



Review

# Programming of the endocrine pancreas by the early nutritional environment

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Available online 10 November 2005

## Abstract

A substantial body of evidence now suggests that poor intrauterine milieu elicited by maternal nutritional disturbance or placental insufficiency may programme susceptibility in the foetus to later develop chronic degenerative diseases, such as obesity, hypertension, cardiovascular diseases and diabetes. Further data showing the developmental programming of the metabolic syndrome are now available thanks to animal studies in which the foetal environment has been manipulated. This review examines the developmental programming of glucose intolerance by disturbed intrauterine metabolic condition in rats. It focuses on the alteration of the endocrine pancreas at birth. Long-term consequences, deterioration of glucose tolerance and even transgenerational effects are reported. Maternal protein, caloric restriction and diabetes during gestation/lactation lead to altered  $\beta$ -cell mass. This review also tempts to identify cellular and molecular mechanisms involved in this process.

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**Keywords:** Maternal diet; Development; Pancreas; Diabetes; Animals

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## 1. Introduction

The growth and the development of the foetus are dictated by its genetic potential which can strongly be modulated by intrauterine environmental factors that may exert inhibitory or stimulatory effects. The supply of nutrients to the foetus depends on the health and the metabolic status of the mother. The metabolic and nutritional environment of the foetus is modified when the mother is malnourished due to an imbalance or a lack of certain nutrients or when the mother suffers from diabetes or from placental alteration like pre-eclampsia. Metabolic intrauterine environment may also be modified in case of maternal high fat diet, obesity or smoking. As shown in a review of 32 international studies, a large number of epidemiological studies in human suggest that a poor intra-uterine environment elicited by maternal dietary insufficiency or imbalance may programme susceptibility in the foetus to later development of type-2 diabetes, obesity, hypertension and cardiovascular diseases (Hales & Barker, 2001). Not only under-nutrition during foetal life may have lasting consequences but abundant supply of glucose to the foetus as it occurs in gestational diabetes may have a dramatic impact for the progeny as well. Indeed, intrauterine exposure to diabetes conveys risk factors for insulin resistance, type-2 diabetes and obesity (Plagemann, Harder, Franke, & Kohlhoff, 2002; Silverman, Rizzo, Cho, & Metzger, 1998). In the Pima Indian population, where diabetes has the highest prevalence, the risk of diabetes was higher in siblings born from diabetic mothers than in those born before the mother became diabetic (Dabelea, Knowler, & Pettitt, 2000).

The mechanism by which adverse intrauterine environment increases the susceptibility to develop glucose intolerance and type-2 diabetes is not well understood. Besides the insulin resistance that has been postulated to develop as a consequence of the lack of nutrients availability during early life, a primary insult in to the  $\beta$ -cell mass has been observed. Foetal under- or over-nutrition would have led to inappropriate development of endocrine pancreas resulting in a population of insulin secreting cells that will not be able to face metabolic and oxidative stress in later life. The question is how the

memory of these events is stored and later expressed. Aiming to answer that question, several animal models have been established and tend to propose some cellular and molecular mechanisms.

## 2. Normal development of the endocrine pancreas in the rodent

Development of the pancreas starts from a pool of common progenitor cells (multipotent endodermal progenitors) which will commit into duct cell, endocrine or acinar cell lineage. Thereafter, within the endocrine compartment, the cells will have to further differentiate into  $\alpha$ ,  $\beta$ ,  $\delta$  or PP cells producing, respectively, glucagon, insulin, somatostatin or the pancreatic polypeptide. This is regulated by the expression of distinct genes, under the control of an hierarchy of various and specific networks of transcription factors.

The development of the pancreas in rodents shows similarities to that in humans. However, while foetal  $\beta$  cells are functioning as true endocrine cells at the end of the first trimester in human (Piper et al., 2004), this occurs only during the last third of gestation in rats. In response to signals coming from the mesodermal tissues, pancreatic morphogenesis begins at 9.5 days with two evaginations of foregut endoderm to form a dorsal, and then a ventral pancreatic bud. The dorsal and ventral buds fuse at E16.5. A branched structure is already distinguishable at E14.5 where endocrine cells can be identified by E15.5. The endocrine tissue is derived from epithelial duct cells (neogenesis) (Fig. 1). After divisions, the cells will form small clusters budding out the pancreatic ducts. The vascularisation begins to invade these immature endocrine cell clusters that co-express several pancreatic hormones and neuropeptides and will become “islets of Langerhans” (Reusens, Hoet, & Remacle, 2000).

The classic way of specifying a particular cell fate of the pancreatic cell within the field of initially equivalent cells is the lateral specification mediated by the Notch-Delta serrate pathway (Fig. 2). In the pancreas, blocking the activation of the Notch receptor results in high Neurogenin-3 gene (*ngn3*) expression, and promotes the endocrine fate. In contrast, cells with active

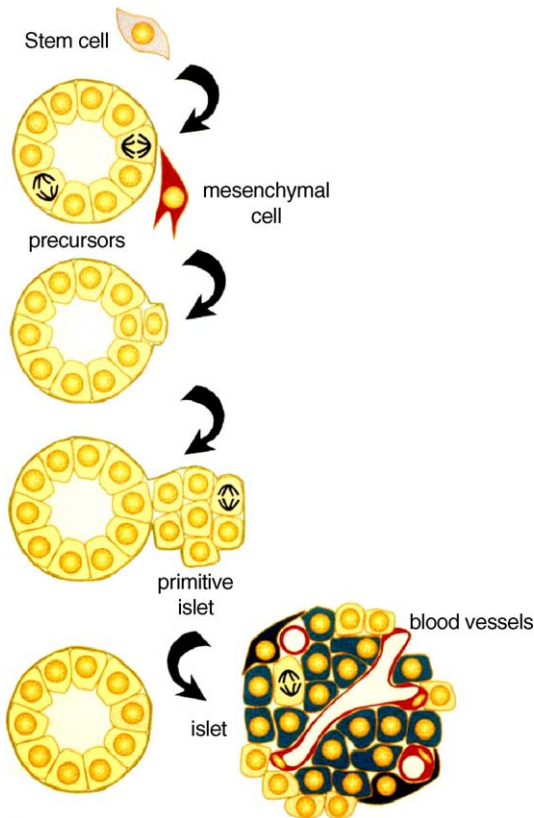


Fig. 1. Schematic view of the differentiation of the islets cells and islet formation.

