Enhanced Cholinergic Response in Pancreata of Nonhuman Primates with Impaired Glucose Tolerance Shown on [^{18}F]Fluorobenzyltrozamicol Positron Emission Tomography

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Abstract

Background: Islet cell adaptation to insulin resistance in type 2 diabetes mellitus may be due in part to increased stimulation of beta cells by the autonomic nervous system. The parasympathetic neurotransmitter acetylcholine (ACh) mediates insulin release via M3 muscarinic receptors on islet beta cells. The vesicular ACh transporter (VACHT) receptor correlates with cholinergic activity in vivo. The positron emission tomography (PET) radiotracer (+)-4-[^{18}F]fluorobenzyltrozamicol ([^{18}F]FBT) binds to the VACHT receptor on presynaptic cholinergic neurons and can be quantified by PET. In this study, we utilize [^{18}F]FBT PET to demonstrate pancreatic cholinergic activity before and after dextrose infusion in nonhuman primates with normal (NGT) and impaired (IGT) glucose tolerance.

Methods: Seven adult female vervet (*Chlorocebus aethiops*) monkeys were maintained on an atherogenic Western diet. They were divided into two groups: four with NGT and three with IGT. Each subject underwent [^{18}F]FBT PET twice: first, a baseline PET under fasting conditions; and second, PET under fasting conditions but after intravenous infusion of dextrose solution. Quantitative analysis of pancreatic uptake at 60 min post-injection was performed.

Results: There was no difference in pancreatic uptake of [^{18}F]FBT on baseline scans between the two groups. Pancreatic uptake of [^{18}F]FBT increased in every subject after dextrose infusion ($P = 0.03$). On post-dextrose PET scans, pancreatic uptake of [^{18}F]FBT was significantly higher in IGT subjects compared with NGT subjects ($P = 0.03$). The post-dextrose to pre-dextrose uptake ratios were higher in IGT subjects ($P = 0.08$).

Conclusions: Acute increases in pancreatic cholinergic activity in vivo were detected in the pancreata of nonhuman primates with NGT and IGT after intravenous dextrose infusion on [^{18}F]FBT PET. In subjects with IGT, this activity was significantly higher, suggesting increased autonomic nervous system stimulation of the pancreatic islets in insulin-resistant subjects.

Introduction

THE AUTONOMIC NERVOUS SYSTEM plays an important role in the response of the endocrine pancreas to insulin insensitivity, a hallmark of type 2 diabetes mellitus. The parasympathetic nervous system control of the endocrine pancreas occurs via the vagus nerve and abundant cholinergic receptors in the islets of Langerhans.¹ Specifically, the parasympathetic neurotransmitter acetylcholine (ACh) mediates insulin release via M3 muscarinic receptors on islet beta cells.^{2–6} Islet cell adaptation to insulin resistance in type 2

diabetes mellitus may be due in part to increased stimulation of beta cells by the autonomic nervous system.^{7,8}

Positron emission tomography (PET) is a radiotracer-based method of imaging that shows physiological processes in vivo. The PET radiotracer (+)-4-[^{18}F]fluorobenzyltrozamicol ([^{18}F]FBT) binds to active vesicular ACh transporter (VACHT) receptors on presynaptic cholinergic neurons. The VACHT is responsible for loading presynaptic vesicles with the neurotransmitter ACh and has been shown to be a reliable marker of ACh activity in vivo.⁹ This tracer has been most extensively studied in the brain, where increased uptake of [^{18}F]FBT

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FIG. 1. [^{18}F]FBT PET of a nonhuman primate showing [^{18}F]FBT uptake in a normal pancreas (arrow).

reflects increased activation of VACHT receptors.^{10–15} Reproducibility of measurements of cholinergic activity in brain on PET has been demonstrated.¹⁶

In addition to localization in the brain, [¹⁸F]FBT localizes in the pancreas and can be quantified on PET. Ex vivo studies have shown that pancreatic [¹⁸F]FBT binding is increased in the pancreata of prediabetic mice that are normoglycemic and have lost 15–25% of pancreatic beta cells compared with controls.¹⁷ In this study, we utilize [¹⁸F]FBT PET to evaluate cholinergic response to dextrose infusion in the pancreas and compare responses of nonhuman primates with normal (NGT) and impaired (IGT) glucose tolerance.

