Molecular Approaches to Study Control of Glucose Homeostasis

Nicole Neubauer and Rohit N. Kulkarni

Abstract

Type 2 diabetes is a polygenic disease that can lead to severe complications in multiple tissues. Rodent models have been used widely for investigating the pathophysiology underlying type 2 diabetes and for examining the potential link with obesity, largely due to the limitations of invasive testing and of studying detailed molecular mechanisms in human tissues. Among rodents, the mouse model is especially popular because mice are easy to manipulate genetically, have a short generation time, and are relatively inexpensive. The most commonly used inbred mouse strains are reviewed in addition to several genetically engineered mouse models that have been generated to study type 2 diabetes in the context of obesity, with a focus on insulin, leptin, and peroxisome proliferator-activated receptor (PPAR) signaling pathways.

Key Words: inbred mouse strains; insulin; genetically engineered mouse models; leptin; obesity; peroxisome proliferator-activated receptor; signaling; type 2 diabetes

Introduction: Diabetes—Problems and Challenges

Light control of energy metabolism is critical for optimal functioning of all tissues in mammals. Among nutrients, glucose is the major source of fuel for energy metabolism, and a minimum level of circulating blood glucose is necessary for the normal functions of organs. A small increase in blood glucose, which is sustained over a prolonged period, or a significant increase in blood glucose even over short periods, can induce cellular toxicity in susceptible tissues including pancreatic β-cells, neurons, renal cells, and retinal cells. Patients with diabetes are therefore at high risk for developing severe complications that result from hyperglycemia including blindness, kidney failure, amputations, and cardiovascular disease. Therefore, knowledge of the factors and pathways that are essential for maintaining blood glucose within the physiological range is crucial for understanding the pathophysiological basis of diabetes and for planning therapeutic strategies to prevent the disease and/or limit its complications.

The two most common forms of diabetes are type 1 and type 2 diabetes. Type 1 diabetes (insulin-dependent diabetes mellitus) is also known as juvenile onset diabetes, is generally diagnosed in younger patients, and constitutes approximately 8 to 10% of all cases of diabetes. It is caused by irreversible autoimmune destruction of insulin-producing β-cells in the pancreas and requires lifelong insulin replacement therapy (Mathis et al. 2001; McDevitt 2004). Other, rare forms of diabetes have also been characterized, including maturity-onset diabetes of the young (MODY) (Giuffrida and Reis 2005), gestational diabetes (Buchanan and Xiang 2005), and latent autoimmune diabetes in adults (LADA) (Fourlanos et al. 2005). MODY has a strong genetic component and has been described to be secondary to mutations in genes coding for transcription factors and glucose-sensing proteins (reviewed in Shih and Stoffel 2002). Humans carrying single gene mutations of predominantly transcription factors have revealed important insights into β-cell development and the cause of MODY (Fajans et al. 2001; Servitja and Ferrer 2004). Thus, mutations in hepatocyte nuclear factor (HNFα)-4α, which is crucial for liver and β-cell differentiation, cause MODY1 in humans (Yamagata et al. 1996a). Mutations in HNF-1α, which is regulated by HNF4α during development, underlie MODY3 (Yamagata et al. 1996b). Mutations in HNF-1β, which is highly homologous to HNF1α, cause MODY5 (Horikawa et al. 1997); and mutations in insulin promoter factor-1/PDX-1 and NeuroD have been identified in patients with MODY4 and MODY6 (Malecki et al. 1999; Stoffers et al. 1997). A very common form—MODY2—is caused by mutations in the gene coding for glucokinase (Gidh-Jain et al. 1993).

Abbreviations used in this article: βIGFIRKO, β-cell-specific insulin-like growth factor 1 receptor knockout; β-IRKO, β-cell-specific insulin receptor knockout; Akt, v-akt murine thymoma viral oncogene homolog 1; GLUT, glucose transporter; HNF, hepatocyte nuclear factor; IGF-1, insulin-like growth factor-1; IRS, insulin receptor substrate; LADA, latent autoimmune diabetes in adults; MODY, maturity-onset diabetes of the young; NSY mouse, Nagoya-Shibata-Yasuda mouse; PGC-1, peroxisome proliferator-activated receptor-γ coactivator-1; P13K, phosphatidylinositol 3′-kinase; PPAR, peroxisome proliferator-activated receptor; SNP, single nucleotide polymorphism; TZD, thiazolidinedione.

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Gestational diabetes occurs during pregnancy and is usually reversed after delivery (Ben Haroush et al. 2004). LADA, also referred to as type 1.5 diabetes, is characterized by late onset and the presence of islet cell antibodies. LADA patients are typically older than 25 yr of age when they manifest symptoms of the disease. The residual insulin secretion in these patients makes them insulin independent for at least 6 mo after diagnosis. However, these patients also carry islet cell autoantibodies, although the destruction of β-cells is slow (Naik and Palmer 2003). Type 2 diabetes (also known as non-insulin-dependent diabetes mellitus) is generally diagnosed in adult individuals and constitutes more than 90% of all cases of diabetes (CDC 2005). Recently, this form of diabetes has also been reported to correlate with increased prevalence of obesity in young individuals, implicating an early onset of type 2 diabetes due to obesity (Deckelbaum and Williams 2001; Drude and Bloom 2006; Ludwig 2003; Quarry-Horn et al. 2003; Spiegelman and Flier 2001).

