Metabolic Programming: Causes and Consequences

The normal programmed development of a multicellular organism from the germ cell is a synchronized series of events driven by genetic instructions acquired during conception. During the early critical periods in life the organism also has the ability to respond to environmental situations that are alien to normal development by adaptations at the cellular, molecular, and biochemical levels. Such early adaptations to a nutritional stress/stimulus permanently change the physiology and metabolism of the organism and continue to be expressed even in the absence of the stimulus/stress that initiated them, a process termed “metabolic programming” (1). A brief summary of the findings from human epidemiological and animal studies is presented below in support of the concept of metabolic programming induced by nutritional experiences during critical periods in development with consequences later in adulthood. For detailed accounts the reader is referred to excellent reviews on this subject (2–5). This minireview will focus on metabolic programming with reference to a novel rat model developed in our laboratory.

Evidence for Metabolic Programming from Human Epidemiological Data

Extensive epidemiological findings indicate that metabolic programming occurs in humans. Barker (6) was the first to suggest from epidemiological studies that the disproportionate size of the newborn resulting from maternal malnutrition correlated with an increased risk for adverse health outcomes (type II diabetes, hypertension, and cardiovascular diseases) later in adult life. These primary observations resulted in the now widely recognized “fetal origins” hypothesis emphasizing the importance of adequate maternal nutrition during pregnancy (4).

Evidence for Metabolic Programming in Animals

Nutritional programming has been demonstrated in animal studies. In pioneering studies with rodents, McCance (7) demonstrated by adjusting litter size that the quantity of food consumed during early periods of postnatal life has long term consequences on growth. The consequences of maternal malnutrition induced by either a low protein diet or caloric restriction during gestation and lactation cause major changes in the structure and function of several organs in the offspring. Preg-
drawal of the HC milk formula at the time of weaning (15, 16) (Figs. 1 A and 2 A). During the suckling period there are no differences in body weights and in plasma glucose levels between the HC and age-matched control groups (17). The overlap of the critical window for postnatal pancreatic development with the high carbohydrate nutritional intervention in the HC rat suggests that the endocrine pancreas is a target organ for significant adaptations. We have observed significant alterations at the cellular, molecular, and biochemical levels in islets isolated from neonatal HC rats and have also observed the programming of these adaptations into adulthood.

Cellular adaptations induced by dietary intervention during the suckling period include an increased number of smaller sized islets in the HC pancreas compared with controls (18). Such HC islets have a larger immunopositive area for insulin resulting in a net increase in the insulin-producing mass in HC islets (18). Additionally, the rate of apoptosis and the expression of proliferating cell nuclear antigen are inversely altered in islets and the ductal epithelium of the HC rat (18). One of the factors contributing to the altered ontogeny has been attributed to the change in the expression of insulin-like growth factor II (18).

Several molecular adaptations are observed in neonatal islets in response to the HC dietary intervention. Increases in insulin biosynthesis and in gene expression of preproinsulin are observed in islets of neonatal HC rats (19) (Fig. 2 B). Additionally, mRNA levels of transcription factors such as pancreatic duodenal homebox factor-1 (PDX-1, also known as somatostatin transcription factor-1) (Fig. 2 B), islet factor-1 (Isl-1), upstream stimulatory factor-1, regenerating factor-3 (reg-3) (19, 20), and Beta2/NeuroD and hepatocyte nuclear factor are significantly increased in neonatal HC islets. mRNA levels of stress-activated protein kinase-2 (SAPK-2), phosphatidylinositol 3-kinase, acetyl-CoA carboxylase, glucose transporter 2, and insulin receptor substrate-1 and -2 are also significantly higher in these HC islets (19, 20). PDX-1 is an important transactivator of the insulin gene and is an essential component of the mechanisms whereby glucose modulates insulin promoter activity (21). DNA binding activity of PDX-1 has been proposed to be modulated by glucose via a phosphorylation cascade(s) involving SAPK-2 and PI3-kinase, which facilitates the translocation of the 46-kDa, phosphorylated active form to the nucleus resulting in increased insulin gene transcription (21) (Fig. 3). The possible correlation between the observed effects of the HC dietary intervention in neonatal HC rats and preproinsulin gene transcription is shown in Fig. 3. In addition PDX-1 is an important transcription factor that com-

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Fig. 1. Diet-induced metabolic programming in first and second generation HC rats. Metabolic adaptations in first generation (A) and second generation (B) HC rats in response to feeding a HC milk formula to first generation neonatal rat pups are shown. GTT, glucose tolerance test.

