

Impact of Genetic Background on Development of Hyperinsulinemia and Diabetes in Insulin Receptor/Insulin Receptor Substrate-1 Double Heterozygous Mice

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Type 2 diabetes is a complex disease in which genetic and environmental factors interact to produce alterations in insulin action and insulin secretion, leading to hyperglycemia. To evaluate the influence of genetic background on development of diabetes in a genetically susceptible host, we generated mice that are double heterozygous (DH) for knockout of the insulin receptor and insulin receptor substrate-1 on three genetic backgrounds (C57BL/6 [B6], 129Sv, and DBA). Although DH mice on all backgrounds showed insulin resistance, their phenotypes were dramatically different. B6 DH mice exhibited marked hyperinsulinemia and massive islet hyperplasia and developed early hyperglycemia, with 85% overtly diabetic by 6 months. By contrast, 129Sv DH mice showed mild hyperinsulinemia and minimal islet hyperplasia, and <2% developed diabetes. DBA mice had slower development of hyperglycemia, intermediate insulin levels, and evidence of islet degeneration, with 64% developing diabetes. Thus, mice carrying the same genetic defects on different backgrounds exhibited the full spectrum of abnormalities observed in humans with type 2 diabetes, which allowed for identification of potential loci that promote development of the diabetic phenotype. *Diabetes* 52:1528–1534, 2003

Several common human disorders, including obesity, diabetes, hyperlipidemia, atherosclerosis, and hypertension, have complex patterns of inheritance with superimposed effects created by diet, level of exercise, and other environmental factors (1–3). Several monogenic forms of diabetes, including mutations in genes for insulin (4), the insulin receptor (5), maturity-onset diabetes of the young (6), and mitochondrial diabetes (7), have been identified and constitute <5% of all cases of type 2 diabetes. The genetic basis for the more common form(s) of type 2 diabetes remains elusive but is thought to be the result of interactions between

genes controlling insulin resistance and β -cell function, environmental factors, and variations in other background genes (8).

One approach toward defining the primary and background genes involved in type 2 diabetes and obesity is the use of naturally occurring rodent models (9,10) and inbred strains of mice (11–13). Indeed, Hummel et al. (9) and Coleman and Hummel (14) have shown that when mutations in leptin found in the *ob/ob* mouse are bred into different backgrounds, there is a difference in the severity of obesity and diabetes. A more recent strategy to define the role of genetic alterations and candidate genes for type 2 diabetes has been the creation of transgenic and knockout mice with alterations in insulin action (15) and in insulin secretion (16). Whereas knockout and transgenic mice often show diabetic or insulin-resistant phenotypes, by their very nature, the development of knockout mice leads to progeny that are born with a mixed genetic background and provide a unique tool for evaluation of the impact of background genes on phenotype (17,18).

We have reported the creation and characterization of mice double heterozygous (DH) for deletion of insulin receptor (IR) and IR substrate (IRS)-1 (19). On a mixed (C57BL/6 \times 129Sv) genetic background, some of the DH mice manifest early insulin resistance, severe hyperinsulinemia, and massive islet hyperplasia, whereas others remain near normal. Overall, 50% of the male mice progress to develop diabetes by 6 months, and this fraction seemed to remain stable thereafter, suggesting depletion of a genetically susceptible pool of mice from the mixed population. To determine the effect of genetic background on this phenotype, we backcrossed DH mice on to C57BL/6 (B6), 129Sv, or DBA/2 (DBA) for six generations. Examination of the DH progeny from the three different strains revealed dramatic differences in hyperinsulinemia, glucose tolerance, and islet hyperplasia between the B6 and 129Sv strains, indicating a role for the genetic background in the development of hyperinsulinemia and diabetes.

RESEARCH DESIGN AND METHODS

Breeding and growth curves. IR/IRS-1 DH mice originally created on a mixed genetic background (19) were backcrossed for six generations on the B6, 129Sv, and DBA backgrounds using genetic markers to track the mutant alleles (Fig. 1). The DH progeny were estimated to have ~98% of the background genes derived from each strain, representing near-congenic DH

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Received for publication 6 August 2002 and accepted in revised form 19 February 2003.

DH, double heterozygous; FKHR, forkhead transcription factor; IR, insulin receptor; IRS, IR substrate; PKB, protein kinase B; WT, wild-type.

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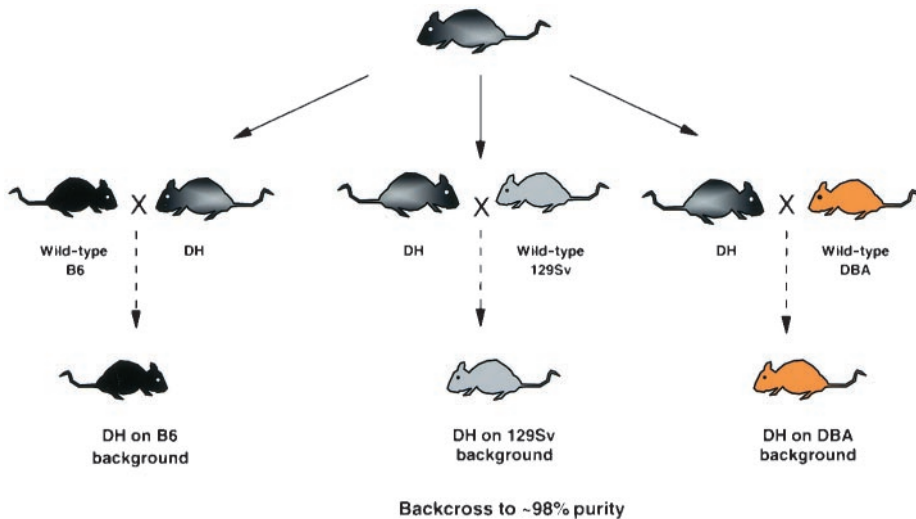