Notch signalling adopt the acinar fate and/or remain as undifferentiated progenitor cells (Edlund, 2001). Pdx1 plays a central role in the  $\beta$ -cell differentiation. It initiates endocrine lineage commitment for cell within the pancreatic ducts. It becomes restricted to differentiating  $\beta$ ,  $\delta$  and PP cell, is switched-off in presumptive  $\beta$  cell and becomes restricted to mature  $\beta$  cell. The final fate of the individual endocrine cells is determined by the expression of a series of transcription factors specific for each type of endocrine cell. Some of them are early markers, such as Pax4, Nk2.2 and Nkx6.1, co-expressed with neurogenin-3, others are late markers, such as Pax6, Isl1, Hb9 and Pdx-1 for the  $\beta$  cells (Fig. 2).

Peptide growth factors, such as PDGF, VEGF, FGF-7 that are expressed within the pancreatic stroma adjacent to the ductal epithelium contribute to endocrine cell formation and islet expansion. The ongoing proliferation and developmental differentiation of  $\beta$  cell, once formed is highly dependent on the expression of the insulin like growth factors (IGFs) within the islets.

In rat and mouse, it is at the end of the foetal period that the  $\beta$ -cell mass increases the most (Kaung, 1994).

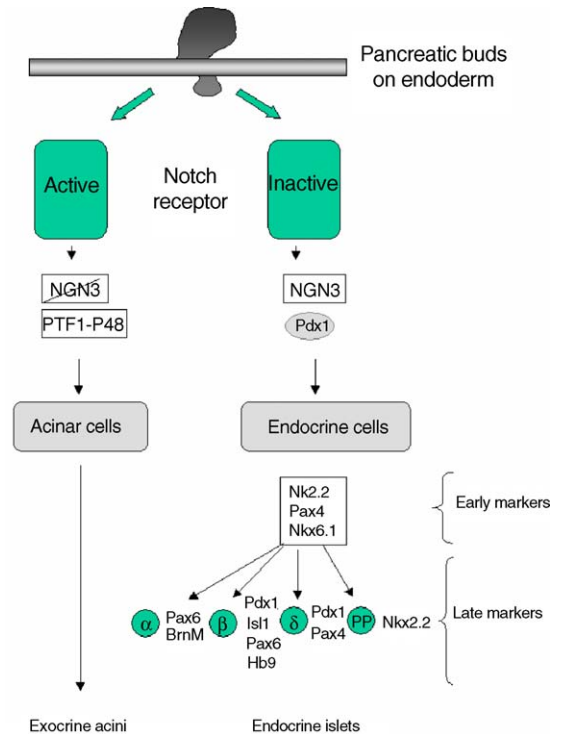


Fig. 2. Schematic mechanism of exocrine and endocrine specification.

Following birth, this growth declines although the proliferation rate maintains. Therefore, a wave of apoptosis is suspected which should occur between 2 and 3 weeks postnatally (Scaglia, Cahill, Finegood, & Bonner-Weir, 1997). The loss of  $\beta$  cells during that time is compensated by neogenesis. Although it was thought that in adult, new  $\beta$  cells derive mainly from stem cells, it seems now that they arise abundantly from pre-existing  $\beta$  cells that multiply (Dor, Brown, Martinez, & Melton, 2004). They may also be generated from trans-differentiation of acinar cell (Lardon & Bouwens, 2005). Thus, the  $\beta$ -cell mass at the end of development will determine the islet cell mass in adulthood. Anyway, the production of new  $\beta$  cells in adulthood is low. It is thus obvious that any deficiency occurring in utero or soon after birth will jeopardize the  $\beta$ -cell mass contributing to  $\beta$ -cell failure or excess favouring glucose intolerance later in life.

### 3. Programming of the endocrine pancreas by adverse intrauterine environment

Alterations of the  $\beta$ -cell mass by maternal malnutrition were already demonstrated in humans about 40 years ago (Winick & Noble, 1966), but the mechanism

involved in these alterations and their long-term consequences remained unknown. Although foetal or neonatal malnutrition in animal may not always adequately represent the human situation, they have helped to identify key events to better understand the foetal origin of  $\beta$ -cell malfunction in adult.

Disturbances in the development of the endocrine pancreas have been observed when the availability of nutrients was increased or decreased. In rats, intrauterine metabolic perturbations induced by several means, like manipulation of the maternal diet, such as protein or calorie restriction, or alteration in the availability of the nutrients by placental insufficiency, or maternal diabetes alter the islet development at the perinatal period and provoke lasting consequences.

### 3.1. Maternal low protein diet

#### 3.1.1. Early alteration

We have developed a rat model of protein-restriction in which pregnant rats were fed on a low protein but normocaloric diet containing 8% of protein instead of 20% during gestation or gestation and lactation (low protein, LP). Such a diet modified the process of islet cell expansion (Boujendar et al., 2002; Petrik et al., 1999; Snoeck, Remacle, Reusens, & Hoet, 1990) leading at birth to a smaller endocrine and  $\beta$ -cell mass. When the protein restriction was maintained until weaning, the deficiency was even more pronounced. The replication rate of islet cells was reduced by almost 50% in the protein-restricted offspring, preferentially in the  $\beta$  cells. Further analysis revealed that the cell cycle was lengthened in these LP islets (Petrik et al., 1999). This may reflect a cell cycle block or lengthening in  $\beta$  cells that already differentiated, in which cells are not able to progress to DNA synthesis. It may also result from a permanent re-programming of cell kinetics which may be imprinted on a precursor cell population before the differentiation of the pancreas has begun. Indeed, the pancreatic population of the endocrine precursor cells that were immuno-positive for markers like nestin, CD34 and c-Kit was decreased at the perinatal period in the LP offspring (Joanette et al., 2004). An increased rate of apoptosis was reported in the LP foetal and neonatal islets contributing to the reduced endocrine cell mass (Boujendar et al., 2002; Petrik et al., 1999).

