Advancements and challenges in generating accurate animal models of gestational diabetes mellitus

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Pasek RC, Gannon M. Advancements and challenges in generating accurate animal models of gestational diabetes mellitus. Am J Physiol Endocrinol Metab 305: E1327–E1338, 2013. First published October 1, 2013; doi:10.1152/ajpendo.00425.2013.—The maintenance of glucose homeostasis during pregnancy is critical to the health and well-being of both the mother and the developing fetus. Strikingly, approximately 7% of human pregnancies are characterized by insufficient insulin production or signaling, resulting in gestational diabetes mellitus (GDM). In addition to the acute health concerns of hyperglycemia, women diagnosed with GDM during pregnancy have an increased incidence of complications during pregnancy as well as an increased risk of developing type 2 diabetes (T2D) later in life. Furthermore, children born to mothers diagnosed with GDM have increased incidence of perinatal complications, including hypoglycemia, respiratory distress syndrome, and macrosomia, as well as an increased risk of being obese or developing T2D as adults. No single environmental or genetic factor is solely responsible for the disease; instead, a variety of risk factors, including weight, ethnicity, genetics, and family history, contribute to the likelihood of developing GDM, making the generation of animal models that fully recapitulate the disease difficult. Here, we discuss and critique the various animal models that have been generated to better understand the etiology of diabetes during pregnancy and its physiological impacts on both the mother and the fetus. Strategies utilized are diverse in nature and include the use of surgical manipulation, pharmacological treatment, nutritional manipulation, and genetic approaches in a variety of animal models. Continued development of animal models of GDM is essential for understanding the consequences of this disease as well as providing insights into potential treatments and preventative measures.

gestational diabetes mellitus; pregnancy; β-cell; hyperplasia; hypertrophy; glucose-stimulated insulin secretion

THE FAILURE OF SUFFICIENT INSULIN PRODUCTION or signaling during pregnancy results in gestational diabetes mellitus (GDM), a condition affecting approximately 7% of all pregnancies in the United States (3). GDM is defined as diabetes appearing specifically during pregnancy, typically during the second trimester, as women diagnosed with GDM show no signs of diabetes prior to the pregnancy (80). While 3–5% of GDM patients remain diabetic post-pregnancy, most women regain euglycemia following parturition (29). Risk factors for GDM include a family history of type 2 diabetes (T2D), obesity, and certain ethnic backgrounds, including Asian and Hispanic (13, 89). While abnormal glucose homeostasis has immediate health concerns for a pregnant woman, it is the long-term health consequences that often prove the most deleterious. Women diagnosed with GDM are six times more likely to develop T2D later in life than women who maintain normal blood glucose levels during pregnancy (10). Other maternal health concerns have also been associated with GDM, including preeclampsia and increased probability of cesarean section (80). Children born to mothers with GDM have an increased risk of developing perinatal hypoglycemia, hypocalcemia, polycythemia, jaundice, respiratory distress syndrome, and macrosomia and have an increased risk of obesity and T2D as adults (23, 80).

The transitory nature of GDM makes the study of its physiology in humans difficult, since by definition the disease exists only during pregnancy. Study of pancreatic tissue from pregnant women with and without GDM would advance our understanding of the islet defects that contribute to the disease. However, the scarcity of post mortem tissue samples and the lack of imaging modalities to noninvasively measure β-cell mass in vivo hinder this process. For these reasons, animal models provide an attractive alternative to studying the mechanisms behind, and possible therapeutics for, GDM. In this review, we explore the various techniques and animal models that are currently available for the study of this disease, highlight their respective strengths and weaknesses, and discuss what these models reveal about the β-cell in GDM.
As is true with most biomedical research, many of the animal models for GDM utilize either mice or rats; thus, rodent models will be the primary focus of this review. However, other vertebrates, including dogs, pigs, sheep, and nonhuman primates, have been used in the study of GDM, and these models will also be discussed when appropriate.

Finally, many models of diabetes during pregnancy are often more appropriate for studying the health consequences of hyperglycemia on the offspring of mothers with GDM rather than the physiology of GDM itself. In this review, we emphasize the utility of animal models that focus on the maternal aspects of GDM, in order to better understand the etiology and effects of the disease in human patients.

Relevance of Animal models to Human GDM

In humans, pregnancy is accompanied by a variety of maternal hormonal and metabolic changes that allow for optimal fetal growth. Global hormonal changes, including increased production of prolactin and placental lactogen, decrease maternal insulin sensitivity, thus ensuring sufficient amounts of glucose crossing the placenta to nourish the developing fetus. Maternal glucose production is also increased via gluconeogenesis and glycogenolysis in order to increase available energy stores. Likewise, late pregnancy is characterized by increased plasma concentrations of free fatty acids (FFA) (81). While increased FFA serve as a supplemental energy source, they also contribute to the increase in insulin resistance associated with pregnancy (99).

