MINIREVIEW

Neonatal Nutrition: Metabolic Programming of Pancreatic Islets and Obesity[1]

MALATHI SRINIVASAN,* SUZANNE G. LAYCHOCK,† DAVID J. HILL,‡ AND MULCHAND S. PATEL[2]*

*Departments of *Biochemistry and †Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, State University of New York, Buffalo, NY 14214; and ‡Departments of Physiology and Pediatrics, Lawson Research Institute, St. Joseph's Health Center, London, Ontario, Canada N6A 5A5*

Obese individuals are more likely to suffer from diseases termed the "metabolic syndrome," which includes type 2 diabetes. It is now recognized that early life dietary experiences play an important role in the etiology of such diseases. In this context, the consequences of a high carbohydrate (HC) dietary intervention in neonatal rats is being studied in our laboratory. Artificial rearing of 4-day-old rat pups on a HC milk formula up to Day 24 results in the immediate onset of hyperinsulinemia, which persists throughout the period of dietary intervention. Several adaptations at the biochemical, cellular, and molecular levels in the islets of these HC rats support the onset and persistence of the hyperinsulinemic condition during this period. Some of these adaptations include a distinct leftward shift in the insulin secretory capacity, increased hexokinase activity, increased gene expression of preproinsulin and related transcription factors and specific kinases in 12-day-old HC islets, and alterations in the number and size of islets. These adaptations are programmed and expressed in adulthood thereby sustain the hyperinsulinemic condition in the postweaning period and form the basis for adult-onset obesity. HC females spontaneously transmit the HC phenotype (chronic hyperinsulinemia and adult-onset obesity) to their progeny. Collectively, our results indicate that even a mere switch in the nature of the source of calories (from fat rich in rat milk to carbohydrate rich in the HC milk formula) during critical phases of early development in the rat results in metabolic programming of islet functions leading to chronic hyperinsulinemia (throughout life) and adult-onset obesity. This metabolic programming, once established, forms a vicious cycle because HC female rats spontaneously transmit the HC phenotype to their progeny. The results from our laboratory in the context of metabolic programming due to neonatal nutritional experiences are discussed in this review. Exp Biol Med 228:15–23, 2003

Key words: high carbohydrate; early nutrition; islets; insulin; obesity; metabolic programming

The past two decades have seen an explosive increase in the number of diagnosed diabetics worldwide. The worldwide frequency of the occurrence of diabetes is expected to grow at a rate of 6% per annum, with the potential to reach a total of approximately a quarter billion patients by the year 2010 (1). The main force accelerating the increased incidence of diabetes is the staggering occurrence of obesity, the single most effective contributor to the pathogenesis of type 2 diabetes. Mokdad *et al.* (2) have shown that in the year 2000, approximately one in five American adults were obese (~20% of the adult population), and 56% of the American population was over weight (body mass index [BMI] $\geq$ 25 kg/m^2) compared with 45% in 1991 (2). It has been implicated that obese adults are nearly twice as likely to suffer from chronic illness (such as type 2 diabetes, coronary heart diseases, dyslipidemia, hypertension, glucose intolerance, insulin resistance, etc.) than adults of normal weight, making obesity a high risk factor for chronic illness. It is now apparent that genetic predisposition, sedentary life styles, and high caloric food intake alone do not satisfactorily account for the sudden boom in its occurrence.

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[2] To whom requests for reprints should be addressed at Department of Biochemistry, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, 140 Farber Hall, 3435 Main Street, Buffalo, NY 14214. E-mail: mspatel@buffalo.edu

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Epidemiological studies have suggested that metabolic programming is one of the critical factors contributing to the etiology of obesity. Metabolic programming is the phenomenon whereby a nutritional stress/stimulus applied during critical periods of early development permanently alters an organism's physiology and metabolism, the consequences of which are observed much later in life in the absence of the stress/stimulus that initiated them (3). Metabolic programming is possible because of the organism's potential to modulate "biological switches" when encountering an altered nutritional environment during early periods in life. The immediate goal being survival, this process enables the organism to adapt to this altered environment during critical periods of development, but once these biological switches are programmed, the physiology of the organism is permanently altered and so too are its responses to various stimuli later in life.

