Rodent Models of Diabetes

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Abstract

Currently, diabetes affects approximately 29 million Americans (http://www.cdc.gov/diabetes/basics/index.html) and 380 million people worldwide (IDF Diabetes Atlas: www.idf.org/diabetesatlas). The significant progress in understanding diabetes and its clinical management is, in part, the result of research using rodent models of diabetes. Parallels between humans and rodents make these diabetes models practical tools for studying the characteristic features of diabetes and preclinical evaluation of potential treatments. This chapter describes major rodent models of type 1 and type 2 diabetes and highlights some of the latest developments based on selective genetic modifications in rodents. While these models allow providing further mechanistic insight into disease pathogenesis and testing novel diagnostic and treatment approaches, the strengths and limitations of each model should be considered when designing experiments and interpreting results.

Keywords

Diabetes • Insulin sensitivity • Insulin resistance • Glucose intolerance • Rodent models • Genetically • Modified mice • Cre/LoxP system • Pancreas • Beta cells

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Introduction

Animal models have been vital to diabetes research even prior to the discovery of insulin [1]. Today, rodent models sharing genetic, pathogenic, metabolic, and pathophysiological features typically observed in patients with diabetes are used in laboratories throughout the world. Parallels between humans and rodents make these diabetes models practical tools for research. While each model presents characteristic features of diabetes, the strengths and limitations of each model must be considered when designing experiments and interpreting results. This chapter describes the major rodent models of type 1 diabetes (T1D) and type 2 diabetes (T2D) and highlights the general advantages and disadvantages of these models.

Rodent Models of Type 1 Diabetes (T1D)

T1D is a complex disease which develops through autoimmune-mediated destruction of the pancreatic beta (β) cells in the islets of Langerhans, followed by insufficient insulin production and hyperglycemia [2]. T1D progression and severity are influenced by genetic and environmental factors [2]. For decades, rodent models of T1D have assisted in revealing disease pathogenesis and have led to the development of treatment approaches used to alleviate disease severity and disease progression. As outlined in Table 1, the following section will focus on describing the key experimentally induced and spontaneous rodent models of T1D.

Table 1 Rodent models of type 1 diabetes

Type 1 diabetes models	
Categories	Examples
Chemically induced	Streptozotocin Alloxan
Virus associated Virus antigen associated	Coxsackie B virus (CVB4) Encephalomyocarditis (EMC) virus Kilham rat virus (KRV) RIP-LCMV
Surgically induced	Pancreatic excision
Spontaneous	NOD mice BB rats LETL/KDP rats and substrains LEW.1AR1/Ztm-iddm

Experimentally Induced Models

The use of cytotoxic agents to model features of T1D in rodents has been instrumental in numerous preclinical studies. Cytotoxic agent-induced models are appropriate for expedited investigation of potential treatment modalities. When administered to rodents, these agents that are toxic toward insulin-producing pancreatic β-cells can rapidly generate a diabetes-like phenotype with a relatively high reproducibility. Unlike the pathogenesis of T1D in humans, these cytotoxic models lack signature genetic biomarkers of susceptibility, such as variants of major histocompatibility complexes (MHC), as well as *Ctla4*, *Ptpn22*, and *Cd25/Il2ra* autoimmune genes which are commonly associated with human T1D [3, 4]. Today, the most frequently used cytotoxic agents for inducing T1D in rodents are the glucose analogues, streptozotocin and alloxan. While both agents produce β-cell destruction, the mechanisms of β-cell destruction by high doses of these cytotoxic agents are quite different when compared to the human condition (i.e., chemical cytotoxicity vs. autoimmune).

Streptozotocin-Induced Model

The most commonly used agent to induce diabetes in rodents is streptozotocin (Table 2). First discovered in *Streptomyces achromogenes* during the 1950s, streptozotocin was later identified to be a diabetogenic agent promoting DNA damage to insulin-producing β-cells [5, 6]. As a glucose analogue, streptozotocin gains intracellular access via glucose transporter 2 (GLUT2) proteins found abundantly on β-cells [7]. β-cell toxicity following a single high dose is mediated through its intracellular accumulation and the intercalation

Table 2 Features of streptozotocin for inducing T1D in rodents

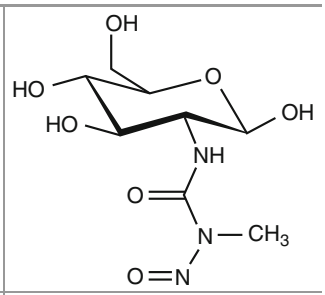
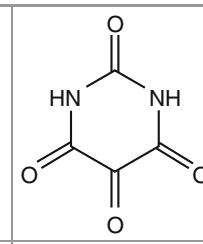
Streptozotocin	
Chemical structure	
Mechanism of action	Alkylating agent
Target	β-cells via GLUT2
Source	Exogenous only
Susceptible species	Mice and rats
Dosing regimen	Multiple low-dose injections Single high-dose injection

Table 3 Features of alloxan for inducing T1D in rodents

Alloxan	
Chemical structure	
Mechanism of action	Oxidizing agent
Target	β-cells via GLUT2
Source	Exogenous for induction, but found endogenously
Susceptible species	Mice and rats
Dosing regimen	Single high-dose injection

of DNA followed by DNA fragmentation leading to β-cell death [8].

A single high-dose injection of streptozotocin promotes massive β-cell toxicity, terminating insulin production and leading to hyperglycemia within 1–2 days [9–11]. The streptozotocin model is quite variable in rodents, affected by gender (with males more affected than females), strain (DBA/2 > C57BL/6 > MRL/MP > 129/SvEv > BALB/c), as well as dose and diet (Reviewed in [12]). Susceptible mice treated with high-dose streptozotocin must be carefully monitored to avoid moribund conditions. Alternatively, streptozotocin administered in multiple low doses to mice reduces injury to other organs when compared to the single high-dose injection; multiple low doses of streptozotocin have been shown to stimulate the induction of autoantigens (e.g., glutamic acid decarboxylase, or GAD) implicated in Th1-dependent inflammation and produce limited β-cell death similar to that observed in human T1D [13]. Streptozotocin-treated animals develop hyperglycemia and other T1D symptoms including insulinopenia, weight loss, and polyuria [10, 14, 15]. Streptozotocin-induced symptoms can progress to further complications such as nephropathy, retinopathy, cardiovascular damage, cataracts, and polyneuropathy, typically observed in human T1D progression [16]. Finally, streptozotocin can be combined with other chemicals (e.g., nicotinamide) or high fat diet to produce models of T2D in rodents [17]. Although not optimal for studying the etiology of T1D (particularly the high-dose regimen), the streptozotocin-induced models are particularly useful for examining novel therapeutic options in nongenetically altered animals.

Alloxan-Induced Model

Alloxan, another cytotoxic glucose analogue, was first identified in 1943 [18]. Like streptozotocin, alloxan is preferentially transported via GLUT2 transporters, predominantly expressed by pancreatic β-cells. However, unlike streptozotocin, alloxan is an endogenous molecule produced during uric acid metabolism and is reported to be elevated in the circulation of children with T1D [19], supporting its potential role in the pathogenesis of T1D (Table 3). As an oxidizing agent, alloxan promotes β-cell necrosis in mice and rats through the production of reactive oxygen species [9]. In addition, alloxan suppresses glucokinase activity, which inhibits insulin secretion from β − cells [9]. Rodents exposed to a single dose of alloxan present with common manifestations of T1D, including β-cell loss, insulinopenia, hyperglycemia, polyuria, hyperphagia, and weight loss

[20]. When compared to streptozotocin, alloxan has a narrower diabetogenic range and can cause kidney damage [17]. Similar to the streptozotocin models, the results of alloxan depend on the dose, route of administration, and strain of animal used (Reviewed in [21]). Alloxan-treated animals also must be vigilantly monitored and treated with insulin to avoid ketoacidosis.

Viral Models

Under sterile housing conditions, several environmental factors have been employed to induce T1D in rodents, including infectious agents [22–24]. The most common infectious agents used to induce models of diabetes in rodents are viruses. To date, numerous viruses have been implicated in promoting and/or preventing autoimmune diabetes in mice including picornaviruses, arteriviruses, parvoviruses, cardioviruses, reoviruses, and retroviruses, among others [22, 24]. Evidence for viral participation in T1D in humans stems from epidemiological studies reporting a correlation between viral infections and subsequent appearance of anti-β-cell autoantibodies [22, 25]. Thus, there appears to be a link between certain viral infections and autoimmune diabetes. However, the relationship between viruses and T1D is complex and controversial as viruses can both induce and protect against T1D [22, 23].

Coxsackie B Virus-Induced Model

Enteroviruses, members of the picornavirus family, have been implicated in the pathogenesis of T1D [26]. In 1978, it was reported that the B4 strain of the coxsackie virus (or CVB4) induced a T1D phenotype in mice [27]. A year later, a CVB4-like virus was isolated from human pancreatic β-cells of a pediatric diabetic patient [28]. The precise role of CVB4 in T1D pathogenesis remains unclear. However, several epidemiological studies report evidence of CVB4 infection in both children and adults with T1D [29, 30]. Results of studies with CVB4 inoculation of nonobese diabetic (NOD) mice suggest that insulitis is required for the viral exacerbation of diabetes [31]. Furthermore, vaccination of young mice against CVB4 prevents the development of diabetes [32]. This model in NOD mice requires inoculation with CVB4 and results in the development of hyperglycemia within 14 days, which eventually resolves in approximately 60 days. While the exact molecular mechanism(s) by which CVB4 and other enteroviruses promote T1D is not completely understood, there is some evidence that in some patients CVB4-specific antibodies induce β-cell apoptosis to promote T1D [33].

Encephalomyocarditis Virus-Induced Model

Craighead and McLane were the first to report that encephalomyocarditis (EMC) induced diabetes. Like the coxsackie B virus, EMC is a member of the picornavirus family and depending on the strain is associated with myocarditis, encephalitis, and other neurological conditions, as well as endocrine disorders [34]. Following infection with the D strain of EMC, susceptible rodents exhibit hyperglycemia, with timing dependent upon several variables, including viral variant, dosing, and the genetic background of the rodent [35, 36]. EMC-induced diabetes involves acute β-cell infection followed by either cell lysis (high dose) or recruitment of macrophages (low dose) [37]. Limitations of this model include exocrine tissue damage and a lack of autoantibodies [38]. However, similarities between the EMC-T1D model and fulminant T1D, including a lower incidence of insulitis, make this model potentially more useful than the popular nonobese diabetic (NOD) model [38].

Kilham Rat Virus-Induced Model

The Kilham rat virus (KRV) is a rat parvovirus used to induce an autoimmune diabetic phenotype in the typically diabetes-resistant biobreeding (DR-BB) rats and the mostly resistant LEW1. WR1 rats [39, 40]. The pathogenesis of this model believed to involve insulitis and β-cell necrosis [39–41], leading to autoimmune reactions following macrophage recruitment and perturbation of regulatory T cells [41]. Although early reports indicated that KRV did not infect

β-cells, more recent studies demonstrate β-cell infection by KRV in vitro and in vivo [42]. This model induces diabetes in only 30% of DR-BB rats versus 100% of LEW1.WR1 rats [42]. Additionally, coinfection with KRV and rat cytomegalovirus (RCMV) increases the development of autoimmune diabetes in LEW1.WR1 rats [43].

RIP-LCMV-Induced Model

Human T1D is associated with the presence of T lymphocytes reactive to β-cell antigens. Although few studies have examined the positive association between T1D and lymphocytic choriomeningitis virus (LCMV) infection alone in rodents, numerous investigations have employed LCMV in a transgenic mouse model where the lymphocytic choriomeningitis virus glycoprotein is under the control of the rat insulin promoter (RIP-LCMV) and, hence, expressed in their β-cells [44]. This mouse model is designed to break tolerance to autoantigens of β-cells via viral infection. RIP-LCMV transgenic mice develop T1D following the induction of LCMV-induced pancreatic lymphocytic infiltration and inflammation. Distinct from other viral T1D models, which typically require simple inoculation with live virus, this model requires specific transgenic mice, live virus, and autoreactive CD4 and CD8 T cells [45, 46] that ultimately destroy the β-cells. As with other rodent models, there is variability depending on the transgene used (LCMV-GP vs. NP) and dose and timing of virus inoculation.

