# Age- and Diabetes-related Changes in Tissue Glucose Uptake and Estradiol Accumulation in the C57BL/KsJ Mouse

DAVID R. GARRIS, DOUGLAS L. COLEMAN, AND CARL R. MORGAN

**SUMMARY**

**The effect of the diabetes (*db/db*) mutation on the age-related changes in glucose uptake and estradiol incorporation in peripheral tissues were investigated in C57BL/KsJ mice between 2 and 16 wk of age. Glucose uptake in the uterus, ovaries, pancreas, lung, liver, heart, kidney, and spleen were markedly increased in diabetic mice after the development of the hyperglycemic condition, as compared with control mice. The age-related increase in glucose uptake observed in control mice was enhanced in hyperglycemic (i.e., ≥4 wk of age) animals. In contrast, the diabetes mutation caused a decreased estradiol uptake by the uteri, ovaries, and mesometrial fat pads at 16 wk, while having little effect in nontarget tissues of diabetic mutants. These data indicate that the diabetes mutation enhances glucose uptake, especially in estradiol target tissues (i.e., uterus, ovary), at the same time that estradiol incorporation is depressed. These results suggest that an alteration in glucose utilization by steroid-sensitive reproductive tract tissue may underlie the impaired reproductive ability in these animals. Other peripheral tissues did not demonstrate any remarkable changes in estradiol uptake, but the enhanced carbohydrate metabolism observed may relate to the subsequent age- and diabetes-related changes in tissue structure and function in these animals. DIABETES 1985; 34:47–52.**

Glucose is a primary metabolic substrate for most aerobic cells.[1] In most cell types, glucose utilization is both an insulin- and energy-dependent event,[1-5] and the accumulation rate of radiolabeled glucose has been used as a general index of cellular metabolic activity.[2,6-8] Several reports have documented the diabetes-associated changes in cellular energy metabolism, structure, and function in the C57BL/KsJ diabetic (*db/db*) mouse.[4,11-18] Of particular interest was the observation that brain glucose uptake was suppressed in diabetic mice as compared with controls.[19] These mutant mice develop a non-insulin-dependent type of diabetes that is characterized by hyperglycemia, obesity, and pancreatic islet hypertrophy, with higher-than-normal circulating insulin levels coupled with severe insulin resistance.[9,11] It has been hypothesized that the CNS insensitivity to a satiety factor (possibly glucose) by hypothalamic glucoregulatory neurons may underlie many of the secondary changes that result from chronic exposure of various cell types (e.g., renal, uterine) to the hyperglycemic condition.[2,4,19-20] It remains to be determined if peripheral tissues, which are recognized to be insulin dependent with respect to glucose uptake, demonstrate changes in glucose utilization rates after the expression of the diabetes mutation in C57BL/KsJ mice.

Steroid hormones, in particular estradiol, have been recognized to have some protective effects in the treatment of hyperglycemia.[4,21-25] Many peripheral tissues possess estrogen receptors[25-28] and recent studies have indicated that chronic treatment with either estradiol or dehydroepiandrosterone effectively suppresses the hyperglycemia and islet hypertrophy associated with the diabetes mutation in the C57BL/KsJ mouse.[21-22] The ability of these steroids to modulate insulin-dependent cellular events has been related to their regulation of intracellular energy generation and substrate metabolism.[23-25] However, any relationship between changes in tissue sensitivity to estradiol and glucose utilization rates in cells from various peripheral organs remains to be elucidated.

The present studies were undertaken to determine the diabetes-related changes in glucose uptake and the associated alterations in estradiol accumulation rates in peripheral tissues. In turn, the effects of aging and the hyperglycemic condition were evaluated with respect to the time of onset of expression of the diabetes mutation in the C57BL/KsJ mouse.

From the Department of Anatomy, East Carolina University School of Medicine, Greenville, North Carolina (D.R.G., C.R.M.); and the Jackson Laboratory, Bar Harbor, Maine (D.L.C.).
Address reprint requests to Dr. David R. Garris, Department of Anatomy, East Carolina University School of Medicine, Greenville, North Carolina 27834.
Received for publication 17 January 1984 and in revised form 26 March 1984.



Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/1/47/353229/34-1-47.pdf by guest on 13 October 2024

TABLE 1
Age-related changes in serum glucose and body weight in normal and diabetic mice

| Group | Condition | Age (wk) 2 | 4 | 8 | 16 |
|---|---|---|---|---|---|
| Control | Serum glucose (mg/dl) | 137.6 ± 7.1 (3) | 141.5 ± 3.0 (4) | 136.0 ± 3.6 (8) | 141.4 ± 3.05 (4) |
| | Body wt (g) | 6.18 ± 0.2 (5) | 17.2 ± 0.14 (5) | 19.1 ± 0.26 (8) | 21.3 ± 0.31 (3) |
| Diabetic | Serum glucose (mg/dl) | 121.3 ± 6.1 (3) | 237.0 ± 21.0 (5)* | 507.0 ± 5.60 (9)* | 452.0 ± 18.0 (5)* |
| | Body wt (g) | 6.54 ± 0.3 (5) | 22.8 ± 0.15 (5)* | 32.2 ± 3.4 (9)* | 53.8 ± 1.17 (5)* |

All values are represented as group means (± SEM) for (N) animals per group. Intergroup differences ($P \leq 0.05$) for serum glucose and body weight at each age are denoted by asterisks.

## MATERIALS AND METHODS

**Animals.** Both control (+/+ and +/? genotypes) and match-paired, diabetic (*db/db* and *mdb/mdb* genotypes) female mice of the C57BL/KsJ strain were obtained from the Jackson Laboratory (Bar Harbor, Maine). All animals were housed three or four per cage with food (Purina Mouse Chow or Old Guilford) and water available ad libitum. No males were housed with these females, thus all mice were either acyclic or exhibited irregular estrous cycles. All groups were maintained under controlled environmental conditions (23–25°C) with an established photoperiod of 12 h light/day (lights on: 0600 h). Body weights and blood glucose (Technicon Autoanalyzer) concentrations were monitored and all animals that exhibited obesity and/or hyperglycemia (Table 1) at the time of killing (i.e., ≥4 wk of age) were considered diabetic.

**Steroid pulse tracing.** Radiolabeled 2,4,6,7,-$^3$H[N]estradiol ($E_2$) was purchased from New England Nuclear (Boston, Massachusetts) and purified by thin-layer chromatography (sp act: 100 Ci/mM). Preliminary experiments indicated that a pulse of 10 μCi of $^3$H-$E_2$ administered either by intracardiac injection or by intraperitoneal (i.p.) injection gave maximal peripheral tissue labeling by 30 min postinjection.[41] In the present studies, each mouse (i.e., normal and diabetic) received a 10-μCi injection (i.p.) of $^3$H-$E_2$ between 0800 and 1100 h and at 30 min postinjection; each mouse was then perfused with 10 cc of saline by intracardiac puncture and subsequently decapitated. At the time of perfusion, 0.1 ml of trunk blood was collected for the estimation of circulating $^3$H-$E_2$. In young (2–4-wk-old) mice in which perfusion of the tissues was not easily performed, each piece of tissue was washed in three changes of physiologic saline to remove any nonspecific, blood-borne radioactivity. Samples of various peripheral organ tissues were collected, cleaned, and weighed to the nearest 0.1 mg. After weighing each piece of tissue, 1 ml of Scintigest Tissue Solubilizer (Fisher) and 0.2 ml of $H_2O$ were added to the tissue vials, and each sample was subsequently dissolved for 18 h at 50°C. Sub-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/1/47/353229/34-1-47.pdf by guest on 13 October 2024