Human epidemiological and animal studies provide supporting evidence for the phenomenon of metabolic programming. The results from several human epidemiological studies emphasize the importance of adequate nutrition during fetal development. The fetal origins hypothesis proposed by Barker (4) suggests that disproportionate size at birth of the newborn due to an adverse intrauterine environment correlates well with increased risk of adult-onset ill health outcomes (type 2 diabetes, hypertension, and cardiovascular diseases). In animal studies, McCance (5) demonstrated that metabolic programming could be induced by early nutrition. By adjusting litter size in rats such that pups in small litters received more milk than pups in large litters, he showed that lifetime growth trajectory was programmed by just 3 weeks of such a dietary intervention (5). Malnutrition induced by either protein restriction or caloric restriction during gestation and lactation in the female rat resulted in metabolic programming of several target organs in the offspring, including pancreatic islets, hypothalamus, liver, and muscle, and were accompanied by adult-onset diseases in those offspring (6–9). Hyperglycemia, even of a mild degree, during pregnancy has been shown to cause glucose intolerance in the progeny (10).

Most of the studies cited above deal with an altered intrauterine environment (mostly malnourishment), resulting in the altered growth pattern of the fetus and subsequently in adult-onset diseases. Experimental difficulties in rearing newborn pups away from their dams limit investigation with nutritional modifications during the suckling period. The artificial rearing technique as described by Hall (11) circumvents this difficulty and enables one to rear rat pups on a modified milk formula during the suckling period. This technique has been adapted in our laboratory to evaluate the consequences of a switch in the nature of calories from fat-rich rat milk to carbohydrate-rich (high carbohydrate [HC]) formula during the suckling period (Fig. 1) (12).

Our results, as described below, show that a mere change in the quality of nutrition without affecting total caloric intake in the immediate postnatal period (suckling period) causes metabolic programming. In the rat, the suckling period constitutes an important phase of pancreatic ontogeny (13). The overlap of the critical window of pancreatic development with the HC dietary intervention in this model results in several adaptive changes in the islets of these rats. During the suckling period in the rat, the major source of calories is fat and hence insulin secretion is suppressed in mother-fed (MF) rat pups. The increase in carbohydrate-derived calories in the HC formula increases the demand for insulin in neonatal rats raised on such a formula, and compensatory adaptations occur in islets to meet this immediate demand to facilitate the development of these pups on this diet. However, because these adaptations occur during the critical postnatal development period of the pancreas, they are programmed and are expressed throughout life with adverse consequences in adult life (14).

The HC Rat ("Pup in a Cup") Model for Neonatal Dietary Modification

Artificial rearing of 4-day-old rat pups via intragastric feeding (pups have no further access to their dams) on an HC milk formula (caloric distribution in HC milk formula: carbohydrate 56%, protein 24%, and fat 20%; and in rat milk: carbohydrate 8%, protein 24%, and fat 68%) results in the immediate onset (within the first 24 hr) of hyperinsulinemia that persists into adulthood despite withdrawal of the nutritional stimulus on postnatal Day 24 (12, 15). After an increase in the growth rate from Day 55 onward, full-blown obesity is evident from approximately Day 100 (15). In contrast, rats fed a high-fat formula (caloric distribution: carbohydrate 8%, protein 24%, and fat 68%), a control group for the artificial rearing protocol, did not develop hyperinsulinemia or adult-onset obesity. Thus, the evidence indicates that the artificial rearing per se of neonatal rats does not induce metabolic programming (15). Figure 2 briefly summarizes the major adaptive changes in the HC rat model.

This review will summarize the findings from our studies at three stages: immediate adaptations (focusing on changes in 12-day-old HC rats during the preweaning period when the dietary modification is in progress), consequences in adult life (focusing on changes in 100-day-old HC rats), and generational effect (focusing on changes in the HC rat progeny; second generation HC rats).