The reduced islet cell proliferation, the increased apoptotic rate and the lower insulin release in response to secretagogues were still apparent even when the LP foetal islets were cultured for 7 days and withdrawn from the disturbed metabolic environment, testifying the programming of these cells (Cherif, Reusens, Dahri, &

Remacle, 2001; Dahri, Snoeck, Reusens, Remacle, & Hoet, 1991). To reveal specific pathways involved in the altered  $\beta$ -cell mass, a proteome analysis was performed on these islets and the expression of 70 proteins was detected as being changed by the maternal diet. The analysis confirmed modification in expression of proteins involved in the cell cycle and growth control as well as in exocytosis. In addition, proteins responsible for the folding and chaperoning of the protein and proteins involved in cellular defence were also differently expressed in LP foetal islets (Sparre et al., 2003). Such modifications may participate to the increased vulnerability reported in the LP foetal  $\beta$  cells in presence of nitric oxide (NO) donor or interleukin-1 (IL-1 $\beta$ ) (Merezak, Hardikar, Yajnick, Remacle, & Reusens, 2001).

The endocrine pancreas is a richly vascularised tissue. Blood vessels that provide metabolic sustenance are also involved early in endocrine pancreas development (Lammert, Cleaver, & Melton, 2001). Islet blood vessel development is very sensitive to the lack of protein availability in utero, since both the volume occupied by blood vessels and the blood vessel number were lower in protein-restricted foetal islets (Boujendar, Arany, Hill, Remacle, & Reusens, 2003; Snoeck et al., 1990). This was associated with an altered expression of the vascular endothelial growth factor (VEGF) and its receptor Flk-1 in the foetal LP islets (Boujendar et al., 2003). VEGF is a growth factor for endothelial cell and is a major factor of endothelium–endocrine cell interactions. It remains however unknown if the reduced  $\beta$ -cell mass is the cause or consequence of the perturbed vascularisation.

#### 3.1.2. Long-term consequences

Poor development of the endocrine pancreas as a response to intrauterine malnutrition may be worthwhile for survival in early life. It may however be a risk factor for glucose intolerance and diabetes later on, as suggested already by many epidemiological studies.

The long-term consequences of the programming of islet cells and insulin sensitive-tissues, as well as some possible mechanism involved in this programming have been extensively reported in animals models (see reviews from Aerts & Van Assche, 2005; Holemans, Aerts, & Van Assche, 2003; Ozanne & Hales, 2002; Reusens & Remacle, 2001a) and will not be detailed again in this review. Results may be different depending on the time window that the diet was applied, the postnatal nutrition and hormonal environment, the age at which the investigation was done and the gender of the animals.

Briefly, if the low protein diet was limited to pregnancy, 3-month-old females but not males had a lower

plasma insulin level after a glucose challenge (Dahri et al., 1991). When the low protein diet was maintained during lactation and replaced by a normal diet only after weaning, females as well as males exhibited a lower growth rate. Plasma insulin was significantly reduced in females at weaning and adulthood (Reusens & Remacle, 2001a) but insulin response to an oral glucose challenge was highly depressed in both genders. Both presented however a better glucose tolerance (Reusens & Remacle, 2001b). Normal glucose tolerance in presence of low insulin secretion in these juvenile animals has been explained by an adaptation of the peripheral tissue through an increased number of insulin receptors in the liver, adipose tissue and muscle (Holness, Fryer, & Sugden, 1999; Ozanne, Smith, Tikerpa, & Hales, 1996; Ozanne, Wang, Coleman, & Smith, 1996; Shepherd, Crowther, Desai, Hales, & Ozanne, 1997) but also through other peripheral adaptations.

In the LP foetal islet cells, since the apoptotic rate was increased and the defence mechanism appeared to be altered, the susceptibility of these cells to cytokines (molecules involved in the selective destruction of the  $\beta$  cells in type-1 diabetes) was investigated.  $\beta$  cells from foetus of protein-deprived mother exhibited a higher apoptotic rate than the control foetal  $\beta$  cells when incubated in the presence of IL-1 $\beta$  (Merezak et al., 2001). Moreover, the increased susceptibility was maintained throughout life, despite adequate feeding after weaning, since adult islets were also more vulnerable in presence of cytokines (Merezak, Reusens, Ahn, & Remacle, 2004). The lack of protein during development has thus generated a  $\beta$ -cell population that is more sensitive to cytokines.

However, the exhaustion of the low  $\beta$ -cell mass generated early in life may only be clearly revealed in situations of increased insulin demand, such as obesity, pregnancy and aging. In LP offspring, the adaptation of the peripheral tissues to the lower insulin secretion observed at 3 months declined with age and insulin resistance appeared at 15 months (Hales, Desai, Ozanne, & Crowther, 1996). At 17 months, the males suffered from diabetes (Petry, Dorling, Pawlak, Ozanne, & Hales, 2001; Petry, Ozanne, Wang, & Hales, 2001). Age-dependent changes are apparent in adipocytes and skeletal muscle of the LP progeny as they become resistant both to the action of insulin to stimulate glucose uptake and to inhibit lipolysis. This appeared to result from a molecular defect that lies downstream of the insulin receptor (Ozanne & Hales, 2002). It is consistent with the animals becoming glucose intolerant with age.

### 3.2. Maternal low calorie diet

#### 3.2.1. Early alteration

Not only protein deprivation is harmful for the development of the endocrine pancreas. Reducing the food intake by 50% during the last week of gestation in rats (calorie restriction, LC) reduced the birth weight of the progeny. The foetus featured a lower  $\beta$ -cell mass 1 day before birth and a decreased pancreatic insulin content (Blondeau, Lesage, Czernichow, Dupouy, & Bréant, 2001; Garofano, Czernichow, & Bréant, 1997). In contrast to the low protein effect, the reduced  $\beta$ -cell mass was not attributed to lower proliferation or increased apoptosis rates but rather to alteration in the islet neogenesis (Garofano et al., 1997). Therefore, the cellular and molecular mechanisms involved in the reduced  $\beta$ -cell mass after low protein or low calorie diet may be different. In these experiments, however, the duration of exposure was shorter in the calorie restriction than in protein restriction. Interestingly, when calorie restriction occurred during the last week of gestation, the foetal islet vascularisation was not affected (unpublished data).

