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Impaired glucose homeostasis and neonatal mortality in hepatocyte nuclear factor 3α-deficient mice

(hypoglycemia/proglucagon/neuropeptide Y/pancreatic islet cells)

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ABSTRACT **Hepatocyte nuclear factors 3 (HNF-3) belong to an evolutionarily conserved family of transcription factors that are critical for diverse biological processes such as development, differentiation, and metabolism. To study the physiological role of HNF-3α, we generated mice that lack HNF-3α by homologous recombination in embryonic stem cells. Mice homozygous for a null mutation in the HNF-3α gene develop a complex phenotype that is characterized by abnormal feeding behavior, progressive starvation, persistent hypoglycemia, hypotriglyceridemia, wasting, and neonatal mortality between days 2 and 14. Hypoglycemia in HNF-3α-null mice leads to physiological counter-regulatory responses in glucocorticoid and growth hormone production and an inhibition of insulin secretion but fails to stimulate glucagon secretion. Glucagon-producing pancreatic alpha cells develop normally in HNF-3α−/− mice, but proglucagon mRNA levels are reduced 50%. Furthermore, the transcriptional levels of neuropeptide Y are also significantly reduced shortly after birth, implying a direct role of HNF-3α in the expression of these genes. In contrast, mRNA levels were increased in HNF-3 target genes phosphofructo-2-kinase/fructose-2,6-bisphophatase, insulin growth factor binding protein-1, and hexokinase I of HNF-3α-null mice. Mice lacking one or both HNF-3α alleles also show impaired insulin secretion and glucose intolerance after an intraperitoneal glucose challenge, indicating that pancreatic beta-cell function is also compromised. Our results indicate that HNF-3α plays a critical role in the regulation of glucose homeostasis and in pancreatic islet function.**

The hepatocyte nuclear factor 3 (HNF-3)/forkhead (fkh) family of transcription factors in mammals include three distinct genes designated HNF-3α, HNF-3β, and HNF-3γ. They all have in common a highly conserved 100-aa winged-helix motif that is responsible for monomeric recognition of specific DNA target sites (1). The structure of the highly conserved DNA-binding domain of the HNF-3 proteins is similar to that of linker histones H1 and H5 (1). However, in contrast to linker histones that compact DNA in chromatin and repress gene expression, HNF-3 proteins have been shown to decompact DNA from the nucleosome and are associated with transcriptionally active chromatin (2, 3).

In the adult, HNF-3 genes have overlapping patterns of tissue expression including gut, central nervous system, neuroendocrine cells, lung, and the liver [in which they were first identified (4–6)]. During development, HNF-3α and HNF-3β are sequentially expressed in the definite endoderm, notochord, and floor plate of the neural tube, with HNF-3β being expressed first (7, 8). Targeted disruption of HNF-3β in mice produces an embryonic-lethal phenotype that lacks a notochord and exhibits defects in foregut and neural-tube development (7, 8). In contrast, HNF-3α- and HNF-3γ-deficient embryos develop normally, suggesting that they have distinct roles from HNF-3β during development (9–11).

Liver-specific expression is controlled primarily at the transcriptional level and relies on the activity of multiple tissue-enriched transcription factors such as HNF-1, HNF-3, CCAAT/enhancer-binding protein, HNF-4, and HNF-6 (12, 13). Many genes involved in the regulation of glucose, lipid, and amino acid metabolism have HNF-3-binding sites in their promoters (13, 14). Mice deficient in HNF-3γ have reduced transcription rates in several genes, including phosphoenolpyruvate carboxykinase, transferrin, and tyrosine aminotransferase (11). We have recently shown in visceral endoderm derived from HNF-3α−/− and HNF-3β −/− embryonic stem (ES) cells that expression of several metabolic proteins is dysregulated (9). We have also shown that HNF-3α and HNF-3β can regulate the expression of the early-onset diabetes genes HNF-1α (*MODY3*) and HNF-4α (*MODY1*), suggesting that HNF-3 transcription factors have a critical role in pancreatic islet function (9). A recent study by Kaestner *et al.* (10) has shown that HNF-3α plays a critical role for pancreatic alpha-cell function, including glucagon gene expression. In this study, we investigated the role of HNF-3α using genetic, molecular, and physiological approaches. We demonstrate that mice lacking HNF-3α expression develop a complex metabolic syndrome characterized by neonatal persistent hypoglycemia, hormonal insufficiencies, pancreatic alpha- and beta-cell dysfunction, and increased expression of HNF-3α target genes.

