Published in final edited form as:

Drug Discov Today Dis Models. ; 10(2): e71–e77. doi:10.1016/j.ddmod.2013.01.003.

Intrauterine Growth Retardation – A Developmental Model of Type 2 Diabetes

Sara E. Pinney, MD, MS

Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

Abstract

Intrauterine growth retardation has been linked to the development of type 2 diabetes later in life and the mechanisms underlying this phenomena are unknown. Epidemiological studies in humans show a distinct link with the exposure to an intrauterine insult that results in low birth weight and the development of type 2 diabetes in adulthood. Intrauterine growth retardation can be induced in rodent models by exposing the pregnant rat to a low protein diet, total calorie restriction, high dose glucocorticoids or inducing uteroplacental insufficiency, all which result in abnormalities in glucose homeostasis in the offspring later in life. Animal models of intrauterine growth retardation allow for a better characterization of changes in glucose homeostasis and corresponding changes in gene expression that can provide insight in the mechanisms by which intrauterine growth retardation leads to type 2 diabetes.

Introduction

Intrauterine growth retardation (IUGR), a common complication of pregnancy, has been linked to the later development of diseases in adulthood such as type 2 diabetes, obesity and metabolic syndrome [1]. Adverse prenatal and postnatal nutritional environments can have long term effects on the development of organs and tissues that are critical for maintaining health as the organism ages. The incidence of type 2 diabetes (T2DM) has rapidly increased over the past several decades and is now reaching epidemic proportions. Genome-wide association studies have now identified up to 75 genetic loci associated with T2DM, suggesting that T2DM is a complex genetic disorder influenced by interactions between multiple susceptible genetic loci and environmental perturbations [2]. Environmental contributions to the development of T2DM potentially include exposures such as a

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Address correspondence to: Sara E. Pinney, MD, MS, Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Philadelphia, PA 19104. pinneys@email.chop.edu, Phone: 215-590-3174, Fax: 215-590-3053.

Conflict of Interest:

The author had no conflicts of interest to declare.

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suboptimal in utero environment, low birth weight, obesity, inactivity and advancing age [3].

The epidemiological literature provides several examples where human exposure to an abnormal intrauterine milieu leads to abnormalities in glucose homeostasis and ultimately T2DM. For example, pregnant women exposed to the Dutch Hunger Winter, the period in late World War II during which daily caloric intake was limited to 400–800 kcal, delivered infants with lower birth weights. By age 50, these offspring had impaired glucose tolerance compared to offspring who were in utero either the year before or after the famine [4]. Another study from Hertfordshire, UK found that men who were the smallest at birth (<2.5 kg) were seven times more likely to have glucose intolerance or T2DM than those who were heaviest at birth [5]. Although the epidemiological studies show clear associations between the adverse intrauterine milieu, they do not provide any insight to the mechanism responsible for the development of diabetes in the adult offspring.

The abnormal intrauterine milieu associated with IUGR limits the supply of critical substrates and hormones to the fetus and affects its development by permanently modifying gene expression and function of susceptible cells such as the pancreatic beta cell, the hepatocyte, the adipocyte and the myocyte. Here, we will review the various animal models of IUGR and their specific effects on metabolic gene expression. In addition we will discuss a recent example providing evidence that epigenetic mechanisms contribute directly to the malprogramming of gene expression during the critical fetal and neonatal periods and this period may represent a critical time for intervention.

Experimental Models of IUGR

Animal models based on an outbred genetic background offer an opportunity to examine the effects of environmental insults on gene expression during gestation or early postnatal life. Established models of IUGR exist in rodents, sheep, pigs and non-human primates; however, rodents are often used for models of fetal programming due to their shorter lifespan and shorter gestational periods, allowing for detailed study of the long-term effects resulting from an in utero exposure. The most common rodent models used for inducing IUGR to study the development of T2DM are models that employ protein calorie restriction, total calorie restriction, glucocorticoid exposure or induction of uteroplacental insufficiency in the pregnant rodent.