Alterations in glucose production and insulin sensitivity during pregnancy necessitate compensatory actions in order to maintain maternal euglycemia. Research using a variety of animal models indicates that one of the most important determinants of euglycemia during pregnancy is achieved through physiological changes in the maternal pancreatic β-cell. Notably, these changes include increased β-cell mass via proliferation and hypertrophy, increased insulin production, and enhanced glucose-stimulated insulin secretion (GSIS) (Fig. 1) (77, 78, 108). These adaptations are not uniform throughout pregnancy, however, as increased β-cell replication, β-cell size, and GSIS peak midway through gestation and return to pregestational levels close to parturition. Although these events are firmly established in rodents, the compensatory changes that occur in the human β-cell during pregnancy remain much more controversial. An early report concluded that pregnancy was accompanied by a more than twofold increase in the fraction of pancreas occupied by β-cells (103). This finding makes it tempting to speculate that rodents and humans have similar mechanisms to maintain glucose homeostasis during pregnancy. In contrast, a more recent study found a more modest increase in β-cell mass expansion during human pregnancy, with only an approximately 1.4-fold increase in volume density of β-cells, and no evidence of the increased β-cell replication and size that has been documented in pregnant rodents (6). Due to the scarcity of subjects, both studies included samples from women of varying age and at different stages of pregnancy. In addition, the cause of death for the pregnant women in these studies varied and included accidental death and death from diseases, some of which were inflammatory in nature. These confounding variables make direct measurements of changes in β-cell mass during human preg-

![Fig. 1. Maternal β-cell compensation during pregnancy. During pregnancy, euglycemic conditions are maintained largely through β-cell hypertrophy, hyperplasia, and increased glucose-stimulated insulin secretion (GSIS). These changes in β-cell biology are transient, as β-cell size, replication, and GSIS return to pregestational levels postparturition. Conversely, changes in α-cell size and replication have yet to be reported. For reasons that remain poorly understood, these compensatory mechanisms do not occur properly in all pregnancies, resulting in GDM. While euglycemia is generally reestablished in women with GDM immediately after childbirth, the disease is associated with increased incidence of type 2 diabetes (T2D) later in life. It is currently unclear whether this is due to detrimental effects on the maternal β-cells during pregnancy, is a reflection of continued β-cell dysfunction, or is due to other unknown factors.](http://ajpendo.physiology.org/)

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nancy difficult, as age, gestational stage, and disease all affect β-cell mass and function. Admittedly, factors not directly related to the β-cell may influence the development of GDM in humans, but current animal models and genome-wide association studies indicate that loss of proper β-cell function is the single largest determinant in pathogenesis of the disease.

Diagnosing GDM

Currently, the diagnostic criterion for GDM is somewhat controversial. Using classic diagnostic standards, abnormally high blood glucose levels on a 1-h 50-g oral glucose tolerance test (OGTT) would be followed by a 2-h 75-g or a 3-h 100-g OGTT test (Table 1) (65, 74, 80). Two or more abnormal values on the follow-up test would be indicative of GDM. In contrast, guidelines laid forth in 2010 by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommend a diagnosis of GDM if any one of three blood glucose levels is abnormally high on a 2-h 75-g OGTT (Table 2) (66). The American Diabetes Association (ADA) has already adopted this diagnostic standard, and if adopted by physicians nationwide, the IADPSG guidelines will create a dramatic rise in GDM diagnoses, with some estimates placing it as high as one in five pregnancies (60). While it is beyond the scope of this article to comment on which diagnostic criteria should be used in human patients, anyone interested in studying GDM using animal models should carefully decide on diagnostic criteria that are appropriate for their selected model before initiating any studies.

Surgical Models to Study Diabetes During Pregnancy

As endocrine pancreas function is the single most important determinant of glucose homeostasis, it is unsurprising that surgical reduction (partial pancreatectomy; Ppx) of this organ is arguably the most direct way to induce diabetes during pregnancy in animal models. Ppx results in dramatic reduction in the insulin-producing β-cells (as well as all other pancreatic cell types) and, as a consequence, impairs the ability of the body to regulate glucose homeostasis. In the early 20th Century, total pancreatectomy was employed in dogs both before and during various stages of pregnancy (9 wk gestation) to induce diabetic symptoms. While these early reports pioneered research in diabetes and pregnancy, they often proved contradictory. For example, Markowitz and Soskin (62) reported that total pancreatectomy in the dog during advanced stages of pregnancy resulted in hyperglycemia and glycosuria within days, while Carlson and Drennan noted an absence of glycosuria after performing a similar operation (8). Others performed Ppx prior to pregnancy and reported the presence of glycosuria during early gestation, which lessened as the pregnancy progressed (12). However, as is true for similar studies at that time, glucose homeostasis in pancreatectomized nonpregnant controls was not reported, making uncertain whether pregnancy exacerbated, ameliorated, or had no effect on the diabetic phenotypes. Other caveats to these studies include the very small sample sizes (typically 1–3 dogs) used in these experiments as well as administration of exogenous insulin to varying degrees to keep the dogs healthy and allow the pregnancy to come to term, complicating analysis of diabetic phenotypes. These early experiments were less than ideal for other reasons, since maternal death and spontaneous abortion proved a common occurrence within days after the procedure.

Although Ppx is not commonly used in rodent models of GDM, one report used a 95% Ppx technique to induce diabetes in rats prior to pregnancy (42). Once diabetes was confirmed with measurement of elevated blood glucose, Ppx female rats were mated to control males to induce pregnancy. This strategy was used to determine whether the uterus of pregnant rats was affected by diabetes. In those studies, production of secreted pro- and anti-vasoconstrictive signaling molecules was significantly altered compared with controls, as demonstrated by an increase in thromboxane and a corresponding decrease in prostacyclin. These findings support the notion that the intrauterine environment is altered in pregnant diabetic women, potentially affecting fetal development and contributing to the increased health risks of children born to mothers with GDM. However, the utility of this model is unclear, as diabetes took up to three months to develop, in contrast to other published studies, where 90% Ppx resulted in overt diabetes within two weeks of the procedure (98).

