
Perspectives in Diabetes

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The Struggle for Mastery in Insulin Action: From Triumvirate to Republic

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Type 2 diabetes arises from a combination of impaired insulin action and defective pancreatic β -cell function. Classically, the two abnormalities have been viewed as distinct yet mutually detrimental processes. The combination of impaired insulin-dependent glucose metabolism in skeletal muscle and impaired β -cell function causes an increase of hepatic glucose production, leading to a constellation of tissue abnormalities that has been referred to as the diabetes “ruling triumvirate.” Targeted mutagenesis in mice has led to a critical reappraisal of the integrated physiology of insulin action. These studies indicate that insulin resistance in skeletal muscle and adipose tissue does not necessarily lead to hyperglycemia, so long as insulin sensitivity in other tissues is preserved. Additional data suggest a direct role of insulin signaling in β -cell function and regulation of β -cell mass, thus raising the possibility that insulin resistance may be the overarching feature of diabetes in all target tissues. I propose that we replace the original picture of a ruling triumvirate with that of a squabbling republic in which every tissue contributes to the onset of the disease. *Diabetes* 53: 1633–1642, 2004

The classic view of the development of type 2 diabetes is that skeletal muscle (1) and adipose tissue (2) are resistant—be it on a genetic or on an environmental basis—to insulin-dependent glucose uptake and nonoxidative disposal (3). However, the onset of hyperglycemia reflects two separate events occurring at different sites, namely a failure of the β -cell’s compensatory ability (4) with an attendant rise in hepatic glucose production (5). In this context, hyperglycemia is

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Fox, forkhead box-containing protein; Irs, insulin receptor substrate; Insr, insulin receptor; Neurog3, Neurogenin3; PGC, peroxisome proliferator-activated receptor- γ coactivator; PI3K, phosphatidylinositol 3-kinase.

Mouse loci are indicated by lowercase italics; human loci are indicated by uppercase italics.

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viewed as a vicarious mechanism to promote peripheral glucose utilization by mass action, in the face of insulin resistance (Fig. 1) (6). The paradox implicit in this view is that, whereas insulin resistance occurs primarily in muscle and adipose tissue, diabetes is actually a disease of liver and β -cells, which do not appear to be primary targets of insulin resistance. One school of thought contends that metabolic mediators engendered by the insulin-resistant muscle and fat cause metabolic abnormalities in liver and β -cells (Fig. 2). There is ample experimental evidence to support this view (7,8) and no shortage of candidate mediators of insulin resistance (9). However, a different mechanism has begun to emerge from studies of mice with targeted gene mutations, namely that liver and β -cell are primary sites of insulin resistance. This question bears on the therapeutic approach to diabetes, for if the response of the liver and the β -cell observed in insulin-resistant individuals is indeed a compensatory one, then we should endeavor to preserve it. If, on the other hand, the liver and β -cell response is detrimental to long-term preservation of insulin sensitivity, then we should redouble our efforts to treat diabetes by acting on insulin resistance at those sites.

The elusive genetics of insulin resistance. Insulin resistance is genetically determined in many individuals. Epidemiological studies indicate that having a diabetic first-degree relative causes a fourfold increase in one’s risk of developing diabetes. Moreover, the concordance rate for type 2 diabetes in monozygotic twins exceeds that of dizygotic twins, suggesting that genes, more than environment or maternal factors, account for the excess risk (10). Studies of monogenic forms of insulin-resistant diabetes (11) as well as maturity-onset diabetes of the young (MODY) (12) have revealed a surprisingly broad cast of diabetes susceptibility genes. However, the common clinical form of insulin resistance has proven largely intractable in human genetic studies. Several factors have concurred to thwart genetic studies of insulin resistance in humans: phenocopies, i.e., the ability of environmental conditions (diet, sedentary lifestyle) to cause insulin resistance; genetic heterogeneity, i.e., the ability of mutations in different genes to bring about the same metabolic trait (for example, fasting hyperinsulinemia); and polygenic inheritance, i.e., interactions among multiple diabetes susceptibility alleles in the same individual (13). To complicate things further, diabetes susceptibility alleles are likely

Primary sites of insulin resistance

Primary sites of onset of hyperglycemia

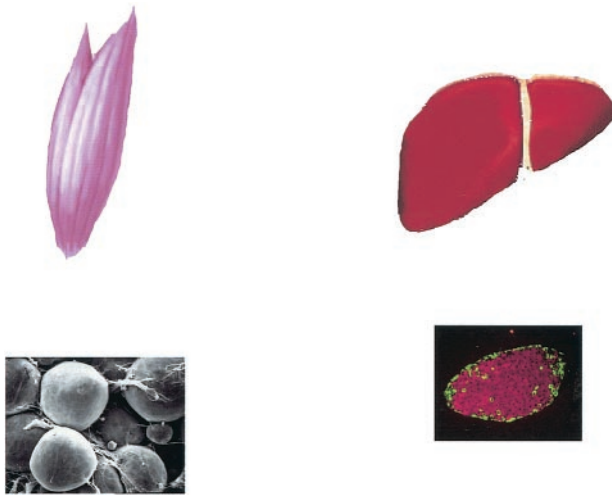


FIG. 1. Development of diabetes. Resistance to insulin action on glucose disposal in muscle and fat precedes the onset of overt diabetes, which is caused by impaired β -cell ability to restrain hepatic glucose production. The rise in glucose levels should be viewed as a compensatory mechanism to allow glucose utilization in an insulin-independent manner.