METHODS

Glucose, Glycogen, Amino Acid, Triglyceride, Insulin, Glucagon, Glucocorticoid, and Growth Hormone Measurements. Plasma for insulin, glucagon, cortisol, and leptin measurements were obtained by collecting whole blood in heparinized capillary tubes, immediately centrifuging, and storing at −20°C. Insulin and glucagon levels were measured by using radioimmunoassay (RIA) kits with rat insulin and glucagon standards (Linco). Glucocorticoid levels were measured with a cortisol RIA kit (Diagnostic Systems Laboratories), and growth hormone (GH) was assayed by using a ^{125}I-GH assay system with magnetic separation (Amersham Pharmacia). Glucose was measured by using an enzymatic (glucose oxidase, trinder reagent) assay (Sigma). Serum triglyceride levels were analyzed by using colorimetric test (GPO-Trinder, Sigma). Liver glycogen contents were measured after homogenization in ice-cold perchloric acid and

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Abbreviations: HNF-3, hepatocyte nuclear factor 3; NPY, neuropeptide Y; MODY, maturity-onset diabetes of the young; ES, embryonic stem.

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glycogen hydrolysis with amyloglucosidase as described (15). Quantitation of amino acids was performed on a Beckman Model 6300 ion-exchange instrument.

Immunohistochemical Analysis. Pancreata were fixed with 4% formaldehyde and 0.1% glutaraldehyde in PBS (pH 7.4) overnight at 4°C, dehydrated, and embedded in paraffin. Fifty-micrometer sections were deparaffinized, rehydrated, and permeabilized in 0.2% Triton X-100 for 10 min, blocked with 0.3% H_2O_2 for 20 min, and 4% normal goat serum for 30 min. IgG-fractionated anti-insulin and anti-glucagon antibodies (Linco Research Immunoassay, St. Charles, MO) were diluted 1:100 in PBS containing 3% goat serum. Slides were incubated for 1 hr at room temperature and washed in PBS before incubation with a secondary biotinylated anti-IgG antibody. Sections were developed with the ABC kit (Vector Laboratories) and AEC substrate (red) and counterstained with hematoxylin.

Gene Expression Analysis. Total RNA was extracted from tissues by Trizol reagents following the manufacturer's instructions (GIBCO/BRL). Contaminating genomic DNA was removed by using DNase I treatment (20 units/10 μg of total RNA). cDNA was synthesized by using Moloney murine leukemia virus reverse transcriptase with dNTPs and random hexamer primers (Stratagene). The cDNAs provided templates for PCRs with specific primers in the presence of dNTPs, [α-^{32}P]dCTP, and *Taq* DNA polymerase (16). The primer sequences used are available on request.

Glucose Tolerance Test. Glucose tolerance tests were performed by intraperitoneal glucose injection (2 mg/g body weight). Whole blood was obtained from 2- to 3-day or 6- to 8-week-old mice by decapitation or tail bleeds 1 hr after glucose injection. Blood glucose was measured by using an enzymatic assay (Trinder, Sigma).

Statistical Analysis. Results are given as mean ± SE if not otherwise indicated. Statistical analyses were carried out by using a two-tailed Student's unpaired *t* test, and the null hypothesis was rejected at the 0.05 level.

RESULTS

Generation of HNF-3α-Null Mice. The HNF-3α gene was disrupted in ES cells by homologous recombination using a targeting vector in which most of exon 2 of the HNF-3α gene, including the DNA-binding domain, was deleted and fused in-frame to the *Escherichia coli lacZ* gene and a pgk-neomycin cassette (Fig. 1*A*). One ES cell clone that carried the targeted allele was used to generate chimeric male animals that passed the mutant allele to the offspring (Fig. 1*B*). HNF-3α heterozygous mice were phenotypically indistinguishable from wild-type mice and were inbred to produce HNF-3α-null mice. No HNF-3α mRNA could be detected in HNF-3α−/− mice by reverse transcription–PCR analysis (Fig. 4*A*).

Persistent Hypoglycemia, Other Complex Metabolic Abnormalities, and Neonatal Mortality in HNF-3α-Null Mice. Heterozygous HNF-3α mice were intercrossed to generate a total of 460 offspring that had genotypes of an expected Mendelian ratio. HNF-3α−/− neonates were phenotypically indistinguishable from their littermates; the gross and histological morphologies of the gut, liver, and pancreas were normal. However, all HNF-3α-null mice died between 2 and 14 days postnatally. Mutant newborn mice were as active as their littermates, exhibiting normal suckling behavior, and they had milk in their stomachs. However, 1–2 days postnatally, HNF-3α−/− mice showed signs of growth retardation (Fig. 2*A*). Although milk could be detected in the stomach throughout the life of HNF-3α−/− mice, milk volume in the stomach was drastically decreased after 1 day (Fig. 2*B*). Blood glucose levels and triglyceride levels were reduced 20–60%, whereas serum cholesterol was normal in 2- to 3-day old mutant mice compared with wild-type littermates (Fig. 2 *C* and *D*).