Although Ppx can successfully result in diabetes during pregnancy, the procedure is nonspecific in its actions, removing both endocrine and exocrine tissue. Surgery also results in inflammation, and other changes in the pancreatic microenvironment not necessarily related to diabetes, including upregulation of growth factors such as EGF, that facilitate mild pancreatic regeneration following the procedure (36, 71). Furthermore, human GDM is a spontaneous event that is caused by a combination of both genetic and environmental factors, not a disease that occurs due to a sudden insult to the pancreas. As such, Ppx is not a true model of GDM and does not accurately reflect the etiology of the disease. Rather, it is more useful for studying fetal outcomes of diabetic mothers and changes in the uterine environment that accompanies diabetes during pregnancy.

Although rarely employed in the context of pregnancy, Ppx does induce diabetes in a variety of other animals, including

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Fasting ≥92</th>
<th>Fasting ≥180</th>
<th>Fasting ≥153</th>
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<tr>
<td>1 h 130</td>
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<td>2 h 155</td>
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<td>3 h 140</td>
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Table 1. Classic standards for the diagnosis of GDM

Table 2. 2010 Standards for the diagnosis of GDM

75-g OGTT

Time of Measurement | Fasting ≥92 | Fasting ≥180 | Fasting ≥153 |
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<td>No. of elevated time points for diagnosis</td>
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</tbody>
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pigs, sheep, and nonhuman primates (28, 41, 55). Despite the drawbacks mentioned previously, for model organisms in which genetic or drug-induced GDM are either not practical or possible, Ppx remains a viable option for study of the effects of diabetes during pregnancy on maternal health and the subsequent health of the offspring.

Chemical Models To Study Diabetes During Pregnancy

A variety of chemicals are currently available that either result in the death of insulin-producing $\beta$-cells or otherwise impair $\beta$-cell function. As a result, these drugs can be used to disrupt glucose homeostasis in a time-dependent manner, including during pregnancy. In this section, we will discuss some of the more common drugs that have been used to generate animal models to study diabetes during pregnancy.

Streptozotocin. One of the most widely used diabetogenic drugs is streptozotocin (STZ), a glucosamine-nitrosurea compound that selectively kills $\beta$-cells after being transported into the cells through the GLUT2 transporter (92). As an alkylation agent, it causes cell death largely through DNA damage, ultimately leading to necrosis and/or apoptosis (2, 72, 91). Susceptibility to STZ and the amount of time required to induce diabetes are subject to a variety of factors, including species, strain, age, sex, and dose (7, 61, 87, 106). As such, no universally agreed-upon protocol and dosing strategy exists for its use. Thus, a protocol for STZ-mediated induction of diabetes needs to be determined empirically after a consultation of the published literature.

In rodents, the timing of STZ administration to induce diabetes during pregnancy varies, with some researchers administering the drug before pregnancy, some treating with STZ immediately after successful mating, and others treating in the last week of gestation (1, 63, 94). While induction before pregnancy may be preferred so as to avoid potential toxic effects on the embryos, STZ administration before pregnancy adversely affects mating behavior, at least in part due to the diabetic phenotypes it produces (104). STZ can cross the placenta, but its short half-life of less than five minutes makes it unlikely to affect the earliest stages of embryonic development (84, 90). Furthermore, by the last week of pregnancy in rats and mice, maternal $\beta$-cell size and replication are already beginning to return to pre-pregnancy status; thus, STZ administration at this late time point does not really interrogate the $\beta$-cell compensatory mechanisms and physiology that underlie GDM (78, 85). STZ treatment late in gestation also risks harming the developing fetal $\beta$-cells, since GLUT2 expression increases during late gestation in preparation for birth (76). For these reasons, STZ administration on the day of successful mating is often the preferred time point.

Rats and mice remain the most common models for STZ-based studies of diabetes during pregnancy, but larger animals have been successfully used as well. Pigs, sheep, and nonhuman primates are each susceptible to STZ-induced diabetes and have been used to study how diabetes during pregnancy affects growth and metabolism of the mother and fetus (17, 18, 21, 50). Not surprisingly, studies in larger animals, although potentially more relevant to human physiology, are more limited due to the increased cost of studying large cohorts.

Alloxan. Alloxan, a pyrimidine derivative, is used with similar success to STZ to induce diabetes in pregnant animals (58). Originally derived in the first half of the 19th Century during an investigation into the chemical nature of uric acid, alloxan was not widely known to be diabetogenic until 1943 when it was reported to cause $\beta$-cell death after injection into rabbits (20). Like STZ, alloxan is transported into $\beta$-cells through the GLUT2 transporter (31). Once in the cytosol, alloxan induces reactive oxygen species (ROS) formation, resulting in $\beta$-cell necrosis and consequent disruption of normal glucose homeostasis (11). The dosage of alloxan necessary to induce diabetes, as well as the physiological outcomes, is affected by extraneous factors, including species, age, diet, and gestational time point of administration (16, 47, 57, 100). Even the method of delivery can affect alloxan’s diabetogenic properties in certain species, as alloxan injection directly into the pancreas of the guinea pig causes $\beta$-cell death, whereas systemic administration had less effect on $\beta$-cell viability (32).