#### 3.2.2. Long-term consequences

Long-term consequences were also fully apparent in offspring of mothers on calorie restriction during the last week of gestation and lactation (Garofano, Czernichow, & Bréant, 1998). At 3 months of age, male offspring had fewer  $\beta$  cell and secreted less insulin in response to an oral glucose challenge. However, as in the low protein offspring, no glucose intolerance was observed at that age. As in the LP offspring, deterioration of the glucose tolerance appeared in older age (Blondeau, Garofano, Czernichow, & Bréant, 1999).

### 3.3. Uterine arteries ligation

#### 3.3.1. Early alteration

Uteroplacental insufficiency, the most common cause of intrauterine growth retardation limits the supply of critical substrates, such as oxygen, glucose and amino acids to the foetus and results in poor growth of the foetus. No measurement of the  $\beta$ -cell mass was performed at birth but at 14 days postnatally, intrauterine growth retarded pups exhibited a reduced cell proliferation but a normal apoptotic rate. The level of Pdx1 mRNA, a critical regulator of pancreatic and  $\beta$ -cell development was dramatically decreased in the foetal  $\beta$  cell and the reduced expression persisted after birth until 3 months (Boloker, Shira, & Simmons, 2002; Stoffers, Desai, DeLeon, & Simmons, 2003).

### 3.3.2. Long-term consequences

Early programming of the endocrine pancreas after uterine arteries ligation was manifest at adulthood since  $\beta$ -cell mass remained lower. It led to altered glucose metabolism later in life. The intrauterine growth retarded offspring developed marked fasting hyperglycemia and hyperinsulinemia at 10 weeks of age which deteriorated into glucose intolerance and insulin resistance at 15 weeks. After that age, severe lack of insulin secretion appeared at 26 weeks of age (Boloker et al., 2002).

## 3.4. Maternal diabetes

### 3.4.1. Early alteration

Maternal diabetes affects foetal tissues and more specifically the endocrine pancreas development in human (Holemans et al., 2003) as well as in experimental animals (Aerts, Holemans, & Van Assche, 1990; Holemans et al., 2003). Macrosomic human neonates have an increased percentage of pancreatic endocrine tissue with  $\beta$ -cell hyperplasia and high vascularisation (Van Assche, Gepts, & Aerts, 1976) while intrauterine growth retarded infants have a reduced volume density of the endocrine tissue (Holemans et al., 2003). In rat, diabetes may be induced experimentally by streptozotocin injection, that selectively destroys  $\beta$  cells and mild or severe diabetes ensue depending on the dose used. At birth, the progeny of mild diabetic mothers was macrosomic and the development of the endocrine pancreas was enhanced by increased blood glucose concentration which resulted in an enhanced percentage of endocrine tissue due to hyperplasia and hypertrophy of the islets of Langerhans (Aerts et al., 1990). Islet cell proliferation was increased by 42% (Reusens-Billen, Remacle, Daniline, & Hoet, 1984), leading to higher  $\beta$ -cell mass that was hyper-vascularized (Reusens & Remacle, 2001b). The pancreatic insulin content and insulin secretion were raised in these foetuses (Kervran, Guillaume, & Jost, 1978). On the other hand, foetuses from severe diabetic dams were small at birth. Foetal pancreatic weight was decreased but the percentage of endocrine tissue was higher (Aerts, Vercruysse, & Van Assche, 1997). Due to an over-stimulation by the excessive glucose concentration, the  $\beta$  cells were almost degranulated, leading to lower pancreatic content and plasma secretion at the perinatal period (Kervran et al., 1978).

### 3.4.2. Long-term consequences

The long-term consequences have been extensively evaluated in this progeny (see Review from Aerts & Van Assche, 2005). Impaired glucose tolerance was observed in the offspring of mild diabetic rats due to lower insulin

secretion, while insulin resistance was reported in the offspring of the severe diabetic mother.

## 4. Evidence of a transgeneration effect

During pregnancy, the endocrine pancreas of the mother has to adapt in response to the higher demand of insulin required for the foetal growth. Indeed, the threshold at which glucose stimulates insulin secretion decreased, insulin secretion increased and  $\beta$ -cell mass doubled (Aerts et al., 1997) due to a strong rise in  $\beta$ -cell proliferation (Sorenson & Breltje, 1997). Such adaptations to gestation were perturbed in case of disturbed metabolic milieu during foetal life. Whatever the pregnant mother was the offspring of a protein or caloric restricted dam, a diabetic or ligated mother, it became glucose intolerant and even hyperglycaemic. The second generation featured a diabetogenic tendency. A transgenerational development of glucose intolerance has thus been demonstrated (Aerts et al., 1990; Blondeau et al., 1999; Boloker et al., 2002; Dahri, Reusens, Remacle, & Hoet, 1995; Reusens & Remacle, 2001b)

## 5. Perturbed nutritional and hormonal environment are responsible for the altered $\beta$ -cell mass

What are the factors that may be responsible for the dramatic picture of the endocrine pancreas observed at birth in these different offspring? The  $\beta$  cells are particularly vulnerable to changes in substrate and hormone availability.

IGFs potentiate  $\beta$ -cell growth, maturation and function and are expressed by  $\beta$  cell in early life. Foetal malnutrition induced by maternal low protein diet reduced circulating levels and tissue expression of IGFs (El-Khattabi, Grégoire, Remacle, & Reusens, 2003). IGF-II which is predominant during foetal life in rodents can functionally act as growth factor but also as a survival factor preventing apoptosis in  $\beta$  cells (Petrik, Arany, McDonald, & Hill, 1998). A reduced expression of IGF-II mRNA in the pancreas and a lower number of cells positive for IGF-II in the islets of the LP pups were found suggesting that the higher apoptosis and the lower  $\beta$ -cell proliferation reported in islets of LP rats may be functionally linked to this reduction of islet IGF-II expression (Petrik et al., 1999).