to be common variants, as opposed to rare mutations (14). This adds another level of complexity, as the ability to detect allele-specific risk decreases with increasing allele frequencies (15), and because functional testing of common allelic variants poses an unmet challenge.

It is because of this daunting complexity that investigations have increasingly relied on gene-targeted and transgenic mice to examine the contribution of genetic variants to insulin resistance and identify new diabetes-causing alleles.

I will review experiments in mice carrying heterozygous mutations of *Insulin receptor* (*Insr*) as an example of how mouse genetics can provide insight into the genetic interactions leading to type 2 diabetes (16,17). A first step in this direction was the development of an oligogenic 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 het-

erozygous for both *Insr* and *Irs1* null alleles develop severe hyperinsulinemia and pancreatic β -cell hyperplasia, and, by 4–6 months of age, a sizable fraction of these mice becomes 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 (18,19). The main lesson from these studies is that 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 (20). Indeed, when the double heterozygous knockout mice are bred onto different genetic backgrounds, the prevalence of diabetes can vary from <2 to 85% (21,22). Interestingly, the relative risk of diabetes in double heterozygous offspring (*Insr/Irs1*^{+/-}) of single heterozygous *Insr* or *Irs1* parents increases fourfold, similar to the excess risk of diabetes in first-degree relatives of humans with type 2 diabetes (10). The findings in the *Insr/Irs1*^{+/-} mouse are consistent with an oligogenic mode of inheritance of type 2 diabetes and are supported by human genetic studies in which the susceptibility to diabetes in carriers of the *NIDDM1* mutation (*CALPAIN10*) is increased by alleles on chromosome 15 (23).

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 (19). **Probing the sites of insulin resistance.** Experimental evidence supporting the need for a reassessment of the primary site(s) of insulin resistance came from two studies in genetically modified mice (24,25). In the first study, our group set out to critically test the hypothesis that insulin resistance in adipose tissue and skeletal muscle is necessary and sufficient to cause diabetes. To this end, we generated mice that were resistant to insulin action in

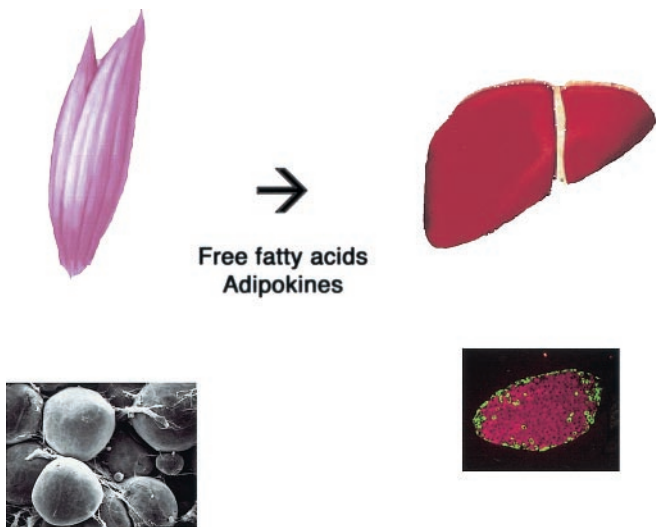


FIG. 2. Insulin resistance in muscle and fat indirectly causes liver and β -cell defects. There is ample support for the notion that insulin resistance in muscle and fat can indirectly impinge upon liver and β -cell function. For example, free fatty acid release from insulin-resistant adipocytes can affect hepatic glucose output, as can adipocyte-secreted hormones like resistin and adiponectin.