FIG. 1. Strategy for generating IR/IRS-1 DH mice purified on the C57BL/6J, 129Sv, and DBA/2 genetic backgrounds. DH mice on the original mixed background were mated with WT mice from C57BL/6J, 129Sv, or DBA/2 backgrounds for six generations. Mice were selected for heterozygosity in disruption of the IR and IRS-1 alleles. Both male and female mice were used for breeding. At the sixth generation, the colony was expanded to obtain at least 100 DH mice of each sex.

lines. Animals were housed in virus-free facilities on a 12-h light/12-h dark cycle (0700 on, 1900 off) and were fed standard rodent food at the Foster Animal Laboratory (Brandeis University, Waltham, MA). At least 100 male and 100 female DH mice and an equal number of wild-type (WT) littermates were studied for 6 months, and a small group ($n = 14-20$) was followed up to 1 year. Body weights were recorded every month for 6 months in both males and females. Genotyping was performed by PCR using genomic DNA isolated from tail snips (19). All protocols for animal use were approved by the Institutional Animal Care and Use Committee of Joslin Diabetes Center and Brandeis University and were in accordance with National Institutes of Health guidelines.

Analytical procedures. Blood glucose and plasma insulin levels were measured in the random-fed state between 8:00 and 10:00 A.M. or after a 14-h overnight fast, and blood was collected in chilled heparinized tubes for estimation of insulin and leptin by enzyme-linked immunosorbent assay (Crystal Chem, Chicago, IL) (20). Triglycerides levels in serum from fasted animals were measured by colorimetric enzyme assay (GPO-Trinder Assay; Sigma, St. Louis, MO). Free fatty acid levels were measured in serum from fasted animals using the NEFA-Kit-U (Amano Enzyme). Glucose and insulin tolerance tests were performed essentially as described previously (21).

Immunoprecipitation and Western blot analysis. Immunoprecipitations and Western blot analyses of IR and IRS-1 proteins were performed on liver homogenates as previously described (22). Western blotting for IRS-2 and forkhead transcription factor (FKHR) protein levels was performed by blotting with either an anti-IRS-2 or an anti-FKHR antibody (Cell Signaling, Beverly, MA). Results are expressed as the percentage of signal intensity seen in samples from WT B6 animals. For estimation of Akt activity, liver was removed at 5 min after insulin stimulation and instantly frozen in liquid nitrogen. Tissue homogenates were extracted with buffer A containing 25 mmol/l Tris-HCl (pH 7.4), 10 mmol/l Na_3VO_4 , 100 mmol/l NaF, 50 mmol/l $\text{Na}_4\text{P}_2\text{O}_7$, 10 mmol/l EGTA, 10 mmol/l EDTA, 5 $\mu\text{g/ml}$ leupeptin, 5 $\mu\text{g/ml}$ aprotinin, 2 mmol/l phenylmethylsulfonyl fluoride, and 1% Nonident-P 40. Tissue homogenates were subjected to immunoprecipitation with goat polyclonal anti-Akt antibody (Santa Cruz Biotechnology) followed by Akt kinase assay using Crosstide, as described previously (23). Briefly, the immunoprecipitates were washed and resuspended in 50 mmol/l Tris-HCl (pH 7.5), 10 mmol/l MgCl_2 , and 1 mmol/l dithiothreitol, to which 20 $\mu\text{mol/l}$ ATP, 5 μCi [γ - ^{32}P]ATP, and 5 μg Crosstide had been added. After 20 min at 30°C, the reaction was stopped, and the aliquots were spotted on squares of P-81 paper and washed with 0.5% of phosphoric acid, and radioactivity was counted.

DNA sequencing. For IR sequencing, RNA was prepared from liver homogenates using Tri Reagent (Sigma), and cDNA was synthesized using the ProStar RT-PCR system from Stratagene (La Jolla, CA). IRS-1 sequencing was performed on genomic DNA extracted from tail snips. All sequencing reactions were analyzed on an Applied Biosystems 373 DNA Sequencer (Perkin Elmer, Wellesley, MA).

Immunohistochemistry and body composition. Mice were anesthetized; pancreas was rapidly dissected, weighed, fixed in Bouin's solution, sectioned, and stained; and morphometric analysis and β -cell mass was calculated as described previously (20). Livers were dissected and fixed in 10% buffered formalin, and sections were stained with periodic acid-Schiff reagent using standard protocols. For body composition, mice were anesthetized, and the fat pads were dissected and weighed before returning to the carcass, which was treated with 1 N KOH overnight.