Surgically Induced Models

Surgical excision of the pancreas from dogs by Banting and Best led to the discovery of insulin [47]. Pancreatectomy models involving the surgical removal of between 60% and 90% of the pancreas in rodents have been widely used for studying T1D. This model is generally used to identify alternative ways to maintain glucose homeostasis, with recent studies focused on islet-cell transplant and regeneration. However, it is important to note that pancreatic excision eliminates numerous pancreatic digestive enzymes and increases the risk of infections and death (as a result of surgery). There is also evidence that partial pancreatectomy can serve as a model for T1D-myopathy, shedding insight into the developmental impairments of patients with T1D [48].

Spontaneous Models

Spontaneous rodent T1D models share the greatest homology to human T1D and therefore are commonly used in the study of autoimmunity in diabetes. Similar to human disease, rodents possess genetic risk factors typically associated with T1D susceptibility [2, 49]. For these reasons, spontaneous models are excellent for investigating the etiology, pathogenesis, and progressive complications of T1D. Spontaneous models have helped elucidate the role of immune cells, particularly T lymphocytes, monocytes/macrophages, and dendritic cells, in promoting insulitis and the progression of autoimmunity, characteristic features of T1D. The major limitation of these models is their spontaneity in disease development, making them less reproducible and more time-consuming than other T1D models. Difficulties in standardizing these models are largely due to environmental factors, and as a result, rodents must be maintained under pathogen-free conditions to prevent exposure to infectious agents (reviewed in [50]), which can modulate disease susceptibility and progression. Nevertheless, spontaneous T1D models offer opportunities for investigating genetic components of T1D and for testing new therapeutics. The following section will focus on the most common spontaneous models of T1D, including NOD mice, biobreeding (BB) rats, LETL/KDP, and Lewis rats.

NOD Mice

The nonobese diabetic (NOD) mouse model was developed in Osaka, Japan, by selective breeding of the offspring of JcI-ICR mice prone to cataract development [51]. As observed in human T1D, NOD mice share polygenic risk factors for developing T1D-like characteristics, making it a

popular model of T1D. Approximately 10–30% of male NOD mice develop autoimmune T1-like diabetes versus 60–80% of females. The NOD model is characterized by insulitis, β-cell apoptosis, insulinopenia, and hyperglycemia, which if left untreated would result in death [52]. There are several known insulin-dependent diabetes (*Idd*) susceptibility loci associated with the diabetogenicity of NOD mice, including *Idd1* and *Idd3* [63]. *Idd1* is linked to the MHC and acts as a dominant gene with variable degrees of penetrance for insulitis [53]. This locus is critical for the expression of glycoproteins responsible for distinguishing between self versus nonself antigens. The *Idd3* locus is associated with reduced production of IL-2, a mediator of T-cell tolerance and autoimmunity [64], whereas the *Idd5.1* locus is associated with *Ctla4* [54], whose gene product attenuates β-cell-specific T-cell autoimmune responses [55]. Further congenic mapping revealed that interactions between *Idd3/Il2*, *Idd5.1/Ctla-4*, and a novel *Ctex* interval on chromosome 1 promote autoimmune T1D in NOD mice [56].

Pathogen-free and germ-free NOD mice (lacking intestinal microbiota) were initially reported to develop increased incidence of T1D characterized by earlier immune cell infiltration into pancreatic islets progressing to severe insulitis by 10 weeks of age [49]. This observation suggests that host-microbial interactions modulate T1D pathogenesis. More recent research revealing that female NOD mice maintained in a germ-free environment exhibited no difference in the incidence of T1D challenges this viewpoint [57] and suggests that changes in intestinal microbiota impart beneficial effects on the development of autoimmune T1D. Finally, it is important to note that although commonly studied as a reflection of the pathogenesis of human T1D, insulitis in NOD mice is considerably more pronounced as compared to that observed in human disease [58].

BB Rats

Biobreeding (BB) rats originated from a colony of Wistar rats at BioBreeding Laboratories in Ottawa, Canada. The two existing colonies of diabetes-prone (DP) BB rats are the inbred BBDP/Wor from Worcester, Massachusetts, and the outbred BBdp rats from Ottawa, Canada [58]. BB rats have been among the most commonly used T1D rat models, with biobreeding diabetic-resistant (BBDR) rats used as the negative controls. T1-like diabetes spontaneously occurs in more than 85% of BB rats between 8 and 16 weeks of age, as demonstrated by severe hyperglycemia, hypoinsulinemia, weight loss, polyuria, polydipsia, glycosuria, and ketosis [59]. In addition to these common T1D symptoms, BB rats spontaneously develop autoimmune-mediated β-cell destruction and T-cell lymphopenia, as a result of a GTPase immunity-associated protein family member 5 (*Gimap5* or *Iddm2*) gene mutation [60, 61]. T-cell lymphopenia is unique to the BB rat T1D model and is not observed in humans with T1D. Also similar to both the NOD mice model and human T1D, BB rats exhibit genetic polymorphisms in multiple genes, including the MHC II haplotype (RT1.B^u D^u or *Iddm1* in rats) [62]. Because of the severity of T1D in this model, BB rats have been useful for studying complications of T1D and interventional strategies.

LETL/KDP Rats

Developed in Japan, the Long-Evans Tokushima lean (LETL) rat and the substrains, Komeda diabetes-prone (KDP) and Komeda nondiabetic (KNP) rats, have been used for more than a decade in diabetes research. The incidence of diabetes in LETL rats is approximately 20% [63]. However, this model resembles human diabetes because of the lack of lymphopenia and gender differences in susceptibility [63]. The KDP substrain of rats develops diabetes with 70% incidence of insulitis by 4 months of age [63]. Like the LETL rat, KDP rats do not develop lymphopenia [63]. In addition to MHC genes, the *Cblb* gene in the KDP rat was discovered to be a major susceptibility marker for T1D [64].

LEW.1AR1/Ztm-iddm Rats

A less common spontaneous model of T1D is the Lew.1AR1/Ztm-iddm rat model [65], which was developed at the Institute of Laboratory Animal

Science of Hannover Medical School (Ztm) through inbreeding of LEW1.AR1 rats, which have a defined MHC haplotype [66]. Further inbreeding has produced a strain exhibiting 60% incidence of T1D in both males and females and both β-cell apoptosis and insulinopenia [67]. The LEW.1AR1/Ztm-iddm model is relatively recent, and its complex genetic features are not well characterized.

Rodent Models of Type 2 Diabetes (T2D)

Type 2 diabetes (T2D) affects about 95% of all diabetic patients in the USA and 9% of the total US population [68]. T2D, typically accompanied by obesity, is characterized by hyperglycemia, hyperinsulinemia with insulin resistance, and the lack of dependence on exogenous insulin and the absence of autoimmune antibodies. Based on its widespread and increasing prevalence and adverse health consequences, it is critically important to provide better insight into the pathogenesis of T2D and to evaluate new therapeutic strategies using relevant animal models. Numerous rodent models of T2D are available, including spontaneous and experimentally induced models (Table 4). No single model of T2D in rodents represents all aspects of T2D flawlessly, and therefore, investigators must choose among available models based on their needs and interests. We highlight the advantages and limitations of rodent T2D models and briefly describe how the latest research utilizing some of these models has advanced our understanding of the pathogenesis of T2D.

Table 4 Rodent models of type 2 diabetes

Type 2 diabetes models	
Categories	Examples
Spontaneous, obesity associated Monogenic	 ob/ob mice db/db mice
Polygenic	KK mice NZO mice NSY mice TALLYHO/JngJ mice
Spontaneous, nonobese models	GK rats Spontaneously diabetic Torii (SDT) rats Akita (Ins2Akita) mice
Experimentally induced Diet induced	 High fat diet Israeli sand rats Nile grass rats
Chemically induced	Streptozotocin Alloxan
Surgically induced Gestational diabetes mellitus (GDM)[a]	Partial pancreatectomy and duct ligation Streptozotocin Genetic-based models High fat diet/high fat + high sugar diet
Genetic modification	General gene knockouts Tissue- and cell-specific knockouts Optogenetics and CRISPR/Cas9 based

[a]GDM increases risk of T2D in the future

Spontaneous Models

Rodent models of spontaneous T2D can be categorized into those with genetic alterations coupled with obesity versus nonobese models.

Models Associated with Obesity

T2D in the setting of obesity is considerably more common than T2D in the absence of obesity. Therefore, more obesity-associated models of T2D are available than nonobese T2D models. T2D phenotypes can be produced in rodents by utilizing genetic mutations, including monogenic and polygenic mutations. Interestingly, many of these models were developed and employed before recognizing and understanding the underlying genetic mutations.

Monogenic Models

Although monogenic mutations are not commonly found in humans, numerous rodent models targeting single genes produce features of T2D in the setting of obesity, including *Lep*$^{ob/ob}$ (ob/ob) mice, *Lepr*$^{db/bb}$ (db/db) mice, and Zucker diabetic fatty (ZDF-*Lepr*$^{fa/fa}$ or fa/fa) rats.

ob/ob mice: C57BL/6 J mice homozygous for the recessive obese *Lep*$^{ob/ob}$ mutation (aka ob/ob) are among the earliest reported obese mouse

models [69]. At birth these mice are identical to their littermates, but exhibit an early and rapid increase in body weight when compared to wild-type mice. The *ob/ob* gene was later described, mapped [70–72], and shown to encode leptin. Leptin, known as the "satiety hormone," interacts with leptin receptors found on cells in the hypothalamus to control appetite [73]. While mutations in the *OB* gene are quite rare in obese humans [74], mice with mutations in the *ob* gene have been intensely studied in the context of obesity and T2D. $Lep^{ob/ob}$ mice exhibit hyperphagia and reduced energy expenditure, along with hyperglycemia and impaired glucose tolerance. This phenotype can be significantly improved by administering exogenous leptin [75]. Genetic background significantly influences the $Lep^{ob/ob}$ gene and, thus, needs to be considered when planning experiments. $Lep^{ob/ob}$ mice bred on the C57BL/6J background are commercially available (Charles River, JAX/Jackson Laboratory, Taconic, Harlan, etc.) and exhibit transient and mild hyperglycemia (peaking at 3–5 months) with hyperinsulinemia and some β-cell hypertrophy until 14–16 weeks [76, 77]. The $Lep^{ob/ob}$ mice on the C57BLKS/J background exhibit weight gain, chronic hyperglycemia, hypoinsulinemia, and β-cell atrophy [76, 78]. In addition, FVB/N-$Lep^{ob/ob}$ mice show more severe liver insulin resistance than C57BL/6 J-Lep^{ob} mice [79]. Thus, the genetic background for $Lep^{ob/ob}$ mice significantly influences disease severity and must be considered when designing rodent T2D studies. In addition, $Lep^{ob/ob}$ mice are sterile; fertility can be restored with exogenous leptin treatment [80, 81].

db/db mice: As described above, the effect of leptin on satiety is mediated by binding to high-affinity leptin receptors found on neurons in the hypothalamus [73]. The first report of obese diabetic (or *db/db*) mice of the C57BLKs/J strain, characterized by excessive weight gain with persistent hunger, was in 1966 [82]. This mutation, now referred to as $Lepr^{db/db}$, produces hyperglycemia, hyperinsulinemia, and early insulin resistance (by 3–4 months of age), and unlike $Lep^{ob/ob}$ mice, $Lepr^{db/db}$ mice, which are also commercially available, are nonresponsive to exogenous leptin. Based on a recent PubMed search, the $Lepr^{db/db}$ or *db/db* mouse model (yielding 1358 hits) is more frequently used as a preclinical model of T2D with obesity when compared to the $Lepr^{ob/ob}$ or *ob/ob* mouse model (yielding 483 hits) [search terms: ob/ob [or db/db] mouse AND T2D, January 7, 2015]. Rodents bearing the homozygous mutant $Lepr^{db/db}$ are infertile due to hypogonadotropic hypogonadism and, therefore, must be bred as heterozygotes.

