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Effects of Estrogen on Food Intake, Body Weight, and Temperature of Male and Female Obese Mice[1] (42204)

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Abstract. Estradiol benzoate (EB) treatment of male and female C57BL/6J ob/ob mice for 32 days led to decreased body weight (20%), percentage body fat (8%) and carcass protein content (12%) when compared with non-EB-treated obese control mice. Estradiol reduced the caloric intakes of both genders by 25–35%, but did not affect body temperature regulation. Circulating glucose and insulin concentrations were also lowered by estrogens, although hyperinsulinemia persisted. Since post-treatment body weight changes correlated with daily food intakes ($r = 0.81$) rather than to rectal temperatures ($r = -0.19$), it appears that hypophagia provided a greater contribution to the estrogen-mediated reductions of growth and carcass fat than did altered energy expenditure for thermoregulation. While these data show that EB treatment does reduce the severity of some metabolic disturbances in a genetic model of type II diabetes, long-term estrogens do not appear to offer substantial advantages in the treatment of obesity or diabetes when compared with the effects of caloric restriction alone.

In both rats and mice an inverse relationship exists between circulating estrogens and growth. Bilateral ovariectomy leads to weight gain with the distribution of growth about equally divided between increased fat content and lean body mass (1–4). Replacement with estrogens causes weight loss and reduced growth rates until normal values are achieved (1, 2, 5).

C57BL/6J mice having the ob/ob mutation exhibit many metabolic and growth disturbances including obesity, diabetes, hyperphagia, and chronically depressed rates of energy expenditure (6). Obese mice of both sexes are sterile and have low levels of circulating gonadal hormones (6–9). Little is known of the role which depressed sex hormones may play in the obesity of ob/ob mice. Previous studies show that exogenous androgens depress growth and reduce fat content in male ob/ob mice, even though it is known that androgens increase body mass and orchidectomy elicits weight loss in normal males (10–13). In the present study we extended these earlier findings by examining the long-term effects of a female sex steroid, estradiol (benzoate), on growth, carcass composition, and diabetes in mature male and female ob/ob mice.

Materials and Methods. A total of 49, five- to six-month-old male and female C57BL/6J obese mice were randomly divided by sex into experimental groups receiving either Silastic capsules containing 1 mg crystalline estradiol benzoate (Sigma Chemical, St. Louis, Mo.) or empty capsules. Approximately 50% of each male or female subgroup was allowed free access to a dry mash of Purina mouse diet in order to determine daily food intake. The remaining mice had *ad libitum* access to the standard Purina pellet diet. All mice were housed individually in commercially available grid floor cages in a colony room having a 12-hr diurnal light cycle and a temperature of 22 ± 2°C. After 2 weeks adaptation to the feeding schedule, capsules constructed of silicone tubing (0.125 in o.d. × 0.078 in i.d. × 0.25 in, Dow-Corning No. 602-305) were subcutaneously implanted in the intrascapular region. In previous work we have found that these capsules provide sustained estradiol delivery to weanling rats for a minimum of 2 months (5). The body weights and rectal temperatures (YSI telethermometer) of mice in all groups were determined at regular intervals during the 32-day post-treatment period. At the end of the experiment, mid-AM blood samples were taken from the retroorbital plexus (ether anesthesia) for glucose and insulin determinations. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, Calif.). Insulin

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values were determined using a commercially available solid-phase radioimmunoassay (Micromedic Systems, Horsham, Pa.). The carcasses were then prepared for analysis of body composition. The contents of the stomach, intestinal tract, and urinary bladder were removed and discarded. The carcasses were autoclaved, frozen, and lyophilized (72 hr) to constant weight (total body water). The dry carcasses were minced and extracted (ether: methanol, 1:2) to constant weight in a Soxhlet apparatus to determine total lipid content. The fat-free carcass was then oxidized completely in a muffle furnace (900°C) and protein and ash contents were determined gravimetrically.

Results. *Survival.* Three of the EB-treated mice died during the experiment. These mice had empty capsules at necropsy indicating that the entire 1-mg EB dose had leaked from the implant shortly after surgical placement. All other hormone-treated mice still had EB remaining in the capsule at the end of the experiment.

Growth effects of pelleted vs mash diets. There were no consistent differences in body weight gain, nasoanus length, carcass protein, carcass ash, or percentage carcass fat between subgroups of obese mice fed the Purina diet as pellets or as a dry mash. Therefore, the values from mice in the two feeding conditions have been combined for presentation below.

