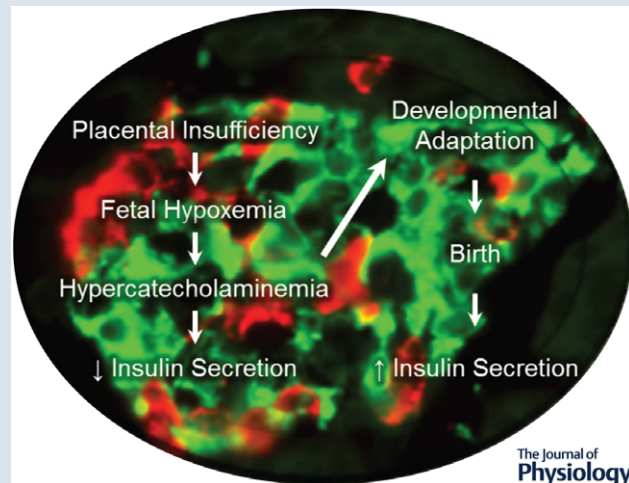SYMPOSIUM REVIEW

Fetal adaptations in insulin secretion result from high catecholamines during placental insufficiency

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Abstract Placental insufficiency and intrauterine growth restriction (IUGR) of the fetus affects approximately 8% of all pregnancies and is associated with short- and long-term disturbances in metabolism. In pregnant sheep, experimental models with a small, defective placenta that restricts delivery of nutrients and oxygen to the fetus result in IUGR. Low blood oxygen concentrations increase fetal plasma catecholamine concentrations, which lower fetal insulin concentrations. All of these observations in sheep models with placental insufficiency are consistent with cases of human IUGR. We propose that sustained high catecholamine concentrations observed in the IUGR fetus produce developmental adaptations in pancreatic β-cells that impair fetal insulin secretion. Experimental evidence supporting this hypothesis shows that chronic elevation in circulating catecholamines in IUGR fetuses persistently inhibits insulin concentrations and

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secretion. Elevated catecholamines also allow for maintenance of a normal fetal basal metabolic rate despite low fetal insulin and glucose concentrations while suppressing fetal growth. Importantly, a compensatory augmentation in insulin secretion occurs following inhibition or cessation of catecholamine signalling in IUGR fetuses. This finding has been replicated in normally grown sheep fetuses following a 7-day noradrenaline (norepinephrine) infusion. Together, these programmed effects will potentially create an imbalance between insulin secretion and insulin-stimulated glucose utilization in the neonate which probably explains the transient hyperinsulinism and hypoglycaemia in some IUGR infants.

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Abstract figure legend Adrenergic programming of fetal islets. Placental insufficiency reduces the delivery of oxygen and nutrients to the fetus resulting in low blood oxygen concentrations. Fetal hypoxaemia raises plasma catecholamine concentrations, which inhibits insulin secretion. Furthermore, experimental evidence from sheep fetuses with placental insufficiency and intrauterine growth restriction (IUGR) shows that chronic adrenergic stimulation causes β-cell adaptations. These adaptations lead to a hyper-secretion of insulin following the decline of sustained high catecholamines or inhibition of adrenergic signalling. We postulate that the hyper-responsiveness in β-cells will persist following parturition and potentially explain the hyperinsulinism and hypoglycaemia observed in IUGR infants following delivery and stabilization.

Abbreviations   AGA, appropriate-for-gestational-age; GPAIS, glucose-potentiated arginine-induced insulin secretion; GSIS, glucose-stimulated insulin secretion; HG-IUGR, hypoglycaemia-induced intrauterine growth restriction; IUGR, intrauterine growth restriction; PI-IUGR, placental insufficiency-induced IUGR; SGA, small-for-gestational age.

