*Rapid Publication*

# Mice Transgenic for an Expanded CAG Repeat in the Huntington's Disease Gene Develop Diabetes

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**The autosomal dominant neurological syndrome of Huntington's disease has been modeled in transgenic mice by the expression of a portion of the human huntingtin gene together with 140 CAG repeats (the R6/2 strain). The mice develop progressive chorea with onset at ~9 weeks of age and with death at ~13 weeks. Associated symptoms include weight loss and polyuria in the absence of eating or drinking deficits. We have found that these mice have insulin-responsive diabetes. Fasting glucose was 211 + 19 mg/dl in R6/2 mice compared with 93 + 5 mg/dl in C57/B6 controls ($n$ = 12, both groups; $P$ < 0.01). Administration of insulin intraperitoneally led to a reduction in blood glucose. At 12.5 weeks, animals were killed and pancreas weighed and analyzed for insulin and glucagon. Pancreatic mass in R6/2 mice was the same as controls, and islets appeared normal in morphology without lymphocytic infiltration. Immunohistochemical staining showed dramatic reductions in glucagon in the α-cells and in insulin in the β-cells. Direct tissue assays showed glucagon and insulin content were reduced to only 10 and 15% of controls, respectively. Diabetes has been reported as being more common in Huntington's disease and other triplet repeat disorders. The R6/2 mouse should prove useful for elucidating the mechanism of diabetes in these genetic diseases.** ***Diabetes*** **48:649–651, 1999**

Transgenic mice (R6/2 strain) have been developed to model human Huntington's disease (HD) by expression of a portion of the human HD gene under human gene promoter elements (1 kb of 5′ UTR sequence and exon 1 together with ~140 CAG repeats). Expression of this amino-terminal fragment of the huntingtin protein with its polyglutamine expansion is sufficient to reproduce the phenotype of human HD (1). The mice show a progressive, neurological syndrome with onset at ~9 weeks of age, which includes involuntary and choreic movements, tremor, limb dyskinesia, handling-induced seizures, weight loss, polyuria, and abrupt sudden death of unknown cause. The neuropathology of R6/2 mice is similar to human HD at the cellular level with development of nuclear huntingtin protein deposits before the onset of symptoms (2,3). We now report that R6/2 mice, like many human HD patients, develop diabetes. The R6/2 mice have insulin-responsive hyperglycemia and reduced pancreatic insulin and glucagon.

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Received for publication 11 August 1998 and accepted in revised form 11 January 1999. Posted on the World Wide Web at www.diabetes.org/diabetes on ?? January 1999.

HD, Huntington's disease; RIA, radioimmunoassay.

## RESEARCH DESIGN AND METHODS

**Animal model.** The 6-week-old R6/2 animals were obtained from Jackson Laboratories (Bar Harbor, ME) and maintained on a standard laboratory diet and fed ad libitum. Founder animals were generated from C57BL/6 embryos and maintained by crossing carrier males to CBAxC57BL/6 F1 females. Experimental protocols were approved by the Animal Care and Use Committee of the University of Colorado Health Sciences Center.

**Sample collection.** Animals were housed in 12-h light/dark cycle environment and given food pellets and water ad libitum. Blood glucose was measured by the One-Touch system (LifeScan, Milpitas, CA). One drop of blood was collected from a single cut into the mouse tail with a lancet. Larger volumes of blood for insulin determination and other laboratory measures were collected retroorbitally with heparinized capillary tubes and placed into tubes containing heparin. For fasting blood glucose, mice were fasted 6 h with free access to water before blood collection. Animals were anesthetized at 12.5 weeks with a lethal dose of chloral hydrate and the organs harvested. The pancreas was removed and the wet weight determined. Half of the pancreas was used for pathology and the other half for determination of hormone content. For histology, the pancreas was placed into 10% formalin for 5 days before parafin embedding and processing. Five micron (μm) sections were cut and mounted for immunohistochemistry.

**Insulin and glucagon measurements.** Insulin and glucagon were measured by radioimmunoassay (RIA) (RIA kit by Linco Research, St. Louis, MO). Insulin and glucagon were extracted from weighed pancreas samples in an acid-ethanol solution (ethanol:water:hydrochloric acid, 50:16:1). Samples were cut into small pieces, homogenized in hand-held glass homogenizers, and sonicated for 20 s. Samples were centrifuged at 3,000$g$ for 10 min. Supernatant was diluted for the RIA according to manufacturer guidelines.

