

UNDERSTANDING THE PATHOGENESIS AND TREATMENT OF INSULIN RESISTANCE AND TYPE 2 DIABETES MELLITUS: WHAT CAN WE LEARN FROM TRANSGENIC AND KNOCKOUT MICE?

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SUMMARY - The development of type 2 diabetes is linked to insulin resistance coupled with a failure of pancreatic β -cells to compensate by adequate insulin secretion. Here, we review studies obtained from genetically engineered mice that have helped dissect the pathophysiology of this disease. Transgenic/knockout models with monogenic impairment in insulin action and insulin secretion have highlighted potential molecular mechanisms for insulin resistance and suggested a mechanism for the development of MODY in humans. Polygenic models have strengthened the idea that minor defects in insulin secretion and insulin action, when combined, can lead to diabetes, pointing out the importance of interactions of different genetic loci in the production of diabetes. Tissue-specific knockouts of the insulin receptor have challenged current concepts on the regulation of glucose homeostasis and have highlighted the importance of insulin action in pancreatic β -cells and brain. The impact of the genetic background on insulin action, insulin secretion and the incidence of diabetes is also evident in these models. These findings highlight potential new therapeutic targets in the treatment of type 2 diabetes.

Key-words: type 2 diabetes, transgenic mice, knockout mice, insulin resistance.

RÉSUMÉ - Pathogénie et traitement de l'insulino-résistance et du diabète de type 2 : que nous apportent les souris transgéniques ?

Le diabète de type 2 est une maladie polygénique caractérisée par des anomalies qui touchent à la fois la sécrétion d'insuline et son action au niveau des tissus cibles. Récemment, l'utilisation de souris transgéniques a permis, en invalidant ou en surexprimant certains gènes candidats, de déterminer leur fonction dans le maintien de la glycémie et de préciser leur rôle dans le développement du diabète de type 2. Ainsi, l'invalidation monogénique des gènes codant des molécules impliquées dans l'action de l'insuline et la sécrétion de l'insuline a permis de préciser les mécanismes moléculaires de l'insulinorésistance et a suggéré une mécanistique au développement du diabète MODY que l'investigation humaine ne permet pas. Les modèles de diabète polygéniques ont conforté l'idée que des anomalies de la sécrétion et de l'action de l'insuline, infracliniques lorsqu'elles sont isolées, pouvaient entraîner un diabète lorsqu'elles étaient combinées, ce qui confirme l'importance de l'interaction épistatique de différents gènes dans le développement du diabète de type 2. L'invalidation tissu-spécifique du récepteur de l'insuline dans les principaux tissus insulinosensibles a apporté des éléments nouveaux sur les mécanismes de régulation de la glycémie et ouvert de nouvelles perspectives sur les mécanismes physiopathologiques du diabète de type 2. Le rôle du terrain génétique dans le développement du diabète est également discuté. Enfin, le rôle des animaux transgéniques dans la découverte de nouvelles molécules ou de nouvelles cibles thérapeutiques dans le diabète de type 2 est également envisagé.

Mots-clés : diabète de type 2, souris transgéniques, invalidation de gènes, insulinorésistance.

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Type 2 diabetes mellitus is a polygenic disease characterized by impaired insulin stimulated glucose uptake in skeletal muscle and adipose tissue, increased hepatic glucose production and inadequate compensation of insulin secretion from pancreatic β -cells, ultimately leading to fasting hyperglycemia [1, 2]. To fully understand the pathogenesis of type 2 diabetes and to develop new approaches for treatment or prevention of this disease one must understand the relative order and the relative importance of these defects in the progressive deterioration of glucose homeostasis seen in the disease. In addition one must elucidate the molecular mechanisms of insulin resistance and impaired β -cell function and identify the genetic factors that might influence them.

Over the past decade, the mouse has become one of the most useful tools in the study of the basis of mono- and polygenic disorders, as well as in the understanding of complex diseases with gene-gene and gene-environment interactions such as type 2 diabetes. One major scientific advance has been the ability to modulate the expression of candidate diabetogenes in mice. This review will briefly discuss the methods used to produce genetically engineered mice and will focus on recent advances in understanding the mechanisms of maintenance of euglycemia and the molecular basis of type 2 diabetes provided by transgenic/knockout mice. In addition, we will highlight prospects for new therapeutic targets provided by these animal models.

■ TECHNIQUES

Transgenic technology

Transgenic animals have been used for simulating diseases and testing new therapies. The overexpressed transgene can be a normal gene product, a mutant gene product (increasing or decreasing the activity of the protein), or an antisense RNA which hybridizes with native RNA and decreases the expression of the normal gene product. Transgenic technology can also be used to eliminate a specific tissue by overexpression of a gene encoding toxins which will destroy the target tissue in which they are expressed. By using specific promoters one can drive tissue-specific or developmental-specific expression of the transgene. For example, a transgene can be expressed in all tissues of the transgenic animal using a promoter from a ubiquitously expressed gene, such as that of β -actin or of the simian virus 40T antigen. Alternatively, selective expression in fat cells or in skeletal muscle can be achieved using the aP2 or MCK promoters respectively, while the insulin promoter restricts the expression of the transgene to pancreatic β -cells. Inducible promoters allow transgene expression at a time chosen by the investigator. The most commonly used method

of gene transfer to create transgenic mice is the direct microinjection of the DNA construct into the pronuclei of fertilized eggs [3]. The injected eggs are then implanted in the reproductive system of a pregnant mouse. If successful, the transgene integrates randomly into the genome in the one-cell stage and can be transferred to the next generation via germ cells. The level of expression of the transgene and the resulting phenotype of the transgenic mouse are dependent upon a variety of factors, including the number of copies integrated, the position of integration into the genome and the strength of the promoter used.

Gene knockout technology

A second approach to analysing the function of a gene product in intact animals is to eliminate its expression via homologous recombination targeted gene knockout. A targeting vector is created by flanking a mutated DNA sequence (the gene of interest) with DNA sequence homologous to the endogenous gene. This vector is then introduced into mouse embryonic stem (ES) cells where the mutant DNA replaces the native gene via homologous recombination. ES cells that have correctly incorporated the mutant DNA are then injected into the blastocyst of a pregnant mouse where they participate in the formation of the tissues of a chimeric animal [4]. Those chimeras that carry the mutation in their germ cells can be bred to obtain mice heterozygous and homozygous for the mutant gene. As the mutant gene encodes a major deletion or missense mutation, mice homozygous for the targeted allele do not express the native gene product and can be used to study the effect of a total lack of a given protein. Heterozygotes usually express the protein at levels 50% of normal allowing the study of the effect of gene dosage. Breeding of various heterozygous and/or homozygous transgenic/knockout animals can be used to combine alterations in the expression of multiple genes and to develop animal models of polygenic diseases. Recently, a new variation of this technology has been developed in order to inactivate a gene in a tissue-specific fashion [5]. Mice in which the target gene is flanked by bacterial DNA sequences at which crossing-over occurs (lox sites) are bred with transgenic mice expressing Cre recombinase enzyme which promotes crossing over, under the control of a tissue-specific promoter. This results in tissue-specific knockouts of gene expression. Inducible knockout can be also be generated by expression of adenovirus in the liver (see below).