Low Protein Model

Initially established by Snoeck et al, offspring born to protein restricted dams had lower birth weights, and developed age-dependent glucose intolerance that progressed to overt diabetes [6]. In this model, dams were fed a diet containing 8% protein throughout gestation and lactation (LP) and compared to offspring of a control dam fed an isocaloric diet containing 20% protein. There were no effects on conception rates or litter size but placental and offspring birth weights were consistently reduced in this model. Pups of mothers on the LP diet had 5.5% lower birthweights than controls. [6,7]. Neonates of LP dams had impaired pancreatic development and islet vascularization. Beta-cell mass, islet size and insulin content were reduced due to reduced beta cell proliferation and increased apoptosis and

there was a reduction in the pancreatic transcription factor *Pdx-1* (pancreatic duodenal homeobox-1), a homeodomain-containing transcription factor that regulates early development of both endocrine and exocrine pancreas and later the differentiation into beta-cells [6,8–11]. Targeted homozygous disruption of *Pdx-1* in mice results in pancreatic agenesis and homozygous mutations yield a similar phenotype in humans [12]. The *Pdx-1*(+/-) mouse has normal beta-cell mass but a defect in glucose stimulated insulin secretion [12]. In addition, in the LP model there was a decrease in the expression of IGF-II (insulin like growth factor -II) in fetal and neonatal islets of pups born to LP fed pregnant rodents [13,14]. IGF-II is thought to act as a beta-cell mitogen and prevents apoptosis. Taurine supplementation of the LP diet given to the pregnant rats partially prevented beta-cell mass reduction by restoring IGF-II expression and thus normalizing the proliferation and apoptosis rates [10,13].

In general, male offspring had altered insulin secretory capacity and reduced beta-cell mass through a reduction in beta-cell proliferation rate and an increase in apoptosis [6–8,10,15–17]. The 15 month old male offspring of mothers fed a LP diet had hyperinsulinemia [18]. At 17 months of age, male offspring had fasting hyperglycemia and post-prandial hyperinsulinemia. Female offspring at 21 months of age had elevated fasting insulin levels compared to controls but no fasting hyperglycemia. Glucose tolerance during IVGTT was comparable in the female offspring and controls but plasma insulin area under the curve was 1.9 times higher in the female offspring of LP fed dams [18].

Maternal protein restriction has been shown to have profound long-term effects on the liver of the offspring. The ability of glucagon to stimulate hepatic glucose output is impaired in *ex vivo* experiments at 3 months of age and is related to a reduction in the expression of glucagon receptors [19] as well as being associated with an increase in the activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and reduced levels of glycolytic glucokinase [20,21]. These changes persisted at age 11 months despite the fact that the pups were fed a standard diet [20,22]. At 3 month of age in the *ex vivo* livers from male offspring of LP fed pregnant rats displayed evidence of hepatic insulin resistance despite an increase in expression of insulin receptors [19]. There was a decrease in circulating IGF-1 and IGF-1 mRNA expression in hepatocytes derived from LP fetuses [23].

In the male offspring at 15 months, insulin-stimulated glucose uptake is reduced in both muscle and adipose tissue [24,25]. Maternal protein restriction results in reduced skeletal muscle mass but young adult LP offspring have improved insulin sensitivity at the level of the skeletal muscle [20]. Despite an early increase in insulin receptor expression in adipose tissue, at 15 months of age in male offspring of LP fed dams, there was no change in insulin receptor expression indicating that the mechanism for insulin resistance is downstream of the receptor. Finally Ozanne, *et al* showed decreased expression of specific insulin-signaling proteins in skeletal muscle in low birthweight human subjects and low birthweight rats born to LP dams [24,26]. Low birthweight human males had decreased expression of protein kinase c (PKC) zeta, p85 alpha, p110beta and GLUT4, while the male offspring of rats fed a low protein diet showed decreased PKC zeta, GLUT4 and p85 [24]. The similarity of the protein expression profile of the men with low birthweight and the rats suggest that the low-

protein model of IUGR is an appropriate model to study the development of peripheral insulin signaling defects that may lead to insulin resistance in IUGR [26].