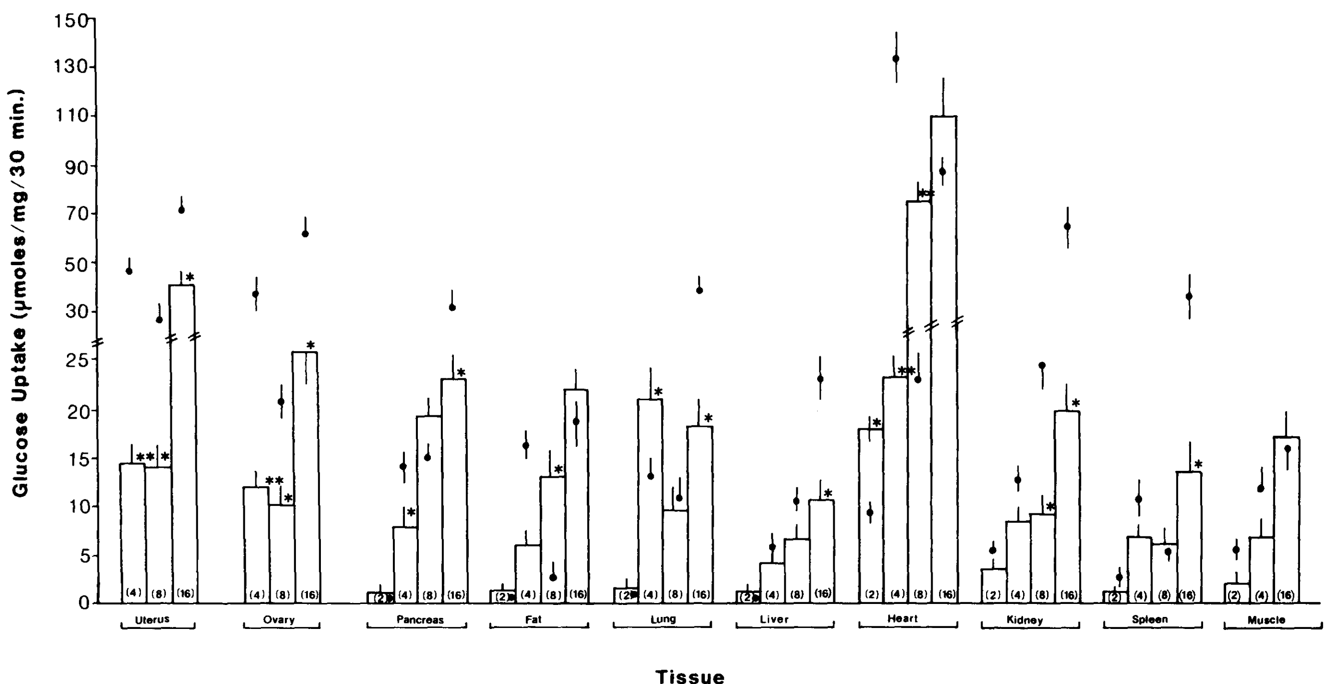


**FIGURE 1. Summary of age- and diabetes-related changes in $^3$H-2-DOG incorporation by the indicated tissues after a 30-min pulse of 10 μCi of the radiolabeled compound. All values are expressed as glucose uptake rates (μmol/mg/30 min) and are represented as group means (± SEM) for control (open bars) and diabetic (black dots within open bars) animals (N = 5–7 pair/group) at 2, 4, 8, and 16 wk of age (indicated at bottom of open bars). Asterisks denote significant (*$P \leq 0.05$, **$P \leq 0.01$) differences between corresponding control and diabetic groups.**

sequently, 15 ml of scintillation fluid was added to each vial and mixed, then placed in a scintillation counter for estimation of radioactivity. The amount of estradiol uptake was expressed as cpm $^3$H-estradiol/mg tissue.

**Analysis of tissue glucose uptake.** Radiolabeled 1,2-$^3$H[N]2-deoxyglucose (2-DOG, sp act: 37.3 Ci/mM) was purchased from New England Nuclear and purified by thin-layer chromatography. In the present studies, each animal (i.e., matched normal and diabetic) received a 10–15 $\mu$Ci injection (depending on the calculated specific activity of blood glucose) of $^3$H-2-DOG between 0800 and 1100 h. By altering the amount of $^3$H-2-DOG injected, blood-borne radioactivity was stabilized and comparable (approximately $5 \times 10^3$ cpm/0.1 ml blood) in all mice during the course of the experiment (30 min), which allowed for accurate intergroup comparisons to be made.[19] At 30 min postinjection, each mouse was perfused with 10 cc of saline by intracardiac puncture and subsequently decapitated. At the time of perfusion, 0.1 ml of trunk blood was collected for the estimation of circulating $^3$H-2-DOG, which was used for the calculation of glucose specific activity. A sample of the various peripheral organ tissues (Figure 1) was collected, cleaned, frozen on dry ice, and weighed to the nearest 0.1 mg. After weighing, each tissue was solubilized as described above and the amount of 2-DOG uptake was estimated. The glucose uptake for each piece of peripheral tissue was calculated based on blood glucose levels, corrected for specific activity (i.e., sp act = cpm ml blood/blood glucose [mg/dl] = cpm/mg glucose), and expressed as $\mu$mol glucose/mg tissue/30 min (i.e., cpm mg/SA = [mg glucose/mg tissue]/mol wt glucose = $\mu$mol glucose/mg tissue/30 min).

**Hormone analysis.** Blood samples were collected by decapitation from corresponding age groups and allowed to clot at 5°C overnight. Serum was collected after centrifugation and stored at −20°C until assayed. Serum estradiol-17β ($E_2$) levels were estimated by radioimmunoassay as previously described[35] and appropriately validated. All samples were assayed in duplicate or as pooled group samples depending on the amount of serum available. Inter- and intra-assay variability approximated 10% for $E_2$ estimations, with an assay sensitivity of 5 pg. All values were expressed as corrected for procedural loss.