Zucker fa/fa rats: The most commonly used T2D obese rat model, the monogenic Zucker diabetic fatty (ZDF-$Lepr^{fa/fa}$) *fa/fa* rat model, was derived from inbreeding the original nondiabetic Zucker fatty rats [83, 84]. Similar to the $Lepr^{db/db}$ mice, these ZDF rats inherit two mutant leptin receptor genes (*fa/fa* or $Lepr^{fa/fa}$) [85]. ZDF-$Lepr^{fa}$ rats exhibit hyperphagia and consequent morbid obesity, even when fed a normal diet, as well as overt T2D/insulin resistance, hyperlipidemia, hypertension, and mild hyperglycemia [86, 87]. Diabetes onset occurs early (at approximately 10 weeks of age) and progresses with time. Thus, the ZDF-$Lepr^{fa}$ model is useful for studying microvascular injuries and diabetic nephropathy in adult animals [88, 89]. Homozygous ZDF-$Lepr^{fa}$ rats are infertile and must be bred and maintained on the heterozygous background (*fa/+*). For best results, commercial vendors (e.g., Charles River) recommend feeding ZDF-$Lepr^{fa}$ males Purina #5008 and ZDF-$Lepr^{fa}$ females Research Diet D12468 to consistently produce T2D.

Polygenic Models

Human T2D is considered mostly polygenic. Thus, polygenic rodent models may be more informative when investigating the pathogenesis of human T2D and its complications and when exploring novel treatments for human T2D. Numerous polygenic rodent models exist, and each offers a unique set of characteristics to consider (e.g., timing, severity, metabolic abnormalities, and associated complications). However, unlike the monogenic rodent models, there are no heterozygotes or wild-type "controls" available for rodent polygenic models.

KK mice: The Kuo Kondo (or KK) mouse strain was originally developed in Japan. Male KK mice develop T2D (with hyperglycemia and hyperinsulinemia) following consumption of an obesogenic diet, or by either chemical induction or aging [90, 91]. These mice are hyperphagic, hyperinsulinemic, insulin resistant, and obese. Appearance of diabetes peaks at 4–5 months (Reviewed in [92]). In addition, these mice exhibit signs of diabetic nephropathy [91].

The KK-A^y or KK/Upj-A^y/J strain was created by introducing the yellow, obese A^y gene, which imparts a yellow coat, into KK mice [93]. These mice are commercially available (Jackson Laboratory). Heterozygote KK-A^y (or yellow obese) mice develop mature onset insulin resistance, with severe hyperinsulinemia, and obesity between 8 and 17 months of age [94]. Similar to the KK mice, obesity is more prominent in male KK-A^y mice (Reviewed in [92]). In addition, while KK-A^y mice consume between 10% and 36% more calories than their lean littermates [95–97], they exhibit some level of satiety [98]. The obesity in these mice has been hypothesized to be due to improved storage of calories as fat [99]. Thus, heterozygote KK-A^y (or yellow obese) mice differ significantly from the *Lepr*$^{db/db}$ and *Lep*$^{ob/ob}$ mice because they are mildly hyperphagic, display reasonable satiety, and exhibit mature onset obesity and insulin resistance. The KK and KK-A^y mouse strains are commercially available.

NZO mice: New Zealand obese mice (NZO), introduced in the 1950s, represent another model of polygenic T2D in the setting of obesity [100]. These inbred mice are large at birth and become severely obese and hyperleptinemic. Because NZO mice were difficult to breed and only recently became commercially available in the USA [101], they are not as well characterized as other models. While neither male nor female NZO mice show signs of hyperinsulinemia [102], males fed a high fat diet develop hyperinsulinemia, hypercholesterolemia, and hypertension [103]. Like other polygenic models, matched nonobese "control" strains are not available for the NZO strain. NZW and NZB models are similar but may not be ideal "controls" [104]. Several obese NZO substrains have been developed, including the NZO/HI and NZO/HILt models. Both male and female NZO/HI mice exhibit impaired glucose tolerance; however, only about half of the males develop overt T2D by 12–20 weeks of age [105]. Finally, the older NZO/HI males, which develop diabetes, show pancreatic β-cell destruction with B lymphocytic infiltration [106].

NSY mice: The inbred Nagoya-Shibata-Yasuda (NSY) mouse model, developed by selective breeding for glucose intolerance from outbred JcI:ICR mice (from which NOD mice were derived), is a relatively newer model of polygenic spontaneous "diabesity" [107, 108]. Progression to moderate obesity and moderate diabetes (without extreme hyperinsulinemia) occurs with age; approximately 98% of males and 31% of females exhibit spontaneous diabetes by 48 weeks of age [108]. In this mouse model, no hypertrophy, pancreatic inflammatory infiltrate, or β-cell destruction is observed, suggesting that insulin secretion in response to glucose might be dysfunctional [108]. As noted, the NSY mouse model is derived from NOD mice, which are commonly used as a T1D model, and thus may be useful for studying potential genetic overlap between T1D and T2D [109].

TALLYHO/JngJ mice: One of the more recently described mouse models of T2D with obesity is the TALLYHO (or TH) mouse model, which was introduced in the early 2000s [110]. TH mice display obesity, hyperinsulinemia, and hyperlipidemia, regardless of gender, and only males exhibit hyperglycemia. Genetic analyses have implicated multiple loci on chromosomes 16, 18, and 19 [110]. Further characterization revealed that young female and male mice (<8 weeks) weigh 45–60% more than age- and gender-matched C57BL/6 mice and both males and females display hypercholesterolemia and hypertriglyceridemia [111], with more prominence among the males. At 8 weeks of age, male mice begin to exhibit glucose intolerance, which progresses through 16 weeks of age. By contrast, female mice do not become diabetic, i.e., they maintain glucose tolerance through 16 weeks of age [111]. Pancreas samples obtained from male TH mice post diabetes (>16 weeks) show limited

β-cell injury [111]. In addition, the kidneys of 6-week-old males (prediabetic) show histologic injury which worsens with age [112]. Thus, the TH model of obesity and insulin resistance in male mice emerges early during the transition to T2D, with aberrant lipid metabolism and glucose intolerance preceding significant hyperglycemia [113].

NONcNZO10/LtJ mice: Another recently described mouse model of polygenic T2D was developed by combining the New Zealand obese (NZO/HILt) and the nonobese nondiabetic (NON/LtJ) strains. The resulting polygenic NONcNZO10/LtJ (RCS10) strain exhibits mature onset obesity, hyperglycemia, and insulin resistance in males [114]. At 8 weeks of age, NONcNZO10/LtJ mice are not obese but have mild insulin resistance in the skeletal muscle, which is associated with reduced GLUT4 expression. The progression to severe diabetes occurs between 8 and 13 weeks of age with increased insulin resistance in the skeletal muscle, liver, and heart, and is accompanied by dyslipidemia, suggesting that different mechanisms of insulin resistance occur in the hyperglycemic obese state when compared to the nonobese state [114]. These mice have been used to investigate pathways of wound healing in obese diabetic individuals [115] and biochemical profiling to identify regulators of insulin secretion [116].

OLETF rats: The Otsuka Long-Evans Tokushima Fatty (OLETF) strain of rats was derived from an outbred colony of Long-Evans rats maintained at the Tokushima Research Institute in the 1980s. The subsequently established OLETF line spontaneously develops mild obesity with late-onset hyperglycemia, accompanied by progressive β-cell degeneration and kidney damage in males (>18 weeks of age) [35, 117]. One gene implicated in this T2D model is *Cckar*, which encodes the cholecystokinin A receptor (or CCK1) [118]. OLETF rats lack CCK1 which mediates the CCK's satiety-inducing effects, and as such, they are hyperphagic. Additional genetic analyses revealed that this model of T2D with mild obesity was polygenic and complex, with highly significant linkages between phenotype, fasting glucose, hyperglycemia, and body weight found on multiple chromosomes [119] and involving more than 14 quantitative trait loci [120].

Nonobese Models

Although considerably less common, T2D can occur in the absence of obesity. Atypical forms of nonobese T2D have been reported in Europe and Asia [121]. The nonobese T2D phenotype is characterized by lower circulating insulin levels or impaired β-cell function and reduced insulin resistance when compared to obese T2D, along with similar risks for cardiovascular disease and other comorbidities. Numerous factors are proposed to contribute to T2D in nonobese individuals, including environment, genetics, and in utero exposures [121]. Rodent models have been employed to elucidate how these factors influence the pathogenesis of nonobese T2D and to explore potential treatments of nonobese T2D.

GK rats: Goto-Katazaki (GK) rats represent a well-characterized model of nonobese T2M. GK rats exhibit insulin resistance in the skeletal muscle and liver, with impaired insulin release and hyperglycemia [122]. Although these rats exhibit some characteristic features of T2D without obesity, they are not routinely employed to study nonobese T2D because they display reduced fetal pancreatic β-cell proliferation, as well as reduced neonatal β-cell numbers and function [123, 124], features not believed to be common in humans.

Spontaneously diabetic Torii (SDT) rats: The spontaneously diabetic Torii (or SDT) rats, an inbred strain of Sprague Dawley rats, represent a new model of spontaneous nonobese T2D [125, 126]. More than 90% of male and female SDT rats survive through 65 weeks of age. However, T2D develops earlier and more severely in SDT males, with 100% of males achieving a diabetic state by 40 weeks of age versus 33% of females by 65 weeks of age [125]. SDT males are not obese but display both hyperglycemia and hypoinsulinemia after 25 weeks and hyperlipidemia after 35 weeks [125]. Genetic analyses revealed that glucose intolerance in SDT rats is associated with multiple genes on chromosomes 1, 2, and X [127]. This model has been employed

by many groups investigating diabetic retinopathy and other diabetic complications (e.g., neovascular glaucoma, peripheral and autonomic neuropathy, and diabetic nephropathy) [125, 126, 128–131].

Akita (Ins2Akita) mice: The *Ins2*^{Akita} (or Akita) mice, bred on the C57BL/6 background in Akita Japan, spontaneously develop diabetes in the absence of obesity and following early loss of pancreatic β-cells [132, 133]. Diabetes is more severe in Akita males than females [134]. A missense mutation in the insulin 2 (*Ins2*) gene in these mice results in the production of proinsulin with Cys^{96Tyr}, which impairs its processing and leads to the intracellular accumulation of mutant insulin A and B chains and β-cell apoptosis and, hence, hypoinsulinemia with hyperglycemia in 3–4-week-old mice [135, 136]. Most early studies employing Akita mice investigated early-onset insulin-dependent diabetes (or T1D). However, these nonobese Akita mice display chronic hyperglycemia and insulin resistance in several organs (e.g., liver, skeletal muscle, adipose tissue), without intracellular lipid accumulation [137]. Thus Akita mice exhibit several aspects of nonobese T2D.

Experimentally Induced Models

T2D can be induced in rodents by using numerous approaches, including obesogenic diets, chemical exposure that lead to pancreatic injury, and partial pancreatectomy.

Diet-Induced Models

As described above, obesity is a major contributing factor for the development of T2D. In rodents this can be mimicked by dietary modifications that promote weight gain/obesity and metabolic dysfunction. Typical obesogenic diets include a higher percentage of fat, predominated by saturated fats, with or without increased amounts of sugar.

C57BL/6 mice: Diet-induced obesity (DIO) models commonly employ C57BL/6 mice fed a 60% high fat diet (consisting of saturated fat [e.g., lard]) ad libitum for 6–8 weeks or more weeks versus C57BL/6 lean mice fed a typical 10% fat diet ad libitum for the same timeframe. Hyperglycemia is typically found after 4 weeks on the high fat diet [138, 139]. Significant metabolic consequences, including hyperlipidemia, pre-T2D symptoms, and hypertension, are observed after approximately 16 weeks on the high fat diet when weight gain is more than 20–30% of the controls (i.e., obesity) [140, 141]. With chronic feeding C57BL/6 males a high fat diet (60% calories from fat, Research Lab Diet D12492) for 30 weeks, we observed >80% weight increase when compared to lean controls fed normal rodent chow for 30 weeks, along with evidence of metabolic syndrome and significantly reduced circulating adiponectin concentrations [142]. C57BL/6 mice are the most susceptible to DIO, followed by 129X1, DBA/2, and FVB/N strains, whereas the AKR/J, DBA/2 J, BALB/c, and C57BL/KsJ strains are comparatively resistant to DIO [143, 144]. With long-term feeding, DIO-C57BL/6 mice exhibit prediabetic symptoms, including hyperinsulinemia, hyperglycemia, and hypertension [145]. Male mice are much more sensitive to diet-induced weight gain and subsequent metabolic syndrome than females [146, 147]. These metabolic changes reflect those observed in chronically obese humans. Estrogen has been postulated to protect against diet-induced obesity and metabolic changes [148]. Interestingly, estrogen protects premenopausal women from DIO [149, 150], and polymorphisms in the estrogen receptor (*ESR1*) have been identified in several cohorts in France and Sweden [151]. In summary, long-term DIO in C57BL/6 mice is accompanied by pre-T2D and T2D symptoms (Reviewed by [50]). However, other environmental challenges or genetic alterations can be included to produce robust T2M models.