## RESULTS

R6/2 pups were born normal in size and weight but showed progressive neurological signs, weight loss, and polyuria beginning at age 9 weeks. Because the animals appeared to have no eating or drinking deficits, we sought an alternative explanation for the polyuria and weight loss. Four of six neurologically asymptomatic R6/2 animals at age 7 weeks exhibited higher random blood glucose concentrations (226 ± 14.8 mg/dl, $n$ = 4) than age-matched C57BL/6 controls (164 ± 5.8 mg/dl, $n$ = 5, $P$ < 0.05, Student's $t$ test). As the HD phenotype progressed (age 9 weeks), the severity of hyperglycemia in the four diabetic R6/2 animals increased to 318 ± 28.5 mg glucose/dl, while the remaining two R6/2 animals were euglycemic. Plasma insulin levels in hyperglycemic R6/2 mice (0.28 ± 0.04 ng/ml, $n$ = 3) were lower than those in control mice (0.87 ± 0.07 ng/ml, $n$ = 2). The hyperglycemic animals were sensitive to insulin; a dose of 2.5 U/kg insulin (75% Lente

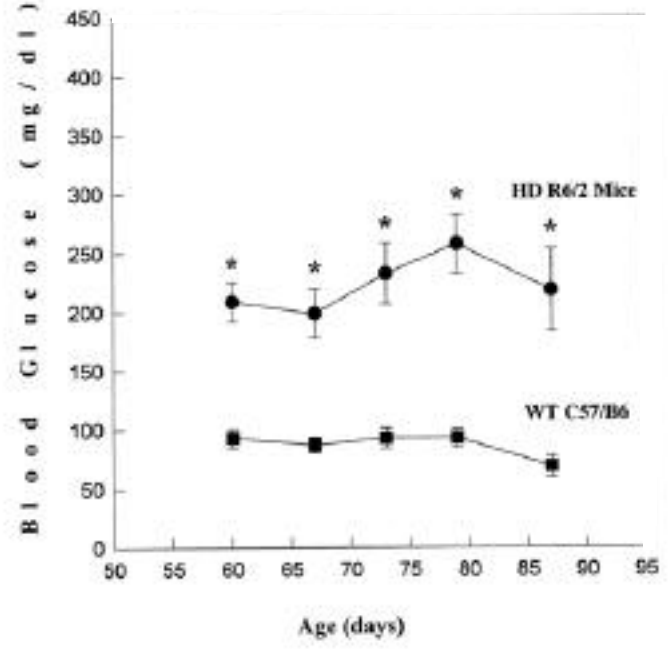


**FIG. 1. Fasting blood glucose concentrations in R6/2 and normal mice. R6/2 mice (●) were hyperglycemic in fasting blood glucose measurements compared with the C57BL/6 controls (■) at all ages tested (*$P$ < 0.01, Student's $t$ test, compared with controls at each time point. $n$ = 12 each group).**

insulin/25% regular insulin, Lilly, i.p.) reduced blood glucose concentrations from >300 mg/dl to 106 ± 17.9 mg/dl after 2 h ($n$ = 2 hyperglycemic R6/2 animals, repeated four times).

In a larger study with 12 R6/2 mice and 12 age-matched C57BL/6 mice, fasting blood glucose concentrations were