Adenoviral gene transfer technology

A third approach is the adenoviral gene transfer technology. A cDNA sequence to be expressed is introduced into an adenoviral vector. The adenovirus provides an excellent *in vivo* gene transfer vehicle for

efficient hepatocyte transduction and it can be used to introduce the gene efficiently in the liver after systemic administration [6]. This technique can be used to overexpress a protein in the liver of a normal mouse, to rescue the function of a deleted gene by liver gene therapy, or to create inducible liver-specific gene knockouts using the *Cre-loxP* strategy.

■ MONOGENIC DEFECTS IN INSULIN ACTION

Type 2 diabetic patients and their first degree relatives show insulin resistance to glucose transport in skeletal muscle and adipose tissue, and impaired insulin stimulation of glycogen synthesis in muscle [1, 2]. Potential sites for this insulin resistance include any of the molecules in the insulin signaling cascade, although so far no specific defects in these pathways have been clearly associated with the common forms of type 2 diabetes. Transgenic animals represent an alternative approach to investigate the role of candidate genes in the development of insulin resistance.

Insulin receptor and insulin receptor substrate (IRS) proteins

After insulin binds to its receptor, the activated receptor tyrosine kinase catalyses autophosphorylation of the receptor and subsequent phosphorylation of intracellular substrates on tyrosine residues. The major substrates of the insulin receptor (IR) kinase in most cells are IRS-1 and -2. When phosphorylated on tyrosine residues, these proteins serve as docking platforms for src homology (SH)-2 domain containing proteins, such as Grb2 or phosphatidylinositol (PI) 3-kinase that mediate a variety of insulin's biological effects [7].

Heterozygous disruption of the IR gene ($IR^{+/-}$) results in diabetes in only 10% of adult mice, while mice homozygous for the IR knockout gene ($IR^{-/-}$) die within the first week of life from diabetic ketoacidosis, preventing detailed analysis of glucose homeostasis and insulin action at a tissue specific level [8, 9]. Attempts to rescue $IR^{-/-}$ pups by treatment with insulin-like growth factor I (IGF-1) result in a sustained decrease in plasma glucose due to increased peripheral glucose disposal and inhibition of hepatic gluconeogenesis presumably by IGF-1 acting on its own receptor [10]. However, IGF-1 was not able to stimulate lipogenesis, prevent ketosis or rescue the lethal phenotype [10]. This model also points out differences between genetic defects in mice and humans since patients with leprechaunism in which the IR is mutant or missing exhibit relatively mild hyperglycemia [11, 12].

Mutations in IRS-1 have been described in 10-20% of type 2 diabetic patients in several populations sug-

gesting that this may be an important candidate gene in the development of insulin resistance [7]. In addition, IRS-1 expression and phosphorylation are decreased in skeletal muscle from obese and type 2 diabetic patients [7]. Nevertheless, disruption of IRS-1 in mice leads to growth retardation and insulin resistance, but not diabetes. Rather, these mice develop β -cell hyperplasia resulting in increased insulin secretion which compensate for the mild insulin resistance [13, 14]. The residual insulin action in IRS-1 knockout mice also led to the discovery of an alternative signaling pathway via a second insulin receptor substrate: IRS-2. In contrast to IRS-1, disruption of IRS-2 in mice impairs both peripheral insulin action and pancreatic β -cell function. IRS-2-deficient mice show progressive deterioration of glucose homeostasis in early life, with insulin resistance in the liver and skeletal muscle and a lack of β -cell compensation both leading to overt diabetes [15]. This was the first animal example of how a monogenic mutation in an insulin signaling protein might affect both insulin secretion and insulin action leading to a phenotype of type 2 diabetes. However, so far no mutation of IRS-2 has been identified in human type 2 diabetes [16].

IRS-3 is expressed in fat, but IRS-3 knockout mice show no abnormality of glucose homeostasis [17]. Interestingly, disruption of both the IRS-1 and IRS-3 genes in mice leads to decreased white adipose tissue (WAT) mass and glucose intolerance pointing out the importance of these two molecules in mediating insulin action in fat [18]. In humans, no IRS-3 homologue has been identified suggesting that IRS-1 may be the major mediator of insulin action in adipose tissue, and thus IRS-1 variants in humans may have a more profound deleterious effect on insulin action than in mice.

Phosphoinositide 3-kinase (PI 3-kinase)

Another critical aspect of insulin action is the activation of the intracellular enzyme phosphoinositide 3-kinase (PI 3-kinase) [19]. PI 3-kinase is a heterodimer composed of a 110 kDa catalytic subunit and a regulatory subunit. In insulin sensitive tissues most of PI 3-kinase activation is mediated by the regulatory subunit $p85\alpha$ and its shorter splice variants $p50\alpha$ and $p55\alpha$. A large body of evidence obtained in cultured cells indicates that PI 3-kinase has a central role in the metabolic effects elicited by insulin, including glucose transport in muscle and fat, stimulation of glycogen synthesis and inhibition of hepatic glucose production [19]. Mice lacking only the long form ($p85\alpha$) [20] or all forms ($p85\alpha$, $p55\alpha$, $p50\alpha$) [21, 22] of regulatory subunits surprisingly show increased insulin sensitivity and hypoglycemia suggesting that $p85\alpha$ plays a negative regulatory role on insulin-dependent PI 3-kinase activation. Indeed, decreasing the expression of $p85\alpha$ seems to release this inhibitory input and improves insulin sensitivity [22]. Furthermore, decreasing $p85\alpha$ expression in animal models of genetic

insulin resistance and diabetes improves insulin resistance and almost reverses the diabetic phenotype [22]. Thus, modulation of p85 α expression is one potential therapeutic approach to the treatment of insulin resistance and type 2 diabetes.

Glucose transporters

GLUT4, the insulin-responsive glucose transporter is expressed in skeletal muscle, heart and adipose tissue, and plays a crucial role in postprandial glucose disposal [23]. Altered GLUT4 activity has been suggested to be one factor responsible for decreased glucose uptake in muscle and adipose tissue in obesity and type 2 diabetes [24]. Male mice heterozygous for the GLUT4 gene knockout (GLUT4^{+/-}) with a 50% decrease in GLUT4 expression in adipose tissue and skeletal muscle exhibit insulin resistance and diabetes [25]. They also develop hypertension and diabetic histopathologies in the heart and liver similar to those of humans with type 2 diabetes mellitus [25]. In this model, type 2 diabetes can arise from a decreased glucose transport in muscle and fat without insulin resistance in liver and without an alteration in insulin secretion [26]. Surprisingly, mice homozygous for the GLUT4 mutation (GLUT4^{-/-}) exhibit only moderate insulin resistance and are not overtly diabetic [27], suggesting that these homozygous mice compensate by the production of another yet unidentified glucose transporter.