The development of the  $\beta$  cell is also dependent on glucose and the latter is one of the key elements for the observations made in the mild diabetic offspring in rats. Amino acids appear however to be also potent in stimulating  $\beta$ -cell proliferation and differentiation (de

Gasparo, Milner, Norris, & Milner, 1978). Amino acids potentiate the release of immuno-reactive IGF-II from isolated foetal rat islets (Hogg, Han, Clemmons, & Hill, 1993), and correlatively less IGF-II was observed in the pancreas of the foetus that grew in an intrauterine milieu poor in amino acids. In protein-restricted animals, plasma glucose levels were normal, but the amino acid profile was perturbed in the foeto-maternal unit (Reusens et al., 1995), several amino acids being significantly decreased. Taurine, that does not participate in protein synthesis, but that is important during development for several tissues (Sturman, 1993), was the most reduced. During mild and severe diabetic pregnancy, total amino acids concentration was lower in the foetal plasma (Aerts, Van Bree, Feytons, Rombauts, & Van Assche, 1989). Like during protein-restricted gestation, the amino acid profile was altered in mild diabetic mothers as well as in their foetuses: branched-chain amino acids and taurine were reduced compared to normoglycemic mother (Aerts & Van Assche, 2001).

Restoration of a normal level of taurine by its supplementation in the drinking water of the protein-restricted pregnant rats prevented the reduction of the  $\beta$ -cell mass, enhancing  $\beta$ -cell proliferation and decreasing islet cell apoptosis. This was achieved, in part, by the normalisation of the number of islet cells expressing IGF-II (Boujendar et al., 2002). The islet blood vessels and the VEGF expression were also sensitive to the depletion of taurine that occurs in low protein foetuses, since supplementation of this amino acid to the protein-restricted diet of the dams was sufficient to recover a well-vascularised endocrine pancreas in the progeny (Boujendar et al., 2003). It is however unclear, if a primary effect of taurine is on the endothelial or the endocrine cells of the pancreas. The preventive effect of taurine was also apparent when the foetal islets were challenged in vitro with cytokines (Merezak et al., 2001). The preventive effect was maintained throughout life, as the increased susceptibility of the low protein islets to cytokines and the lower insulin secretion observed at adulthood were prevented at 3 months (Merezak et al., 2004). An adequate level of taurine during foetal and early life seems thus a critical parameter in order to achieve a normal development and function of the endocrine pancreas. Should that not be present,  $\beta$ -cell vulnerability will ensue and will remain throughout life.

In a study comparing the effect of maternal isocaloric low protein, low calorie and low protein/low calorie diets, Bertin et al. (2002) demonstrated that, although the three approaches inducing foetal malnutrition led to a reduction in the foetal  $\beta$ -cell mass, plasma taurine was only depleted in the case of protein deficiency. This

means that taurine cannot be involved in the reduced  $\beta$ -cell mass observed in calorie-restricted foetuses. Other factors have been suggested. During development, a negative role of glucocorticoids on the development of foetal  $\beta$  cell has been demonstrated (Blondeau et al., 2001). Maternal calorie restriction increased maternal and foetal plasma corticosterone levels (Lesage, Blondeau, Grino, Bréant, & Dupouy, 2001). The normalisation of glucocorticoid levels in IUGR calorie-restricted foetus restored a normal  $\beta$ -cell mass that was associated with the correction of the decreased  $\beta$ -cell neogenesis (Blondeau et al., 2001). Glucocorticoids have indeed been demonstrated to play an important role in pancreatic  $\beta$ -cell lineage and the reduced  $\beta$ -cell mass. Gesina et al. (2004) showed that rat pancreatic buds treated with dexametasone, a glucocorticoid agonist, exhibited less cells expressing insulin and Pdx1 and featured a doubling of acinar component area, suggesting that glucocorticoids favour the development of the exocrine pancreas at the detriment of the endocrine tissue.

Thus, in the two models of maternal malnutrition, although the  $\beta$ -cell mass is diminished in both experimental conditions, the cellular and molecular mechanisms may be completely different (Fig. 3). The  $\beta$ -cell mass is deficient in the LC pancreas because the neogenesis is reduced rather than the vascularisation and the proliferation. The high glucocorticoid level may participate to the poor differentiation of these  $\beta$  cells. By contrast, the reduced proliferation rate and vascularisation as well as the increased apoptotic rate are responsible for the reduced  $\beta$ -cell mass and the low level taurine may be a key factor. Addition of taurine to maternal low protein diet, or the normalisation of the maternal plasma glucocorticoid level in the calorie-restricted mother prevent the alterations in the endocrine pancreas and show that early intervention may be beneficial.

## 6. Programming of the mitochondrial function

Disturbance in the metabolic intrauterine environment alters the development of the endocrine pancreas and the insulin sensitive tissues. It modulates the gene expression in both susceptible pluripotential cells and fully differentiated  $\beta$  cell that collectively alter their long-term programming and will favour a “prediabetic” phenotype.

The molecular mechanism responsible for permanent changes in genes expression is not clear. It is however critical to understand how early development can programme the health and disease later in life. Given the link between reduced mitochondrial DNA

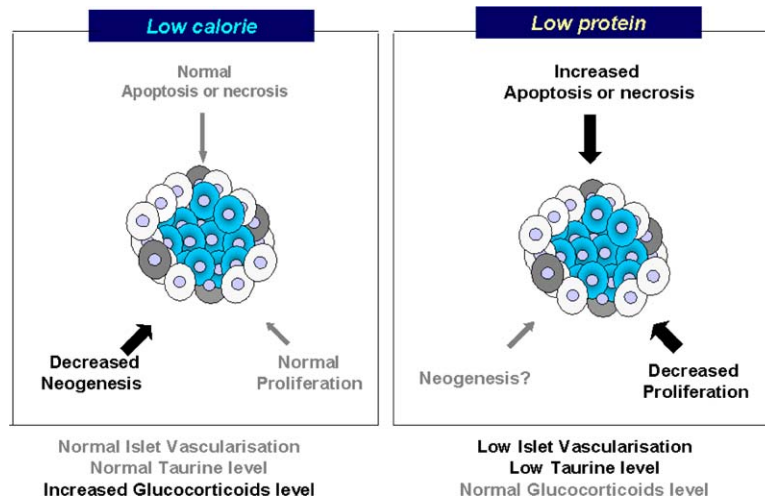


Fig. 3. Mechanisms by which maternal low calorie and low protein diets may reduce the  $\beta$ -cell mass in the progeny.