Chemical destruction of $\beta$-cells confers the advantage of rapidly inducing diabetes, with elevated blood glucose evident within two days of STZ administration in rodents (101). Although alloxan was established as a diabetogenic drug before STZ, the latter remains the method of choice for many laboratories. This is at least in part due to the higher mortality rate and nephrotoxicity attributed to alloxan (5, 37). Likewise, alloxan has a circulating half-life of less than three minutes after intravenous injection, almost half that of STZ, making proper dosing more challenging (105). Despite these caveats, alloxan has also proved effective in generating models of diabetes during pregnancy in a wide variety of species, including mice, rats, rabbits, pigs, and sheep (27, 67, 83, 102, 112).

Similarly to Ppx, STZ or alloxan administration in adult animals creates a permanent state of diabetes through widespread destruction of $\beta$-cells. This results in a different physiological state than GDM, where the disease is a result of maternal insulin resistance and/or deficiency. The permanent nature of the STZ/alloxan treatment, and the lack of extensive pancreatic regeneration in treated adults, also means that the animal will remain diabetic after parturition, in contrast to GDM patients, where glucose homeostasis typically returns to pregestational levels. It is most likely for these reasons that STZ/alloxan-based models often do not recapitulate many of the clinical outcomes of GDM, including macrosomia (26). For these reasons, STZ- and alloxan-based GDM models are useful for studying how diabetes during pregnancy affects fetal development but less so in studying the pathogenesis of GDM itself.

Additionally, STZ/alloxan-induced models of diabetes during pregnancy often result in severe elevation of blood glucose, whereas GDM is characterized by more mild glucose intolerance (61). While islet transplants into STZ-treated mice can ameliorate the extreme elevation in blood glucose, the procedure naturally increases the difficulty and cost of the model (88). Thus, both surgical and chemical induction can be used to assess the consequences of hyperglycemia on mother and offspring but do not truly mimic human GDM.

Nutritional Manipulation to Induce GDM

Obesity and high-fat diet (HFD) are known risk factors for GDM among pregnant women across various ethnic backgrounds. As the ability of HFD to disrupt $\beta$-cell function and confer insulin resistance and diabetes is also a well-established phenomenon in animal models, several models of GDM have
been created using high-fat feeding in pregnant animals. Hole-mans et al. (38) demonstrated that virgin female rats main-
tained on a HFD became obese after four weeks but displayed
normal glucose clearance in response to a glucose challenge. In
contrast, after successful mating and maintenance on the HFD,
an overt GDM phenotype was present by the end of gestation
(gestational day 20), as demonstrated by significantly elevated
levels in an intravenous glucose tolerance test (IVGTT). Seemingly, HFD before pregnancy primed the fe-
males for a diabetic state during gestation. It is unclear how
long it takes GDM to develop in this model, since glucose
challenges earlier in gestation were not reported. Maternal
overnutrition in rats has also been achieved through glucose
infusions directly into pregnant dams. Gauguiuer et al. (25)in-
duced hyperglycemia and hyperinsulinemia through continu-
ous glucose infusions during the last week of pregnancy and
observed that the offspring born to these mothers displayed
impaired insulin secretion and glucose intolerance as adults,
replicating the finding that human children born to GDM
mothers are more likely to develop T2D.

HFD treatment has also been carried out in mice to examine
maternal pathologies during GDM that may affect fetal out-
comes. Liang et al. (59) placed mice on a HFD initiated one
month before pregnancy and continued throughout gestation.
In agreement with other HFD models, the treatment resulted in
elevated blood glucose and insulin levels during pregnancy.
Significantly, HFD also caused placental pathologies, includ-
ing endothelial cell damage due to oxidative stress, and loss of
embryo-nourishing trophoblast cells. While fetal outcomes
were not reported in this publication, it highlights the changes
in the placenta that occur during GDM that have the potential
to affect offspring health. However, in this model, elevated
blood glucose and insulin levels were observed before onset of
pregnancy, and thus this model does not accurately represent
most cases of the human disease.

While rodents are widely used in the study of GDM, HFD has
also been used to induce GDM in larger model organisms. One
such study placed ewes on an obesogenic diet from 60
days prior to mating until gestational day 75, the time point
halfway through the approximately 147-day pregnancy of
sheep (22). At gestational day 75, pregnant ewes fed the
obesogenic diet displayed insulin resistance and increased
circulating glucose. Fetuses showed an increase in pancreatic
weight as a percentage of fetal weight, a significant increase in
β-cell area, and a significant increase in proliferating β-cells.
The authors speculated that physiological changes during de-
velopment could contribute to premature β-cell dysfunction
later in the life of the offspring, ultimately resulting in in-
creased disposition to obesity and diabetes.

HFD has also proven successful in generating GDM models
in dogs (70). Pregnant dogs placed on a high-fat, high-fructose
diet starting approximately halfway through their nine-week
pregnancy displayed impaired glucose clearance by the seventh
week of pregnancy as determined by an OGTT. Despite these
advantages, a HFD model of GDM does not take into account
the strong impact genetic background plays in disease. Fur-
thermore, all HFD-based models of GDM create a condition
similar to T2D, where peripheral insulin resistance and in-
creased adiposity are strong drives of disease. While some
patients may present with GDM due to preexisting obesity,
many manifest with the disease despite being lean prior to
pregnancy. For example, one study found that the fraction of
GDM cases attributable to obesity was only 15.1% among
Asians/Pacific Islanders (53).