Total Calorie Restriction Model

The total calorie restriction model of IUGR approximates a generalized poor nutritional state during pregnancy and the effects on the offspring. Three month old pregnant rats had food intake limited to 50% of ad-lib during pregnancy and lactation which resulted in offspring with significantly lower birth weights who continued to remain small even in adulthood [27]. There was no difference in litter size or in the male-to-female ratio between control and IUGR offspring. When calorie restriction was limited to 50% during gestation only, the offspring were born with reduced birth weights but their body weights increased and were greater than control offspring by postnatal day 20 [27]. When the time period during which the maternal nutritional deprivation (50% restriction) was limited to the last week to 10 days of gestation, the pups were born small; some models found impaired beta-cell development, and reduced plasma glucose and insulin concentrations during the neonatal period [8,28], while other models found no statistical difference in pancreas weight, beta-cell fractional area, beta-cell mass, beta-cell replication or apoptosis in the neonate [29]. In the neonate, *Pdx-1* expression was not reduced in IUGR islets [29]. At 21 days, the male pups had a 15% reduction in body weight and a 30% reduction in pancreatic weight, while there was no difference in female pups at this age [29]. There was a 50% reduction in absolute beta-cell mass and a 30% reduction in beta-cell replication but no change in rates of apoptosis in offspring at 21 days. IUGR offspring had an 80% reduction *Pdx-1* ductal immunoreactivity compared to control at 21 days [29]. At 3–4 months, the offspring continued to have reduced beta-cell mass along with altered insulin response and sensitivity at 3–4 months of age [28,30]. At 8 months of age, the reduction of beta-cell mass in this model was still apparent and the beta-cells had a 40% lower insulin content compared to controls [28]. The offspring were not overtly hyperglycemic but did have decreased fasting insulin levels [28].

Maternal Glucocorticoid Exposure Model

Late gestation treatment with high dose glucocorticoids are used in obstetric practice to accelerate the rate of maturation of fetal lungs and other organs when there is concern about possible premature delivery. Treatment of pregnant rats with 100 mg of dexamethasone per day during the last week of pregnancy retards fetal growth and lead to a 10% decrease in birthweight and a smaller adult rat, while earlier gestational exposures to dexamethasone did not affect birthweight [31]. At 6 months of age, the offspring of rats exposed to dexamethasone in late gestation had impaired glucose tolerance and post-prandial insulin resistance. Fasting insulin levels were not significantly different from controls [31]. At 8 months of age, hepatic mRNA expression of PEPCK and the glucocorticoid receptor (GR) were significantly increased. Insulin content of fetal beta-cells was reduced and was associated with a reduction of *Pdx-1* expression in the beta-cell [32]. These observations suggest that excessive glucocorticoid exposure in late pregnancy predisposes the offspring to glucose intolerance and insulin resistance.

Uteroplacental Insufficiency Model

Uteroplacental insufficiency (UPI), caused by disorders such as preeclampsia, maternal smoking, abnormalities of uteroplacental development, is one of the most common causes of fetal growth retardation worldwide. The resultant abnormal intrauterine milieu restricts the supply of crucial nutrient to the fetus, thereby limiting fetal growth. In one model of UPI in Sprague Dawley rats, pregnant dams undergo bilateral uterine artery ligation surgery on gestational day 18 (term is 22) and deliver spontaneously [33]. UPI fetal rats have critical features of a metabolic profile characteristic of growth retarded human fetuses: decreased levels glucose, insulin, IGF-1, amino acids and oxygen [33–36]. Birth weights of UPI offspring were significantly lower than those of controls until approximately 7 weeks of age, when weights of UPI offspring caught up. Between 7 and 10 weeks of age, the growth rate of UPI offspring accelerated and UPI weights surpassed that of controls, and by 26 weeks UPI rats were obese [33]. There were no significant differences in blood glucose and insulin levels at 1 week of age. However, by 7–10 weeks of age IUGR rats developed mild fasting hyperglycemia and hyperinsulinemia. UPI male offspring were glucose intolerant and insulin resistant by 15 weeks of age. First phase insulin secretion in response to glucose was also impaired by 7 weeks of age in UPI males before the onset of hyperglycemia. There were no significant differences in beta-cell mass, islet size, or pancreatic between 1–7 weeks of age of offspring. In 15 week old male offspring from UPI affected moms, relative beta-cell mass was 50% of that of controls, and by 26 weeks of age, beta-cell mass was less than 33% of controls. The loss of beta-cell mass was accompanied by a reduction in expression of *Pdx-1* [37]. By 6 months of age UPI offspring developed diabetes, which was similar to Type 2 diabetes seen in adult humans with progressive dysfunction of insulin secretion and insulin action [33].