Introduction

Placental insufficiency, defined as a failure of the placenta to deliver adequate oxygen and nutrients to maintain fetal growth rates at the genetically determined potential, results in intrauterine growth restriction (IUGR). This is associated with small-for-gestational age (SGA) infants and is estimated to affect 8% of all births (McCowan *et al.* 2010; Monteith *et al.* 2017). Considerable epidemiological evidence in humans and experimental evidence from animal models of IUGR demonstrate that fetal under-nutrition, usually associated with placental insufficiency, produces developmental adaptations that predispose offspring to metabolic disorders of insulin secretion and glucose utilization (insulin sensitivity) at later stages of life (Barker *et al.* 1993; Fernandez-Twinn & Ozanne, 2006; Harder *et al.* 2007; Owens *et al.* 2007*b*; Green *et al.* 2010; Zimmermann *et al.* 2015). SGA children develop glucose intolerance more frequently, and in adulthood exhibit impaired insulin secretion and a greater risk for developing Type 2 diabetes (Hales *et al.* 1991; Phipps *et al.* 1993; Yajnik *et al.* 1995; Mericq *et al.* 2005). However, glucose intolerance appears to be preceded in some formerly IUGR infants by a period of hyperinsulinism which transitions over the first few months to years of life (Bazaes *et al.* 2003; Soto *et al.* 2003; Milovanovic *et al.* 2014). Furthermore, evidence from human data is accumulating which shows that some formerly IUGR fetuses demonstrate neonatal hypoglycaemia associated with the transient form of hyperinsulinism, implying increased insulin concentrations and secretion relative to their insulin sensitivity (Miralles *et al.* 2002; Stanley *et al.* 2015). In severe cases this transient form of hyperinsulinism and hypoglycaemia may persist for several months (Hoe *et al.* 2006; Arya *et al.* 2013).

Observational data from humans indicate that the developmental adaptations of both β-cell function and insulin sensitivity to IUGR result in a lifelong pathophysiological process which allows the fetus to maintain normal metabolic rates for survival to term at the expense of normal fetal growth but produces glucose intolerance and diabetes later in life (Fall *et al.* 1998; Jensen *et al.* 2002, 2007; Newsome *et al.* 2003). Similarly, in sheep, surgical removal of placental attachment sites, or caruncles, before pregnancy produces placental restriction and fetal IUGR that lead to deficiencies in insulin secretion in the fetus, month-old lambs, and yearling sheep (De Blasio *et al.* 2007; Owens *et al.* 2007*a*; Gatford *et al.* 2008). The uterine carunclectomy model of placental restriction shows that fetal complications, such as low oxygen and glucose concentrations, lead to the development of glucose intolerance in adulthood, a finding consistent with several rodent models of fetal undernutrition as well (Robinson *et al.* 1979; Jones & Robinson, 1983; Green *et al.* 2010; Liu *et al.* 2015). While the development of diabetes in later life is well established in humans and animal models of IUGR, what has been less well studied are the mechanisms responsible for the period of increased insulin sensitivity

and secretion immediately after birth and how these fit into the developmental pathophysiological processes which align IUGR and the later development of Type 2 diabetes. Identification of the mechanisms responsible for these developmental adaptations to IUGR will allow for targeted interventions to prevent both short and long term complications of placental insufficiency.

Factors contributing to IUGR and developmental programming of insulin secretion

In the literature, human studies generally compare outcomes in SGA neonates (usually defined as <10th percentile for birth weight) to appropriate-for-gestational-age neonates (AGA) because birth weight is the most accessible proxy for fetal growth restriction. Although this statistical classification introduces heterogeneity by including newborns who are genetically predetermined to be small along with cases of IUGR where pathological fetal vascular patterns can be identified with Doppler assessments of the middle cerebral and umbilical artery, aorta and the ductus venosus, these studies provide important observations on insulin secretion and sensitivity which have been confirmed experimentally in animal models of placental insufficiency (Platz & Newman, 2008; Monteith *et al.* 2017). Such observations include those made by umbilical cord blood sampling in human SGA fetuses prior to delivery or the onset of labour. Human SGA fetuses develop hypoxaemia defined as low blood oxygen concentrations, hypo-glycaemia and hypoinsulinaemia relative to AGA controls (Economides *et al.* 1989; Nicolini *et al.* 1990). Additionally, human SGA fetuses are characterized by increased plasma catecholamine concentrations (Greenough *et al.* 1990). In human fetuses, prior to the onset of labour or maternal anaesthesia, glucose-stimulated insulin secretion (GSIS), measured by placing an umbilical vein catheter for fetal blood sampling and glucose infusion, is absent in IUGR fetuses. This is in contrast to a robust GSIS response in control fetuses (Nicolini *et al.* 1990). Clinical studies determining the mechanisms responsible for impaired GSIS in IUGR fetuses are limited to histological evaluation of the pancreas. One study found that fetuses with severe SGA (<5th percentile) have smaller islets and a lower fraction of β-cells within the islets compared to AGA newborns at ∼34 weeks of gestation (Van Assche *et al.* 1977). In a different study with less severe SGA cases (<10th percentile), there was no difference in the pancreatic β-cell area (Beringue *et al.* 2002). These data indicate that insulin secretion is blunted in human IUGR fetuses, yet reduced insulin secretion is not exclusively explained by less β-cell mass. Therefore, impairments in insulin secretion are postulated to result not just from structural defects of the pancreatic islet and fewer β-cells, but also from extrinsic factors such as hypoxaemia, hypoglycaemia, hypercatecholaminaemia, or the combination of these conditions.

