Peripheral Insulin Insensitivity in the Hyperglycemic Athymic Nude Mouse: Similarity to Noninsulin-Dependent Diabetes Mellitus (43216)

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Abstract. **Previous studies in the homozygous athymic nude mouse (USC colony) have indicated a diabetic state characterized by spontaneous hyperglycemia, abnormal glucose tolerance, and normal or relatively decreased plasma insulin levels. Pancreatic islet cell population assessed by morphometric and immunohistochemical studies demonstrated normal insulin-secreting cells in the hyperglycemic nude mouse. To further elucidate the pathogenesis of the hyperglycemic state in the athymic nude mouse, we have studied the effects of insulin on the transport of glucose in skeletal muscle by measuring basal and insulin-stimulated uptake of a nonmetabolizable glucose analogue, 2-deoxy-D-glucose by using the perfused hindquarter preparation. Although basal 2-deoxy-D-glucose uptake by peripheral skeletal muscle was similar in the hyperglycemic and control mice, the insulin-stimulated uptake of 2-deoxy-D-glucose was significantly decreased in the athymic nude mouse at both 0.1 milliunits/ml and supraphysiologic concentrations of insulin (1 milliunit/ml) when compared with control mice ($P < 0.05$ and $P < 0.001$, respectively). This form of peripheral insulin insensitivity with normal pancreatic β cell reserve, in addition to the lean body mass of the diabetic animal, mimics in part the peripheral insulin insensitivity seen in non-insulin-dependent diabetes mellitus.** [P.S.E.B.M. 1991, Vol 196]

Previous studies have indicated that peripheral tissue (muscle) insensitivity to insulin is an important factor in the pathogenesis of glucose intolerance in noninsulin-dependent diabetes mellitus (NIDDM) (1). While the primary defect in (NIDDM) may develop at the level of the pancreatic B cell and present itself as an impairment in insulin secretion, peripheral tissue insensitivity may subsequently become apparent or may be present simultaneously before glucose intolerance ensues (2–4).

Our previous studies using the hyperglycemic athymic nude mouse (USC) demonstrated spontaneous hyperglycemia, impaired carbohydrate tolerance, and normal or relatively decreased plasma immunoreactive insulin levels in a lean animal model (5–7). Immunohistochemical and morphometric studies on pancreatic islets in hyperglycemic athymic nude mice have shown normal insulin- and glucagon-containing cells without any evidence of insulitis and increased numbers of somatostatin-containing cells (8). In humans the pathogenesis of NIDDM appears to be related to peripheral unresponsiveness to insulin action, variably associated with either normal or impaired insulin reserve (2). To further determine the pathogenesis of the diabetic state in our colony of athymic nude mice, the present study was undertaken to assess peripheral insulin sensitivity by using 2-deoxy-D-glucose uptake by skeletal muscle in the hyperglycemic nudes compared with normal BALB/c mice.

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Experimental Animals

Eight-week-old hyperglycemic athymic nude mice were obtained from our ongoing colony at the University of Southern California. Details about the maintenance and handling of the colony have been described

previously (5). Because female athymic nude mice do not develop the diabetic syndrome, only male mice were used in these experiments. The athymic nude mouse colony is raised and kept under sterile conditions in a temperature-controlled room with a 12-hr light:dark cycle (0700–1900 hr). Normal BALB/c mice matched for age (8 weeks old) and sex were used as controls. Both the controls and the nude mice were raised on similar diets of Wayne Sterilizable Lab Blox (Applied Mills, Chicago, IL). All mice were caged separately the day before the experiment and were fasted for 12 hr. Blood samples were obtained in the fasting state between 0800 and 1000 hr by paraorbital puncture for plasma glucose levels. The animals were allowed free access to food and water for the ensuing 12 hr before further experimentation was done. All animals were maintained and used in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and all of the procedures involving animals have been approved by the University of Southern California Animal Care and Use Committee.