Fig. 2. Biochemical adaptations in pancreatic islets from neonatal HC rats. A represents plasma insulin levels (pM) in neonatal HC rats. B is the relative levels of preproinsulin and PDX-1 mRNAs in islet from 12-day-old HC and mother-fed (MF) rats. C represents the insulin secretory response to a glucose (Glu) stimulus by islets from 12-day-old first generation HC rats at 60 min. Insulin secretion is expressed as femtomoles of insulin/30 islets/60 min. D represents the activity of the low K_m hexokinase activity in supernatant (S) and pellet (P) fractions of islet extracts of 12-day-old HC rats and mother-fed control rats. Activity is expressed as milliunits/mg of protein.

Fig. 3. Putative pathway for the regulation of preproinsulin gene transcription in neonatal HC islets. This scheme indicates the various responses induced in the HC rat to the high carbohydrate dietary intervention, the possible interactions among them, and the consequent regulation of preproinsulin gene transcription. USF-1, upstream stimulatory factor-1.

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mits the progression of the pluripotent ductal cell into an endocrine cell (22). The significant increase in the gene expression, DNA binding activity, and protein content of PDX-1 in neonatal HC islets suggests that it plays a pivotal role in the cellular adaptations as well as in the onset and maintenance of hyperinsulinemia in this rat model (19). Transcription factors like Isl-1, Beta2/NeuroD, reg-3, and hepatocyte nuclear factor β-3 also contribute to pancreatic organogenesis (22–24). The increases in the gene expression of these factors suggest that they too contribute to the modification of the islet architecture in the neonatal HC rats. cDNA array analysis has indicated significant changes in global gene expression patterns in neonatal HC islets as well as in adult HC islets suggesting that a wide range of molecular alterations is essential for the onset and persistence of the HC phenotype in HC rats (20).

Significant biochemical adaptations summarized below initiate and sustain hyperinsulinemia in neonatal HC rats. A characteristic feature of neonatal HC rats is about a 6-fold increase in the concentration of circulating insulin (16, 25) (Fig. 2A). There is a distinct leftward shift (increased sensitivity) in the glucose-stimulated insulin secretory response in HC islets (Fig. 2C), and this is associated with increases in the low $K_m$ hexokinase activity (Fig. 2D) and an increase in glucose transporter 2 protein content (25). Insulin secretion by islets is regulated by three pathways (the $K_{ATP}$ channel-dependent pathway, the $K_{ATP}$ channel-independent augmentation pathway, and the Ca$^{2+}$ channel-independent augmentation pathway (26)), and these pathways are up-regulated in HC islets (27). Circulating levels of glucagon-like peptide-1 (GLP-1) are significantly higher in HC rats suggesting that GLP-1-mediated events contribute significantly to the hyperinsulinemia of HC rats (27). In addition to its insulinotropic effects, GLP-1 stimulates transcription of the preproinsulin gene and PDX-1 gene and also the proliferation and neogenesis of β cells from ductal epithelium in rodents (28–30). In light of the above, the significant increase in circulating levels of GLP-1 in 12-day-old HC rats has important implications for the HC phenotype in suckling HC rats.

Because of chronic hyperinsulinemia the capacity for hepatic lipogenesis is enhanced in neonatal HC rats (16). Enzymes such as glucokinase and malic enzyme that normally appear in the liver at weaning are precociously induced by this dietary treatment suggesting that their appearance is not development-dependent but is controlled by the presence of the stimulus (diet-induced hyperinsulinemia) (16). It is evident from the above that feeding rats an HC milk formula, instead of mother’s milk, during the suckling period elicits significant alterations in islet functions as well as in metabolic responses of peripheral tissues, which are important for the development of adult-onset obesity (Fig. 4, Adaptive Phase).