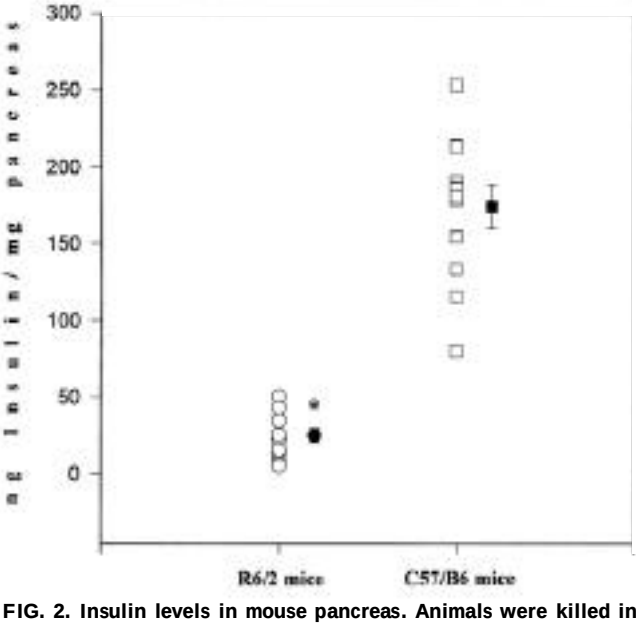


**FIG. 2. Insulin levels in mouse pancreas. Animals were killed in the fasted state, the pancreas rapidly removed, weighed, and placed in acid:ethanol solution. Insulin concentration was in nanograms insulin per milligram pancreas wet weight as measured by RIA. R6/2 mice (●) had ~15% of the pancreatic insulin content of the C57BL/6 controls (■); individual animals (○ □) and group averages (● ■) (*$P$ < 0.01, Student's $t$ test. $n$ = 11 R6/2 mice and $n$ = 12 C57BL/6 mice).**

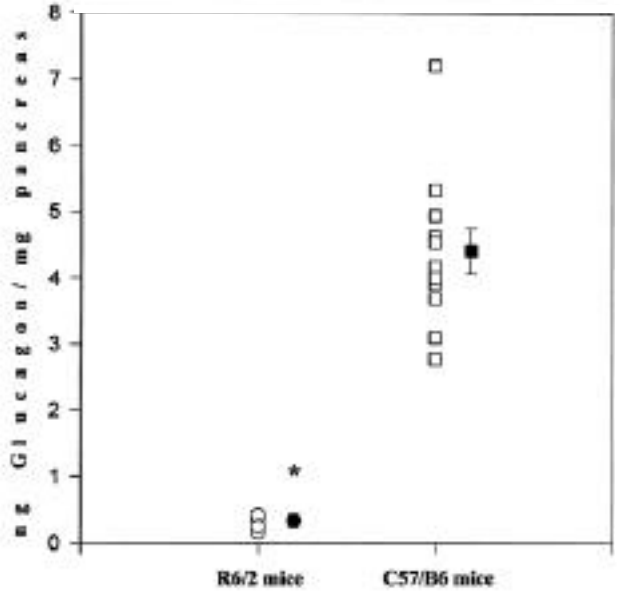


**FIG. 3. Glucagon levels in mouse pancreas. Glucagon was measured by RIA in the same pancreatic homogenates used for insulin assay. R6/2 mice (●) had ~10% of the pancreatic glucagon content compared with C57BL/6 controls (■); individual animals (○ □) and group averages (● ■) (*$P$ < 0.01, Student's $t$ test. $n$ = 11 R6/2 mice and $n$ = 12 C57BL/6 mice).**

determined at five time points between the ages 8.5 and 12.5 weeks. R6/2 mice showed fasting hyperglycemia compared with controls (Fig. 1). R6/2 mice with blood glucose levels >300 mg/dl were also glycosuric. At 12.5 weeks of age, before the R6/2 animals died from the HD phenotype, animals were killed and the pancreas removed for determination of hormone content and histology. The pancreatic wet weight in both groups was comparable (R6/2 mice 134 ± 9.2 mg and C57BL/6 mice 134 ± 9.0 mg). RIA of pancreatic insulin and glucagon showed that the R6/2 animals had only 15% of the pancreatic insulin and 10% of the pancreatic glucagon of the control mice (Figs. 2 and 3). Histology showed no apparent abnormality in the exocrine pancreas. However, immunocytochemical staining of the islets showed reduced insulin and glucagon (Fig. 4). No lymphocytic infiltrate was seen, suggesting that there was no autoimmune mechanism for this disorder.

## DISCUSSION

The endocrine pancreas is of endodermal origin, yet it expresses many genes associated with neuronal tissue such as GAD, tyrosine hydroxylase, and synaptophysin (4–6). Huntingtin is expressed in most tissues, including the pancreas (7). Knockout of a developmentally important neural gene, NeuroD/Beta2, results in abnormal development of the pancreas without formation of mature islets (8). Similarly, knockout of a gene important in forebrain development, Nkx 2.2, leads to β-cells, which fail to differentiate into mature insulin-producing cells (9).