Immediate Adaptations

At the physiological level, hyperinsulinemia is evident within 24 hr after the pups are fed the HC milk formula and persists during the entire suckling period (12). The plasma insulin concentration is about 6-fold higher in 12-day-old HC rats compared with age-matched MF rats (Fig. 3A) (16). Although the HC pups are hyperinsulinemic during this period, their plasma glucose levels (Fig. 3B) and body weights are not significantly different from those of pups of MF rats (16).

Because hyperinsulinemia is an immediate event in the

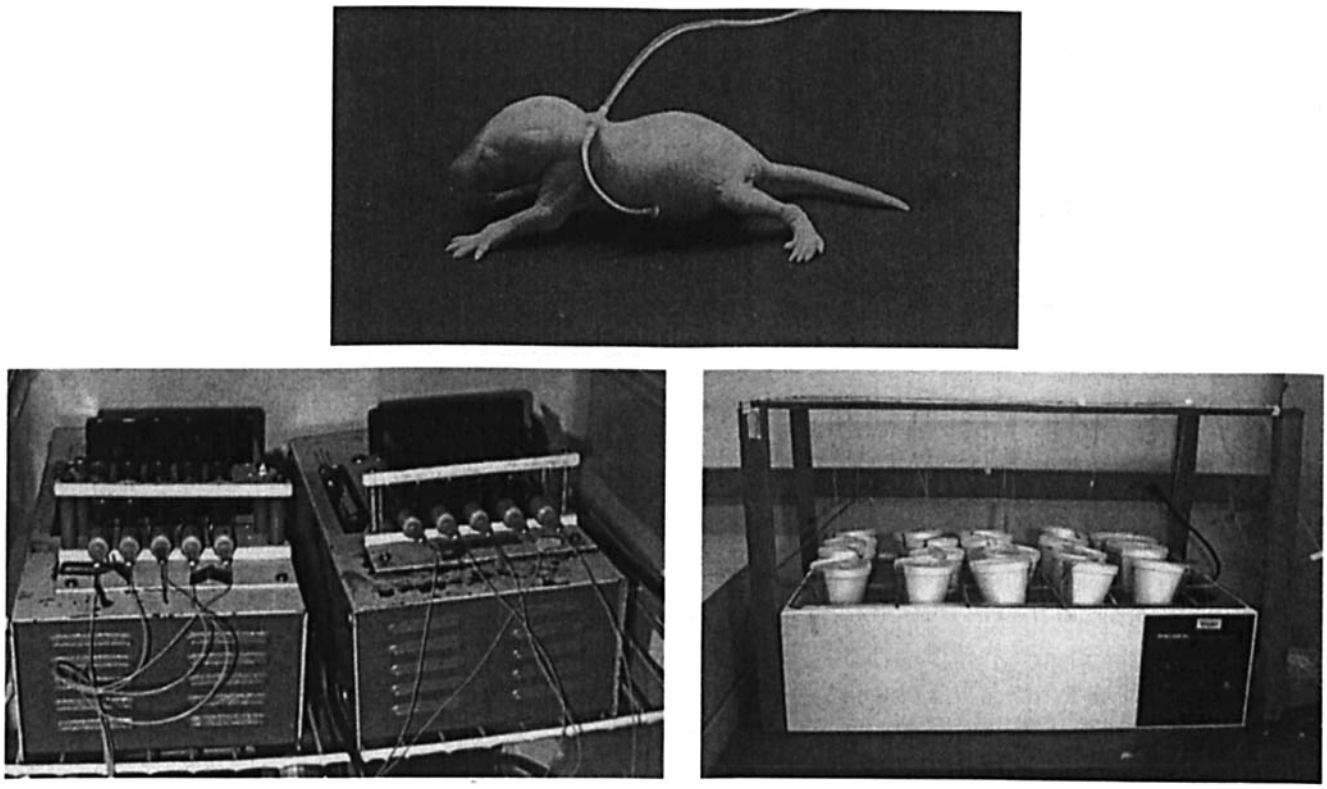

Figure 1. The artificial rearing technique for the neonatal HC rats. The top panel depicts a 4-day-old rat pup after cannulation, and the bottom panel depicts the feeding and housing systems used for the artificial rearing of these pups.