**Statistical analysis.** All data were expressed as group means (± SEM). Intergroup differences were determined

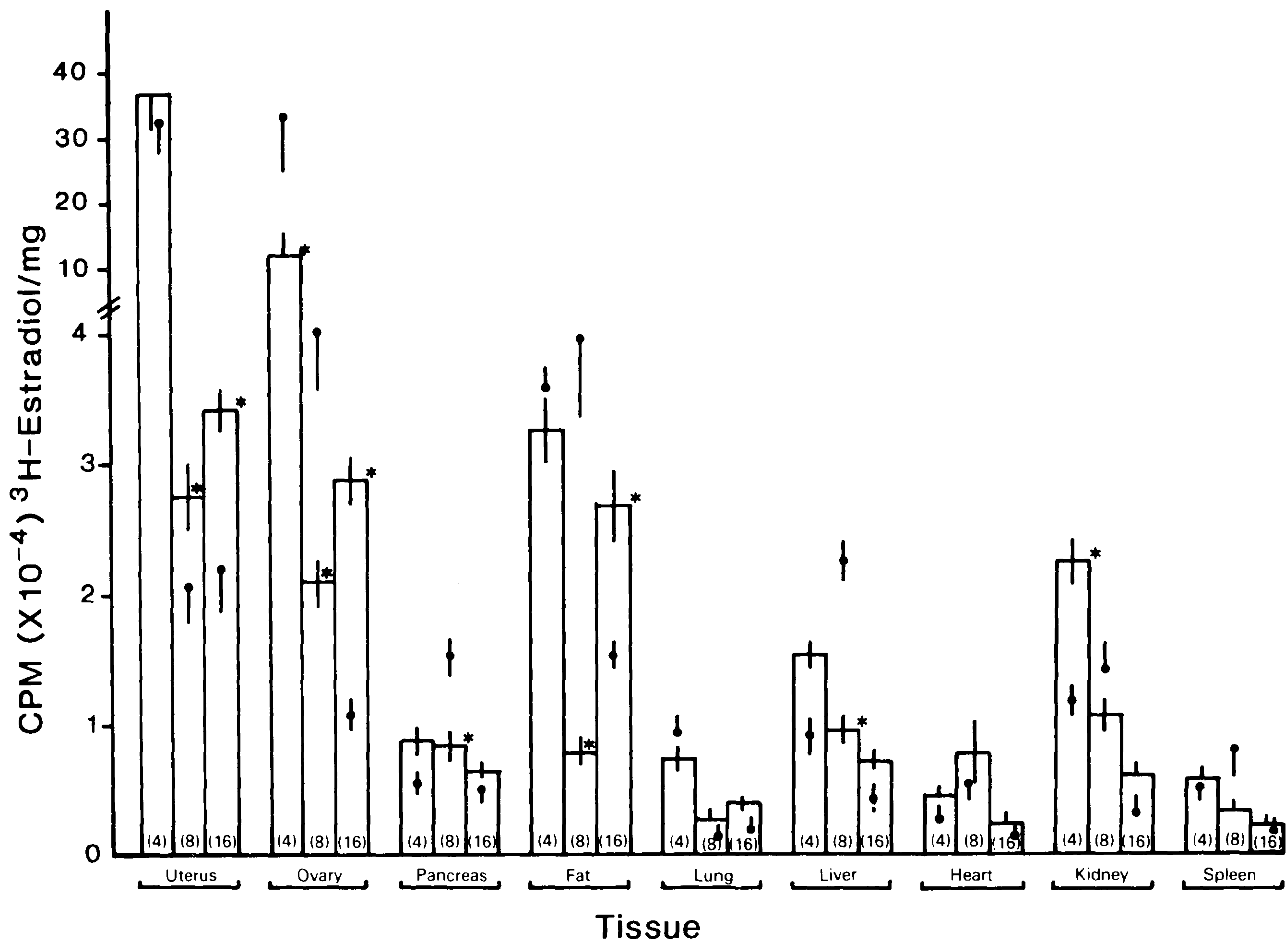


FIGURE 2. Summary of age- and diabetes-related changes in $^3$H-estradiol incorporation by the indicated tissues after a 30-min pulse of 10 $\mu$Ci of the radiolabeled compound. All values are expressed as cpm ($\times 10^{-4}$) $^3$H-estradiol/mg tissue and are represented as group means (± SEM) for control (open bars) and diabetic (black dots in line with open bars) animals (N = 5–7 pair/group) at 4, 8, and 16 wk of age (indicated at bottom of open bars). Asterisks denote significant (*$P \leq 0.05$) differences between corresponding control and diabetic groups.



Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/1/47/353229/34-1-47.pdf by guest on 13 October 2024

using the paired Student's *t*-test or ANOVA exams, where appropriate, with $P \leq 0.05$ accepted as denoting significant differences between groups.

