REVIEW

Consequences of a compromised intrauterine environment on islet function

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Abstract

Low birth weight is an important risk factor for impaired glucose tolerance and diabetes later in life. One hypothesis is that fetal β -cells inherit a persistent defect as a developmental response to fetal malnutrition, a primary cause of intrauterine growth restriction (IUGR). Our understanding of fetal programming events in the human endocrine pancreas is limited, but several animal models of IUGR extend our knowledge of developmental programming in β -cells. Pathological outcomes such as β -cell dysfunction, impaired glucose tolerance, and diabetes are often observed in adult offspring from these animal models, similar to the associations of low birth weight and metabolic diseases in humans. However, the identified mechanisms underlying β -cell dysfunction across

models and species are varied, likely resulting from the different methodologies used to induce experimental IUGR, as well as from intraspecies differences in pancreas development. In this review, we first present the evidence for human β -cell dysfunction being associated with low birth weight or IUGR. We then evaluate relevant animal models of IUGR, focusing on the strengths of each, in order to define critical periods and types of nutrient deficiencies that can lead to impaired β -cell function. These findings frame our current knowledge of β -cell developmental programming and highlight future research directions to clarify the mechanisms of β -cell dysfunction for human IUGR.

Journal of Endocrinology (2010) **205**, 211–224

Introduction

The incidence of type 2 diabetes mellitus (T2DM) is growing worldwide. It is now well established that interactions between an individual's genetic makeup and environment contribute to the development of T2DM (Gerich 1998). Evidence continues to mount showing that T2DM is more prevalent among subjects that were intrauterine growth restricted (IUGR) during fetal development, indicating that the defects in glucose homeostasis originate *in utero* (Barker *et al.* 1993, Ravelli *et al.* 1998, Newsome *et al.* 2003). The reciprocal relationship between insulin secretion and sensitivity (Table 1) suggests that defects occur in either insulin secretion capacity or insulin action at target tissues, or both in the case of T2DM (Robertson 1992, Kahn *et al.* 1993, Weir 1993a,b). Despite differences in the type, timing, and duration of intrauterine insult, most animal models of IUGR have similar outcomes of impaired glucose tolerance or T2DM. However, because the etiology of these phenotypes can involve a variety of mechanisms, they are relatively nonspecific endpoints. Therefore, care is required when interpreting animal studies and planning interventions to

ameliorate β -cell dysfunction. In this review, we discuss the evidence for β -cell dysfunction in human IUGR and identify several potential mechanisms found in animal models that begin to explain these outcomes.

β -Cell defects in small-for-gestational-age-infants

Human studies generally compare outcomes in small-for-gestational-age-neonates (SGA; usually defined as <10th percentile for birth weight, although the statistical cut-off can vary) to appropriate-for-gestational-age-neonates (AGA). Although birth weight is the most accessible proxy for fetal growth restriction, the statistical nature of this classification may introduce heterogeneity into human studies, whereas the term IUGR describes a pathophysiological process. In most cases, IUGR occurs in genetically normal fetuses that are restrained from reaching their full growth potential. This is usually associated with placental insufficiency and almost always with fetal undernutrition, which causes asymmetric fetal growth (Platz & Newman 2008). IUGR is rarely diagnosed before 22–24 weeks of gestation, but detection is

Table 1 Measurements of glucose metabolism and insulin secretion

	Definition	Test
Term		
Insulin secretion	Capacity of β -cells to release insulin in response to a secretagogue	Glucose-stimulated insulin secretion (GSIS) – insulin response to an i.v. glucose infusion Intravenous arginine stimulation tests (IVAST) – insulin response to an i.v. arginine bolus Glucose-potentiated arginine-induced insulin secretion (GPAIS) – insulin response to a continuous i.v. glucose infusion followed by an i.v. arginine bolus
Insulin sensitivity	Capacity of insulin-responsive tissues (i.e. muscle, adipose, and liver) to extract glucose from circulation at a given insulin concentration	Hyperinsulinemic–euglycemic clamp (HEC) – insulin concentration is raised with a constant infusion, and glucose is adjusted to maintain euglycemia Intravenous glucose tolerance tests (IVGTT) – measure clearance of a glucose bolus
Insulin resistance	A condition in which responsive tissues do not respond adequately to normal amounts of insulin	Homeostatic model assessment (HOMA) or quantitative insulin sensitivity check index (QUICKI) – estimates insulin resistance by fasting insulin and glucose concentrations
Insulin disposition	An index that includes measurements for both insulin secretion and insulin sensitivity in order to assess β -cell function	Calculated as insulin responsiveness (e.g. $AUC_{\text{insulin}}/AUC_{\text{glucose}}$ during OGTT) multiplied by an insulin sensitivity index
Impaired glucose tolerance	Prediabetic state that is demonstrated by an inability to clear glucose or slowed return to glucose homeostasis after administration (glucose disposal) Affected by both insulin secretion and insulin sensitivity	Oral glucose tolerance test (OGTT) or intravenous glucose tolerance tests (IVGTT) – glucose disposal after the administration of glucose bolus. IVGTT can also be used to measure first-phase insulin secretion

improving with increasing use of ultrasonography (Ferrazzi *et al.* 2002, Creasy *et al.* 2004). Despite the limitations of the SGA classification, the human studies discussed below provide substantial evidence for relationships between birth weight and metabolic syndromes resulting from β -cell dysfunction.

Based on umbilical cord blood sampling, SGA fetuses have lowered blood oxygen levels; decreased plasma amino acids, glucose, and insulin (Economides *et al.* 1989, Setia *et al.* 2006); and increased catecholamine concentrations (Greenough *et al.* 1990) relative to AGA controls. Glucose-stimulated insulin secretion (GSIS; Table 1) has been measured in IUGR fetuses (diagnosed by ultrasound) prior to the onset of labor or maternal anesthesia. They demonstrated an almost complete absence of first-phase insulin secretion compared with a robust response in control fetuses (Table 2; Nicolini *et al.* 1990). Elucidation of the mechanisms responsible for impaired GSIS in IUGR fetuses has been limited to measurements of pancreatic morphology. One study found no differences in the β -cell population between SGA (<10th percentile) and AGA fetuses (50–75th percentiles) at 36 weeks of gestation (Beringue *et al.* 2002). However, newborns with severe SGA (<3rd percentile) had a lower fraction of β -cells and smaller islets with less pronounced vasculature than AGA newborns (~40th percentile) at ~34 weeks of gestation (Van Assche *et al.* 1977). Together, these studies demonstrate

that, at least in severe IUGR fetuses, the pancreatic β -cell population and insulin secretion are reduced (Table 2).