"pup in a cup" model, adaptations that cause and support this condition were investigated in islets isolated from 12-day-old HC rats. Investigation of glucose-stimulated insulin secretion (GSIS) at 1, 2.8, 5.5, and 16.7 m*M* glucose at 10 and 60 min in pancreatic islets isolated from 12-day-old HC and MF rats detected a distinct leftward shift in the response to glucose in HC islets (Fig. 4A) (16). Islets isolated from 12-day-old HC rats secrete significantly more insulin after both 10 and 60 min of glucose stimulation compared with islets isolated from MF rats at each of the glucose concentrations tested (Fig. 4A) (16). There occurs several biochemical correlates of this leftward shift in GSIS. HC rat islets show a significant increase in the low K_m hexokinase activity (increase of 100% and 60% in the supernatant and pellet fractions, respectively) and its protein content in islets from HC rats (16). Significant increases in the content of glucose transporter protein 2 (GLUT 2) and in the activities of glyceraldehyde-3-phosphate dehydrogenase and in the active and total forms of the pyruvate dehydrogenase complex indicate an increased flux through the glycolytic pathway and mitochondrial metabolism in islets from 12-day-old HC rats (16). The upregulation of glucose metabolism,

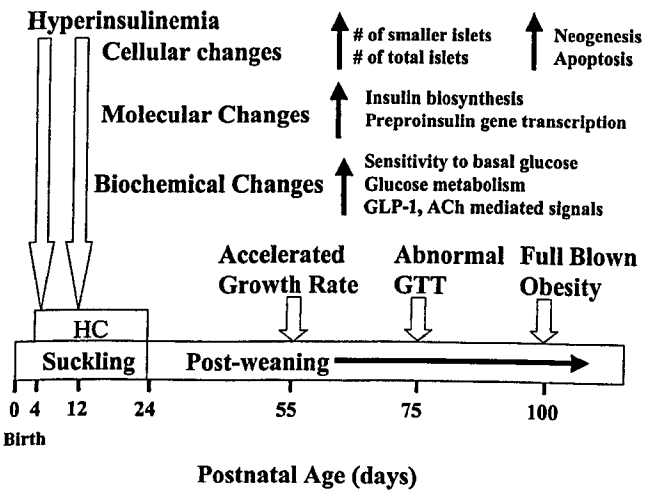

Figure 2. A brief summary of the adaptations in first generation HC rats both during the preweaning (up to postnatal Day 24) and postweaning periods.

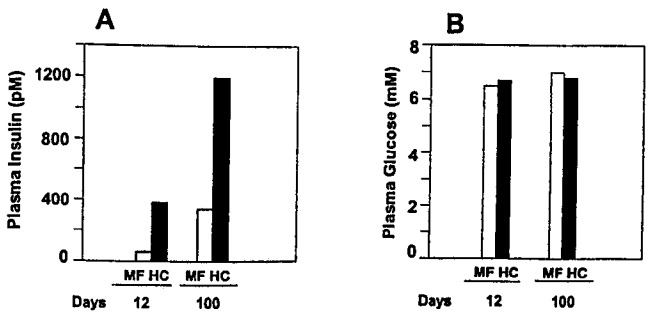

Figure 3. The insulin (A) and the glucose (B) concentrations in the plasma of 12-day-old and 100-day-old HC (male) rats.