Isolating IUGR complications that impair fetal insulin secretion

Due to ethical considerations, mechanistic studies in humans are limited. IUGR models in sheep offer a unique advantage to investigate the fetal β-cell physiology both *in vivo* and *in vitro* because of the ability to catheterize sheep fetuses for experimental challenges and to study islets isolated from the same fetus. Using different experimental approaches in pregnant sheep, potential mechanisms responsible for the β-cell dysfunction in placental insufficiency and IUGR can be tested (Green *et al.* 2010). For example, comparing β-cell dysfunction in IUGR sheep fetuses created with hyperthermia-induced placental insufficiency (PI-IUGR) and maternal insulin infusion-induced chronic hypoglycaemia (HG-IUGR) is particularly informative (Bell *et al.* 1987; DiGiacomo & Hay, 1990). Fetal glucose concentrations are reduced to equivalent levels (∼50%) in these two sheep models of IUGR, but there are stark differences in duration of nutrient restriction, blood oxygen concentrations, and plasma catecholamine concentrations. Placental insufficiency restricts transfer of oxygen, glucose and amino acids to the fetus resulting in hypoglycaemia and hypoxaemia as early as 0.7 of gestation, prior to notable differences in fetal weight (Thureen *et al.* 1992; Ross *et al.* 1996; de Vrijer *et al.* 2004; Limesand *et al.* 2013). The fetal hypoglycaemia and hypoxaemia identified at 0.7 of gestation progressively worsen as the pregnancy gets closer to term and the IUGR becomes prominent (Limesand *et al.* 2007, 2013). In contrast, maternal hypoglycaemia experimentally restricts placental transport of glucose but does not affect placental oxygen and amino acid transfer to the fetus (DiGiacomo & Hay, 1990; Limesand *et al.* 2009). Therefore, while these fetuses are hypoglycaemic, they are not hypoxaemic and their degree of fetal IUGR is much less than in the PI-IUGR model (DiGiacomo & Hay, 1990). Another difference is that PI-IUGR sheep fetuses have higher circulating catecholamine concentrations compared to HG-IUGR sheep fetuses, whereas cortisol concentrations tend to increase in both models (Narkewicz *et al.* 1993; Limesand & Hay, 2003; Limesand *et al.* 2006; Thorn *et al.* 2012).

By integrating findings from different sheep models of IUGR, we can identify mechanisms linking specific conditions of IUGR and β-cell dysfunction (Fig. 1). While fetal GSIS is impaired in both models, glucose-stimulated insulin concentrations are much lower in PI-IUGR fetuses than in HG-IUGR fetuses (Limesand & Hay, 2003; Limesand *et al.* 2006). Other important differences between the models can be identified with *in vitro* experiments using isolated islets from fetal sheep. In

isolated islets from PI-IUGR fetuses GSIS is reduced due to less insulin production by the β-cell. However, the fraction of insulin present that is released in response to stimulatory glucose concentrations (secreted insulin/total islet insulin content) is actually greater in PI-IUGR islets compared to controls. PI-IUGR islets also have a deficiency in glucose metabolism. Compared to control isolated islets, PI-IUGR islet oxidative glucose metabolism is significantly lower in hyperglycaemic conditions that stimulated insulin release (Limesand *et al.* 2006). In summary, PI-IUGR islets have decreased insulin secretion due to reduced insulin biosynthesis and storage, which might be in response to impaired islet oxidative metabolism of glucose. However, *in vitro* PI-IUGR islets compensate for these deficits to some extent by augmenting their capacity to release meager insulin stores. In this respect, the PI-IUGR islets appear to be hyper-responsive to glucose with regards to their ability to release the insulin that is produced and stored by the β-cell. These findings contrast with the situation for islets isolated from HG-IUGR fetuses (Rozance *et al.* 2006, 2007). In HG-IUGR islets, GSIS also is reduced, but this is not due to reduced islet insulin content or glucose metabolism; instead the reduced GSIS results from a significantly lower fraction release of insulin present in the β-cell in response to secretagogues. Essentially, the HG-IUGR islets are hypo-responsive to glucose. Therefore, PI-IUGR islets have impaired *in vitro* insulin secretion due to decreased islet insulin production and storage, which is partly compensated for by their hyper-responsiveness to glucose in terms of their greater capacity to release the insulin that is present. In contrast, HG-IUGR *in vitro* islet insulin secretion is impaired due to an intrinsic inability of the β-cell to release the insulin