To investigate the physiological basis for postnatal persistent hypoglycemia in HNF-3α−/−mice, we measured the major hormones such as insulin, glucagon, cortisol, and growth hormone that are known to regulate blood glucose concentrations. Serum insulin levels in mutant mice were reduced to ≈50%, which is that found in wild-type mice after 24 hours of starvation (Fig. 2*E*). Glucagon levels were paradoxically de-

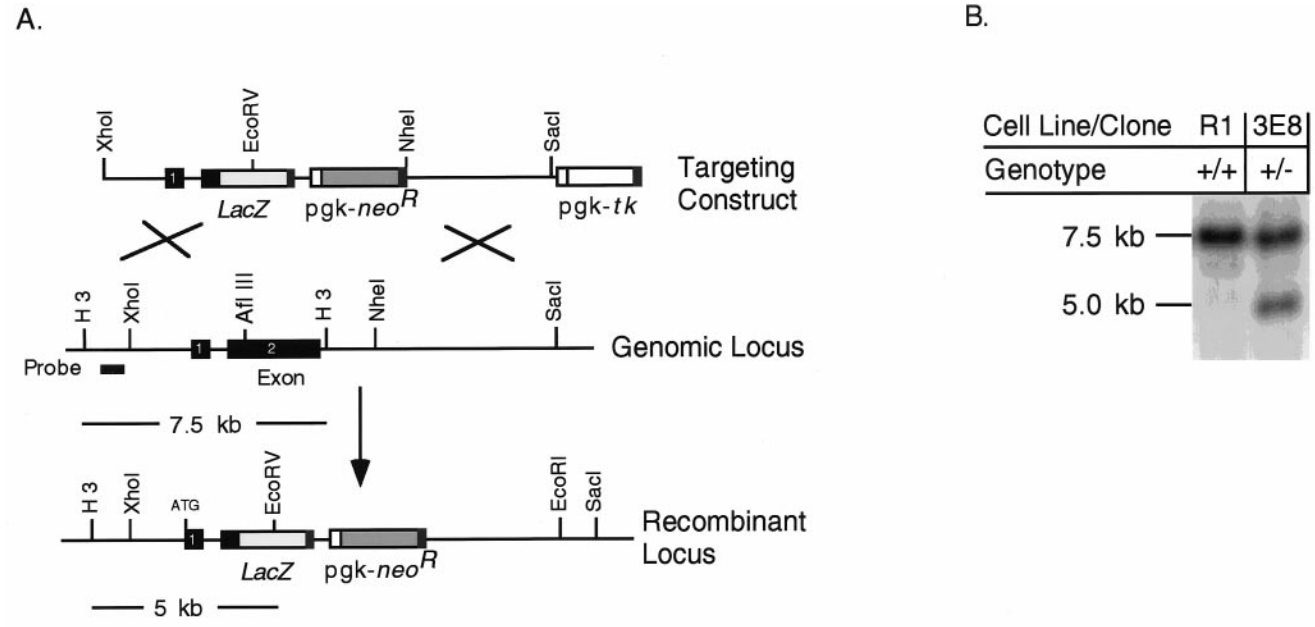

FIG. 1. Disruption of the HNF-3α gene by homologous recombination. (*A*) Strategy for targeted disruption of the murine HNF-3α gene in ES cells. The genomic structure and restriction map of the mouse HNF-3α gene locus and targeting vector pPNT-2 used to disrupt the HNF-3α gene are shown. The targeting vector was constructed by deleting exon 2, including the DNA-binding domain downstream from the *Afl*III site and fusing it in-frame to the *E. coli lacZ* gene. The probe 3′ of the left targeting arm was used for Southern blot analysis and is shown as a bar. (*B*) Southern blot analysis of transfected ES cells and HNF3α+/− ES cells grown in the presence of G418. Genomic DNA was digested with *Hin*dIII (H3) and *Eco*RV. The wild-type allele shows a 7.5-kilobase band, and the targeted allele shows a 5.0-kilobase band.

