# The Effects of Estradiol and Progesterone on Reproductive Tract Atrophy and Tissue Adrenergic Indices in Diabetic C57BL/KsJ Mice (42987)

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*Abstract.* **The diabetes-associated changes in tissue norepinephrine (NE) concentrations and related adrenergic receptor types were correlated with changes in blood glucose and serum insulin levels in 8- to 16-week-old C57BL/KsJ-*db/db* mice relative to corresponding age-matched control (+/?) parameters. In addition, the ability of estradiol and progesterone treatments to modify the diabetes-related adrenergic imbalance was investigated. Tissue (i.e., ovarian, uterine, pancreatic, and adrenal) NE levels were determined by high-performance liquid chromatography and compared with the associated changes in tissue $\alpha_{1,2}$ and $\beta$-adrenergic membrane receptor populations. All *db/db* mice exhibited overt hyperglycemia, hyperinsulinemia, and obesity relative to controls between 8 and 16 weeks of age. Tissue NE levels in diabetics were either similar to, or elevated, as compared with those of age-matched controls. Although the $\alpha_1$ and $\beta$ receptor populations (except liver) were similar in 16-week-old groups, $\alpha_2$ receptor populations in *db/db* mice were elevated relative to controls. Chronic estradiol therapy effectively counteracted the diabetes-induced elevations in tissue NE and influenced all adrenergic receptor populations, normalizing both parameters to control levels as well as modifying the hyperglycemia, but not the hyperinsulinemic component, of the diabetes-obesity syndrome in this species. Chronic progesterone treatment was found to be less effective in modulating these systemic and adrenergic parameters in diabetics relative to oil- or estradiol-treated mice. These data demonstrate that a marked modification in tissue adrenergic parameters occur in association with the overt expression of the diabetes mutation in this species. The ability of estradiol treatment to normalize both blood glucose levels and tissue adrenergic parameters in C57BL/KsJ-*db/db* mice indicates that a direct association between systemic and cellular counterregulating influences, relative to the severity of the Type II diabetic condition, exists in this species. The therapeutic correction of these metabolic problems by ovarian steroid hormones suggests the existence of a causal relationship between cellular glucose homeostasis and steroid action in the diabetic model.** [P.S.E.B.M. 1990, Vol 193]

The genetically diabetic (*db/db*) C57BL/KsJ mouse model exhibits an overt diabetes-obesity (Type II) syndrome as compared with age-matched controls (+/?) (1–5). Characterized by an increase in body weight, blood glucose, and insulin levels (1–5), this model demonstrates a variety of the metabolic and cellular alterations which are clinically recognized to occur in diabetic humans (2, 6–8). Recent studies have demonstrated that the diabetes-induced imbalance in glucose homeostasis influences both brain (9–17) and peripheral tissue (1–3, 18–31) structural and functional indices, including premature female reproductive tract adiposity and atrophy (1–3, 30, 32–35). Peripheral tissue glucose uptake rates are chronically depressed (18) and have been temporally associated with alterations in intracellular lipid deposition (1–3), cell surface characteristics (1–3), hormonal sensitivity and responsivity (1–3, 10–11), endocrine milieu (1–5), as well as cellular dysfunction characterized by premature cellular degeneration and atrophy (1–3). These data suggested that the genomic mutation responsible for the expression of the diabetes-obesity syndrome in this model induces an imbalance in carbohydrate metabolism modulating agents, resulting in a systemic disruption in glucose homeostasis (3, 18).

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Recent studies have demonstrated that the expressed diabetes-obesity syndrome in C57BL/ksJ-*db/db* mice is also accompanied by adiposity and atrophy of the female reproductive tract (1–3). Alterations in follicular growth and recruitment patterns, ovarian steroid production, corpus luteum formation, cellular and interstitial structure, uterine epithelial activity, endometrial sensitivity to exogenous steroid therapy, and ovarian refractoriness to pituitary gonadotropin stimulation are well-recognized consequences of the diabetes-obesity syndrome in this mutant murine model (1–3, 17, 18, 20, 34–36). In addition, the accompanying changes in pancreatic, hepatic, renal, neuronal, and peripheral nerve structure and function have been associated with the expression of the altered carbohydrate metabolism and imbalanced glucose homeostasis in this model, characterized by overt hyperglycemia and hyperinsulinemia (4–5, 9, 10, 13–15, 21–29). Changes in utero-ovarian glucose uptake rates (2, 18), as well as noradrenergic counterregulatory influences (35), exacerbate the diabetes-associated depression in intercellular processing, responsivity, sensitivity, structure, and function (1–3, 18, 33).