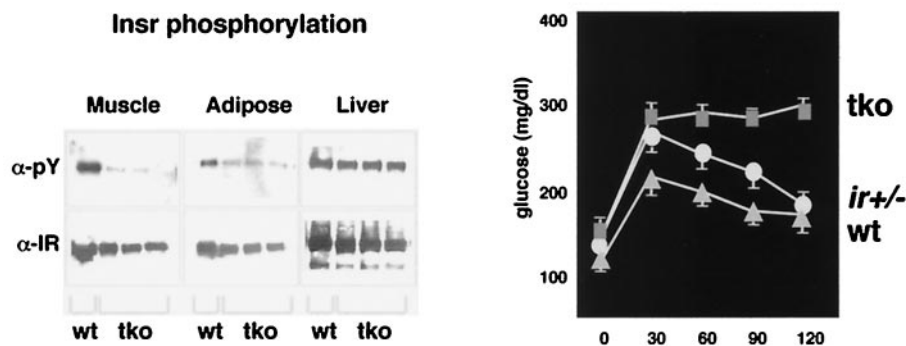


FIG. 3. Insulin resistance in muscle and adipose tissue. To test whether insulin resistance in muscle and fat is sufficient to cause diabetes, Lauro et al. (24) generated transgenic knockout mice with targeted impairment of insulin action in these two tissues. The Western blot on the left shows impaired tyrosine phosphorylation of the Insr in muscle (*left panel*) and fat (*middle panel*) but not liver (*right panel*), while Insr protein levels were similar in all mice (*lower panels*). The metabolic abnormality in these mice is shown by the glucose tolerance test on the right: the transgenic knockouts (tko) are glucose intolerant, whereas one of the parental strains (*Insr*^{+/-}) and the wildtype controls (wt) are not.

these two tissues and asked whether they developed hyperglycemia. This task was achieved by crossing mice haploinsufficient for an *Insr* null allele (*Insr*^{+/-}) with transgenic mice bearing a kinase-inactive *Insr* transgene (K1030M) expressed in muscle and fat but not in liver and β -cells. Biochemical and metabolic data indicated that the double-mutant mice (*Insr*^{+/-} K1030M) had impaired insulin-dependent glucose uptake in muscle and adipocytes and developed impaired glucose tolerance without ever becoming diabetic (Fig. 3). Their β -cell function was preserved throughout life, despite the increased metabolic demands (24). Thus, these findings indicate that mice can overcome the insulin resistance of muscle and adipose tissue as long as insulin action in liver is preserved and there are no additional strains on the β -cell.

The second piece of evidence came from mice bearing a conditional inactivation of *Insr* in skeletal muscle (25), generated by tissue-specific recombination with the Cre/loxP system (26) to specifically ablate the *Insr* gene product in skeletal muscle cells. Conditional *Insr* knockout in skeletal muscle (so-called “MIRKO” mice) leads to impaired insulin signaling and decreased glucose transport in isolated muscle strips in response to insulin, without systemic insulin resistance. This decrease in muscle me-

tabolism is partly compensated for by a concurrent increase in insulin-stimulated glucose transport in adipose tissue (27), which may explain the lack of a more severe impairment of glucose metabolism (Fig. 4).

These observations are unexpected. Whereas it is generally accepted that insulin resistance per se—in muscle or elsewhere—is not sufficient to cause overt diabetes, the prediction was that an impairment of insulin signaling in muscle and/or adipose tissue would lead to systemic insulin resistance. This apparent discrepancy is discussed below.

Alternative pathways to glucose metabolism in muscle. The failure to develop diabetes in mice with a selective impairment of insulin signaling in muscle can be explained by two compensatory pathways: the IGF1 receptor (Igf1r) pathway and the contraction-activated pathway.

The Igf1r shares extensive structural similarities with *Insr*. Like *Insr*, it is composed of two homodimeric subunits ($\alpha_2\beta_2$) and is activated upon ligand binding, the main distinguishing feature of *Insr*-like receptors from other receptor tyrosine kinases (28). Moreover, the *Insr* and Igf1r engage in heterodimer formation, yielding “hybrid” receptors (29). Although the interactions of *Insr* and Igf1r have mostly been thought to regulate embryonic growth

