Maternal malnutrition programs the endocrine pancreas in progeny1–4

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ABSTRACT
Type 2 diabetes arises when the endocrine pancreas fails to secrete sufficient insulin to cope with metabolic demands resulting from β cell secretory dysfunction, decreased β cell mass, or both. Epidemiologic studies have shown strong relations between poor fetal and early postnatal nutrition and susceptibility to diabetes later in life. Animal models have been established, and studies have shown that a reduction in the availability of nutrients during fetal development programs the endocrine pancreas and insulin-sensitive tissues. We investigated several modes of early malnutrition in rats. Regardless of the type of diet investigated, whether there was a deficit in calories or protein in food or even in the presence of a high-fat diet, malnourished pups were born with a defect in their β cell population, with fewer β cells that did not secrete enough insulin and that were more vulnerable to oxidative stress; such populations of β cells will never completely recover. Despite the similar endpoint, the cellular and physiologic mechanisms that contribute to alterations in β cell mass differ depending on the nature of the nutritional insult. Hormones that are operative during fetal life, such as insulin, insulin-like growth factors, and glucocorticoids; specific molecules, such as taurine; and islet vascularization have been implicated as possible factors in amplifying this defect. The molecular mechanisms responsible for intrauterine programming of β cells are still elusive, but among them the programming of mitochondria may be a strong central candidate. Am J Clin Nutr doi: 10.3945/ajcn.110.000729.

INTRODUCTION
Type 2 diabetes and associated pathologies such as cardiovascular disease and hypertension are major health issues in the 21st century. Indeed, >250 million people worldwide, in both developed and developing countries, currently suffer from type 2 diabetes. This rapid rise in the prevalence of type 2 diabetes suggests that the environment must be an important factor. Epidemiologic studies have indicated that a suboptimal fetal environment may be a determinant in the increased risk of developing diabetes. In 1992, Hales and Barker (1) published convincing data obtained in a group of men living in Hertfordshire, United Kingdom, who at the time of the study were 64 y old. These studies showed that men who had the lowest birth weight were 6 times more likely to have type 2 diabetes than those who were heavier at birth. A few years later, data collected from individuals exposed in utero to the Dutch famine between 1944 and 1945 showed that maternal malnutrition, especially during the last trimester of pregnancy, led to intrauterine growth retardation (IUGR) and was associated with impaired glucose tolerance and insulin resistance in 50-y-old offspring (2). Subsequently, many other studies have reported similar findings in various populations and ethnic groups worldwide (3, 4). It is now widely accepted that there is a relation between maternal malnutrition and diabetes; however, the mechanistic basis of this relation remains to be established. Hales and Barker (1, 5) coined the term thrifty phenotype hypothesis, which suggests that in the case of poor fetal nutrition resulting from either poor maternal nutrition or poor delivery of nutrients to the fetus due to other causes (eg, placental dysfunction), an adaptive response occurs that favors the growth of key organs, such as the brain, at the detriment of other tissues, such as muscles, kidneys, and the pancreas.

TYPE 2 DIABETES
Type 2 diabetes arises when the endocrine pancreas fails to produce sufficient insulin to cope with metabolic demand due to β cell secretory dysfunction and/or decreased β cell mass. Still increased metabolic demand may result from pregnancy, obesity, or aging. Views regarding the pathogenesis of type 2 diabetes have evolved over the last decade. Whereas islet β cell dysfunction was considered to be the final determinant for the switch from normal glucose tolerance to glucose intolerance, a lower acquired β cell mass was later introduced into the schema based on careful analyses of human and animal data (6). Now, β cell dysfunction is believed to play a role much earlier in development, perhaps even at birth (7). Indeed, a series of susceptibility loci for type 2 diabetes that affect the development and ongoing homeostasis of the β cell mass, as well as insulin secretion and biosynthesis, have been identified in recent genome-wide scans (8).