**Experimental protocol.** Match-paired C57BL/KsJ control (+/?) and diabetic (*db/db*) mice at 2, 4, 8, and 16 wk of age were used in these studies. In each case, animals were pulsed with either the $^3H$-$E_2$ or $^3H$-2-DOG tracers and at 30 min postinjection, the tissues were collected, microdissected, solubilized, and the amount of estradiol incorporated or glucose taken up by each tissue sample was analyzed. Blood samples from age and group-paired mice were analyzed for endogenous levels of circulating estradiol. Data were analyzed for the effects of both aging and diabetes on estradiol incorporation and glucose uptake in all tissues that were examined.

## RESULTS

The age- and diabetes-related changes in glucose uptake by various peripheral organ tissues are depicted in Figure 1. Circulating concentrations of $^3H$-2-DOG were found to be stable ($5 \times 10^3$ cpm/0.1 ml blood) for the duration of the experiment in all age groups and genotypes. In tissues from normal mice, a general, age-related increase in the rate of glucose uptake was observed between 2 and 16 wk of age, during which time blood glucose concentrations remained within a constant, limited range (Table 1). Except for the variable rates demonstrated for lung tissue, all tissues demonstrated maximal $^3H$-2-DOG incorporation at 16 wk of age. Heart tissue (left ventricle) exhibited the highest glucose uptake rates of all the tissues examined. Uterine, ovarian, and pancreatic tissues also exhibited elevated glucose uptake as compared with such tissues as liver and spleen. The presence of the diabetes mutation produced a further increase in the uptake of $^3H$-2-DOG between 2 and 16 wk of age in most tissues examined. The rise in tissue uptake of the $^3H$-2-DOG paralleled the elevations in blood glucose concentrations in the diabetic mice between 4 ($237 \pm 21$ mg/dl) and 16 ($452 \pm 18$ mg/dl) wk of age. Of particular interest were the striking increases in glucose uptake exhibited by the uterus and ovary of diabetic mice as compared with controls at all ages studied. In other tissues (e.g., kidney, liver, lung, pancreas, and spleen), significant increases in glucose uptake were calculated at 16 wk of age as compared with controls. The diabetes mutation had minimal effects on glucose uptake rates by other tissues between 2 and 16 wk of age.

Most tissues from control (+/?) mice demonstrated a maturation-associated decrease in estradiol uptake (Figure 2) between 4 and 16 wk of age, during which time circulating estradiol levels were constant and comparable in both (+/?) and (*db/db*) mice (Table 2). By 16 wk of age, only the uterus, ovary, and mesometrial fat pad of control mice demonstrated an enhanced affinity for estradiol, but the total uptake of the radiolabel was reduced as compared with the 4-wk values. In contrast, the diabetic (*db/db*) mutation decreased the amount of estradiol accumulated in most peripheral tissues, including uterine and ovarian tissues, as compared with controls between 4 and 8 wk of age. By 16 wk of age, the amount of estradiol accumulated by peripheral tissues was uniformly decreased in diabetic mice as compared with controls, but was only significantly ($P \leq 0.05$) depressed in target tissues such as the uterus, ovary, and mesometrial fat pads.

## DISCUSSION

The results of the present studies demonstrate that the age-related changes in tissue glucose uptake and estradiol incorporation are markedly affected by the diabetes condition produced by the diabetes (*db*) mutation in the C57BL/KsJ mouse. Most tissues in control (+/?) mice exhibited a maturation-associated increase in glucose uptake, and the presence of the diabetes mutation further increased the rate of glucose uptake in most peripheral tissues. This increase in glucose uptake occurred by 4 wk of age in association with marked hyperglycemia. These changes in peripheral tissue glucose uptake are in marked contrast to the decreased uptake of glucose seen in most brain regions of *db/db* mice.[19] Both hyperglycemia and hyperinsulinemia have been demonstrated to induce CNS as well as peripheral nerve damage,[30-39] while the hyperglycemic condition has also been implicated in peripheral organ dysfunction,[9,11,35,39,40,42,43] especially in estradiol-sensitive target tissues (i.e., uterus, ovary, pancreas).[44] In the C57BL/KsJ mouse, the diabetes syndrome is characterized by hyperglycemia in the presence of increased circulating insulin concentrations and severe insulin resistance.[9-11] Since the CNS tissue is freely permeable to glucose,[7,8,19] the constant hyperglycemia may selectively impair neuronal function in the *db/db* mice, while the accompanying hyperinsulinemia may increase glucose uptake and metabolism in peripheral tissues sufficient enough to cause a metabolic impairment in the tissues. Interestingly, not all peripheral tissues reacted to these metabolic conditions in a similar manner. After the full expression of the hyperglycemic condition at 8 wk of age, the uterus, ovary, and kidney all demonstrated a significant enhancement of glucose uptake, whereas the pancreas, lung, liver, heart, spleen, and skeletal muscle tissues had either a comparable or depressed glucose uptake rate relative to controls. These results strongly suggest that a tissue-specific sensitivity to insulin action exists in the *db/db* mouse, and as receptor sensitivity to the circulating insulin concentration changes, so does the rate of glucose uptake. The exact mechanisms and events associated with the changing tissue glucose uptake rates in relation to insulin sensitivity remain to be elucidated in this animal model.