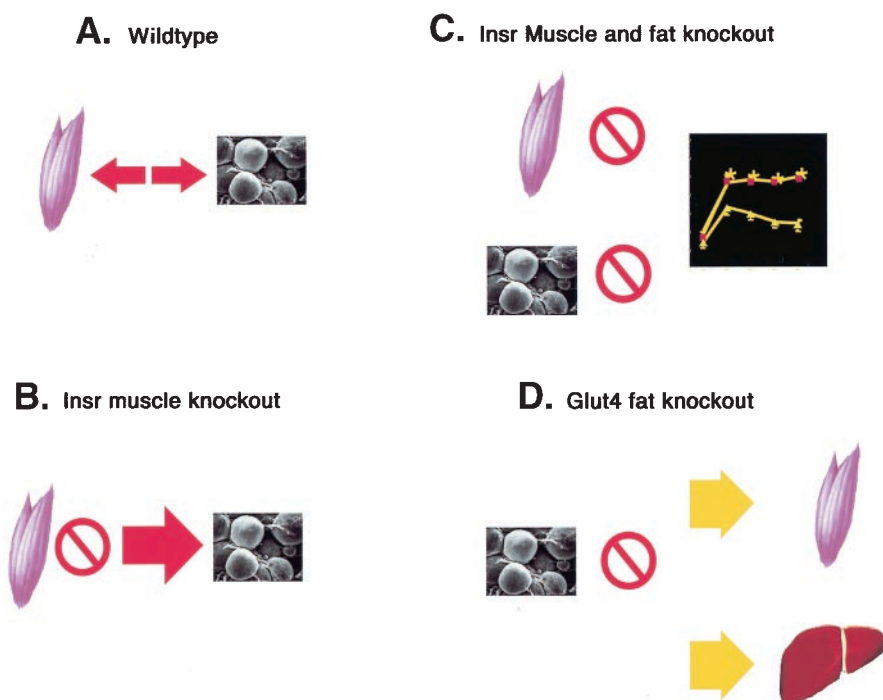


FIG. 4. The metabolic balance of power. Tissue-to-tissue communication affects insulin sensitivity. These interactions are remarkably complex. In normal mice (A), insulin-dependent glucose disposal (indicated by the arrows) is partitioned between adipose and muscle. Mice lacking *Insr* in muscle (B) do not develop significant insulin resistance and are able to shunt some of their glucose utilization to fat (indicated by a larger arrow). Accordingly, mice with double inactivation of *Insr* in muscle and fat (C) develop impaired glucose tolerance, a more severe form of insulin resistance (indicated by the chart with normal and abnormal glucose tolerance tests). These are examples of compensation in which defective signaling in one tissue can be rescued by signaling in another tissue. Conversely, mice lacking *Glut4* in fat (D) develop insulin resistance in muscle and liver (arrows), indicating that the inability to utilize glucose in fat cells negatively affects insulin sensitivity in other organs.

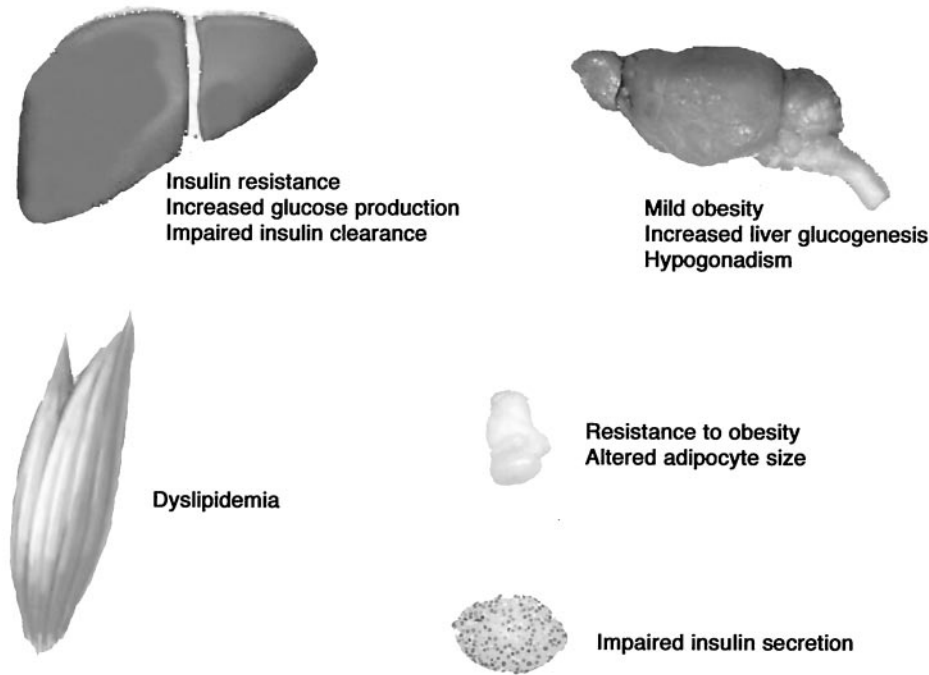


FIG. 5. Metabolic phenotypes associated with tissue-specific inactivation of insulin receptors. Mice with conditional null alleles of *Insr* have shed light onto the roles of different tissues in the pathogenesis of insulin resistance. Selected inactivation of *Insr* in different tissues causes mild forms of insulin resistance. The main metabolic phenotype is indicated next to the organ.

(30,31), the metabolic role of *Igf1r* is emphasized by experiments in which a dominant-negative *Igf1r* expressed in skeletal muscle of transgenic mice was able to cause diabetes, presumably through inhibition of *Insr* receptor function (32). These data are consistent with the ability of IGF1 to decrease glucose levels when injected in *Insr*-deficient mice (33).

Muscle contraction is another powerful trigger for glucose metabolism (34, 35) and provides an additional, potential compensatory mechanism whereby mice deficient in muscle *Insr* signaling can overcome insulin resistance. Indeed, contraction-stimulated glucose uptake in *MIRKO* mice is normal (36). The conclusion from these different datasets is that alternative biochemical pathways can bypass the requirement for insulin signaling to promote glucose utilization in muscle.

These data should not be construed as dismissive of the muscle's role in glucose metabolism. For example, selective disruption of the insulin-sensitive glucose transporter *Glut4* in muscle results in a profound reduction of both insulin- and contraction-stimulated glucose transport, with early-onset insulin resistance and glucose intolerance (37). In conclusion, these studies do not challenge the fact that muscle glucose disposal is central to fuel metabolism, but indicate that *Insr* signaling is only one of the pathways leading to *Glut4*-mediated glucose uptake.