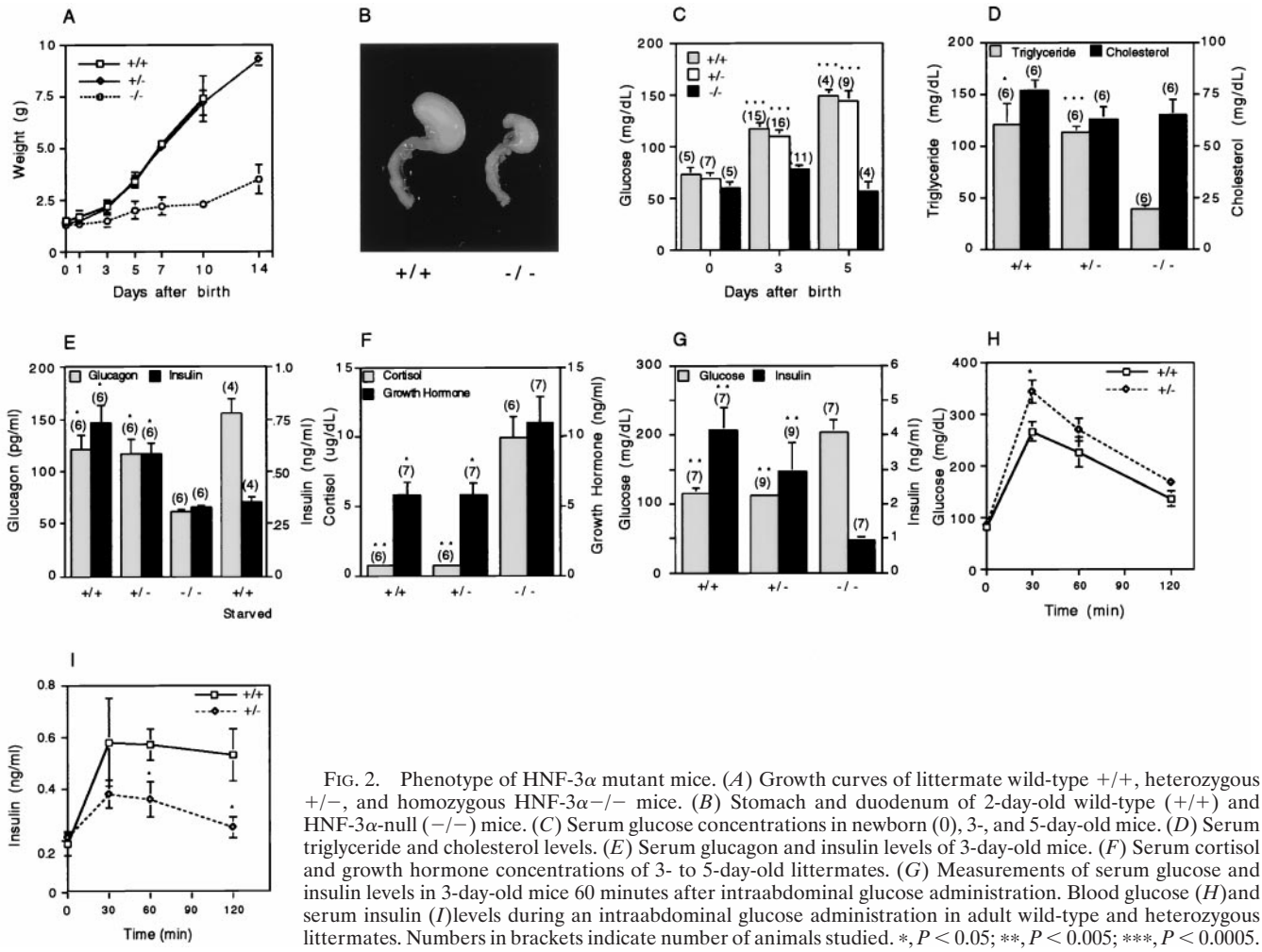

FIG. 2. Phenotype of HNF-3α mutant mice. (*A*) Growth curves of littermate wild-type +/+, heterozygous +/−, and homozygous HNF-3α−/− mice. (*B*) Stomach and duodenum of 2-day-old wild-type (+/+) and HNF-3α-null (−/−) mice. (*C*) Serum glucose concentrations in newborn (0), 3-, and 5-day-old mice. (*D*) Serum triglyceride and cholesterol levels. (*E*) Serum glucagon and insulin levels of 3-day-old mice. (*F*) Serum cortisol and growth hormone concentrations of 3- to 5-day-old littermates. (*G*) Measurements of serum glucose and insulin levels in 3-day-old mice 60 minutes after intraabdominal glucose administration. Blood glucose (*H*)and serum insulin (*I*)levels during an intraabdominal glucose administration in adult wild-type and heterozygous littermates. Numbers in brackets indicate number of animals studied. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$.

creased (≈50%) in hypoglycemic HNF-3α−/− mice when compared with wild-type littermate controls or starved animals (Fig. 2*E*). Total pancreatic glucagon content of HNF-3α-null mice was also reduced 50% compared with wild-type littermates (3.2 vs. 6.4 μg/g pancreas, respectively). Serum growth hormone and cortisol concentrations were increased ≈2- and 8-fold, respectively, in HNF-3α−/− mice compared with control animals (Fig. 2*F*). These results suggest that pancreatic alpha cells from HNF-3α−/− mice have lost their ability to respond to hypoglycemia by increasing glucagon secretion. In contrast, counter-regulatory responses to hypoglycemia of the pituitary gland, adrenal cortex, and pancreatic beta cells that stimulate growth hormone and corticosteroid release and inhibit insulin secretion, respectively, appeared normal.