Because glucose transport is the rate limiting step for glucose uptake in muscle and adipose tissue, the overexpression of glucose transporters has been used to improve glucose tolerance in various models. Overexpression of GLUT4 both in muscle and adipose tissue of normal mice results in enhanced insulin action with increased basal and insulin stimulated glucose transport [28-31]. More interestingly, diabetic db/db mice overexpressing GLUT4 in muscle and adipose tissue show improved glucose homeostasis [32]. Overexpression of GLUT4 specifically in muscle of diabetic mice enhances insulin action and whole body glucose disposal, thus reversing the diabetic phenotype without increasing fat mass [33, 34]. Surprisingly, overexpression of GLUT4 exclusively in adipose tissue also results in improvement of fed glucose and insulin levels, despite the fact that only about 10% of glucose uptake can be accounted for by the adipose tissue. This improvement in glucose tolerance is seen at the expense of an increase in total body fat and fat cell number [35].

GLUT1 is ubiquitously expressed and is responsible for basal glucose transport [36]. This role has been confirmed in transgenic mice with muscle specific GLUT1 overexpression. These mice exhibit reduced fasting and fed glucose, despite normal insulin levels, but resistance to insulin-stimulated glucose uptake in vivo [30, 31].

Taken together, these data suggest that manipulations of glucose transporters number especially in muscle (since it does not lead to obesity), may provide a therapeutic means to lower blood glucose.

Positive and negative modulators of insulin action

Protein-tyrosine phosphatases

Protein-tyrosine phosphatases (PTPases) act as physiological negative regulators of insulin signaling by dephosphorylating the IR and the IRS proteins thereby limiting the insulin signal. Increased expression and activation of PTPases have been observed in muscle and fat from obese and diabetic humans and rodents and are believed to be involved in the pathogenesis of insulin resistance [37, 38]. Protein tyrosine phosphatase-1B (PTP-1B) is a cytosolic PTPase that dephosphorylates and thus inhibits the IR. Disruption of the PTP-1B gene in mice (PTP-1B^{-/-}) leads to improved glucose tolerance and enhanced insulin sensitivity as a result of increased insulin-induced phosphorylation of the IR in liver and muscle [39]. Insulin stimulated glucose uptake is elevated in muscle from PTP-1B^{-/-} whereas adipose tissue is unaffected [40]. In addition, PTP-1B^{-/-} mice have decreased adiposity and are protected from diet-induced obesity [39, 40].

The leukocyte antigen-related tyrosine phosphatase (LAR) is a transmembrane PTPase expressed in insulin-sensitive tissues. LAR deficient mice show enhanced IR activation with increased basal insulin sensitivity. On the other hand, these mice exhibit resistance to insulin-stimulated glucose disposal and suppression of hepatic glucose output in euglycemic, hyperinsulinemic clamp studies [41].

Thus, inhibition of PTP-1B provides a potential therapeutic approach in the treatment of type 2 diabetes and obesity, whereas further studies are needed to define the precise role of LAR in insulin action.

G proteins

Crosstalk between the insulin signaling cascade and G-protein pathways have also been described and it has been suggested that G proteins can positively regulate insulin action. Consistent with this hypothesis, mice deficient for G_{i α 2} subunit represent a model of type 2 diabetes in which glucose intolerance develops as a result of impaired glucose transport, insulin resistance to counterregulation of lipolysis and to activation of glycogen synthase [42].

Tumor necrosis factor- α (TNF- α)

TNF- α is a potent cytokine secreted by adipocytes and mononuclear cells that exerts a myriad of biological actions in numerous different tissues, including adipocytes, through its two distinct cell surface receptors. There is now substantial evidence linking TNF- α

to the development of insulin resistance in humans and animals [43]. In studies of TNF- α or TNF- α receptor deficient mice (TNF- $\alpha^{-/-}$) with diet-induced obesity, or when these mice are crossed with genetically obese ob/ob mice, there is an improvement in glucose tolerance and insulin sensitivity. This finding suggests that deletion of TNF- α action leads to decreased insulin resistance [44]. How TNF- α -related insulin resistance is mediated is not fully understood, although an increase in insulin-stimulated serine phosphorylation of the IR in the muscle and adipose tissue of TNF- $\alpha^{-/-}$ mice is observed. Other effects include increased phosphorylation of serine residues on IRS-1, which turns down the insulin signal and elevates FFA levels via stimulation of lipolysis, and negative regulation PPAR γ [45].

Peroxisome proliferator-activated receptor- γ (PPAR γ)

PPAR γ belongs to a superfamily of nuclear hormone receptors involved in adipogenesis and other cellular processes of lipid accumulation. The ligands for PPAR γ include prostaglandins, oxidized LDL particles and the insulin-sensitizing, antidiabetic drugs, thiazolidinediones [46]. In humans, mutations in PPAR γ have been associated with certain forms of obesity and extreme insulin resistance syndromes [47, 48]. Interestingly, mice heterozygous for PPAR γ knockout gene (PPAR $\gamma^{+/-}$) show obesity resistance (on a high fat diet) [49] and enhanced insulin sensitivity [49, 50]. Elegant studies of PPAR $\gamma^{+/-}$ mice from Kadowaki's group have revealed that PPAR γ seems to play a dual role in the regulation of insulin sensitivity: Pharmacological activation of PPAR γ by thiazolidinediones enhances adipocyte differentiation, which generates small, insulin sensitive adipocytes thereby improving insulin sensitivity. On the contrary, high levels of expression of PPAR γ (when both alleles of the gene are present) promotes adipocyte hypertrophy on a high fat diet leading to the formation of large adipocytes which results in insulin resistance [49]. Thus, if the use of PPAR γ agonists like thiazolidinediones improves insulin sensitivity, reducing the level of active receptors in cells (by PPAR γ antagonist) could prove successful to treat obesity and obesity-linked insulin resistance.

CCAAT/enhancer-binding proteins(C/EBP)

C/EBP α and β are transcription factors controlling the expression of genes important for lipid synthesis and gluconeogenesis. The use of transgenic technologies has provided important insights into the role of these transcription factors in fat and liver metabolism. C/EBP α deficient mice (C/EBP $\alpha^{-/-}$) are born without lipid and glycogen reserves and die of hypoglycemia within the first hours of life because of the absence of enzymes for gluconeogenesis and glycogen synthase in liver [51]. These mice show no discernable WAT

depots. Indeed, fat specific inhibition of C/EBP α activity in a transgenic mouse leads to essentially no white fat and dramatically reduced amounts of brown fat [52]. On the other hand, C/EBP β deficient mice (C/EBP $\beta^{-/-}$) are viable and show hypoglycemia secondary to decreased hepatic cAMP levels in response to stress hormone counterregulation. This results in a 40% decrease in hepatic glucose production, mainly due to impaired glycogenolysis, without affecting gluconeogenic genes [53]. In addition these mice show decreased circulating FFA levels due to a resistance to β -adrenergic-induced lipolysis and to a decrease fat mass [53]. Given that increased hepatic glucose production and elevated circulating FFA are two cardinal abnormalities of type 2 diabetes, the design of antagonists that decrease C/EBP β activity could lead to potential new anti-diabetic therapies.