Approximately 150 million people worldwide have diabetes mellitus, and the number is expected to double by the year 2025 due to a combination of population growth, aging, unhealthy diets, obesity, and lifestyle (Zimmet et al. 2001). In the United States alone, more than 18.2 million people are currently estimated to have diabetes (13 million diagnosed cases; 5.2 million undiagnosed cases) (http://www.cdc.gov/diabetes), which corresponds to 6.3% of the population. The prevalence of obesity shows remarkably similar increasing trends (Flegal et al. 1998, 2002), and the increase in obesity is thought to contribute significantly to the growing incidences of diabetes (Haslam and James 2005). Although it is difficult to precisely determine the financial burden caused by the different forms of diabetes and the treatment of its complications, a recent estimate placed the figure at 132 billion dollars in the United States alone (Hogan et al. 2003). The precise environmental, lifestyle, and/or one or more genetic factors that promote the development of type 2 diabetes in susceptible individuals is still unclear; however, it is evident that both insulin resistance and pancreatic β-cell dysfunction are essential for full development of the disease (Accili 2004; DeFronzo 1997; Kahn 1994, 2003; Polonsky et al. 1996).

Although all forms of diabetes are characterized by uncontrolled hyperglycemia, other hormonal parameters are also frequently dysregulated in type 2 diabetes and contribute to the pathology of the disease. These parameters include insulin, glucagon, and metabolites (e.g., free fatty acids and triglycerides) (Dunning et al. 2005; Erdmann 2005). For example, abnormal accumulation of lipids in different tissues has been associated with reduced insulin sensitivity (Dresner et al. 1999; Kim et al. 2001). The causal interaction between glucose and lipid metabolism is not fully understood; however, the family of nuclear hormone receptors known as peroxisome proliferator-activated receptors (PPARs) has been a focus of considerable study in this context over the last several years. Three PPARs have been identified: α, β (also termed δ), and γ (Evans et al. 2004).

All three receptors form dimers with another nuclear receptor, the retinoid X receptor. Upon binding to an endogenous ligand, PPAR activates the transcription of a variety of cellular genes. PPAR-α can be activated by different activators like fatty acids or steroids and is the target of fibrate antithrombotic and hypolipidemic agents. PPAR-γ is expressed predominantly in adipocytes and is necessary for adipocyte differentiation. The endogenous ligand for PPAR-γ is presumed to be fatty acids and their derivatives, and PPAR-γ has been identified as a target for thiazolidinediones (TZDs), which are known insulin sensitizers (Eibl et al. 1996). Transcriptional activity of PPAR can be enhanced significantly by binding to coactivators such as PPAR-γ coactivator-1 (PGC-1) (Puigserver et al. 1998).

In addition to metabolic and environmental factors, there is evidence that development of type 2 diabetes includes a genetic component. In the search for diabetes-susceptibility genes, patients with type 2 diabetes have been examined for single nucleotide polymorphisms (SNPs) to identify candidate genes using linkage analyses. Genome-wide linkage analysis is a complex approach that has also been used with some success in obesity research to link appetite regulation, metabolism, and insulin signaling. However, empirical data that prove to be significant determinants for development of diabetes in one population can be completely absent in other populations and can make this approach very difficult. In addition, large pedigrees are difficult to find for linkage analysis studies due to the late onset of the disease, so that older generations are deceased while younger generations have not yet developed the disease (O’Rahilly et al. 1988). Nevertheless, it is becoming increasingly clear that most cases of type 2 diabetes likely result from a combination of gene mutations (polygenic) and environmental factors (Hansen and Pedersen 2005). One such example includes a recent study that described the inheritance of severe insulin resistance in a family with mutations in both the protein phosphatase 1 and PPAR-γ genes (Savage et al. 2002), supporting the notion that a combination of factors contributes to development of insulin resistance and hyperglycemia.

Because a considerable number of studies investigating the pathophysiology of diabetes have focused on the insulin/insulin-like growth factor (IGF) system, leptin signaling, and the pathways related to PPARs, we focus in this review on mouse models that have been studied and/or created to investigate these pathways (Figure 1). The discussion below is intended to complement the accompanying reviews in this issue of ILAR Journal that are focused on other rodent models.

Models in Diabetes Research

Due to the limitations of performing invasive in vivo studies in humans, several laboratories have focused on creating and studying animal models that mimic the human disease. These models provide a unique opportunity to study the
onset, development, and course of the disease and, more importantly, to understand the molecular mechanisms that lead to diabetes. Among mammals, the mouse model has several advantages, which include a complete knowledge of the genome, ease of genetic manipulation, a relatively short breeding span, and adaptability to physiological and invasive testing.

