# Mouse Models of Insulin Resistance

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tion to insulin resistance results from genetic and environmental factors. The search for gene variants that predispose to insulin resistance has been thwarted by its genetically heterogeneous pathogenesis. However, using techniques of targeted mutagenesis and transgenesis in rodents, investigators have developed mouse models to test critical hypotheses on the pathogenesis of insulin resistance. Moreover, experimental crosses among mutant mice have shed light onto the polygenic nature of the interactions underlying this complex metabolic condition.

I. INTRODUCTION

Type 2 diabetes results from a combination of insulin resistance and impaired insulin secretion (203). The two abnormalities are intertwined, but in most individuals the onset of insulin resistance precedes overt β-cell dysfunction and is thought to arise from genetic predisposition. Nonetheless, subclinical β-cell dysfunction can be demonstrated in nonobese first-degree relatives of diabetic patients, consistent with the hypothesis that β-cell failure is also genetically programmed (186). The predisposition to insulin resistance and/or β-cell dysfunction is inherited in a non-Mendelian fashion (22), due to their genetic heterogeneity and multigenic pathogenesis. Moreover, environmental factors such as inappropriate diet and inactivity can modulate the manifestations of the genotype (incomplete penetrance) or mimic the genetic contribution, resulting in phenocopies (139). Thus human genetic studies of the predisposition to insulin resistance are fraught with uncertainties and complicated by the considerable variations of the human genetic pool. To dissect the complex genetics of insulin resistance and β-cell dysfunction, investigators have generated transgenic and knockout mice bearing mutations in genes required for insulin action and/or insulin secretion (Table 1). This review focuses on lessons learned from these mouse models.

II. OVERVIEW OF INSULIN ACTION

Insulin acts through a cell surface receptor that belongs to a subfamily of growth factor receptor tyrosine kinases. This subfamily is comprised of three members: insulin receptor (Insr), type 1 insulin-like growth factor (IGF) receptor (Igf1r), and Insr-related receptor (Irr) (172, 209, 232, 233). Interestingly, although these receptors are highly conserved from a structural standpoint, their functions are quite distinct, with Insr being primarily important for fuel metabolism and Igf1r for growth. No clear-cut function has been ascribed thus far to Irr, in part because it is unable to bind any of the ligands that are known to activate the other two receptors: insulin, Igf1, and Igf2 (172). The three receptors also share common intracellular signaling pathways. There are two main pathways that
propagate the signal generated through insulin and Igf1 receptors: the insulin receptor substrate (Irs) → phosphatidylinositol 3-kinase (PI3K) pathway (39), and the Ras → mitogen-activated protein (MAP) kinase pathway (111) (Fig. 1). The Irs → PI3K pathway leads to the activation of a cascade of PI3K-dependent kinases, such as Pdk1 (7). Pdk1, in turn, phosphorylates and activates additional serine/threonine kinases, the most prominent of which are the three Akt isoforms (37). Akt phosphorylates glycogen synthase kinase 3 (Gsk3) (47), cGMP-inhibitable phosphodiesterase 3b (121), and Foxo transcription factors (26, 33, 170), leading to stimulation of glycogen synthesis and inhibition of lipolysis and gene expression, respectively. There is also evidence linking Akt activation to stimulation of glucose transport (125, 126). In contrast, activation of protein kinase C (PKC) in response to insulin remains controversial. Interest in the role of this family of kinases has been rekindled by the observations that dominant negative inhibitors of its atypical isoforms results in partial inhibition of glucose uptake in adipocytes (130) and that increased PKC activity is associated with increased intramyocellular triglycerides, a hallmark of insulin resistance (249). Insulin also activates the Ras → MAP kinase pathway through the formation of complexes between the exchange factor Sos and Grb2 (212). Grb2 can be activated by Irs or Shc, two direct substrates of the Insr kinase. The emerging consensus is that the acute metabolic effects of insulin require activation of the Irs → PI3K pathway, whereas the Ras → MAP kinase pathway plays a role in certain tissues to stimulate the actions of insulin on growth and proliferation. This is an oversimplification. For example, whereas inhibition of PI3K can effectively blunt insulin action on glucose transport (210), agents that mimic insulin’s effect to activate PI3K are unable to promote glucose transport, suggesting that PI3K may be necessary, but not sufficient, to promote glucose transporter translocation (98). Based on these observations, a new cascade emanating from Insr has been identified that leads to insulin stimulation of glucose transport in a PI3K-independent fashion. This pathway is based on the activation of the protooncogene c-Cbl (19, 41). There is evidence that this or a related pathway may lead to glucose transporter recycling via remodeling of cortical actin filaments within the cell (229), potentially involving atypical myosin isoforms (32, 99).

III. INSULIN RECEPTOR KNOCKOUT

The generation of mice bearing null Insr mutations has been instrumental to dissecting the pathogenesis of insulin resistance, diabetes, and growth retardation (4, 101). Mice lacking insulin receptors are born at term with slight growth retardation (>10%) (156). After birth, there is a progressive increase in glucose levels, accompanied by a transient, robust increase in insulin levels up to 1,000-fold above normal. However, β-cell “failure” occurs within a few days, characterized by extensive degranulation of β-cells (172) and followed by death of the animals in diabetic ketoacidosis. This phenotype indicates that Insr is necessary for postnatal fuel homeostasis, but not for prenatal growth and metabolic control.

The Insr null mouse is remarkably different from humans carrying similar mutations (102, 131, 191, 222, 238). Unlike mice, humans lacking insulin receptors show severe intrauterine growth retardation, failure to thrive,
lipodystrophy, and hypoglycemia (93, 102, 103, 131, 191, 220, 223, 238). We examine the mechanism of these differences in the next three sections.

A. Insulin Receptor’s Growth Effects

Although the primary focus of this review is insulin resistance, the known correlation between birth weight and predisposition to diabetes indicates a complex interaction between metabolism and growth (48). The lack of significant growth retardation in Insr-deficient mice appears to be due to differences in developmental timing between humans and rodents, rather than to the absence of growth-promoting effects of insulin (156).

Evidence for a role of Insr in promoting embryonic growth derives from studies of mice with combined mutations in various elements of the insulin/Igf system. These experiments indicate that Insr is the receptor that mediates Igf2’s effects on embryonic growth. Thus single mutations ablating Insr function result in embryos that are 90% of normal weight, while single Igf1r mutations result in small embryos (45% of normal). Combined ablation of Insr and Igf1r, however, results in smaller embryos than either mutation alone (30% of normal), suggesting that the two receptors have partially overlapping growth-promoting functions. The same extent of growth retardation (30% of normal weight) is associated with combined mutations of Igf1 and Igf2. Combined mutations inactivating Igf2 and Igf1r also result in 30% embryos, suggesting that Igf2 signals through both Igf1r and Insr. In contrast, double mutants of Igf1 and Igf2r have the same phenotype as single mutants of either gene, indicating that their functions are entirely overlapping in promoting embryonic growth. This genetic evidence indicates that Insr acts as an Igf2 receptor during embryogenesis. Biochemical evidence supports this conclusion. Insr is expressed as two alternatively spliced isoforms, which differ by the presence or absence of a 12-amino acid peptide at the carboxy terminus of the extracellular α-subunit, encoded by exon 11. It has been shown that the exon 11-containing (A) isoform is enriched in embryonic tissues and that it binds Igf2 with high affinity (68).

If Insr is indeed a growth-promoting receptor, why are mice lacking Insr considerably less growth retarded than humans lacking Insr? The clue lies in the differing developmental patterns of humans and rodents. Mice are born at an earlier developmental stage than humans. This is reflected, among other features, in differences in body composition. Lipid content at birth represents 16% of total body weight in humans, but only 2% in rodents (240). Adipose tissue development is very sensitive to insulin, as demonstrated by the excessive adiposity of fetuses exposed to hyperinsulinemia of maternal (183, 231) or fetal origin (51), erythroblastosis fetalis (18, 88), and persistent hyperinsulinemic hypoglycemia of infancy (194). Indeed, while it is difficult to apportion the growth effects of insulin due to nutrition versus cell proliferation, the observations that fetuses exposed to hyperinsulinemia primarily increase adipose mass are consistent with the notion that the latter are predominant. Because the acquisition of adipose mass occurs postweaning in rodents, growth retardation in Ins1/Ins2- or Insr-deficient mice is not as severe as in children with leprechaunism. We have confirmed this interpretation using mosaic analysis to introduce partial ablations of Insr function in mice. This was accomplished by conditional mutagenesis, using mice in which floxed Insr alleles have been deleted to various degrees using Cre recombinase driven by the heat shock protein 70 gene promoter. These experiments indicate that, when >80% of somatic cells lose Insr expression, mice develop a phenotype similar to human leprechaunism, with severe postnatal growth retardation and hypoglycemia (122). The growth retardation appears to be due to a >50-fold increase in expression of Igfbp1, a known modulator of Igf bioavailability (44, 71, 72). These data are consistent with two important conclusions: 1) that insulin acting through Insr promotes growth and 2) that it does so by inhibiting hepatic expression of Igfbp1. It should be noted that, postnatally, the growth-promoting actions of Insr are mediated by insulin in rodents, because Igf2 expression virtually ceases after birth (52, 159). In contrast, Igf2 secretion persists throughout life in humans (85), providing an additional element of diversity between the two species (172).