Catecholamines, especially $\alpha$ and $\beta$ receptor stimulators, are recognized to influence glucose homeostasis through their direct modulation of pancreatic insulin and glucagon secretion, peripheral tissue and brain glucose metabolism, as well as through counterregulatory mechanisms (37). Recent studies have demonstrated that the onset of the overt diabetic (Type II) condition in C57BL/KsJ-*db/db* mice is accompanied by a remarkable elevation in serum insulin levels and tissue norepinephrine concentrations (35), which cannot be attributed to the associated obesity component of the disease syndrome. Since prediabetic mice exhibited obesity, but not hyperglycemia or elevated tissue norepinephrine (NE) levels, it was recognized that the diabetic state must be manifested in this model prior to the stimulation of noradrenergic synthesis or alteration of tissue adrenergic parameters (35). To date, however the influence of the diabetic state on cellular adrenergic receptor populations remains to be determined for this mutant murine model. In addition, the recognized therapeutic effects of estrogens and progesterone on cellular structure and function in the *db/db* mice (11, 33, 38, 39) have not been determined with respect to their modulatory effects on adrenergic parameters in this species. The present studies were undertaken in order to elucidate the effects of the diabetic state on tissue NE levels and related $\alpha$- and $\beta$-adrenergic receptor populations in the reproductive tract tissues of C57BL/KsJ-*db/db* mice, as well as the effects of estradiol and progesterone therapies on these diabetes-modulated cellular parameters.

## Materials and Methods

**Animals.** Adult female C57BL/KsJ mice derived from The Jackson Laboratory (Bar Harbor, ME) stock were used in these studies. Each normal (*+/?*) mouse was age-matched with a genetically diabetic (*db/db*) littermate. All mice were housed five per cage under controlled environmental (23°C) conditions, with an established photoperiod of 12-hr light/day lights on 0700 hr) and an *ad libitum* supply of Purina Mouse Chow. Blood glucose levels (Ames Glucometer method) and body weights were monitored for all mice. Animals exhibiting both obesity and hyperglycemia relative to controls (Table I) were regarded as overt diabetics.

**Hormone Treatment.** 17$\beta$-Estradiol (1 $\mu$g/3.5 days) and progesterone (2 mg/3.5 days) were dissolved in sesame oil (0.1 ml) for subcutaneous injections. The oil vehicle (0.1 ml) served as the sham (control/*+/?*) injection procedure as described previously (33).

**Serum Insulin Assay.** Serum insulin concentrations were assayed from duplicate samples by radioimmunoassay as described previously (30, 33, 35) using mouse insulin (17.21 units/mg; Novo Industries) standards. Assay sensitivity approximated 80 pg with an intraassay variability of $<10\%$. All serum insulin values were expressed as pg/ml.

**Tissue Collection and Norepinephrine Analysis.** Mice were sacrificed by decapitation, trunk blood was collected for serum glucose and insulin analyses, and the pancreas, adrenal glands, ovaries, and uterus were collected, cleaned, and weighed to the nearest 0.01 mg. These tissues were selected for analysis since they are well recognized to be modulated by the adrenergic nervous system and demonstrate diabetes-associated changes in tissue structural and functional parameters (1–5). Each tissue was subsequently prepared for NE analysis.