Mice with Naturally Occurring Mutations

Mice with naturally occurring mutations have provided a unique resource to study the development of several diseases including type 2 diabetes and obesity (Leiter and Reifsnyder 2004; Loskutoff et al. 2000). A widely used mouse model for type 1 diabetes is the nonobese diabetic mouse, which develops diabetes spontaneously (Anderson and Bluestone 2005; Makino et al. 1980). To study type 1 diabetes, mouse models can also be generated by streptozotocin or alloxan injection (Szkudelski 2001). A single high dose of streptozotocin can be sufficient for the total destruction of the pancreatic β-cells resembling the insulin-dependent phenotype whereas low doses have been used to study insulitis (Like and Rossini, 1976; Rees and Alcolado 2005).

Among the most widely studied models for type 2 diabetes are the Lep“ob” (obese) and Lep“db” (diabetes) mice. These obese models have mutations either in the leptin gene or in the leptin receptor gene, respectively (Chung et al. 1996; Zhang et al. 1994). The Lep“db” mouse becomes hyperinsulinemic within 2 wk of age followed by obesity at 3 to 4 wk and hyperglycemia at age 4 to 8 wk (Bates et al. 2005). The Lep“ob” mouse is a suitable model for studying type 2 diabetes because the hyperglycemia is rather mild in these animals (Bandsma et al. 2004). In the Lep“ob” model, hyperinsulinemia manifests at 3 to 4 wk of age and is coupled with hyperphagia and insulin resistance (Folli et al. 1993; Saad et al. 1992). Characteristically, the obesity predisposes these mice to diabetes and has been used to examine the impact of obesity on development of diabetes.

Using point mutagens such as N-ethyl-N-nitrosourea, investigators have introduced mutations that have caused a diabetic phenotype with the aim of identifying new genes that may have an impact on the development of diabetes (Toye et al. 2004). This approach is similar to studies in humans wherein genome linkage analysis is used to identify mutated genes causing the hyperglycemic phenotype.

Many established mouse models are available commercially. The Jackson laboratory (http://jaxmice.jax.org/models/diabetes_obesity.html) is an excellent source that provides detailed background information of the different strains used in diabetes and obesity research.
Inbred Mouse Models

Similar to humans, the phenotype of a mouse model also depends on the genetic background, gender, and age (Cheverud et al. 2004; Goren et al. 2004; Ueda et al. 2000). For example, mice on the C57Bl/6 background are more susceptible to obesity and diabetes when fed a high-fat diet (Black et al. 1998) whereas mice on the dilute brown non-agouti 2/2 (DBA/2) background manifest islet failure earlier than other strains (Kulkarni et al. 2003). The first proof of a synergistic impact of several pro-diabetic genes has been made by generating hybrid mice strains (Cahill et al. 1967) and in studies using different background strains (Hummel et al. 1972). The use of models to enable examination of the double heterozygote models for insulin receptor and insulin receptor substrate (IRS) 1 null alleles takes a similar approach, and such studies provide an opportunity to study molecular mechanisms behind genetically complex diseases such as diabetes in more detail (Bruning et al. 1997).

The sex of the mouse also plays a role, with males being more susceptible to manifesting phenotypes in studies relating to glucose homeostasis (Toye et al. 2005). Finally, the age of the mouse can also affect the outcome (Kodama et al. 1994; Wyse and Dulin 1970). The phenotype of young versus old mice varies between rodent models. For example, in Lepdb mice, hyperinsulinemia occurs at an early age and the subsequent β-cell failure leads to hyperglycemia by age 8 wk. However, the Nagoya-Shibata-Yasuda (NSY) 1 mice develop diabetes at a slower pace, with insulin resistance not manifesting until after 12 wk of age (Ueda et al. 2000).

Selected inbreeding has yielded the KK mouse, which shows moderate obesity, hyperinsulinemia, and hyperglycemia (Reddi and Camerini-Davalos 1988). The NSY mouse, which manifests hyperglycemia (200 mg/dL) at 120 min during an intraperitoneal glucose tolerance test, has been defined as “diabetic” by the investigators (Ueda et al. 1995, 2000). These mice also develop increased epididymal fat pads in an age-dependent manner and develop an accelerated diabetic phenotype that is characterized by impaired insulin secretion and altered insulin sensitivity in response to a high-fat diet and administration of sucrose (Ikegami et al. 2004).

Genetically Engineered Mouse Models

Since the mid-1990s, transgenic and knockout mice have become powerful tools to study the role of gene coding for individual proteins in the development of diabetes (Kahn et al. 2000; Kulkarni and Okada 2002; Leiter 2002; Mauvais-Jarvis et al. 2002).

Global Knockouts and Transgenics

Insulin and IGF 1 belong to the growth factor family and are known to play roles in metabolism and growth of virtually every tissue in mammals. It is therefore not surprising that studies directed toward creating mouse models of diabetes have focused on targeting proteins in the insulin/IGF-1 signaling pathways. Furthermore, although most studies have focused on classic target tissues (e.g., skeletal muscle, liver, and adipose), other studies in noncanonical tissues (e.g., the pancreatic islets and brain) have yielded unexpected phenotypes. We review some of these experiments and provide references of more comprehensive reviews for interested readers.