TABLE 2
Serum estradiol levels in 2–16-wk C57BL/KsJ (+/+) and (*db/db*) mice

| Age (wk) | Group | N | Estradiol (pg/ml) |
|---|---|---|---|
| 2 | +/+ | 7 | 11.8 ± 1.21 |
| | *db/db* | 5 | 19.4 ± 2.13 |
| 4 | +/+ | 7 | 12.2 ± 1.34 |
| | *db/db* | 6 | 12.9 ± 1.42 |
| 8 | +/+ | 5 | 17.0 ± 1.87 |
| | *db/db* | 4 | 11.8 ± 1.30 |
| 16 | +/+ | 8 | 15.9 ± 1.75 |
| | *db/db* | 3 | 11.6 ± 1.28 |

All values are represented as the group mean (± SEM) for the indicated number of mice.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/1/47/353229/34-1-47.pdf by guest on 13 October 2024

The enhanced glucose uptake seen in such tissues as the pancreas, uterus, ovary, and kidney of diabetic mice, as compared with controls, may be associated with both the age-related and diabetes-associated occurrence of organ dysfunction in this model[9,16-18] (unpublished observations). The shortened life span (6–10 mo) of the diabetic mice[9,11] as compared with controls (24–30 mo) could, in fact, be a direct result of the deleterious effects of chronic exposure of the peripheral organs to the hyperglycemic conditions that have been associated with cell structural changes (e.g., basement membrane thickening, glycogen deposition) recognized in the diabetic state[9,16-18,40-44] of several animal models. These structural changes would be expected to severely curtail normal tissue function and development in these animals.

Estradiol receptors have been localized in many peripheral tissues, although reproductive, pancreatic, and brain cells appear to be the most specific target sites for the steroid.[26-29] The anabolic and the gluconeogenesis-suppressing properties of estradiol have been attributed to its insulin-agonist effects.[24] The results of the present studies indicate that, except for the uterus, ovary, and mesometrial fat pad, the diabetes mutation did not dramatically alter estradiol incorporation into peripheral tissues. However, both the uterus and ovary undergo early (i.e., 4 wk) atrophy in the *db* mice, resulting in reproductive dysfunction,[9,40] while the mesometrial fat pads increase in size dramatically in association with elevated blood glucose levels (unpublished observations). The tissue atrophy and alterations in intracellular lipid metabolism that occur in the uterus and ovaries of hyperglycemic animals[40,42] may be related to the impairment of estradiol uptake. The estradiol and glucose uptake rates observed in peripheral tissues vary substantially from those exhibited by various brain regions in which the neuronal uptake of both compounds in diabetic mice was found to be depressed, especially in such estradiol target regions as the hypothalamus and amygdala.[19,41] In the present study, estradiol sensitive tissues, such as the uterus and ovary, demonstrated a depressed uptake of the steroid at 16 wk of age, whereas glucose uptake was exaggerated relative to controls. Both the uterus and ovaries of the *db* mice undergo structural atrophy in association with hyperglycemia[40] (unpublished observations), similar to the changes observed in other diabetic models.[35,42-44] In the *db* mouse, uterine and ovarian atrophy is associated with an increase in intracellular lipid deposition, suggesting that the increased glucose uptake is being metabolically shunted into lipogenesis, rather than carbohydrate metabolism. Estrogen therapy has dramatic effects on lipid accumulation in the *db* mouse,[21,22] suggesting that estrogens may participate in the regulation or modulation of insulin-dependent glucose uptake in these sensitive tissues.[19,41] Considering the severe insulin resistance that develops in *db/db* mice,[9,11] it was surprising that glucose uptake in peripheral tissues was not decreased, rather than increased, in older diabetics. These data suggest that either minimal insulin bioactivity in *db/db* mice is sufficient to allow for maximal glucose uptake by peripheral tissues or that the insulin-resistant condition is tissue specific and can be compensated for by the markedly enhanced glucose concentrations.