RESULTS

Effects on growth and fat mass. The DH mice were all born normally and, as expected, both males and females showed some growth retardation compared with controls on each background, as indicated by reduced body weights (Fig. 2A-F) and shorter body lengths (Fig. 2G). DH mice on all backgrounds showed a significant decrease in fat pad weights, even when expressed as a percentage of body weight and a decrease in circulating leptin levels (Fig. 3A and B). Among WT mice, both fatty acids and triglyceride levels were highest in DBA mice and lowest in 129Sv groups (Fig. 3C and D). DH mice in both the B6 and DBA strains showed significantly lower triglycerides compared with the WT controls, which corresponded with the lower fat mass (Fig. 3A and C). Fasting serum free fatty acids were also lower in general in the DH mice than the WT mice, but this difference was only significant in the 129Sv strain (Fig. 3D).

Effects on blood glucose, serum insulin concentrations, and incidence of diabetes. The impact of background genes on the control of blood glucose, insulin levels, and β -cell function was evaluated at 2, 4, and 6 months and in a small group of mice at 1 year of age. Because the metabolic phenotype was more profound in the male mice, only data on males will be presented. Among the WT groups, a significantly higher circulating fed insulin level was consistently observed in the B6 and DBA groups compared with the 129Sv mice (Fig. 4D-F). Fed blood glucose levels also tended to be higher in both the B6 and DBA mice (Fig. 4A-C), although almost all WT mice maintained blood glucose levels in the normal range during the entire study, with ~5% of B6 and 2% of DBA mice being defined as diabetic at 6 months, based on a fed blood glucose level >200 mg/dl (Fig. 4G and I). Interestingly, none of the WT mice in the 129Sv strain showed fed blood glucose levels >140 mg/dl, even at the end of 1 year (data not shown). Among the WT controls, no significant differences were observed in fasting insulin levels, whereas fasting blood glucose levels were highest in the B6 group and lowest in the DBA animals (Fig. 5G and H).

In the fed state, high circulating insulin levels were detected in DH mice on the B6 background as early as 2 months (Fig. 4D). At this age, ~20% of the DH mice had also developed diabetes, and the incidence progressed to

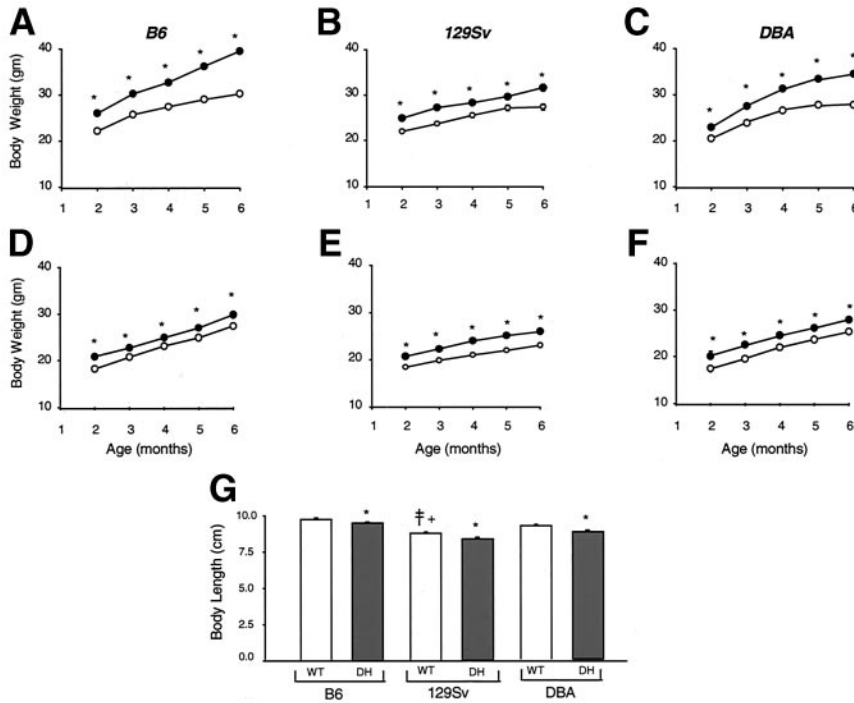


FIG. 2. Male and female DH mice on all three backgrounds show growth retardation. Body weights of male (A–C) and female (D–F) WT controls (●) and DH mice (○) from the three backgrounds are plotted over 6 months. Body lengths of 6-month-old male WT (□) and DH (■) mice on the three backgrounds are shown in G. * $P < 0.01$, DH vs. WT; ‡ $P < 0.05$, WT (129Sv) vs. WT (B6); + $P < 0.05$, WT (129Sv) vs. WT (DBA). $n = 100$ –110. All values are expressed as means \pm SE. The error bars, if not visible, lie within the symbols.

~85% by 6 months (Fig. 4G) and ~94% by age 1 year (data not shown), indicating the high penetrance of diabetes in the genetically insulin-resistant B6 strain. Although DH mice in the DBA strain showed hyperinsulinemia, it was not as severe as that observed in the B6 mice (Fig. 4F), and ~64% of the DH mice developed diabetes by 6 months (Fig. 4I). By sharp contrast, DH mice on the 129Sv background showed very modest increases in circulating insulin levels at 4 and 6 months of age (Fig. 4E). However, by age 6 months, only 2% of the mice became diabetic compared with WT controls (Fig. 4E and H), and this did not progress even at 1 year (data not shown). Interestingly, despite the various degrees of hyperglycemia, the mild fasting hyperinsulinemia resulted in significantly lower fasting blood glucose levels in the DH mice of all the strains compared with their respective controls (Fig. 5G and H). Further, we observed reduced glycogen stores in

the liver in the B6 DH mice compared with B6 WT controls (data not shown).