**Programming of Early Onset Adaptations into Adulthood**

Hyperinsulinemia persists into adulthood of HC rats despite withdrawal of the HC nutritional intervention on day 24 (Fig. 1A). Alterations in the insulin secretory pathway observed in the neonatal HC islets are programmed into adult islets (31, 32). The molecular changes (increase in the preproinsulin gene transcription) observed in islets from neonatal HC rats are also observed in adult HC islets (32). There is an increase in the insulin-producing mass of the adult HC pancreas (17). Chronic hyperinsulinemia is accompanied by an increase in the body weight of the animals from day 55 onward and full blown obesity by day 100 (15) (Fig. 1A). Although the adult HC rats maintain normoglycemia, an abnormal response to an oral glucose tolerance test is observed in HC animals on approximately day 75 (17) (Fig. 1A). In addition, liver and adipose tissue show increased lipogenic capacities in adulthood (15). An increase in the cell size in epididymal adipose tissue reflects the adiposity observed in adulthood (15). Glycogen content and the glycogen synthase activation cascade are down-regulated in liver and skeletal muscle (33) and up-regulated in epididymal adipose tissue of adult HC rats (34). Taken together these findings indicate that early adaptations are programmed and are accompanied by additional changes probably triggered by adult-onset factors (Fig. 4, Persistence Phase).

**Nutritionally Induced Generational Effect on Metabolic Programming**

An important and unexpected consequence of the nutritionally induced metabolic programming noted above is the transmission of the phenotype from the HC mother to the progeny (see Fig. 1B). Female rats fed an HC milk formula during their suckling period spontaneously transmitted their metabolic characteristics to their progeny without the pups themselves having to undergo any nutritional treatment (35). The second generation HC rats display hyperinsulinemia and an altered insulin secretory pattern within 48 h after weaning them onto laboratory chow on day 24. Molecular adaptations (increases in mRNA levels of preproinsulin, PDX-1, upstream stimulatory factor-1, SAPK-2, and PI3-kinase) are also programmed in second generation HC rats. The growth pattern of HC rats in the second generation parallels that of first generation HC rats (35) (Fig. 1B). Cross-breeding experiments have demonstrated that only HC females transmit these traits to the progeny, suggesting that the intrauterine experience may be essential for the transmission (Fig. 4, Transmission Phase).
Potentially induced hyperinsulinemia in neonatal rats during the critical period of brain development results in alterations in body weight, blood pressure, and glucose metabolism in adult life because of disorganization of the ventromedial hypothalamic nuclei (12). The HC milk formula induces responses in pancreatic islets and the small intestine as evidenced by increases in the circulating plasma levels of insulin and GLP-1 in neonatal HC rats. In the context of reports on the effects of a nutritional intervention on the brain and the effects of the HC dietary intervention on the islets and gut in neonatal HC rats, it is tempting to suggest that cross-talk may occur among the pancreas, small intestine, and brain resulting in the onset and persistence of hyperinsulinemia in HC neonates.

Epigenetic mechanisms that mediate phenomena such as genomic imprinting may also contribute to programming (36). Epigenetic modification is triggered by changes in environment and can occur in both somatic and germ cell lineage during development (37). Altered DNA methylation patterns have been shown to be caused by protein deficiency and folate depletion (38). Nutritional alterations early in life may modify cell-specific DNA methylation patterns leading to altered levels of gene expression in specific tissues. In addition, because the altered DNA methylation patterns in specific cells are transmitted to the daughter cells by replication, the initial modifications are immortalized (Fig. 4).

Concluding Remarks

The “pup in a cup” rat model is unique in that it permanently programs the metabolism of an adult rat by merely modifying the composition of the milk fed to the animal during its suckling period. In addition, this type of metabolic programming is transmitted to the next generation by the mother. It is evident from our studies with rats that the nature and the timing of the dietary treatment programs the onset of pathological conditions in the adult that mimic major metabolic diseases noted in humans, such as obesity and type II diabetes. Evidence points to diet-induced hyperinsulinemia as the primary event in metabolic programming in the HC rat.

In a recent report Mokdad et al. (39) have pointed out that the epidemic of obesity is a critical public health threat in the United States (nearly one of five Americans is obese). Because there has been a significant change in the gene pool in the United States in recent decades (39), it is unlikely that genes related to obesity are involved in the increased incidence in this disease. There has also been a 33% increase in the incidence of diagnosed diabetes in the past decade, and this has been highly correlated with the increase in the incidence of obesity, suggesting that obesity is a major risk factor for chronic diseases (39). It is becoming clear that the origins of chronic diseases are not limited to only inherited genes and/or sedentary life styles. The results from the HC rat model suggest that nutritional experiences of infants during the immediate postnatal life such as overfeeding of formula and early introduction of supplemental weaning foods high in carbohydrates (e.g., cereals, fruits, juices, etc.) may contribute to metabolic programming leading to adult-onset diseases like obesity and diabetes. The newly emerging field of metabolic programming therefore offers an additional route to examine the etiology of adult-onset chronic diseases.

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REFERENCES