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Reproductive Tract and Pancreatic Norepinephrine Levels in Pre- and Overt-Diabetic C57BL/KsJ Mice: Relationship to Body Weight, Blood Glucose, Serum Insulin, and Reproductive Dysfunction (42783)

DAVID R. GARRIS

Department of Anatomy, Cleveland Research Laboratory, Kansas City, Missouri 64131

Abstract. Diabetes-associated changes in tissue norepinephrine (NE) levels were determined in pre- and overt-diabetic C57BL/KsJ (*db/db*) mice relative to age-matched controls (+/?). At 4 weeks of age, both pre- and overt-diabetic mice exhibited obesity and hyperinsulinemia relative to controls. Prediabetic mice demonstrated normoglycemia and tissue (i.e., pancreatic, ovarian, and uterine) NE levels which were comparable to control levels. However, the hyperglycemic condition that characterized the overt-diabetic mice was found to be associated with significant elevations in tissue NE levels relative to both control and prediabetic values. These data demonstrate that the Type II diabetes–obesity syndrome in this mutant murine model is accompanied by a dramatic imbalance in the adrenergic influence on reproductive tissue glucose homeostasis. The hyperglycemic component of the diabetes–obesity syndrome is temporally linked to the noradrenergic disturbances in this model, which occur independently of insulin- or obesity-related changes that also accompany the expression of the genomic mutation and reproductive tract involution.

The genetically diabetic (*db/db*) C57BL/KsJ mouse model exhibits an overt diabetes–obesity (Type II) syndrome between 3 and 4 weeks of age (1–3). Characterized by an increase in body weight, blood glucose, and insulin levels (1–3), this model demonstrates a variety of the metabolic and cellular alterations which are clinically recognized to occur in diabetic humans (4–6). Recent studies have demonstrated that the genetically induced imbalance in glucose homeostasis in this model affects both brain (7–13) and peripheral tissue (1–3, 14–17) structural and functional indices. Peripheral tissue glucose uptake rates are chronically depressed (14) and have been temporally associated with alterations in intracellular lipid deposition (15), cell surface characteristics (15–17), hormonal sensitivity and responsivity (7, 11, 18), and endocrine milieu (1–4, 16), as well as cellular dysfunction characterized by premature cellular degeneration and atrophy (15). However, many of the altered metabolic and endocrine parameters, including diabetes-associated hyperglycemia, can be normalized in this mutant murine model through the use of novel hormone replacement therapies (11, 18). These data suggest 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 (15–17).

Catecholamines, especially α- and β-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 (19–23). The hyperglycemia characteristic of diabetic or stress states also serves as a positive feedback stimulant to induce the discharge of noradrenergic agents from the sympathetic nervous system (19). As such, alterations in tissue norepinephrine levels serve as an index of intrinsic organ response to abnormal carbohydrate conditions. The present studies were undertaken to determine if the onset of the hyperglycemic component of the diabetes–obesity syndrome in the C57BL/KsJ mouse either precedes or follows the activation of the sympathetic nervous system as indexed by tissue norepinephrine levels measured by high-performance liquid chromatography in pancreatic, ovarian, and uterine tissues collected from obese, prediabetic, and diabetic mice.

Materials and Methods. *Animals.* Control (+/?) and match-paired diabetic (*db/db*) female mice of the C57BL/KsJ strain were ob-

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tained from the Jackson Laboratory (Bar Harbor, ME). All mice were group housed, five per cage, with food (Wayne Mouse Chow) and water available *ad libitum*. All groups were maintained under controlled environmental conditions (23–25°C), with an established photoperiod of 12 hr light/day (lights on: 0600 hr). Body weights and blood glucose concentrations (Ames glucometer method) were monitored weekly between 2 and 4 weeks of age for the first indications of obesity and hyperglycemia relative to controls. Blood samples were collected from nonanesthetized mice by orbital sinus puncture. All mice that exhibited a significant ($P \leq 0.05$) body weight gain relative to controls were considered obese. Diabetic mice exhibited blood glucose levels $\geq$200 mg/dl relative to control values ($\leq$175 mg/dl).

Tissue collection and norepinephrine analysis. At 4 weeks of age (±3 days), *db/db* mice were designated as either prediabetic (i.e., obese and normoglycemic) or diabetic (i.e., obese and hyperglycemic) and grouped accordingly for comparison with age-matched controls (i.e., nonobese and normoglycemic). Mice were sacrificed by decapitation, trunk blood was collected for serum glucose and insulin analysis, and the pancreas, 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 tissue structural and functional alterations (9–14). Each tissue was subsequently prepared for norepinephrine (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 C_{18} reverse phase μBondapak column (Waters and Associates, Milford, CA) as previously described (24). 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 octylsulfonate and 1 m*M* tetrasodium 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 15,000 psi with a dector potential of +0.7 V and a sensitivity range of 1 to 2 nA/V. The injected volume of each sample ranged between 10 and 30 μl. All NE values were expressed as nanogram per gram of fresh tissue corrected for procedural loss on the basis of 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).

Serum insulin analysis. Serum insulin concentrations were determined by radioimmunoassay as previously described (25) using mouse insulin (Novo Industries: 17.2 IU/mg) standards. All serum samples were measured in duplicate, with assay sensitivity approximating 80 pg and an intraassay variability of <10%. All values were expressed as picogram per milliliter.

Statistical analysis. All values were expressed as group means (±SEM) according to the respective control and diabetic groups. Intergroup differences were analyzed using either the Student *t* test or the multiple *t* test exams, where appropriate, following group analysis of variance. A $P \leq 0.05$ was considered as representing significant intergroup differences.