that it has available, or glucose hypo-responsiveness. Comparing the *in vivo* phenotypes of these two different models leads to consideration of a variety of factors in the PI-IUGR fetus, which may be responsible for differences in β-cell dysfunction between the PI-IUGR and HG-IUGR models. Our focus is on factors that differ between the two models, such as elevated plasma catecholamines and lower blood oxygen concentrations.

Actions of hypoxaemia and hypercatecholaminaemia on insulin secretion in IUGR

In fetal sheep, hypoxaemia stimulates release of noradrenaline (norepinephrine) and adrenaline (epinephrine) from adrenal chromaffin cells, which elevates catecholamines in the circulation (Cohen *et al.* 1991; Adams & McMillen, 2000). In human cases of placental insufficiency, there is a sustained elevation of circulating catecholamines in response to chronic fetal hypoxaemia. This tight, negative association between blood oxygen content and plasma noradrenaline concentrations in PI-IUGR sheep fetuses is observed at 0.7 and 0.9 of gestation (Limesand *et al.* 2006; Leos *et al.* 2010; Macko *et al.* 2013). In fact, a negative association between oxygen and noradrenaline is also observed in normally growing sheep fetuses and uterine carunclectomy IUGR fetuses (Cheung, 1990; Simonetta *et al.* 1997). As for acute hypoxaemia, chronic hypoxaemia increases noradrenaline in PI-IUGR fetal sheep, which is postulated to lower oxidative metabolism and spare oxygen (Limesand *et al.* 2013; Milley, 1997). Evidence for this is twofold. First, an experimental infusion of noradrenaline into the fetal circulation increases fetal blood oxygen concentrations
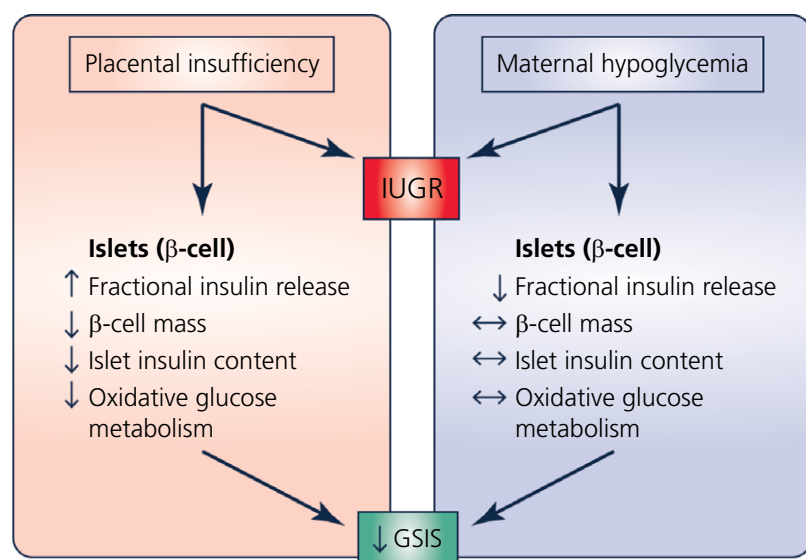

Figure 1. Comparison of insulin secretion defects in sheep models of IUGR
Two sheep models of IUGR, placental insufficiency and maternal insulin infusion-induced chronic hypoglycaemia, exhibit reduced glucose-stimulated insulin secretion (GSIS) *in vivo*. Isolated islets from these treatments distinguish alternative defects that are responsible for the β-cell dysfunction.

(Bassett & Hanson, 1998; Chen *et al.* 2014). Second, acute pharmacological inhibition of adrenergic receptors decreases blood oxygen content in both control and PI-IUGR sheep fetuses (Leos *et al.* 2010; Macko *et al.* 2013). In human fetuses noradrenaline concentrations increase from 16 to 34 weeks of gestation, and similar to the experimental evidence in sheep, hypoxaemia elevates circulating noradrenaline in human fetuses (Greenough *et al.* 1990).