To study whether glucagon deficiency could be explained by a pancreatic islet developmental defect or a loss of pancreatic alpha cells, we looked for the presence of alpha and beta endocrine cell types using immunohistochemistry. Pancreatic islet immunostaining for glucagon and insulin-expressing cells were indistinguishable in HNF-3α mutant and wild-type mice in 3- and 5-day-old mice (Fig. 3). These results indicate that differentiation of the endocrine pancreas is normal in mice lacking HNF-3α.

HNF-3α is highly expressed in pancreatic beta cells and may be a part of a transcriptional network that is essential for normal pancreatic beta cell function (9). To assess pancreatic beta-cell function in HNF-3α−/− mice, we challenged normal and mutant null mice with glucose by parenteral administration. Three-day-old mice were given an intraperitoneal glucose infusion of 2 mg glucose/g total body weight, and glucose and

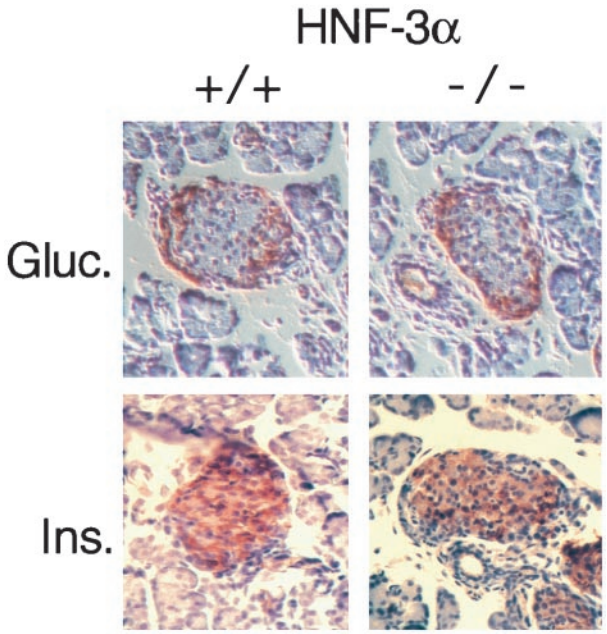

FIG. 3. Normal pancreatic morphology and immunostaining for glucagon and insulin in HNF-3α mice. Pancreatic tissue sections showing representative islets from 3-day-old pancreata of wild-type and HNF-3α mutant mice. Sections were stained with antibodies against glucagon and insulin as described in *Materials and Methods*. Glucagon and insulin are detected in the characteristic cell-lineage patterns. Magnification, ×400.

insulin levels were measured after 1 hour. Glucose levels were significantly elevated and insulin levels were markedly reduced in HNF-3α−/− mice as compared with wild-type and heterozygous littermates (Fig. 2*G*). Because hypoglycemia and starvation can have adverse effects on glucose-stimulated insulin secretion, we also performed intraperitoneal glucose tolerance tests in 6- to 8-week-old wild-type and heterozygous HNF-3α mice that have indistinguishable body weight and normal blood glucose levels. Fig. 2 *H* and *I* show that HNF-3α+/− mice had significantly higher blood glucose levels and reduced serum insulin concentrations after an intraperitoneal glucose challenge. These data indicate that HNF-3α haploinsufficiency causes a mild insulin-secretory defect and that HNF-3α is also critical for normal pancreatic beta-cell function.

Liver glycogen is important for newborn pups as an energy source and later in life for glucose homeostasis (17). To investigate whether glycogen synthesis or degradation was impaired in the livers of HNF-3α−/− animals, we measured total liver glycogen content in fed and fasted states. Glycogen content was similar overall in mutant and wild-type mice, suggesting that there was no intrinsic defect in glycogen synthesis or metabolism. Furthermore, mutant HNF-3α mice were able to increase their hepatic glycogen stores after intraperitoneal glucose administration, providing further support that glycogen synthesis in HNF-3α-null animals is unimpaired (data not shown).

Ketone bodies are produced by the liver from free fatty acids at times of hypoglycemia, low insulin, and high serum glucagon concentrations. We therefore measured β-hydroxybutyrate serum concentrations to study whether HNF-3α mice maintain the ability to metabolically adapt to hypoglycemia by increasing fatty acid degradation. As expected, β-hydroxybutyrate concentrations were moderately elevated in the serum of HNF-3α−/− animals and reached levels that were comparable to wild-type mice that had been starved for 24 hours (data not shown).

HNF-1α-null mice develop a Fanconi-like syndrome characterized by excessive loss of glucose and amino acids in their urine (18). We therefore investigated whether a defect in renal reabsorption of glucose contributes to hypoglycemia by performing a quantitative urinalysis of glucose and amino acids. No glucose was detected in the urine of mutant HNF-3α mice, and amino acid concentrations were similar to littermate control animals, demonstrating that hypoglycemia and wasting cannot be explained by loss of glucose or amino acids in the urine (data not shown).