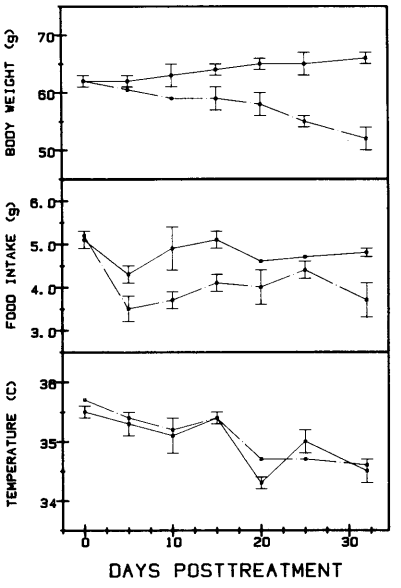

FIG. 1. Body weights (means ± SEM), food intakes, and rectal temperatures of male EB-treated (N = 13, dotted lines) and sham-treated (N = 12), solid lines) obese mice. Food intakes presented as daily, means over each 5-day interval.

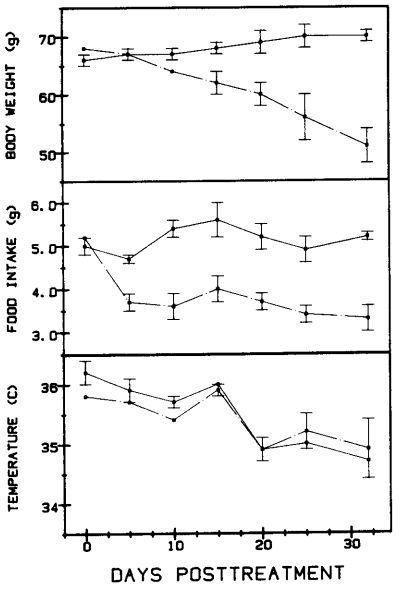

FIG. 2. Body weights, food intakes, and body temperatures of female EB-treated (N = 11, dotted lines) and sham-treated (N = 10, solid lines) mice. All data presented as means ± SEM.

Somatic growth. Figures 1 and 2 summarize the body weight, food intake, and body temperature responses of male (Fig. 1) and female (Fig. 2) obese mice to EB treatment. Female ob/ob mice (66.1 ± 1.0 g) were slightly heavier than males (62.4 ± 0.8 g, $P < 0.05$, Student's t test) before capsule implantation. Two-way analysis of variance of the body weight changes during the 32-day observation period revealed that EB administration led to lower body weights in both male and female mice. A significant gender × treatment interaction ($P < 0.05$) indicated that females were more responsive than males to the weight-suppressing effects of the hormone. Linear growth, as determined by nasoanus length, generally mirrored the changes in body weight. EB-treated mice were shorter than untreated controls at sacrifice ($P < 0.01$) and females were more sensitive than males to the hormone's effect on linear growth ($P_{interaction} < 0.01$, Table I).

Food intake. No gender differences in daily

TABLE I. FINAL CARCASS COMPOSITION OF EB- AND SHAM-TREATED OBESE MICE

Group	Body wt	NA length	% Fat	Ash	Protein	Uterus
Male, EB						
$N = 13$	51.3 ± 1.7	104.3 ± 1.0	45.6 ± 1.1	0.98 ± 0.02	5.7 ± 0.1	—
Male, sham						
$N = 12$	65.1 ± 0.9	106.7 ± 0.7	54.9 ± 0.4	0.95 ± 0.01	6.4 ± 0.1	—
Female, EB						
$N = 11$	48.7 ± 2.7	98.7 ± 2.2	53.7 ± 1.8	0.97 ± 0.03	5.0 ± 0.2	3.0 ± 0.3
Female, sham						
$N = 10$	70.2 ± 1.2	105.8 ± 0.8	61.5 ± 0.8	0.89 ± 0.02	5.7 ± 0.1	1.3 ± 0.2
ANOVA results	*b,c*	*b, c*	*a, b*	*b, c*	*a, b*	*b*

Note. Values presented as means ± SEM; N = number of mice in group. Body weight, ash, and protein values in grams; nasoanus length and uterus width in millimeters.

[a] Denotes a main effect of gender within column values ($P < 0.05$, two-way ANOVA).

[b] Denotes a main effect of estrogen treatment within column values.

[c] Denotes a significant gender × treatment interaction within column values.

food intake were seen in the 2-week pretreatment period (males, 5.2 ± 0.2 g/day females, 5.4 ± 0.2 g/day). Food intakes fell immediately after surgery in both drug- and sham-implanted mice (Figs. 1 and 2, middle panel). In the control groups, intake returned to normal within the first postsurgery week and thereafter remained in the range of pretreatment values. Two-way ANOVA of the differences between pre- and post-treatment values of the four subgroups revealed a significant effect of EB on food intake as both male and female treated groups displayed reduced intake throughout the observation period. No main effects of gender on these responses were detected, but a significant treatment × gender interaction ($P < 0.01$) reflected the tendency of sham-implanted females to have higher, and EB-treated females to have lower, daily food intakes relative to the appropriate male groups.