In fetal sheep, as in several other species, catecholamines inhibit insulin secretion from the pancreatic β-cells (Yates *et al.* 2012). Adrenergic antagonists have shown that catecholamines mediate this effect via α_2-adrenergic receptors (Sperling *et al.* 1980; Jackson *et al.* 1993, 2000). Surgical ablation of the fetal chromaffin cells in the adrenal medulla prevents acute hypoxaemia-induced noradrenaline and adrenaline secretion and inhibition of fetal GSIS (Yates *et al.* 2012). Furthermore, this experimental paradigm comparing GSIS under normoxaemic and hypoxaemic conditions indicates that catecholamines from the adrenal gland are responsible for the suppression of insulin secretion and not the fetal hypoxaemia. This is important because in this study the degree of hypoxaemia achieved is equivalent to the concentrations seen in PI-IUGR fetuses, suggesting that hypoxaemia inhibits insulin secretion indirectly through adrenergic stimulation.
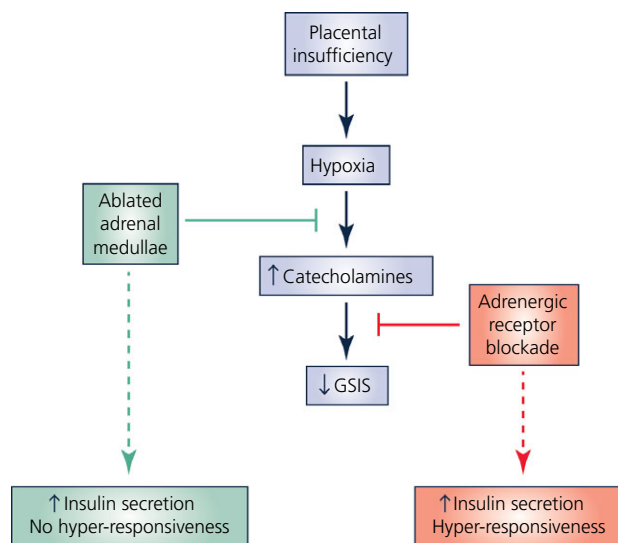
Figure 2. Adrenergic programming of insulin secretion in PI-IUGR fetuses
The proposed actions and responses for placental insufficiency leading to decreased glucose-stimulated insulin secretion (GSIS) are depicted. Experimental approaches used to disrupt the proposed actions of catecholamines are indicated along with the major conclusions for the induction of hyper-responsiveness in insulin secretion.

To test this, adrenergic receptor antagonists were administered to PI-IUGR fetuses and GSIS was measured (Fig. 2). The results show that elevated catecholamines persistently inhibit insulin secretion between 0.7 and 0.9 of gestation (Leos *et al.* 2010; Macko *et al.* 2013). Importantly, during the pharmacological adrenergic blockade GSIS in PI-IUGR fetuses is increased compared to control sheep fetuses. This is despite lower blood oxygen concentrations. The *in vivo* hyper-responsiveness of the PI-IUGR fetus to glucose stimulation occurs despite markedly lower pancreatic β-cell mass and significantly less insulin per β-cell (Limesand *et al.* 2006, 2013; Leos *et al.* 2010; Macko *et al.* 2013). The findings are consistent with the hyper-responsiveness of the PI-IUGR islet to glucose stimulation when tested *in vitro* without exposure to exogenous catecholamines. Another notable finding is that at 0.7 of gestation plasma concentrations of noradrenaline in the PI-IUGR fetuses are substantially lower compared to the near-term PI-IUGR fetuses. The concentrations are actually similar to noradrenaline concentrations found in the near-term control fetus. This shows that higher concentrations of catecholamines are effective throughout the final third of gestation, but progressively increase due to the maturation of the adrenal gland responsiveness and worsening hypoxaemia conditions in PI-IUGR sheep fetuses. Taken together, these *in vitro* and *in vivo* findings across the latter third of gestation indicate that, compared to control sheep fetuses, fetal β-cells in PI-IUGR fetuses develop a hyper-responsiveness for insulin secretion to stimulatory glucose concentration independent of low blood oxygen concentrations. However, increased GSIS *in vivo* is suppressed to levels lower than control fetuses by chronic adrenergic signalling.