Exocrine pancreas insufficiency can lead to chronic malabsorption because of decreased synthesis or secretion of amylase, proteinases, and lipases. We therefore tested whether hypoglycemia in HNF-3α−/− mice might be caused by exocrine insufficiency. We assayed the enzymatic activities of amylase, trypsin, chymotrypsin, and phospholipase from gut homogenate of wild-type and mutant mice. No significant differences were noted in these activities, suggesting that wasting is not caused by malabsorption because of decreased capacity to hydrolyze nutrients in the intestine.

Impaired Gene Expression of Pancreatic Proglucagon and Neuropeptide Y in HNF-3α-Null Mice. To study the molecular mechanisms that are responsible for the complex metabolic abnormalities in HNF-3α mice, we analyzed steady-state mRNA levels in the brain, liver, gut, and pancreas of newborn (3–6 hours) and 3-, 5-, and 7-day-old mice. Gene expression of wild-type, heterozygous, and null mice were assayed by using reverse transcription–PCR. Each sample contained similar amounts of mRNA, as shown by the equal amplification of hypoxanthine phosphoribosyltransferase mRNA. We analyzed the expression of 126 genes that have either been reported to be regulated by HNF-3 transcription factors or are known to affect glucose and energy homeostasis and have similar expression patterns as HNF-3α. Genes tested in the expression screen included hormones, peptide neurotransmitters and their receptors, glucose, amino acid and peptide transporters, transcription factors regulating metabolic pathways, enzymes of glycolysis, gluconeogenesis, glycogen synthesis and degradation, urea cycle, cholesterol and fatty acid synthesis and metabolism, intracellular storage proteins, and serum factors (the list of genes tested is available on request from M.S.). No abnormalities in gene expression were detected in 3-day-old mutant mice that would indicate primary defects in gluconeogenesis, glycogen synthesis, or degradation and fatty acid metabolism. Furthermore, most mRNA transcript levels of genes that have been shown to be regulated by HNF-3 transcription factors *in vitro* including enzymes of gluconeogenesis (phospho*enol*pyruvate carboxykinase, glucose 6-phosphatase) were unchanged in HNF-3α−/− mice. However, we identified two genes with reduced expression shortly after birth. Proglucagon mRNA was reduced ≈50% in the pancreas of HNF-3α−/−mice and remained low throughout life. Hormones belonging to the same gene family such as secretin, gastric inhibitory peptide, vasoactive intestinal peptide, and cholecystokinin were unchanged. Surprisingly, proglucagon mRNA levels in the gut were indistinguishable in wild-type and HNF-3α−/− mice, suggesting that HNF-3α is not required for expression of proglucagon transcription in intestinal L cells (Fig. 4*A*). Steady-state mRNA levels of neuropeptide Y (NPY) were also reduced by ≈50% shortly after birth, suggesting that HNF-3α is required for transcriptional regulation of this gene. However, in contrast to pancreatic proglucagon mRNA levels, NPY transcription levels normalize after postnatal day 3 (Fig. 4*B*), suggesting that other factors can up-regulate NPY expression in HNF-3α mutant mice.

We previously showed that the expression of genes required for normal metabolism, including apolipoproteins, aldolase B, and L-pyruvate kinase was increased in embryoid bodies derived from ES cells lacking HNF-3α (9). In addition, we found that HNF-3α negatively regulates the expression of the MODY genes HNF-1α and HNF-4α in embryoid bodies. To test whether this is true in liver, gut, and pancreas of HNF-3α-null mice, we measured steady-state mRNA levels of these genes. In contrast to our results in embryoid bodies, regulation of these genes was not affected in HNF-3α−/− mice (data not shown). However, steady-state mRNA levels of three genes, not previously analyzed, were found to be increased in HNF-3α-null mice after postnatal day 3. Hexokinase I and insulin growth factor binding protein-1 were significantly increased in 5-day-old mutant animals, and phosphofructo-2-kinase/fructose-2,6-bisphosphatase was increased 2-fold in 7-day-old HNF-3α null-mice compared with their wild-type littermates (Fig. 4*B*).

DISCUSSION

We have used a genetic approach to study the function of HNF-3α by generating mutant mice with a loss-of-function mutation in the HNF-3α gene. Mice that lack HNF-3α expression develop neonatal persistent hypoglycemia. Persistent low blood-glucose levels can be caused by conditions of extreme malabsorption, underproduction of glucose (liver-enzyme defects of glucose metabolism, hormone deficiencies, and acquired liver abnormalities), and overutilization of glucose because of hyperinsulinemia. Reduced glucagon levels in HNF-3α -null mice are likely to cause hypoglycemia. However, other mechanisms may contribute to the wasting syndrome, because parenteral glucose and glucagon administration twice daily did not lead to weight gain or prolonged survival in HNF-3α-null mice. One of the most dramatic phenotypes in HNF-3α−/− mice is an altered feeding behavior, as evidenced by reduced milk intake that is followed by malnutrition. The