Tissue NE concentrations in fresh tissue were determined by high-performance liquid chromatography using dihydroxybenzylamine as an internal standard (added prior to tissue homogenization) and a C18 reverse phase $\mu$-Bondpack column (Waters and Associates, Milford, MA) as described previously (35, 40). The column was attached to a LC-4 electrochemical detector with a glassy carbon electrode coupled with an Ag+/AgCl reference electrode (Bioanalytical Systems, West Lafayette, IN). The mobile phase was 0.05 *M* sodium acetate buffer (pH 5.0), to which 0.05 m*M* sodium octyl sulfonate and 1 *mM* tetra sodium EDTA were added. Separation was accomplished at ambient temperature (22–24°C) at a flow rate of 1.5 ml/min which generated a back pressure of 1000 to 1500 psi with a dector potential of +0.7 V and a sensitivity range of 1–2 nA/V. The injected volume of each sample ranged between 10 and 30 $\mu$l. All NE values were expressed as ng/g of fresh tissue corrected for procedural loss based on the recovery of the internal standard. Final calculations of tissue NE levels used the external standard quantitation technique utilizing a Data Module 720 (Waters and Associates).

**Measurement of Uterine $\alpha$- and $\beta$-Adrenergic Receptor Content.** *Preparation of membranes.* Each

tissue sample was placed in 5 ml of ice-cold homogenate buffer (250 m*M* sucrose, 50 m*M* Tris-HCl, and 5 m*M* $MgCl_2$, pH 7.5) and homogenized for three 30-sec pulses with a Brinkmann (Westbury, NY) polytron PT-10 tissue homogenizer at half-maximal speed. The homogenate was centrifuged at 4°C, 2000*g* for 20 min. The pellet containing connective tissue and cellular debris was discarded and the supernatant was centrifuged at 4°C (40,000*g*) for 30 min. The supernatant was then discarded and the pellet resuspended in homogenate buffer to a final concentration of approximately 6 mg of protein/ml. The membrane preparations containing cellular mitochondria, microsomes, and membrane fragments were divided into aliquots and stored below −25°C until receptor binding was performed. Protein concentrations were determined according to the method of Lowry *et al.* (41) with suitable blanks used and corrected for sucrose levels.

β-Adrenergic receptor binding was estimated using tritiated dihydroalprenolol, [$^3$H]DHA, according to the method of Alexander *et al.* (42) as described previously (40). α-Adrenergic receptors were assessed with titrated dihydroepiandrosterone, [$^3$H]DHE, as described previously (40). Binding took place at 30°C for 30 min with nonspecific binding being determined in the presence of 1 μ*M* phentolamine-HCl and expressed corrected for nonspecific binding. Total adrenergic receptor numbers were determined from equilibrium binding curves at a saturation concentration. The affinity $K_d$ values were determined from displacement curves.

**Statistical Analysis.** All values were expressed as group means (±SEM). Intergroup differences with respect to genotype, age, and hormone treatment were analyzed using Student's *t* test or Newman-Keuls (paired) test followed by analysis of variance tests, with a $P \leq 0.05$ accepted as representing statistical intergroup significance.

**Experimental Protocol.** Starting at 4 weeks of age, match-paired *+/?* and *db/db* C57BL/KsJ mice were treated with either oil, estradiol, or progesterone every 3.5 days. Body weight and blood glucose concentrations were monitored on a biweekly basis. At either 8 or 16 weeks of age, mice were sacrificed and tissues collected, as indicated, for analysis of tissue adrenergic indices and compared with body weight, blood glucose, and serum insulin concentrations. The effects of estradiol and progesterone treatments on reproductive tissue adrenergic parameters were analyzed and compared with the corresponding control parameters.

## Results

The age- and diabetes-related changes in body weights, blood glucose, and serum insulin levels are summarized in Table I for control and diabetic groups. At 8 weeks of age, all control groups (i.e., oil, estradiol, and progesterone treatment groups) exhibited comparable intergroup values for all examined parameters. By comparison, all diabetic groups exhibited significant ($P \leq 0.05$) elevations in body weight, blood glucose, and serum insulin levels relative to corresponding control treatment groups. Both estradiol- and progesterone-treated *db/db* mice exhibited elevated serum insulin concentrations as compared with oil-treated diabetics. In 16-week-old control mice, body weights and blood glucose levels were found to be comparable to the 8-week parameters, but serum insulin levels were elevated significantly ($P \leq 0.05$). In 16-week-old *db/db* mice, all parameters were elevated relative to corresponding controls in all treatment groups, except estradiol-treated mice which demonstrated normalized blood glucose levels relative to age-matched controls (Fig. 1). Hyperinsulinemia characterized all 16-week-old *db/db* mice regardless of treatment regimen.