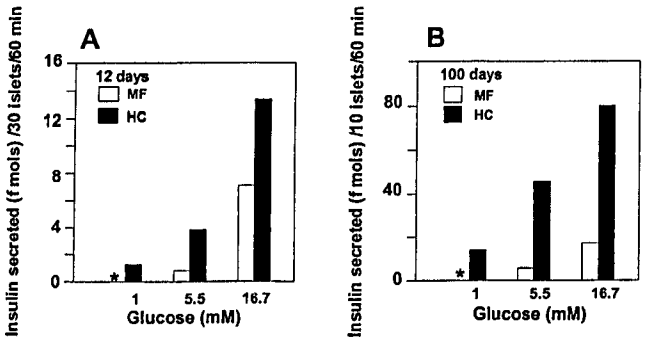

Figure 4. The insulin secretory response to 1, 5.5, and 16.7 m*M* glucose by islets from 12-day-old MF and HC rats (A) and by islets from 100-day-old male MF and HC rats (B). An asterisk indicates insulin secretion not detected.

correlated with the increases in low K_m hexokinase activity and perhaps glucose transport likely contribute to the lowering of the glucose threshold for insulin secretion by HC islets and support, in part, the development of the hyperinsulinemic condition in the HC rat. It appears that the early HC dietary intervention modifies islet function in HC islets such that the response of the HC islets to basal glucose is augmented to the extent that it approximately mimics the response of MF islets to high glucose.

The lowering of the threshold for glucose sensitivity and the increased glucose metabolism do not completely account for the chronic hyperinsulinemic condition (~6-fold higher plasma insulin levels) observed in 12-day-old HC neonates compared with age-matched MF rats. Hence, other primary adaptations that would support the hyperinsulinemic condition were investigated. The pancreatic islet *in vivo* is stimulated not only by glucose, but also by a variety of other agonists including amino acids, fatty acids, and neuroendocrine and incretin factors (17). Hence, it is now widely accepted that in addition to metabolism of glucose resulting in an increase in intracellular Ca^{2+} concentrations and exocytosis of insulin (K_{ATP} channel-dependent pathway), signals derived from factors such as acetylcholine (ACh), pituitary adenylate cyclase activating polypeptide (PACAP), glucose-dependent insulinotrophic peptide, glucagon-like peptide-1 (GLP-1), etc., act in synergy to augment insulin secretion (K_{ATP} channel-independent and Ca^{2+} channel-independent augmentation pathway) (17). In 12-day-old HC islets, several of these factors significantly augment insulin secretion (Fig. 5) (18). GLP-1 and acetylcholine augment insulin release to a larger extent in HC islets (18). Protein kinase C activity is significantly higher in HC