(mtDNA) content and the development of type-2 diabetes, an interesting hypothesis of the reprogramming of the mitochondrial function has been proposed in case of a limited energy environment (Peterside, Selak, & Simmons, 2003). Especially in the  $\beta$  cell which has a high energy requirement and poor antioxidant defence (Tiedge, Lortz, Drinkgern, & Lenzen, 1997), Simmons, Suponisky-Kroyter, and Selak (2005) showed that IUGR due to uterine arteries ligation induced mitochondrial dysfunction in the foetal  $\beta$  cell, that will lead to increased ROS production and will damage the mitochondrial DNA. This may progressively deteriorate the mitochondrial and the  $\beta$ -cell function and diabetes will ensue.

Thus, reprogramming of the mitochondrial function might also occur in case of early malnutrition. Protein malnutrition is associated with depressed antioxidant defence system and increased oxidative stress (Huang & Fwu, 1993). The proteome analysis of the foetal LP islets revealed that the expression of proteins involved in the mitochondrial energy transfer, glucose metabolism, RNA and DNA metabolism was changed. In particular, antioxidant protein-2 which is able to reduce  $H_2O_2$  and to protect the pancreas against injury was down-regulated (Sparre et al., 2003). Increased susceptibility to NO and IL-1 of these foetal islets as well as in adult islets was reported and prevention can be achieved by maternal taurine supplementation to the low protein diet (Merezak et al., 2001). Interestingly, it has been shown that taurine could critically affect mitochondrial function. Suzuki, Suzuki, Wada, Saigo, and Watanabe (2002) found two novel taurine containing modified uridines in

mitochondrial DNA. When Uridines modification was not present, defective mitochondrial function occurs and disease may ensue (Lee, Park, Pak, & Lee, 2005). Programming of mitochondrial anomalies in the progeny are also described in case of high fat diet during gestation (Taylor et al., 2005). Kidney mtDNA was reduced in this 3-month-old offspring and aorta showed reduced expression of the mitochondrial genome.

## 7. Conclusion

Alteration of the intrauterine metabolic environment does perturb the development of the endocrine pancreas and particularly the  $\beta$  cells. The consequences of this disturbed development may lead to diabetes later in life. Type-2 diabetes should be consecutive to abnormal insulin response and premature exhaustion of the  $\beta$ -cell mass in the offspring, but also type-1 diabetes as a consequence of a higher vulnerability of  $\beta$  cells to cytotoxic cytokines and to oxidative stress. Cellular and molecular mechanisms involved in the modified programming of the endocrine pancreas are subject of intense research in the varied animal models of maternal malnutrition or placental deficiency. Several clues are now open: alteration in growth factors and hormones like glucocorticoid axis, IGF system or insulin itself, lack in specific sensors or small specific molecules like taurine, and mitochondrial deficit. Today an unifying hypothesis cannot be put forward and moreover, it remains possible that several pathways would finally concur even to a same issue at the level of the foetal endocrine pancreas, resulting from different maternal metabolic anomalies.

## Acknowledgements

We are grateful to the Parthenon Trust (London, UK), the Belgian FNRS and The European Commission (QLK1-2000-00083, Frame Programme 5) for their financial support.