B. Metabolic Effects

The development of diabetes in Ins or Insr null mice at an early postnatal stage suggests that an insulin-dependent fuel-sensing apparatus develops perinatally in rodents. This is an additional developmental difference with humans, in whom insulin responsiveness is established during the last trimester of gestation. Similar to Ins1/Ins2 and Insr knockouts, mutations in other key genes required for glucose metabolism give rise to early neonatal diabetes, for example, glucokinase (80, 188, 225), Glut2 (83), and Pepck (207), as well as genes encoding transcription factors required for insulin gene transcription and/or pancreatic β-cell development (3).

However, the most important and seemingly paradoxical difference between Insr-deficient mice and humans is that mice are steadily hyperglycemic, whereas humans develop postprandial hyperglycemia and fasting hypoglycemia. There is no direct demonstration of a mechanism that would explain this difference. However, some physiological considerations and a new mouse model can help us formulate an educated guess. In humans with INSR mutations, hypoglycemia develops dur-
Insulin secretion deteriorates with age (155, 195). Muscle-specific Igf1r in this process in more detail in the section on the muscle-specific Insr knockout.

The different compensatory abilities of human and murine β-cells result from developmental differences between the two species. Insulin secretion in rodents begins at E15.5, and islets do not become organized until E18.5 (89, 163, 248). Insulin secretion rises about threefold between E18.5 and birth (77, 109, 193). In rodents, the early postnatal period is a time of intense β-cell proliferation, and an appropriate β-cell mass is generally achieved by 3–4 wk postnatally (65). In contrast, INS transcripts appear comparatively earlier (8 wk of gestation) in human embryos (30, 185). β-Cell clusters can be observed at 12–16 wk (150, 215) and become organized into functioning islets by 25 wk, after which plasma insulin concentrations increase steadily (58). We have shown that pancreatic β-cells are morphologically abnormal in Insr knockouts by postnatal day 4, with depleted insulin storage granules and abnormal mitochondria (172). Therefore, mice simply lack sufficient β-cell reserve to compensate for insulin resistance. Newborn humans have a greater β-cell reserve, which may contribute to hypoglycemia. This explanation is consistent with the clinical observation that hypoglycemia subsides and is gradually replaced by hyperglycemia in children with INSR mutations as insulin secretion deteriorates with age (155, 195).

There are also species-specific differences in the role of different tissues in metabolic control, which can partly account for the different presentation of the human and murine phenotypes. Newborn rodents are mostly dependent on liver for fuel metabolism (76), whereas humans rely on muscle and adipose tissue, in addition to the liver. Thus, if the extreme hyperinsulinemia leads to activation of muscle Igf1r and promotes glucose uptake, there is greater potential to cause hypoglycemia in humans than in mice, since muscle glucose disposal is quantitatively more important in humans. This speculation is borne out by studies in which Igf1 treatment of mice lacking Insr results in a rapid decrease of glucose levels, suggesting that Igf1 can indeed stimulate muscle glucose uptake through its receptor. However, this decrease is not sufficient to rescue mice from death (56), presumably because the liver does not possess Igf1 receptors, and thus Igf1 does not rescue hepatic insulin action (114, 181, 199).

Finally, evidence from mice with mosaic-patterned Insr ablation is also consistent with the view that the difference between hypoglycemia and hyperglycemia may hinge upon muscle glucose utilization. Using the cre/loxP system, we have generated mice with variable degrees of mosaicism for Insr null alleles. In these mice, each tissue is a mixture of Insr-expressing and Insr-knockout cells. Mice in which 80% of cells are devoid of Insr (referred to as Δ80 mice) develop hypoglycemia. In contrast, mice in which 98% of cells are devoid of Insr (Δ98) develop hyperglycemia. In liver, both groups of mice are equally insulin resistant. Hepatic insulin resistance causes inability to synthesize and store glycogen, which explains the fasting hypoglycemia in Δ80 mice. In contrast to liver, muscle insulin sensitivity is normal in Δ80, but reduced in Δ98 mice. Sensitivity to insulin in muscle would be expected to exacerbate hypoglycemia in Δ80 mice, because glucose is cleared from the blood and taken up by skeletal muscle. In contrast, resistance to insulin action in muscle of Δ98 mice results in impaired glucose uptake and hyperglycemia. In summary, the development of hypoglycemia appears to depend on the preservation of muscle insulin sensitivity in the face of hepatic insulin resistance.

### IV. CONDITIONAL MUTAGENESIS OF INSULIN RECEPTOR

The lethal phenotype of Insr knockouts precludes a detailed analysis of insulin receptor function in different tissues in adult mice. Since an impairment of insulin action in one or more insulin target cells is a likely cause of diabetes, several mouse models have been developed to circumvent this limitation (Table 2). These experi-

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<th>Insr knockout</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
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<tr>
<td>Constitutive</td>
<td>Diabetic ketoacidosis</td>
<td>4, 101</td>
</tr>
<tr>
<td>Muscle</td>
<td>Dyslipidemia</td>
<td>35</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>Reduced heart size and performance</td>
<td>21</td>
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<tr>
<td>Muscle/adipose tissue</td>
<td>Impaired glucose tolerance</td>
<td>140</td>
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<tr>
<td>Adipocyte</td>
<td>Protection against obesity, longevity, β-Cell failure</td>
<td>28, 29, 82</td>
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<tr>
<td>Brown adipose tissue</td>
<td></td>
<td></td>
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<tr>
<td>Liver</td>
<td>Moderate insulin resistance, transient hyperglycemia</td>
<td>164</td>
</tr>
<tr>
<td>β-Cell</td>
<td>Impaired glucose tolerance</td>
<td>134</td>
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<tr>
<td>Vascular endothelium</td>
<td>Protection from hypoxia-induced neovascularization</td>
<td>237</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Obesity, subfertility</td>
<td>34</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>Growth retardation, lipoatrophy, hypoglycemia</td>
<td>122</td>
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ments exploit a binary system, in which a mutation of \( \text{Insr} \) is generated in a restricted pattern by breeding Cre-producing and Cre-responding mice. The system has been reviewed elsewhere (136). It bears emphasizing that, although these experiments are described for simplicity as “tissue-specific” knockouts, they should be more properly defined as “Cre promoter-specific.” In other words, the pattern and extent of tissue recombination is dependent on the specificity of the promoter used to drive Cre expression, which is rarely, if ever, limited to a single tissue or cell type and, even within a given cell type, may be limited to a specific developmental stage.

A. Skeletal Muscle

In humans, skeletal muscle is the primary site of insulin-dependent glucose disposal (64). Resistance of skeletal muscle to insulin-dependent glucose uptake and phosphorylation is an early step in the development of type 2 diabetes (45). To examine the role of insulin receptors in muscle metabolism, and how the latter affects the development of diabetes, we inactivated \( \text{Insr} \) in this tissue using MCK-Cre transgenics to excise \( \text{Insr} \) exon 4, leading to a null allele (skeletal muscle insulin receptor knockout, MIRKO). Conditional \( \text{Insr} \) knockout in skeletal muscle leads to impaired insulin signaling and decreased insulin-dependent glucose transport, without systemic insulin resistance (35). This is similar to the phenotype of mice carrying a dominant negative \( \text{Insr} \) transgene in muscle and a heterozygous systemic \( \text{Insr} \) knockout. These mice fail to develop diabetes, despite a >90% decrease in insulin receptor kinase activity and a sizable decrease in insulin-dependent glucose uptake in muscle (140).