PROGRAMMING OF β CELL MASS BY A DISTURBED INTRAUTERINE ENVIRONMENT
It is now well established that low birth weight predisposes to type 2 diabetes, prompting the hypothesis that impaired growth

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in early life programs metabolic disease in adulthood. To understand the cellular and molecular mechanisms that underlie early programming, investigators have induced impaired growth in utero in rodents by giving a calorie- or protein-restricted diet to the pregnant dam, by prenatal overexposure to glucocorticoids, or by reducing blood flow after uterine artery ligation and analyzing the effect on the development of the endocrine pancreas. Other research groups have investigated islet cell development and function in sheep by using different techniques to induce growth retardation (for reviews, see references 9–11). Despite differences in the type, timing, and duration of the intrauterine insult involved, most of the animal models for IUGR have produced similar outcomes. This article mainly covers data collected from the offspring of dams that were fed a protein- or calorie-restricted diet during gestation.

Animal studies have shown that insulin target tissues are programmed by early events. Indeed, the young offspring (6 wk to 3 mo old) of dams fed a low-protein (LP) diet had a better glucose tolerance when plasma insulin concentrations were reduced, which suggests an improved insulin sensitivity at this stage (12, 13). However, age reversed this trend such that by 15 mo, the offspring had impaired glucose tolerance; by 17 mo, frank diabetes with insulin resistance was observed in male LP offspring (14), whereas only glucose intolerance was observed in female LP offspring (15). Pregnancy is a physiologic situation that requires adaptation of the endocrine pancreas to overcome insulin resistance. Studies have shown that both LP and low-calorie (LC) diets given to the mother during gestation seem to impede this adaptation in the progeny, because neither LP nor LC offspring were able to increase their insulin secretion in response to pregnancy (16, 17).

The data emerging from animal studies clearly show that the β cell mass is also affected and may be the first target programmed during fetal life. In 1970, Winick (18) demonstrated that poor nutrition during gestation irreversibly led to reduced cell numbers in tissues such as the pancreas. From a biological point of view, this is not surprising because from the zygote stage onward, the growth and development of an organ involve cell proliferation, and cells usually undergo several steps of commitment that progressively restrict the variety of the differentiation potential. During this period of coordinated growth and progressive acquisition of function, one might expect alterations in the milieu to have permanent consequences, and this would obviously be true for the endocrine pancreas.

The various pancreatic cell lineages originate from a pool of common progenitors and are controlled by a transcription factor network that is very complicated because several factors are expressed more than once during the differentiation process and play more than a single role (19, 20). Pancreatic and duodenal homeobox gene 1 (Pdx1) is a key transcription factor. Cells expressing Pdx1 between E9.5 and E11.5 give rise to pancreatic duct cells, acinar cells, and endocrine islets. The specification for exocrine or endocrine cells occurs between the 10th and 15th day of gestation in rats and depends on the Notch-Delta-Serrate pathway. Cells with an active Notch receptor will commit to an exocrine lineage or become duct cells, due to inactivation of the neurogenin 3 gene (Ngn3). The endocrine compartment that expresses Ngn3 will further differentiate into insulin, glucagon, somatostatin, or pancreatic polypeptide cells depending on which transcription factors are expressed (19, 20). The endocrine cells make islets that subsequently leave the connection with the ducts and become vascularized.

Therefore, at birth, the β cell mass is determined by the recruitment of undifferentiated precursors, as well as the replication of differentiated cells and apoptosis of the β cells. Obviously, any disturbance in the environment of the endocrine cells at a specific developmental time point may perturb the balance of controlling factors, thereby contributing to β cell abnormalities and diabetes later in life.