Materials and Methods

Institutional Animal Care and Use Committee approval was obtained. Seven adult female vervet (*Chlorocebus aethiops*) monkeys 15–18 years old were maintained on an atherogenic Western diet to promote insulin secretion and resistance. Females were chosen as they were part of a breeding colony screened for naturally occurring IGT. Due to the nature of a breeding colony, females are the population majority.

The Western diet was used to aid in obesity development and further challenge the naturally occurring glucose intolerance. Dietary macronutrient breakdown was as follows: 19.0% of calories were protein, 35.3% of calories were lipids, and 45.8% of calories were carbohydrates. Fatty acids supplied by the diet were as follows: 41.8% saturated, 35.6% monounsaturated, and 22.6% polyunsaturated. Laboratory and other parameters were obtained as previously described; Table 1 gives subject characteristics.¹⁸ Glucose tolerance tests were normal in four; three showed IGT on glucose tolerance tests. In no subject was type 2 diabetes mellitus induced. Normal glycated hemoglobin was defined as <5.7%. Homeostasis model assessment (HOMA) was calculated from the product of glucose (mM) and insulin (μUI/mL)/22.5 and used as an indicator of insulin resistance. HOMA >6 was considered predictive of insulin resistance. Obesity was defined as waist circumference of >40.5 cm.¹⁸

Each subject underwent [¹⁸F]FBT PET twice. Subjects were held without food and water overnight before each PET. Sedation and anesthesia were achieved using intramuscular ketamine (12 mg/kg) and inhaled isoflurane. First, a PET scan under basal fasting conditions was performed. Second, a PET scan was performed under fasting conditions and after intravenous infusion of dextrose solution. [¹⁸F]FBT was synthesized as previously described.^{14,17} Approximately 5 mCi of [¹⁸F]FBT was injected intravenously into each subject. Each PET scan was acquired in a dynamic fashion from time of injection through 90 min post-injection. Prior to the second PET scan, 500 mg/kg dextrose was administered intravenously 20 min prior to [¹⁸F]FBT injection. Region-of-interest analysis of pancreatic uptake at 60 min post-injection was performed by a single reviewer (P.B.C.) who defined the pancreas on PET images by freehand tracing of each axial pancreatic slice. Quantitative values, or maximum standardized uptake values (SUV_{max}), were calculated for each slice of pancreas using the following formula: measured activity from region of interest × body weight of subject/injected dose of [¹⁸F]FBT.

Data were subject to analysis by nonparametric methods because of the small sample size using Statistica software

TABLE 1. BASAL FASTING PARAMETERS OF AGE-MATCHED FEMALE VERVET MONKEYS THAT UNDERWENT FBT-PET IN THIS STUDY

	NGT	IGT
Glycated hemoglobin (%)	5.27 (0.19)	8.3 (0.40)
Glucose (mg/dL)	60.5 (4.73)	104.7 (7.42) ^a
Insulin (μIU/mL)	20.81 (5.19)	26.52 (2.82)
HOMA	3.04 (0.61)	6.76 (0.41) ^a
K _g (h ⁻¹)	3.58 (0.33)	1.92 (0.30) ^a
Waist circumference (cm)	38.37 (0.36)	43.33 (2.17) ^a
Triglycerides (mg/dL)	49.25 (10.5)	118 (40.15)

Data are mean ± standard error values of four NGT subjects and three IGT subjects. HOMA is an indicator of insulin resistance; K_g, rate constant calculated for glucose disappearance following intravenous glucose tolerance tests.

^aP < 0.05 for non-parametric comparison between groups.

(Statsoft, Tulsa, OK). Data were computed using the Mann-Whitney U test for differences in means. The Wilcoxon Matched Pairs test was used to evaluate pancreatic SUV_{max} on pre- and post-dextrose PET.

Results

[¹⁸F]FBT activity was evident in the brain, salivary glands, lungs, liver, biliary system, pancreas, bowel, and bladder in all study subjects. Qualitatively, the pancreas was easily distinguished from adjacent abdominal organs (Fig. 1).