Sterol regulatory element-binding protein 1c (SREBP-1c)

Insulin increases the mRNAs encoding lipogenic enzymes, including glucokinase (which initiates the conversion of glucose to fatty acids), acetyl CoA carboxylase, and fatty acid synthase. Recent evidence indicates that this increased transcription is mediated by an insulin-regulated transcription factor designated SREBP-1c which activates transcription of multiple genes encoding enzymes responsible for the synthesis of cholesterol and fatty acids in cells [54]. In liver, evidence for the role of SREBP-1c in modulating insulin action can be summarized as follows:

- insulin treatment increases the amount of mRNA encoding SREBP-1c in livers and hepatocytes of rats [55];
- overexpression of SREBP-1c in livers of transgenic mice [56] or in isolated rat hepatocytes [57, 55] bypasses the insulin requirement and activates the same genes that are known to be activated by insulin;
- overexpression of SREBP-1c in livers of transgenic mice prevents the decline in lipogenic mRNAs that normally occurs when insulin declines during fasting [56].

Transgenic mice that express a dominantly active form of SREBP-1c specifically in adipose tissue show severe impairment in adipose tissue differentiation and a syndrome that resembles congenital lipodystrophy in humans [58].

Leptin

The role of leptin in glucose homeostasis is discussed below.

Monogenic knockout models of molecules involved in insulin action are summarized in *Table 1*.

TABLE I. Monogenic knockout mice that affect insulin action.

| Monogenic knockout mice | Phenotype | Reference |
|--|--|-----------|
| IR ^{-/-} | Early postnatal death by ketoacidosis | [8, 9] |
| IR ^{+/-} | Diabetes in 10 % of adults [22, 85, 87] | |
| IRS-1 ^{-/-} | Growth retardation Insulin resistance, normoglycemia β -cells hyperplasia, increased insulin secretion | [13, 14] |
| IRS-2 ^{-/-} | Severe insulin resistance β -cells hypoplasia, decreased insulin secretion Diabetes in early life | [15] |
| IRS-3 ^{-/-} | No abnormality of glucose homeostasis | [17] |
| p85 α ^{-/-} (long form only) | Enhanced insulin action, hypoglycemia | [20] |
| pik3r1 ^{-/-} (p85 all forms) | Hypoglycemia and perinatal death | [21] |
| pik3r1 ^{+/-} | Enhanced insulin action, hypoglycemia | [22] |
| Glut-4 ^{-/-} | Mild insulin resistance and glucose intolerance [27] Decreased fat mass. | |
| Glut-4 ^{+/-} | Insulin resistance, diabetes, hypertension | [25, 26] |
| PTP-1B ^{-/-} | Improved insulin sensitivity, obesity resistance | [39, 40] |
| G α s _i ^{-/-} | Insulin resistance, glucose intolerance | [42] |
| TNF- α ^{-/-} | Improved insulin sensitivity in obese models | [44] |
| PPAR γ ^{+/-} | Improved insulin sensitivity | [46] |
| C/EBP α ^{-/-} | Neonatal death, hypoglycemia, lack of PEPCK, G6Pase, glycogen synthase, no WAT | [51] |
| C/EBP β ^{-/-} | Hypoglycemia, decreased cellular cAMP, decreased response to counterregulation, decreased HGP, decreased lipolysis | [53] |

■ MONOGENIC DEFECTS IN INSULIN SECRETION

Glucose-stimulated insulin secretion from pancreatic β -cells is complex, involving glucose transport through the glucose transporters GLUT2, followed by phosphorylation of glucose in glucose 6-phosphate by glucokinase. The subsequent glucose metabolism results in elevation of the ATP/ADP ratio with closure of ATP-sensitive-sensitive K⁺ channels leading to depolarization of the cell with a consequent influx of extracellular calcium, triggering exocytosis of insulin secretory granules [59]. Over the past few years, evidence has accumulated suggesting that early defects in insulin secretion and the ability of the β -cells to compensate for insulin resistance are genetically determined. All signaling molecules involved in glucose metabolism within the β -cells are potential candidate genes for the insulin secretory defect of type 2 diabetes.

GLUT-2

GLUT-2 is a low-affinity transporter present in the plasma membrane of pancreatic β -cells and hepatocytes [36]. In β -cells, GLUT-2 is involved in the control of glucose-stimulated insulin secretion, and its expression is strongly reduced in glucose-unresponsive islets from different animal models of diabetes [60, 61]. In transgenic mice in which GLUT-2 expression is reduced in β -cells either by use of an antisense RNA or by insertion of a neo cassette in the gene, glucose mediated insulin secretion is impaired, but overt diabetes does not develop [62, 63].

Glucokinase (GK)

The secretion of insulin is controlled by the rate of glucose metabolism in the pancreatic β -cells. As phosphorylation of glucose by GK is the rate-limiting step for glucose catabolism in β -cells, this enzyme is referred to as the glucose sensor [64]. Mice either globally deficient in GK, or lacking GK only in β -cell, die soon after birth from severe diabetes, pointing out the importance of this enzyme in insulin secretion and glucose homeostasis [65, 66]. Transgenic rescue by overexpressing GK exclusively in β -cells of the GK-deficient mice, reverts the diabetic phenotype in 50% of the mice, the others being only mildly hyperglycemic. Thus, β -cells GK seem to play a greater role in glucose homeostasis than hepatic GK [65]. Heterozygous animals that have a 50% decrease in GK, either globally or in β -cells, develop impaired insulin secretion and mild diabetes, a phenotype in agreement with patients with maturity onset diabetes of the young Type 2 (MODY2) who have heterozygous mutations in the GK gene [67]. Mice lacking GK specifically in liver are only mildly hyperglycemic, but interestingly,

they also show impaired insulin secretion in response to glucose. The finding that alterations in glucose metabolism in liver can influence glucose metabolism in β -cells suggests an hepato-insular axis of communication involved in the regulation of insulin secretion [68, 69].

Hepatocyte nuclear factor-1 α (HNF-1 α)

HNF-1 α is a transcription factor involved in the tissue-specific regulation of genes in the liver and other tissues, including the pancreatic islets. Mutations in the HNF-1 α gene cause MODY 3 in humans [70], and animal models have shed light on the mechanism of impaired insulin secretion observed in this form of diabetes. Mice lacking the HNF-1 α gene show impaired insulin secretion in response to glucose and arginine, but normal response with non-nutrient stimuli such as potassium chloride [71, 72]. This occurs without alteration in β -cell mass. In these mice, diabetes results from defective β cell fluxes of glucose through glycolysis. This defect is potentially correctable using substrates that bypass the defect [71, 72].