Spiny mice: The first reports of spiny mice (*Acomys cahirinus*), native to Israel, which exhibit fur bristles on their backs, date back to the 1960s. When fed normal rat chow ad libitum, approximately one half of these mice become obese and diabetic, with mild hyperglycemia, hyperglycosuria, and hyperinsulinemia, which progresses to more severe disease with advanced age [152]. Older spiny mice develop diabetes in the absence of marked insulin resistance,

irrespective of gender [153]. When fed rodent chow supplemented with fatty seeds, these mice eventually progress to obesity, mild hyperglycemia, glucose intolerance, and hyperinsulinemia, along with initial pancreatic β-cell hyperplasia followed by loss of insulin production and β-cell apoptosis [153]. Feeding a high fat diet promotes β-hypertrophy and proliferation with β-cell loss leading to overt diabetes [153].

Israeli sand rats: Although originally named Israeli sand rats (*Psammomys obesus*), these animals belong to the Gerbillinae family. Also known as desert gerbils, they were first found in the sandy deserts of the Middle East where they consume a native vegetable diet and maintain a lean phenotype [153, 154]. A portion of Israeli sand rats housed under laboratory conditions and fed standard rodent chow (consisting of grains) ad libitum become obese and exhibit T2D [153]. By 16 weeks of age, approximately one third of these rodents develop diabetes, one third exhibit hyperinsulinemia/normoglycemia, and one third show normal glucose tolerance [155]. Similarly, a wide range of weights are observed among Israeli sand rats [156]. Israeli sand rats with body weight greater than 75th percentile showed obesity and an increased risk of developing T2D [156]. Hepatic insulin resistance is believed to precede hyperglycemia and hyperinsulinemia [154] in these rodents and is most likely due to impaired insulin-insulin receptor signaling [157, 158]. Thus, this model of polygenic T2D exhibits a wide range of body weights that correlate with the incidence of T2D and reflects the human condition.

Nile grass rats: Nile grass rats (*Arvicanthis niloticus*) are native to the dry regions of northern Africa, where they consume a vegetarian diet [159]. One distinguishing feature of these animals in their natural habitat is that they are exclusively diurnal, unlike the common laboratory rat (*Rattus norvegicus*) which is nocturnal [160]. Recent reports indicate that when fed standard rodent chow ad libitum, most Nile grass rats exhibit characteristic features of metabolic syndrome including obesity, dyslipidemia, hyperinsulinemia, and hyperglycemia by 1 year [161]. Approximately 90% of males and 50% of females develop T2D, accompanied by increased abdominal fat, elevated cholesterol and triglyceride levels, hypertension, reduced islet mass, and hepatic steatosis, which is more severe in the males [161]. With disease progression, abdominal fat declines as ketosis progresses, and there is a high correlation between plasma triglycerides and glycated hemoglobin (HbA1c) levels, supporting a link between diabetic state and dysfunctional lipid metabolism similar to that observed in humans with T2D and metabolic syndrome [161]. Recent studies have used the Nile grass rat model to study diabetic retinopathy, as these animals display retinal endothelial cell injury, particularly in the microvessels (e.g., vascular tortuosity, pericyte ghosts, and damaged acellular capillaries) by 1 year [162]. Finally, it is important to note that Nile grass rats do not belong of the genus *Mus* or *Rattus*, and thus, their use in the laboratory is regulated by the USDA, similar to rabbits.

Chemically Induced Models

In addition to their use in experimental models of T1D, streptozotocin and alloxan can be used in modeling features of T2D [163]. Streptozotocin has a much broader scope of use related to possibilities to induce different levels of hyperglycemia and other diabetes manifestations without generating ketosis and high mortality more commonly observed with alloxan (see sections "Streptozotocin-Induced Model" and "Alloxan-Induced Model"). Administration of streptozotocin to Sherman or Wistar rats is used to generate the neonatal streptozotocin model of T2D, which is characterized by dysregulated insulin release and sensitivity. In this model, streptozotocin administration after birth leads to almost immediate hyperglycemia, evident 2 days later. However, blood glucose levels normalize after the first week, accompanied by β-cell restoration. This regeneration is seemingly non-efficient or sustained, because mild hyperglycemia appears at 6 weeks [164]. By 8 weeks of age, this model is characterized by hyperglycemia and a 50% decrease in pancreatic insulin content, which occurs without alterations in pancreatic glucagon levels. The neonate model can be altered by utilizing streptozotocin administration at a

different time after birth, most commonly on postnatal day 2 or 5 [165]. The different timings of streptozotocin administration result in different levels of disease severity in the adult rats. While the 0- and 2-day model rats do not significantly differ, the 5-day model rats develop hyperglycemia with glucose intolerance, increased HbA1c, and markedly lower pancreatic insulin store, associated with about 50% reduction in basal plasma insulin levels and a lack of plasma insulin response to glucose [165].

Characteristic features of T2D such as hyperglycemia, glycosuria, and polydipsia also can be generated by utilizing low doses of alloxan administration. Rodents administered alloxan also develop symptoms of T2D, along with neuropathies, cardiomyopathy, and retinopathy, which provide a useful model to study T2D and the efficacy of new therapeutics on these complications [163].

Surgically Induced Models

A major step in diabetes research and treatment is islet transplantation. However, this approach is constrained by the scarcity of available islets and poor viability of transplanted islets due to autoimmunity and allorejection. Based on the need for alternative approaches, a great deal of research has focused on pancreatic β-cell regeneration and neogenesis. The insights generated can be relevant for both T2D and T1D. Classical rodent models utilized to study pancreatic regeneration and islet-cell growth are based on partial surgical removal of the pancreas (partial pancreatectomy) and duct ligation. These pancreatic injury models are predominantly performed in rats, because of the difficulties associated with surgical manipulations in mice. They provide a valuable tool for studying pancreatic β-cell regeneration and β-cell progenitors [50]. Removal of 60–90% of the pancreas is usually used in partial pancreatectomy models. Sixty percent pancreatectomy triggers regenerative processes resulting in marked restoration of the endocrine and exocrine pancreas at 4 weeks [166], whereas 90% pancreatectomy is shortly followed by hyperglycemia and noticeable pancreatic regeneration, which is associated with the formation of duct-enriched parts as early as 3 days after pancreatectomy [167]. Following partial rat pancreatic duct ligation, a replacement of exocrine acini by ductal complexes and significant growth of islet β-cells has been observed. The β-cell and α-cell populations significantly increase 1 week after the procedure. In addition, small islets and islet-cell clusters, indicating islet neogenesis, have been observed mainly in the pancreatic tail [168]. These observations support a hypothesis suggesting that islet-cell neogenesis can be reactivated by stimulation of pancreatic duct cells [168]. The models based on surgically induced pancreatic injury/pancreatectomy provide a platform for studying the regenerative processes in the pancreas, and the knowledge generated can be utilized in strategizing new treatments for diabetes. However, a general limitation of these models is their invasiveness and loss of other important pancreatic components.

Rodent Models of Gestational Diabetes

One important area of diabetes research often overlooked is gestational diabetes mellitus (GDM), defined as impaired glucose tolerance with onset or first diagnosis during pregnancy (typically during the 2nd trimester). The prevalence of GDM in the USA is estimated to be between 4% and 9% of pregnant women, and this continues to increase [169]. Pregnant women with GDM are at increased risk for preeclampsia and cesarean sections, as well as T2D and cardiovascular disease later in life [170]. Consistent with the concepts of fetal programming, babies exposed to GDM in utero are at increased risk for developing T2D later in childhood and adulthood [171], as well as numerous long-term metabolic, neurological, and endocrine disorders [172]. Because GDM is a major public health concern, numerous rodent models have been developed and employed to better understand its pathogenesis, as well as to investigate the short- and long-term consequences of in utero exposure to GDM and to test interventions.

Rodent models of GDM include streptozotocin (administered prior to pregnancy or early–mid-late pregnancy (reviewed in [173])) and dietary

manipulation (e.g., high fat diet, [173, 174]). Despite the plethora of monogenic and polygenic models of diabetes, most are not suitable for studying GDM because they either significantly impair fertility or lead to overt infertility (e.g., *ob/ob* and *db/db* mice), effect males more than females, or model diabetes prior to pregnancy. For more details, we refer the readers to a recent review on GDM models [173]. Herein, we highlight one model of rodent GDM, which mimics several aspects of the human condition [175]. This model of GDM is induced following administration of a high fat/high sugar "cafeteria" diet (prepared by mixing standard rat chow with 33% full fat sweetened condensed milk, 7% sucrose, and 27% water) to female Wistar rats 4 weeks prior to pregnancy and throughout pregnancy [175]. This model is characterized by impaired maternal glucose tolerance, elevated insulin levels, and insulin resistance, which was worsened by pregnancy [175]. This model has been used by our laboratory, as well as several other labs [176–179], to explore the effects of GDM on maternal, fetal, and offspring outcomes and assess various interventions (e.g., metformin).

Models Based on Genetic Manipulation

Selective manipulation of the mammalian genome by gene targeting has significantly advanced diabetes research and consequently our understanding of both T1D and T2D. Although we include these approaches under rodent models of T2D, we need to clarify that manipulation of genes implicated in diabetes pathogenesis and complications does not result in distinct and complete modeling of T2D as observed in humans. Instead, these models provide valuable insights related to the physiological role of the gene product(s), consequences of gene-environment interactions, and their pathophysiological deviations. In addition to T2D, this information can be analyzed from the perspective of T1D.

General Gene Knockout Models

Using mice lacking whole-body expression of a certain gene or genes has been instrumental for determining gene function in the context of diabetes. However, germline mutations of some genes encoding molecules with important roles in metabolism and diabetes pathogenesis can be lethal, because these genes are indispensable for embryonic and postnatal development. Therefore, some of these general gene knockout (KO) models provide a very short, if any, time window for evaluation. For instance, mice with a global KO of the insulin receptor (*Ir*) die within 4–5 days post-birth [180]. The information gathered during this extremely short time reveals a phenotype characterized by ketoacidosis, elevated plasma free fatty acids, triglycerides, and reduced hepatic glycogens. A general KO of the insulin receptor substrate-1 gene (*Irs1)* is not lethal, but mice with this gene ablation have embryonal and postnatal growth retardation [181]. Targeted disruption of *Irs1* also results in muscle insulin resistance and insulin hypersecretion associated with increased β-cell mass, in the absence of diabetes [181]. The lack of dramatic effect of *Irs1* gene disruption might be due to possible redundancy within the insulin signaling cascade, associated with compensatory gene overexpression [182]. Possible alterations in other gene expression as a compensatory reaction to specific gene manipulation are a general limitation of KO and transgenic models. Insulin receptor substrate-2 gene (*Irs2)* deficient mice have reduced β-cell mass resulting in insufficient insulin secretion and glucose intolerance manifested by fasting hyperglycemia at 6 weeks of age [183]. These mice show peripheral insulin resistance, characteristic diabetic polydipsia, and polyuria and die at 10 weeks of age due to hyperosmolar coma [183].