Glucose tolerance and insulin sensitivity. Evaluation of glucose tolerance in the WT controls showed a higher glucose excursion in the B6 mice compared with the 129Sv and DBA groups (Fig. 5D–F), indicating relative intolerance in the B6 inbred strain. The hyperglycemic and hyperinsulinemic DH mice on the B6 background exhibited even greater intolerance compared with their WT controls, with blood glucose levels staying elevated at ~300 mg/dl at the end of 2 h (Fig. 5D). By contrast, the presence of heterozygosity for IR and IRS-1 did not alter the tolerance in the DH mice on the 129Sv or DBA backgrounds (Fig. 5E and F). The reduced insulin sensitivity was evident in all three groups, as indicated by fasting hyperinsulinemia (Fig. 5H) and an abnormal insulin tolerance test (Fig. 5A–C). Both the hyperinsulinemia

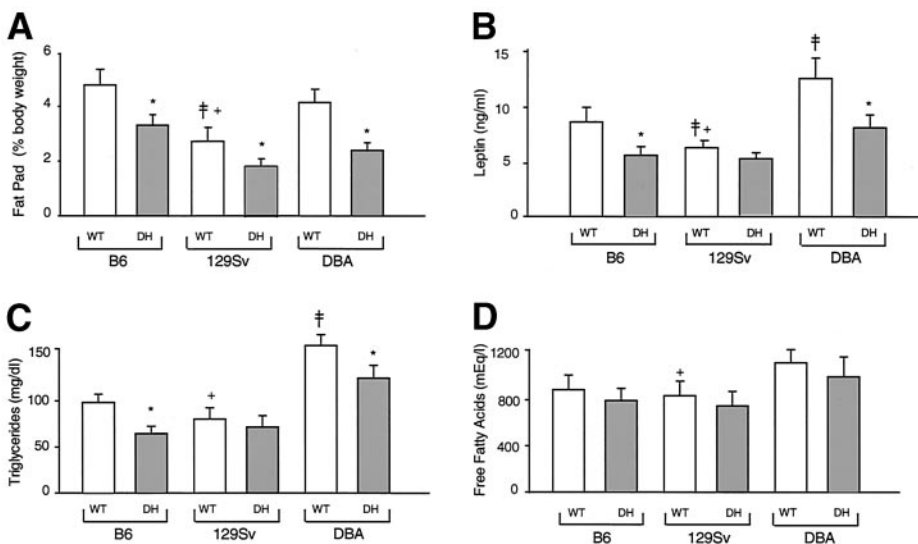


FIG. 3. Alterations in parameters of lipid metabolism and serum leptin in DH mice. A: Fat pad mass is expressed as the percentage body weight in WT (□) and DH (■) male mice. $n = 7$ –11. * $P < 0.02$, DH vs. WT; ‡ $P < 0.05$, WT (129Sv) vs. WT (B6); + $P < 0.05$, WT (129Sv) vs. WT (DBA). B: Serum leptin was determined in the fed state in WT (□) and DH (■) male mice. $n = 6$ –8. * $P < 0.05$, DH vs. WT; ‡ $P < 0.05$, WT (129Sv) or WT (DBA) vs. WT (B6); + $P < 0.05$, WT (129Sv) vs. WT (DBA). C: Serum triglycerides were measured in 14-h overnight-fasted male WT (□) and DH (■) mice. $n = 8$ –10. * $P < 0.02$, DH vs. WT; ‡ $P < 0.01$, WT (DBA) vs. WT (B6); + $P < 0.01$, WT (129Sv) vs. WT (DBA). D: Serum free fatty acids were measured in 14-h overnight-fasted male WT (□) and DH (■) mice. $n = 8$ –10. + $P < 0.05$, WT (129Sv) vs. WT (DBA).