There was no difference in pancreatic uptake of [¹⁸F]FBT on baseline scans between NGT and IGT subjects (P = 0.29). Pancreatic uptake of [¹⁸F]FBT increased in every subject, regardless of glucose tolerance, after dextrose infusion (P = 0.03). On post-dextrose PET scans, pancreatic uptake of [¹⁸F]FBT was significantly higher in IGT subjects compared with NGT subjects (P = 0.03). The uptake ratios (post-dextrose:pre-dextrose uptake) were higher in subjects with IGT (P = 0.08). Table 2 gives mean [¹⁸F]FBT uptake values for both groups.

Discussion

The pancreas is a complex organ, providing both endocrine and exocrine functions. Laboratory-based measures of the health of the endocrine pancreas are imprecise measurements of beta cell secretory capacity and/or destruction. As the pancreas is a heterogeneous organ, biopsy is not a reliable indicator of beta cell mass or function. Noninvasive, in vivo measurements of pancreatic endocrine health and physiological changes associated with it are needed to better delineate the

TABLE 2. MEAN PANCREATIC UPTAKE (SUV_{max}) OF [¹⁸F]FBT ON PET IN FEMALE VERVET MONKEYS WITH NGT AND IGT

	NGT	IGT	P
Baseline	10.04 ± 2.09	11.10 ± 3.27	0.29
Post-dextrose	11.10 ± 2.25	17.43 ± 2.38	0.03
Ratio post-dextrose:baseline	1.11 ± 0.10	1.65 ± 0.41	0.08

Data are mean ± standard error values of four NGT subjects and three IGT subjects.

normal pancreas and pathophysiology involved in type 1 and type 2 diabetes mellitus.¹⁹

Imaging of neurofunctional changes in the normal endocrine pancreas and the endocrine pancreas in diabetes has been explored by our group and others in an effort to provide information on the neurophysiology of the endocrine pancreas noninvasively.^{19–23} In the laboratory, direct manipulation and measurement of pancreatic neuronal changes in animal subjects often require highly invasive measures, such as surgery. In human studies, indirect measures of parasympathetic activity on the endocrine pancreas are often relied upon (e.g., serum pancreatic polypeptide measurements). No direct measurement of cholinergic activity is available for use in humans to validate the hypothesis of the role of cholinergic input into islet adaptation to insulin resistance or autoimmune beta cell loss.⁸

Beta cell insulin production is increased as insulin sensitivity of peripheral tissues is decreased in type 2 diabetes. Several mechanisms have been hypothesized for these changes, including stimulation of beta cells by circulating glucose, circulating free fatty acids, and increased input from the autonomic nervous system.⁸

The evidence for the autonomic nervous system's role in increasing insulin production is strong, particularly in rodent studies. Insulin secretion in rodents is increased by vagal nerve stimulation and decreased by the cholinergic antagonist atropine.⁸ Islet hypertrophy in a mouse model of type 2 diabetes can be prevented by vagotomy. Insulin-resistant humans show increases in insulin production when administered a cholinergic agonist.²⁴ Because of these and similar studies, diabetes treatments that enhance cholinergic stimulation of beta cells are being considered.²⁵

In this study, we evaluated nonhuman primates fed an atherogenic Western diet in an effort to promote the development of type 2 diabetes. Glucose tolerance tests were normal in four; three had insulin resistance as evidenced by abnormal glucose tolerance tests. Baseline pancreatic cholinergic activity measured by [¹⁸F]FBT uptake in fasting subjects was similar between the NGT and IGT groups. [¹⁸F]FBT uptake increased in all subjects—regardless of glucose tolerance status—after intravenous dextrose infusion, mimicking findings in rodent studies.⁸ Significantly higher pancreatic cholinergic activity was evident on [¹⁸F]FBT PET in IGT subjects after intravenous dextrose challenge. These findings support several lines of evidence that the parasympathetic nervous system is a major factor in islet adaptation to insulin resistance.

Noninvasive, FBT-PET-based analysis of neurofunctional changes in the pancreas in diabetes should be validated with further research, including comparison with results of other concomitant laboratory-based functional maneuvers and longitudinal studies in nonhuman primate colonies with and without diabetes mellitus. To date, FBT-PET has been used in animal and human studies without adverse incident. Ultimately, FBT-PET could provide a valuable tool for noninvasive evaluation of preventive and therapeutic measures for type 1 and type 2 diabetes.

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Author Disclosure Statement

No competing financial interests exist.

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