How the expanded CAG repeat in the huntingtin gene causes loss of insulin and glucagon production is uncertain. Equally unknown is the mechanism by which the GABAergic medium spiny neurons of the striatum are selectively destroyed in the human HD patient. The fact that the GABA synthetic enzyme GAD is present in islets and that GABA

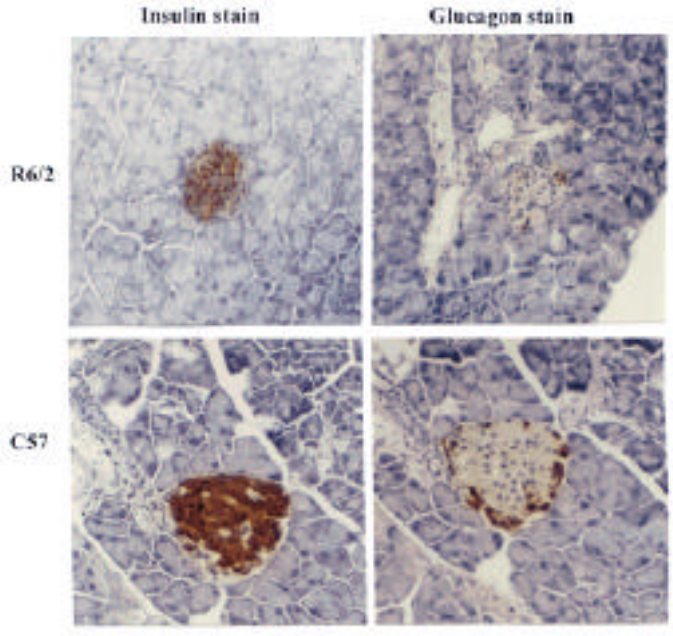


**FIG. 4. Immunohistochemistry of insulin and glucagon in islets of R6/2 and C57BL/6 mice. R6/2 mice had reduced insulin staining of the β-cells and reduced glucagon staining in α-cells compared with control mice. Nonetheless, the distribution of insulin in the R6/2 mice was uniform across the islet, and glucagon was found in a circumferential distribution as in normal animals.**

exerts a paracrine effect on the α-cell may be a clue. Intranuclear deposits of huntingtin have been seen in the pancreatic islet cells of the R6/2 mouse (personal communication, Gillian Bates, November 1998). Whether the huntingtin protein with polyglutamine expansion is directly toxic to pancreatic islet cells remains to be elucidated. Because regulation of insulin production is a complex process involving the sympathetic nervous system and the hypothalamic/pituitary axis, HD transgene effects at other sites might play a role in the insulin deficiency of the R6/2 mouse.

Symptoms of weight loss, polyuria, and diabetes have been noted in HD patients with up to a fourfold increase of diabetes (10). Some studies have found insulin-resistant hyperglycemia with elevated insulin levels, while other studies have shown no abnormalities in glucose tolerance (11–14). No pathologic studies of human Huntington pancreas have been reported. Our observation that diabetic R6/2 mice are responsive to exogenous insulin and have depleted stores of pancreatic hormones argues that the phenotype is of pancreatic origin. We have seen no evidence of β-cell loss or amyloid deposition such as occurs in type 2 diabetes in humans or β-cell hyperplasia that occurs in the insulin-resistant ob/ob mouse. The absence of inflammatory cells distinguishes the R6/2 model from the common autoimmune type 1 diabetic phenotype. The R6/2 Huntington diabetic model in many ways resembles the single-gene disorders found in maturity-onset diabetes of the young (MODY) patients. Regardless of the mechanism of the pancreatic pathology in the R6/2 mouse or human HD, our results demonstrate that expanded polyglutamine repeats can produce diabetes. Other triplet repeat disorders such as Friedreich's ataxia (GAA repeat) and myotonic dystrophy (CTG repeat) have an increased incidence of diabetes, suggesting that there may be a common mechanism causing reduced pancreatic insulin production (15,16). The R6/2 transgenic mouse should prove to be a useful model for elucidating the mechanism of diabetes in Huntington's and other triplet repeat diseases. If the CAG-repeat disorders produce pathology by inhibiting gene expression or protein synthesis, there may be a common mechanism for the deficiency in both insulin and glucagon seen in the R6/2 mouse.

## ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grants NS-18639 and GM-07635, The National Parkinson Foundation, the Program to End Parkinson's Disease, and the Children's Diabetes Foundation. The University of Colorado is a National Parkinson Foundation Center of Excellence.

We thank Pat Bell, Cindy Hutt, and Joe Hensley for their technical assistance.

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