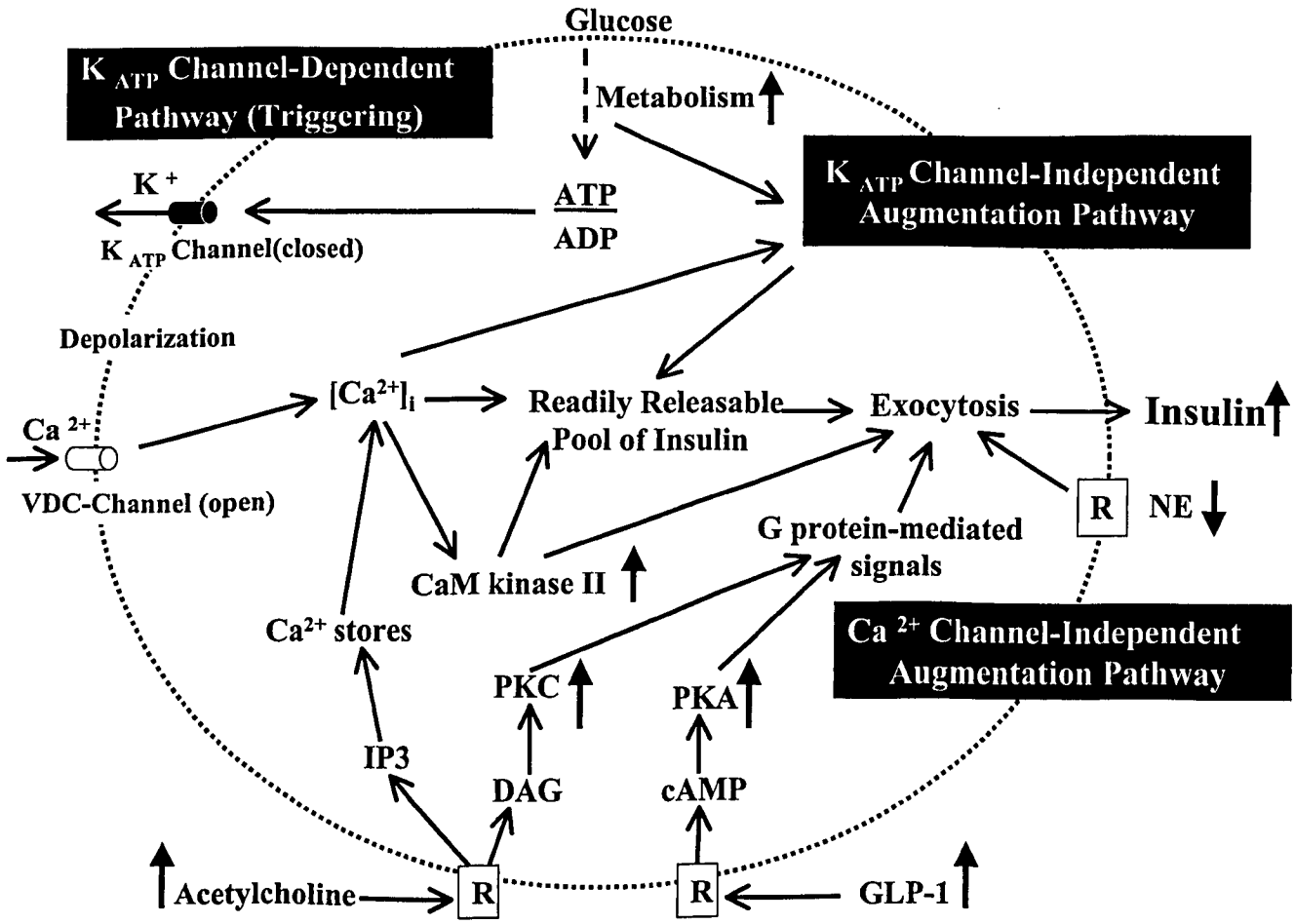

Figure 5. A summary of the role of the signaling pathways induced by nutrient, neuronal, and incretin inputs on insulin secretion by HC islets. R, receptor; IP3, inositol-3-phosphate; DAG, diacylglycerol; NE, norepinephrine; ↑ indicates increase in enzyme activity, mRNA, and/or protein content. (adapted from Refs. 17 and 21)

islets (18). Additionally, levels of adenylyl cyclase type VI mRNA and activities of protein kinase A and calcium calmodulin kinase II are increased in HC islets (18). The augmented insulin release by islets in response to ingested glucose by the incretin effect plays a significant role, enabling coupling of the glucose absorbed and the gastrointestinal tract hormone responses on insulin secretion (19). GLP-1 is one of the principal incretin hormones and promotes insulin secretion by islets via the second messenger cAMP (20). Increased plasma GLP-1 levels and islet GLP-1 receptor mRNA in the 12-day-old HC rat strongly suggest that incretins play a role in the enhanced insulin secretion in this model (Fig. 5) (18).

A balance between signals for stimulation and inhibition of secretion maintains circulating insulin levels. α-Adrenergic receptor stimulation acting through an inhibitory G-protein-coupling mechanism in the β-cell of the islet exerts an inhibitory effect on insulin release (21). In HC islets, a 10-fold increase in norepinephrine concentration is required to inhibit insulin secretion compared with MF islets, indicating reduced sensitivity to adrenergic signals (18).