Notably, diet-based GDM models confer several advantages
over other models of the disease. Most importantly, obesity is a
known contributor to the development of GDM in human patients.
Furthermore, HFD-based models have been successfully used to
study both the etiology and the pathology of GDM in maternal
tissue as well as the health consequences on fetal offspring of
diabetic mothers. In larger models such as sheep, they also allow
the study of GDM in an animal with a more comparable body
mass to humans than rodents and also share the human trait of
being generally monotocous. Additionally, like surgically induced
models of diabetes during pregnancy, they allow the study of
GDM in animals where genetic manipulation is not available
and/or practical but have a significant advantage over surgical
models by not requiring expertise in difficult surgical procedures
or causing significant mortality issues.

Genetic Models of GDM

Genetic susceptibility is a critical factor in the development
of diabetes, and numerous mutant rodent lines are available
that display diabetic phenotypes during pregnancy (Table 3). While
many of these models have contributed to our understand-
ing of how diabetes affects both maternal and fetal health,
the overwhelming majority of these mutants display severe
glucose intolerance even in a nonpregnant state, thus limiting
their use as an accurate model of human GDM. In this section,
we discuss several mutant and transgenic mouse models where
pronounced diabetic phenotypes develop as a result of preg-
nancy and are thus excellent models for studying the maternal
pathogenesis and etiology of GDM.

Genetic manipulation of cellular receptors to induce GDM.
The db/db mouse is a classic model for the study of obesity and
diabetes (40). These mice are mutant for the leptin receptor
(ObR), a member of the cytokine receptor family, and are
characterized by an inability to adequately suppress feeding
behavior (9). As a result, they become morbidly obese and
develop T2D. db/db females are sterile (44). In contrast,
females heterozygous for the mutant allele (db/+ are fertile,
do not display perturbed glucose homeostasis in the nonpreg-
nant state, and show normal fasting serum glucose and insulin
levels (48). However, during pregnancy, db/+ females display
hyperphagic feeding behavior and increased adiposity, contrib-
uting to the development of insulin resistance and a GDM
phenotype characterized by moderate glucose intolerance (48,
110, 111). Offspring of db/+ mothers also show characteristics
commonly reported for infants of GDM mothers, including
macrosomia, regardless of fetal genotype (48). ObR has a
direct role in islet growth in addition to its important role in
satiation. Conditional loss of the leptin receptor from β-cells re-
sulted in impaired glucose homeostasis in mice fed a HFD
compared with weight-matched controls despite no change in
food intake. This finding raises the possibility that ObR also has
a direct role in β-cell development or growth during pregnancy,
but this possibility has yet to be thoroughly explored, and it is
complicated by the fact that in this model ObR was also deleted
from parts of the hypothalamus. The GDM phenotype of the db/+ 
mouse makes it one of the first truly accurate genetic mimics of
human GDM in which the diabetic phenotype is not present in
healthy nonpregnant animals. Additionally, this model is useful for studying both the pathogenesis of GDM and the health consequences of children born to GDM mothers.

Genetic mouse models of GDM can also be generated as a direct result of defects in the β-cell to appropriately respond to pregnancy hormones. A notable example of this includes mice deficient for the prolactin receptor (PrlR), another member of the cytokine receptor family (49). Increased production of prolactin is one of many physiological changes that occur maternally during pregnancy in both rodents and humans. A key consequence of elevated prolactin is increased β-cell proliferation through PrlR signaling mediated by Jak2/Stat5 activation (24, 39). While homozygous PrlR mutants (PrlR−/−) are unable to carry pregnancy to term, heterozygous PrlR (PrlR+/−) females are fertile. In a nonpregnant state, PrlR+/− females display a decrease in islet mass (possibly reflecting developmental abnormalities) compared with controls but maintain euglycemia. During pregnancy, PrlR+/− females display moderate glucose intolerance and fail to upregulate β-cell proliferation. This mouse model provides in vivo evidence for the importance of prolactin signaling in β-cell adaptation to pregnancy, and suggests that failure of maternal prolactin signaling could be a contributing factor to the development of GDM. Such speculation was validated when an association between polymorphisms in the 5’UTR and promoter region of the human PRLR gene and GDM was found in a population of Chilean patients (56).

Table 3. Current genetic mouse models of GDM

<table>
<thead>
<tr>
<th>Targeted Gene</th>
<th>Mouse ID</th>
<th>Allele Description</th>
<th>Virgin Characteristics</th>
<th>Pregnant Characteristics</th>
<th>Ref. Citation</th>
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<td>ObR</td>
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<td>Euglycemic</td>
<td>Moderate glucose intolerance</td>
<td>(48, 110, 111)</td>
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<td>Hyperphagic</td>
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<td>Increased adiposity</td>
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<td>PrlR</td>
<td>PrlR+/−</td>
<td>Global heterozygote</td>
<td>Euglycemic</td>
<td>Moderate glucose intolerance</td>
<td>(39)</td>
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<td></td>
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<td>Decreased β-cell proliferation</td>
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<tr>
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<td>PancMet KO</td>
<td>Conditional KO</td>
<td>Euglycemic</td>
<td>Mild glucose intolerance</td>
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<td>Normal islet architecture</td>
<td>Decreased β-cell mass</td>
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<td>Disruption of prolactin receptor signaling</td>
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<td>Decreased β-cell proliferation</td>
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<td>Decreased β-cell mass</td>
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<td>Mild glucose intolerance</td>
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<td>(33)</td>
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<td>Normal β-cell mass</td>
<td>Decreased β-cell mass</td>
<td></td>
</tr>
<tr>
<td>FoxD3</td>
<td>Foxd3+/−; Pdx1-Cre</td>
<td>Conditional KO</td>
<td>Euglycemic</td>
<td>Severe glucose intolerance</td>
<td>(82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decreased β-cell mass</td>
<td>Decreased β-cell proliferation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Decreased β-cell mass</td>
<td></td>
</tr>
<tr>
<td>FoxM1</td>
<td>FoxM1−/−</td>
<td>Conditional KO</td>
<td>Euglycemic</td>
<td>Mild glucose intolerance</td>
<td>(114)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decreased β-cell mass</td>
<td>Decreased β-cell mass</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Decreased β-cell proliferation</td>
<td></td>
</tr>
</tbody>
</table>