Preventing high catecholamines partially restores GSIS in PI-IUGR sheep fetuses

To fully elucidate the effects of catecholamines in PI-IUGR fetuses, we surgically ablated the adrenal medullae before 0.7 of gestation to prevent the onset of chronically elevated catecholamines (Fig. 2; Macko *et al.* 2016). As expected, PI-IUGR fetuses with the adrenal medullae ablation had lower circulating noradrenaline and adrenaline concentrations compared to intact PI-IUGR fetuses near term despite being hypoxaemic and hypoglycaemic. At 0.9 of gestation GSIS is greater in PI-IUGR fetuses which have the adrenal medullae ablation compared to intact PI-IUGR fetuses. The maximum glucose-stimulated insulin concentrations are similar to control fetuses with adrenal medullae ablation, though lower than intact control fetuses. Moreover, PI-IUGR fetuses did not display hyper-responsiveness to glucose stimulation following adrenal medullae ablation at 0.7 of gestation. Comparing GSIS in PI-IUGR fetuses with chronically low circulating catecholamine concentrations

with GSIS in the PI-IUGR fetuses following acute pharmacological adrenergic blockade demonstrates a principal role for chronically elevated catecholamines in IUGR to induce hyper-responsive insulin secretion that is independent of hypoxaemia, hypoglycaemia, and other fetal conditions associated with placental insufficiency. Therefore, adaptations to chronic adrenergic stimulation such as compensatory gain in β-cell responsiveness were not present in PI-IUGR fetuses that had undergone adrenal medullae ablation, indicating that chronic adrenergic stimulation produces this effect.

In addition to the profound effects of chronically high circulating catecholamines on GSIS in PI-IUGR fetuses, elevated plasma catecholamines also promote growth restriction and asymmetric growth in PI-IUGR fetuses, at least in tissues other than the endocrine pancreas (Davis *et al.* 2015). Approximately 50% of the growth restriction is associated with chronically elevated plasma catecholamines in PI-IUGR fetuses because ablation of the adrenal medullae increases fetal weights by this amount despite hypoxaemia and hypoglycaemia. Sparing of brain and heart weights relative to fetal weight is a hallmark of asymmetric growth in IUGR and is present in intact PI-IUGR fetuses. The asymmetric growth characteristics of these tissues are lost in PI-IUGR fetuses with adrenal medullae ablation at 0.7 of gestation. However, the increase in fetal and pancreas weight does not translate to greater numbers of pancreatic endocrine cells because β-cell mass is similar between intact and adrenal medulla-ablated PI-IUGR fetuses (Davis *et al.* 2015). This finding is the result of less immunopositive area for β-cells in PI-IUGR fetuses with adrenal medullae ablation. Furthermore, rates of β-cell replication are not different between PI-IUGR surgical treatments, which are both less than intact controls. These data indicate that high circulating catecholamines suppress somatic growth but do not constrain proliferation or neogenesis of pancreatic endocrine cells. Factors other than catecholamines are likely to be the cause of reduction in β-cell mass. These other factors include growth factors, islet vascularity, amino acids, hypoglycaemia and hypoxaemia to name a few that have been investigated in PI-IUGR fetuses (Chen *et al.* 2012; Lavezzi *et al.* 2013; Rozance *et al.* 2015; Brown *et al.* 2016; Hay *et al.* 2016; Macko *et al.* 2016; Benjamin *et al.* 2017).

Enhanced GSIS persists following sustained high noradrenaline

Integration of *in vivo* and *in vitro* data from studies on PI-IUGR and HG-IUGR fetuses leads to the hypothesis that chronic exposure to elevated catecholamines enhances β-cell responsiveness to secretagogues after cessation of the adrenergic signalling. To study the specific effects of chronically elevated noradrenaline on β-cell