Peripheral tissue NE levels were comparable in all oil-, estradiol- and progesterone-treated 8-week-old controls (Table II). By comparison, oil-treated *db/db*

**Table I.** Age- and Diabetes-Related Changes in Body Weight, Blood Glucose, and Serum Insulin Levels in C57BL/KsJ Mice: Effects of Estradiol and Progesterone Treatment[a]

| Age (weeks) | Group | Treatment | *n* | Body weight (g) | Blood glucose (mg/dl) | Serum insulin (pg/ml) |
|---|---|---|---|---|---|---|
| 8 | *(+/?)* | Oil | 5 | 20.0 ± 0.8 | 91.0 ± 3.7 | 312.0 ± 15.4 |
| | | Estradiol | 5 | 20.8 ± 0.6 | 78.0 ± 5.3 | 375.3 ± 32.1 |
| | | Progesterone | 5 | 21.2 ± 0.2 | 76.6 ± 7.8 | 530.1 ± 23.8 |
| | *(db/db)* | Oil | 5 | 40.0 ± 1.7[b] | 341.0 ± 22.5[b] | 2,056.4 ± 43.0[b] |
| | | Estradiol | 5 | 37.4 ± 0.9[b] | 217.8 ± 52.4 | 11,975.9 ± 426.8[b] |
| | | Progesterone | 4 | 39.0 ± 1.2[b] | 275.5 ± 37.4 | 6,175.0 ± 355.8[b] |
| 16 | *(+/?)* | Oil | 4 | 24.0 ± 0.7 | 90.3 ± 9.6 | 1,202.8 ± 125.1 |
| | | Estradiol | 6 | 22.7 ± 0.9 | 95.8 ± 4.8 | 1,964.2 ± 132.1 |
| | | Progesterone | 3 | 21.7 ± 1.2 | 82.0 ± 5.0 | 875.2 ± 65.4 |
| | *(db/db)* | Oil | 5 | 50.8 ± 0.7[b] | 502.2 ± 13.2[b] | 4,114.6 ± 271.5[b] |
| | | Estradiol | 6 | 57.7 ± 1.3[b] | 159.0 ± 16.2 | 11,615.8 ± 707.0[b] |
| | | Progesterone | 3 | 46.3 ± 1.8[b] | 584.0 ± 9.0[b] | 2,020.8 ± 69.8[b] |

[a] All values are represented as group means (±SEM) for control (*+/?*) and diabetic (*db/db*) mice.
[b] Significant differences ($P \leq 0.05$) between control and corresponding diabetic mice for both age and hormone treatment group. Both estradiol (1 μg/3.5 days) and progesterone (2 mg/3.5 days) treatments are noted.