A unique characteristic of insulin secretion from HC islets is that basal insulin secretion is at least partially independent of metabolic and calcium ion dependency. The HC islets secrete significant amounts of insulin in the absence of glucose and under a simultaneously stringent Ca^{2+}-depleted condition (~1.8 fmol /30 islets /60 min), which is more than three times the amount secreted by MF islets at 5.5 m*M* glucose (18). Under identical conditions, MF islets do not secrete any measurable amount of insulin (18). Such data imply that mechanisms regulating the biochemical and biophysical elements of secretion may be affected during metabolic programming to modulate the docking and fusion of insulin secretory granules. Collectively, the above findings suggest that significant alterations at proximal and distal sites of the insulin secretory pathway in HC islets support the hyperinsulinemic condition of these rats.

At the molecular level, insulin biosynthesis and transcription of the preproinsulin gene in islets are enhanced in islets from 12-day-old HC rats (22). The immediate replenishment of insulin stores to compensate for the insulin secreted occurs via an increase in insulin biosynthesis. Insulin biosynthesis measured by the incorporation of ^{3}H-leucine in freshly isolated islets is markedly increased (~4-fold) in 12-day-old HC rats compared with age-matched MF rats (22). The chronic hyperinsulinemic condition of the HC rats suggests alterations in insulin biosynthetic capacity at the transcriptional level as well. An increase in preproinsulin mRNA levels (~5-fold) in islets from 12-day-old HC rats (22) results, in part, from an increase in preproinsulin gene transcription. Pancreatic duodenal homeobox transcription factor-1 (PDX-1) is an important transactivator of the preproinsulin gene and is an essential component of the mechanisms whereby glucose modulates preproinsulin promoter activity (23). PDX-1 mRNA levels, its DNA binding activity, and protein content are significantly increased in islets from 12-day-old HC rats compared with islets from age-matched MF rats (22). The DNA-binding activity of PDX-1 is modulated by glucose via a phosphorylation cascade involving phosphatidylinositol 3-kinase (PI3 kinase) and stress-activated protein kinase-2 (SAPK-2) (24). PI3 kinase and SAPK-2 contribute to the phosphorylation of PDX-1 (an inactive 31-kDa form in the cytoplasm) to an active 46-kDa form that is translocated to the nucleus, resulting in increased preproinsulin gene transcription (Fig. 6) (24). The significant increase in the mRNA levels of both PI3 kinase (~3-fold) and SAPK-2 (~4-fold) and also in the activity of SAPK-2 (~1.5-fold) in islets from 12-day-old HC rats supports the important role of PDX-1 in the regulation of preproinsulin gene transcription in the HC islets (22). Additionally, upstream stimulatory factor-1 (USF-1) plays a functional role in supporting transcription of the PDX-1 gene (25). The upregulation of USF-1 gene transcription (~2-fold) (22) may also contribute to the increased PDX-1 mRNA level observed in islets from 12-day-old HC rats. These results clearly indicate that the sustained hyperinsulinemia is supported by significant increases in the preproinsulin gene transcription process (Fig. 6).

Recently, several lines of evidence have suggested the possibility of an autocrine action of insulin on β cells (26). Functional insulin receptors and insulin receptor substrates identical to those found in peripheral tissues have been identified in both clonal and primary β cells (27). The mRNA levels of both insulin receptor substrate-1 (IRS-1) and IRS-2 are significantly increased in islets from 12-day-old HC rats (Fig. 7) (28). PI3 kinase is also a downstream component of the insulin-signaling pathway (29). The increase in the gene expression of these factors in HC islets suggests that an autocrine effect of insulin may be an essential component of the mechanism that sustains the hyperinsulinemic condition in the HC rat.