These observations, reported by different laboratories using independent experimental approaches, are unexpected. Whereas it is generally accepted that insulin resistance per se, in muscle or elsewhere, is not sufficient to cause overt diabetes, the expectation was that an impairment of insulin signaling in muscle would lead to systemic insulin resistance. Although this was not the case, MIRKO mice develop a metabolic syndrome with increased fat stores and hypertriglyceridemia. Several recent models of targeted gene knockouts have helped us to clarify this apparent conundrum. First, it appears that there are at least three different pathways regulating GLUT4 transport in adipose tissue (116). This suggests that changes in glucose metabolism in adipose tissue may in part compensate for the muscle-specific insulin resistance in MIRKO mice. This phenomenon also explains the finding of more severe insulin resistance with disruption of insulin signaling in both muscle and adipose tissue (140). MIRKO mice do not demonstrate differences in the expression of signaling proteins such as Irs1 or p85\( \alpha \), suggesting that upregulation of these genes is not important in the compensatory mechanism (35). Likewise, muscle Glut4 levels in MIRKO mice are not altered, indicating alternate regulatory pathways leading to translocation of Glut4 transporters.

A potential compensatory pathway is that mediated by Igf1r (Fig. 2). Although changes in Igf1r expression or insulin-stimulated Igf1r phosphorylation are not noted in this case, transgenic mice with dominant negative mutations of Igf1r targeted to skeletal muscle do indeed develop diabetes, insulin resistance, and \( \beta \)-cell dysfunction. The dominant negative Igf1r transgene inhibits both Igf1 and insulin receptors by forming homo- and heterodimers (“hybrid receptors”) with endogenous receptors and thereby preventing their autophosphorylation (63).

The notion that inactivation of \( \text{Insr} \) in muscle may not suffice to impair glucose metabolism is further supported by results from muscle-specific inactivation of the Glut4 (254). The reduction in insulin- and contraction-stimulated glucose transport seen in these mice is accompanied by early development of insulin resistance and glucose intolerance. Surprisingly, these mice also demonstrate

![Diagram of glucose transport and signaling pathways](image-url)
impaired insulin-stimulated glucose uptake in adipose tissue and suppression of gluconeogenesis in the liver. Phloretin treatment leads to a restoration of insulin sensitivity in adipocytes and liver, indicating that these defects are probably secondary to hyperglycemia or glucose toxicity (117).

Therefore, although MIRKO mice do not demonstrate significant insulin resistance or diabetes, the phenotypes of mice expressing dominant negative Igf1r and muscle-specific knockout of Glut4 confirm the critical role of muscle tissue in glucose metabolism. Various compensatory mechanisms, including increased glucose transport by adipose tissue, suppression of gluconeogenesis in the liver, and activation of Igf1r are able to prevent the development of insulin resistance and diabetes. These studies also indicate that insulin receptor signaling is only one of the pathways leading to Glut4 translocation and glucose uptake in skeletal muscle.

B. Heart

Defects in cardiac metabolism are among the most common comorbidities in type 2 diabetes, but the role of insulin resistance in their pathogenesis, as opposed to hyperglycemia and dyslipidemia, is unclear. The question has been addressed by generating mice in which Insr knockout is restricted to the heart by way of the cardiac-specific a-myosin heavy chain promoter (CIRKO). Ablation of insulin signaling in cardiomyocytes resulted in smaller hearts, due to reduced cardiomyocyte size. Interestingly, glucose uptake and glycolysis were increased when measured in perfused hearts, probably due to a compensatory increase in Glut4 expression. In contrast, the heart’s ability to oxidize fatty acids was decreased, and cardiac performance was moderately impaired (21, 211). Tellingly, the CIRKO hearts are also more prone to injury and failure when subjected to pressure overload (96). These findings indicate that insulin resistance may affect cardiac performance independently of changes in lipid and glucose levels.

C. Adipose Tissue

Adipose tissue accounts for a small percentage of total glucose uptake, 20% of an oral glucose load in humans (78, 160) and 3–5% of glucose uptake during euglycemic hyperinsulinemic clamps in rats (198). Nevertheless, an early component of insulin resistance in type 2 diabetes involves increased lipolysis and decreased glucose transport in the adipocyte. Furthermore, insulin has been implicated as an important player in the maintenance of key adipocyte functions, including adipogenesis, stimulation of glucose uptake as well as lipid synthesis, and inhibition of lipolysis (197). To further identify the specific role of insulin signaling in adipocyte function and its contribution to glucose metabolism, mice with Insr knockout limited to white and brown fat tissues (FIRKO) have been generated using a Cre transgene driven by the adipose-specific aP2 promoter (29).

FIRKO mice demonstrate significant defects in insulin-mediated glucose uptake and in the ability of insulin to inhibit isoproterenol-stimulated lipolysis. Although basal glucose uptake remains intact, there is evidence of increased basal lipolysis. These findings are associated with decreased fat mass and whole body triglyceride content. This difference is not secondary to a decrease in adipocyte number, but rather corresponds to a polarization of adipocyte size into large and small cells. Leptin levels in these mice are comparable to the wild-type strain, indicating an unexpectedly high level for the apparent fat mass. To assess the effects of hyperphagia on adipocyte development in these mice, gold thioglucose (GTG) treatment was utilized to introduce specific lesions in the ventromedial hypothalamus. Interestingly, despite increased food intake, FIRKO mice do not demonstrate the expected increase in adipose tissue mass. Moreover, intraperitoneal glucose tolerance tests indicate that hyperphagic FIRKO mice fail to develop insulin resistance or abnormal glucose tolerance.

In summary, it appears that insulin signaling in adipose tissue is not critical for the maintenance of euglycemia in mice but is required for the development and maintenance of normal triglyceride stores, inhibition of lipolysis, and insulin-stimulated glucose uptake.

As seen in muscle, selective knockout of Glut4 in adipose tissue has a more profound effect, indicating a major difference in the whole body response to insulin resistance at different steps of the insulin action cascade (2). A further interesting phenotype noted in the FIRKO mice is a 20% increase in mean life span, with parallel increases in median and maximum life spans. These data support the notion that a decreased fat mass can affect life span independently of caloric restriction (29). These findings are of interest in the context of the association between longevity and mutations in the insulin/IGF signaling pathway in Caenorhabditis elegans (81, 108, 118, 146, 228).

D. Brown Adipocyte

Brown adipose tissue is thought to play an important role in determining peripheral insulin sensitivity (157), as well as thermal adaptation. Insr has been inactivated selectively in this tissue (BATTIRKO) using the uncoupling protein-1 (UCP1) promoter to drive Cre expression. Although brown adipose tissue develops normally in these mice, it hypotrophies gradually with age, with a 50% reduction at 3 mo, and ~75% by 6–12 mo. Interestingly, the
age-dependent loss of brown adipose tissue is associated with deterioration of β-cell function and decrease of β-cell mass, giving rise to hyperglycemia (82). This observation suggests that the maintenance of an adequate β-cell mass requires brown adipose tissue. It remains to be determined whether this is an endocrine effect of factors produced in brown adipose tissue, or whether it reflects a broader metabolic change. It is also unclear why this phenotype is not observed in FIRKO mice, which carry Insr mutations in both brown and white adipocytes.

E. Hepatocytes

The liver plays a central role in the control of glucose homeostasis and is subject to complex regulation by substrates, insulin, and other hormones. The liver appears to be even more important in insulin sensitivity in rodents, as indicated by the fact that, as long as hepatic insulin action is preserved, mice are protected from diabetes (140). The physiological basis of this observation remains unclear but can potentially relate to species-specific differences in glycogen storage. In fact, rodents store a larger proportion of total body glycogen in liver, compared with humans (95). Insulin resistance in the liver, in particular the loss of its ability to suppress hepatic glucose output, is closely correlated with fasting hyperglycemia in type 2 diabetes. Insulin affects hepatic glucose suppression initially by inhibiting glycolysis. With prolonged fasting, however, insulin reverts to the alternate pathway of inhibiting gluconeogenesis. There is considerable debate as to whether this latter phenomenon is secondary to a direct effect of insulin on the liver or rather an indirect effect mediated by insulin’s action on muscle and fat to decrease the supply of gluconeogenic precursors (40). In addition to their effect on suppression of glucose production, the hepatic insulin receptors are believed to play an important role in the degradation and clearance of insulin by the liver. Thus hepatic insulin resistance may in part account for the hyperinsulinemic state found in type 2 diabetes.

With the use of the Cre-loxP system, with an albumin promoter to mediate liver specific Cre expression (187), it has been possible to investigate the direct effects of loss of insulin receptor function in the liver. By 2 mo age, liver-specific Insr knockout (LIRKO) mice exhibit dramatic elevations in blood glucose levels. Intraperitoneal glucose tolerance tests confirm severe glucose intolerance. In addition, they develop marked hyperinsulinemia due to a combination of increased β-cell mass and decreased insulin clearance, as well as failure of insulin to suppress hepatic gluconeogenesis (164). Euglycemic, hyperinsulinemic clamp studies reveal that this loss of regulation of gluconeogenesis is due to the direct effects of insulin on the liver, rather than the indirect pathway of action (66), suggesting that hepatocyte insulin signaling is required for both direct and indirect control of hepatic glucose production.