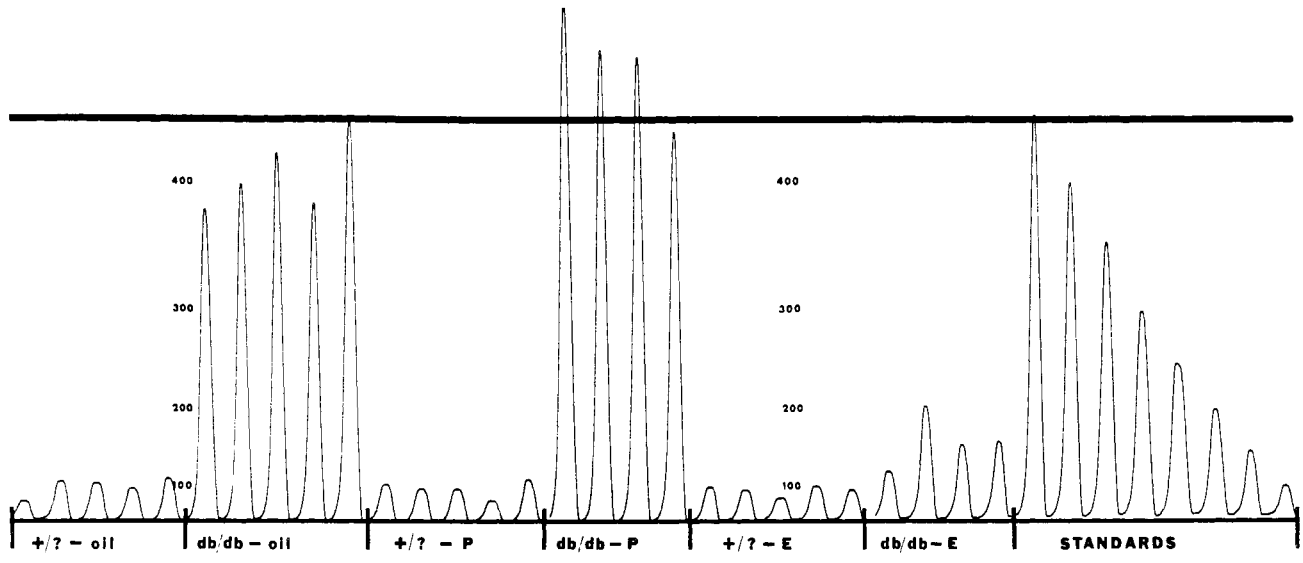


**Figure 1.** Chart recording of blood glucose levels obtained from 16-week-old control (*+/?*) and diabetic (*db/db*) C57BL/KsJ mice following treatment with either oil vehicle, estradiol (E) or progesterone (P) twice a week starting at 4 weeks of age. Standard glucose levels ranging from 50 (far right) to 450 (left) mg/dl are indicated for reference. The dark line at the top denotes the 450 mg/dl blood glucose level, while 100 mg/dl increments are indicated in columns. None of the treatment regimens affected blood glucose levels in control groups. However, estradiol-treated (*db/db*) mice exhibited normoglycemia (see Table I) as compared with the hyperglycemic states which characterized both oil- and progesterone-treated (*db/db*) mice.

**Table II.** Effects of Progesterone and Estradiol Therapy on Tissue NE Concentrations in C57BL/KsJ Mice[a]

| Age | Genotype | Treatment | *n* | Pancreas (ng/g) | Tissue NE | | |
|---|---|---|---|---|---|---|---|
| | | | | | Ovary (ng/g) | Uterus (ng/g) | Adrenal (μg/g) |
| 8 | (*+/?*) | Oil/estradiol/progesterone | 9 | 1052.2 ± 92.9 | 1020.3 ± 217.9 | 438.9 ± 55.5 | 819.7 ± 63.7 |
| 8 | (*db/db*) | Oil | 5 | 1197.9 ± 81.7 | 1792.4 ± 513.4[b] | 2418.4 ± 314.4[b] | 1325.2 ± 94.2[b] |
| | | Estradiol | 5 | 1187.0 ± 170.8 | 358.0 ± 115.7[b] | 267.0 ± 28.9 | 948.3 ± 186.0 |
| | | Progesterone | 5 | 1119.2 ± 83.5 | 896.0 ± 64.8 | 1627.8 ± 139.3[b] | 1024.1 ± 114.4[b] |
| 16 | (*+/?*) | Oil/estradiol/progesterone | 9 | 1291.7 ± 146.0 | 1485.6 ± 119.7 | 741.0 ± 125.6 | 1096.6 ± 155.0 |
| 16 | (*db/db*) | Oil | 5 | 1133.6 ± 148.4 | 970.2 ± 96.3 | 2165.1 ± 420.3[b] | 1850.5 ± 423.4[b] |
| | | Estradiol | 4 | 1266.1 ± 186.5 | 1214.8 ± 303.5 | 149.4 ± 9.3[b] | 732.4 ± 122.8[b] |
| | | Progesterone | 5 | 1241.3 ± 89.1 | 1936.9 ± 604.8 | 986.6 ± 63.2[b] | 1290.6 ± 264.7 |