## References

- Aerts, L., Holemans, K., & Van Assche, F. A. (1990). Maternal diabetes during pregnancy: Consequences for the offspring. *Diabetes Metabolic Review*, *16*, 147–197.
- Aerts, L., & Van Assche, A. (2001). Low taurine, gamma-aminobutyric acid and carnosine levels in plasma of diabetic pregnant rats: Consequences for the offspring. *Journal of Perinatal Medicine*, *29*, 81–84.
- Aerts, L., & Van Assche, F. A. (2006). Animal evidence for the trans-generational development of diabetes mellitus. *The International Journal of Biochemistry and Cell Biology*, *38*, 894–903 (PHID: 16118061).
- Aerts, L., Van Bree, R., Feytons, V., Rombauts, W., & Van Assche, F. A. (1989). Plasma amino acids in diabetic pregnant rats and in their fetal and adult offspring. *Biology of the Neonate*, *56*, 31–39.
- Aerts, L., Verduyck, L., & Van Assche, A. (1997). The endocrine pancreas in virgin and pregnant offspring of diabetic pregnant rats. *Diabetes Research and Clinical Practice*, *38*, 1–19.
- Bertin, E., Gangnerau, M. N., Bellon, G., Bailbe, D., Arbelot de Vaquer, A., & Portha, B. (2002). Development of beta-cell mass in fetuses of rats deprived of protein and/or energy in last trimester of pregnancy. *American Journal: Regulatory Integrative and Comparative Physiology*, *1283*, R623–R630.
- Blondeau, B., Garofano, A., Czernichow, P., & Bréant, B. (1999). Age-dependant inability of the endocrine pancreas to adapt to pregnancy: A long term consequence of perinatal malnutrition in the rat. *Endocrinology*, *40*, 4208–4213.
- Blondeau, B., Lesage, J., Czernichow, P., Dupouy, J. P., & Bréant, B. (2001). Glucocorticoids impair fetal  $\beta$  cell development in rat. *American Journal of Physiology*, *281*, E592–E599.
- Boloker, J., Shira, J. G., & Simmons, R. (2002). Gestational diabetes leads to the development of diabetes in adulthood in the rat. *Diabetes*, *51*, 1499–1506.
- Boujendar, S., Arany, E., Hill, D., Remacle, C., & Reusens, B. (2003). Taurine supplementation of a low protein diet fed to rat dams normalized the vascularization of the fetal endocrine pancreas. *Journal of Nutrition*, *133*, 2820–2825.
- Boujendar, S., Reusens, B., Merezak, S., Ahn, M. T., Arany, E., Hill, D., et al. (2002). Taurine supplementation to a low protein diet during fetal and early postnatal life restores a normal proliferation and apoptosis of rat pancreatic islets. *Diabetologia*, *45*, 856–866.
- Cherif, H., Reusens, B., Dahri, S., & Remacle, C. (2001). A protein restricted diet during pregnancy alters in vitro insulin secretion from islets of fetal Wistar rats. *Journal of Nutrition*, *131*, 155–159.
- Dabelea, D., Knowler, W. C., & Pettitt, D. J. (2000). Effect of diabetes in pregnancy on offspring: Follow-up research in the Pima Indians. *Journal of Maternal Fetal Medicine*, *9*, 83–88.
- Dahri, S., Reusens, B., Remacle, C., & Hoet, J. J. (1995). Nutritional influences on pancreatic development and potential links with non-insulin dependent diabetes. *Proceedings of the Nutrition Society*, *54*, 345–356.
- Dahri, S., Snoeck, A., Reusens, B., Remacle, C., & Hoet, J. J. (1991). Islet function in offspring of mothers on low protein diet during gestation. *Diabetes*, *40*, 115–120.
- de Gasparo, M., Milner, G. R., Norris, P. D., & Milner, R. D. G. (1978). Effect of glucose and amino acids on foetal rat pancreatic growth and insulin secretion in vitro. *Journal of Endocrinology*, *77*, 241–248.
- Dor, Y., Brown, J., Martinez, O., & Melton, D. (2004). Adult pancreatic beta cells are formed by self-duplication rather than stem-cell differentiation. *Nature*, *429*, 41–46.
- Edlund, H. (2001). Factors controlling pancreatic cell differentiation and function. *Diabetologia*, *44*, 1071–1079.
- El-Khattabi, I., Grégoire, F., Remacle, C., & Reusens, B. (2003). Isocaloric maternal lowprotein diet alters IGF-I, IGFs, and hepatocyte proliferation in the fetal rat. *American journal of Physiology and Endocrinology Metabolism*, *285*(5), E991–E1000.
- Garofano, A., Czernichow, P., & Bréant, B. (1997). In utero undernutrition impairs rat  $\beta$ -cell development. *Diabetologia*, *40*, 1231–1234.
- Garofano, A., Czernichow, P., & Bréant, B. (1998). Beta-cell mass and proliferation following late fetal and early postnatal malnutrition in the rat. *Diabetologia*, *34*, 373–384.
- Gesina, E., Tronche, F., Herrera, P., Duchene, B., Tales, W., Czernichow, P., et al. (2004). Dissecting the role of glucocorticoids on pancreas development. *Diabetes*, *53*, 2322–2329.
- Hales, C. N., & Barker, D. J. P. (2001). The thrifty phenotype hypotheses. *British Medicine Bulletin*, *60*, 5–20.
- Hales, C. N., Desai, M., Ozanne, S. E., & Crowther, N. J. (1996). Fishing in the stream of diabetes: From measuring insulin to the control of fetal organogenesis. *Biochemical Society Transactions*, *24*, 341–350.
- Hogg, J., Han, V. K. M., Clemmons, D., & Hill, D. J. (1993). Interactions of glucose, insulin like growth factors (IGFs) and IGF binding proteins in the regulation of DNA synthesis by isolated fetal rat islets of Langerhans. *Journal of Endocrinology*, *138*, 401–412.
- Holemans, K., Aerts, L., & Van Assche, F. A. (2003). Lifetime consequences of abnormal fetal pancreatic development. *Journal of Physiology*, *547*, 11–20.
- Holness, M. J., Fryer, L. G., & Sugden, M. C. (1999). Protein restriction during early development enhances insulin responsiveness but selectively impairs sensitivity to insulin at low concentrations in white adipose tissue during a later pregnancy. *British Journal of Nutrition*, *81*, 481–489.
- Huang, C. J., & Fwu, M. L. (1993). Degree of protein deficiency affects the extent of the depression of antioxidative enzyme activities and the enhancement of tissue lipid peroxidation in rats. *Journal of Nutrition*, *123*, 803–810.
- Joanette, E. A., Reusens, B., Arany, E., Thyssen, S., Remacle, C., & Hill, D. J. (2004). Low-protein diet during early life causes a reduction in the frequency of cell immunopositive for nestin and CD34 in both pancreatic ducts and islets in the rat. *Endocrinology*, *145*, 3004–3013.
- Kaung, H. L. (1994). Growth dynamic of pancreatic islet cell population during fetal and neonatal development of the rat. *Development Dynamics*, *200*, 163–175.
- Kervran, A., Guillaume, M., & Jost, A. (1978). The endocrine pancreas of the foetus from diabetic pregnant rat. *Diabetologia*, *15*, 387–393.
- Lammert, E., Cleaver, O., & Melton, D. (2001). Induction of pancreatic differentiation by signals from blood vessels. *Science*, *294*, 564–567.
- Lardon, J., & Bouwens, L. (2005). Metaplasia in the pancreas. *Differentiation*, *73*, 278–286.