In studies examining the effects of diet in gestating rats, the LC diet was mostly given during the last week of gestation (ie, after initiation of the endocrine fate commitment) (21), whereas rats given an LP diet were analyzed after the diet had been given throughout gestation (22–24). However, both models revealed a lower β cell mass in the fetus just before birth. The mechanisms leading to defective development are suspected to be different, but it is conceivable that the different time windows would perturb the endocrine pancreas in different ways. Therefore, we carefully analyzed several parameters that contribute to lower β cell mass at birth in a well-standardized protocol by giving an isocaloric diet containing 8% compared with 20% protein (LP) or 50% of the control energy intake (LC) during the last week or throughout gestation to pregnant rats (25). As shown in previous experiments, both the LP diet (22–24) and the LC diet (21) reduced the β cell mass by half whether it was given from the first day of gestation or during the last week; however, different mechanisms led to this deficiency (25). The LC diet affected β cell differentiation, because at 15.5 d of gestation, the number of cells that were positive for Ngn3 and PDX-1 was lower in the pancreatic duct, whereas a normal number was found in the pancreas of the LP group. When β cell proliferation was analyzed at 21.5 d of gestation, a period in which β cell expansion is important, it was reduced by half in the LP groups independently of the time window during which the LP diet was given, but the proliferation rate was not affected in the LC groups. Further analysis of the lower proliferation rate in LP islets revealed that proliferation was lower because of a lengthening of the cell cycle, as evidenced by higher cyclin D1 (a marker of the G1 phase) and lower NEK2 (never in mitosis gene A–related kinase 2; an indicator of cells in G2 and mitosis), as well as a reduction in the expression of insulin-like growth factor (IGF)–II, a growth factor for β cells (23).

Thus, it is clear that pups exposed to nutritional restriction in utero have few β cells, but does this imply that insulin secretion is also altered? We measured insulin release from isolated fetal control and LP islets after stimulation by glucose or several amino acids, and found that LP islets secreted half the amount of insulin secreted by control islets (26).

Because apoptosis also controls expansion of the cell mass, we investigated the apoptotic rate in the pancreas of LP fetal and neonatal offspring, and found it to be increased (23). It is known that β cells are exceedingly sensitive to different molecules, including streptozotocin (27), nitric oxide (NO), and cytokines involved in β cell destruction in insulin-dependent diabetes mellitus (28). This may be due in part to weak antioxidant defense activity (29, 30). Several studies showed that a maternal LP diet during gestation increased this vulnerability, because LP fetal and adult islets had an increased rate of apoptosis compared with control islets when incubated in the presence of cytokines, even when a normal diet was given after weaning
exocrine tissue to the detriment of endocrine tissue (42). Restoration of a normal concentration of this hormone by adrenalectomy and glucocorticoid implant prevented the decrease in β cell mass in LC pups (43).

Role of vascularization

Studies in humans have shown that very-small-for-gestational-age neonates have a lower fraction of β cells and smaller islets with less vasculature than neonates who are considered to be appropriate for gestational age (44), as well as alterations in insulin secretion (45). Throughout development to adulthood, endothelial cells are responsible for paracrine and blood-borne signals that contribute greatly to pancreas development, endocrine cell differentiation and proliferation, and secretory function (46). Indeed, vascular signals coming from the dorsal aorta and cardiogenic mesoderm, which are near the dorsal pancreatic endoderm, induce budding of the dorsal and ventral pancreas and allow the expression of transcription factors such as PDX1 and PTF1α (pancreatic specific transcription factor 1α), and later the expression of insulin and glucagon. Peptide growth factors such as platelet-derived growth factor, vascular endothelial growth factor (VEGF), and fibroblast growth factor 7 (FGF-7), which are expressed within the pancreatic stroma adjacent to the ductal epithelium, contribute to endocrine cell formation and islet expansion (47). In particular, VEGF-A is necessary for endothelial fenestration (48). There is convincing evidence that VEGF-A may be a coordinator between blood vessel density and islet cell mass during pancreatic development. A reduction in islet vascularization was observed upon alteration of the metabolic intrauterine environment, such as occurs with an LP diet during fetal life (21, 22, 39). LP fetuses showed a decreased number of islet cells expressing VEGF and its receptor Flk-1. In 1-mo-old LP pups, VEGF-positive cells remained decreased. As noted above, β cell proliferation was also reduced in these LP islets (21, 22). A similar association between the proliferation rate and islet vascularization was observed when dexametasones was given to the mother throughout gestation or only during the last week. Again, a lower β cell mass was observed independently of the duration of the treatment. However, islet vascularization was only reduced when the treatment was given throughout gestation and was accompanied by a decreased β cell proliferation (O Dumortier, N Theys, C Remacle, B Reusens, unpublished data, 2009).