Insulin promoter factor-1 (IPF-1)

IPF-1 (also called STF-1 or PDX1) is a homeodomain transcription factor expressed in pancreatic β -cells and is involved in early pancreatic development. IPF-1 also regulates the expression of a variety of pancreatic endocrine genes including insulin, somatostatin, GK, islet amyloide polypeptide (IAPP) and Glut2 [73]. In humans, heterozygosity for a point mutation in the *Ipf1* gene is associated with MODY4 [74]. Interestingly, mice lacking one allele of the *Ipf1* gene specifically in pancreatic β -cells develop an early loss of glut2 and a gradual decrease in the insulin gene expression in β -cells, resulting in impaired glucose tolerance. In addition, heterozygous disruption of IPF-1 in animal models of genetic insulin resistance with β -cell hyperplasia, impairs the ability of the islets to grow and their ability to compensate for insulin resistance leading to diabetes [75]. This model provides a mechanistic for the development of MODY4 in haploinsufficient individuals.

K_{ATP} channels

The regulation of glucose-induced insulin secretion depends on the electrical activity of the pancreatic β -cell controlled by the various ion channels present on the cell surface. Among these, K_{ATP} channels are critical in linking glucose metabolism to the electrical activity of the cells. The K_{ATP} channels in pancreatic β -cells are composed of two subunits: an inward rectifier α -subunit Kir6.2 and the sulfonylurea receptor SUR-1. To determine the role of K_{ATP} channels in pancreatic endocrine function, Takashi and co-workers

generated two kinds of genetically-engineered mice: one expressing a dominant negative form of Kir6.2 specifically in pancreatic β -cells and the other with a knockout of Kir6.2 [76]. Studies of these mice have elucidated the role of the K_{ATP} channels in glucose and sulfonylurea-induced insulin secretion. These channels are important for β -cell survival, architecture of the islets and differentiation of the islet cells. Interestingly, Kir6.2 knockout mice show only moderate glucose intolerance despite severe defects in glucose-stimulated insulin-secretion. However, as the mice become obese with age, diabetes develops. Thus the Kir6.2 knockout mice, provide a typical model of type 2 diabetes in which hyperglycemia results from the interaction between genetic defects in insulin secretion and acquired insulin resistance due to environmental factors.

Gastric inhibitory polypeptide and Glucagon-like peptide 1

Incretins are gastrointestinal polypeptides that potentiate the insulin response after an oral glucose load, which exceeds that after an I.V. injection of a similar amount of glucose. Two incretins have been identified: gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GIP is released from duodenal endocrine K cells after absorption of glucose or fat and further promotes glucose induced insulin secretion from pancreatic β -cells. GLP-1 results from post-translational processing from pro-glucagon in the L cells of the lower intestinal tract. It is believed that glucose and other nutrients directly or indirectly stimulate secretion of GLP-1 via release of GIP.

Mice with a targeted mutation of the GIP receptor gene (*GIPR*^{-/-}) exhibit glucose intolerance with impaired initial insulin response to an oral glucose load. On a high-fat diet, these mice also lose the compensatory higher insulin secretion characteristic of normal mice, and glucose homeostasis deteriorates [77].

GLP-1 receptor knockout mice (*GLP-1R*^{-/-}) exhibit hyperglycemia following an oral glucose challenge as a consequence of impaired insulin secretion [78]. However, on a high fat diet, unlike *GIPR*^{-/-} mice, *GLP-1R*^{-/-} mice maintain normal glucose tolerance. Thus, in normal mice, both carbohydrates and fat promote GIP secretion from the gastrointestinal tract, which enhances insulin secretion to compensate for the insulin resistance induced by fat, thereby maintaining glucose homeostasis. This effect is lost in *GIPR*^{-/-} mice, but not in *GLP-1R*^{-/-} mice. This is consistent with human studies of type 2 diabetes in which the insulinotropic effect of GIP is impaired while the effect of GLP-1 is preserved [79]. Taken together, these studies suggest that while GLP-1 may serve as a therapeutic agent in type 2 diabetes, the GIP/insular axis could have an important role in the pathogenesis of type 2 diabetes.

Islet amyloid polypeptide (IAPP)

Pancreatic islet amyloid (IAPP) (also called amylin) deposits are a characteristic histopathological finding of type 2 diabetes. As a result, the amylin gene has been postulated to be involved in the alterations that contribute to type 2 diabetes. However, when the human IAPP gene was overexpressed in mice, no diabetogenic effect on the β -cells was observed. Indeed, chronic overproduction and secretion of islet amyloid polypeptide was associated with increased insulin storage and enhanced secretion of insulin [80-83].

Insulin and insulin like growth factor (IGF)-1 actions in pancreatic β -cells

Insulin and IGF-1 bind to similar, but distinct, cell surface tyrosine kinase receptors, and following activation phosphorylate IRS-1 and IRS-2. The development of transgenic and knockout animals has allowed direct *in vivo* studies on the role of insulin and IGF-1 signaling on pancreatic β -cells development and function.

The most dramatic example of this is found in β -cell insulin receptor knockout (β IRKO) mice. These mice exhibit a selective loss of acute insulin release in response to glucose that results in a progressive and severe impairment of glucose tolerance, a defect similar to that seen in type 2 diabetes [84]. Thus, the IR is critical for glucose sensing by pancreatic β -cells. This finding suggests that defects in insulin action at the level of the β -cell may contribute to the impaired insulin secretion in type 2 diabetes. Subsequent studies of islets from IRS-1 and IRS-2 knockout mice and the development of mice with combined heterozygous mutations in the IR, the IGF-1 receptor, the IRS-1 and the IRS-2 molecules have shed new light into the mechanisms of β -cell insulin and IGF-1 signaling. Thus, there is increasing evidence that the IR, acting through IRS-1, is important for insulin secretion from the β -cells [85-87], whereas IGF-1 receptor acting through IRS-2 is required for β -cell growth and development [15, 88].

Monogenic knockout models of molecules involved in insulin secretion are summarized in *Table II*.