responsiveness, we continuously infused noradrenaline intravenously into normally grown sheep fetuses for 7 days near the end of gestation (Chen *et al.* 2014). During the infusion, noradrenaline concentrations are increased and insulin concentrations are decreased to levels similar to those in PI-IUGR fetuses, but, unlike PI-IUGR fetuses, glucose and oxygen concentrations are increased in noradrenaline-infused fetuses. To simulate the acute adrenergic blockade performed in the PI-IUGR fetuses, the noradrenaline infusion was terminated 3 h before measuring insulin secretion responsiveness. In the noradrenaline-infused fetuses, GSIS and glucose-potentiated arginine-induced insulin secretion (GPAIS) are approximately fourfold greater than age-matched control fetuses or pretreatment assessment of GSIS and GPAIS in the noradrenaline-infused fetuses. The hyper-responsiveness in insulin secretion is present despite the fact that plasma noradrenaline concentrations remain modestly elevated compared to controls. Lower mRNA expression of α_{2A}-adrenergic receptor (α_{2A}-AR), α_{2C}-adrenergic receptor (α_{2C}-AR) and G protein subunit alpha i2 ($G_{\alpha i\text{-}2}$), and adrenergic desensitization in islets from noradrenaline-infused fetuses explains increased insulin secretion after termination of the infusion despite persistently elevated noradrenaline concentrations (Chen *et al.* 2014, 2017). Although no differences are seen in insulin, pancreatic and duodenal homeobox 1, and glucose transporter 2 mRNA concentrations, expression of uncoupling protein 2 is lower in islets from noradrenaline-infused fetuses, which indicates improved insulin stimulus–secretion coupling (Zhang *et al.* 2001). It is also worth mentioning that uncoupling protein 2 expression is lower in PI-IUGR islets as well, indicating a potential shared programming mechanism for both models with hypercatecholaminaemia (Kelly *et al.* 2017). The findings from the noradrenaline-infused fetuses show that following acute removal from the chronically elevated adrenergic stimulation, fetal insulin secretion becomes hyper-responsive to secretagogues. This is associated with adrenergic desensitization and greater potential of stimulus–secretion coupling in pancreatic islets.
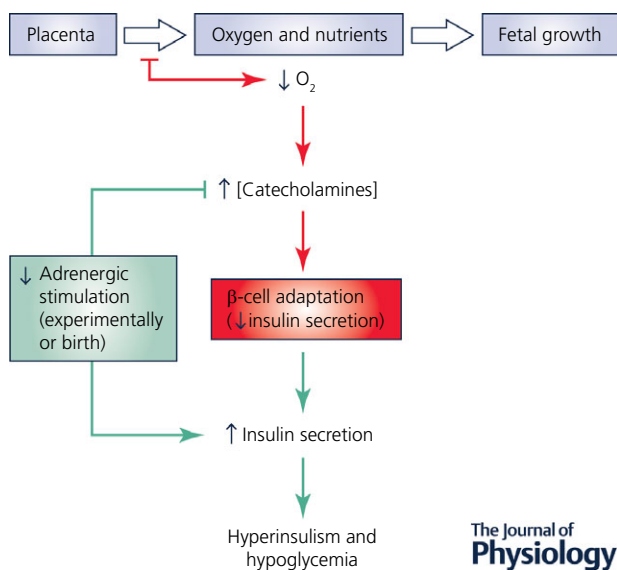
In a follow-up study, we tested the hypothesis that the compensatory increase in insulin secretion following chronic elevation of noradrenaline is independent of hyperglycaemia and persists in conjunction with adrenergic desensitization of islets (Chen *et al.* 2017). The noradrenaline infusion was performed as explained above, but in this cohort fetal euglycaemia was maintained with a maternal insulin infusion. The noradrenaline infusion was terminated at 7 days, 1 and 5 days prior to measuring GSIS and GPAIS respectively. In noradrenaline fetuses GSIS is approximately twofold greater than controls at both time points, indicating that the significant enhancement in insulin secretion persisted. The GPAIS

is also significantly enhanced in noradrenaline-infused fetuses. Adrenergic sensitivity was measured in pancreatic islets collected from these fetuses, and the half-maximal inhibitory concentration for noradrenaline is greater in noradrenaline-infused islets. The *in vitro* analysis also shows that the maximum GSIS in islets isolated from noradrenaline-infused fetuses is significantly greater than controls, but islet insulin content and intracellular calcium signalling are not different between treatments. These findings show that chronic noradrenaline exposure and not hyperglycaemia produce persistent adaptations in pancreatic islets that augment β-cell responsiveness in part through decreased adrenergic sensitivity.