The metabolic balance of power. The use of techniques of gene manipulation in mice has breathed new life into our approach to the integrated physiology of insulin action, the metabolic "balance of power" among various organs in determining insulin sensitivity (or lack thereof). There have been several studies in which investigators have genetically inactivated insulin signaling or glucose transport in selected tissues and then asked what the metabolic consequences of these manipulations would be. A detailed analysis of this work (38) is beyond the scope of the present review, but I will summarize some of the conclusions (Fig. 5). A common theme emerging from

these investigations is that inactivation of insulin signaling in tissues that exhibit insulin-dependent glucose uptake and utilization, such as muscle and fat, generally results in milder metabolic phenotypes than would be predicted (18,39). On the other hand, inactivation of insulin signaling in tissues that are not dependent on insulin for their glucose uptake, such as brain, liver, and pancreatic β -cells, is associated with significant metabolic abnormalities, suggesting that these tissues play a primary role in insulin resistance (40–42). Let me then turn to insulin action in two of these cell types, the hepatocyte and the pancreatic β -cell.

Insulin action on liver gene transcription. The liver is an important site of inhibition of gene expression by insulin. There are acute effects of insulin on hepatic glucose production and uptake that may be mediated via both direct and indirect mechanisms. These effects are very rapid and do not require regulation of gene transcription (43). However, there are potent effects of insulin in modulating the gene expression of key metabolic enzymes. These effects may account for the long-term inhibition of hepatic glucose output by insulin (44). Pioneering work by Granner (45) and Hanson (46) has demonstrated that insulin inhibits the expression of the gene encoding phospho-enolpyruvate carboxykinase (*Pck1*). Similarly, glucose-6-phosphatase (*G6pc*) gene expression is inhibited by insulin (47). A liver with altered expression of these enzymes has altered responses not only to the acute direct and indirect effects of insulin, but also to the important effects of hyperglycemia per se on glucose production and uptake (48). Thus, alterations in insulin's ability to regulate hepatic gene expression are likely to play a pivotal role in the pathophysiology of type 2 diabetes.

The mechanism by which insulin regulates gene expression in liver has until recently remained unclear, despite tremendous progress in understanding how insulin works (49). Genetic epistasis experiments in the roundworm *C. Elegans* identified *Daf16*, a transcription factor of the

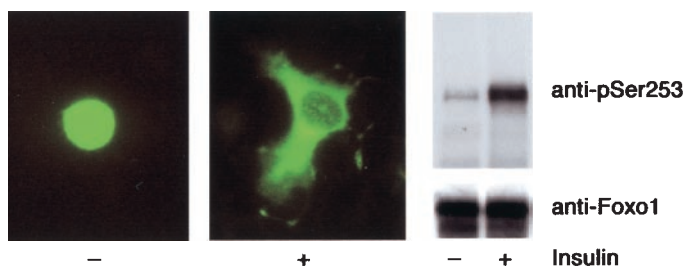


FIG. 6. Foxo nuclear exclusion. In the absence of insulin, the forkhead transcription factor Foxo1 is dephosphorylated and nuclear (left panel). Upon insulin stimulation, Akt catalyzes Ser253 phosphorylation (right upper panel), resulting in nuclear exclusion (middle panel). Through this mechanism, insulin is able to curtail hepatic glucose production.

forkhead family, as a key effector of insulin signaling (50,51). The prediction from the *C. Elegans* studies was that the mammalian ortholog of *Daf16*, *FoxO* (*FORKHEAD box-containing gene, O subfamily*), would act as a negative regulator of insulin signaling and that loss-of-function *Foxo* mutations would rescue diabetes due to *Insr* mutations. Foxo proteins are Akt substrates in vivo (52). Upon phosphorylation, Foxo1 is inhibited by nuclear exclusion, thus providing a mechanism for negative control of gene expression by insulin and other growth factors that signal through phosphatidylinositol 3-kinase (PI3K) and Akt (Fig. 6).

Foxo1 plays an important role in insulin control of hepatic glucose production. Evidence from studies in cultured cells indicates that Foxo1 can single-handedly confer insulin-inhibitable transcription of *G6pc* (53). In the fasting state, Foxo1 is largely dephosphorylated and localizes to the nucleus, where it binds the peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α (54) and Cbp/p300 (55) to promote *Pck1* and *G6pc* gene transcription. Insulin-dependent phosphorylation dissociates the Foxo1/PGC-1 α complex and causes Foxo1 redistribution to the cytoplasm. 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 results in diabetes arising from increased hepatic glucose production (56). 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 (57).