Disturbed vascularization may be one of the mechanisms involved in alterations of β cell mass. In a recent study examining the process of β cell loss in IUGR in rat offspring, it was shown that the reduction in β cell mass observed at 7 wk was preceded by a diminution in islet vascularization and VEGF protein expression that could be normalized by exendin-4 administration (49). Of interest, islet vascularization dysfunction has been suggested to play a role in the onset of type 2 diabetes in Zucker diabetic fatty rats (50). The islet vascular integrity was altered in these rats, and it was proposed that such vascular alteration plays a role in the β cell loss. However, no modification in β cell proliferation or islet blood vessel density was observed in the fetus when an LC diet was given to the mother (21), suggesting that other mechanisms are involved in the lower β cell mass in this model.
Role of epigenetic regulation

It has been hypothesized that the molecular mechanisms underlying this altered β cell mass may be in part related to epigenetic modulation of the expression of key developmental genes (51). In mammals, DNA methylation and histone modifications represent major epigenetic mechanisms that have been implicated in the regulation of gene transcription. A number of studies have suggested that maternal disorders during early life may induce epigenetic modifications in the progeny. More data on epigenetic regulation can be found in the chapter written by Gabory et al (52) in this issue.

Lillycrop et al (53) showed that administration of a protein-restricted diet to pregnant dams induced hypomethylation of PPARγ (peroxisome proliferator-activated receptor γ) and the glucocorticoid receptor gene, and increased the expression of these genes in the liver of weaning offspring. Epigenetic regulation in β cell development is poorly documented. Simmons (54) examined epigenetic regulation of PDX-1 in β cells of IUGR offspring and found that PDX-1 expression progressively declined in the IUGR animals. The proximal promoter of PDX-1 is required for transcription of the gene, and histones H3 and 4 in this region are heavily acetylated in normal β cells (55). In islets of IUGR rats, H3 and 4 in this region of the PDX-1 promoter were shown to be deacetylated (56). To our knowledge, epigenetic regulation has not yet been investigated in β cells from the offspring of malnourished LP and LC dams.

Role of the mitochondria

In mammalian cells, each mitochondrion contains 2–10 copies of mtDNA, which codes for 13 polypeptides, 2 rRNAs, and 22 tRNAs that are necessary for protein synthesis within mitochondria. However, most of the proteins and enzymes present in mitochondria are nuclear gene products. The mitochondria are responsible for producing energy by oxidizing pyruvate through the tricarboxylic acid cycle, and lipids through β-oxidation. These processes produce reducing equivalent that drive the electron transport chain enclosed in the inner membrane to produce ATP. The mitochondria are also the major site of ROS production, which can damage macromolecules (57). In addition, the mitochondria play a major role in the regulation of apoptosis (58, 59).

Mitochondrial dysfunction may contribute to the development of diabetes (60), and 1.5% of diabetic patients exhibit mutations in mtDNA (61). Moreover, type 2 diabetic patients feature a down-regulation of nDNA-encoded mitochondrial genes leading to alterations in the concentrations of regulators of mitochondrial biogenesis, such as PGC-1α (peroxisome proliferator-activated receptor γ, coactivator 1α) and NRF-1 (NADPH-dependent flavin reductase) (62).

It has been proposed that programming of mitochondrial function may be a key adaptation that enables a fetus to survive in a limited-energy environment (63–65). Such an adaptation may be positive in utero but can lead to metabolic disease in adulthood. Uteroplacental insufficiency provoked by uterine artery ligation induces an alteration in pyruvate oxidation in muscle (63) and liver (64) from IUGR young adult rats, contributing to insulin resistance and hyperglycemia of type 2 diabetes. The concept of mitochondrial programming may apply especially to cells that have a high energy requirement, such as β cells. Uteroplacental insufficiency induces mitochondrial dysfunction in the endocrine pancreas, leading to increased production of ROS and reduced ATP production due to a decline in the functional activity of complexes I and III of the electron transport chain. In turn, such alterations will damage mtDNA content (65), which may progressively deteriorate the mitochondrial and β cell functions and precipitate the occurrence of diabetes.