■ RECONSTRUCTION OF DIABETES AS A POLYGENIC DISEASE

The monogenic models of insulin resistance described above have provided important insights into the role of each of these proteins in insulin action but have been of limited importance to the understanding of the pathophysiology of the common form of type 2

TABLE II. Monogenic knockout mice that affect insulin secretion.

| Monogenic knockout mice | Phenotype | Reference |
|---|---|-----------|
| Glut-2 ^{-/-} | Impaired glucose-stimulated insulin secretion Glucose intolerance | [62, 63] |
| GK ^{-/-} and GK ^{+/-} (β -cells) | Perinatal death from severe diabetes | [65, 66] |
| GK ^{+/-} and GK ^{+/-} (β -cells) | Impaired insulin secretion, mild diabetes | [65, 66] |
| GK ^{-/-} (liver) | Mild hyperglycemia, impaired insulin secretion in response to glucose | [68, 69] |
| HNF-1 α ^{-/-} | Diabetes, impaired insulin secretion, defective β -cells glycolysis | [61, 72] |
| IPF-1 ^{+/-} (β -cells) | Loss of Glut-2, decreased insulin gene expression, impaired glucose tolerance | [74] |
| Kir6.2 ^{-/-} | Impaired glucose-induced insulin secretion, glucose intolerance. Diabetes in obese mice | [76] |
| GIP-R ^{-/-} | Glucose intolerance on oral glucose load Hyperglycemia on high fat diet | [77] |
| GLP-1-R ^{-/-} | Glucose intolerance on oral glucose load Normal glucose levels on high fat diet | [78] |

diabetes. On the other hand, insulin resistance in humans involves defects at multiple levels in the insulin signaling cascade, thus we and others have developed polygenic models of diabetes in mice.

For example, heterozygous IRS-1 knockout mice do not develop diabetes [13, 14], while heterozygous disruption of the IR results in diabetes in a small percentage of the animals [85]. However, mice double heterozygous for null alleles in the IR and IRS-1 genes (IR/IRS-1^{+/-}) exhibit a synergistic impairment of insulin action and develop severe insulin resistance in muscle and liver with compensatory β -cell hyperplasia leading to hyperinsulinemia to compensate for insulin resistance. In midlife, half of these mice be-

come overtly diabetic [85]. This mouse model in which diabetes arises in an age-dependent manner from the interaction between two genetically-determined, subclinical defects in the insulin signaling cascade, demonstrates the role of epistatic interactions in the pathogenesis of type 2 diabetes.

In another model, when IRS-1 deficient mice (that are euglycemic) are crossed with heterozygous β -cell glucokinase deficient mice (that show mild and non progressive glucose intolerance), the resulting double knockout mice develop overt hyperglycemia despite β -cell hyperplasia providing direct evidence that non-diabetogenic abnormalities in insulin action and insulin secretion can act together to cause type 2 diabetes when they coexist [89].

Mice double heterozygous for the IR and IRS-2 knockout genes have also been generated. In these mice, diabetes develops at a frequency similar to that of the IR/IRS-1^{+/-} mice, but while they develop severe insulin resistance in liver, there is minor insulin resistance in muscle and modest β -cell hyperplasia [87]. Triple heterozygotes IR/IRS-1/IRS-2^{+/-} develop early diabetes associated with severe insulin resistance in liver and muscle with marked β -cell hyperplasia [87].

Polygenic knockout models of type 2 diabetes are summarized in *Table III*.

■ ROLE OF INDIVIDUAL TISSUES IN THE REGULATION OF GLUCOSE HOMEOSTASIS

More recently, we and others have developed genetic models to assess the contribution of individual insulin sensitive tissues in the regulation of glucose homeostasis and to the development of type 2 diabetes.

Skeletal muscle

Using the Cre-loxP-mediated recombination strategy, Kahn and coworkers have been able to inactivate the insulin receptor gene in a tissue-specific fashion. Although insulin resistance in skeletal muscle is among the earliest detectable defects in humans with type 2 diabetes [1, 2], muscle-specific insulin receptor knockout (MIRKO) mice, which exhibit no early insulin signaling events in muscle, show no alteration in glucose homeostasis [90]. Rather, these mice have elevated serum triglycerides, free fatty acids and develop increased visceral fat mass [91]. These results are consistent with a previous report of a transgenic mouse overexpressing a kinase deficient insulin receptor (resulting in decreased receptor activity) in skeletal muscle [92]. Although, MIRKO mice have reduced glucose uptake *in vitro* and during a euglycemic clamp [93], interestingly, during a glucose tolerance test, these mice have near normal glucose uptake in muscle

[94]. This, coupled with the shift in substrates to adipose (see below) may explain why these animals do not become diabetic [94]. On the other end, MIRKO mice show an impaired insulin activation of muscle glycogen synthase resulting in a decreased muscle glycogen content [93, 94]. Thus, although insulin is necessary for storage of glucose into glycogen, insulin action in muscle is not necessary to maintain post-prandial glucose disposal in mice. Furthermore, exercise can activate postreceptor insulin signaling and increase glucose transport in muscle from MIRKO mice independently of insulin [95]. Thus, contraction of postural muscles, a non insulin-dependant mechanism of glucose uptake, could also be a factor which helps in the maintenance of glucose uptake in muscle of MIRKO mice.

Another unexplained finding is that MIRKO mice show a shift of glucose utilization from muscle towards adipose tissue with a resulting increased fat mass [94]. Indeed, this phenomenon is accentuated during hyperinsulinemic conditions [93]. This suggests that muscle insulin resistance sensitizes adipose tissue to insulin thereby promoting adiposity and that

TABLE III. Polygenic models of type 2 diabetes.

| Mice | Phenotype | Reference |
|--|---|-----------|
| IR/IRS-1 ^{+/-} | Severe muscle and liver insulin resistance, Selective β -cells hyperplasia, Development of diabetes in 50 % of adults | [85, 87] |
| IR/IRS-2 ^{+/-} | Severe liver insulin resistance, Modest β -cells hyperplasia, Development of diabetes in adults | [87] |
| IR/IRS-1/IRS-2 ^{+/-} | Early and severe diabetes Severe insulin resistance in muscle and liver Severe β -cell hyperplasia | [87] |
| IRS-1/IRS-3 ^{-/-} | Decreased fat mass, low circulating leptin levels Diabetes | [18] |
| IRS-1 ^{-/-} GK ^{+/-} | Insulin resistance, β -cell hyperplasia, Development of diabetes in adults | [89] |
| IR/p85 ^{+/-} IR/IRS-1/p85 ^{+/-} | Protection from diabetes | [22] |

muscle somehow communicates with fat to maintain euglycemia. Indeed, when transgenic mice are made with insulin resistance in both muscle and fat, hyperglycemia develops [96]. In agreement with the notion of communication between muscle and other insulin-sensitive tissues, selective insulin resistance in muscle seems to modify glucose sensing from pancreatic β -cells by a yet unknown mechanism [94]. Indeed, double tissue-specific knockout β IRKO-MIRKO mice show improved glucose tolerance compared to β IRKO mice due to improved glucose-stimulated acute insulin release [94].

Unlike the MIRKO mice, selective disruption of the Glut4 glucose transporter in muscle (MIGKO mice) leads to glucose intolerance and insulin resistance secondary to a marked decrease in muscle glucose uptake [97]. This finding, along with recent work in humans [98], suggests that glucose uptake rather than early insulin signaling events is of critical importance for the maintenance of normal glucose homeostasis by this tissue.