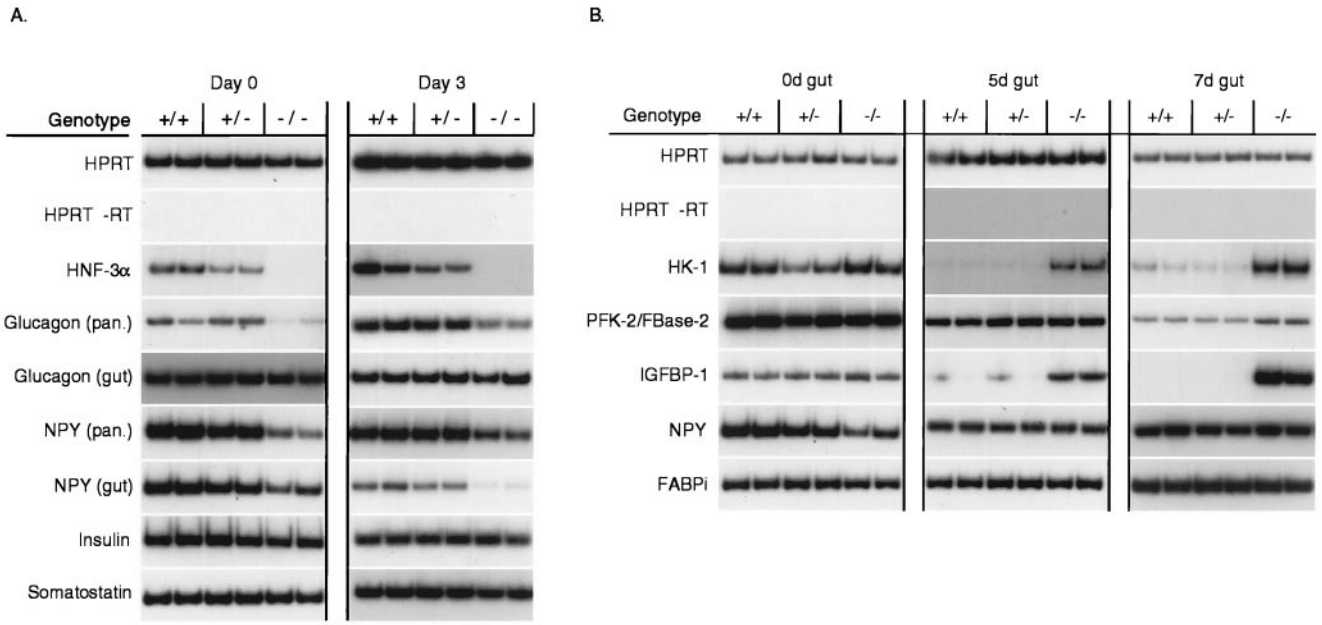

FIG. 4. Steady-state mRNA levels of HNF-3 target genes. Steady-state mRNAs were measured by reverse transcription–PCR by using [α-^{32}P]dCTP (16). PCR products were separated by PAGE, and bands were visualized by autoradiography. (*A*) Steady-state mRNA levles of genes that are reduced shortly after birth. (*B*) Steady state mRNA levels of hexokinase I, bifunctional enzyme phosphofructo-2-kinase/fructose-2,6-bisphosphatase-2 (PFK-2/FBase-2), and insulin growth factor binding protein-1 (IGFBP-1) are up-regulated in HNF-3α-null mice.

molecular and physiological mechanisms by which HNF-3α regulates food intake and participates in glucose sensing are currently not understood. The hypothalamus plays a major role in normal control of appetite behavior, sensing hypoglycemia and mediating counter-regulatory responses (19, 20). We have found HNF-3α to be highly expressed in the hypothalamus (unpublished results), and we cannot rule out that the defect in feeding behavior is caused by a central nervous system defect. We have also tested the expression of hypothalamic modulators of food intake such as galanin, orexin, peptide YY, cholecystokinin, and proopiomelanocortin and found the mRNA levels to be indistinguishable in mutant and wild-type HNF-3α newborn mice. Interestingly, expression of the orexigenic peptide NPY was normal in the hypothalamus but was significantly reduced in the pancreas and gut of newborn and 3-day-old HNF-3α-null mice. NPY mRNA levels increased after day 3 to normal levels, suggesting that elevated serum glucocorticoid levels and hypoinsulinemia, both known to increase NPY gene expression in the rat, can compensate for the transcriptional defect in NPY expression (21).