Body temperature. Body temperatures of female obese mice (35.9 ± 0.1 °C) were slightly higher than males before implantation (35.5 ± 0.1, $P < 0.05$, Student's t test). Three-way repeated measures ANOVA of the body temperatures during the 32-day observation period revealed that female mice continued to have higher temperatures than males, but there were no effects of EB on temperature in either gender ($P > 0.05$). All groups exhibited parallel declines in temperature from pretreatment values and the final values were about 0.6°C lower (Figs. 1 and 2, bottom panel). This pre- to post-treatment reduction in temperature was highly significant ($P < 0.001$) and apparently reflected either adaptation to the measurement procedures or an effect of age on thermoregulation.

Carcass composition. The effects of EB treatment on carcass composition are summarized in Table I. Analysis of the carcass fat contents of the experimental groups revealed that both EB-treated and untreated females had larger fat stores than males. Estrogen reduced obesity in both sexes and the responses of the two genders were similar ($P_{\text{gender×treat}} > 0.05$). Separate groups of *ad libitum*-fed male and female obese mice matched in age to the experimental groups were sacrificed to determine initial carcass composition. Before the experiment, male obese mice had 32.5 ± 1 g carcass fat ($N = 5$) and females 39.0 ± 1 g ($N = 5$). Thus, of the body weight losses seen in both EB-treated male and female mice about 75% was accounted for by loss of fat stores. Fat-free residue, largely carcass protein (14), was higher in male mice than in female mice at both the beginning and the end of the experiment ($P < 0.01$, two-way ANOVA). Protein content was reduced by EB in both sexes and the responses of the two genders were similar ($P_{\text{gender×treatment}} > 0.05$). Skeletal mass, as defined by carcass ash content, was increased by estrogens. Females frequently exhibited greater EB-elicited increases in ash than males (Table I), but the gender × treatment interaction term did not reach significance ($P = 0.08$). The uterus of the ob/ob mouse is persistently atrophied, and corresponds to the low circulating estrogens (7). EB treatment led to a two- to threefold increase in uterine size (Table I) indicating that capsule

estrogen release was in the range of normal physiological concentrations. The apparently normal responsiveness of the uterus to EB is consistent with earlier reports implicating the hypothalamic–pituitary axis as the site of reproductive dysfunction in these mice (7, 8).

Glucose and insulin. The hyperglycemia associated with the ob/ob mutation becomes progressively less severe after about 3 months of age and glycemia approaches that of lean mice 2 to 3 months later (15, 16). Although glucose values from lean mice were not included in the present study, *ad libitum*-fed glycemia in untreated ob/ob mice was within the range generally seen in 5- to 6-month-old lean mice (Table II). Estradiol lowered glycemia to the same degree in both genders ($P < 0.01$, two-way ANOVA). Immunoreactive insulin values generally paralleled the glycemic effects, as lower values were seen in both EB-treated males and females. A small number of lean mouse plasma samples were included in the insulin assay for comparative purposes. The IRI concentrations determined in these mature lean mice samples (18.6 ± 4.8 SEM, $N = 4$) showed that the insulinopenic effect of EB administration was small in comparison with the severe hyperinsulinemia which characterizes the mature ob/ob mouse.

Discussion. Previous reports have described the effects of sex steroids on body weight regulation in rodents. Ovariectomy leads to weight gain in female rats and mice (1–4), while replacement therapy in females or estrogen treatment of males causes weight loss (1, 2, 5, 11). Most animal models of genetic obesity, including the Zucker fa/fa rat (17, 18) yellow obese mouse (19), db/db, or ob/ob mouse (11) also respond to estrogen or dehydroepiandrosterone (DHEA) with lowered rates of body weight gain.

TABLE II. GLUCOSE AND INSULIN VALUES OF EB- AND SHAM-TREATED OBESE MICE AT SACRIFICE

Group	*N*	Plasma glucose	Plasma IRI
Male, EB	13	103.0 ± 13.0*	92.4 ± 8.0**
Male, Sham	12	146.5 ± 7.9	198.8 ± 14.5
Female, EB	11	81.1 ± 8.0	89.5 ± 4.8
Female, Sham	10	150.5 ± 12.5	148.8 ± 7.4

Note. N = number of animals, glucose in mg/dl, insulin in μU/ml.

* Glucose, significant main effect of estrogen treatment ($P < 0.05$, two-way ANOVA).

** Insulin, significant main effects of treatment and gender ($P < 0.05$, two-way ANOVA).

The mechanisms by which estrogens exert their effects on growth remain to be fully described. Obviously, reduced food intake would be the first consideration in mediating the body weight responses, but interactions between circulating estrogens and energy expenditure (as motor activity and thermogenesis) have also been reported. Diestrus in rats is characterized by elevated food intake coupled with low body temperatures and reduced activity. At estrus, food intakes decline and temperatures and activity are elevated (20, 21). Thus, the positive energy balance seen after ovariectomy stems from an increased "set-point" of body weight which is achieved by both transient elevations of food intake and reduced energy expenditure. Conversely, the loss of energy stores during estrogen replacement is associated with increased energy expenditure and reduced caloric intake (11, 22–24).