In summary, the diabetes mutation either had little effect, or enhanced, the normal developmental increases in glucose uptake rates in peripheral tissues in C57BL/KsJ mice between 2 and 16 wk of age. Estradiol uptake by peripheral tissues was similar, except for specific target tissues, such as the uterus, ovaries, and fat pads, where the diabetes mutation effectively reduced estradiol incorporation as compared with controls. The effects of the diabetes mutation on glucose and estradiol uptake were less obvious, and occurred later in peripheral tissues than has been observed previously in brain tissue in which there was a diabetes-associated impairment of estradiol and glucose uptake.[19,41] These results suggest that the diabetes-related, CNS disturbances may cause many of the secondary metabolic alterations and abnormalities that occur in peripheral tissues and that are characteristic features of older diabetic mice.

## REFERENCES


1 Klip, A.: Regulation of glucose transport by insulin and non-hormonal factors. Life Sci. 1969; 31:2537–48.

2 Young, J. K.: Current evidence for a role of glucose as a regulator of hypothalamic function and caloric homeostasis. Psychoneuroendocrinol. 1981; 6:281–99.

3 Woods, S. C., and Porte, D., Jr.: The central nervous system, pancreatic hormones, feeding and obesity. Adv. Metab. Disord. 1978; 9:283–312.

4 Bray, G. A., and York, D. A.: Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 1979; 59:719–809.

5 Straus, D. S.: Effects of insulin on cellular growth and proliferation. Life Sci. 1981; 29:2131–39.

6 Lautala, P., and Martin, J. M.: Glucose metabolism in rat hypothalamus. Acta Endocrinol. 1981; 98:481–87.

7 Sokoloff, L.: Relation between physiological function and energy metabolism in the central nervous system. J. Neurochem. 1977; 29:13–26.

8 Sokoloff, L.: Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. J. Cerebral Blood Flow Metab. 1981; 1:7–36.

9 Coleman, D. L.: Diabetes-obesity syndromes in mice. Diabetes 1982; 31 (Suppl. 1):1–6.

10 Herberg, L., and Coleman, D. L.: Laboratory animals exhibiting obesity and diabetes syndromes. Metabolism 1977; 26:59–98.

11 Coleman, D. L.: Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 1978; 14:141–48.

12 Sima, A., and Robertson, D. M.: Peripheral neuropathy in the mutant diabetes mouse BL/Ks *db/db*. Acta Neuropathol. (Berl.) 1979; 41:85–89.

13 Hanker, J. S., Ambrose, W. W., Yates, P. E., Koch, G. G., and Carson, K. A.: Peripheral neuropathy in mouse diabetes mellitus. I. Acta Neuropathol. (Berl.) 1980; 51:145–54.

14 Moore, S. A., Peterson, R. G., Fellow, D. L., Cartwright, T. R., and O'Connor, B. L.: Reduced sensory and motor conductance velocity in 24-wk-old diabetic (C57BL/Ks *db/db*) mice. Exp. Neurol. 1980; 70:548–55.

15 Like, A. A., Lavine, R., Paffenbarger, L., and Chick, W.: Studies in the diabetic mutant mouse. VI. Evolution of glomerular lesions and associated proteinuria. Am. J. Pathol. 1972; 66:193–207.

16 Gartner, K.: Glomerular hyperfiltration during the onset of diabetes in mice. Diabetologia 1979; 15:59–63.

17 Bower, C., Brown, B. M., Steffes, M. W., Vernier, R. L., and Mauer, S. N.: Studies of the glomerular mesangium and the juxtaglomerular apparatus in the genetically diabetic mouse. Lab. Invest. 1980; 43:333–46.

18 Meade, C. S., Brandon, D. R., Smith, W., Simmons, R. G., Harris, S., and Sowter, C.: The relationship between hyperglycemia and renal immune complex deposition in mice with inherited diabetes. Clin. Exp. Immunol. 1981; 43:109–20.

19 Garris, D. R., Williams, S. K., Coleman, D. L., and Morgan, C. R.: Glucose utilization by the mouse brain: influence of age and diabetes. Dev. Brain Res. 1984; 15:141–46.

20 Coleman, D. L.: Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 1973; 9:294–98.

21 Coleman, D. L., Leiter, E. H., and Schwizer, R. W.: Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. Diabetes 1982; 31:830–33.

22 Coleman, D. L., Schwizer, R. W., and Leiter, E. H.: Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and in aged normal mice. Diabetes 1984; 33:26–32.


Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/1/47/353229/34-1-47.pdf by guest on 13 October 2024

[23] Baily, C. J., and Ahmed-Sorour, H.: Role of ovarian hormones in the long-term control of glucose homeostasis: effects on insulin secretion. Diabetologia 1982; 19:475–81.
[24] Kalkhoff, R. K.: Effects of oral contraceptive agents and sex steroids on carbohydrate metabolism. Annu. Rev. Med. 1972; 23:429–38.
[25] Paik, S.-G., Michelis, M. A., Kim, Y. T., and Shin, S.: Induction of insulin-dependent diabetes by streptozotocin: inhibition by estrogens and potentiation by androgens. Diabetes 1982; 31:724–29.
[26] Baulieu, E. E., Atger, M., Best-Belpomme, M., Corvol, P., Courvalin, J. C., Mester, J., Milgram, E., Robel, P., Rochefort, H., and de Catalogne, D.: Steroid hormone receptors. Vitam. Horm. 1975; 33:649–736.
[27] Jensen, E. V., Suzuki, T., Numata, M., Smith, S., and DeSombre, E. R.: Estrogen-binding substances of target tissues. Steroids 1969; 13:417–27.
[28] Cidlowski, J. A., and Muldoon, T. G.: The dynamics of intracellular estrogen receptor regulation as influenced by 17-β-estradiol. Biol. Reprod. 1978; 18:234–46.
[29] Boctor, A. M., Band, P., and Grossman, A.: Analysis of binding $^3$H-estradiol to the cytosol fraction of rat pancreas: comparison with sites in the cytosol of uterus. Endocrinology 1983; 113:453–62.
[30] Reske-Nielsen, E., Lundbaek, K., and Rafaelson, O. J.: Pathological changes in the central and peripheral nervous system of young long-term diabetes. Diabetologia 1965; 1:233–41.
[31] Olsson, Y., Soderbergh, J. S., Sourander, P., and Angervall, L.: A patho-anatomical study of the central and peripheral nervous system in diabetes of early onset and long duration. Pathol. Eur. 1968; 3:62–79.
[32] Hilsted, J.: Pathophysiology in diabetic and autonomic neuropathy: cardiovascular, hormonal and metabolic states. Diabetes 1982; 31:730–37.
[33] Smith-West, C., and Garris, D. R.: Diabetes-associated hypothalamic neuronal depopulation in the aging Chinese hamster. Dev. Brain Res. 1983; 9:385–89.
[34] Garris, D. R., Diani, A. R., Smith, C., and Gerritsen, G.: Depopulation of the ventromedial hypothalamic nucleus in the diabetic Chinese hamster. Acta Neuropathol. 1982; 56:63–66.
[35] Garris, D. R., Smith, C., Davis, D., Diani, A. R., and Gerritsen, G.: Morphometric analysis of the hypothalamic-ovarian axis of the ketonuric-diabetic Chinese hamster: relationship to the reproductive cycle. Diabetologia 1982; 23:275–79.
[36] Sidenius, P.: The axonopathy of diabetic neuropathy. Diabetes 1982; 31:356–63.
[37] Sima, A. F.: Peripheral neuropathy in the spontaneously diabetic BB-Wistar rat. Acta Neuropathol. 1980; 51:223–27.
[38] DeJong, R. N.: CNS manifestations of diabetes mellitus. Postgrad. Med. 1977; 61:101–107.
[39] Brownlee, M., and Cerami, A.: The biochemistry of the complications of diabetes mellitus. Annu. Rev. Biochem. 1981; 50:385–432.
[40] Garris, D. R.: Diabetes-associated alterations in uterine structure in the C57BL/KsJ mouse: relationship to changes in estradiol accumulation, circulating ovarian steroid levels and age. In press. Anat. Rec. 1985.
[41] Garris, D. R., and Coleman, D. L.: Diabetes-associated changes in estradiol accumulation in the aging C57BL/KsJ mouse brain. Neurosci. Lett. 1984; 49:285–90.
[42] Garris, D. R., Williams, S., Smith-West, C., and West, L.: Diabetes associated endometrial disruption in the Chinese hamster: structural changes in relation to progressive hyperglycemia. Gynecol. Obstet. Invest. 1984; 17:293–300.
[43] Garris, D. R.: Effects of progressive hyperglycemia on ovarian structure and function in the spontaneously diabetic Chinese hamster. In press. Anat. Rec. 1984.
[44] Garris, D. R., Whitehead, D. S., and Morgan, C. R.: Effects of alloxan-induced diabetes on corpus luteum function in the pseudopregnant rat. Diabetes 1984; 33:611–15.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/34/1/47/353229/34-1-47.pdf by guest on 13 October 2024