After insulin is synthesized and released upon glucose stimulation of the islet β cells, it acts by binding to its receptor in metabolic tissues that include the liver, skeletal muscle, and adipocyte. Upon binding to its receptor, insulin leads to autophosphorylation of the tyrosine kinase, which in turn activates the IRS/phosphoinositide 3′-kinase (PI3-K) pathway and the Ras-Raf/mitogen-activated protein kinase pathway (Backer et al. 1992; Folli et al. 1992; Kasuga et al. 1982; Sun et al. 1991). Global insulin receptor knockout mice show hyperglycemia and hyperketonemia within hours after birth (Accili et al. 1996) and die within days after birth from diabetic ketoacidosis (Table 1). This phenotype is in contrast to patients with mutations in the insulin receptor who show severe growth retardation and only mild diabetes (Taylor 1992).

Mice carrying null mutations for different IRS genes have also been generated and manifest distinct phenotypes. Mice lacking IRS-1 protein globally exhibit intrauterine growth retardation, reduced body mass, and mild hyperinsulinemia without overt diabetes (Araki et al. 1994; Tatemoto et al. 1994).

Interestingly, studies on islets and β-cell lines, derived from IRS-1 knockouts, have revealed a β-cell defect characterized by altered Ca2+ flux and expression of sarcoplasmic reticulum Ca2+-ATPase (SERCA) genes (Kulkarni et al. 1999b, 2004). In IRS-1 knockouts, the presence of a β-cell secretory defect even when coupled with mild insulin resistance failed to promote the development of overt diabetes, which could be due to compensatory upregulation of IRS-2 (Hemmige et al. 2005). Disruption of the IRS-2 gene in mice caused an insulin-resistant phenotype in which the β-cells could not compensate due to a failure of islet hyperplasia leading to a mild or severe diabetes phenotype depending on the genetic background of the founder mice (Kubota et al. 2000; Withers et al. 1998) (Table 1). These data are consistent with multiple observations that the severity of phenotypes in genetically engineered rodent models is dependent on the background strain used (Goren et al. 2004 and references therein).

Two more IRS proteins have been identified in the mouse, but disruption of genes coding for these proteins have shown little or no effect. The knockout of the IRS-3 gene, which is abundantly expressed in mouse adipocytes but has not been identified in humans, showed virtually no effect on growth and glucose homeostasis (Bjornholm et al. 2002; Liu et al. 1999). Furthermore, the knockout of IRS-4 led to minimal or no effects on growth and glucose homeostasis (Fant et al. 2000) (Table 2).
Knockout studies in which PI3K was targeted by eliminating its regulatory subunit p85 or its shorter splice variant p55 and p50 led to increased insulin sensitivity in mice (Chen et al. 2004; Terauchi et al. 1999; Ueki et al. 2002). In contrast, mice heterozygous for the null allele of the catalytic subunit of PI3K—p110—were slightly glucose intolerant, were hyperinsulinemic, and showed reduced expression of the regulatory subunit p85 (Brachmann et al. 2005). Deletion of the p38 MAP kinase subunit p38α has been described to be embryonic lethal whereas heterozygote mice show no obvious abnormalities (Allen et al. 2000). In another study, some p38α knockout animals survived and were anemic (Tamura et al. 2000).

Downstream of the IRS/PI3K pathway, v-akt murine thymoma viral oncogene homolog 1 (Akt1) is activated Akt (also referred to as protein kinase B). Three Akts that have high sequence homology (Akt-1, -2, and -3) have been identified. Targeted disruption of the most abundantly expressed Akt-1 revealed no diabetic phenotype (Chen et al. 2001). In contrast, disruption of Akt-2, which is expressed at higher levels than Akt-1 in insulin-responsive tissues, resulted in a phenotype characterized by hyperinsulinemia and hyperglycemia in both sexes, indicating that Akt-2 rather than Akt-1 is the predominant mediator of the insulin signal. Interestingly, Akt-2 null mice showed no apparent growth defects (Cho et al. 2001). The hyperplasia of islets in this animal model indicates a potential cross-talk between tissues that allows a compensatory response. It also indicates that the ability of insulin to promote islet growth and/or anti-apoptosis is maintained in islets while insulin action is impaired largely in peripheral tissues.

Other factors that are of therapeutic interest in diabetes research are glucokinase, often referred to as the islet/liver glucose sensor of liver and islets, and the insulin-sensitive glucose transporter (GLUT-4). Null mice for glucokinase die perinatally due to severe hyperglycemia. In contrast, mice that carry only one allele for glucokinase are born normally with body weights similar to control mice but develop hyperglycemia (from day of birth) and reduced insulin secretion (Grupe et al. 1995), suggesting an essential role for glucokinase in glucose homeostasis. In the same study, it was reported that re-expression of glucokinase exclusively in the β-cell is sufficient for survival, indicating a critical tissue-specific role for the protein (Table 1). GLUT-4 is predominantly expressed in muscle and adipose tissue, the major sites for postprandial glucose disposal. Knockout mice for GLUT-4 display growth retardation, decreased longevity associated with cardiac hypertrophy, and small fat depots (Katz et al. 1995). Altered glucose homeostasis, characterized by postprandial hyperinsulinemia, is evident only in males. This rather mild phenotype in GLUT-4 null mice can be explained by compensatory actions of GLUT-2 and GLUT-1, which are upregulated in liver and heart, respectively, in these animals.