Effects on energy expenditure are also seen during sex hormone treatment of genetically obese rodents. Bray *et al.* (18) reported that reduced food intake could not account for the decreased growth of EB-treated Zucker "fatty" rats. A later paper indicates that the effects of estrogen on growth may be mediated by elevated metabolism in brown adipose tissue (25). In a comprehensive study of several genetic, dietary, and hypothalamic obese models, Massoudi *et al.* (26) found that ethinyl estradiol slightly depressed oxygen consumption/body weight$^{3/4}$ in both Zucker fa/fa rats (93% of normal) and ob/ob mice (94% of normal), but by far the largest effects of estrogens were in depressing food intake (75% of normal). Although the regulation of body temperature is only one avenue of energy expenditure, and may not always be equivalent to heat production, several lines of evidence indicate that the thermoregulatory deficits and depressed energy expenditure of ob/ob mice frequently coincide. These mice are hypothermic shortly following birth and the lower body temperatures are associated with both a diminution of oxygen consumption and an increase in carcass energy content (27, 28). There are deficits in brown fat thermogenesis (29–31) which apparently culminate in the inability to maintain adequate nonshivering heat production and

body temperatures under ambient conditions even slightly below thermoneutrality (32). Since we found no elevations of body temperature in EB-treated ob/ob mice and Massoudi *et al.* (26) found oxygen consumption to be slightly lower in estrogen-treated obese mice, while caloric intakes were persistently depressed in both studies, it appears that hypophagia is primarily responsible for the depressed growth observed during estrogen administration. Furthermore, regression analysis of food intake and temperature on body weight revealed very high positive correlations between intake and body weight responses ($r = 0.81, N = 23$) but little relationship between temperature and growth ($r = -0.19$).

Few techniques significantly reduce the fat content of ob/ob mice. Limited availability of food (33, 34), increased energy expenditure (35, 36), or combinations of these procedures (36) result in a loss of fat-free body mass at nearly the same rate as carcass fat. Even when growth is limited to one-half normal (36), obese mice continue to maintain a severe obesity. Exogenous estrogen increased percentage carcass water and decreased fat and protein content to about the same degree as food restriction (34, 36). However, with limited food intake the nonoxidizable residue which is mostly skeleton (14) remains the same as before treatment or declines slightly with weight loss. In estrogen-treated mice we saw significant increases in this carcass component. Even with severe weight loss, EB-treated mice exhibited absolute increases of skeletal mass. When expressed as a percentage of body weight, the skeleton was about 35% larger in both males and females. Rats also have increased bone mass after estrogen administration (37). This increase is coupled with a depressed longitudinal growth (37, 38) which may account in part for our findings that EB increased bone mass but depressed skeletal growth as defined by nasoanus length (Table I).

Diabetes was ameliorated by EB treatment as indicated by the substantial reductions of both circulating insulin and glucose levels. Although the insulin values were in the range typically found with food restriction alone (34, 36), a comparable hypoglycemia seldomly is observed during caloric restriction. DHEA treatment of either ob/ob or db/db mice on the 6J background also causes glycemia to fall to levels equal to or below those of nonmutant controls, while insulin is lower but remains well above normal (11). Thus, both DHEA and estradiol apparently have effects on glucose metabolism which are independent of both hypophagia and insulin concentrations and which result in lowered circulating levels. Whether the steroids affect target tissue sensitivity to insulin or they alter hepatic glucose production will require additional study.

In summary, the present results show that similar to androgens and DHEA, long-term EB treatment reduces body weight gain in genetically obese diabetic male and female mice. Although there were some benefits of hormone administration when compared with food restriction alone (increased carcass ash content and lowered glycemia), the relatively small (8%) effects on percentage fat content, the associated losses in lean body mass and the hormone's ineffectiveness to normalize insulin levels suggests that EB offers no substantial advantages over caloric restriction in the treatment of obesity or diabetes in these mice. Finally, it is notable that the effectiveness of sex steroids to depress food intake may be unique to the C57BL/6J strain of mice. Our data and those of Massoudi *et al.* (26) clearly demonstrated estrogen elicited hypophagia in C57BL/6J mice. Similarly, DHEA, (11) testosterone (10), and androstanolone (10) depress food intake in this strain. DHEA, however, accomplishes similar beneficial effects on metabolism and diabetes without substantially affecting food intake in A^{vy} mice (19), ob/ob and db/db mice on the Ks background (11), and in Zucker fatty rats (17, 18, 25).

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