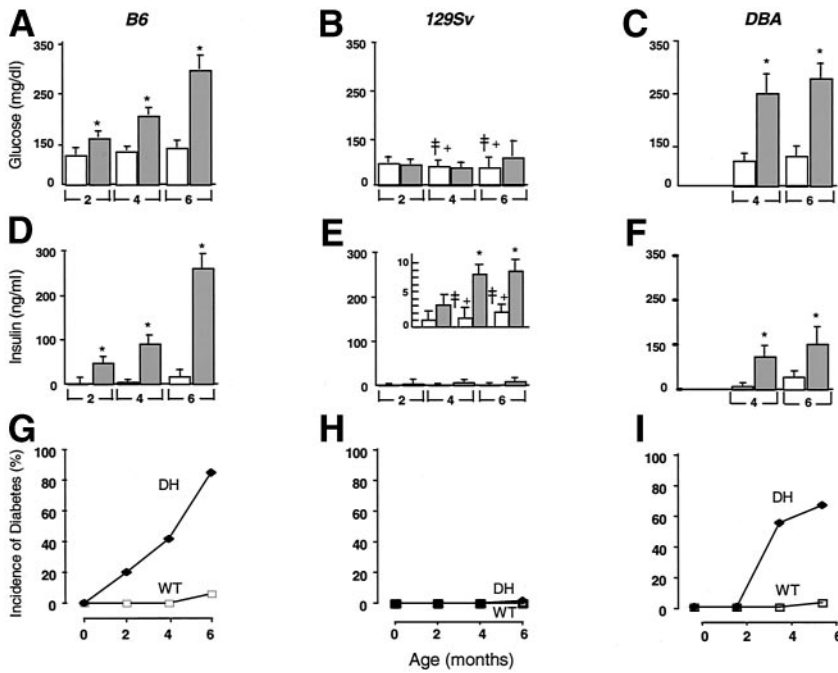


FIG. 4. Massive hyperinsulinemia and hyperglycemia and a higher incidence of diabetes in DH mice on the B6 and DBA strains. Fed blood glucose (A–C) and serum insulin (D–F) in 2-, 4-, and 6-month-old WT (□) and DH (■) male mice on the three backgrounds. E: Serum insulin levels in mice in the 129Sv strain on an expanded scale. Incidence of diabetes in WT (□) and DH (■) mice on the B6 (G), 129Sv (H), and DBA (I) backgrounds. Diabetes is defined as fed blood glucose >200 mg/dl. $n = 100$ –110. * $P < 0.001$, DH vs. WT; ‡ $P < 0.01$, WT (129Sv) vs. WT (B6); + $P < 0.05$, WT (129Sv) vs. WT (DBA). No data are available at 2 months in the DBA mice.

and the loss of sensitivity to injected insulin were greatest in the B6 DH mice and very mild in the 129Sv strain (Fig. 5B and H).

Insulin signaling events. Alterations in signaling events in the insulin/IGF-I signaling pathway in the liver, skeletal muscle, and adipose tissue contribute to the insulin resistance observed both in rodents and in humans with type 2 diabetes (1,24). To detect potential signaling defects in these pathways, we stimulated WT and DH mice with insulin and examined phosphorylation of IR and IRS-1 in the liver. Although a significant phosphorylation was detected in both these proteins in all WT groups, no significant differences were observed between the three

backgrounds (Fig. 6A–F). Among the DH groups, the DBA mice showed a slightly higher basal IR phosphorylation and a minimal increase in phosphorylation after insulin stimulation that was not significantly different from that observed in the B6 and 129Sv groups (Fig. 6C). Similarly, a blunted insulin-stimulated IRS-1 phosphorylation was detected in the B6 DH mice, which was not significantly different when compared with IRS-1 phosphorylation in the other groups (Fig. 6D). To further rule out the possibility of sequence variations in the remaining allele of IR (25) or IRS-1 (26), which might affect the level of insulin resistance, we analyzed coding sequences for these proteins at the level of DNA in the three genetic backgrounds

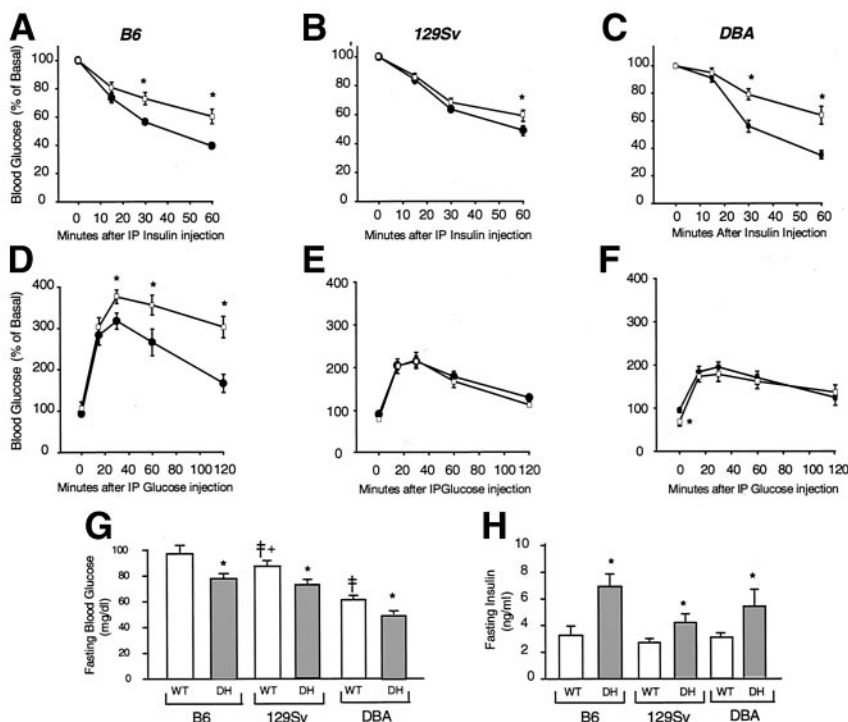


FIG. 5. Altered insulin resistance, glucose intolerance, fasting serum insulin, and blood glucose levels in DH mice. Insulin tolerance tests were performed on random-fed male WT (●) and DH (□) mice on the B6 (A), 129Sv (B), and DBA (C) backgrounds. Animals were injected with 1 unit/kg body wt of human regular insulin (Humulin; Lilly) i.p. Blood glucose was recorded from tail vein samples at 0, 15, 30, and 60 min. Glucose tolerance tests were performed on 14-h overnight fasted male WT (●) and DH (□) mice on the B6 (D), 129Sv (E), and DBA (F) backgrounds. Animals were injected with 2 g/kg body wt glucose i.p. Blood glucose was recorded from tail vein samples at 0, 15, 30, 60, and 120 min. $n = 8$ –12. * $P < 0.01$, WT vs. DH. Fasting blood glucose (G) and serum insulin (H) was measured after a 14-h overnight fast in WT (□) and DH (■) male mice. $n = 35$ –76. * $P < 0.01$, DH vs. WT; ‡ $P < 0.02$, WT (129Sv) or WT (DBA) vs. WT (B6); + $P < 0.01$, WT (129Sv) vs. WT (DBA). IP, intraperitoneal.