Foxo controls insulin-dependent cellular differentiation. Foxo1 plays an important role in adipocyte development and physiology. In fact, Foxo1 haploinsufficiency has been shown to decrease adipocyte size and increase expression of genes that promote lipid metabolism (57). This effect is not simply secondary to improved insulin sensitivity in liver, but 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 regulated (57) during differentiation of 3T3-F442A

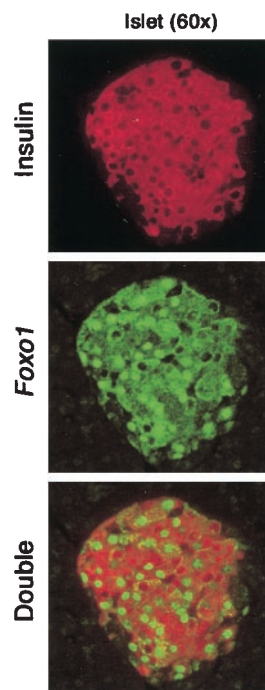


FIG. 7. Foxo in β -cells. Immunohistochemical studies indicate that Foxo1 localizes to insulin-producing β -cells. However, the subcellular localization of Foxo1 is variable, with some cells showing predominant nuclear staining and some cells showing predominant cytoplasmic staining. Upper panel, insulin staining; middle panel, Foxo1 staining; lower panel, overlay.

cells. 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 restores adipocyte differentiation of murine embryonic fibroblasts from *Insr*-deficient mice (57). The emerging model of Foxo1 function in fat cells is that it may control the coupling of extracellular (hormonal) cues that activate adipocyte differentiation with the cell cycle machinery. A similar paradigm appears to be at play in myoblast differentiation (58).

Foxo1 in β -cells. While Foxo1 function in liver was wholly predictable, the finding that Foxo1 immunoreactivity is abundant in pancreatic β -cells and ducts raises the question of its role in these cells (Fig. 7). Mice lacking *Irs2* develop β -cell failure, suggesting that insulin signaling is required to maintain an adequate β -cell mass (59,60). 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 (56). Based on the localization of Foxo1 to a subset of insulin-positive cells abutting pancreatic ducts, we have suggested that insulin/IGFs regulate β -cell differentiation by relieving Foxo1 inhibition of Pdx1 expression in these cells (61). The implication of these studies is that *Foxo1* expression is a marker of a pancreatic cell subpopulation with the potential to give rise to mature β -cells.

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. The maintenance of an adequate β -cell mass is the result of a physiologic balance between β -cell proliferation and apoptosis. Diabetes ultimately results from the failure to maintain β -cell mass (62). Unlike other terminally differentiated cell types, pancreatic islet β -cells maintain the ability to undergo proliferation in both physiologic (growth, gestation) and disease conditions (obesity, insulin resistance). At the same time, there is evidence for de novo generation of β -cells from undifferentiated progenitor cells, possibly arising from the epithelial lining of pancreatic ducts (63–65) or from a nonrenewable pool of Neurogenin3 (Neurog3)-positive endocrine progenitor cells (66). The factors that drive β -cell neogenesis and proliferation under normal or pathological conditions are unknown (67).

The association of peripheral insulin resistance with compensatory β -cell hyperplasia is extremely well documented in animal models and, to a lesser extent, in humans (12). We do not know why β -cells replicate in response to insulin resistance. One possibility is that they are responding to changes in insulin secretory patterns, although insulin resistance initially causes only subtle abnormalities of insulin secretion. Other authors have suggested that β -cells proliferate in response to transient oscillations in glucose levels, occurring before the onset of overt hyperglycemia (62). The alternative and not mutually exclusive view is that they are activated to proliferate by circulating β -cell growth factors (68).

Signaling pathways regulating β -cell function. Classically, β -cell function has been viewed as a response to intracellular glucose metabolism triggered by variations in plasma glucose levels (69). In recent years, signaling by receptor tyrosine kinases has been integrated among the β -cell regulatory mechanisms, impinging on such diverse functions as glucose sensing, insulin release, and cellular proliferation (67). Based on observations in genetically engineered mice lacking various components of the insulin/IGF signaling pathway, it has been proposed that insulin and IGF receptors regulate two key processes in the life of a β -cell: proliferation and hormone secretion (Fig. 8). Ablation of *Insr* in β -cells by site-specific recombination leads to altered glucose sensing and impairs glucose tolerance (40). A similar phenotype results from generalized ablation of *Irs1* (70). Similar results have been obtained in mice lacking Igf1 receptors in β -cells (71,72), consistent with the observation that IGF1 is a negative regulator of insulin secretion (73). On the other hand, it has been shown that ablation of *Irs2* results in decreased β -cell mass (59,74), and that this phenotype is exacerbated by *Igf1r* (75) or *Insr* haploinsufficiency (61). Complex alterations of insulin secretion result also from mutations of PI3K (76,77). Furthermore, ablation of p70^{S6K1}, an Akt substrate, is associated with decreased β -cell size (78). Conversely, overexpression of a constitutively active mutant Akt increases β -cell mass and protects from streptozotocin-induced diabetes without affecting insulin secretion (79,80).