Other pathways that can influence glucose homeostasis include signaling via the PPARs and the adipose-derived hormone leptin. Several mouse models depleted for individual PPARs have been generated. Whereas the global

### Table 1 Mouse models manifesting overt diabetes

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Phenotype</th>
<th>Body weight</th>
<th>Sex examined</th>
<th>Reference (see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin receptor (IR) knockout</td>
<td>Severe hyperglycemia and hyperketonemia; the mice die within days after birth due to ketoacidosis</td>
<td>Normal development, no growth retardation</td>
<td>No difference reported</td>
<td>Accili et al. 1996</td>
</tr>
<tr>
<td>Insulin receptor substrate-2 (IRS-2) knockout</td>
<td>Hyperglycemia, insulin resistance, and hyperinsulinemia with a failure of islet hyperplasia</td>
<td>10% growth retarded</td>
<td>NS, but males die from dehydration and hyperosomolar coma whereas females do not</td>
<td>Witters et al. 1998</td>
</tr>
<tr>
<td>Glucokinase knockout</td>
<td>Null allele mice die perinatally with severe hyperglycemia</td>
<td>ND</td>
<td>No difference reported</td>
<td>Grupe et al. 1995</td>
</tr>
<tr>
<td>Insulin receptor (IR) in β-cell knockout (βIRKO)</td>
<td>Reduced β cell mass and decreased insulin secretion lead to hyperglycemia; 25% of animals develop diabetes</td>
<td>Normal body weight</td>
<td>No difference reported</td>
<td>Kulkarni and Kahn 2001; Kulkarni et al. 1999; Mauvais-Jarvis et al. 2000; Otani et al. 2004</td>
</tr>
</tbody>
</table>

*ND, not determined; NS, not specified.*
knockout model for PPAR-α showed no gross phenotype (Lee et al. 1995), pharmacological inhibition of the mitochondrial import of long-chain fatty acids led to hepatic and cardiac lipid accumulation, hypoglycemia, and death in all of the males but only 25% of females in this model (Djouadi et al. 1998). In contrast, the PPAR-α knockout model developed profound placental defects that were lethal (Barak et al. 1999). Heterozygote PPAR-γ 1 or 2 knockouts showed increased insulin sensitivity and normal body weights (Miles et al. 2000) and were protected from insulin resistance when fed a high-fat diet, presumably due to adipocyte hypertrophy (Kubota et al. 1999). Studies performed in mice carrying the leptin receptor gene that is specifically disrupted for the tyrosine residue that activates signal transducer and activator of transcription 3 (STAT3\(^1\)), showed improved insulin sensitivity and glucose tolerance compared with db/db mice that manifested an overt obese phenotype (Bates et al. 2005). Together, these studies support an adiposity-independent role of leptin in glucose homeostasis. At the level of the islet, leptin has been suggested to be a component of the adipo-insular axis and to link obesity with β-cell dysfunction (Kieffer and Habener 2000; Poitout et al. 1998b). Several studies have reported a direct inhibitory effect of leptin on insulin release in human and rodent islets and β-cell lines (Kulkarni et al. 1997; Poitout et al. 1998a; Seufert et al. 1999). Further work is necessary to delineate the precise pathways and proteins involved in the

### Table 2 Mouse models exhibiting minimal effects on glucose homeostasis

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Phenotype</th>
<th>Body weight</th>
<th>Sex studied</th>
<th>Reference (see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin receptor in adipose tissue (FIRKO)</td>
<td>Protected against age related glucose tolerance</td>
<td>Protected against age-related and hyperphagia-associated obesity</td>
<td>Glucose tolerance studied in males</td>
<td>Bluher et al. 2002</td>
</tr>
<tr>
<td>Insulin receptor in muscle (MIRKO)</td>
<td>Normal glucose homeostasis, metabolism appears to compensate for the insulin resistance of the muscle</td>
<td>Elevated fat mass at normal body weight</td>
<td>No difference reported</td>
<td>Bruning et al. 1998</td>
</tr>
<tr>
<td>Insulin receptor substrate-3 (IRS-3) knockout</td>
<td>No effect on glucose homeostasis, reduced fasted insulin levels only in young males</td>
<td>Normal body weight</td>
<td></td>
<td>Liu et al. 1999</td>
</tr>
<tr>
<td>Insulin receptor substrate-4 (IRS-4) GLUT-4 knockout</td>
<td>Mild hypoglycemia, mild glucose intolerant</td>
<td>Mildly growth retardation</td>
<td>Phenotype more pronounced in males</td>
<td>Fantin et al. 2000</td>
</tr>
<tr>
<td>Akt(^a) knockout</td>
<td>Mild glucose intolerance, mild insulin resistance</td>
<td>Growth retardation and reduced adipose tissue</td>
<td>Phenotype more pronounced in males</td>
<td>Katz et al. 1995</td>
</tr>
<tr>
<td>GLUT-4 knockout</td>
<td>Growth retardation</td>
<td>Growth retardation</td>
<td>No difference reported</td>
<td>Chen et al. 20001</td>
</tr>
<tr>
<td>PPAR-γ(^a) in β-cell</td>
<td>Normal glucose homeostasis and islet hyperplasia</td>
<td>Normal body weight</td>
<td>Males studied</td>
<td>Rosen et al. 2003</td>
</tr>
</tbody>
</table>