As LIRKO mice age, there is a concomitant improvement in the metabolic phenotype. This is not secondary to an amelioration of hepatic insulin resistance, but it appears to be due to functional and morphological changes in the liver itself, mediated by the absence of hepatic insulin receptors (164). Thus insulin receptors in hepatocytes are critical in regulating glucose homeostasis, facilitating insulin clearance, and maintaining normal hepatic function.

F. Vascular Endothelium

Vascular endothelium is a target of Insr signaling, and several physiological functions have been ascribed to Insr in this cell type (16). However, these functions are sometimes at odds. For example, insulin has been shown to promote nitric oxide (NO)-mediated relaxation of smooth muscle cells and to protect these cells from apoptosis. However, it has also been shown to act as a potent stimulator of vascular epidermal growth factor (VEGF) and endothelin expression, which would be expected to cause vasoconstriction and potentially lead to hypertension. Moreover, it has been suggested that Insr in vascular endothelium may contribute to delivery of insulin to target cells through a transcytosis process (119, 216). Finally, there is evidence that insulin is a vasodilator in vivo (17, 31) and that some of its effects on glucose uptake may be secondary to increased tissue blood flow (247). Mice lacking Insr in vascular endothelium display slight reductions of arterial blood pressure under both low- and high-salt diets and are mildly insulin resistant. Expression of endothelial NO synthase (eNOS) and endothelin-1 are reduced by ~30–60% in endothelial cells, without changes in Vegf expression (237). The failure of these mice to develop hypertension, either basally or in response to a high-salt diet, refutes the notion that the association between insulin resistance and hypertension can be explained by insulin’s action on the vascular endothelium. Similarly, the lack of significant insulin resistance indicates that insulin transport across the endothelial barrier is not limiting for peripheral insulin action. It remains to be seen whether the changes in vasoactive peptide expression predispose to altered vascular reactivity under conditions of increased genetic susceptibility to vascular injury.

Interestingly, when these mice are subjected to chronic hypoxia, they display a decrease in retinal neovascularization, the primary cause of diabetic retinopathy and blindness (128). These data, along with similar, albeit less pronounced data in mice lacking Igf1r in vascular endothelium, suggest that increased activation of insulin/
G. Neurons

Insulin and Igf1 receptors are expressed at high levels in many areas of the brain and in different cell types, including glial and neuronal cells (87). Because neurons metabolize glucose in an insulin-independent manner, the role of Insr in the brain has remained somewhat arcane (205). The question as to the potential function of neuronal Insr has been addressed by generating neuron-specific Insr knockout mice (NIRKO) using nestin/Cre-mediated ablation (34). Loss of Insr in nestin-positive neurons does lead to mild insulin resistance and hypertriglyceridemia. Interestingly, there is also a dramatic increase in food intake in female mice, while increases in adipose tissue depots occur in both male and female mice. This suggests that, in addition to glucose metabolism, Insr is important in brain nutrient sensing and regulation of energy expenditure. A further, unexpected result in NIRKO mice is impaired male and female fertility. Initial assessment indicates hypogonadotrophic hypogonadism in relation to defective spermatogenesis and follicular maturation. Further studies have indicated normal pituitary luteinizing hormone (LH) content and response to exogenous gonadotropin, suggesting a hypothalamic defect leading ultimately to altered gonadotropin expression. Evaluation of hypothalamic hormones that affect fertility and food intake has revealed increased proopiomelanocortin (POMC) without significant changes in neuropeptide Y (NPY) levels (J. Bruning, personal communication). It is notable that Irs2 knockout mice, in addition to developing diabetes, also demonstrate abnormalities in food intake, increased obesity despite elevated leptin levels and infertility, particularly in the females (38). The reproductive abnormalities are associated not only with fewer ovarian follicles but also decreased pituitary size and gonadotroph numbers. Although these mice represent a generalized lack of Irs2 protein, the phenotypic similarities with NIRKO mice suggest that Irs2 is important in modulating insulin’s role in regulation of hypothalamic functions in energy expenditure, food intake, and fertility.

Studies to define the specific role of hypothalamic Insr signaling have utilized short-term intracerebroventricular infusions of antisense oligonucleotides directed against the Insr in rats. The subsequent downregulation of Insr expression in the medial portion of the arcuate nucleus leads to hyperphagia and increased fat mass, similar to the NIRKO mice (176). Furthermore, insulin clamp studies in these rats have shown ineffective suppression of hepatic gluconeogenesis in the setting of hyperinsulinemia (177). Interestingly, unlike the findings in the NIRKO mice, short-term inhibition of Insr in the arcuate nucleus increases NPY and Agouti-related peptide (AGRP) levels, but does not alter POMC levels. Further analysis of the mechanisms by which hypothalamic Insr may affect insulin-mediated suppression of hepatic gluconeogenesis suggests that ATP-sensitive potassium channels rather than central melanocortin receptors are important in this process.

Although the role of brain Insr in coordination of fuel metabolism, nutrient sensing, and fertility could be considered surprising, it does appear to serve a teleological function by connecting these various functions, thus limiting reproduction in times of food deprivation. These findings corroborate earlier work in C. elegans demonstrating the importance of insulin/Igf signaling in the nervous system on reproduction and life span (10).

H. Pancreatic β-Cell

Although peripheral insulin resistance is one of the hallmarks of type 2 diabetes, development of diabetes requires the onset of β-cell dysfunction. Multiple defects in insulin secretion and β-cell mass have been noted in patients with type 2 diabetes and also during the insulin-resistant prediabetic stage. There appears to be a genetically determined tendency to β-cell dysfunction. However, the particular mechanisms leading to these defects have not yet been completely elucidated (186).

Classically, insulin secretion has been thought to be regulated by the products of glucose metabolism in the β-cell (161). To address the role of insulin signaling in these processes, Insr has been inactivated in mature β-cells using a minimal Insulin2 promoter to drive cre-dependent recombination (βIRKO). These mice demonstrate a selective impairment in the first phase of glucose-stimulated insulin secretion, a phenotype reminiscent of that seen in type 2 diabetes patients (Fig. 3). This defect ultimately leads to age-dependent glucose intolerance and, in some mice, to overt diabetes. These data suggest that the insulin-resistant state in type 2 diabetics may, in part, also be responsible for the defect in insulin secretion that is seen in this disease (134). It is unclear how Insr controls insulin secretion. The pancreatic portal system is designed to provide for insulin control of glucagon secretion, but more work is required to determine the mechanism of Insr regulation of insulin secretion. Nevertheless, the idea that Insr signaling regulates insulin production and exocytosis is teleologically attractive, in that it would provide a unifying mechanism for insulin resistance and impaired β-cell function.
Similar to Insr, Igf1r ablation in β-cells affects insulin secretion and results in fasting hyperinsulinemia with impaired glucose tolerance (Fig. 3). The likeliest cause of these combined abnormalities is that basal insulin secretion is increased, but glucose-stimulated insulin secretion is impaired. Indeed, electron microscopic analysis of Igf1r-deficient β-cells revealed a depletion of insulin storage granules, and an increase in the amount of rough endoplasmic reticulum and Golgi stacks, consistent with a state of constitutively active secretion. These findings are consistent with the known effect of Igf1 to inhibit insulin secretion from β-cells (252) and perfused rat pancreas (145).

Notably, despite the fact that it is expressed at higher levels in β-cells than either insulin or Igf1 receptors (90), the Irr does not appear to be involved in β-cell function. In fact, metabolic analyses and insulin release studies from purified islets of Irr knockouts have failed to demonstrate a role for this receptor in β-cell function (120).

I. What Is the Role of Insulin Signaling in Pancreatic β-Cells?

As noted above, β-cell dysfunction is a sine qua non for development of the diabetes phenotype and may also play a role in the insulin-resistant state. In states of metabolic dysregulation, defects are seen in both insulin secretion and compensatory changes in β-cell mass.