Materials and Methods

Hindquarter Perfusion Procedure. Twenty-one athymic nude mice and 18 control mice were used for this study. The mouse hindquarter perfusions were performed with the procedure previously described by Chan and Dehaye (9), which is a modification of the method of Ruderman *et al.* (10) for rats. Mice were anesthetized by intraperitoneal injection of Equi-thesin (3.5 ml/kg body wt) before being placed in the perfusion apparatus. The abdomen was opened, and the testis, spermatic cord, and bladder were ligated with silk suture and removed. The hypogastric vessels, epigastric branches of the femoral artery, and the vessels to the external oblique muscles and inferior mesenteric artery were tied. The sigmoid colon was then tied and transected. After an injection of sodium heparin (10 mg/kg body wt) into the hepatic portal vein, the superior mesenteric artery, celiac axis, and portal vein were tied, as were the renal, adrenal, and iliolumbar arteries and veins. Cannulas were placed in the abdominal aorta and the inferior vena cava, and a nonrecirculating perfusion was started with a Harvard peristaltic pump (Harvard Apparatus, Quincy, MA) with a flow rate of 0.9 ml/min. The standard perfusion medium was Krebs/Henseliet bicarbonate buffer (pH 7.3) containing 3% bovine serum albumin (Pentax, Fraction V; Miles Laboratories, Elkhart, IN) and a suspension of thoroughly washed (with saline) aged human erythrocytes at a final concentration of 20%. When needed, insulin was added to the perfusion medium at the required concentration. The entire chamber and buffer were kept at 37°C, and the perfusion medium was constantly gassed with a mixture of 95% O_2-5% CO_2.

2-Deoxy-D-Glucose Uptake Measurement. 2-Deoxy-D-glucose uptake rates were determined by measuring the accumulation of 2-deoxy-D-[^{3}H]glucose-6-phosphate (2DGP) as follows. After a 30-min equilibration with the standard perfusion medium, 10 m*M* (10 μgCi/ml) 2-deoxy-D-[^{3}H]glucose (2DG) (New England Nuclear, Boston, MA) was infused into the arterial line at a rate of 30 μl/min during the following 15 min with a Harvard parallel reciprocal infusion pump. At the end of the perfusion period, an arterial sample of the perfusion medium was obtained to measure the specific activity of the infused 2DG. The hind limbs were quickly dissected and frozen rapidly by clamping the tissue between aluminum blocks precooled in liquid nitrogen. The frozen muscles were stored at −70°C until assayed for 2DGP according to the method of Olefsky (11). Frozen muscles were pulverized in liquid nitrogen; a 0.2-g aliquot of the pulverized muscles was then boiled in 1 ml of water, and the extracts were chromatographed through an ion-exchange column (Dowex 1 × 8; chloride form). After the column was washed with 9 ml of water to remove the free 2DG, 2DGP was eluted with 6 ml of 0.2 *M* formic acid in 0.5 *M* ammonium acetate buffer (pH 4.9). A 1.5-ml aliquot of the eluted sample was transferred to a scintillation vial, and the radioactivity was determined in a liquid scintillation counter after addition of scintillation cocktail.

Measurement of Plasma Glucose. Plasma glucose was measured using a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA).

Statistical Analysis of Data. Levels of significance were determined by Student's *t* test (12). Data were expressed as mean ± SE.

Source of Reagents. Glucose was purchased from Mallinckrodt (St. Louis, MO). Cold 2-deoxy-D-glucose and insulin were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade and purchased from Fisher Scientific (Tustin, CA).

Results

Body weights and fasting plasma glucose levels for the athymic nude and control mice used in this study are shown in Table I. The athymic nude mice and the BALB/c control mice had similar body weights. Fasting plasma glucose levels were significantly higher in athymic nude mice compared with controls ($P < 0.05$).

2-Deoxy-D-glucose uptake by skeletal muscle from athymic nude and control mice was measured in the basal state and at three different insulin concentrations (50 microunits/ml, 100 microunits/ml, and 1 milliunit/ml). Results from these experiments are shown in Figure 1.