\(^a\)Akt, acute transforming retrovirus thymoma homolog; PPAR-γ, peroxisome proliferators-activator receptor γ; NS, not specified.

knockout model for PPAR-α showed no gross phenotype (Lee et al. 1995), pharmacological inhibition of the mitochondrial import of long-chain fatty acids led to hepatic and cardiac lipid accumulation, hypoglycemia, and death in all of the males but only 25% of females in this model (Djouadi et al. 1998). In contrast, the PPAR-γ knockout model developed profound placental defects that were lethal (Barak et al. 1999). Heterozygote PPAR-γ 1 or 2 knockouts showed increased insulin sensitivity and normal body weights (Miles et al. 2000) and were protected from insulin resistance when fed a high-fat diet, presumably due to adipocyte hypertrophy (Kubota et al. 1999). Studies performed in mice carrying the leptin receptor gene that is specifically disrupted for the tyrosine residue that activates signal transducer and activator of transcription 3 (STAT3\(^1\)), showed improved insulin sensitivity and glucose tolerance compared with db/db mice that manifested an overt obese phenotype (Bates et al. 2005). Together, these studies support an adiposity-independent role of leptin in glucose homeostasis. At the level of the islet, leptin has been suggested to be a component of the adipo-insular axis and to link obesity with β-cell dysfunction (Kieffer and Habener 2000; Poitout et al. 1998b). Several studies have reported a direct inhibitory effect of leptin on insulin release in human and rodent islets and β-cell lines (Kulkarni et al. 1997; Poitout et al. 1998a; Seufert et al. 1999). Further work is necessary to delineate the precise pathways and proteins involved in the

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cross-talk between leptin and growth factor signaling in the regulation of β-cell biology (Figure 2).

**Tissue-specific Knockouts and Transgenics**

Although the mouse models described above have provided novel insights into biological functions of genes involved in insulin action in vivo, one or more functions of most of these genes vary considerably between tissues, necessitating a tissue-specific approach. Another limitation to interpreting data from global knockout models is the ability of the organism to compensate and mask the precise phenotype. For example, compensatory effects are evident in IRS-1 or GLUT-4 null mice. Finally, embryonic lethality, secondary to knocking out a specific protein, precludes studying the function of the target protein in adult physiology.

Tissue-specific knockout approaches take advantage of the Cre-loxP or the Flp-FRT system. Several excellent reviews are available that outline the details regarding the approach for the interested reader (Gossen and Bujard 2002; Kos 2004; Lewandoski 2001). Briefly, in these systems, Cre (from bacteriophage P1) or Flp (from *Saccharomyces cerevisiae*) recombinase excises DNA fragments that are flanked by so-called loxP/FRT sequences (O’Gorman et al. 1991; Sauer and Henderson, 1989). To generate tissue-specific knockouts, mice carrying the gene of interest flanked by loxP/FRT sites are crossed with mice expressing the Cre/Flp recombinase under a tissue-specific promoter. Following activation by recombinase, the gene of interest is excised in the specific tissue expressing the promoter. The Cre-loxP system is one of the most frequently used approaches followed by the more recently developed Flp-FRT system.

One classic example of the need for tissue-specific knockouts in diabetes research is the early lethal phenotype of global insulin receptor knockouts. In such a scenario, it is advantageous to create tissue-specific knockouts of the insulin receptor, with spatial and temporal control, to learn its role in specific tissue types. For example, although the presence of insulin-signaling molecules has been described in the brain (Folli et al. 1994), the neuron-specific disruption of the insulin receptor gene in mice (NIRKO model) has yielded novel data that the brain is indeed a major target for insulin action (Bruning et al. 2000) (Table 3). Female NIRKO mice were hyperphagic, and both males and females developed diet-sensitive obesity. Rescue experiments revealed that the insulin signal in the brain is crucial for fuel homeostasis and to prevent diabetes. Fat-specific disruption of the insulin receptor in mice (FIRKO mice) exhibited a...
lean phenotype and protection from age-related obesity, suggesting that altered insulin signaling in adipose tissues is critical in the pathogenesis underlying obesity (Bluher et al. 2002). Transgenic mice overexpressing a dominant negative insulin receptor in muscle weighed normally but developed increased fat pads and modest hyperinsulinemia (Møller et al. 1996). A similar alteration in body composition was evident in muscle-specific insulin receptor knockout (MIRKO) mice, while circulating insulin levels were surprisingly unaffected (Bruning et al. 1998) (Table 2). A more detailed description of these knockouts is available in the following reviews (Kahn et al. 2000; Mauvais-Jarvis and Kahn 2000).