- Lee, Y. Y., Park, K. S., Pak, Y. K., & Lee, H. K. (2005). The role of mitochondrial DNA in the development of type-2 diabetes caused by fetal malnutrition. *Journal of Nutritional Biochemistry*, *16*, 195–204.
- Lesage, J., Blondeau, B., Grino, M., Bréant, B., & Dupouy, J. P. (2001). Maternal undernutrition during gestation induces fetal overexpression to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. *Endocrinology*, *142*, 1692–1702.
- Merezak, S., Hardikar, A., Yajnick, C. S., Remacle, C., & Reusens, B. (2001). Intrauterine low protein diet increases fetal  $\beta$  cell sensitivity to NO and IL-1- $\beta$ : The protective role of taurine. *Journal of Endocrinology*, *171*, 299–308.
- Merezak, S., Reusens, B., Ahn, M. T., & Remacle, C. (2004). Effect of maternal low protein diet and taurine on the vulnerability of wistar rat islets to cytokines. *Diabetologia*, *7*, 669–675.
- Ozanne, S. E., & Hales, C. N. (2002). Pre- and early postnatal non-genetic determinants of type 2 diabetes. *Expert Review Molecular Medicine*, 1–14.
- Ozanne, S. E., Smith, G. D., Tikerpaie, J., & Hales, C. N. (1996). Altered regulation of hepatic glucose output in the male offspring of protein malnourished rat dams. *American Journal of Physiology*, *270*, E559–E564.
- Ozanne, S. E., Wang, C. L., Coleman, N., & Smith, G. D. (1996). Altered muscle insulin sensitivity in the male offspring of protein malnourished rats. *American Journal of Physiology*, *271*, E1128–E1134.
- Peterside, I. E., Selak, M. A., & Simmons, R. A. (2003). Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. *American Journal of Physiology-Endocrinology and Metabolism*, *285*, E1256–E1258.
- Petrik, L., Arany, E., McDonald, T. J., & Hill, D. J. (1998). Apoptosis in the pancreatic islet cells of neonatal rat is associated with a reduced expression of insulin-like growth factor II that may act as a survival factor. *Endocrinology*, *139*, 2994–3004.
- Petrik, J., Reusens, B., Arany, E., Remacle, C., Coelho, C., Hoet, J. J., et al. (1999). A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor II. *Endocrinology*, *140*, 4861–4873.
- Petry, C. J., Dorling, M. R., Pawlak, D. B., Ozanne, S. E., & Hales, C. N. (2001). Diabetes in old male offspring of rat dams fed a reduced protein diet. *International Journal of Experimental Diabetes Research*, *2*, 139–143.
- Petry, C. J., Ozanne, S. E., Wang, C. L., & Hales, C. N. (2001). Early protein restriction and obesity independently induce hypertension in 1-year-old rats. *Clinical Science (London)*, *93*, 147–152.
- Piper, K., Brickwood, L., Turnpenny, L., Cameron, I., Ball, S., Wilson, D., et al. (2004). Beta cell differentiation during human pancreas development. *Journal of Endocrinology*, *181*, 11–23.
- Plagemann, A., Harder, T., Franke, K., & Kohlhoff, R. (2002). Longterm impact of neonatal breast-feeding on body weight and glucose tolerance in children of diabetic mothers. *Diabetes Care*, *25*, 16–22.
- Reusens-Billen, B., Remacle, C., Daniline, J., & Hoet, J. J. (1984). Cell proliferation in pancreatic islets of rat fetus and neonates from normal and diabetic mothers. An in vitro and in vivo study. *Hormone and Metabolic Research*, *16*, 565–571.
- Reusens, B., Dahri, S., Snoeck, A., Bennis-Taleb, N., Remacle, C., & Hoet, J. J. (1995). Long-term consequences of diabetes and its complications may have a fetal origin: Experimental evidence. In Richard Cowett (Ed.), *Diabetes* (pp. 187–198). New York: Raven-Press.
- Reusens, B., Hoet, J. J., & Remacle, C. (2000). Anatomy, developmental biology and pathology of the pancreatic islets. In L. J. De Groot, et al. (Eds.), *Endocrinology* (pp. 5024–5035). Philadelphia: Saunders Co.
- Reusens, B., & Remacle, C. (2001a). Effects of maternal nutrition and metabolism on the developing endocrine pancreas. In D. Barker (Ed.), *Fetal origins of cardiovascular and lung disease* (pp. 339–358). New York: Marcel Dekker.
- Reusens, B., & Remacle, C. (2001b). Intergenerational effects of adverse intrauterine environment on perturbation of glucose metabolism. *Twin Research*, *4*, 406–411.
- Scaglia, L., Cahill, C. J., Finegood, D. T., & Bonner-Weir, S. (1997). Apoptosis participates in the remodelling of the endocrine pancreas in the neonatal rat. *Endocrinology*, *138*, 1736–1741.
- Shepherd, P. R., Crowther, N., Desai, M., Hales, C. N., & Ozanne, S. E. (1997). Altered adipocyte properties in the offspring of protein malnourished rats. *British Journal of Nutrition*, *78*, 121–129.
- Silverman, B. L., Rizzo, T. A., Cho, N. H., & Metzger, B. E. (1998). Long-term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center. *Diabetes Care*, *21*, B142–B149.
- Simmons, R. A., Suponisky-Kroyter, I., & Selak, M. A. (2005). Progressive accumulation of mtDNA mutations and decline in mitochondrial function lead to  $\beta$ -cell failure. *Journal of Biological Chemistry*, *280*, 28785–28791.
- Snoeck, A., Remacle, C., Reusens, B., & Hoet, J. J. (1990). Effect of low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biology of the Neonate*, *57*, 107–118.
- Sorenson, R. L., & Bretlje, T. C. (1997). Adaptation of islets of Langerhans to pregnancy: Beta-cell growth enhanced insulin secretion and the role of lactogenic hormones. *Hormone and Metabolic Research*, *29*, 301–307.
- Sparre, T., Reusens, B., Cherif, H., Larsen, M. R., Roepstroff, P., Fey, S. J., et al. (2003). Intrauterine programming of fetal islet gene expression in rats—effects of maternal protein restriction during gestation revealed by proteome analysis. *Diabetologia*, *46*, 1497–1511.
- Stoffers, D. A., Desai, B. M., DeLeon, D. D., & Simmons, R. (2003). Neonatal Exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes*, *52*, 734–740.
- Sturman, G. A. (1993). Taurine in development. *Physiological Review*, *73*, 119–147.
- Suzuki, T., Suzuki, T., Wada, T., Saigo, K., & Watanabe, K. (2002). Taurine as a constituent of mitochondria diseases. *EMBO Journal*, *21*, 6581–6589.
- Taylor, P. D., McConnell, J., Khan, I. Y., Holemans, K., Lawrence, K. M., Asare-Anane, H., et al. (2005). Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. *The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *288*, 134–139.
- Tiedge, M., Lortz, S., Drinkgern, J., & Lenzen, S. (1997). Relation between antioxidant enzyme gene expression and antioxidative defence status of insulin-producing cells. *Diabetes*, *46*, 1733–1742.
- Van Assche, F. A., Gepts, W., & Aerts, L. (1976). The fetal endocrine pancreas in diabetes (human). *Diabetologia*, *12*, 423–424.
- Winick, M., & Noble, A. (1966). Cellular response in rats during malnutrition at various ages. *Journal of Nutrition*, *89*, 300–306.