction

TABLE II. EFFECT OF EB ADMINISTRATION ON CYTOPLASMIC PROGESTIN BINDING IN H-POA AND PARAMETRIAL ADIPOSE TISSUES OF LEAN AND OBESE ZUCKER RATS[a]

	Lean (Fafa)		Obese (fafa)	
	Oil (N = 4)	EB (N = 4)	Oil (N = 4)	EB (N = 4)
Tissue				
Parametrial adipose	0.160 ± 0.036	2.076 ± 0.257*	0.050 ± 0.022	0.288 ± 0.013*
H-POA	1.07 ± 0.20	2.22 ± 0.40*	1.08 ± 0.25	3.70 ± 0.10*

[a] Data are presented as means ± SEM, in units of fmol 3H-R5020 specifically bound per mg protein.
* $P < 0.05$, Student's t, Oil vs EB within genotype.

of protein synthesis, the obese Zucker adipose and uterine cells are functional and responsive to some of the regulatory effects of the ovarian hormones. These data further suggest that there might be either differential systems of hormone responsivity within the same tissue, or different thresholds of hormone concentrations necessary for the initiation of different subcellular responses to estrogens (e.g., modulation of specific enzyme activities vs induction of protein synthesis).

The presence of estrogen-sensitive LPL activity was recently demonstrated in uteri from OVX Sprague–Dawley rats (7). It was suggested that the ovarian hormones might serve a gating function in the directing of metabolic fuels to a variety of tissues, including tissues directly involved in reproduction, so as to provide these tissues with optimal levels of utilizable energy at times when the tissues would be most active (7). The lack of an appropriate estrogen response for uterine LPL activity in the obese animals suggests that the metabolic regulatory deficits are not limited to tissues involved mainly in the maintenance of metabolic homeostasis, but extend to at least one major component of the female reproductive tract (the uterus).

Although LPL activity was increased in the whole uterus of estrogen-treated, heterozygous Zucker rats (Fafa; Fig. 1C), the increase in enzyme activity was less specific than the increase in uterine LPL activity which was found in Sprague–Dawley rats (7). We have previously reported EB-induced increases in uterine LPL activity when data were expressed both as activity per whole tissue, as well as activity per milligram protein, suggesting a specific induction of LPL synthesis and/or activation which was greater than the general increase in uterine protein found following estrogen administration. That the Zucker rats who were lean displayed an estrogen effect intermediate between those of the Sprague–Dawley rats and the obese animals suggests a possible expression of the heterozygous gene makeup. More detailed documentation for an explanation based upon the heterozygous genes for intermediate effects in the lean littermate of the obese Zucker rat has been suggested, but not extensively explored, for other systems (14, 26).

In summary, the obese Zucker rat, which has both metabolic and reproductive dysfunctions, has a complex pattern of responsivity to estrogen. Although a robust induction of progestin receptors is found in uterus, H–POA and parametrial adipose tissues ((21, 22), Table II), uterine and adipose tissues LPL activities are insensitive to the normally observed regulation by the gonadal steroid (Fig. 1). It is currently unknown whether these metabolic deficits, and the possible resultant irregularities in shunting of metabolic fuels to appropriate reproductive tissues, might be reflected in the reproductive deficiencies observed in this obese rat.

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