In the literature, there are conflicting reports describing neonatal insulin secretion in SGA subjects, spanning from β -cell dysfunction to overcompensation. One report has shown that β -cell dysfunction persists in SGA infants at 48 h, but they also tend to be more sensitive to insulin, which may mask impaired glucose tolerance (Bazaes *et al.* 2003). The relationship between insulin secretion and insulin sensitivity is hyperbolic in nature. Thus, for a given degree of glucose tolerance, the product of insulin secretion and insulin sensitivity is constant, and the changes are reciprocal (i.e. insulin secretion decreases in response to greater insulin sensitivity; Kahn *et al.* 1993). Therefore, it is important to account for insulin sensitivity when measuring insulin secretion. Another report found that by 72 h, SGA infants had greater plasma insulin concentrations compared with AGA infants, but glucose concentration was not different (Wang *et al.* 2007). In this study, SGA subjects were not as growth restricted as those examined in the 48-h study, making direct comparisons difficult, but also suggesting a trend for degrees of IUGR. Several case series identify SGA newborns presenting with hyperinsulinemic hypoglycemia (HG). Although retrospective and selective, these cases support β -cell overcompensation in some SGA newborns (Collins & Leonard 1984, Collins *et al.* 1990, Hoe *et al.* 2006).

Table 2 Fetal and pancreatic characteristics in humans and animal models of intrauterine growth restriction (IUGR)

	Rodent models of IUGR			Ovine models of IUGR		
	Human SGA/IUGR subjects	Uterine artery ligation	Caloric restriction	Low-protein diet	Hyperthermic placental insufficiency	Carunclectomy-placental restriction
Fetal characteristics						
Timing of insult ^a	Variable, but >0.55G	0.82–0.91G	0.68–1G	0–1G	>0.47–1G	0.8–0.93G
Hypoxemia	Yes	Yes, 0.82–0.86G Normal at birth	None	None	↓ 34–59%	None
Hypoglycemia	Yes	Yes, 0.82–0.86G Normal at birth	NR	NR	↓ 31–52%	↓ 46–54%
Hypoinsulinemia	Yes	Yes, 0.82–0.91G Normal at birth	↔	↔	↓ 69%	↓ 26–36%
Fetus weight	↓ ≥2 s.d.	↓ 15–20%	↓ 15–20%	↓ 5–8%	↓ 24–57%	↓ 17–24%
Other factors defined	↑ Catecholamines	NR	↑ Corticosterone ↓ IGF2 ↓ VEGF	↔ Corticosterone ↓ Taurine	↔ Corticosterone ↑ Taurine ↑ Catecholamines	↑ Corticosterone ↑ Taurine ↔ Catecholamines
Fetal pancreatic characteristics						
β-Cell mass	NR	↔	↓	↓	↓ 76%	↔
β-Cell fraction	↓/↔	NR	↓	↓	↓ 42%	↔
Islet vascularization	↓	↓	↔	↓	↓ 33% ^b	NR
β-Cell proliferation	NR	↓	↔	↓	↓ 72%	NR
β-Cell neogenesis	NR	NR	↓	↔	↔	NR
β-Cell apoptosis	NR	↔	↔	↑	↔	NR
<i>In vitro</i> β-cell responsiveness	NR	↓ at P7	NR	↓ (amino acids)	↓ 82%	↓
<i>In vivo</i> β-cell responsiveness	↓ 89%	NR	NR	NR	↓ 76%	↓ 45%

NR, not reported in the literature to the authors' knowledge; ↔, factor was measured, but no change was found; G, fraction of gestation; P, postnatal days.

^aThe timing of the insult for humans and sheep with placental insufficiency is based on initial diagnoses for IUGR or lower index of placental function.^bRozance & Limesand, unpublished data.

More thorough evaluation is required to determine which infants have persistent β -cell dysfunction and which develop hyperresponsive β -cells, as well as to the underlying mechanisms responsible for these differences.

Many SGA children exhibit catch-up growth and reach normal height and weight by 3 years of age (Colle *et al.* 1976, Bazaes *et al.* 2004, Iniguez *et al.* 2006). Those showing catch-up growth, especially for length, have greater insulin secretion compared with those without catch-up growth (Colle *et al.* 1976, Hofman *et al.* 1997, Crowther *et al.* 2000, Soto *et al.* 2003, Bazaes *et al.* 2004). Catch-up growth is also associated with increased adiposity, raising the incidence of insulin resistance by the time the children reach 5 years of age (Hofman *et al.* 1997, Ong *et al.* 2000). This places a greater burden on a β -cell population already compromised by fetal malnutrition, perhaps accelerating the progression towards β -cell failure (Meriq *et al.* 2005).

In SGA children and young adults, reports of insulin secretion and β -cell function are discordant. Interpretation of results is complicated by the development of insulin resistance (Hofman *et al.* 1997, Soto *et al.* 2003, Bazaes *et al.* 2004, Meriq *et al.* 2005), in addition to subject population heterogeneity, and confounding environmental variables (e.g. diet and exercise). Even with these limitations, studies that have measured insulin secretion and accounted for insulin sensitivity have shown that insulin secretion is impaired in SGA subjects as children (up to 14 years of age; Li *et al.* 2001) and in otherwise healthy, glucose-tolerant, 19-year-old men without a family history of diabetes (Jensen *et al.* 2002). These studies contrast to several reports indicating that β -cell function was unaffected (Crowther *et al.* 2000, Flanagan *et al.* 2000, Soto *et al.* 2003, Veening *et al.* 2003). However, these negative studies did not measure insulin sensitivity. Therefore, β -cell dysfunction relative to insulin resistance is apparent, and over time, these factors combine to cause T2DM in adults born with SGA.