Existing genetic mouse models that display gestational diabetes phenotypes. KO, knockout.

During pregnancy, expression of HGF and c-Met is upregulated in the maternal pancreatic endothelial cells of the blood vessels and the β-cells, respectively (15, 43). In conditional knockout mice lacking c-Met in the pancreas (PancMet KO), normal glucose homeostasis and normal islet architecture is observed in virgin females, indicating that c-Met is not necessary for β-cell development during embryogenesis (15). However, by gestational day 15, PancMet KO females have a significant decrease in β-cell mass compared with controls, and the mice display mild glucose intolerance, as determined by intraperitoneal glucose tolerance test (IPGTT). These phenotypes were accompanied by a decrease in PrlR expression and a decrease in Stat5 localization to the nuclei of β-cells. As PrlR-induced Stat5 dimerization and nuclear localization is a critical event in β-cell expansion during pregnancy, the authors speculate that this process may be regulated by HGF/c-Met signaling (4).

Genetic manipulation of serotonin signaling to induce GDM. In addition to prolactin and HGF, other secreted factors are associated with β-cell expansion during pregnancy. Circulating maternal prolactin and placental lactogen during pregnancy induce expression of the enzymes tryptophan hydroxylase-1 (Tph1) and tryptophan hydroxylase-2 (Tph2), and the 5-hydroxytryptamine (serotonin) receptor 2B (5Htr2b) in β-cells by signaling through phosphorylated Stat5 (52, 93). Tph1 drives serotonin synthesis in β-cells, which signals in an autocrine/paracrine fashion to increase β-cell mass expansion through 5Htr2b. Interruption of this pathway can result in insufficient β-cell mass expansion during pregnancy and GDM. In mice, Kim et al. (52) showed that female Htr2b KO mice (Htr2b−/−) are euglycemic as virgins but develop moderate glucose intolerance by gestational day 13 as well as having a significant...
reduction in β-cell mass compared with pregnant controls. Pharmacological methods were also used to substantiate these genetic findings, including inhibition of serotonin signaling using p-chlorophenylalanine (PCPA), an inhibitor of Tph1, and the Htr2b antagonists SB-204741 and methysergide. The authors also used diet manipulation to inhibit serotonin signaling. Serotonin is generated from tryptophan in a multistep enzymatic process, and since tryptophan is an essential amino acid, rodents, humans, and many other animals must acquire it from their diet (69). Kim et al. took advantage of this fact to disrupt serotonin signaling by feeding pregnant mice a tryptophan-free diet from gestational days 6 to 12 (52). An IPGTT at gestational day 13 revealed severe glucose intolerance. Significantly, tryptophan deprivation did not have an effect on glucose homeostasis in virgin females.

While disruption of the serotonin signaling pathway has pronounced detrimental effects on maternal euglycemia, limitations with these models exist. A significant number of Htr2b−/− mice die embryonically due to defects in heart development, creating practical issues in generating the mice phenotype, limiting the utility of these mice as a model for GDM (73). A conditional allele of Htr2b would circumvent these problems and potentially yield a more accurate model of human GDM, but it has yet to be reported. Although tryptophan deprivation induces GDM in rodents, it is not a suggested cause of the disease in humans. Tryptophan deficiency also produces a more extreme glucose intolerance phenotype than what is typically seen in human GDM patients.

Regardless of these limitations, it is clear that serotonin signaling is important in β-cells during pregnancy. Intriguingly, the use of selective serotonin reuptake inhibitors (SSRI) is linked to increased incidence of T2D in humans, arguing that disruptions of serotonin signaling can contribute to human diabetes (54, 75). The long-term consequences of such treatments to maternal and fetal health are currently unclear. One can speculate that such treatment would cause an increase in long-term GDM-associated phenotypes in treated females and their offspring; however, no such study has yet been conducted.