White adipose tissue (WAT)

White adipose tissue accounts for less than 10% of glucose uptake following an oral glucose load or during hyperinsulinemic conditions. In addition, WAT is an endocrine tissue that releases in the circulation various modulators of insulin secretion and insulin action [99]. The most striking example is leptin, an adipocyte-derived hormone that plays a critical role in the regulation of energy homeostasis. Leptin acts primarily as a satiety factor acting on the hypothalamus, decreasing food intake and increasing energy expenditure, thereby preventing excessive increase in body weight.

Selective insulin resistance in WAT in a tissue-specific knockout of the IR (FIRKO mice) in that tissue, does not alter glucose homeostasis, despite a 90% decrease in fat mass, but rather protects mice from obesity-induced glucose intolerance [100]. However, these mice still have detectable levels of circulating leptin. On the contrary, in double IRS-1/IRS-3 knockout mice in which WAT mass is also dramatically decreased but leptin levels are very low, diabetes develops in midlife [18]. In addition, other transgenic mice without WAT depots and with undetectable leptin levels develop insulin resistance, elevated lipid levels, and diabetes, a phenotype similar to that of generalized lipoatrophic diabetes in humans [52, 58]. In this later model, restoration of circulating leptin levels either by leptin infusion [101] or by surgical implantation of WAT [102], reverses the diabetic phenotype. These results indicate that insulin resistance and diabetes in at least some of these lipodystrophic mice can be caused by a deficiency of leptin secondary to a failure of adipocyte differentiation.

As noted above, leptin is a modulator of whole body glucose homeostasis, apparently via its direct

effects on the hypothalamus and by indirect mechanisms on muscle and liver [103, 104]. The importance of leptin in the maintenance of glucose disposal, is even more apparent by the finding that chronic overexpression of leptin in mice results in complete disappearance of adipose tissue with increased insulin action in muscle and liver [105]. Thus, leptin acts as an adipocyte derived anti-diabetic hormone *in vivo*, and leptin deficiency appears to lead to insulin resistance and diabetes. Understanding the mechanisms of leptin-induced glucose disposal in peripheral tissue is of critical importance to the understanding of the regulation of glucose homeostasis and to the development of new hypoglycemic agents.

Brown adipose tissue (BAT)

Brown adipose tissue (BAT), because of its capacity for uncoupled mitochondrial respiration, is an important site of facultative energy expenditure. Thus, this tissue plays a critical role between food intake and obesity. Lowell *et al.* used a transgenic toxigen approach to specifically eradicate BAT in mice [106]. These mice which have decreased brown fat, develop obesity early in life. Interestingly, in mice in which BAT subsequently regenerates, obesity resolves. Mice with persistent BAT deficiency develop obesity in absence of hyperphagia, indicating increased metabolic efficiency in absence of brown fat [106]. When marked obesity is reached in adults, mice show severe insulin resistance in muscle and fat, and diabetes develops [107]. When BAT-deficient mice are fed a Western diet, there is a synergistic effect of high fat food and decreased BAT to cause marked obesity and its metabolic related disorders [108]. Taken together, this work provides evidence for a role of BAT in the protection from diet-induced obesity and type 2 diabetes. Recently Benito *et al.* reported in abstract form that in BAT insulin receptor knockout mice (BAT-IRKO), hyperglycemia develops without obesity [109].

Liver

The development of fasting hyperglycemia in type 2 diabetes is believed to be secondary to an increased hepatic glucose production, mainly through an increase in gluconeogenic pathways. The importance of the liver in the maintenance of normoglycemia has been studied by different approaches. Mice overexpressing phosphoenolpyruvate carboxykinase (PEPCK) or glucose-6-phosphatase (G6Pase), the key enzymes of gluconeogenesis, show unsuppressed hepatic glucose production, despite hyperinsulinemia, resulting in diabetes [110, 111]. This confirms the central role of the liver in propagating fasting hyperglycemia in type 2 diabetes.

Disruption of insulin action in liver by tissue specific knockout of the insulin receptor (LIRKO mice) also leads to severe glucose intolerance and resistance to the blood glucose-lowering effect of insulin. This provides evidence of a direct role of liver in post-prandial glucose homeostasis, and suggests that a considerable portion of the decrease in blood glucose following insulin administration is due to a suppression of hepatic glucose production rather than an increase in muscle glucose uptake. In these LIRKO mice, because of absence of insulin receptor-mediated clearance of insulin by the liver, insulin concentrations are extremely high. In addition LIRKO mice exhibit a six fold increase in pancreatic β -cell mass [112].

The ultimate development of fasting hyperglycemia in type 2 diabetes results from a β -cell failure to suppress hepatic glucose output. The three genetic models presented here demonstrate that the ability of the β -cell to compensate for insulin resistance in the liver and to prevent the resulting hyperglycemia is limited. In addition, the compensatory hyperinsulinemia that occurs in insulin-resistant states may further increase insulin resistance in the liver via downregulation of the insulin signaling cascade itself. Indeed, transgenic mice overexpressing insulin in the liver develop hyperinsulinemia and glucose intolerance with aging as a result of insulin receptor downregulation without any defect in pancreatic insulin secretion [113, 114]. In addition, in lipoatrophic mice, chronic hyperinsulinemia leads to a decrease IRS-2 expression in liver with insulin resistance to gluconeogenic pathways (unsuppressed hepatic glucose production) but hyperstimulation of lipogenic pathways (increased triglycerides production) [90]. Thus, hepatic insulin resistance in type 2 diabetes may be an acquired phenomenon secondary to chronic hyperinsulinemia.

The possibility of controlling hepatic glucose utilization and production as a treatment of type 2 diabetes has been explored by Ferre *et al.* using a transgenic approach to overexpress GK in the liver of diabetic mice under the control of the PEPCK promoter [115]. In the liver of these diabetic mice expressing GK, glycolysis and glycogenesis were induced while gluconeogenesis and ketogenesis were blocked. This was associated with normalization of blood glucose, ketone bodies, triglycerides, and free fatty acids, even in the absence of insulin [115]. Treatment of mice made diabetic by high fat diet or obese ob/ob mice by injection adenovirus expressing the human hepatic GK in liver leads to suppression of hepatic glucose output and correction of hyperglycemia [116]. These results suggest that overexpression of GK by gene therapy in liver is a potential approach in the treatment of type 2 diabetes.

Brain

IR are also expressed in "insulin-insensitive tissues" and are widely distributed throughout the central

nervous system (CNS), especially in the hypothalamus and the pituitary where they share specific patterns of distribution. Mice with tissue specific disruption of the IR gene in the CNS (NIRKO mice) show no abnormality of brain development or neuronal survival but surprisingly develop diet-induced obesity and insulin resistance [117]. These mice show increased fat mass and plasma leptin levels, hyperinsulinemia and hypertriglyceridemia. Thus, insulin acting in the CNS through its receptor appears to provide a negative feedback loop for post-prandial inhibition of food weight and plays a role in the regulation of body weight at a central level. NIRKO mice also exhibit dysregulation of pituitary LH secretion resulting in impaired spermatogenesis in males and ovarian follicle maturation in females. One possibility is that IR expressed on GnRH-producing neurons induces GnRH synthesis or secretion [117]. This study reveals an important link between brain insulin action and reproductive endocrinology. Indeed, in severe insulin resistance syndromes, the hypothalamic-pituitary-ovarian axis is perturbed and polycystic ovarian disease can develop.