There is substantial evidence for a role of receptor tyrosine kinases in insulin synthesis and release, as well as β-cell proliferation and survival (Fig. 3). Inactivation of Insr (134), Igf1r (135, 245), and Irs1 (137) leads to defective insulin secretion in response to glucose and amino acids. On the other hand, inactivation of Irs2 leads to reduced β-cell mass (242), presumably for lack of neogenesis or increases in apoptosis. Overexpression of Akt in β-cells increases neogenesis and results in increased β-cell mass and size, without affecting insulin secretion (24, 230), while ablation of its substrate p70s6k results in decreased cell size and hypoinsulinemia. In contrast, mutations of the eukaryotic translation initiation factor 2α (eIF2α) (204) and its kinase Perk (86) impair the metabolic stimulus/secrection coupling, presumably by affecting Insulin mRNA translation.

While it is clear that tyrosine kinase signaling is important for β-cell proliferation and survival, evidence that Insr and/or Igf1r are the receptors that promote β-cell growth is, at this point, inconclusive. The main supportive data derive from the Irs2 knockout and from observations that haploinsufficiency for either Igf1r (241) or Insr (123) in the Irs2 null background accelerates β-cell demise and results in rapid-onset diabetes. On the other hand, it is disappointing that neither the Insr nor the Igf1r β-cell specific knockout results in alterations of β-cell mass or proliferation. Derivation of mice bearing a double mutation of both genes should address whether this is a result of compensation by the cognate receptor. A possible interpretation of these data is that Irs2 is activated in β-cells in response to other cues, such as β-cell specific growth factors (67, 73, 235, 236). This explanation would be consistent with the observation that neither Insr nor Igf1r appears to be necessary for embryonic growth of the endocrine pancreas, while they have a major effect on exocrine pancreas development (112). However, as stated above, embryonic development of the endocrine pancreas is rather limited in mice, since β-cell proliferation occurs mostly postnatally (65, 245). Thus it is possible that Insr and Igf1r affect postnatal, but not embryonic, β-cell proliferation/survival.

An alternate explanation, which we tend to favor (3, 123), is that Insr and/or Igf1r are important for β-cell neogenesis through proliferation and terminal differentiation of β-cell precursors. If that were the case, ablation of the two receptors induced by the β-cell-specific insulin

![Growth factor receptors](http://physrev.physiology.org/)

**FIG. 3.** Mutations of Insr and Igf1r signaling in pancreatic β-cells. Insr and Igf1r knockouts in β-cells have been obtained using the insulin promoter to drive cre expression. In both instances, insulin secretion is impaired, albeit the mechanisms appear to differ. The effect of Irs1 knockout on β-cells is similar to that of Igf1r, suggesting that Irs1 lies downstream of Igf1r. Alterations of insulin secretion result also from mutations of PI3K. Although ablation of Irs2 has a profound effect on β-cell proliferation, neither receptor knockout affects this aspect of β-cell physiology. Thus it is possible that Irs2 is activated by additional receptors. A prime candidate was Irr, but studies of Irr knockouts rule out an effect on β-cell proliferation and glucose-induced insulin secretion.
promoter would not affect neogenesis, since this promoter should be inactive in precursor cells. As our understanding of the elusive β-cell precursor increases, it should be possible to address the function of the two receptors in the β-cell differentiation process.

The role of PI3K also presents a complex picture (Fig. 3). Pharmacological inhibition of PI3K signaling by wortmannin is associated with increased insulin release from purified islets (251). Indeed, Eto et al. (61) have shown that islets from mice lacking the PI3Kr subunit have increased insulin secretion, due to elevation in cytosolic calcium levels. This is in contrast to data in Insr, Igf1r, and Irs1 knockouts, in which insulin secretion appears to be blunted and calcium signaling unaffected (14, 134, 135, 137, 147, 148, 245). This discrepancy may reflect different experimental conditions and different measurements performed in each study. However, as pointed out by Eto et al. (61), it is also possible that different steps of the exocytotic process may be affected in opposite ways by defects in PI3K signaling.

V. Igf1r Knockout

Lack of Igf1r impairs growth (mice weigh 45% of normal) but is compatible with fetal development (59). Mice are born with multiple abnormalities, including hypoplastic muscles, delayed bone development, and thin epidermis and die from respiratory failure immediately after birth (153). Compared with the extreme metabolic phenotype due to lack of insulin receptors, it appears that Insr and Igf1r play very different roles. However, there exists some functional overlap. This is more evident in the ability of Insr to substitute for the growth-promoting actions of Igf1r than in the latter's ability to substitute for the metabolic actions of Insr. For example, ablation of both Igf1r and Igf2r results in normal mice (158), presumably due to the ability of insulin receptors to stimulate growth and substitute for the actions of Igf1r. It is important to emphasize that this is possible by virtue of an increase in Igf2 levels caused by the ablation of Igf2r (158). On the other hand, administration of Igf1 to Insr-deficient mice does not result in prolonged survival, although it does decrease glucose levels by increasing muscle glucose uptake (56). Withers et al. (241) have reported an increase in glucose levels and β-cell hypoplasia in Igf1r-deficient mice. However, it is unlikely that the magnitude of the hyperglycemia would suffice to account for the early postnatal death, considering that even insulin-deficient mice survive a few days (57).

With respect to the issue of diverging signaling properties between Insr and Igf1r, in vitro studies with hepatocytes derived from Insr-deficient mice have begun to provide some mechanistic insight into this phenomenon. These experiments suggest that there are intrinsic signaling differences between Insr and Igf1r that make Insr more suited to metabolic signaling than Igf1r (113, 170, 181, 199).

VI. Insulin Resistance Caused by Mutations of Genes Encoding Proteins in the Insulin Signaling Pathway

A. Irs Signaling

Irs proteins mediate insulin, IGF, and cytokine actions (239) (Fig. 1). The Irs family is composed of four closely related members (Irs1 to -4) (142, 143, 218, 219) and a more distantly related homolog, Gab1 (92). It is conceivable that the presence of a unique complement of phosphotyrosines in each molecule would allow for differential signaling. In addition, there are distinctive patterns of tissue expression, such that each Irs protein may play a different role in different tissues (110, 239). The results of gene inactivation experiments are consistent with the view that Irs proteins have different specificities and functions (Fig. 4).

B. Irs1 Knockout

The main consequences of Irs1 inactivation in mice are intrauterine and postnatal growth retardation, associated with mild insulin resistance without diabetes (13, 221). These findings indicate that Irs1 mediates the growth-promoting actions of Igf1r, but plays a secondary role in metabolic signaling. Nonetheless, lack of Irs1 has also been shown to impair insulin secretion from β-cells (137), suggesting that insulin/IGF1 signaling through Irs1 is required for proper β-cell function. Moreover, combined heterozygosity for Insr and Irs1 null alleles causes a severe impairment of insulin action associated with a steep rise in the incidence of diabetes in the resulting progeny (36, 110), suggesting that Irs1 has an important role on insulin action. These experiments are reviewed below.

C. Irs2 Knockout

Mice lacking Irs2 develop diabetes due to a combination of insulin deficiency and impaired insulin action. The disease is lethal in some genetic backgrounds (242) and milder in others (132). The metabolic syndrome is due to combined insulin resistance in peripheral tissues (110, 190) and impaired growth or increased apoptosis of pancreatic β-cells (123, 138). The findings in the Irs2-
deficient mouse recapitulate the natural history of type 2 diabetes and have led to the suggestion that IRS2 mutations may predispose to diabetes in humans, a conclusion that is not borne out by the genetic analyses carried out thus far (8, 20, 23). These data are consistent with an important role of Irs2 in fuel homeostasis, but also underline the conclusion that multiple substrates are required to mediate insulin action, since the phenotype due to lack of insulin receptors is substantially more severe than that due to lack of Irs2.

D. Irs3 Knockout

Irs3 is the most abundant Irs protein in adipocytes (213, 244), but is also found in other tissues (143, 206). Lack of Irs3 has no apparent effect on mouse development and metabolism, or on insulin-dependent glucose uptake in isolated adipose cells (154). However, this may be due to compensation by Irs1 in adipose cells, as described below. It should be noted that humans lack IRS3 (27).

E. Irs4 Knockout

Ablation of Irs4 results in a mild phenotype with reduced growth, impaired glucose tolerance, and reproductive abnormalities (62). This phenotype is reminiscent of the Irs1 knockout phenotype, albeit considerably milder.