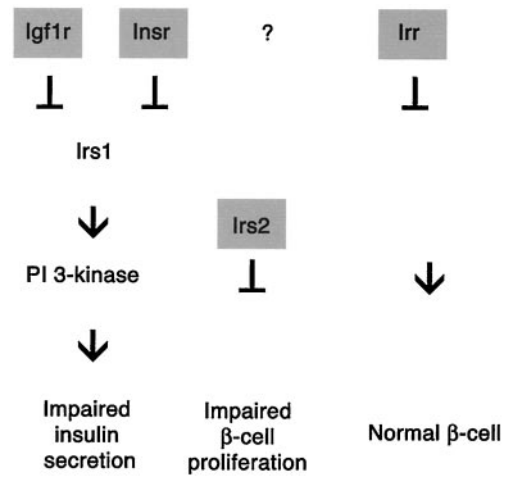


FIG. 8. Mutations of *Insr* and *Igf1r* signaling in pancreatic β -cells. *Insr* and *Igf1r* knockouts in β -cells have been obtained using site-specific recombination. In both instances, insulin secretion is impaired, albeit the mechanisms appear to differ. The effect of *Irs1* knockout on β -cells 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 *Insr* nor *Igf1r* knockout affects this aspect of β -cell physiology. Thus, it is possible that *Irs2* is activated by additional receptors. A prime candidate was the insulin receptor-related receptor (*Irr*), but studies of *Irr* knockouts rule out an effect on β -cell proliferation and glucose-induced insulin secretion (90).

The controversial role of pancreatic duct cells in β -cell neogenesis. As stated above, there is convincing evidence for β -cell neogenesis, but the source of new β -cells is unknown. Insulin-producing cells can be commonly visualized in the vicinity of pancreatic ducts (81), and the number of duct-associated, hormone-positive cells increases in insulin resistance or following partial pancreatectomy (82). Increased duct-associated β -cell neogenesis has also been described in mice expressing constitutively active Akt (79,80). Bonner-Weir et al. (83) have shown that the purification procedure commonly used to obtain pancreatic islets from cadaver donors for transplantation purposes yields a mixture of hormone-producing islets and insulin/pancytokeratin double-positive cells, which they have suggested to represent insulinogenic duct cells. The implication of these studies is that terminally differentiated epithelial ducts are physiologically able to yield hormone-producing cells by a trans-differentiation process (84). The identity of the cell type that gives rise to insulin-producing cells within ducts has remained elusive. Several authors have suggested that these progenitor cells are epithelial in nature, based on the expression of various cytokeratin isoforms, carbonic anhydrase II, and lectins. It is uncertain whether they also express Nestin, another potential pancreatic stem cell marker (85–87).

Recent evidence from lineage-tracing studies appears not to substantiate the alleged role of duct epithelia as a source of endocrine progenitor cells (Fig. 9). Gu et al. (66) have used an inducible Cre transgene driven by either the *Neurog3* or *Pdx1* promoters to indelibly label pancreatic cells. The conclusions of these studies are that, whereas Neurog3-positive cells act as nonrenewable endocrine progenitor cells, Pdx1-positive cells give rise to exocrine and endocrine cells, as well as epithelial duct cells. The latter, however, fail to develop into islets, indicating that