To assess whether maternal malnutrition without restriction of the oxygen supply leads to mitochondrial programming in islets, Reusens et al (40) analyzed parameters of mitochondrial biogenesis and function in fetal and adult offspring of dams fed an LP diet. Poor protein supply during gestation affected the mitochondrial function in fetal islets, as revealed by a micro-array analysis. Maternal LP diet changed the expression of >10% of the genes, 11% of which encode for mitochondrial protein. The tricarboxylic acid cycle and ATP production were highly targeted. Theys et al (66) investigated mitochondrial function in isolated islets from 3-mo-old LP offspring of dams fed an LP diet throughout gestation. They studied both male and female progeny because it is becoming obvious that programming is a sex-specific phenomenon. Both male and female offspring exhibited blunted ATP production and a lower response to glucose challenge compared with control offspring, due in part to a lower expression of malate dehydrogenase and subunit 6 of the ATP synthase encoded by the mitochondrial genome (mtDNA). However, mtDNA content was unchanged in LP islets compared with C islets. Male offspring seemed to be more affected than females, in that the males showed a lower β cell mass and an increase in ROS production associated with a higher expression of mitochondrial subunits of the electron transport chain ND4L, and overexpression of PPARγ and UCP-2 (66).

Further studies were then undertaken in parallel in 3-mo-old male and female offspring of mothers fed an LC or high-fat (HF) diet during gestation (67). Similarly to the case of LP offspring, an LC or HF diet during pregnancy led to an absence of increased insulin release and ATP content in response to glucose stimulation. In addition, for each progeny, consequences downstream from the entry of glucose were apparent. Expression of genes involved in glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation were altered in LC and HF rats in a sex- and diet-dependent manner. Moreover, prenatal malnutrition affected mitochondrial biogenesis. The mtDNA content and expression of PGC-1α, a transcriptional regulator of mitochondrial biogenesis, were higher in islets from LC rats (67). Maternal malnutrition also programs the mitochondria of other organs. In one study (68), an HF diet during gestation and lactation induced long-term alterations in mtDNA in the kidney and aorta. In an investigation of the offspring of dams fed an LP diet during pregnancy and weaning (69), both mtDNA content and mtDNA-encoded gene expression were reduced in the liver and skeletal muscle, and lower mtDNA amounts were found in the total pancreas.

These findings indicate that programming of mitochondrial dysfunction is a consequence of maternal malnutrition, which may predispose to glucose intolerance in adult offspring.

CONCLUSIONS

Placental insufficiency leads to severe IUGR resulting from reduced blood flow, which suggests a reduced transfer of both
nutrients and oxygen to the fetus. In a general population, a nutritional imbalance in the presence of an adequate amount of calories and oxygen is obviously less drastic, but is probably more frequent. In such instances, some alterations may go unnoticed; however, data collected from the different models described above point to a subtler mechanism of programming involving plasmalytes, vascularization, and mitochondria. Less availability of nutrients and growth hormones to the β-cells, a small repeated increase in ROS production, lower production of ATP, sporadic light hyperglycemia, and insults resulting in the destruction of a limited number of β-cells during growth, adolescence, and adult life can jeopardize the ability of the β-cell mass to function, leading to a prediabetic state.

The authors’ responsibilities were as follows—BR and CR: designed the research and obtained the funding; NT: performed the experiments investigating mitochondrial programming; OD: collected and analyzed the data comparing the effects of LP and LC diets and glucocorticoids on β-cell development; and KG: investigated the mechanisms involved in the increased vulnerability of adult islets and their capacity to regenerate. All of the authors participated in writing the manuscript. None of the authors declared a conflict of interest.

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