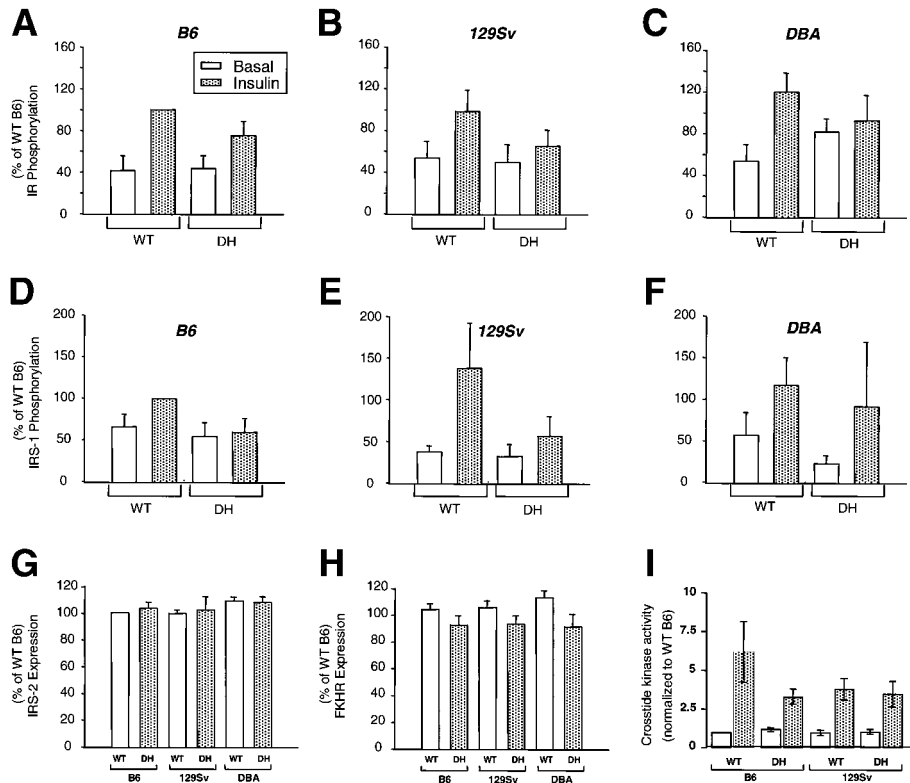


FIG. 6. Insulin-stimulated phosphorylation of hepatic IR, IRS-1, and PKB/Akt2 and hepatic content of FKHR and IRS-2 in WT and DH mice. Bar diagrams show quantification of basal and insulin-stimulated phosphorylation of IR and IRS-1 in liver tissue isolated from WT and DH mice of the B6 (A and D), 129Sv (B and E), and DBA (C and F) genetic backgrounds. Hepatic expression of IRS-2 and FKHR is shown in G and H, respectively. *n* = 2–4. Insulin-stimulated hepatic PKB/Akt2 activity in the B6 and 129Sv groups is shown in I. *n* = 3.

and found no differences. IRS-2 and FKHR also play a role in the maintenance of insulin sensitivity in the liver (27). Again, no significant differences were observed in the hepatic content of FKHR or IRS-2 among the WT or DH mice in the B6 and 129Sv strains (Fig. 6G and H). To investigate alterations in protein kinase B (PKB)/Akt2, which plays a pivotal role in insulin sensitivity and hepatic glucose production (28), we examined insulin-stimulated Akt activity in lysates extracted from liver tissue (Fig. 6I). A robust response was detected in B6 WT mice compared with the 129Sv WT group, but the differences did not reach statistical significance. Further, the stimulated Akt activity in the B6 DH group was lower compared with the response in the B6 WT mice. In contrast, Akt activity in the livers from both the 129Sv DH and the 129Sv WT mice showed similar responses to insulin stimulation. No significant differences were evident among groups.

Islet hyperplasia. Most animal models of insulin resistance, including the DH mice, show compensatory islet hyperplasia (16,19). DH mice on the B6 background exhibited the greatest level of islet hyperplasia with an ~22-fold increase in β -cell mass at 6 months of age (Fig. 7A–C). By contrast, islet size and β -cell mass were in the normal range in the 129Sv mice (Fig. 7A and B). Pancreas sections from DBA mice showed hyperplasia, but not as dramatic as in the B6 DH mice, with an approximately ninefold elevation of β -cell mass by 6 months (Fig. 7A and B). Furthermore, by 10 months of age, the hyperplastic islets in the DBA DH mice began to show vacuoles, dilated blood vessels, and infiltration of acinar-like cells (Fig. 7C), suggesting degeneration of β -cells, as observed in other studies of this strain (29). The different islet compensatory response in these two strains was evident when plasma insulin was plotted against the corresponding fed blood glucose levels (Fig. 7D). In both B6 and DBA mice, there

was a linear relationship between glucose and insulin levels, but with a relatively steep slope in the B6 mice and a flatter response in the DBA animals, indicating poor compensation in the latter group.