[a] All values are represented as the group means (±SEM) for the indicated number of mice treated with either oil, estradiol (1 μg) or progesterone (2 mg) twice a week (i.e., every 3.5 days) starting at 4 weeks of age. Control (*+/?*) concentrations are represented as a single value since the therapies failed to significantly alter the NE levels in any of these groups relative to oil-treated mice.
[b] Intergroup differences ($P \leq 0.05$) relative to oil-treated controls (*+/?*).

mice exhibited elevated ovarian, uterine, and adrenal NE concentrations relative to controls. Following progesterone treatment, ovarian NE levels were normalized to control values. In a similar manner, estradiol treatment normalized both uterine and adrenal NE levels to control levels as compared with controls (Table II). In 16-week-old *db/db* mice, uterine and adrenal NE levels were found to be elevated relative to corresponding controls. Both progesterone and estradiol treatments failed to influence pancreatic and ovarian NE concentrations, but significantly ($P \leq 0.05$) depressed uterine NE levels below that of oil-treated *db/db* mice. In addition, estradiol treatment effectively depressed adrenal NE concentrations in diabetics as compared with both oil-treated *db/db* and *+/?* mice.

In 16-week-old mice, $\alpha_1$ receptor concentrations in cardiac, pancreatic, uterine, and ovarian tissue samples from both *+/?* and *db/db* mice were found to be comparable regardless of treatment regimen (Table III). In contrast, the hepatic $\alpha_1$ receptor population was significantly elevated in all treated *db/db* groups relative to corresponding controls. $\alpha_2$-Receptor concentrations (Table IV) were found to be comparable between all (*+/?*) treatment groups for cardiac, pancreatic, hepatic, uterine, and ovarian tissue samples. In corresponding *db/db* oil-treated mice, cardiac, pancreatic, and ovarian

$\alpha_2$ receptor populations were significantly elevated relative to controls. Both estradiol and progesterone treatments normalized peripheral tissue $\alpha_2$ receptor populations to control levels in 16-week-old *db/db* mice (Table IV).

Treatment-related changes in tissue $\beta$ receptor populations were noted in both control and diabetic 16-week-old mice (Table V). All *+/?* treatment groups demonstrated similar tissue-specific receptor populations, except for estradiol-treated hepatic samples which had depressed receptor concentrations. Tissues from *db/db* mice exhibited either similar or depressed $\beta$ receptor concentrations relative to oil-treated controls (Table V). The estradiol and progesterone treatment regimens either depressed or were ineffective in modulating peripheral tissue $\beta$ receptor populations in *db/db* mice relative to corresponding controls.

## Discussion

The results of this study indicate that both age- and diabetes-related changes in peripheral tissue noradrenergic parameters characterize the overt, diabetes-obe-

**Table III.** Effects of Diabetes on $\alpha_1$ Receptors in Peripheral Tissues of 16-Week-Old C57BL/KsJ Mice[a]

| Genotype | *n* | Treatment | Heart | $\alpha_1$ Receptors (fmol/mg protein) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Pancreas | Liver | Uterus | Ovary |
| *(+/?)* | 5 | Oil | 31.2 ± 1.5 | ND[b] | 159.6 ± 1.4 | 25.3 ± 0.9 | 163.0 ± 1.0 |
| | 5 | Estradiol | 29.4 ± 1.6 | ND | 161.8 ± 2.8 | 27.7 ± 2.4 | 159.0 ± 1.0 |
| | 5 | Progesterone | 25.0 ± 2.0 | ND | 160.3 ± 2.6 | 28.5 ± 1.0 | 159.0[d] |
| *(db/db)* | 5 | Oil | 36.6 ± 2.4 | ND | 188.0 ± 2.2[c] | 25.5 ± 2.5 | 170.0 ± 9.0 |
| | 5 | Estradiol | 33.4 ± 1.6 | ND | 184.8 ± 1.9[c] | 27.5 ± 2.5 | 167.5 ± 2.5 |
| | 5 | Progesterone | 37.0 ± 2.0 | ND | 204.8 ± 8.2[c] | 27.5 ± 3.0 | 176.0[d] |

[a] All values are represented as group means (±SEM) for the indicated number of mice.
[b] Nondetectable (ND) levels are indicated for some samples.
[c] Intergroup differences ($P \leq 0.05$) between genotypes with the same type of oil, estradiol, or progesterone treatments.
[d] Single point determination values from pooled samples.