Critical windows for fetal islet development

Before comparing and contrasting islet deficiencies in animal models of IUGR, we must first consider the timing of events of pancreas morphogenesis and differentiation

of endocrine cells. Pancreas development could be impacted differently depending on the timing and duration of restricted nutrition relative to the stage of development. For example, when rat dams are calorie restricted during gestation and lactation, pup β -cell mass and function are reduced, but this reduction is less severe if the dam is fed adequate calories during lactation. Such postnatal interventions may impact outcomes more markedly in altricial species such as the rat, which undergo extensive islet maturation after birth, compared with precocial species, such as the human and the sheep. Moreover, in rodents, pancreas development appears to happen in discrete transitions, which allows us to dissect out stage-specific complications and relate them to the multifocal developmental progression found in the human and sheep pancreas (Table 3; Sarkar *et al.* 2008, Cole *et al.* 2009).

Organogenesis of the pancreas is a highly coordinated process with structural and cytological aspects. Early morphological events of pancreas formation appear to be phylogenetically conserved among mammals (Pictet *et al.* 1972a). The developmental process begins when the endoderm is specified to ‘the pancreatic state’, and after the primary transition, these protodifferentiated cells expand to form the pancreatic anlagen (Pictet *et al.* 1972a). The pancreas originates from the dorsal and ventral region of the foregut endoderm at embryonic day (E) 9.5 in the mouse, E11 in the rat, and 25–26 days gestational age (dGA) in the human (Wessells & Cohen 1967, Pictet *et al.* 1972a,b, Piper *et al.* 2004). Pancreatic precursor cells expand, forming a dense outgrowth that begins to lobulate and then elongates into branches (Rutter 1980, Jensen 2004, Piper *et al.* 2004, Cole *et al.* 2009). In rodents, the elongation is associated with a condensed mesodermal covering (Wessells & Cohen 1967). In contrast, human and sheep pancreatic buds appear to elongate into a loose mesenchymal bed, which occurs between 26 and 41 dGA in the human (Piper *et al.* 2004) and between 24 and 29 dGA in the sheep (Cole *et al.* 2009). Reasons for these morphological differences are undefined, but we expect that the mesenchymal cells provide factors to support pancreas development in all mammals (Wessells & Cohen 1967, Bhushan *et al.* 2001, Norgaard *et al.* 2003). Finally, the progenitor cell expansion culminates with the

Table 3 Species comparison of critical periods for pancreas development

	Mouse	Rat	Sheep	Human
Primary transition	E9.5 (0.5G)	E11 (0.5G)	<24 dGA (<0.16G)	25–26 dGA (0.09G)
Secondary transition	E13.5–E15.5 (0.71–0.82G)	E15–18 (0.68–0.82G)	24–147 dGA (0.16–1.0G)	7.5–40 wGA (0.19–1.0G)
Isletogenesis	E15–19 (0.79–1.0G)	E17–21 (0.77–0.95G)	33–147 dGA (0.22–1.0G)	1–40 wGA (0.28–1.0G)
Endocrine cell proliferation	E18.5–P30 (0.97–1.0G)	E21.5–22 (0.97–1.0G)	33–147 dGA (0.22–1.0G)	8–40 wGA (0.20–1.0G)
Term gestation	E19	E22	147 dGA	40 wGA

E, embryonic day; dGA, days gestational age; wGA, weeks gestational age; G, fraction of gestation.

secondary transition ‘differentiated state’ when mature secretory products are identified, occurring between E13.5 and 15.5 in the mouse and between E15 and 18 in the rat (Wessells & Cohen 1967, Pictet *et al.* 1972b). The secondary transition begins before 24 dGA in the sheep and at 52 dGA in the humans, and it proceeds in conjunction with progenitor cell expansion, but defined endpoints are not evident during gestation in either species (Polak *et al.* 2000, Piper *et al.* 2004, Cole *et al.* 2007, 2009).

Studies using genetically engineered mice have defined a hierarchy of transcription factors that coordinates pancreas organogenesis and endocrine cell specification from pluripotent progenitors (for a comprehensive review see Jensen (2004)). For brevity, we will only focus on transcription factors that have also been examined in humans and sheep. A homeodomain protein, pancreatic and duodenal homeobox-1 (*PDX1*), initiates differentiation and morphogenesis of the pancreatic epithelial progenitor cells and later becomes restricted to mature β -cells, where it is involved in transactivation of β -cell-specific genes (Offield *et al.* 1996, Sander & German 1997). In humans and sheep, a similar pattern of cellular expression of *PDX1* is apparent (Piper *et al.* 2004, Cole *et al.* 2009). At the secondary transition in mice, neurogenin 3 (*Ngn3* also known as *Neurog3*) is essential for committing pancreatic epithelial progenitor cells to an endocrine cell lineage (Gradwohl *et al.* 2000, Jensen *et al.* 2000). In humans, however, evidence for an absolute requirement of *NGN3* is unclear (del Bosque-Plata *et al.* 2001, Wang *et al.* 2006). Despite this, the temporal and spatial expression of *NGN3* indicates that it is involved in endocrine cell specification in humans and sheep (Sugiyama *et al.* 2007, Sarkar *et al.* 2008, Cole *et al.* 2009).