Interestingly, compared with the rather mild phenotype in whole body GLUT-4 knockouts, mice with a musclespecific knockout for GLUT-4 revealed normal body weights, glucose intolerance, and an insulin-resistant phenotype that was more pronounced in males, but suggesting that the GLUT-4 transporter in muscle is of major importance for glucose homeostasis (Zisman et al. 2000). In addition, the absence of GLUT-4 selectively in adipose tissue had virtually no effect on fat mass in the mutants, but the mice developed insulin resistance in adipose tissue, liver, and skeletal muscle (Abel et al. 2001). In this context, it is worth exploring whether defects in one metabolic tissue allow for development of similar defects in other metabolic tissues by cross-talk via circulating factors.

Another tissue-specific approach that provided an unexpected phenotype is exemplified by knockout of the insulin or IGF-1 receptors in pancreatic β-cells. In these studies, the β-cell-specific knockout model for the insulin receptor (BIRKO) or IGF-1 receptor (BIGF1RKO) surprisingly showed normal development of β-cells but an impaired ability to respond to glucose challenges (Kulkarni et al. 1999a, 2002; Otani et al. 2004; Xuan et al. 2002) indicating a role for insulin/IGF-I signaling in glucose sensing of β-cells (Table 3). Furthermore, BIRKO but not BIGF1RKO mice exhibited reduced β-cell mass in an age dependent manner suggesting a prominent role for insulin signaling in maintenance of adult β-cell mass. Interestingly, overexpression of IGF-2 in pancreatic β-cells, using the rat insulin 1 promoter, revealed an important role for IGF-2 in islet formation (Devedjian et al. 2000). Animals in this study showed islet hyperplasia, increased insulin content and secretion, and insulin resistance.

The contradictory phenotypes of mice null for PPAR-γ1 and PPAR-γ2 raised the question of the precise role of PPAR-γ in insulin sensitivity in metabolic tissues. Both isoforms are transcribed from the same gene using different promoters, leading to an extension of the 5’ end of the PPAR-γ2 transcript (Elbrecht et al. 1996). Mice with muscle-specific deletion for exon 1 and 2 of the PPAR-γ gene, lacking PPAR-γ1 and the functional PPAR-γ2 isoform, exhibit normal body weights and normal glucose uptake in muscle, but whole body insulin resistance in combination with enlarged epididymal fat pads indicating potential tissue cross-talk (Hevener et al. 2003; Norris et al. 2003). A critical role for PPAR-γ in the development and maintenance of adipose tissue was confirmed by an adipose tissue-specific knockout of exon 1 and 2 of the PPAR-γ gene (He et al. 2003). These mice developed increased hepatic gluconeogenesis and insulin resistance despite normal whole body glucose homeostasis. Interestingly, treatment of mutant mice with TZDs reversed the insulin resistance in liver but failed to lower plasma free fatty acids. Additionally, mice with a liver-specific knockout for PPAR-γ1 and 2 exhibited increased adipose mass, insulin resistance, and hyperlipidemia (Gavrilova et al. 2003). The β-cell-specific PPAR-γ knockout model showed islet hyperplasia but impaired islet growth response to high-fat feeding indicating a role for PPAR-γ in β-cell growth (Rosen et al. 2003) (Table 2). The specificity of effects mediated by PPAR-dependent pathways is further modified by the actions of PPAR coactivators. Whole body knockout for the PPAR-γ coactivator PGC-1α showed a surprisingly mild phenotype (Lin et al. 2004) while, in contrast, adenovirus-mediated depletion of PGC-1 in hepatocytes enhanced insulin sensitivity in the liver leading to fasting hypoglycemia (Koo et al. 2004). However, adenovirus-mediated overexpression of PGC-1 in β-cells led to a marked inhibition of glucose-induced insulin secretion in the islets (Yoon et al. 2003). Together, these data illustrate another example of contrasting phenotypes between mice with global disruption of genes versus the effects of tissue-specific modulation of the same protein.

### Inducible Knockouts

General and tissue-specific knockouts have shown that the function of one protein can be highly dependent on the tissue in which it is expressed. However, these deletions are irreversible and occur as soon as the Cre or Flp driving promoter is first activated. Another factor that can be crucial in determining the role of a specific protein is the time of expression because most questions addressed to animal models in type 2 diabetes research require adult animals. There are many reasons why it can be desirable to control the time of expression or to cause a transient depletion by an inducible system. Different systems have been described that can be controlled by an administrable agent that acts as an experimental switch. One such agent is doxycyline, which can be administered to mice to control gene expression in the TetR system (Tet-off) and its reverse, rtTA (Tet-on), allowing inactivation and activation of a gene, respectively. The disadvantages of this system are that after cessation of the doxycyline administration, time is required for the doxycyline to be cleared from the system. Moreover, leakiness of the system can occur especially when inadequate concentrations of doxycyline reach the tissue of interest. The tet-on system has been used to investigate the role of the homeodomain protein PDX-1 and HNF1α in diabetes (Thomas et al. 2001; Wang et al. 2002).