DISCUSSION

The present study shows major differences in the metabolic phenotype when the same genetic mutation is introduced into three different strains of mice. The metabolic phenotype of the WT B6 mice was particularly different when compared with the 129Sv group. The B6 mice showed greater glycemia, a larger fat pad mass, and a higher incidence of diabetes compared with the 129Sv mice. Earlier reports have also shown that WT B6 mice have a tendency toward mild glucose intolerance and higher glycosylated hemoglobin levels than WT DBA and WT C3H/HeJ mice (30), suggesting an “inbred susceptibility” to develop diabetes in the B6 strain. The higher fat pad mass and higher leptin levels in the WT B6 mice indicate a greater percentage of body fat, which makes these mice susceptible to obesity, diet-induced type 2 diabetes (31), atherosclerosis (31), and possibly a greater effect of genetic insulin resistance.

Consistent with earlier observations (19), the DH mice showed growth retardation compared with controls on each background. This represents the influence of IRS-1 heterozygosity on growth responses, as illustrated in the male 129Sv mice and most of the female mice as reported earlier (22,32,33), and in older mice growth retardation may be due in part to the development of diabetes, especially in DH males on the B6 and DBA backgrounds. The introduction of the IR/IRS-1 mutation led to striking hyperinsulinemia and hyperglycemia in the DH mice on the B6 and DBA backgrounds compared with the ex-