Two other processes that occur during pancreagenesis include the onset of mature islet cell replication and isletogenesis, both of which also exhibit temporal differences among species. In rodents, the newly formed endocrine cells are mitotically quiescent until near term (E19 mouse and E21.5 rats; Pictet *et al.* 1972b, Jensen *et al.* 2000, Sander *et al.* 2000), whereas human β -cells begin to proliferate at 8 weeks gestational age, albeit at low levels, and continue replicating throughout gestation (Bouwens *et al.* 1997, Bocian-Sobkowska *et al.* 1999, Kassem *et al.* 2000, Polak *et al.* 2000). A similar time frame of onset and duration is found in sheep (Limesand *et al.* 2005, Cole *et al.* 2007). The formation of islet-like structures also happens earlier in humans and sheep than in rodents. In humans and sheep, aggregates begin forming shortly after the onset of differentiation, resulting in primitive islets by week 11 in humans (Bocian-Sobkowska *et al.* 1999, Nieto 2002, Piper *et al.* 2004) and 33 dGA in sheep (Cole *et al.* 2009). In contrast, islets do not form until 0.78 of gestation in rodents (Slack 1995, Jensen *et al.* 2000). During isletogenesis, vascularization of the islets is also observed (Pictet *et al.* 1972b), and recent findings indicate that endothelial cells augment β -cell proliferation (Lammert *et al.* 2001, Nikolova *et al.* 2006).

In the human fetus, β -cells exhibit robust insulin secretion in response to insulin secretagogues (Adam *et al.* 1969, Nicolini *et al.* 1990). In rats, β -cells begin to be responsive near term, but the response is not robust until a week after birth (Kervran *et al.* 1979, Kervran & Randon 1980). In the sheep fetus, β -cells respond to glucose and arginine as early as mid-gestation, and the response improves toward term (Molina *et al.* 1993, Aldoretta *et al.* 1998). Strikingly, newborn lambs have a GSIS response that is almost 5 times that of the late-term fetus (Philipps *et al.* 1978). This enhanced GSIS occurs within 6 h of parturition, suggesting that the changes are induced by the postnatal internal milieu, not by a sudden β -cell expansion; GSIS potentiation has not been confirmed in human infants.

Morphological and cytological processes for pancreas development appear to be conserved among humans and species that are commonly used as models for IUGR. However, the timing and progression of pancreas development are different in precocious species compared with rodents (Table 3). The distinct developmental transitions observed in rats later in gestation provide insight into how pancreas development is affected by IUGR, while the sheep provides a model system more reminiscent of human pancreas development (Table 3). Regardless of species, critical windows of islet development include differentiation, proliferation, development of vasculature, and functional maturation of metabolism (Fig. 1); all of these are potentially affected by fetal nutrient restriction.

β -cell deficiencies in animal models of IUGR

In addition to obvious ethical issues, studies in humans are difficult because of our long life span, during which time complex environmental and genetic factors may cloud associations between fetal growth and adulthood diseases. Therefore, animal models of IUGR play a critical role in building our understanding of developmental programming of β -cells. Although no model perfectly replicates human circumstances, all of the rodent and sheep models discussed here show a clear association between fetal growth restriction and β -cell dysfunction (Table 2). However, the mechanisms for these adverse outcomes differ (Fig. 1), which may reflect the type, timing, and duration of fetal insults.

Rodent models of IUGR

Uterine artery ligation model Abrupt global nutrient and oxygen restriction is created by uterine artery ligation (UAL) in rats at E18–E19 (term is 22 days), causing IUGR (Table 2; Wigglesworth 1964, Ogata *et al.* 1986, Simmons *et al.* 1992). The period of fetal insult is relatively brief, with reductions in fetal pH and oxygen lasting for 1–2 days, and plasma glucose and insulin being reduced for 2–3 days prior to normalizing by birth (Ogata *et al.* 1986, Peterside *et al.* 2003). Simmons *et al.* (2001) have shown that UAL rat pups exhibit

Figure 1 Mechanisms for β -cell dysfunction in models of IUGR. A schematic representation of pancreas development and processes controlling β -cell function is depicted. Identified β -cell impairments are shown for each of the rat (above) and sheep (below) models of IUGR. Rat IUGR models include uterine artery ligation (UAL), low-protein diet (LP) and caloric restriction (CR). Sheep IUGR models include uterine carunclectomy-placental restriction (UC-PR), chronic hypoglycemia (HG), and hyperthermia-induced placental insufficiency (HT-PI). A fetal sheep islet at 103 dGA is shown in the micrograph; it is immunostained for insulin (β -cell, blue), other endocrine cells (glucagon, somatostatin, and pancreatic polypeptide cells, red), and vasculature (*Griffonia simplicifolia* 1, green). Solid lines indicate processes sensitive to nutrient restriction in the fetus; dashed lines indicate postnatal effects. Lines ending with arrows represent enhancement, and lines ending with bars represent inhibition of the defined processes. ER, endoplasmic reticulum; ROS, reactive oxygen species; Ca_v1D , voltage-gated calcium channel α 1D.

catch-up growth and surpass controls, becoming obese by 26 weeks. No irregularities in pancreas β -cell morphology are observed before 7 weeks of age, but by 15 weeks, β -cell mass is 50% of controls and continues to decline thereafter (Simmons *et al.* 2001). β -Cell proliferation is lower at

postnatal day (P) 14, which is associated with reductions in islet vascular density or other underlying β -cell defects that take several weeks to manifest (Stoffers *et al.* 2003, Ham *et al.* 2009). Interestingly, when UAL is conducted on rats at E17, just 1 day earlier than in the studies by Simmons *et al.* (2001),

β -cell mass is reduced at P1 (De Prins & Van Assche 1982). Thus, earlier timing of UAL targets a different stage of pancreas development, causing reductions in β -cell mass and differentiation rather than impairments in maturation.

Fasting glucose and insulin concentrations are normal in UAL rats at P7, but they develop mild fasting hyperglycemia and hyperinsulinemia by 7 weeks and frank diabetes by 26 weeks of age. Insulin secretion stimulated with either glucose or leucine is impaired in newborns and progressively worsens with age, but responsiveness to arginine remains intact (Simmons *et al.* 2001, 2005). These findings indicate that the β -cell defect is in metabolic coupling rather than a deficiency in the releasable pool of insulin (Simmons *et al.* 2001).