Vascular endothelium

Endothelial cells mediate the accessibility of hormones and nutrients to tissues. Endothelial cells also express the insulin receptor and respond to the metabolic and growth-promoting effects of this hormone. Insulin has also been shown to promote vasodilatation and increase blood flow by an endothelial-dependent mechanism. This effect is impaired in IRS-1 knockout mice [118] suggesting that hypertension associated with type 2 diabetes or obesity might result from insulin resistance in the vascular endothelium itself. The physiological relevance of the vascular effects of insulin on glucose homeostasis is currently being assessed in mice with a vascular endothelial cell specific insulin receptor knockout (VENIRKO mice).

Tissue-specific knockout mice are summarized on Table IV.

■ IMPACT OF THE GENETIC BACKGROUND ON INSULIN ACTION, INSULIN SECRETION AND THE INCIDENCE OF DIABETES

Insulin resistance is known to have an important genetic component, but efforts to identify the exact predisposing genes have thus far been unsuccessful. When β IRKO mice are generated on a mixed genetic background, a significant number of the animals develop peripheral insulin resistance leading to overt diabetes ([94] and F.Mauvais-Jarvis, C.R.Kahn: unpublished observations), a phenotype much stronger

TABLE IV. *Tissue-specific knockout mice.*

| | Phenotype | Reference |
|--------------------|---|--------------|
| MIRKO | Normal glucose and insulin levels Dyslipidemia and increased adiposity | [91, 93-95] |
| LIRKO | Severe insulin resistance Fasting hyperglycemia Hyperplastic islets | [112] |
| FIRKO | Normal glucose and insulin levels Decreased fat mass Protection from obesity-induced diet | [100] |
| β IRKO | Loss of glucose-induced first phase insulin release Age dependent glucose intolerance Decreased islet size and pancreas insulin content Diabetes in obese mice | [84] [94] |
| β IRKO-MIRKO | Mild glucose intolerance | [94] |
| NIRKO | Diet-induced obesity, insulin resistance, dyslipidemia Dyregulation of LH secretion, impaired spermatogenesis, Impaired ovarian follicle maturation | [117] |
| Muscle Glut4 KO | Insulin resistance Glucose intolerance | [97] |

than previously reported [84]. Moreover, when double heterozygous knockout IR/IRS-1 mice are generated on different genetic strains of mice, the incidence of diabetes for the same given mutation varies from almost 100% penetrance to protection from diabetes depending on the genetic background used [119]. To begin to identify such genetic loci predisposing to insulin resistance, Accili and coworkers performed a genome wide scan with polymorphic markers between different strains of mice with a targeted null allele of the IR gene. They have tentatively identified a locus on chromosome 2 that shows linkage to elevated plasma insulin levels [120]. Thus there may be a modifier gene that influences β -cell function, degree of insulin resistance and the incidence of diabetes.

This may also help explain why phenotypical features in the onset and outcome of type 2 diabetes vary among the various human populations.

■ CONCLUSION AND PERSPECTIVES

Although the precise determinants of type 2 diabetes are still unclear, major advances have been provided through the use of genetically-modified animal models in our understanding of the *in vivo* mechanisms of regulation of insulin action, the determinants of insulin secretion and the potential dysregulation that lead to hyperglycemia. What is the cause of the common form of type 2 diabetes? Polygenic models have strengthened the idea that minor defects in insulin secretion and/or insulin action can lead to diabetes when combined, pointing out the importance of epistatic interactions of different genetic loci in the production of diabetes. The experience of tissue-specific disruption of insulin action has shed light on the possible contribution of insulin-sensitive and "insulin-insensitive" tissues in the development of hyperglycemia. Thus, insulin resistance in muscle does not alter muscle glucose uptake but rather, promotes obesity and dyslipidemia, a phenotype similar to the metabolic syndrome in humans. White adipose tissue, previously considered as a static fat store, is now envisioned as a dynamic endocrine tissue playing a critical and central role in the regulation of glucose homeostasis and energy metabolism, through release of modulators of insulin action, glucose uptake and insulin secretion. The finding that insulin actions in pancreatic β -cells and in brain are important for normal glucose sensing and prevention of obesity, brings a unifying hypothesis in which insulin resistance at the level of muscle, adipose, liver, pancreatic β -cells and brain, may act synergistically to induce obesity, insulin resistance, dyslipidemia and the full-blown metabolic phenotype of type 2 diabetes. Also of interest is the emerging idea that these tissues can communicate between each other to regulate glucose homeostasis. Muscle seems to communicate with fat and liver, and muscle, liver and fat can all modulate insulin secretion from pancreatic β -cells possibly via endocrine or metabolic mechanisms. Thus, impaired communication between insulin-sensitive tissues could also contribute to the pathogenesis of type 2 diabetes.

Another interesting application of the transgenic/knockout technology is the discovery of new drug targets, which when altered may improve the disease process. Such potential therapeutic targets are summarized in Table V. These mutant mice are also of potential interest for testing drugs aimed at correcting specific metabolic abnormalities that are a primary cause of hyperglycemia rather than a secondary change.

It is likely that transgenic knockout technology will improve and that animal models combined with gene expression analysis technologies, such as DNA mi-

TABLE V. Potential therapeutic targets in insulin resistance and type 2 diabetes.

| Therapeutic approach | Phenotype | Reference |
|---|--|------------|
| Increase peripheral glucose uptake | | |
| Glut-4 overexpression in muscle and fat | Increased insulin action and glucose in normal mice (muscle and fat) | [28-31]. |
| | Improved glucose homeostasis in diabetic db/db Mice (muscle and fat) | [32] |
| | Increase insulin action, glucose disposal, improves diabetes (muscle) | [33, 34] |
| PTPases antagonists | Increased muscle insulin action in normal mice Resistance to diet-induced obesity | [39, 40] |
| TNF- α antagonists | Improved insulin action and glucose homeostasis in obese TNF- α KO mice. | [44] |
| Decreasing PPAR γ expression | Improvement of insulin sensitivity, resistance to obesity in PPAR $\gamma^{+/-}$ mice | [49, 50] |
| Decreasing p85 α expression | Improves insulin action in normal mice, improves diabetes in insulin resistant diabetic mice | [22] |
| Decrease hepatic glucose production | | |
| Overexpression of GK in liver | Suppression of HGP, correction of the diabetic phenotype in lean and obese diabetic mice | [115, 116] |
| C/EBP β antagonists | Suppression of HGP, decreased circulating FFA | [53] |

croarrays, will prove to be a powerful tool for the classification of the function of genes, and for the exploration of the role of these genes in diagnosis and treatment of diabetes.

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