Targeted disruption of the receptor for the glucagon-like peptide 1 gene (*Glp1*r) has provided valuable information about the role of GLP1R-mediated signaling in glucose homeostasis and feeding behavior [184]. These KO mice are viable, but develop hyperglycemia, in parallel with decreased blood insulin levels. Somewhat surprisingly, *Glp1r*KO mice have a normal body weight and feeding behavior. The role of the brain GLP1R in feeding behavior is demonstrated by the observation that intracerebroventricular injection of GLP1 suppresses

feeding in the wild-type controls, but not in the KO mice [184].

Gene manipulation can be combined with other "classical" approaches used in diabetes modeling. For instance, important insight related to the role of glucagon in diabetes pathogenesis has been revealed by expressing glucagon receptors in livers of glucagon receptor-null ($GcgR^{-/-}$) mice before and after administering high-dose streptozotocin to cause β-cell destruction [185]. In contrast to wild-type mice, $GcgR^{-/-}$ mice with β-cell destruction do not display hyperglycemia, impaired glucose tolerance, or hepatic glycogen depletion. However, restoration of receptor expression (by using adenovirus containing the *GcgR* cDNA) and hepatic GcgR signaling results in severe hyperglycemia. The spontaneous disappearance of *GcgR* mRNA is associated with a significant alleviation of hyperglycemia. This study suggests that glucagon suppression should be considered in diabetes treatment [185].

Models Based on Tissue- and Cell-Specific Gene Manipulation

The development of the Cre-*lox*P system of DNA recombination has allowed tissue- and cell-specific gene inactivation, *de novo* induction of select gene-coding sequences, as well as other types of spatial and temporal gene manipulation [186]. These approaches overcome limitations of the standard homologous recombination technology. Cre is a bacteriophage P1 recombinase enzyme that recognizes specific sequences of DNA 34-bp long (LoxP sites). When two of these sites are close to each other, Cre cleaves DNA sequences between them. The use of cell type-specific promoters (for instance, the insulin promoter) to drive expression of Cre recombinase provides a high level of cell specificity. These promoters can be also designed to incorporate drug-responsive elements, allowing Cre recombinase expression to be switched on by drugs such as tamoxifen (CreERT). There are numerous transgenic Cre mice with cell- or tissue-specific promoters, which facilitate their use in diabetes research [187]. Useful information about transgenic mouse Cre lines is available at http://www.findmice.org/index.jsp, and http://www.informatics.jax.org/. Some important considerations for using pancreas-specific Cre driver lines have been recently summarized [188].

The Cre-loxP system has been used to inactivate the insulin receptor gene (*Ir)* in a tissue-specific manner, which overcomes limitations related to the general *Ir* KO model and provides specific insights. The skeletal muscle-specific *Ir* KO reveals a phenotype with some features of the metabolic syndrome, including increased fat mass and increased triglycerides, but without glucose intolerance [189]. Pancreatic β-cell-specific *Ir* KO mice have a defect in insulin secretion, resembling one of the cardinal features of T2D and impaired glucose tolerance [190]. A tissue-specific knockout of IR in the brain showed the role of the brain receptor in controlling body weight and reproduction [191]. Interestingly, brown adipose tissue-specific *Ir* KO mice display a diabetic phenotype without insulin resistance [192].

Targeted cell-specific genetic modification has been used in rodent models to study β-cell regeneration capacity and for identifying β-cell precursors/progenitors [50]. These models complement the pancreatic injury models of β-cell regeneration described above. They provide additional advantages related to studying β-cell regeneration in the absence of confounding autoimmunity-related factors, recovery of dysfunctional β-cells, or damage to other cell types. For instance, a useful mouse model has been created by administering doxycycline to transgenic mice that expressed diphtheria toxin in β-cells [193]. The subsequent expression of diphtheria toxin A leads to apoptosis of 70–80% of β-cells, destruction of islets, and hyperglycemia. Subsequent withdrawal of doxycycline leads to β-cell mass recovery following proliferation of surviving β-cells, restoration of islet architecture, and normoglycemia [193]. In this model, treatment with sirolimus and tacrolimus immunosuppressants (commonly used according to the Edmonton protocol for human islet transplantation) suppresses β-cell regeneration and prevents normoglycemia [193]. These somewhat surprising observations suggest that regenerative therapy for diabetes might be improved in the context of adequate autoimmunity suppression

and drugs that promote β-cell regeneration [193]. Another interesting transgenic mouse model with inducible and reversible β-cell ablation is the so-called PANIC-ATTAC (pancreatic islet β-cell apoptosis through targeted activation of caspase 8) model [194]. In this model, β-cell death is induced by administration of a chemical dimerizer, AP20187, to 2–3-month-old transgenic mice, containing a mutated FK506 binding protein (FKBP) that is fused to caspase 8 and expressed under control of the insulin promoter. The diabetes phenotype and β-cell loss in these mice are entirely reversible, and significant β-cell recovery and normoglycemia are evident after 2 months. In this model of β-cell regeneration, a significant population of $GLUT2^{+}$/$insulin^{-}$cells has been detected and proposed to serve as β-cell precursors [194]. Directing Cre expression to specific cell populations has been utilized in analyzing the cell lineage in the pancreatic islets [195]. Irreversibly tagging all the progeny of pancreatic cells using the Cre-loxP approach and then studying adult islet β- and α-cells for derivation from these "tagged" cells have indicated that β-cell and α-cell lineages arise independently during ontogeny, most likely from a common precursor [195]. The use of a combination of targeted cell-specific gene manipulations revealed that in response to injury, progenitor cells give rise to glucagon-expressing α-cells, which then differentiate into β-cells [196, 197]. These models of ablating β-cells, which can be manipulated by changing the timing of dimerizer treatment, the dose, and frequency of dimerizer treatment and by varying dietary and/or environmental exposures, have been useful for investigating islet-cell physiology and β-cell regeneration methods.

Models Utilizing Optogenetics and the CRISPR-Cas9 System

Optogenetics combine genetic and optical elements to generate cell-specific gain or loss of function [198]. Initially optogenetic manipulation was almost exclusively used as a valuable tool in brain studies. This technology is based on the expression of light-sensitive proteins, known as opsins, in specific neurons or regions and selective activation or silencing of these targets by light exposure [199]. The opsin expression can be achieved by in vivo injection of Cre-dependent viral vectors to specific regions or by generating transgenic mice with stable expression of opsins, for instance, channelrhodopsin-2 (ChR2) in specific neuronal populations [200]. The use of optogenetic tools led to significant advances in defining specific neuronal function and evaluating neuronal circuitry and its role in behavior. Some important principles of using optogenetics and potential confounds in this field have been recently summarized [199]. In addition to studying neurocircuitry, optogenetics can be used in addressing important questions in a much broader scope of biological systems [198]. Exploration of this technology has also started in diabetes research, for instance, in studying mechanisms of insulin secretion [201, 202]. Initial in vitro observations have shown that laser light (470 nm) exposure of *Chr2*-transfected mouse pancreatic β-cell line (ChR2-MIN6 cells) results in enhanced insulin secretion, associated with increased mRNA levels for calcium-/calmodulin-dependent protein kinase II delta and adenylate cyclase 1 [201]. Laser irradiation of ChR2-MIN6 cells inoculated in mice with streptozotocin-induced diabetes increases ChR2-MIN6 insulin expression and lowers blood glucose levels [201]. This study suggests a new optogenetic alternative for a precise control of β-cell insulin secretion in addition to pharmacological options.

The clustered regularly interspaced short palindromic repeats and the associated nuclease Cas9 (CRISPR-Cas9) system belong to the latest generation of genome-editing technologies. A detailed description of the CRISPR-Cas9 technology is beyond the scope of this chapter, but interested readers are referred to several recent reviews [203–205]. This approach utilizes a short single-guide RNA (sgRNA) to direct the endonuclease Cas9 to a desired point of the genome. Cas9 triggers the formation of DNA double-strand breaks (DSBs) and allows the repair or insertion of mutations, insertion of recombinase recognition sites, or large DNA elements [205]. The CRISPR-Cas9 technology has a number of advantages over other nuclease-based targeting technologies and can be used in all species [205]. Using

the CRISPS-Cas9 system to generate genetic mutations in rodents eliminates many concerns associated with other more "conventional" procedures of gene manipulation, including the presence of single-nucleotide polymorphisms or other genomic variants located in the vicinity of the desired mutation. The scope of potential implications of CRISPR-Cas9 technology for disrupting, modulating, and imaging genetic and epigenetic processes in the context of various physiological and pathophysiological conditions is rapidly expanding. Its utilization in diabetes research has also been initiated. For instance, a knockout of the *Lepr* gene, encoding the leptin receptor in rats has been achieved by using the CRISPR-Cas9 technology [206]. The leptin receptor KO rats show a phenotype characterized by severe obesity, hyperphagia, glucose intolerance, hyperinsulinemia, dyslipidemia, decreased bone mineral density, and diabetes complications. This new model provides some advantages over the existing models, including the lack of transient hyperglycemia reported in *db*/*db* mice and the delayed onset of glucose intolerance in the Zucker rats.

Conclusions

Over 7% of the world's population or 380 million people has diabetes; this number is expected to reach almost 600 million by 2038 (IDF Diabetes Atlas: www.idf.org/diabetesatlas). As the burdens of both T1D and T2D in humans continue to rise, diabetes research is expected to continue to advance our understanding of disease pathogenesis and to explore preventative strategies and potential treatments in pursuing the mission of finding cures. Animal models of diabetes provide the necessary foundation for preclinical studies of the human conditions and will continue to move the field toward breakthrough discoveries. The models described herein have been invaluable in defining genetic and epigenetic aspects of the complex variety of mechanisms implicated in diabetes pathogenesis and complications and examining the efficacy of new treatments. Choosing the appropriate model to address a specific research question is integral to providing relevant insight. Multiple factors should be considered in utilizing a certain model, including age of disease onset; disease incidence; differences in gender susceptibility; the presence of autoantibodies and other autoimmune/immune disorders; insulitis; environmental influences that affect disease incidence, progression, and/or severity; and other related diabetic symptoms. Furthermore, utilizing new approaches of tissue- and cell-specific gene manipulations and genome editing, including the Cre-LoxP system, optogenetics, and the CRISPR-Cas9 technology in studying diabetes in rodents, will further advance the field. Insulin secretion and signaling, glucose metabolism, and other physiological processes, which become dysfunctional in diabetes, are under complex physiological control, involving endocrine, immune, and neural mechanisms [207–209]. Considering and providing insight into these complex regulatory mechanisms by using relevant and specific rodent models is important because it may better define new therapeutic and preventative approaches.