Mitochondrial dysfunction and oxidative stress are potential underlying mechanisms for β -cell dysfunction in UAL rats (Simmons *et al.* 2005). ATP production in response to glucose is lower in UAL islets compared with controls, which is partially explained by deficiencies in the mitochondrial electron transport chain and in greater mitochondrial DNA point mutations (Simmons *et al.* 2005). Reactive oxygen species (ROS) production is also chronically elevated in UAL islets (Simmons *et al.* 2005), probably potentiated by hyperglycemia (Robertson & Harmon 2006). These factors likely create a self-supporting cycle in which abrupt fetal hypoxemia impairs mitochondrial function, which lowers the activity of the electron transport chain and augments ROS production, leading to further damage (Li *et al.* 2004).

Pdx1 mRNA expression is lower in UAL fetuses (50%) and adults (80%), and it has been shown to be downregulated by modification that silences heterochromatin (Park *et al.* 2008). In addition to transactivation of β -cell-specific genes, *Pdx1* also regulates mitochondrial function in mature β -cells (Gauthier *et al.* 2004). In UAL fetuses, histone deacetylase 1 and Sin3A are recruited to the proximal promoter region, deacetylating core histones H3 and H4 and repressing *Pdx1*. Demethylation of lysine 4 and methylation of lysine 9 on histone H3 also occur, reflecting alternative modifications to the core histones that result in the loss of USF-1 binding to PDX1 promoter. Finally, DNA methylation of the *Pdx1* gene further suppresses its expression in adult UAL rats (Park *et al.* 2008). These data provide evidence linking several of the cellular impairments associated with mitochondrial dysfunction to epigenetic modifications.

Neonatal treatment (P0–P6) with exendin-4, a long-acting GLP-1 analog, prevents the loss of β -cell mass and normalizes β -cell proliferation in UAL pups (Stoffers *et al.* 2003). Exendin-4 treatment normalizes islet vascularity and VEGF protein expression (Ham *et al.* 2009), which may explain the improvements in proliferation. Fasting glycemia and glucose tolerance are also restored, and the development of diabetes is averted (Stoffers *et al.* 2003). *Pdx1* expression is maintained with exendin-4 treatment (Stoffers *et al.* 2003), further confirming *Pdx1* as a major point for β -cell programming in the UAL model.

Calorie restriction model Maternal calorie restriction (CR; 50% of control energy intake in most studies) of rat dams during the last week of gestation causes a 12–20% reduction in birth weight in about half of fetuses, which are selected as IUGR (Table 2; Garofano *et al.* 1997, 1998b). One-day-old CR–IUGR pups have reduced pancreas weight, insulin content, islet density, and β -cell mass, but islet size is similar to controls (Garofano *et al.* 1997, 1998a, Dumortier *et al.* 2007). β -Cell proliferation and apoptosis and islet vascularity are not different at E21 or P1. However, CR islets (E15) have fewer *Ngn3* and *Pdx1* positive cells, indicating that β -cell differentiation is impaired (Garofano *et al.* 1997, 1998a, Dumortier *et al.* 2007). However, when CR is extended through lactation, additional declines in β -cell mass, number, and insulin content are observed in adulthood, indicating that β -cells are also susceptible to nutrient restriction after differentiation is complete (Garofano *et al.* 1998a,b, 1999).

Both aging and pregnancy are normal physiological stresses, and β -cells of CR rats appear to be poorly equipped to tolerate these events. During pregnancy, the endocrine pancreas of the mother normally expands by replication to overcome insulin resistance (Blondeau *et al.* 1999, Avril *et al.* 2002). However, 8-month-old CR rats, malnourished through weaning and fed an adequate diet thereafter, display an inability to expand their β -cell population and are hypoinsulinemic at mid-pregnancy (Blondeau *et al.* 1999, Avril *et al.* 2002). Interestingly, 4-month-old CR rats display normal adaptations to pregnancy, implicating an interaction between age and pregnancy in the plasticity of the adult CR pancreas (Blondeau *et al.* 1999). Nonpregnant rats also normally increase β -cell mass by 40% by replication between 3 and 12 months of age; however, CR rats do not show this increase, resulting in reduced insulin secretion (Garofano *et al.* 1999).

CR fetuses have elevated glucocorticoid concentrations, which has been proposed as a mechanism for the observed β -cell dysfunction (Blondeau *et al.* 2001). Studies on pancreas development *in vitro* and in glucocorticoid receptor knockout mice show that glucocorticoids impair normal β -cell differentiation and lower *Pdx1* expression (Gesina *et al.* 2004, 2006). In normal rat fetuses, there is a negative correlation between plasma corticosterone and fetal weight, as well as pancreatic insulin content (Blondeau *et al.* 2001). When CR rat dams are subjected to adrenalectomy and then exogenously supplemented with normal levels of corticosterone, fetal β -cell fraction and mass are restored despite CR (Blondeau *et al.* 2001, Lesage *et al.* 2001). Fetal body weight and pancreas weight are still reduced (Blondeau *et al.* 2001, Lesage *et al.* 2001), indicating that elevated glucocorticoids have a β -cell-specific effect, while malnutrition still impacts other aspects of fetal development.

Low-protein diet model Feeding dams a low-protein (LP) isocalorie diet (8 vs 20% dietary protein) throughout gestation causes IUGR and lowers fetal and postnatal pancreas

weight, β -cell mass, and islet size (Table 2; Snoeck *et al.* 1990, Dahri *et al.* 1991, Petrik *et al.* 1999b, Boujendar *et al.* 2002). In contrast to CR offspring, β -cell differentiation progresses normally in LP fetuses (Dumortier *et al.* 2007). However, at E21.5 islet cell proliferation is lower due to a longer G_1 phase, and apoptosis is increased (Petrik *et al.* 1999b, Boujendar *et al.* 2002). These changes are associated with lower islet insulin-like growth factor 2 (IGF2) expression, which if overexpressed can cause β -cell hyperplasia (Petrik *et al.* 1998, 1999a,b). Moreover, these effects may be influenced by lower islet vascularity and by depressed expression of VEGF and VEGF receptor 2, reminiscent of the UAL model (Snoeck *et al.* 1990, Boujendar *et al.* 2003, Dumortier *et al.* 2007).