Multiple studies have shown that intrauterine growth retardation is associated with increased oxidative stress in the human fetus [37,38]. A key adaption enabling the fetus to survive in a limited energy environment may be the reprogramming of mitochondrial function, but this can have deleterious effects in cells with high energy requirements. Reactive oxygen species production and oxidative stress gradually increased in islets from offspring exposed to UPI [39]. ATP production is impaired and activities of complex I and II of the electron transport chain progressively declined in UPI islets [39]. There was decreased expression of mitochondrially encoded genes in UPI islets and mitochondrial dysfunction resulted in impaired insulin secretion [39].

Studies with the UPI model suggest that the aberrant intrauterine milieu permanently impairs insulin signaling in the liver resulting in an augmentation gluconeogenesis [40]. At age 7–9 weeks, male offspring exposed to UPI had increased hepatic glucose production (HGP) at baseline and insulin suppression of HGP was suppressed. IRS2 and Akt-2 phosphorylation were significantly blunted in the UPI offspring and PEPCK and glucose-6-phosphate mRNA levels were increased 3 – fold [40]. These processes occurred early in the UPI offspring's adult life, before the onset of hyperglycemia indicating that UPI causes a primary defect in hepatic metabolism that contributes to the eventual development of overt hyperglycemia along with the beta-cell defects described above.

Epigenetic Modifications

One mechanism by which environmental perturbations leading to IUGR can lead to hyperinsulinemia and diabetes is by affecting gene expression through epigenetic modifications. Epigenetic changes are defined as mitotically heritable alterations in gene expression that are not related to changes in DNA sequence. Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene expression from one generation of cells to the next [41–45]. There are at least two distinct mechanisms through which epigenetic information can be inherited: histone modifications and DNA methylation.

Histone modifications

The amino termini of histones can be modified by acetylation, methylation, sumoylation, phosphorylation, glycosylation and ADP ribosylation. The most extensively studied histone modifications involve acetylation and methylation of lysine residues in the amino termini of histone 3 and histone 4. Increased acetylation of histones activates transcription, whereas decreased acetylation usually represses transcription. Methylation of histones, by contrast, is associated with both transcription repression and activation. Lysine residues can be mono-, di- or trimethylated in vivo, providing an additional level of regulation [41].

DNA methylation

The second class of epigenetic regulation is DNA methylation, in which a cytosine base is modified by DNA methyltransferase at the C5 position of cytosine. Approximately 70% of CpG dinucleotides in human DNA are constitutively methylated, whereas most of the unmethylated CpGs are located in CpG islands, CG-rich sequences located near coding sequences and they serve as promoters for their associated genes. Approximately half of mammalian genes have CpG islands. The methylation status of CpG islands within promoter sequences works as an essential regulatory element by modifying the binding affinity of transcription factors to DNA binding sites. In normal cells, most CpG islands remain unmethylated; however, under circumstances such as cancer and oxidative stress, they can become methylated de novo. This aberrant methylation is accompanied by local changes in histone modification and chromatin structure, such that the CpG island and its embedded promoter take on a repressed conformation that is incompatible with gene transcription. DNA methylation is commonly associated with gene silencing and contributes to X chromosomal inactivation, genomic imprinting and transcriptional regulation of tissue-specific genes during cellular differentiation [44].

Chromatin remodeling in the beta-cell of UPI rats

As early as 24 hours after the onset of growth retardation, *Pdx-1* mRNA levels are reduced by more than 50% in UPI fetal rats. Suppression of *Pdx-1* expression persists after birth and progressively declines in the UPI exposed offspring, implicating an epigenetic mechanism. A change in histone acetylation is the first epigenetic modification found in beta-cells of UPI rats (Figure 1). Islets isolated from UPI fetuses show a significant decrease in H3 and H4 acetylation and with a loss of binding of USF-1 to the proximal promoter of *Pdx-1* [46].