**Table IV.** Effects of Diabetes, Estradiol, and Progesterone on $\alpha_2$ Receptors in Peripheral Tissues of 16-Week-Old C57BL/KsJ Mice[a]

| Genotype | *n* | Treatment | $\alpha_2$ Receptors (fmol/mg protein) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Heart | Pancreas | Liver | Uterus | Ovary |
| *(+/?)* | 5 | Oil | 13.0 ± 0.6 | 177.5 ± 3.1 | ND[b] | 46.7 ± 1.2 | 51.5 ± 6.5 |
| | 5 | Estradiol | 11.3 ± 1.9 | 157.8 ± 3.3 | ND | 44.3 ± 3.4 | 56.5 ± 5.5 |
| | 5 | Progesterone | 13.5 ± 1.0 | 184.0 ± 2.4 | ND | 46.5 ± 3.8 | 55.3 ± 3.0 |
| *(db/db)* | 5 | Oil | 17.3 ± 1.2[c] | 197.3 ± 9.7[c] | ND | 49.3 ± 3.5 | 71.0 ± 2.0[c] |
| | 5 | Estradiol | 14.6 ± 1.2 | 187.5 ± 4.3 | ND | 44.0 ± 1.5 | 57.5 ± 3.5 |
| | 5 | Progesterone | 20.0 ± 1.0 | 209.9 ± 4.7 | ND | 52.5 ± 3.5 | 62.0 ± 4.0 |

[a] All values are represented as group means (±SEM) for the indicated number of mice treated with oil, estradiol (1 $\mu$g), or progesterone (2 mg) twice a week (i.e., every 3.5 days) starting at 4 weeks of age.
[b] ND, nondetectable.
[c] Significant intergroup differences ($P \leq 0.05$) between genotype groups receiving the same treatment regimen.

**Table V.** Effects of Diabetes, Estradiol, and Progesterone on Peripheral Tissue $\beta$ Receptors in 16-Week-Old C57BL/KsJ Mice[a]

| Genotype | *n* | Treatment | $\beta$ Receptors (fmol/mg protein) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Heart | Pancreas | Liver | Uterus | Ovary |
| *(+/?)* | 5 | Oil | 153 ± 5 | 498 ± 8 | 354 ± 19 | 377 ± 29 | 506 ± 9 |
| | 5 | Estradiol | 149 ± 4 | 493 ± 7 | 277 ± 9[b] | 378 ± 12 | 488 ± 12 |
| | 5 | Progesterone | 153 ± 10 | 481 ± 5 | 346 ± 8 | 373 ± 8 | 497 ± 10 |
| *(db/db)* | 5 | Oil | 172 ± 9 | 498 ± 8 | 285 ± 4[c] | 352 ± 4[b,c] | 416 ± 5[b,c] |
| | 5 | Estradiol | 152 ± 7 | 503 ± 7 | 255 ± 8[b,c] | 352 ± 4[b,c] | 416 ± 5[b,c] |
| | 5 | Progesterone | 175 ± 7 | 495 ± 4 | 286 ± 9 | 349 ± 7[b,c] | 482 ± 11 |

[a] All values are represented as group means (±SEM) for the indicated number of control *(+/?)* and diabetic *(db/db)* mice following oil, estradiol (1 $\mu$g), or progesterone (2 mg) treatments twice a week (i.e., every 3.5 days) starting at 4 weeks of age.
[b] Treatment-related differences within each genotype relative to oil-treated mice.
[c] Significant differences ($P \leq 0.05$) between genotype groups receiving the same treatment regimens.