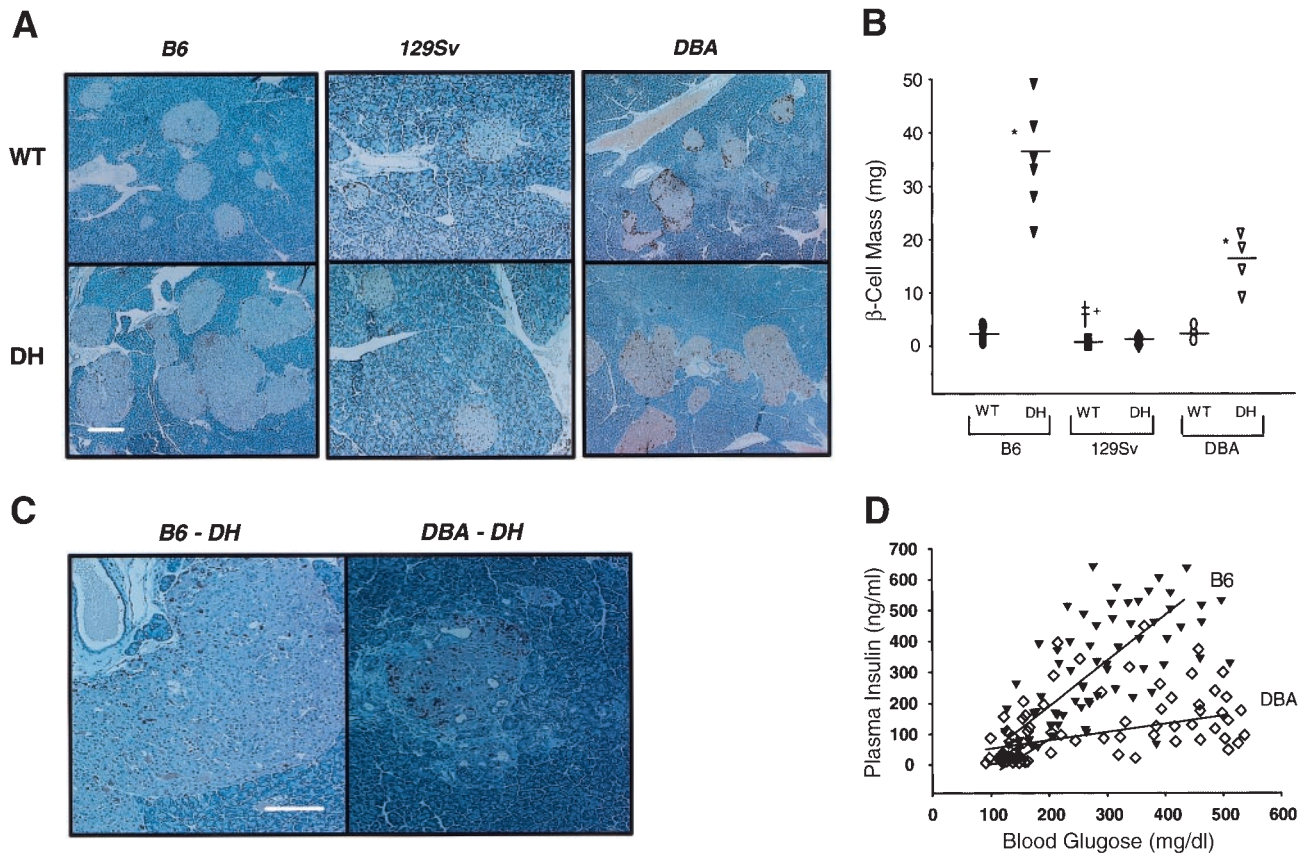


FIG. 7. Massive islet hyperplasia and increase in β -cell mass in DH mice on the B6 and DBA backgrounds but not the 129Sv background. Representative sections from pancreas from (A) 6-month-old WT and DH male mice on the B6, 129Sv, and DBA/2 backgrounds were stained with a cocktail of antibodies against the non- β -cells and counterstained with hematoxylin. Original magnification $\times 10$. Bar = 50 μ m. B: β -Cell mass in 6-month-old mice. * $P < 0.01$, DH (B6) vs. WT (B6) and DH (DBA) vs. WT (DBA); $\ddagger P < 0.05$, WT (129Sv) vs. WT (B6) and WT (DBA); + $P < 0.05$, WT (129Sv) vs. WT (DBA). C: Pancreas sections from 10-month-old DH mice on the B6 and DBA backgrounds stained with a cocktail of antibodies against non- β -cells. Original magnification $\times 20$. Bar = 50 μ m. D: Plot showing relationship between serum insulin levels and blood glucose levels in the fed state in DH mice on the B6 and DBA backgrounds. The lines indicate linear regression.

tremely mild effects in the 129Sv mice. The wide variation observed in random-fed hyperglycemia in the B6 DH males is likely due to alterations in nutrient metabolism and also, in part, due to differences in nutrient loading secondary to variations in the last meal consumed by the mice. The DH mice on all three strains showed fasting hypoglycemia that is likely due to reduced hepatic glycogen stores in the insulin-resistant mice.

DH mice on a mixed genetic background demonstrate epistatic interactions between genes in a common signaling pathway (19). Such interactions and the increased susceptibility in the WT B6 mice would play some role in the high incidence of diabetes in the DH B6 and DBA strains. It is possible that the almost complete lack of disease in the 129Sv mice that carry the *same* mutation indicates the presence of important genetic modifiers in the B6 and DBA mice that allow the genetic insulin resistance to progress to diabetes or that alleles in the 129Sv genome play a protective role.

Clearly, in both B6 and DBA mice, the major modifier lies in the insulin resistance pathway, because DH mice on both backgrounds exhibit marked hyperinsulinemia. However, we observed a difference in the phenotype even between the B6 and DBA DH mice. Thus, hyperinsulinemia was greater in the B6 mice, and this was associated with greater islet hyperplasia and an increase in β -cell mass

that exceeded the levels observed in DBA mice. In fact, the hyperplasia in the B6 mice was greater than our earlier observations in DH mice on the mixed genetic background (19), which may be in part due to the greater ability of B6 islet cells to replicate (34). The milder response in the aging DBA mice may be related to loss of β -cells because of "vacuolation" in the islets, which limits the ability of the β -cells to synthesize and store insulin. In this context, the B6 DH mouse manifests severe hyperglycemia despite a robust islet compensatory response, indicating that defective insulin action is adequate, in this model, to promote full manifestation of diabetes. Together, these mice serve as excellent models to demonstrate the full range of response to genetic insulin resistance and islet growth secondary to insulin resistance, which may be observed in rodents and humans.

The additional lesions in insulin action that contribute to the difference in phenotype between B6 and 129Sv mice do not appear to lie at the level of IR, IRS-1, IRS-2, PKB/Akt2, or FKHR. Although insulin-stimulated PKB/Akt activity was slightly higher in the B6 WT mice than the 129Sv WT group, this did not reach statistical significance. Thus, any differences in insulin sensitivity observed in mice on the different backgrounds appear to be primarily due to differences at steps in the insulin action pathway beyond the PKB/Akt2 proteins. However, the maintenance

of similar levels of insulin-induced Akt2 activity in the 129Sv DH group compared with their corresponding WT, to the same level as the B6 DH group, despite a reduced phosphatidylinositol 3-kinase activity (data not shown), indicates that potential alterations in one or more of the negative regulators for Akt2 activity contributes to maintaining insulin sensitivity in the 129Sv mice in the presence of reduced insulin signaling. Although B6 and DBA mice do have slightly higher free fatty acid levels, it is unlikely that lipid metabolism contributes to the severity in insulin resistance because most parameters examined show a reduction in the mutant mice. Furthermore, the lack of sequence variations in the IR or IRS-1 rules out the possibility of functional mutations in the early signaling pathway that may contribute to the differences in insulin resistance in the B6 and 129Sv animals. Taken together, the increased incidence of diabetes in DH mice may be due to the presence of modifier alleles that increase the severity of insulin resistance on the B6 background and/or decrease the severity in the 129Sv backgrounds. DBA alleles likely influence both insulin resistance and the response of the pancreatic β -cell in growth and function. Further studies using a genome-wide scan will be useful to identify polygenic modifiers that can affect the development and phenotype of type 2 diabetes in these mice with genetic insulin resistance.

ACKNOWLEDGMENTS

This study was supported by the K08 Clinical Scientist Development Award DK 02885 to R.N.K. and National Institutes of Health Grant DK 31036 to C.R.K. K.A. was supported by a grant from the Danish Medical Research Council.

We thank Dr. K.C. Hayes and staff of the Foster Animal Research Laboratories at Brandeis University, Waltham, for housing and maintenance of the mouse colonies. We thank Jens Bruning, Nadia Hashimi, Sarah Flier, Dan Eisenman, Shannon Curtis, Rebecca Quinn, and Beth Fletcher for assistance at various stages of the project and Julie Marr for secretarial assistance.

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