USF-1 is a critical activator of *Pdx-1* transcription and its decreased binding markedly decreases *Pdx-1* transcription [47,48]. After birth, histone deacetylation progresses and is followed by a marked decrease in H3K4 trimethylation (H4K4me3) and a significant increase in dimethylation of H3K9 (H3K4me2) in UPI islets [46]. H3Kme3 is usually associated with active gene transcription, whereas H3K9me2 is usually a repressive chromatin mark. Progression of these histone modifications parallels the progressive decrease in *Pdx-1* expression that manifests as defective glucose homeostasis and increased oxidative stress in aging UPI animals [46]. In offspring exposed to UPI, *Pdx-1* is first silenced as a result of recruitment of co-repressors, including histone deacetylase 1 (HDAC1) and mSin3A [46]. Binding of these deacetylases facilitates loss of H3K4me3, further repressing *Pdx-1* expression [46]. In fact, inhibiting HDAC activity by trichostatin A normalizes H3K4me3 levels on *Pdx-1* in IUGR islets [46]. These data suggest that the association of HDAC1 with *Pdx-1* in IUGR islets probably serves as a platform for recruiting a demethylase to catalyze H3K4me2. These results demonstrate that in the UPI model, IUGR induces a self-propagating epigenetic cycle in which the mSin3A/HDAC complex is first recruited to the *Pdx-1* promoter; histone tails are subjected to deacetylation and *Pdx-1* transcription is repressed. At the neonatal stage, this epigenetic process is reversible through treatment with extendin-4 by increasing histone acetylase activity and might define an important developmental window for therapeutic approaches [49]; however, as H3K9me2 accumulates, DNMT3A is recruited to the promoter and initiates de novo DNA methylation, which locks in the silenced state of *Pdx-1* in the UPI adult pancreas, this leading to permanently suppressed expression of *Pdx-1*.

Given the role that epigenetic modifications play in regulating the expression of *Pdx-1* in UPI islets, Thompson, et al examined the role of DNA methylation in regulating gene expression in islets exposed to UPI on a genome wide scale [50]. Cytosine methylation was altered in 1400 loci in islets from male UPI rats at 7 weeks, preceeding the development of diabetes. The changes in methylation occurred preferentially at conserved intergenic regions, frequently near genes regulating processes known to be altered in IUGR including beta-cell proliferation, vascularization, insulin secretion and cell death and corresponded with concordant changes in mRNA expression [50].

Chromatin remodeling in the beta-cell that is regulated through maternal diet and aging

In the LP model of IUGR, Sandovici et al found that the pancreatic transcription factor Hnf4 α is epigenetically regulated by maternal diet and aging in rat islets from offspring of protein restricted dams [51]. Hnf4 α has been linked to both monogenic diabetes and type 2 diabetes. In the normal rat islets, transcriptional activity of *Hnf4 α* is controlled by the distal P2 promoter and an additional downstream enhancer region. At baseline, the P2 promoter region of *Hnf4 α* in rat islets is associated with minimal DNA methylation as well as enrichment of histone activating marks such as histone 3 acetylation, histone 3 lysine 4 trimethylation [51]. Through *Hnf4 α* promoter- specific quantitative PCR experiments, it was revealed that the decreases in DNA methylation and the enrichment of activating histone modifications corresponded to increased P2 transcript [51]. Using INS-1 (which express the

P2 promoter of *Hnf4a*) and BRIN (which do not express the P2 promoter of *Hnf4a*) cell lines, the authors demonstrated that changes in DNA methylation and histone modifications could be responsible for regulating *Hnf4a* transcription by inducing demethylation with 5-Aza-C and histone acetylation with trichostatin A [51]. Finally, Sandovici, *et al* showed that islets isolated from the LP exposed offspring had significantly decreased expression of the P2 promoter at 3 months of age which correlated with a modest but statistically significant increase DNA methylation at the P2 promoter of *Hnf4a* at both 3 months of age and 15 months of age (before the offspring have developed diabetes) [51]. In addition, the offspring of dams exposed to the LP diet had reductions of activating histone modifications and increases in silencing histone modifications at the *Hnf4a* enhancer in 3 month old islets [51]. There were no diet-associated changes in histone modifications at the P1 promoter of *Hnf4a* at either 3 months or 15 months of age [51]. The authors conclude that exposure to a suboptimal nutritional environment during early development leads to epigenetic silencing at the enhancer region of *Hnf4a*, which weakens the P2 promoter-enhancer interaction and results in a permanent reduction in *Hnf4a* expression [51].