sity syndrome in C57BL/KsJ-*db/db* mice. As demonstrated previously, marked alterations in peripheral tissue glucose homeostasis (18), norepinephrine levels (35), and serum insulin levels (35) accompany the pronounced hyperglycemia which indicates the time of onset of the Type II diabetic condition in this strain. These changes all became manifest without pronounced changes in either cellular insulin uptake rates or membrane insulin receptor populations (16, 31, 36). As such, defects in cellular insulin sensitivity and responsivity accompany, but do not precede, the expression of the pronounced obesity and cellular adiposity which characterize this mutant model (33, 35). The hyperglycemic component of this syndrome, however, does exacerbate cellular adiposity and atrophy in (*db/db*) mice tissue types which do not normally exhibit elevated intracellular lipid stores (1–3). Combined with the pronounced elevations in tissue NE levels and enhanced noradrenergic receptor types as demonstrated in the present studies, it is concluded that the hyperglycemia induces an imbalance in peripheral tissue glucose homeostasis, cellular adiposity, atrophy, and sensitivity to the counterregulatory influences of the noradrenergic indices. As such, these cellular changes result in progressive cellular atrophy associated with enhanced lipid anabolism and depressed carbohydrate catabolism. Recent studies which demonstrated that the reproductive tract tissues of *db/db* mice demonstrate elevated lipogenic enzyme activities relative to controls (3, 34) support these conclusions. The results of the present studies suggest that these novel, steroid therapies may possibly correct, delay, prevent, or compensate for the genomic mutation responsible for the expression of the Type II diabetic condition in this species.

The enhanced uptake of glucose and triglycerides by peripheral tissues of *db/db* contribute to the premature cellular alterations associated with the early death of mutant mice relative to controls (1–3, 33). Since the steroid-induced increase in serum insulin levels do not stimulate an increase in peripheral tissue glucose uptake, it is obvious that minimal insulin bioactivity in *db/db* mice is sufficient to allow for the maximal uptake of circulating glucose (18, 31), resulting in the excess being shunted toward lipogenesis (18). Considering that the peripheral tissues of *db/db* mice demonstrate normal insulin uptake rates (31, 36) and that glucose homeostasis is not affected by serum insulin, cellular insulin receptor concentrations, or uptake rates (31, 36), it is suggested that circulating insulin levels are not a valid index of the severity of the diabetic condition in this model (31). Any insulin-resistant condition may be tissue specific in this species and may be metabolically compensated for by the markedly enhanced glucose concentrations which would effectively modify intracellular metabolism, possibly through gradient diffusion (18, 31).

The demonstrated normalization of utero-ovarian and peripheral tissue structure and function (33), as compared with age- and treatment-matched controls, by ovarian steroid hormones correlates with the observed effectiveness of the treatment regimens to correct intracellular atrophy, adiposity, carbohydrate metabolism, and noradrenergic influences on cellular condition relative to both control and nonhormone-treated diabetic groups. Although estradiol therapy proved to be effective in suppressing the hyperglycemic component of the diabetes-obesity syndrome in 16-week-old diabetics, it was ineffective in suppressing the expression of either the accompanying obesity or hyperinsulinemia. In contrast, progesterone therapy had limited influences on blood glucose or body weight parameters, but did suppress the degree of demonstrated hyperinsulinemia in 16-week-old *db/db* mice relative to controls and oil-treated mutant mice. The selective actions of estradiol and progesterone treatment regimens was demonstrated by the singular ability of estradiol to alter uterine $\beta$ and adrenal $\alpha_1$ receptor populations in *db/db* mice. The additional influences of estradiol on restoring normal ovarian glucose uptake rates (2) and utero-ovarian lipase activities (3, 33) suggest that the imbalanced intracellular glucose metabolism, and not obesity or insulin receptor insensitivity (31, 35, 36), is the primary functional cause for the diabetes-associated cellular atrophy which characterizes this animal model (1–3, 14, 18). The fact that clinical studies demonstrate that similar structural and functional defects exist in female diabetics (6–8), and that steroid-containing oral contraceptives markedly affect diabetes management (43–49), suggests that the results of the present studies may aid in explaining the mechanisms of action of steroids in moderating the expression of the Type II diabetic condition in humans.

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