Both the hyperglycemic athymic nude mice and control mice exhibited similar basal rates of 2DG uptake (expressed as accumulation of 2DGP). Whereas 2DG uptake rates in the athymic nude mice in the

Table I. Body Weights and Fasting Plasma Glucose Levels of Athymic Diabetic Nude and Control BALB/c Mice

Value	Athymic nude mice: *nu/nu* ($n = 21$)[a]	Control mice: BALB/c ($n = 18$)	*P*
Body weight (g)	21.4 ± 1.6[b]	22.5 ± 1.8	NS
Fasting plasma glucose (mg/dl)	195.3 ± 10.1	124.3 ± 3.9	<0.05

[a] *n*, Number of animals studied.
[b] Values are expressed as mean ± SE for the number of mice indicated for each group.

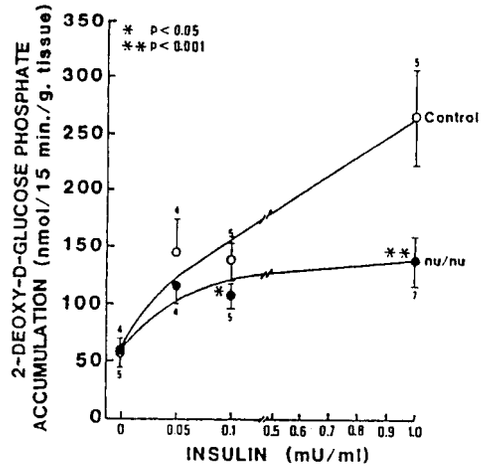

Figure 1. Basal and insulin-stimulated uptake of 2-deoxy-D[^{3}H]glucose by perfused hindquarter skeletal muscles of control (BALB/c) and hyperglycemic athymic nude (*nu/nu*) mice. All values are mean ± SE for the number of animals indicated for each data point.

presence of 50 microunits/ml insulin tended to be lower, the difference from controls was significant only at 100 microunits/ml ($P < 0.05$). At the higher insulin concentration of 1 milliunit/ml, 2DG uptake was markedly decreased in the athymic nude mice compared with the controls ($P < 0.001$). At the highest dose of insulin used, there was a 4.5-fold stimulation (over basal level) of 2DG uptake in muscles from control mice, whereas in athymic nude mice this stimulation was only 2.3-fold.

Discussion

Our initial studies in athymic nude mice (USC colony) exhibited a diabetic syndrome with lean body mass (5). Immunocytochemical studies of the pancreatic islet cell population, including the insulin-, glucagon-, and somatostatin-secreting cells, demonstrated an altered somatostatin cell population with an increase in somatostatin levels, whereas the insulin- and glucagon-secreting cells were similar in the diabetic and control animals (8). These observations therefore suggested the existence of peripheral insulin resistance, which may be partly responsible for the hyperglycemic state in these mice.

Peripheral insulin insensitivity has been described as an important mechanism in the pathogenesis of human NIDDM (2–4). Insulin-stimulated glucose transport has been used to assess peripheral insulin resistance (9). The uptake of 2DG (a nonmetabolizable analogue of glucose) by skeletal muscle has been utilized previously to determine glucose transport into this tissue (9, 10). This process is responsive to stimulation by insulin and reflects the importance of skeletal muscle as a target tissue for insulin action. Our data for basal and insulin-stimulated 2-deoxy-D-glucose uptake obtained in the present experiment for the control mice compares well with our previous measurements in C57Bl mice (13). Although the hyperglycemic athymic nude mice only demonstrated a marked reduction in 2DG uptake at higher insulin levels, the uptake of the glucose analogue in skeletal muscle also tended to be decreased at lower doses of the hormone (Fig. 1), thereby suggesting the existence of a form of peripheral insulin resistance. These findings are similar to previous observations made in other models of NIDDM, such as insulin-resistant diabetes associated with an obese syndrome. A number of studies have reported a similar decrease in insulin-stimulated glucose transport in adipose tissue of humans with NIDDM (14–16). Similar studies of skeletal muscle, which is responsible for approximately 85% of insulin-stimulated glucose disposal (17), are lacking in humans. Recently investigators have shown, using human rectus muscle, that glucose transport decreases in NIDDM compared with studies reported in adipocytes (18). Our present findings, demonstrating insulin insensitivity in peripheral muscles of the athymic nude mouse, may represent a spectrum of NIDDM in humans that offers a possible use of mice to further investigate the pathogenesis of NIDDM.

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