Finally, Radford, *et al* sought to assess the role of imprinted genes in the developmental origins of health and disease [52]. Imprinted genes, a class of functionally mono-allelic genes critical for growth and metabolic development, have been proposed to be uniquely susceptible to environmental change and that perturbations of the epigenetic regulation of the imprinting control regions of such genes may play a role the development of adult disease after an early life insult. The authors analyzed the expression of imprinted genes through microarray and quantitative PCR in two affected generations of IUGR induced by total calorie restriction of the dam [52]. They found that imprinted genes as a class were not particularly susceptible to expression changes following *in utero* undernutrition. Imprinted genes in the developing germline were not affected and imprinted genes are largely stable in the second generation [52].

Conclusion

The four rodent models of IUGR described above demonstrate that exposure to an adverse in utero environment results in fetal adaptations that ultimately have consequences leading to abnormalities in glucose homeostasis and insulin metabolism in the adult animal. Such fetal adaptations lead to changes in gene expression but the specific mechanisms that mediate these processes are still being investigated. IUGR induces changes in the epigenetic regulation of gene expression as one mechanism by which the in utero insult can lead to the development of diabetes in adults. Pancreatic transcription factors *Pdx-1* and *Hnf4a* both appear to be susceptible to epigenetic modifications induced by IUGR that can lead to abnormal expression; however there remains a great deal of work to be done to determine additional mechanisms involved in altering gene expression at specific loci and on a genome wide basis in IUGR which ultimately lead to the development of T2DM.

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**Control:
transcriptional
activation**

**IUGR (UPI):
transcriptional
silencing**

Figure 1.

Summary of epigenetic changes at the *Pdx-1* promoter in UPI islets. In control islets, the *Pdx-1* promoter is normally found in an unmethylated (open circles) open chromatin state allowing access to transcription factors like USF-1 and associated with nucleosomes characterized by acetylated histones H3 and H4 (H3Ac, H4Ac; gray triangles) and with the H3K4me3 (white hexagons). In islets collected from offspring exposed to UPI, histone acetylation is lost through association with a mSin3A-HDAC1-DNMT1 repressor complex. The activating histone modification H3K4me3 disappears while the silencing histone modification H3K9me2 (black diamonds) accumulates. The UPI exposed chromatin is more compressed and extensive DNA methylation (black circles) locks in the transcriptionally silent state of *Pdx-1* in the UPI islets.

Table 1

Rodent models of IUGR: effects on beta-cell phenotype and glucose homeostasis

	Low Protein	Total Calorie Restriction	Uteroplacental Insufficiency
Neonate	<ul style="list-style-type: none"> decreased beta-cell mass, proliferation, islet size and insulin content of beta-cells increased beta-cell apoptosis decreased expression <i>Pdx-1</i> and <i>IGF-II</i> 	<ul style="list-style-type: none"> mixed reports: some models with impaired beta-cell development, low plasma glucose and insulin; others with no change in pancreas weight, beta-cell mass, beta-cell replication or apoptosis <i>Pdx-1</i> expression is not decreased 	<ul style="list-style-type: none"> no difference in beta-cell mass, islet size, pancreatic weight decreased plasma glucose and insulin concentrations first phase insulin secretion and insulin sensitivity impaired. 14 days: reduced beta-cell proliferation; <i>Pdx-1</i> expression decreased by 60%
Adult	<ul style="list-style-type: none"> 15 month males have hyperinsulinemia 17 month males have fasting hyperglycemia 	<ul style="list-style-type: none"> 3–4 months: decreased beta-cell mass; decreased insulin sensitivity 8 months: decreased beta-cell mass; 40% decreased insulin content; elevated fasting insulin but no overt hyperglycemia 	<ul style="list-style-type: none"> 7–10 weeks: mild fasting hyperglycemia and hyperinsulinemia 15 weeks: beta-cell mass reduced 50% 26 weeks: fasting insulin levels decline and first phase insulin secretion absent