VII. DOUBLE Irs GENE KNOCKOUTS

A. Irs1 and Irs2

Mice lacking both Irs1 and Irs2 die before implantation on the C57BL × 129SV background, resulting in one of the most dramatic embryonic lethal phenotypes observed in mice with targeted gene mutations (241). It is important to note that this phenotype is substantially more severe than the phenotype due to combined lack of Insr and Igf1r, suggesting that Irs proteins play additional roles to mediate the actions of other receptors, as predicted by studies of cytokine receptor signaling (239). On the other hand, the metabolic phenotype of Irs1⁻⁻/⁻ Irs2⁻⁻/⁻ mice, bearing the minimum number of Irs1 and Irs2 alleles compatible with live birth, is significantly milder than the Insr knockout phenotype, suggesting that the metabolic actions of insulin may require more than Irs1 and Irs2 signaling (241). However, it should also be noted that double-mutant embryos can be recovered from crosses of single mutants in a different background (DBA × 129SV) (165), suggesting that the actions of Irs1 and Irs2 are dependent on modifier genes.

B. Irs1 and Irs3

An additive effect is seen when Irs1 and Irs3 knockouts are intercrossed. Combined deficiency of these two proteins causes lipoatrophy with insulin resistance. Unlike other lipoatrophic models, Irs1/Irs3 knockouts fail to
develop intrahepatic and intramuscular deposits of triglycerides. As predicted from other models of lipatrophy, leptin administration reverses the hyperglycemia and hyperinsulinemia (141).

C. Irs1 and Irs4

Mice with combined ablation of Irs1 and Irs4 show the same phenotype as Irs1 knockouts, with growth retardation and mild insulin resistance, suggesting that Irs4 is not a primary mediator of insulin and Igf action (141). The lack of additive effects of the two phenotypes indicates that the functions of Irs1 and Irs4 are overlapping.

D. Akt

The primary function of PI3K in insulin and growth factor signaling appears to be the transduction of tyrosine phosphorylation into a signal leading to activation of serine/threonine kinases. This is accomplished via generation of phosphatidylinositol 3-phosphate (PIP3), which in turn acts as a ligand for kinases containing a PH domain. The PIP3-dependent kinase Pdk1 activates a family of serine/threonine kinases. They include p70s6k, Akt, and Sgk isoforms (149, 234). The role of Pdk1 in insulin action awaits further elucidation. A complete knockout is embryonic lethal, while hypomorphic alleles are associated with decreased cell size (144). Among the Pdk1 targets, Akt stands out because of its brisk insulin-dependent activation (127) and ability to mimic many of insulin’s actions when overexpressed (50). A delineation of its precise role in physiologic processes has been hampered by the fact that complete inhibition of its activity appears to be incompatible with cell survival, and by the presence of three highly homologous isoforms in most cell types. However, mice lacking Akt2 have recently been generated. Two phenotypes have been reported: in one instance, mice show evidence of impaired insulin signaling in muscle and adipocytes, which is however insufficient to cause overt diabetes (42). In another experiment, the phenotype is more pronounced, with growth retardation, reduced fat mass, and diabetes (74). The latter phenotype is quite similar to that of Irs-deficient mice. Whether these phenotypic differences are due to compensation by related isoforms remains unclear, but the data are consistent with an important role of Akt in insulin action. When both Akt1 and Akt2 are inactivated, the phenotype resembles closely that of Igf1r-deficient mice (184). Because of the early postnatal lethality, it has not been possible to determine whether metabolic control is also impaired in the double Akt-deficient mice. As detailed elsewhere in this review, Akt appears to be central to β-cell function as well (24, 230).

E. Glut4

Insulin-dependent glucose uptake requires Glut4 translocation from an intracellular compartment to the plasma membrane (175). Thus interest in Glut4 defects as a cause of insulin resistance has been keen. Mice with a complete Glut4 knockout are growth retarded and show cardiac hypertrophy and underdeveloped adipose tissue. They develop moderate insulin resistance, which in male animals is associated with hyperglycemia in the fed state. Female mice do not develop hyperglycemia (106). Somewhat surprisingly, heterozygous males develop insulin-resistant diabetes, without obesity (217). It is not clear why heterozygotes are more severely affected than homozygotes, but it could be due to the fact that additional glucose transporters, such as GlutX/Glut8 (100), can be induced in mice lacking Glut4, but not in heterozygous knockouts, thus leading to metabolic compensation only in homozygous knockouts.

F. Heart-Specific Glut4 Ablation

The presence of cardiac abnormalities in Glut4-deficient mice is of great interest, in view of the increased incidence of cardiac disease in insulin-resistant patients (107). To determine whether the cardiac hypertrophy seen in Glut4-deficient mice is a primary result of Glut4 deficiency in the heart or a consequence of metabolic abnormalities, the cre-loxP system has been used to generate mice lacking Glut4 in the heart. These mice show increased basal levels of glucose uptake but fail to respond to insulin. The metabolic abnormality results in compensated cardiac hypertrophy associated with increased cardiomyocyte size and preserved contractile function (1). In addition, recovery from ischemic damage is impaired in the Glut4-deficient heart (227). The data indicate that Glut4 is important for cardiac function.

G. Muscle-Specific Glut4 Ablation

The phenotype of mice lacking Glut4 indicates that Glut4 is required for insulin-dependent glucose uptake, but not for maintenance of metabolic homeostasis. This surprising conclusion is consistent with the observation that mice with a targeted Insr inactivation in skeletal muscle do not develop obvious metabolic changes (35, 140). To better evaluate the contribution of Glut4 to muscle metabolism, mice lacking Glut4 in skeletal muscle have been generated. Unlike total Glut4 knockouts, muscle-specific Glut4 knockouts are moderately insulin resistant and glucose intolerant, consistent with an important role for Glut4 in glucose metabolism (254). Further analysis of these mice indicates a significant increase in hepatic glycogen storage, an indicator of hepatic glucose
uptake. The compensatory increase in hepatic glycogen levels may prevent higher incidence of frank diabetes in these mice. Glucose uptake in adipose tissue, however, does not show a similar compensatory increase. Hyperinsulinemic-euglycemic clamps demonstrate that muscle-specific Glut4 knockout mice have decreased whole body and insulin-stimulated muscle glucose uptake. In a subset of mice that develops overt diabetes, these studies have revealed also insulin resistance in liver and decreased glucose uptake in adipose tissue. However, the latter defects appear to be secondary to glucose toxicity, since they are reversed by treatment with phlorizin (117). These studies indicate that Glut4 is required for muscle glucose transport. However, since only some of the muscle Glut4 knockout mice develop diabetes, it appears that there are compensatory mechanisms that prevent the onset of hyperglycemia even when muscle glucose utilization is reduced.

H. Fat-Specific Glut4 Ablation

With the use of a similar experimental approach, Glut4 has been selectively ablated in mature adipocytes using the Ap2 promoter to drive cre expression. Mice lacking adipocyte Glut4 fail to increase glucose uptake in response to insulin and develop hyperinsulinemia with hepatic insulin resistance. Because adipocyte-mediated glucose uptake accounts for only ~10% of whole body glucose disposal, the phenotype indicates that a change in insulin sensitivity in adipocytes can disproportionately affect metabolic control (2). As observed in mice with muscle-specific Glut4 ablation, the metabolic consequences display individual variations, and only some mice develop diabetes. Unlike the systemic Glut4 knockout, adipose-selective ablation of this transporter does not lead to growth retardation or reduced adipose tissue mass and adipocyte size. While insulin-stimulated glucose transport in adipocytes is severely blunted, glucose transport in muscle appears to be normal in ex vivo experiments in isolated muscle strips, but is impaired in vivo during hyperinsulinemic-euglycemic clamp studies. Because Glut4 levels in skeletal muscle are normal in these mice, it is likely that an indirect mechanism is responsible for this effect. However, there are no changes noted in three potential mediators of these secondary changes: tumor necrosis factor-α (TNF-α), leptin, or free fatty acids (FFA). Of note, insulin-induced suppression of hepatic gluconeogenesis is also impaired in the fat-specific Glut4 knockouts. Therefore, abnormal glucose transport in the adipocyte can lead to insulin resistance and inadequate glucose disposal in distant tissues through chronic hyperinsulinemia or an unidentified secondary agent. However, despite decreased insulin action at the level of the adipocyte, muscle, and liver, overt diabetes does not develop in these mice. This model supports the importance of Glut4-mediated glucose transport in adipose tissue and the indirect effects of insulin action in adipose cells on insulin action in other tissues.

I. Genes in the Glut4 Translocation Pathway: Syntaxin4

The molecular effectors of Glut4 translocation from the intracellular pool to the plasma membrane are incompletely characterized. Syntaxin4 has been shown to play a role in Glut4-containing vesicle fusion. While homozygous knockouts are embryonic lethal, heterozygous knockouts show no developmental abnormalities but develop impaired glucose tolerance and a 50% decrease in Glut4 translocation in response to insulin in skeletal muscle, but not in adipose tissue (246).