One study attempted to separate temporal effects by feeding the LP diet during either gestation or lactation or throughout both periods (Berney *et al.* 1997). In all circumstances, protein restriction reduced β -cell area and caused islets to be irregularly shaped in weanlings. However, postnatal protein restriction caused greater reductions in β -cell and islet numbers, fitting temporally with the postnatal β -cell expansion and isletogenesis. In addition, feeding an LP diet during just the last week of gestation had a greater impact on fetal β -cell mass than LP feeding throughout gestation, indicating that the dam is able to compensate for protein restriction to some extent when it is initiated earlier (Dumortier *et al.* 2007). These data indicate that timing of protein restriction relative to pancreas development is critical for determining β -cell outcomes in offspring.

Impaired islet insulin responsiveness to amino acids and theophylline is observed *in vitro* in LP fetal islets; however, neither control nor LP fetal islets (E21.5) responded to glucose (Dahri *et al.* 1991, Cherif *et al.* 1996, 1998). As adults, LP rats demonstrate impaired glucose tolerance but do not develop diabetes (Dahri *et al.* 1991). In postnatal LP islets, β -cell metabolism is decreased, likely lowering β -cell response to insulin secretagogues (Sener *et al.* 1996, Goosse *et al.* 2009).

Additional studies have shown that LP islets have increased nitric oxide production in unstimulated islets (Goosse *et al.* 2009) and increased sensitivity to nitric oxide- and IL-1 β -induced apoptosis (Merezak *et al.* 2001). The transcription factor growth arrest and DNA damage inducible-protein 153 (GADD153 that is listed as DDIT3 in the HUGO Database) mRNA and protein expressions are increased in LP islets, implicating endoplasmic reticulum stress pathways in β -cell dysfunction (Goosse *et al.* 2009). Furthermore, LP islets develop an imbalance in antioxidant enzyme expression, likely increasing susceptibility to oxidative stress (Theys *et al.* 2009).

Plasma taurine is lower in LP dams and their fetuses. Taurine has many physiological functions, including serving as an antioxidant (Huxtable 1992). It is considered an essential amino acid for fetal and neonatal development, because *in vivo* synthesis is inadequate at these ages (Huxtable 1992, Sturman 1993, Aerts & Van Assche 2002). Taurine supplementation of LP dams during pregnancy normalizes islet responsiveness, IGF2, and vascularity, as well as β -cell proliferation, apoptosis, and mass (Cherif *et al.* 1998, Boujendar *et al.* 2002, 2003).

However, fetal and pup body weights are not corrected (Boujendar *et al.* 2002). Thus, LP feeding appears to have β -cell-specific effects, which may be caused by a taurine deficiency rather than by global protein malnutrition.

Sheep models of IUGR

Hyperthermia-induced placental insufficiency

Pregnant ewes chronically exposed to elevated ambient temperatures from early to late gestation have fetuses that are growth restricted close to term (135 dGA, term $\sim$ 147; Bell *et al.* 1987, Regnault *et al.* 1999, 2002). Smaller placentae with a decreased capacity to transport oxygen and nutrients from the mother to the fetus cause the fetus to become hypoxemic and hypoglycemic (Table 2; Thureen *et al.* 1992, Limesand *et al.* 2004, 2007, de Vrijer *et al.* 2004), causing growth restriction in the hyperthermia-induced placental insufficiency (HT-PI) model. Biometric parameters and fetal weight begin to diverge from normal after 70 dGA, just after the apex of placental growth, and worsen as gestation continues, demonstrating that the placenta is unable to adequately supply fetal demands for growth (Galan *et al.* 1999, Regnault *et al.* 2002, 2003).

Although asymmetric fetal growth is apparent, fetal adaptations appear to target the β -cells to a great extent (Limesand *et al.* 2005). At 133 dGA (0.9 of gestation), HT-PI pancreas weight parallels the fetal growth reduction (-59%), but β -cell mass is lower (-76%) due to fewer β -cells (Limesand *et al.* 2005). This is the result of an extended cell cycle, because β -cell mitosis is 72% lower in HT-PI islets (Limesand *et al.* 2005). β -Cell differentiation may also be impaired, because β -cells in the extra-islet compartment, which might represent newly formed β -cells, are decreased in the HT-PI pancreas; however, changes in isletogenesis could also explain this difference (Limesand *et al.* 2005). Together, these results indicate that placental insufficiency decreases the number of β -cells (Limesand *et al.* 2005).

HT-PI fetuses also have impaired insulin secretion, measured by GSIS and glucose-potentiated arginine-induced insulin secretion (GPAIS; Table 1; Limesand *et al.* 2006, 2007). Glucose-stimulated insulin release by fetal islets, expressed as a fraction of total insulin content, is greater in HT-PI islets than in controls, but the amount of insulin released per HT-PI islet is significantly less due to lower insulin contents (82%). HT-PI islets also demonstrate a deficiency in glucose metabolism; they are unresponsive to glucose-enhanced oxidative metabolism, but glucose utilization rates are intact (Limesand *et al.* 2006). Therefore, IUGR islets have impaired insulin secretion due to reduced insulin biosynthesis and storage, which might be partially in response to impaired oxidative metabolism. However, IUGR islets appear to compensate for these deficits to some extent by augmenting their capacity to release insulin.