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References

1. Roth J, Qureshi S, Whitford I, Vranic M, Kahn CR, Fantus IG, et al. Insulin's discovery: new insights on its ninetieth birthday. Diabetes Metab Res Rev. 2012;28:293–304.
2. Van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev. 2011;91:79–118.
3. Bakay M, Pandey R, Hakonarson H. Genes involved in type 1 diabetes: an update. Genes. 2013;4:499–521.
4. Mehers KL, Gillespie KM. The genetic basis for type 1 diabetes. Br Med Bull. 2008;88:115–29.
5. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother Rep. 1963;29:91–8.
6. Mansford KR, Opie L. Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by alloxan. Lancet. 1968;1:670–1.
7. Wang Z, Gleichmann H. GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with

multiple low doses of streptozotocin in mice. Diabetes. 1998;47:50–6.
8. Fozzard HA, Beeler Jr GW. The voltage clamp and cardiac electrophysiology. Circ Res. 1975;37:403–13.
9. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51:216–26.
10. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science. 1976;193:415–7.
11. Yamamoto H, Uchigata Y, Okamoto H. Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ri bose) synthetase in pancreatic islets. Nature. 1981;294: 284–6.
12. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, et al. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab Anim. 2011;45:131–40.
13. Reddy S, Wu D, Elliott RB. Low dose streptozotocin causes diabetes in severe combined immunodeficient (SCID) mice without immune cell infiltration of the pancreatic islets. Autoimmunity. 1995;20:83–92.
14. Dekel Y, Glucksam Y, Elron-Gross I, Margalit R. Insights into modeling streptozotocin-induced diabetes in ICR mice. Lab Anim. 2009;38:55–60.
15. Lukic ML, Stosic-Grujicic S, Shahin A. Effector mechanisms in low-dose streptozotocin-induced diabetes. Dev Immunol. 1998;6:119–28.
16. Wei M, Ong L, Smith MT, Ross FB, Schmid K, Hoey AJ, et al. The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. Heart Lung Circ. 2003;12:44–50.
17. Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med. 2012;237:481–90.
18. Dunn JS, Duffy E, Gilmour MK, Kirkpatrick J, McLetchie NG. Further observations on the effects of alloxan on the pancreatic islets. J Physiol. 1944;103:233–43.
19. Mrozikiewicz A, Kielczewska-Mrozikiewicz D, Lowicki Z, Chmara E, Korzeniowska K, Mrozikiewicz PM. Blood levels of alloxan in children with insulin-dependent diabetes mellitus. Acta Diabetol. 1994;31:236–7.
20. Hoftiezer V, Carpenter AM. Comparison of streptozotocin and alloxan-induced diabetes in the rat, including volumetric quantitation of the pancreatic islets. Diabetologia. 1973;9:178–84.
21. Federiuk IF, Casey HM, Quinn MJ, Wood MD, Ward WK. Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. Comp Med. 2004;54:252–7.
22. Coppieters KT, Boettler T, von Herrath M. Virus infections in type 1 diabetes. Cold Spring Harb Perspect Med. 2012;2:a007682.
23. von Herrath M, Filippi C, Coppieters K. How viral infections enhance or prevent type 1 diabetes-from mouse to man. J Med Virol. 2011;83:1672.
24. Schneider DA, von Herrath MG. Potential viral pathogenic mechanism in human type 1 diabetes. Diabetologia. 2014;57:2009–18.
25. Lonnrot M, Korpela K, Knip M, Ilonen J, Simell O, Korhonen S, et al. Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. Diabetes. 2000;49:1314–8.
26. Dotta F, Censini S, van Halteren AG, Marselli L, Masini M, Dionisi S, et al. Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci U S A. 2007;104:5115–20.
27. Yoon JW, Onodera T, Notkins AL. Virus-induced diabetes mellitus. XV. Beta cell damage and insulin-dependent hyperglycemia in mice infected with coxsackie virus B4. J Exp Med. 1978;148:1068–80.
28. Yoon JW, Austin M, Onodera T, Notkins AL. Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. N Engl J Med. 1979;300: 1173–9.
29. Clements GB, Galbraith DN, Taylor KW. Coxsackie B virus infection and onset of childhood diabetes. Lancet. 1995;346:221–3.
30. Andreoletti L, Hober D, Hober-Vandenberghe C, Bela ich S, Vantyghem MC, Lefebvre J, et al. Detection of coxsackie B virus RNA sequences in whole blood sa mples from adult patients at the onset of type I diabetes mellitus. J Med Virol. 1997;52:121–7.
31. Serreze DV, Ottendorfer EW, Ellis TM, Gauntt CJ, Atkinson MA. Acceleration of type 1 diabetes by a coxsackievirus infection requires a preexisting critical mass of autoreactive T-cells in pancreatic islets. Diabetes. 2000;49:708–11.
32. Tracy S, Drescher KM, Chapman NM, Kim KS, Carson SD, Pirruccello S, et al. Toward testing the hypothesis that group B coxsackieviruses (CVB) trigger insulin-dependent diabetes: inoculating nonobese diabetic mice with CVB markedly lowers diabetes incidence. J Virol. 2002;76:12097–111.
33. Bason C, Lorini R, Lunardi C, Dolcino M, Giannattasio A, d'Annunzio G, et al. In type 1 diabetes a subset of anti-coxsackievirus B4 antibodies recognize autoantigens and induce apoptosis of pancreatic beta cells. PLoS One. 2013;8:e57729.
34. Craighead JE, McLane MF. Diabetes mellitus: induction in mice by encephalomyocarditis virus. Science. 1968;162:913–4.
35. Hirasawa K, Jun HS, Maeda K, Kawaguchi Y, Itagaki S, Mikami T, et al. Possible role of macrophage-derived soluble mediators in the pathogenesis of encephalomyocarditis virus-induced diabetes in mice. J Virol. 1997;71:4024–31.
36. Kang Y, Yoon JW. A genetically determined host factor controlling susceptibility to encephalomyocarditis virus-induced diabetes in mice. J Gen Virol. 1993;74(Pt 6):1207–13.
37. Baek HS, Yoon JW. Direct involvement of macrophages in destruction of beta-cells leading to

development of diabetes in virus-infected mice. Diabetes. 1991;40:1586–97.
38. Shimada A, Maruyama T. Encephalomyocarditis-virus-induced diabetes model resembles "fulminant" type 1 diabetes in humans. Diabetologia. 2004;47:1854–5.
39. Ellerman KE, Richards CA, Guberski DL, Shek WR, Like AA. Kilham rat triggers T-cell-dependent autoimmune diabetes in multiple strains of rat. Diabetes. 1996;45:557–62.
40. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, et al. Induction of type I diabetes by Kilham's rat virus in diabetes-resistant BB/Wor rats. Science. 1991;254:1010–3.
41. Zipris D, Lien E, Xie JX, Greiner DL, Mordes JP, Rossini AA. TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. J Immunol. 2005;174:131–42.
42. Alkanani AK, Hara N, Gianani R, Zipris D. Kilham Rat Virus-induced type 1 diabetes involves beta cell infection and intra-islet JAK-STAT activation prior to insulitis. Virology. 2014;468–470:19–27. doi:10.1016/j.virol.2014.07.041. Epub 16 Aug 2014.
43. Tirabassi RS, Guberski DL, Blankenhorn EP, Leif JH, Woda BA, Liu Z, et al. Infection with viruses from several families triggers autoimmune diabetes in LEW*1WR1 rats: prevention of diabetes by maternal immunization. Diabetes. 2010;59:110–8.
44. von Herrath MG, Homann D, Gairin JE, Oldstone MB. Pathogenesis and treatment of virus-induced autoimmune diabetes: novel insights gained from the RIP-LCMV transgenic mouse model. Biochem Soc Trans. 1997;25:630–5.
45. Oldstone MB, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell. 1991;65:319–31.
46. Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, et al. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell. 1991;65:305–17.
47. Banting FG. An address on diabetes and insulin: being the nobel lecture delivered at Stockholm on september 15th, 1925. Can Med Assoc J. 1926;16:221–32.
48. Gordon CS, Serino AS, Krause MP, Campbell JE, Cafarelli E, Adegoke OA, et al. Impaired growth and force production in skeletal muscles of young partially pancreatectomized rats: a model of adolescent type 1 diabetic myopathy? PLoS One. 2010;5:e14032.
49. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature. 2008;455:1109–13.
50. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012;166:877–94.
51. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. Jikken Dobutsu. 1980;29:1–13.
52. Thayer TC, Wilson SB, Mathews CE. Use of nonobese diabetic mice to understand human type 1 diabetes. Endocrinol Metab Clin North Am. 2010;39:541–61.
53. Wicker LS, Todd JA, Peterson LB. Genetic control of autoimmune diabetes in the NOD mouse. Annu Rev Immunol. 1995;13:179–200.
54. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003;423:506–11.
55. Kristiansen OP, Larsen ZM, Pociot F. CTLA-4 in autoimmune diseases – a general susceptibility gene to autoimmunity? Genes Immun. 2000;1:170–84.
56. Lundholm M, Motta V, Lofgren-Burstrom A, Duarte N, Bergman ML, Mayans S, et al. Defective induction of CTLA-4 in the NOD mouse is controlled by the NOD allele of Idd3/IL-2 and a novel locus (Ctex) telomeric on chromosome 1. Diabetes. 2006;55:538–44.
57. King C, Sarvetnick N. The incidence of type-1 diabetes in NOD mice is modulated by restricted flora not germ-free conditions. PLoS One. 2011;6:e17049.
58. Driver JP, Serreze DV, Chen YG. Mouse models for the study of autoimmune type 1 diabetes: a NOD to similarities and differences to human disease. Semin Immunopathol. 2011;33:67–87.
59. Guberski DL. Diabetes-prone and diabetes-resistant BB rats: animal models of spontaneous and virally induced diabetes mellitus, lymphocytic thyroiditis, and collagen-induced arthritis. ILAR J. 1993;35:29–37.
60. Poussier P, Ning T, Murphy T, Dabrowski D, Ramanathan S. Impaired post-thymic development of regulatory CD4 + 25+ T cells contributes to diabetes pathogenesis in BB rats. J Immunol. 2005;174:4081–9.
61. Poussier P, Nakhooda AF, Falk JA, Lee C, Marliss EB. Lymphopenia and abnormal lymphocyte subsets in the "BB" rat: relationship to the diabetic syndrome. Endocrinology. 1982;110:1825–7.
62. Wallis RH, Wang K, Marandi L, Hsieh E, Ning T, Chao GY, et al. Type 1 diabetes in the BB rat: a polygenic disease. Diabetes. 2009;58:1007–17.
63. Komeda K, Noda M, Terao K, Kuzuya N, Kanazawa M, Kanazawa Y. Establishment of two substrains, diabetes-prone and non-diabetic, from Long-Evans Tokushima Lean (LETL) rats. Endocr J. 1998;45:737–44.
64. Yokoi N, Komeda K, Wang HY, Yano H, Kitada K, Saitoh Y, et al. Cblb is a major susceptibility gene for rat type 1 diabetes mellitus. Nat Genet. 2002;31:391–4.
65. Mordes JP, Guberski DL, Leif JH, Woda BA, Flanagan JF, Greiner DL, et al. LEW.1WR1 rats develop autoimmune diabetes spontaneously and in response to environmental perturbation. Diabetes. 2005;54:2727–33.