J. Ceacam1 and Insulin Clearance

Although hyperinsulinemia is generally thought to be a compensatory response to insulin resistance, it can potentially arise as a primary cause of insulin resistance, by triggering a secondary downregulation of the insulin signaling pathway. For example, overexpression of Igf2 in pancreatic β-cells of transgenic mice leads to increased β-cell proliferation, hyperinsulinemia, and secondary diabetes (55). The hepatic membrane protein Ceacam has been shown to participate in the process of receptor-mediated insulin internalization and degradation. Ceacam activation is dependent on the phosphorylation of residues in its intracellular domain (168, 169). To test the hypothesis that defects in Ceacam-dependent insulin internalization result in hyperinsulinemia, transgenic mice expressing a dominant negative Ceacam, in which the key phosphorylation site has been mutated, have been generated. These mice do indeed develop hyperinsulinemia and a metabolic syndrome of increased visceral adiposity and increased triglycerides and FFAs. Thus these findings provide proof of principle for the view that alterations in insulin clearance affect peripheral insulin sensitivity (189).

VIII. MIXING AND MATCHING: MODELING THE COMPLEX GENETICS OF TYPE 2 DIABETES THROUGH EXPERIMENTAL MOUSE CROSSES

A. Insr Haploinsufficiency and Diabetes

Most patients with type 2 diabetes do not have insulin receptor mutations but have acquired a secondary impairment of insulin receptor function (179). In these patients,
normalization of insulin resistance through various regimens (diet, exercise, and medical therapy) leads to a significant recovery of insulin receptor function (68). To better understand the role of Insr mutations in the genetic predisposition to diabetes, we have studied the phenotype of Insr heterozygous knockouts. As predicted from the description of the tissue-specific knockouts, these phenotypes are rather variable, even within the same experimental cross in the same laboratory. For example, Insr+/− mice develop diabetes with a frequency varying between 5 and 10% (36, 110). Back-crosses of the Insr mutation onto different genetic backgrounds indicate that the variability of the metabolic phenotypes due to the Insr mutation is due in part to environmental factors and in part to genetic modifiers, which have been preliminarily mapped (110). The role of genetic background in determining phenotypic differences should be emphasized: with the exception of the insulin receptor itself, virtually all other gene knockouts in the insulin signaling cascade have yielded significantly different phenotypes on different genetic backgrounds. Thus growth retardation in Irs1 knockouts (13, 221) and diabetes in Irs2 (132, 224, 242) and Akt2 knockouts (42, 74) vary in severity depending on the mouse strain in which the experiments were performed.

We have also used the Insr heterozygous mutants to mimic genetic interactions leading to type 2 diabetes. A first step in this direction was the development of a polygenic model of insulin-resistant diabetes by crossing mice with heterozygous null mutations of Insr and Irs1. Whereas mice heterozygous for Irs1 deficiency are normal, mice heterozygous for both Insr and Irs1 null alleles develop severe hyperinsulinemia, pancreatic β-cell hyperplasia and, by 4–6 mo of age, 50% of these mice become frankly hyperglycemic. This process closely resembles the pathogenesis of human diabetes, in which insulin resistance is a chronic process and leads gradually to β-cell failure (36, 110). Thus even a major predisposing allele, such as the null Insr mutation, has a modest effect by itself, but plays a major role in the context of a predisposing background. Indeed, when the double heterozygous knockout mice are bred onto different genetic backgrounds, the prevalence of diabetes can vary from <2 to 85% (9, 133). Interestingly, the relative risk of diabetes in double heterozygous offspring (Irs1+/−) of single heterozygous Insr or Irs1 parents increases fourfold, similar to the excess risk of diabetes in first degree relatives of humans with diabetes (75). The findings in the Insr/Irs1+/− mouse are consistent with an oligogenic mode of inheritance of type 2 diabetes, in which two subclinical defects of gene function, coupled with effects of background genes, can account for virtually the entire genetic susceptibility to the disease. Gene-gene interactions are also likely to play a role in humans, as suggested by the increased susceptibility to diabetes in carriers of the NIDDM1 mutation (CALPAIN10), when they also possess certain alleles on chromosome 15 (46), and when the background genes are also permissive.

While combined mutations of Insr and Irs1 have provided insight into the polygenic nature of type 2 diabetes, comparisons of double mutant mice with Insr/Irs1 or Insr/Irs2 null alleles have shed light onto the issue of genetic heterogeneity. Genetic heterogeneity means that mutations in different genes can result in a similar phenotype. Indeed, both Insr/Irs1 and Insr/Irs2 double mutant mice develop diabetes at similar rates. However, the Insr/Irs1 mice do so because they are primarily insulin resistant in skeletal muscle, whereas the Insr/Irs2 mice do so because they are primarily insulin resistant in liver (110). These data indicate that different insulin receptor substrates play tissue-specific roles, with Irs1 being the primary mediator of insulin action in muscle and Irs2 in liver (190).

IX. GENE KNOCKOUTS ASSOCIATED WITH INCREASED INSULIN SENSITIVITY

In addition to mutations affecting insulin action, mutations in genes required to terminate or modulate insulin signaling have proven very informative in examining the pathophysiology of insulin resistance (Fig. 5).

![Negative regulators of Insr signaling](http://www.prv.org)

**Fig. 5.** Negative regulators of Insr signaling. Several gene knockouts have insulin-sensitizing effects. Mice lacking the tyrosine phosphatase Ptp1b are refractory to diet-induced insulin resistance, possibly resulting from reduced tyrosine dephosphorylation of Insr and its main substrates. Ablation of the regulatory p85 subunit of PI 3-kinase restores insulin sensitivity in Insr and Irs1 knockout mice, due to increased activation of PI3K-dependent responses. Mutations of the inositol-phosphatase Ship2 cause neonatal hypoglycemia, and mice heterozygous for the Ship2 null allele reveal increased glucose tolerance. Pharmacological or genetic inhibition of IKK β-kinase protects mice against diet-induced insulin resistance, as do mutations abating JNK1 function. Haploinsufficiency for the forkhead transcription factor Foxo1, which is normally inhibited by Akt-dependent phosphorylation, results in decreased expression of genes regulating gluconeogenesis.
A. Protein Tyrosine Phosphatase

Several tyrosine phosphatases have been implicated in the regulation of insulin signaling. However, the identification of Insr- or Irs-specific phosphatases has been stymied by the extreme genetic heterogeneity of this gene family. Disruption of the protein tyrosine phosphatase (Ptp1b) gene in mice leads to improved glucose tolerance, decreased glucose levels, and decreased insulin levels, consistent with a state of heightened insulin sensitivity. Increased Insr phosphorylation in muscle and liver is a potential mechanism of enhanced insulin action. Importantly, both homozygous and heterozygous Ptp1b mice are refractory to high-fat diet-induced obesity and insulin resistance. The decreased adipose tissue mass is correlated with increased basal metabolic rate and energy expenditure (60, 124). These data suggest that Ptp1b is one of the tyrosine phosphatases required for termination of insulin action, modulation of energy expenditure, and development of adipose tissue. Therefore, inhibition of this gene product could be of significant benefit for treatment of type 2 diabetes and states of insulin resistance. In fact, treatment of diabetic (253) and obese mice (84) with antisense oligonucleotides directed against Ptp1b results in decreased protein levels and ameliorates the metabolic syndrome, paving the way for therapeutic approaches to diabetes based on inhibition of Ptp1b.

B. PI3K Regulatory Subunits

It is generally agreed that insulin signaling requires the lipid kinase activity of the enzyme PI3K (175). Nevertheless, it remains unclear whether PI3K is both necessary and sufficient for insulin action (98). The lipid kinase activity is dependent on the enzyme’s p110 catalytic subunit, while the regulatory p85 subunit (PI3Kr) binds Irs in an insulin-dependent manner. The gene Pik3r1 encodes three proteins (p85α, p55α, and p50α) that serve as regulatory subunits of class 1A PI3Ks. Mice lacking the p85 isoform of this subunit show increased insulin sensitivity and hypoglycemia due to increased glucose transport in skeletal muscle and adipocytes (226). This unexpected phenotype is associated with a compensatory increase in insulin-dependent binding of Irs proteins to the alternatively spliced products of PI3Kr (especially p50α), which was originally proposed to be the cause of increased insulin sensitivity. However, mice lacking all p85 splice isoforms are also hypoglycemic and insulin sensitive (70), so that this explanation is no longer tenable. Unlike the p85-deficient mice, the pan-PI3Kr1 knockout mice die shortly after birth with multiple abnormalities, consistent with the pleiotropic role of PI3K (70). These experiments provide evidence for a role of PI3K in insulin action but do not address the mechanism by which p85 regulates PI3K activity. Based on the mechanics of the interaction between the catalytic (p110) and regulatory subunits of PI3K (196), these data are consistent with a model in which the regulatory subunits act as inhibitors of PI3K activity under basal conditions, while insulin removes this inhibition via Irs proteins.