Results. The results of the present studies are summarized in Table I. At 4 weeks of age, both prediabetic and overt-diabetic mice exhibited significant increases in body weights relative to controls. However, only overt-diabetic mice exhibited hyperglycemia relative to control, or prediabetic, levels. In contrast, serum insulin levels in both *db/db* groups were elevated relative to controls.

Alterations in tissue NE levels in *db/db* mice, as compared to controls, were found to occur in association with changes in blood glucose levels, irrespective of changes in body weight and serum insulin levels. No differences in pancreatic, ovarian, or uterine NE levels were found to exist between control and prediabetic, obese mice. In contrast, tissue NE levels were dramatically elevated in the overt diabetic group relative to control values. In addition, the tissue NE levels in overt-diabetic mice were significantly ele-

TABLE I. RELATIONSHIP BETWEEN TISSUE NOREPINEPHRINE (NE) LEVELS AND THE ONSET OF THE DIABETES–OBESITY SYNDROME IN C57BL/KsJ MICE

Parameter	Group		
	Control (+/?)	Prediabetic (*db/db*)	Diabetic (*db/db*)
Age (weeks)	4	4	4
Number/Group	10	8	10
Body weight (g)	17.2 ± 0.1	22.3 ± 1.2*	22.8 ± 0.4*
Blood glucose (mg/dl)	159.6 ± 1.3	134.9 ± 21.8	294.5 ± 57.3*,**
Serum insulin (pg/ml)	386.2 ± 31.6	1178.8 ± 121.2*	1864.7 ± 92.4*,**
Pancreatic NE (ng/g)	662.8 ± 99.0	863.8 ± 75.0	1400.0 ± 45.1*,**
Ovarian NE (ng/g)	236.9 ± 63.6	219.0 ± 37.2	955.4 ± 65.1*,**
Uterine NE (ng/g)	1257.0 ± 167.6	1396.7 ± 542.2	2348.0 ± 242.7*,**

Note. All values are represented as group means (±SEM) for the indicated number of mice per group, as denoted by genotype, body weight, and blood glucose parameters. Significant ($P \leq 0.05$) intergroup differences from controls (*), or between pre- and overt-diabetic groups (**), are denoted by superscripts.

vated as compared with corresponding tissue NE levels from the prediabetic group. The elevations in tissue NE in the overt-diabetic mice were recognized to be related to the observed hyperglycemia, but not the obesity or hyperinsulinemia, characterizing the group.

Discussion. The results of the present studies indicate that the overt-diabetic condition which characterizes the C57BL/KsJ (*db/db*) mouse model stimulates a significant elevation in peripheral tissue NE deposition, which may not be attributed to the accompanying obesity syndrome. Since prediabetic mice exhibited obesity, but not hyperglycemia or elevated tissue NE levels, it is recognized that the diabetic state must be manifested in order to stimulate noradrenergic synthesis and tissue accumulation. Recent studies have also indicated that the prediabetic mice will demonstrate similar elevations in tissue NE levels following the overt expression of the diabetes mutations (unpublished observations). These data extend the finding of laboratory and clinical studies of Type I diabetes (19–23)-associated elevations in tissue and plasma NE levels occurring in association with modified glucose or insulin levels. On the basis of the results of the present studies, it may be concluded that obesity is not an adequate sympathetic nervous system stimulant for increasing tissue NE levels in this model. However, the hyperglycemic state is a potent cellular stimulator of NE synthesis, secretion and/or uptake mechanisms in obese–diabetic systems (26). In addition, glucose uptake rates are recognized to be initially enhanced during the development of the diabetes syndrome in *db/db* mice relative to controls (14). As such, both Type I and Type II diabetic conditions share the common feature of an imbalanced adrenergic modulating system, which results in the exacerbation of the poor glucose homeostatic mechanisms characterizing these syndromes.

The elevated serum insulin levels observed in both of the (*db/db*) groups were an expected response to the expressed obesity syndrome in the mutant murine model. The insulin resistance that develops as a result of peripheral and cellular adiposity (26) is well recognized to interfere with normal organ function. Recent studies have demonstrated that the diabetes–obesity syndrome is characterized by both a chronic depression in cellular glucose uptake rates and an increase in intracellular adiposity and tissue atrophy (14–17). These observed changes in cell function are temporarily preceded by the hyperglycemic state and the observed alter-

ations in cellular noradrenergic parameters. The changes in cell structure and function may be recognized by the sensory component of the autonomic nervous system as a type of cellular transformation stress, in which the cells acquire adipocyte-like characteristics as intracellular lipid pools increase in volume (9), thus influencing cellular responsivity and functional characteristics (11–14, 17). The recognized alteration in hormonal modulation of glucose uptake rates in diabetic mice, as compared to controls (14), suggests that an intracellular change in glucose metabolic modulators is involved in the overt development of the syndrome in this species. The transformation of peripheral tissue cell types into adipocyte-like cells, with their insulin-resistant nature, may account for the hyperinsulinemia and hyperglycemia in this model (26). Analysis of the cell receptor subtypes involved with the modulation of cellular insulin, adrenergic, and glucose sensitivity and responsivity are currently under investigation.

In summary, the present studies are the first demonstration that elevations in peripheral tissue NE levels occur in response to the hyperglycemic state that characterizes the Type II diabetes–obesity syndrome in C57BL/KsJ (*db/db*) mice. The increase in tissue NE levels was demonstrated to be independent of both the initial obesity and the hyperinsulinemic components of the syndrome. These data suggest that the imbalance in carbohydrate metabolism stimulates an elevation in tissue NE levels in response to the prominent cellular transformation which characterizes peripheral tissue structure and function in this mutant model system.

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