Hypoglycemia inhibits insulin secretion and usually stimulates rapid increases in the secretion of several hormones, including glucagon, growth hormone, cortisol, and catecholamines, that act in concert to increase plasma glucose concentrations (22). HNF-3$\alpha$$-/-$ mice fail to respond to low blood glucose by increasing glucagon secretion but show normal counter-regulation of hypoglycemia by elevated cortisol and growth hormone levels and hypoinsulinemia. The glucagon gene is expressed as a prohormone in alpha cells of the endocrine pancreas, L cells of the intestine, and in the brain (23). Proglucagon is processed differentially to generate glucagon and glucagon-like peptide-1, which are known to be the main biologically active hormones of the pancreas and intestine, respectively (24). Low serum glucagon levels in HNF-3$\alpha$$-/-$ mice most likely reflect a defect in transcriptional activation of the proglucagon gene in alpha cells, because mRNA levels are reduced shortly after birth. Our results are also consistent with previous studies that have identified an essential HNF-3-binding site in the G2 element of the glucagon promoter (25, 26) and a recent study by Kaestner *et al.* (10) that identified HNF-3α as an essential activator of proglucagon expression *in vitro* and *in vivo*. Surprisingly, we did not find decreased mRNA levels in the intestine of HNF-3$\alpha$$-/-$ mice, indicating that HNF-3α is not required for transcriptional activation of proglucagon in intestinal L cells. Islets of HNF-3$\alpha$$-/-$ mice are morphologically indistinguishable from wild-type mice and show normal size, architecture, and positive immunostaining for glucagon and insulin, providing further evidence that a gene-expression defect rather than an islet developmental abnormality accounts for the low glucagon levels.

HNF-3α is also expressed at high levels in insulin-secreting pancreatic beta cells (10). To study pancreatic beta-cell function in HNF-3α-null mice, we performed glucose tolerance tests by intraperitoneal glucose administration. HNF-3$\alpha$$-/-$ mice have markedly elevated glucose levels after 1 hour and significantly reduced insulin levels compared with wild-type littermate control mice. These results indicated that there may be an insulin secretory defect in pancreatic beta cells of HNF-3α mutant mice that is only revealed after a glucose challenge in the hypoglycemic HNF-3$\alpha$$-/-$ mice. Alternatively, impaired beta-cell function could be caused by an indirect mechanism, because continuous exposure of pancreatic islets to low glucose can impair insulin-secretory responses (27). To address this, we challenged adult wild-type and heterozygous HNF-3α littermates that have indistinguishable fasting blood-glucose levels with an intraperitoneal glucose-tolerance test. Blood glucose concentrations were significantly increased, whereas serum insulin levels were reduced in HNF-3$\alpha$$+/-$animals compared with wild-type mice after a glucose challenge, providing further evidence that HNF-3α is also important for normal pancreatic beta-cell function.

We have identified three genes, hexokinase-1, phosphofructo-2-kinase/fructose-2,6-bisphosphatase, and insulin growth factor binding protein-1, that are up-regulated in HNF-3α mutant mice, suggesting an inhibitory role of HNF-3α on transcription. Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-2 and insulin growth factor binding protein-1 have been shown to contain HNF-3 DNA-binding consensus sequences in their promoter/enhancer sequences that are functional elements in *in vitro* transcriptional activation assays (13). However, we only detect changes in steady-state mRNA

levels of these genes postnatally after day 5, indicating that alterations in gene expression could be adaptive mechanisms for maximal absorption of glucose by maintaining an optimal glucose gradient across the intestinal epithelium.

We did not detect alterations in gene expression in the majority of HNF-3 target genes, including aldolase-B, L-pyruvate kinase, apolipoproteins, and other secreted liver serum factors that we have previously shown to be regulated by HNF-3α and HNF-3β in visceral endoderm of embryoid bodies (9). We believe that transcription of these genes is predominately regulated by HNF-4α and HNF-1α, which are targets of HNF-3α in embryoid bodies. However, in HNF-3α null mice, no differences in HNF-4α and HNF-1α mRNA expression is observed (data not shown). Therefore, targets of HNF-4α/HNF-1α are not affected. It is therefore possible that HNF-3β and HNF-3γ, which are still present in the HNF-3α-null mice, can compensate for the loss of HNF-3α without gross alterations in gene expression of most HNF-3 target genes. This hypothesis can now be tested by studying gene expression in mice in which different allelic combinations of the HNF-3 genes (HNF-3α, -3β, -3γ) are mutated.

Mutations in hepatocyte nuclear factors-1α, -1β, and -4α genes have been associated with pancreatic beta-cell defects in several forms of autosomal-dominant inherited type 2 diabetes (MODY3, 5, and 1, respectively). In this study, we have shown that HNF-3α is essential for normal pancreatic alpha-cell function. No mutations have been described in the HNF-3α gene in humans; however, HNF-3α mice have remarkable similar phenotypes to several reports of patients with neonatal persistent hypoglycemia and glucagon deficiency and should be considered a candidate gene for human congenital glucagon deficiency syndromes (28, 29).

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