Placental insufficiency causes fetal HG and hypoxemia, both of which can independently lower insulin. Chronic HG without hypoxemia, which is discussed as a separate model

below, results in moderate impairment of insulin secretion. Conversely, when glucose is intravenously replaced in HT-PI fetuses for 2 weeks, it is not well tolerated and fails to correct insulin secretion (Rozance *et al.* 2009b), confirming that hypoxemia in addition to HG contributes to the β -cell dysfunction. The suppressive effects of hypoxemia on β -cell function may be directly related to the elevation in catecholamines. Acute bouts of asphyxia cause plasma norepinephrine concentrations to rise in fetal sheep, and via the α_2 -adrenergic receptors, norepinephrine suppresses insulin release (Cheung 1990, Milley 1997, Jackson *et al.* 2000). In human fetuses, norepinephrine concentrations are also increased during hypoxia and are higher in IUGR fetuses (Paulick *et al.* 1985, Greenough *et al.* 1990). We have shown that GSIS can be restored in HT-PI fetuses with adrenergic receptor blockade (Leos *et al.* 2010). This recovery in insulin secretion happens even though fewer β -cells are present, suggesting that the β -cells are hyperresponsive, which is also observed in the isolated islets and persists in the HT-PI lambs at P8 (S W Limesand, R A Leos and M J Anderson, unpublished observations). These findings could begin to explain the mismatch in insulin secretion and glucose disposal rate observed in IUGR infants (Bazaes *et al.* 2003).

Uterine carunclectomy model of placental restriction Another well-established sheep model of IUGR is produced by removing most of the endometrial caruncles from the uterus prior to pregnancy, resulting in placental restriction (UC-PR; Robinson *et al.* 1979). In contrast to humans and rodents, sheep and other ruminants have a cotyledonary placenta that consists of many placentomes. These are discrete units of maternal–fetal interface for gas and nutrient exchange formed by the maternal caruncle and the fetal cotyledon (chorion). Thus, removing the endometrial caruncles lowers the number of sites for placentation in the subsequent pregnancy and reduces placenta size and function (Robinson *et al.* 1979). UC-PR fetuses are smaller compared with controls, hypoxemic (−23 to 35%), and hypoglycemic (−15 to 41%) in late gestation (Table 2; Robinson *et al.* 1979, Owens *et al.* 1989, 2007). Catecholamine (Simonetta *et al.* 1997) and cortisol (Phillips *et al.* 1996) concentrations are also increased during late gestation.

In UC-PR fetuses, Owens *et al.* (2007) have shown that fetal hypoxemia and HG are associated with deficiencies in acute β -cell responsiveness, demonstrated by decreased insulin secretion during intravenous glucose tolerance tests (IVGTT; Table 1) and intravenous arginine stimulation tests (IVAST; Table 1). Importantly, the loss of acute insulin secretion is a hallmark of T2DM (Ward *et al.* 1984, Kahn *et al.* 2001). IVGTT and IVAST measure only first-phase insulin secretion, while the GPAIS test used in the HT-PI model measures maximal insulin release (Robertson 2007). Therefore, reductions in insulin secretion may be even more pronounced in UC-PR fetuses than indicated by the IVGTT/IVAST. No differences are found in β -cell area or mass in UC-PR fetuses compared with controls

(Gatford *et al.* 2008). However, fetal weight does positively correlate with β -cell mass, indicating a similar, though attenuated, response compared with HT-PI fetuses.

UC-PR offspring also suffer postnatal consequences. Young lambs and young adult sheep exhibit impaired glucose tolerance due to β -cell insufficiencies that are associated with reduced body weight or thinness at birth (De Blasio *et al.* 2007). In young IUGR lambs, IVGTT reveal depressed insulin secretion during the first 15 min and a marginally lower insulin disposition that appears to be spared by improved insulin sensitivity (De Blasio *et al.* 2007). In adulthood, however, birth weight is negatively correlated with β -cell mass in males but positively correlated with glucose disposition (Table 1), suggesting that a greater number of β -cells are required to maintain glucose homeostasis in UC-PR adults (Gatford *et al.* 2008). IGF1 and 2, determinants for β -cell growth, are elevated in the pancreas of month-old UC-PR lambs, and these growth factors are postulated to be associated with neonatal catch-up growth (Gatford *et al.* 2008). The expression of the voltage-gated calcium channel α_1D is decreased and is positively correlated with β -cell function, identifying a candidate gene for β -cell dysfunction in the stimulus–secretion coupling (Gatford *et al.* 2008).

Chronic hypoglycemia Experimental HG can be established in fetal sheep to measure β -cell outcomes in a system relatively independent of other common complications usually associated with placental insufficiency, such as hypoxemia. A maternal insulin infusion lowers both maternal and fetal arterial plasma glucose concentrations by about 50% and is maintained for 10–14 days (DiGiacomo & Hay 1990). Maternal insulin does not cross the placenta, and therefore, fetal insulin concentrations decrease markedly in response to HG. Uterine and umbilical blood flows are maintained, as are fetal oxygen, oxygen uptake, and amino acid uptake, but fetuses are smaller than control fetuses (Table 2; DiGiacomo & Hay 1990, Limesand *et al.* 2009). Other changes relevant to β -cell function include alterations in fetal plasma amino acid profiles, with notable increases in taurine and lysine. Cortisol is also elevated, but catecholamines remain normal (Limesand & Hay 2003, Rozance *et al.* 2008).

Fetal GSIS and GPAIS are lower in HG fetuses (Carver *et al.* 1996, Limesand & Hay 2003). Nonlinear modeling of insulin secretion during a square-wave hyperglycemic clamp reveals a delay in insulin release (Limesand & Hay 2003). Morphological analysis of the endocrine pancreas shows that β -cell mass and insulin content are maintained, indicating that the blunted insulin secretion is due to a functional β -cell defect. *In vitro*, despite an ability to utilize and oxidize glucose normally, glucose- and amino acid-stimulated insulin secretions are completely absent, indicating an intrinsic β -cell defect in insulin granule exocytosis (Rozance *et al.* 2006, 2007). Therefore, chronic HG blunts insulin secretion as a result of deficiencies in later steps of stimulus–secretion coupling after glucose metabolism, membrane depolarization,

and calcium entry. The β -cell impairment in chronically HG fetuses is clearly distinct from the HT-PI model, showing that HG alone can create islet dysfunction during late gestation.

Five days of euglycemic correction after 14 days of fetal HG normalizes insulin concentrations during the euglycemic and hyperglycemic clamp periods. However, the time required to reach maximal insulin concentrations remains delayed, and GPAIS does not recover (Limesand & Hay 2003). These data confirm that chronic HG can cause persistent impairments in β -cell function, similar to the findings in the UC-PR model.