A compelling demonstration of the insulin-sensitizing effect of null p85 alleles is seen in heterozygous carriers of the mutation, when they are crossed with insulin-resistant mice. Haploinsufficiency of p85 protects both Insr and Irs1 heterozyotes from insulin resistance and diabetes by improving the efficiency of insulin signaling (162).

C. Ship2

The SH2 domain-containing inositol 5-phosphatase Ship2 is able to dephosphorylate phosphoinositol, resulting in abrogation of insulin signaling. As can be anticipated, decreased expression of this protein allows significant enhancement of insulin action. In particular, Ship2 homozygous null mice develop severe and fatal hypoglycemia early in the neonatal stage (43). The low insulin level in these mice indicates that this effect is secondary to increased insulin sensitivity, rather than increased insulin production. This hypothesis is supported by the finding of decreased levels of gluconeogenic enzymes in the liver. Mice homozygous for the Ship2 null allele live to adulthood but reveal increased glucose disposal during a glucose tolerance test as well as increased Glut4 translocation and glycogen storage in muscle. These data demonstrate both the involvement of phosphoinositides in insulin signaling and the specific role of this class of phosphatases in its negative control.

D. c-Jun Amino-Terminal Kinase

Serine phosphorylation of Insr and insulin receptors has been shown to dampen insulin signaling, potentially contributing to the onset of the metabolic syndrome (53, 105, 182, 201). Identification of the serine kinases that mediate this event has proven to be an elusive target. In recent years, evidence from knockout mice has begun to shed light on this controversial issue. For example, in vitro evidence from cultured cells has shown that the c-Jun amino-terminal kinases (Jnk) can impair insulin action in response to activation by cytokines and FFAs (5), two agents that are known to cause insulin resistance in vivo. Moreover, Jnk1 activity has been shown to be elevated in obesity. Mice lacking Jnk1 appear to be protected against obesity and insulin resistance of both genetic (obese mutation) and environmental (diet) origin (91). Interestingly, Irs1 phosphorylation on Ser307 is associated with decreased interaction with the insulin recep-
tor (6) and is reduced in Jnk1−/− mice (91). Thus Jnk1 appears to be one of the Irs serine kinases that are involved in downregulation of insulin signaling.

E. Salicylates, Ikkβ, and the Metabolic Syndrome

Salicylates improve glucose disposal and increase insulin action. High-dose aspirin treatment decreases fasting plasma glucose and improves the lipid profile of type 2 diabetic patients. Euglycemic-hyperinsulinemic clamp studies indicate that the improvement is associated with a reduction of hepatic glucose output and an increase in insulin-stimulated peripheral glucose uptake (97). The mechanism by which salicylates increase insulin sensitivity has recently been elucidated in some detail using genetically engineered mice. Studies in rodents implicate activation of I kappa B kinase beta (Ikkβ) as a critical step in lipid-induced insulin resistance. Overexpression of Ikkβ in cultured cells leads to altered insulin action, whereas inhibition of this gene improves insulin resistance. Ikkβ knockout mice do not develop insulin resistance in the setting of a lipid infusion, while haploinsufficiency at this locus prevents peripheral insulin resistance in the setting of high-fat diet or leptin deficiency in obese mice (115, 250). These findings identify Ikkβ as a potential target for the treatment of insulin-resistant diabetes.

F. The Forkhead Transcription Factor Foxo1

Insight into distal effectors of insulin signaling has been gleaned from studies of the dauer phenotype in the roundworm C. elegans, a condition characterized by impaired metabolism and prolonged life span. In addition to nutritional and hormonal cues, the dauer stage can be brought about by genetic mutations in insulin signaling (79). Thus mutations of the daf2 (Insr ortholog) (118), age1 (PI3K) (166), and akt1–2 genes (180) cause dauer formation. In contrast, mutations of the daf16 gene rescue the phenotype due to daf2 mutations (151, 152, 178). The mammalian orthologs of daf16 belong to the Foxo subfamily of transcription factors (104). The prediction from the C. elegans studies was that mammalian Foxo would act as a negative regulator of insulin signaling and that loss-of-function Foxo mutations would rescue diabetes due to Insr mutations. In recent publications, Foxo1 has been shown to be a negative regulator of insulin signaling in key tissues for metabolic control. Foxo proteins are Akt substrates. Phosphorylation of key consensus Akt sites in Foxo1, -3, and -4 is associated with nuclear exclusion and suppression of their transcriptional activity (33, 129, 175). It should be emphasized that Foxo1 acts both as a promoter and a repressor of gene expression.

1. Foxo1 in liver

Foxo1 plays an important role in insulin control of hepatic glucose production. Evidence from studies in cultured cells indicates that Foxo1 can mediate insulin-inhibitable transcription of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (174), acting in concert with the PPARγ coactivator Pgc1 (192). Haploinsufficiency of the Foxo1 gene restores insulin sensitivity and rescues the diabetic phenotype in insulin-resistant mice by reducing hepatic expression of glucogenetic genes and increasing adipocyte expression of insulin-sensitizing genes. Conversely, a gain-of-function Foxo1 mutant targeted to liver and pancreatic β-cells results in diabetes arising from a combination of increased hepatic glucose production and impaired β-cell compensation due to decreased Pdx1 expression (171). In addition to restoring insulin sensitivity in a genetic model of insulin resistance, Foxo1 haploinsufficiency protects from diet-induced diabetes in mice, suggesting that strategies aimed at decreasing Foxo1 levels and/or activity represent a new approach to diabetes treatment (173).

2. Foxo1 in β-cells

The localization of Foxo1 immunoreactivity to pancreatic β-cells has led to studies of its role in terminal differentiation and proliferation in a variety of cell systems. Mice lacking Irs2 develop β-cell failure, suggesting that insulin signaling is required to maintain an adequate β-cell mass. Haploinsufficiency of Foxo1 reverses β-cell failure in Irs2−/− mice through partial restoration of β-cell proliferation and increased expression of the pancreatic transcription factor Pdx1. Interestingly, Foxo1 and Pdx1 exhibit mutually exclusive patterns of nuclear localization in β-cells, and constitutive nuclear expression of Foxo1 is associated with reduced Pdx1 expression (171). Based on the localization of Foxo1 to a subset of cells abutting pancreatic ducts, it has been suggested that insulin/IGFs regulate β-cell proliferation by relieving Foxo1 inhibition of Pdx1 expression in these cells (123). The implication of these studies is that Foxo1 expression is a marker of a pancreatic cell population with the potential to give rise to mature β-cells.

3. Foxo1 in adipose cells

Foxo1 haploinsufficiency has been shown to decrease adipocyte size and increase expression of genes that promote lipid metabolism (173). Recent evidence indicates that the effect of Foxo1 haploinsufficiency in fat can be explained by a direct action of this transcription factor in adipocytes. In vitro studies of adipocyte differentiation indicate that Foxo1 expression is induced in the early stages of adipogenesis in 3T3-F442A cells. Interestingly, Foxo1 phosphorylation appears to be tightly regu-
lated (173) in a manner that is consistent with the time course of PI3K activity during adipocyte differentiation (202). Expression of a phosphorylation-defective Foxo1 prevents adipocyte differentiation by altering expression patterns of genes involved in cell cycle control and adipogenesis. In contrast, a dominant negative Foxo1 re-patterns of genes involved in cell cycle control and adipogenesis. In mice, in a manner that is consistent with the time course of PI3K activity during adipocyte differentiation (202).

X. CONCLUSIONS

The availability of techniques of targeted mutagenesis has transformed the experimental approach to metabolism. Investigators can now introduce virtually any mutation in mice and study its metabolic effects. As a result, we have begun to untangle the complex genetic pathways that regulate insulin action. In addition to establishing the role of the three receptors of the insulin family in physiology, these studies have clarified the functions of Irs and PI3K in insulin signaling. Unexpected players, such as the Foxo transcription factors, Cacaam, Ptp-1b, Jnk1, and Ikkβ, have emerged from these studies. In addition, novel roles of established players, such as the insulin receptor and its substrates in pancreatic β-cells and brain, are now more clearly defined.

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