66. Lenzen S, Tiedge M, Elsner M, Lortz S, Weiss H, Jorns A, et al. The LEW.1AR1/Ztm-iddm rat: a new model of spontaneous insulin-dependent diabetes mellitus. Diabetologia. 2001;44:1189–96.
67. Jorns A, Gunther A, Hedrich HJ, Wedekind D, Tiedge M, Lenzen S. Immune cell infiltration, cytokine expression, and beta-cell apoptosis during the development of type 1 diabetes in the spontaneously diabetic LEW.1AR1/Ztm-iddm rat. Diabetes. 2005;54:2041–52.
68. Centers for Disease Control and Prevention (CDC). Awareness of prediabetes – United States, 2005–2010. MMWR Morb Mortal Wkly Rep. 2013;62:209–12.
69. Dickie MMLP. Plus letter to Roy Robinson 7/7/70. Mouse News Lett. 1957;17:52.
70. Bahary N, Siegel DA, Walsh J, Zhang Y, Leopold L, Leibel R, et al. Microdissection of proximal mouse chromosome 6: identification of RFLPs tightly linked to the ob mutation. Mamm Genome. 1993;4:511–5.
71. Friedman JM, Leibel RL, Siegel DS, Walsh J, Bahary N. Molecular mapping of the mouse ob mutation. Genomics. 1991;11:1054–62.
72. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372:425–32.
73. Brennan AM, Mantzoros CS. Drug Insight: the role of leptin in human physiology and pathophysiology – emerging clinical applications. Nat Clin Pract Endocrinol Metab. 2006;2:318–27.
74. Carlsson B, Lindell K, Gabrielsson B, Karlsson C, Bjarnason R, Westphal O, et al. Obese (ob) gene defects are rare in human obesity. Obes Res. 1997;5:30–5.
75. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998;395:763–70.
76. Hummel KP, Coleman DL, Lane PW. The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL-KsJ and C57BL-6J strains. Biochem Genet. 1972;7:1–13.
77. Lindstrom P. The physiology of obese-hyperglycemic mice [ob/ob mice]. Scientific World Journal. 2007;7:666–85.
78. Coleman DL, Hummel KP. The influence of genetic background on the expression of the obese (Ob) gene in the mouse. Diabetologia. 1973;9:287–93.
79. Chua Jr S, Liu SM, Li Q, Yang L, Thassanapaff VT, Fisher P. Differential beta cell responses to hyperglycaemia and insulin resistance in two novel congenic strains of diabetes (FVB- Lepr (db)) and obese (DBA- Lep (ob)) mice. Diabetologia. 2002;45:976–90.
80. Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet. 1996;12:318–20.
81. Mounzih K, Lu R, Chehab FF. Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology. 1997;138:1190–3.
82. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. Science. 1966;153:1127–8.
83. Zucker L, Zucker T. Fatty, a new mutation in the rat. J Hered. 1961;52:275–8.
84. Peterson R, Shaw W, Neel MA, Little LA, Eichberg J. Zucker diabetic fatty rat as a model for non-insulin dependent diabetes mellitus. ILAR News. 1990;32:16–9.
85. Phillips MS, Liu Q, Hammond HA, Dugan V, Hey PJ, Caskey CJ, et al. Leptin receptor missense mutation in the fatty Zucker rat. Nat Genet. 1996;13:18–9.
86. Durham HA, Truett GE. Development of insulin resistance and hyperphagia in Zucker fatty rats. Am J Physiol Regul Integr Comp Physiol. 2006;290:R652–8.
87. Kava R, Greenwood M, Johnson P. Zucker (fa/fa) rat. ILAR J. 1990;32:4–8.
88. Baynes J, Murray DB. Cardiac and renal function are progressively impaired with aging in Zucker diabetic fatty type II diabetic rats. Oxid Med Cell Longev. 2009;2:328–34.
89. Kim J, Sohn E, Kim CS, Kim JS. Renal podocyte apoptosis in Zucker diabetic fatty rats: involvement of methylglyoxal-induced oxidative DNA damage. J Comp Pathol. 2011;144:41–7.
90. Taketomi S, Tsuda M, Matsuo T, Iwatsuka H, Suzuoki Z. Alterations of hepatic enzyme activities in KK and yellow KK mice with various diabetic states. Horm Metab Res. 1973;5:333–9.
91. Ikeda H. KK mouse. Diabetes Res Clin Pract. 1994;24(Suppl):S313–6.
92. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res. 2007;125:451–72.
93. Miltenberger RJ, Mynatt RL, Wilkinson JE, Woychik RP. The role of the agouti gene in the yellow obese syndrome. J Nutr. 1997;127:1902S–7.
94. Roberts DW, Wolff GL, Campbell WL. Differential effects of the mottled yellow and pseudoagouti phenotypes on immunocompetence in Avy/a mice. Proc Natl Acad Sci U S A. 1984;81:2152–6.
95. Frigeri LG, Teguh C, Ling N, Wolff GL, Lewis UJ. Increased sensitivity of adipose tissue to insulin after in vivo treatment of yellow Avy/A obese mice with amino-terminal peptides of human growth hormone. Endocrinology. 1988;122:2940–5.
96. Yen TT, Greenberg MM, Yu PL, Pearson DV. An analysis of the relationships among obesity, plasma insulin and hepatic lipogenic enzymes in “viable yellow obese” mice (Avy/a). Horm Metab Res. 1976;8:159–66.
97. Yen TT, McKee MM, Stamm NB. Thermogenesis and weight control. Int J Obes. 1984;8 Suppl 1:65–78.

98. Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol Rev. 1979;59:719–809.
99. Yen TT, Gill AM, Frigeri LG, Barsh GS, Wolff GL. Obesity, diabetes, and neoplasia in yellow A (vy)/- mice: ectopic expression of the agouti gene. FASEB J. 1994;8:479–88.
100. Bielschowsky M, Bielschowsky F. A new strain of mice with hereditary obesity. Proc Univ Otago Med Sch. 1953;31:29–31.
101. Leiter EH, Reifsnyder PC. Differential levels of diabetogenic stress in two new mouse models of obesity and type 2 diabetes. Diabetes. 2004;53 Suppl 1: S4–11.
102. Bray GA, York DA. Genetically transmitted obesity in rodents. Physiol Rev. 1971;51:598–646.
103. Ortlepp JR, Kluge R, Giesen K, Plum L, Radke P, Hanrath P, et al. A metabolic syndrome of hypertension, hyperinsulinaemia and hypercholesterolaemia in the New Zealand obese mouse. Eur J Clin Invest. 2000;30:195–202.
104. Haskell BD, Flurkey K, Duffy TM, Sargent EE, Leiter EH. The diabetes-prone NZO/HlLt strain. I. Immuno phenotypic comparison to the related NZB/BlNJ and NZW/LacJ strains. Lab Invest. 2002;82:833–42.
105. Leiter EH, Reifsnyder PC, Flurkey K, Partke HJ, Junger E, Herberg L. NIDDM genes in mice: deleterious synergism by both parental genomes contributes to diabetogenic thresholds. Diabetes. 1998;47: 1287–95.
106. Junger E, Herberg L, Jeruschke K, Leiter EH. The diabetes-prone NZO/Hl strain. II. Pancreatic immunopathology. Lab Invest. 2002;82:843–53.
107. Shibata M, Yasuda B. New experimental congenital diabetic mice (N.S.Y. mice). Tohoku J Exp Med. 1980;130:139–42.
108. Ueda H, Ikegami H, Yamato E, Fu J, Fukuda M, Shen G, et al. The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. Diabetologia. 1995;38:503–8.
109. Ikegami H, Fujisawa T, Ogihara T. Mouse models of type 1 and type 2 diabetes derived from the same closed colony: genetic susceptibility shared between two types of diabetes. ILAR J. 2004;45:268–77.
110. Kim JH, Sen S, Avery CS, Simpson E, Chandler P, Nishina PM, et al. Genetic analysis of a new mouse model for non-insulin-dependent diabetes. Genomics. 2001;74:273–86.
111. Kim JH, Stewart TP, Soltani-Bejnood M, Wang L, Fortuna JM, Mostafa OA, et al. Phenotypic characterization of polygenic type 2 diabetes in TALLYHO/JngJ mice. J Endocrinol. 2006;191:437–46.
112. Nakamura N. Reduced aldehyde dehydrogenase activity and arginine vasopressin receptor 2 expression in the kidneys of male TALLYHO/JngJ mice of prediabetic age. Endocrine. 2011;40:379–85.
113. Kim JH, Saxton AM. The TALLYHO mouse as a model of human type 2 diabetes. Methods Mol Biol. 2012;933:75–87. doi:10.1007/978-1-62703-068-7_6.:75-87.
114. Cho YR, Kim HJ, Park SY, Ko HJ, Hong EG, Higashimori T, et al. Hyperglycemia, maturity-onset obesity, and insulin resistance in NONcNZO10/LtJ males, a new mouse model of type 2 diabetes. Am J Physiol Endocrinol Metab. 2007;293:E327–36.
115. Blaber SI, Diaz J, Blaber M. Accelerated healing in NONcNZO10/LtJ type 2 diabetic mice by FGF-1. Wound Repair Regen. 2015;23:538–49.
116. Zhang S, Wang S, Puhl MD, Jiang X, Hyrc KL, Laciny E, et al. Global biochemical profiling identifies beta-hydroxypyruvate as a potential mediator of type 2 diabetes in mice and humans. Diabetes. 2015;64:1383–94.
117. Kawano K, Hirashima T, Mori S, Natori T. OLETF (Otsuka Long-Evans Tokushima Fatty) rat: a new NIDDM rat strain. Diabetes Res Clin Pract. 1994;24 (Suppl):S317–20.
118. Moran TH. Unraveling the obesity of OLETF rats. Physiol Behav. 2008;94:71–8.
119. Nara Y, Gao M, Ikeda K, Sato T, Sawamura M, Kawano K, et al. Genetic analysis of non-insulin-dependent diabetes mellitus in the Otsuka Long-Evans Tokushima Fatty rat. Biochem Biophys Res Commun. 1997;241:200–4.
120. Yamada T, Kose H, Ohta T, Matsumoto K. Genetic dissection of complex genetic factor involved in NIDDM of OLETF rat. Exp Diabetes Res. 2012;2012:582546. doi:10.1155/2012/582546. Epub 15 Oct 2012.
121. Vaag A, Lund SS. Non-obese patients with type 2 diabetes and prediabetic subjects: distinct phenotypes requiring special diabetes treatment and (or) prevention? Appl Physiol Nutr Metab. 2007;32:912–20.
122. Picarel-Blanchot F, Berthelier C, Bailbe D, Portha B. Impaired insulin secretion and excessive hepatic glucose production are both early events in the diabetic GK rat. Am J Physiol. 1996;271:E755–62.
123. Movassat J, Saulnier C, Serradas P, Portha B. Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. Diabetologia. 1997;40:916–25.
124. Portha B, Giroix MH, Tourrel-Cuzin C, Le Stunff H, Movassat J. The GK rat: a prototype for the study of non-overweight type 2 diabetes. Methods Mol Biol. 2012;933:125–59. doi:10.1007/978-1-62703-068-7_9.:125-59.
125. Shinohara M, Masuyama T, Shoda T, Takahashi T, Katsuda Y, Komeda K, et al. A new spontaneously diabetic non-obese Torii rat strain with severe ocular complications. Int J Exp Diabetes Res. 2000;1: 89–100.
126. Sasase T, Ohta T, Ogawa N, Miyajima K, Ito M, Yamamoto H, et al. Preventive effects of glycaemic control on ocular complications of Spontaneously Diabetic Torii rat. Diabetes Obes Metab. 2006;8:501–7.