Genetic manipulation of nuclear factors to induce GDM. Nuclear factors have also been manipulated to generate models of GDM. Menin is a tumor suppressor that forms a complex with myeloid/lymphoid or mixed-lineage leukemia 2 (MLL2) to regulate the latter’s histone methyltransferase activity (46). This in turn inhibits cell proliferation through regulation of the cyclin-dependent kinase inhibitor p27. Karnik et al. (45) have shown that menin expression decreases in maternal β-cells during pregnancy, likely due to placental lactogen-induced inhibition of menin transcription by β-cell lymphoma 6 protein (Bcl-6). To determine whether menin downregulation is essential for β-cell mass expansion during gestation, transgenic mice were generated that conditionally overexpressed menin in β-cells. These mice, referred to as βMen1 mice, displayed no changes in fasting glucose levels or glucose clearance in response to a glucose challenge as virgins. In contrast, pregnant βMen1 females developed hyperglycemia by gestational day 9, which continually worsened throughout gestation; however, even control females in that study displayed elevated blood glucose. After parturition, blood glucose returned to prepregnancy levels. BrdU labeling to mark proliferating cells indicated that βMen1 females have a deficiency in β-cell proliferation during pregnancy. Furthermore, β-cell mass was significantly reduced in pregnant βMen1 females compared with pregnant controls and was indistinguishable from β-cell mass in virgin βMen1 females.

There are also several transcription factors that affect β-cell proliferation during pregnancy. Hepatocyte nuclear factor 4α (HNF-4α) is expressed in α-cells and β-cells as well as nonpancreatic tissues. In β-cells, it induces expression of the Kir6.2 subunit of the ATP-sensitive potassium channel critical for GSIS (34, 68). Virgin mice with a β-cell-specific knockout of HNF-4α (HNF-4αflloxP/loxP; Ins.Cre) display mild glucose intolerance, with normal islet architecture and β-cell mass (33). However, during pregnancy, HNF-4αflloxP/loxP; Ins.Cre females display a significant reduction in β-cell mass and proliferation compared with pregnant controls, indicating that HNF-4α is essential for pregnancy-induced hyperplasia, a critical adaptation for maintaining euglycemia during pregnancy. Pregnant HNF-4αflloxP/loxP; Ins.Cre females also displayed reduced pancreatic insulin content and pronounced glucose intolerance. While the mild glucose intolerance prior to pregnancy may reflect a role for HNF-4α during normal β-cell development, it is clear from the lack of β-cell expansion during pregnancy that the gene is also necessary for the compensatory mechanisms in mice necessary to prevent GDM.

Fig. 2. Examples of cellular mechanisms responsible for β-cell expansion during pregnancy. During pregnancy, prolactin (Prl) and placental lactogen (PL) signal through the prolactin receptor (PrlR) to induce Jak2/Stat5 phosphorylation. Once phosphorylated, Stat5 undergoes dimerization and localizes to the nucleus, where it induces B-cell lymphoma 6 protein (Bcl-6). Bcl-6 inhibits the tumor suppressor menin, which prevents β-cell proliferation through the induction of cyclin-dependent kinase inhibitor p27. Prl/PL-induced Stat5 signaling also stimulates tryptophan hydroxylase-1 (Tph1), tryptophan hydroxylase-2 (Tph2), and 5-hydroxytryptamine receptor 2B (5HT2b) expression. In turn, Tph1 increases serotonin (5HT) synthesis, which is secreted and signals through the protein product of Htr2b, 5HT2b, in an autocrine/paracrine manner to stimulate β-cell proliferation through unknown mechanisms. Binding of hepatocyte growth factor (HGF) to c-Met also modulates expression of PrlR and promotes Stat5 nuclear localization. Additionally, HGF/c-Met signaling increases expression of FoxM1, which directly promotes β-cell proliferation, similarly to FoxD3, but also inhibits menin and p27. HNF-4α is also necessary for β-cell expansion during pregnancy, but its mechanism remains poorly understood.
phenotypes. Among human patients, polymorphisms in HNF-4α are a known cause of maturity onset of diabetes of the young (MODY); however, a conclusive link to GDM has yet to be reported (96, 109).

Like HNF-4α, Forkhead box D3 (FoxD3) is a transcription factor expressed in the pancreatic islets in adult rodents (79). During pregnancy, placental lactogen signaling decreases FoxD3 expression in β-cells. Mice specifically lacking FoxD3 in β-cells (Foxd3<sup>−/−</sup>; Pdx1-Cre) have decreased β-cell mass as virgins, indicative of a role of FoxD3 in normal β-cell development or growth. Despite this, virgin Foxd3<sup>−/−</sup>; Pdx1-Cre females maintain euglycemia (82). In contrast, by gestational day 15.5, pregnant Foxd3<sup>−/−</sup>; Pdx1-Cre dams have severe glucose intolerance and decreased serum insulin levels. Additionally, β-cell mass, proliferation, and size were all significantly decreased. Genes required for β-cell proliferation displayed misregulated expression in the islets of pregnant Foxd3<sup>−/−</sup>; Pdx1-Cre females, including enhancer of zeste homolog 2 (Ezh2), which was significantly downregulated, and S-phase kinase-associated protein-2 (Skp2), which was significantly upregulated. Thus, despite the fact that FoxD3 expression is extinguished in β-cells during pregnancy, its function is required for normal β-cell compensation in pregnancy. These seemingly contradictory results suggest that FoxD3 expression in quiescent β-cells establishes a state in which genes necessary for β-cell compensation can be induced by other factors during pregnancy.