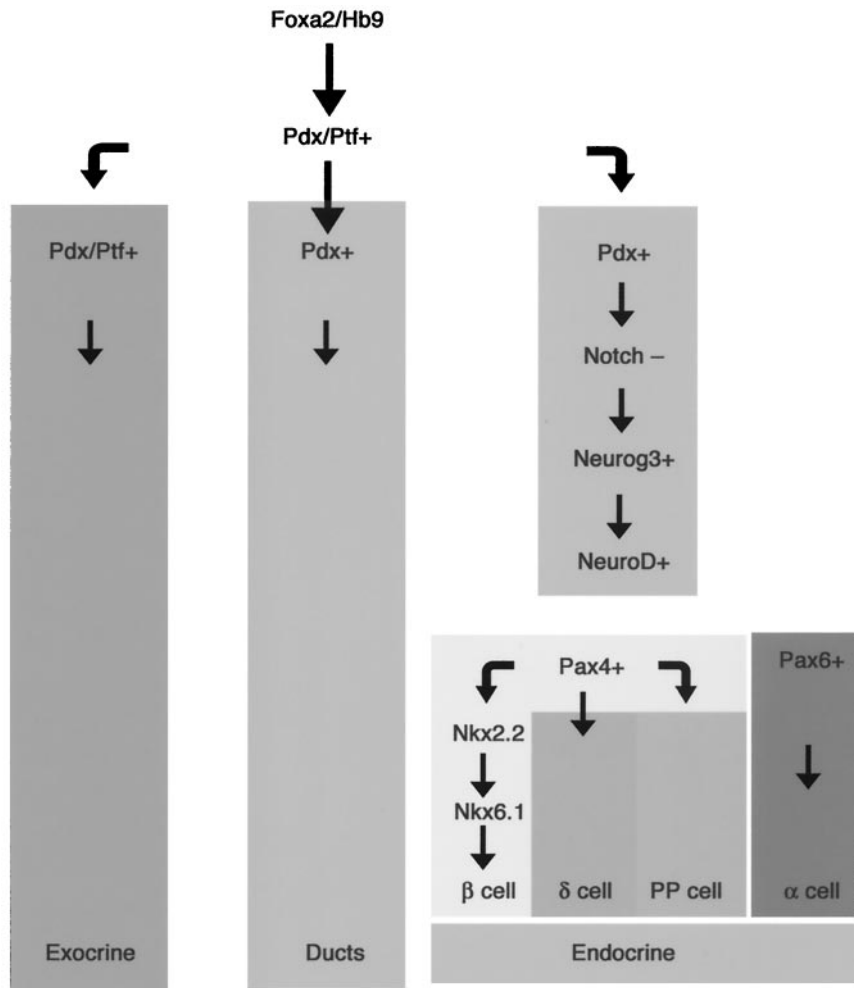


FIG. 9. Synopsis of pancreatic development. Early endodermal commitment to a pancreatic fate requires inhibition of Sonic Hedgehog signaling and activation of *Foxa2* and *Hb9* expression. Expression of *Pdx1* ensues, and during embryonic development a subset of *Pdx1*-positive cells gives rise to ductal structures. Coexpression of *Pdx1* and *Ptf1* characterizes exocrine cell precursors, while islets develop from cells with inactive Notch signaling. In these cells, *Neurog3* is transiently expressed, followed by growth arrest and expression of the bHLH protein *NeuroD*. Thereafter, cells in which *Pax4* is expressed will give rise to α -cells, while β -, δ -, and PP cells develop from *Pax6*-positive precursors (88).

duct epithelia do not act as a physiologic source of endocrine cells. These data do not rule out that duct cells may give rise to endocrine cells with a low frequency or in specific situations (for example, in response to increased metabolic demand) (88).

The insulo-acinar axis and metabolic compensation to insulin resistance. Although it is clear that β -cells can proliferate in response to a host of growth factors, the most parsimonious explanation for insulin resistance-induced β -cell hyperplasia is that insulin itself promotes β -cell neogenesis and proliferation (67). Insulin may have an autocrine effect on β -cell proliferation, but this mechanism would not explain the increase in neogenesis observed in hyperinsulinemic states. The main problem with this explanation is that β -cells are constantly exposed to insulin, so it is unclear how they could discriminate between a physiologic and an abnormal insulin concentration.

To reconcile these seemingly inconsistent observations, we need to reexamine the functional anatomy of the pancreas. It has been suggested that pancreatic hormones are in part released directly into the ductal system, by way of close connections between endocrine cells and ducts (63). It has been known since the late 1970s that sizable quantities of pancreatic hormones, including insulin, glucagons, and somatostatin, can be detected in pancreatic

juice (89). It is therefore tempting to speculate that insulin released into the pancreatic ducts acts directly on a selected population of endocrine progenitor cells that are in close proximity to ducts to stimulate β -cell neogenesis. We hypothesize that these cells are characterized by *Foxo1* expression and are maintained in an undifferentiated state because *Foxo1* acts as a transcriptional brake on the differentiation program. When duct cells are exposed to insulin, *Foxo1* translocates to the cytoplasm, relieving the constraint on terminal differentiation and promoting β -cell neogenesis.

Seeing complexity as an opportunity. Insulin signaling can be viewed as a three-tiered system, in which a metabolic response requires initially tyrosine phosphorylation, then phosphoinositol generation, and ultimately serine/threonine phosphorylation. At each step along the way, checkpoints have been built up to prevent the system from going astray, much like the checkpoints that prevent neoplastic cell transformation. So, we have tyrosine phosphatases, inositol phosphatases, and serine/threonine kinases, the function of which is to dampen insulin signaling (49). Each one of these checkpoints should be viewed as a therapeutic target opportunity to reverse insulin resistance. It should also be pointed out that mouse genetics has enabled us to sift through many different genes with

seemingly related tasks to identify those that are relevant to insulin action.

CONCLUSIONS

The theme of this review is to emphasize how a panoply of tissues contributes to metabolic control. I have grouped them under the moniker of “the republic of insulin resistance,” and in doing so I probably overstepped the modesty of nature, since some of these interactions may reflect more the experimenter’s ingenuity than real-life physiologic drama. Practitioners of basic research bear an eerie resemblance to the character of Mephistopheles in Goethe’s *Faust*, as they conjure up in gruesome detail the spirits of spike-headed flies, ageless worms, and knockout mice (Mephistopheles conjures them up as purveyors of mendacity, not a good precedent for scientists), but it is important to emphasize that sorting out gene function in mammals is no easy task, and my generation is fortunate to be active in science at a time when so much has become technically possible.

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