127. Masuyama T, Fuse M, Yokoi N, Shinohara M, Tsujii H, Kanazawa M, et al. Genetic analysis for diabetes in a new rat model of nonobese type 2 diabetes, Spontaneously Diabetic Torii rat. Biochem Biophys Res Commun. 2003;304:196–206.
128. Matsui K, Ohta T, Oda T, Sasase T, Ueda N, Miyajima K, et al. Diabetes-associated complications in Spontaneously Diabetic Torii fatty rats. Exp Anim. 2008;57:111–21.
129. Katsuda Y, Sasase T, Tadaki H, Mera Y, Motohashi Y, Kemmochi Y, et al. Contribution of hyperglycemia on diabetic complications in obese type 2 diabetic SDT fatty rats: effects of SGLT inhibitor phlorizin. Exp Anim. 2015;64:161–9.
130. Islam MS. Animal models of diabetic neuropathy: progress since 1960s. J Diabetes Res. 2013;2013 149452. doi:10.1155/2013/149452. Epub 29 July 2013.
131. Fujii H, Kono K, Nakai K, Goto S, Komaba H, Hamada Y, et al. Oxidative and nitrosative stress and progression of diabetic nephropathy in type 2 diabetes. Am J Nephrol. 2010;31:342–52.
132. Barber AJ, Antonetti DA, Kern TS, Reiter CE, Soans RS, Krady JK, et al. The Ins2Akita mouse as a model of early retinal complications in diabetes. Invest Ophthalmol Vis Sci. 2005;46:2210–8.
133. Mathews CE, Langley SH, Leiter EH. New mouse model to study islet transplantation in insulin-dependent diabetes mellitus. Transplantation. 2002;73:1333–6.
134. Nemery B, Vanlommel S, Verbeken EK, Lauweryns JM, Demedts M. Lung injury induced by paraquat, hyperoxia and cobalt chloride: effects of ambroxol. Pulm Pharmacol. 1992;5:53–60.
135. Yoshinaga T, Nakatome K, Nozaki J, Naitoh M, Hoseki J, Kubota H, et al. Proinsulin lacking the A7-B7 disulfide bond, Ins2Akita, tends to aggregate due to the exposed hydrophobic surface. Biol Chem. 2005;386:1077–85.
136. Yoshioka M, Kayo T, Ikeda T, Koizumi A. A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. Diabetes. 1997;46:887–94.
137. Hong EG, Jung DY, Ko HJ, Zhang Z, Ma Z, Jun JY, et al. Nonobese, insulin-deficient Ins2Akita mice develop type 2 diabetes phenotypes including insulin resistance and cardiac remodeling. Am J Physiol Endocrinol Metab. 2007;293:E1687–96.
138. Sato A, Kawano H, Notsu T, Ohta M, Nakakuki M, Mizuguchi K, et al. Antiobesity effect of eicosapentaenoic acid in high-fat/high-sucrose diet-induced obesity: importance of hepatic lipogenesis. Diabetes. 2010;59:2495–504.
139. Satapathy SK, Ochani M, Dancho M, Hudson LK, Rosas-Ballina M, Valdes-Ferrer SI, et al. Galantamine alleviates inflammation and other obesity-associated complications in high-fat diet-fed mice. Mol Med. 2011;17:599–606.
140. Speakman J, Hambly C, Mitchell S, Krol E. Animal models of obesity. Obes Rev. 2007;8 Suppl 1:55–61.
141. Inui A. Obesity – a chronic health problem in cloned mice? Trends Pharmacol Sci. 2003;24:77–80.
142. Diaz J, Warren L, Helfner L, Xue X, Chatterjee PK, Gupta M, et al. Obesity shifts house dust mite-induced airway cellular infiltration from eosinophils to macrophages: effects of glucocorticoid treatment. Immunol Res. 2015;63:197–208.
143. Rossmeisl M, Rim JS, Koza RA, Kozak LP. Variation in type 2 diabetes – related traits in mouse strains susceptible to diet-induced obesity. Diabetes. 2003;52:1958–66.
144. Wang CY, Liao JK. A mouse model of diet-induced obesity and insulin resistance. Methods Mol Biol. 2012;821:421–33. doi:10.1007/978-1-61779-430-8_27.:421-33.
145. Collins S, Martin TL, Surwit RS, Robidoux J. Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav. 2004;81:243–8.
146. Begin-Heick N. Of mice and women: the beta 3-adrenergic receptor leptin and obesity. Biochem Cell Biol. 1996;74:615–22.
147. Pettersson US, Walden TB, Carlsson PO, Jansson L, Phillipson M. Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLoS One. 2012;7:e46057.
148. Shi H, Clegg DJ. Sex differences in the regulation of body weight. Physiol Behav. 2009;97:199–204.
149. Saito K, Cao X, He Y, Xu Y. Progress in the molecular understanding of central regulation of body weight by estrogens. Obesity. 2015;23:919–26.
150. Rachon D, Teede H. Ovarian function and obesity – interrelationship, impact on women's reproductive lifespan and treatment options. Mol Cell Endocrinol. 2010;316:172–9.
151. Dahlman I, Vaxillaire M, Nilsson M, Lecoeur C, Gu HF, Cavalcanti-Proenca C, et al. Estrogen receptor alpha gene variants associate with type 2 diabetes and fasting plasma glucose. Pharmacogenet Genomics. 2008;18:967–75.
152. Gonet AE, Stauffacher W, Pictet R, Renold AE. Obesity and diabetes mellitus with striking congenital hyperplasia of the islets of langerhans in spiny mice (*Acomys cahirinus*): I. Histological findings and preliminary metabolic observations. Diabetologia. 1966;1:162–71.
153. Shafrir E, Ziv E, Kalman R. Nutritionally induced diabetes in desert rodents as models of type 2 diabetes: *Acomys cahirinus* (spiny mice) and *Psammomys obesus* (desert gerbil). ILAR J. 2006;47:212–24.
154. Ziv E, Kalman R, Hershkop K, Barash V, Shafrir E, Bar-On H. Insulin resistance in the NIDDM model *Psammomys obesus* in the normoglycaemic, normoinsulinaemic state. Diabetologia. 1996;39: 1269–75.

155. Barnett M, Collier GR, Collier FM, Zimmet P, O'Dea K. A cross-sectional and short-term longitudinal characterisation of NIDDM in *Psammomys obesus*. Diabetologia. 1994;37:671–6.
156. Walder KR, Fahey RP, Morton GJ, Zimmet PZ, Collier GR. Characterization of obesity phenotypes in *Psammomys obesus* (Israeli sand rats). Int J Exp Diabetes Res. 2000;1:177–84.
157. Kanety H, Moshe S, Shafrir E, Lunenfeld B, Karasik A. Hyperinsulinemia induces a reversible impairment in insulin receptor function leading to diabetes in the sand rat model of non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A. 1994;91:1853–7.
158. Shafrir E, Ziv E. Cellular mechanism of nutritionally induced insulin resistance: the desert rodent *Psammomys obesus* and other animals in which insulin resistance leads to detrimental outcome. J Basic Clin Physiol Pharmacol. 1998;9:347–85.
159. Refinetti R. The Nile grass rat as a laboratory animal. Lab Anim. 2004;33:54–7.
160. Blanchong JA, McElhinny TL, Mahoney MM, Smale L. Nocturnal and diurnal rhythms in the unstriped Nile rat, *Arvicanthis niloticus*. J Biol Rhythms. 1999;14:364–77.
161. Noda K, Melhorn MI, Zandi S, Frimmel S, Tayyari F, Hisatomi T, et al. An animal model of spontaneous metabolic syndrome: Nile grass rat. FASEB J. 2010;24:2443–53.
162. Noda K, Nakao S, Zandi S, Sun D, Hayes KC, Hafezi-Moghadam A. Retinopathy in a novel model of metabolic syndrome and type 2 diabetes: new insight on the inflammatory paradigm. FASEB J. 2014;28:2038–46.
163. Srinivas NR. Strategies for preclinical pharmacokinetic investigation in streptozotocin-induced diabetes mellitus (DMIS) and alloxan-induced diabetes mellitus (DMIA) rat models: case studies and perspectives. Eur J Drug Metab Pharmacokinet. 2015;40:1–12.
164. Bonner-Weir S, Trent DF, Honey RN, Weir GC. Responses of neonatal rat islets to streptozotocin: limited B-cell regeneration and hyperglycemia. Diabetes. 1981;30:64–9.
165. Blondel O, Bailbe D, Portha B. Relation of insulin deficiency to impaired insulin action in NIDDM adult rats given streptozocin as neonates. Diabetes. 1989;38:610–7.
166. Leahy JL, Bonner-Weir S, Weir GC. Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. J Clin Invest. 1988;81:1407–14.
167. Bonner-Weir S, Trent DF, Weir GC. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. J Clin Invest. 1983;71:1544–53.
168. Wang RN, Kloppel G, Bouwens L. Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia. 1995;38:1405–11.
169. DeSisto CL, Kim SY, Sharma AJ. Prevalence estimates of gestational diabetes mellitus in the United States, Pregnancy Risk Assessment Monitoring System (PRAMS), 2007–2010. Prev Chronic Dis. 2014;11:E104. doi:10.5888/pcd11.130415.:E104.
170. Cheung NW, Byth K. Population health significance of gestational diabetes. Diabetes Care. 2003;26:2005–9.
171. Dabelea D, Mayer-Davis EJ, Lamichhane AP, D'Agostino Jr RB, Liese AD, Vehik KS, et al. Association of intrauterine exposure to maternal diabetes and obesity with type 2 diabetes in youth: the SEARCH Case–control Study. Diabetes Care. 2008;31:1422–6.
172. Pereira TJ, Moyce BL, Kereliuk SM, Dolinsky VW. Influence of maternal overnutrition and gestational diabetes on the programming of metabolic health outcomes in the offspring: experimental evidence. Biochem Cell Biol. 2014;19:1–14.
173. Pasek RC, Gannon M. Advancements and challenges in generating accurate animal models of gestational diabetes mellitus. Am J Physiol Endocrinol Metab. 2013;305:E1327–38.
174. Liang C, DeCourcy K, Prater MR. High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. Metabolism. 2010;59:943–50.
175. Holemans K, Caluwaerts S, Poston L, Van Assche FA. Diet-induced obesity in the rat: a model for gestational diabetes mellitus. Am J Obstet Gynecol. 2004;190:858–65.
176. Van Mieghem T, van Bree R, Van Herck E, Deprest J, Verhaeghe J. Insulin-like growth factor-II regulates maternal hemodynamic adaptation to pregnancy in rats. Am J Physiol Regul Integr Comp Physiol. 2009;297:R1615–21.
177. Couvreur O, Ferezou J, Gripois D, Serougne C, Crepin D, Aubourg A, et al. Unexpected long-term protection of adult offspring born to high-fat fed dams against obesity induced by a sucrose-rich diet. PLoS One. 2011;6:e18043.
178. Tufino C, Villanueva-Lopez C, Ibarra-Barajas M, Bracho-Valdes I, Bobadilla-Lugo RA. Experimental gestational diabetes mellitus induces blunted vasoconstriction and functional changes in the rat aorta. Biomed Res Int. 2014;2014:329634. doi:10.1155/2014/329634. Epub 28 Dec 2014.
179. Desai N, Roman A, Rochelson B, Gupta M, Xue X, Chatterjee PK, et al. Maternal metformin treatment decreases fetal inflammation in a rat model of obesity and metabolic syndrome. Am J Obstet Gynecol. 2013;209:136–9.
180. Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, et al. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet. 1996;12:106–9.
181. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, et al. Insulin resistance and growth

retardation in mice lacking insulin receptor substrate-1. Nature. 1994;372:182–6.
182. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med. 2005;22:359–70.
183. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, et al. Disruption of IRS-2 causes type 2 diabetes in mice. Nature. 1998;391:900–4.
184. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med. 1996;2:1254–8.
185. Lee Y, Berglund ED, Wang MY, Fu X, Yu X, Charron MJ, et al. Metabolic manifestations of insulin deficiency do not occur without glucagon action. Proc Natl Acad Sci U S A. 2012;109:14972–6.
186. Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis. 2000;26:99–109.
187. Schwartz MW, Guyenet SJ, Cirulli V. The hypothalamus and ss-cell connection in the gene-targeting era. Diabetes. 2010;59:2991–3.
188. Magnuson MA, Osipovich AB. Pancreas-specific Cre driver lines and considerations for their prudent use. Cell Metab. 2013;18:9–20.
189. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, et al. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell. 1998;2:559–69.
190. Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell. 1999;96:329–39.
191. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000;289:2122–5.
192. Guerra C, Navarro P, Valverde AM, Arribas M, Bruning J, Kozak LP, et al. Brown adipose tissue-specific insulin receptor knockout shows diabetic phenotype without insulin resistance. J Clin Invest. 2001;108:1205–13.
193. Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. J Clin Invest. 2007;117:2553–61.
194. Wang ZV, Mu J, Schraw TD, Gautron L, Elmquist JK, Zhang BB, et al. PANIC-ATTAC: a mouse model for inducible and reversible beta-cell ablation. Diabetes. 2008;57:2137–48.
195. Herrera PL. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. Development. 2000;127:2317–22.
196. Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, et al. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell. 2009;138:449–62.
197. Liu Z, Habener JF. Alpha cells beget beta cells. Cell. 2009;138:424–6.
198. Deisseroth K. Optogenetics. Nat Methods. 2011;8:26–9.
199. Allen BD, Singer AC, Boyden ES. Principles of designing interpretable optogenetic behavior experiments. Learn Mem. 2015;22:232–8.
200. Zhao S, Ting JT, Atallah HE, Qiu L, Tan J, Gloss B, et al. Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function. Nat Methods. 2011;8:745–52.
201. Kushibiki T, Okawa S, Hirasawa T, Ishihara M. Optogenetic control of insulin secretion by pancreatic beta-cells in vitro and in vivo. Gene Ther. 2015;22:553–9.
202. Ye H, Daoud-El Baba M, Peng RW, Fussenegger M. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. Science. 2011;332:1565–8.
203. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:819–23.
204. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol. 2014;32:347–55.
205. Pelletier S, Gingras S, Green DR. Mouse genome engineering via CRISPR-Cas9 for study of immune function. Immunity. 2015;42:18–27.
206. Bao D, Ma Y, Zhang X, Guan F, Chen W, Gao K, et al. Preliminary characterization of a leptin receptor knockout rat created by CRISPR/Cas9 system. Sci Rep. 2015;5:15942.
207. Pavlov VA, Tracey KJ. The vagus nerve and the inflammatory reflex – linking immunity and metabolism. Nat Rev Endocrinol. 2012;8:743–54.
208. Scherer T, Lindtner C, Zielinski E, O'Hare J, Filatova N, Buettner C. Short term voluntary overfeeding disrupts brain insulin control of adipose tissue lipolysis. J Biol Chem. 2012;287:33061–9.
209. Shin AC, Fasshauer M, Filatova N, Grundell LA, Zielinski E, Zhou JY, et al. Brain insulin lowers circulating BCAA levels by inducing hepatic BCAA catabolism. Cell Metab. 2014;20:898–909.