Clinical implications to fetal catecholamine exposure

The actions for elevated catecholamines discussed above have direct relevance for the clinical problem of neonatal hypoglycaemia in formerly IUGR newborns. Some human IUGR newborns have evidence of increased insulin sensitivity for glucose (Bazaes *et al.* 2003), a finding supported by hyperinsulinaemic-euglycaemic clamps in PI-IUGR fetal sheep (Thorn *et al.* 2012). When insulin secretion and insulin concentrations

Figure 3. Translation of adrenergic programming in IUGR infants
Normal placental transfer of oxygen and nutrients to the growing fetus is presented in black. Placental insufficiency, represented in red, reduces the delivery of oxygen and nutrients to the fetuses resulting in IUGR. Fetal hypoxaemia raises plasma catecholamine concentrations and subsequently lowers insulin secretion. The chronic elevation in plasma catecholamines causes β-cell adaptations. Loss of the sustained adrenergic signalling following parturition exposes β-cell hyper-responsiveness, which is represented in green. It is hypothesized that hyperinsulinism and hypoglycaemia develop following delivery and stabilization of the neonate that experienced perinatal stress.

are elevated inappropriately for the degree of insulin sensitivity, glucose supply to the newborn from milk feeding and/or intravenous dextrose infusions may not be enough to prevent a transient form of hypo-glycaemia associated with hyperinsulinism (Miralles *et al.* 2002; Hoe *et al.* 2006; Arya *et al.* 2013). The developmental adaptations that result from chronically elevated catecholamines in pregnant sheep models explain how this disturbance might occur. Once sustained adrenergic signalling is alleviated, either experimentally in fetal sheep or by delivery and stabilization in humans, β-cell hyper-responsiveness will be unmasked and lead to inappropriately increased insulin secretion and concentrations (Fig. 3). While the PI-IUGR fetal sheep is exposed to elevated catecholamine concentrations for much of the third trimester (Leos *et al.* 2010; Macko *et al.* 2013), like some human IUGR fetuses (Greenough *et al.* 1990), we have demonstrated that exposure to elevated noradrenaline for as short a time as 7 days is all that is required to establish β-cell hyper-responsiveness (Chen *et al.* 2014). We also have shown that the β-cell hyper-responsiveness persists at least 5 days in this experimental paradigm (Chen *et al.* 2017). It is important to point out that we have not defined the maximum duration that the persistent β-cell hyper-responsiveness will last following either the 7-day noradrenaline infusion or experimental hyperthermia-induced placental insufficiency. Thus, a longer duration of exposure *in utero* may lead to the prolonged courses of transient hyperinsulinism-associated hypoglycaemia demonstrated in some formerly IUGR newborns (Hoe *et al.* 2006; Arya *et al.* 2013). Similarly, we have not defined the minimum period of exposure to elevated noradrenaline concentrations required to establish β-cell hyper-responsiveness. In this respect, it is worth noting that the pathophysiological process we have discussed for placental insufficiency and IUGR could potentially apply to any pregnancy complicated by low fetal oxygen concentrations depending on the degree and duration of this exposure, including chronic perinatal asphyxia, placental abruption, fetal anaemia, and even some pregnancies complicated by maternal diabetes (Madsen, 1986; Pschera *et al.* 1986; Hoe *et al.* 2006; Teramo, 2010; Stanley *et al.* 2015). Collectively, the hyperinsulinism-associated hypoglycaemia present in these conditions and following IUGR has been termed perinatal stress hyperinsulinism and may share a common pathophysiological mechanism as described in the current review (Miralles *et al.* 2002; Hoe *et al.* 2006; Arya *et al.* 2013; Stanley *et al.* 2015; Thornton *et al.* 2015).

Conclusion

Synthesis of the findings from several well-established models of IUGR in fetal sheep reveals that the consequence

of nutrient restriction is β-cell dysfunction, but the type of nutrient restriction causing IUGR also has different effects on the β-cell phenotype. Placental insufficiency reduces nutrient transport globally causing a decrease in β-cell mass, islet metabolism, and insulin production. In contrast, experimentally produced hypoglycaemia impaired distal components in the exocytosis of insulin. A major difference between these models is fetal hypoxaemia and hypercatecholaminaemia, and the latter inhibits insulin secretion and fetal growth to a greater extent than low glucose and insulin concentrations. In addition, chronically elevated noradrenaline makes β-cells hyper-responsive to secretagogues once the adrenergic stimulation subsides by the experimental procedures outlined, or as we propose delivery and stabilization of the IUGR fetus. Although the adaptations are expected to be transient, we have shown the hyper-secretion persists for several days in the fetus following cessation of chronically elevated noradrenaline concentrations. The expectation is that IUGR infants will develop transient hyperinsulinism and hypoglycaemia, which may be asymptomatic but have the potential in severe cases to persist for months and may eventually transition to a state of increased insulin resistance, hyperglycaemia, and eventually Type 2 diabetes.

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Additional information

Competing interests

None declared.