Consequences to β -cells in IUGR models

Animal models of IUGR provide useful insights into potential mechanisms for β -cell programming in response to nutrient or oxygen deprivation (Fig. 1). Advantages of rats as a model species include a short gestation period and lifespan, making them useful for studying adulthood and multigenerational effects of IUGR. Moreover, pancreas development in rodents progresses in relatively discrete stages (Table 3), which allows us to link β -cell defects to distinct developmental transitions. For example, CR throughout gestation targets β -cell differentiation, whereas UAL in late gestation targets β -cell maturation and proliferation (Table 2). Other factors contribute to these outcomes; a primary factor in CR rats appears to be elevated glucocorticoids, whereas epigenetic changes that lower Pdx-1 transcription are central to the UAL β -cell dysfunction. Offspring from all three rat models exhibit reduced β -cell proliferation, which might reflect vascular deficiencies and/or lower growth factor concentrations. Defects in β -cell metabolism have also been identified in UAL and LP offspring, indicating that inefficient insulin secretion responsiveness can continue to take a toll postnatally. Overall, the rodent models show that impaired β -cell function can persist long after the intrauterine complications, and these deficiencies impact a variety of aspects coordinating β -cell mass and function.

The fetal sheep has proven to be a valuable model for studying the pathogenesis of IUGR and will continue to extend our knowledge of how IUGR alters the multifocal pancreas development pattern found in both sheep and humans (Table 3). Of the three ovine models, HT-PI results in the greatest reduction in β -cell mass. The UC-PR model causes a more moderate IUGR (Table 2), but β -cell mass is still correlated with fetal weight. This correlation between the degree of IUGR and the reduction in β -cell mass is reminiscent of the pattern seen in the human studies: severe IUGR cases have lower β -cell mass (Van Assche *et al.* 1977), but there is no evidence for this in moderate IUGR (Beringue *et al.* 2002). Timing of fetal nutrient restriction is also a factor. HT-PI begins after mid-gestation and causes lower β -cell mass than the HG and UC-PR models, for which nutrient restriction occurs only during the final 20% of gestation. The degree of fetal HG is similar in the HT-PI and HG models, but islet responses are different, further

highlighting the importance of discrepancies in the duration and timing of nutrient restriction. However, similar to the rat models, IUGR in sheep impairs a variety of aspects important for β -cell function, including proliferation, insulin storage, glucose metabolism, or exocytosis (Fig. 1).

Future directions in IUGR research

While there is strong evidence for an association between human IUGR and β -cell programming, there is a paucity of data on insulin sensitivity, glucose metabolism, and β -cell function in truly IUGR infants. Therefore, another prospective study with assessments throughout lifespan is needed to verify the results of Mericq and colleagues (Soto *et al.* 2003). In addition to metabolic and morphometric evaluations, it will be critical to include measurements of initiators of β -cell dysfunction described in animal models (e.g. catecholamine and glucocorticoid concentrations). Furthermore, we have little knowledge of β -cell mass in IUGR patients at birth or throughout lifespan. These data could be obtained from autopsy studies, but *in vivo* real-time measurements of β -cell mass would obviously be more advantageous. The emerging technologies of *in vivo* imaging and quantification of β -cell mass (Sweet *et al.* 2004, Souza *et al.* 2006) will be useful for this purpose and could be performed concurrently with a complete *in vivo* assessment.

Future research is also needed to develop interventions that can improve outcomes in IUGR patients by rescuing β -cell function and preventing obesity, insulin resistance, and T2DM. Nutritional interventions in mothers at risk for or with IUGR fetuses have shown mixed results, in some cases worsening perinatal growth and increasing mortality (Rush *et al.* 1980, Harding & Charlton 1989, Kramer & Kakuma 2003). Thus, interventions should be pursued only after appropriate animal testing. The fetal sheep is an excellent model for this purpose, because fetal conditions can be precisely regulated for discrete periods of gestation to mimic features of placental insufficiency-induced IUGR and to test the impact of potential treatments on fetal β -cell function and metabolism. Using the catheterized fetal sheep preparation, the impact of chronic supplementation of fetal glucose (Rozance *et al.* 2009b), amino acids (Rozance *et al.* 2009a), IGF1 (Eremia *et al.* 2007), and insulin (Carson *et al.* 1980) on fetal metabolism has been measured in control and IUGR fetuses. These studies should be expanded to optimize conditions for IUGR fetuses, with the ultimate goal of identifying treatments that can be administered through the mother to rescue fetal β -cell function and mass.

Conclusions

IUGR is associated with impaired glucose tolerance and T2DM later in life, and there is evidence that insulin secretion defects contribute to these outcomes. Animal models of

IUGR are critical for defining potential mechanisms of β -cell dysfunction. They have thus far demonstrated that β -cell dysfunction can be reached by multiple mechanisms (Fig. 1), likely depending on the type, timing, and duration of fetal malnutrition and how these factors coincide with stages of pancreas development. There is currently little data from which to determine which, if any, of the identified mechanisms may cause the insulin secretion defect in humans, highlighting the need for more human studies. Animal studies will continue to be important to expand and refine our knowledge of mechanisms, as well as to test potential therapies that may ultimately be used to improve outcomes in human IUGR patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by Award Number R01DK084842 (Principle Investigator SWL) from the National Institute of Diabetes and Digestive and Kidney Diseases. ASG was supported by an NIH Institutional Training Grant (Interdisciplinary Training in Cardiovascular Research, HL07249) and F32DK088514 from the National Institute of Diabetes and Digestive and Kidney Diseases. PJR was supported by an American Diabetes Association Junior Faculty Award (7-08-JF-51) and a National Institute of Child Health and Human Development Career Development Award (1K08HD060688-01). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Child Health and Human Development, or the National Institutes of Health.

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Received in final form 5 March 2010

Accepted 11 March 2010

Made available online as an Accepted Preprint
11 March 2010