Forkhead box M1 (FoxM1) is another transcription factor involved in β-cell proliferation (113). During pregnancy, FoxM1 is induced by both Prlr- and c-Met-mediated signaling (15, 113). Our group has previously shown that mice with pancreas-specific inactivation of FoxM1 (FoxM1<sup>Δpanc</sup>) display increased menin and p27, which impairs postnatal β-cell proliferation and β-cell mass expansion (114). Despite this phenotype, FoxM1<sup>Δpanc</sup> females maintain glucose homeostasis as virgins, although males become diabetic by nine weeks of age. By gestational day 12.5, females show mild glucose intolerance that worsens as gestation progress. FoxM1<sup>Δpanc</sup> females show no induction of maternal β-cell proliferation and no increase in β-cell mass during pregnancy, contributing to the GDM phenotype.

The list of genetic models available for the study of GDM continues to grow. Each of these animals provides valuable insights into the mechanisms of maternal β-cell adaptation during pregnancy and suggests genetic pathways that may influence the disease in humans (Fig. 2). Unfortunately, the techniques used to create genetic mutations in mice are not possible or practical for many other animals. Additionally, single-gene mutants often create simplistic models of GDM. This stands in stark contrast to GDM in humans, in whom disease susceptibility is most commonly due to complex interactions between polygenetic and environmental factors.

### Genetic factors for GDM in humans

It is becoming increasingly clear that GDM in humans is a heterogeneous disorder strongly influenced by genetic predispositions. Cases of GDM cluster in families, and Asians and Hispanics have an increased risk for the disease compared with Caucasians (19, 89). In further support of a genetic contribution, a strong association between GDM and genetic variants in potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11), mannose-binding lectin (protein C) 2 (MBL2), and transcription factor 7-like 2 (TCF7L2) have been made (64, 95, 97, 107). KCNJ11 is a β-cell potassium channel necessary for the proper control of insulin secretion; thus, mutations in this gene could have profound effects on glucose homeostasis (30). MBL2 is a critical component of pathogen recognition in the innate immune system, and its current physiological link to diabetes remains elusive (51). In contrast, TCF7L2 is a transcription factor whose loss from the pancreas in conditional knockout

### Table 4. Strategies in generating animal models of GDM

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Methods</th>
<th>Examples of Species Used</th>
<th>Major Advantages</th>
<th>Major Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>Pancreatectomy</td>
<td>Dogs</td>
<td>Plausible strategy in animals where other options are not feasible</td>
<td>Requires highly trained personnel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rats</td>
<td></td>
<td>High mortality rate</td>
</tr>
<tr>
<td>Chemically Induced</td>
<td>Streptozotocin</td>
<td>Mice</td>
<td>Affordable</td>
<td>Potential nonspecific consequences</td>
</tr>
<tr>
<td></td>
<td>Alloxan</td>
<td>Rats</td>
<td>Proven technique in many different species</td>
<td>More severe hyperglycemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rabbits, Pigs, Sheep</td>
<td></td>
<td>Not accurate pathogenesis of GDM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonhuman primates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritional Manipulation</td>
<td>High-fat diet</td>
<td>Mice</td>
<td>Affordable</td>
<td>Ignores genetic contribution to disease</td>
</tr>
<tr>
<td></td>
<td>Glucose infusion</td>
<td>Rats, Dogs, Sheep</td>
<td>Plausible strategy for larger animals</td>
<td>Does not reflect cases of GDM not due to diet</td>
</tr>
<tr>
<td>Genetic Manipulation</td>
<td>Gene knockouts</td>
<td>Mice</td>
<td>Spontaneous development of GDM</td>
<td>Not an option for many animals</td>
</tr>
<tr>
<td></td>
<td>Transgenic overexpression</td>
<td></td>
<td>Glucose intolerance specific to pregnancy</td>
<td>Overly simplistic representation for most cases of GDM</td>
</tr>
</tbody>
</table>

Currently available models for the study of GDM include surgery, chemical induction, nutritional manipulation, or genetic manipulation and are viable options for a wide variety of model organisms.
mice results in perturbed GSIS, providing a potential rationale behind association of polymorphisms in the gene to GDM (14).

Significantly, KCNJ11, MBL2, and TCF7L2 have also been linked to T2D (86). It thus remains possible that GDM may not have any exclusive genetic predisposition but rather shares in those that are found in other forms of diabetes. Despite this evidence strongly suggesting a genetic component to GDM, no definitive heritability of GDM has been established, indicating that genetic predisposition is not enough to cause the disease, and other environmental stressors that occur during pregnancy are also required to trigger disease onset. Furthermore, many of the animal models we have discussed demonstrate that changes in the in utero environment complicate the study of GDM inheritance, and studies tracing inheritance of diabetes in human patients indicate that the same is true in humans (35). As such, further studies are needed to clarify the impact of genetics on the inheritance of GDM.

Concluding Remarks

Since the first experimental model of GDM using pregnant Ppx dogs, many alternative animals and techniques have been proposed to continue the study of this disease (Table 4). While each model has advanced our understanding of how maternal diabetes during pregnancy can affect fetal outcomes and long-term maternal health, a thorough understanding of its causes and consequences in humans remains elusive. Undoubtedly, genetics and environmental factors contribute to the pathogenesis of GDM, since both genetic defects affecting β-cell expansion and diet-induced obesity can lead to the disease. As such, a single ideal GDM model may not be possible. A realistic alternative may include using animal models with differing etiologies for developing GDM, including a genetic background that predisposes the animal to environmental modulations in physiology under the right conditions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: R.C.P. and M.G. prepared figures; R.C.P. and M.G. drafted manuscript; R.C.P. and M.G. edited and revised manuscript; R.C.P. and M.G. approved final version of manuscript; M.G. conception and design of research.

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