Alternatively, it is possible to use the GAL4-based system in which a fusion protein of Gal4 and the ligand binding
domain of the progesterone receptor make the gene inducible with RU486 or tamoxifen. This system, known as GLVP, is mainly applicable in adult mice inasmuch as the synthetic steroid inducer has been reported to be abortigenic. It is preferable to use this system for gain of function experiments because the expression level is dependent on the inducer concentrations. However, it has not been popular in models of diabetes, perhaps due to potential side effects of tamoxifen and RU486 on glucose homeostasis (Liu et al. 2005). Nevertheless, the system has been adapted for knockout studies using Cre recombination for studies of mature adipocytes (Imai et al. 2001; Metzger et al. 2005).

### Future Perspectives

Together, animal models have provided major molecular insights into the roles of proteins in multiple pathways that can modulate glucose homeostasis. How and to what extent these genes actually contribute to the human disease obviously requires further investigation. It is worth reiterating that type 2 diabetes is a polygenic disease and that different SNPs, in combination with environmental factors, lead to the complex manifestation of the disease.

## Table 3 Mouse models with glucose intolerance

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Phenotype</th>
<th>Body weight</th>
<th>Sex studied</th>
<th>Reference (see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin receptor substrate-1 (IRS-1) knockout</td>
<td>Glucose intolerant, mild insulin and IGF resistant</td>
<td>Growth retardation</td>
<td>No difference reported&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Araki et al. 1994</td>
</tr>
<tr>
<td>Insulin receptor in liver knockout (LIRKO)</td>
<td>Glucose intolerance, insulin resistance, and perinsulinemia; the phenotype becomes less severe with aging</td>
<td>Moderate growth impairment</td>
<td>Males studied</td>
<td>Michael et al. 2000</td>
</tr>
<tr>
<td>Insulin receptor knockout in brain (NIRKO)</td>
<td>Mild insulin resistance and elevated plasma insulin levels</td>
<td>Mild obesity on normal chow and increased diet-induced weight gain</td>
<td>Phenotype more pronounced in females</td>
<td>Bruning et al. 2000</td>
</tr>
<tr>
<td>IGF-1&lt;sup&gt;a&lt;/sup&gt; receptor β cell knockout</td>
<td>Impaired glucose tolerance and defective glucose-induced insulin secretion</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No difference reported</td>
<td>Kulkarni et al. 2002</td>
</tr>
<tr>
<td>Akt2&lt;sup&gt;a&lt;/sup&gt; knockout</td>
<td>Insulin resistance and glucose intolerance</td>
<td>Normal body weight</td>
<td>No difference reported</td>
<td>Cho et al. 2001</td>
</tr>
<tr>
<td>GLUT-4&lt;sup&gt;a&lt;/sup&gt; knockout in muscle</td>
<td>Severe insulin resistance and glucose intolerance</td>
<td>Normal body weight, reduced weight gain after 6 mo of age</td>
<td>More pronounced insulin resistance in males</td>
<td>Zisman et al. 2000</td>
</tr>
<tr>
<td>GLUT-4 knockout in adipose tissue</td>
<td>Insulin resistance, glucose intolerance, and hyperinsulinemia</td>
<td>Normal body weight</td>
<td>Phenotype more pronounced in females</td>
<td>Abel et al. 2001</td>
</tr>
<tr>
<td>PPAR-γ&lt;sup&gt;a&lt;/sup&gt; knockout in adipose tissue</td>
<td>Adipocyte hypocellularity, high-fat diet-induced hyperinsulinemia, and insulin resistance</td>
<td>Normal body weight with reduced fat</td>
<td>No difference reported</td>
<td>He et al. 2003</td>
</tr>
<tr>
<td>PPAR-γ knockout in muscle</td>
<td>Glucose intolerance, insulin resistance, and hyperinsulinemia</td>
<td>Normal body weight</td>
<td>Males studied</td>
<td>Hevener et al. 2003</td>
</tr>
</tbody>
</table>

<sup>a</sup>IGF-1, insulin-like growth factor 1; Akt, acute transforming retrovirus thymoma homolog; GLUT-4, glucose transporter 4; PPAR-γ, peroxisome proliferator-activator receptor γ; ND, not determined; NS, not specified.

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diabetic phenotype, continued studies with additional genetic mutations are necessary to project the phenotype to humans, especially including proteins that contribute to the pathogenesis of obesity. The rapid advances in genetic engineering techniques including the use of siRNA and stem cell science, to name a few, will continue to play a critical role in the quest for generating therapeutic approaches to prevent and/or cure type 1 and type 2 diabetes.

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References


Anderson MS, Bluestone JA 2005. The NOD mouse: A model of immune pathogenesis of obesity. The rapid advances in genetic en-


Cheverud JM, Ehrich TH, Kenney JP, Pletscher LS, Semenkovich CF. 2004. Genetic evidence for discordance between obesity- and diabetes-related traits in the LGXSM recombinant inbred mouse strains. Diabe-


Cheverud JM, Ehrich TH, Kenney JP, Pletscher LS, Semenkovich CF. 2004. Genetic evidence for discordance between obesity- and diabetes-related traits in the LGXSM recombinant inbred mouse strains. Diabe-


Targeted elimination of peroxisome proliferator-activated receptor gamma in beta cells leads to abnormalities in islet mass without compromising glucose homeostasis. Mol Cell Biol 23:7222-7229.