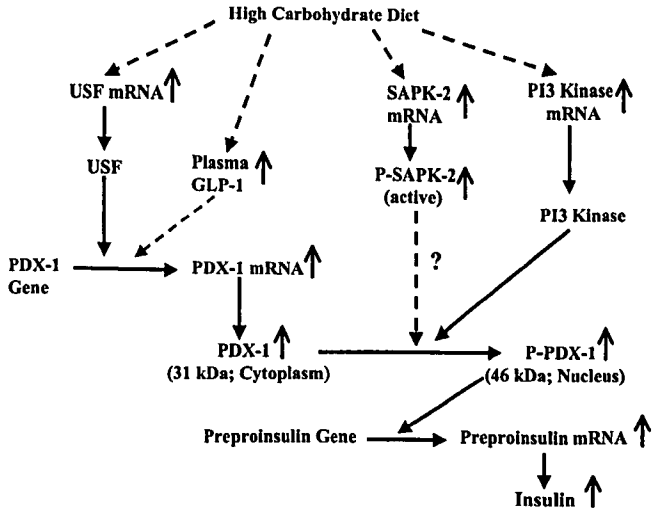

Figure 6. A putative pathway for the regulation of preproinsulin gene expression by the HC dietary intervention in islets of 12-day-old HC rats. The various responses and the interactions between them culminating in increased preproinsulin gene transcription are shown in this figure. ↑ indicates increase in mRNA levels, activity, and/or protein content.

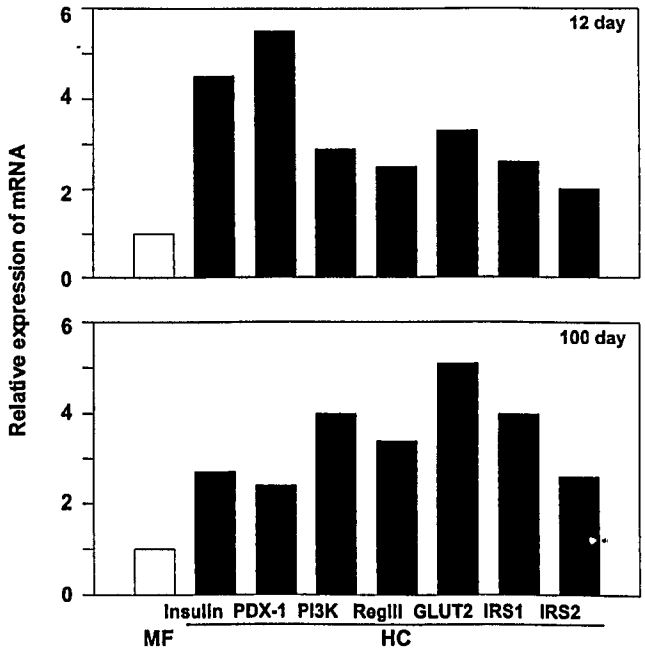

Figure 7. Gene expression patterns of several factors in islets from 12- and 100-day-old HC rats compared to the expression in age-matched MF islets normalized to 1.

Global gene expression changes analyzed by gene array analyses indicate that several clusters of genes involved in a wide array of cellular functions (e.g., cell cycle regulation, protein synthesis, ion channels, and metabolic pathways) are upregulated in HC islets and may contribute to the onset of hyperinsulinemia in HC neonatal rats (28). These results suggest that in addition to the anticipated gene expression pattern (e.g., preproinsulin), changes in the expression of several other genes are also essential for the onset and maintenance of the hyperinsulinemic condition in this rat model (28), and constitute the metabolic programming profile for these islets.

At the cellular level, the HC dietary intervention alters ontogeny of pancreatic islets in neonatal HC rats (30). Immunohistochemical analyses indicates that mean islet size is reduced in HC rats compared with age-matched MF controls (Fig. 8A), although islet number increases markedly throughout the carbohydrate feeding regimen (Fig. 8B) (30) Islets from HC animals also have a significantly increased area occupied by β cells (30). Contributing to the differences in size and islet number between islets from HC and MF rats is a restructuring of the organ. Islets from HC animals have a greater apoptotic rate compared with ductal epithelium, a source of new islets by neogenesis (30). Islet cell replication, measured by insulin and proliferating cell nuclear antigen (PCNA) colocalization showed that approximately 70%–80% of the islet cells staining for PCNA were β cells (30). The expression of islet cell mitogen and survival factor, insulin-like growth factor-II (IGF-II), is reduced in whole pancreas from HC animals (30). An increase in the gene expression of transcription factors (like PDX-1, islet factor-1, β2/Neuro D, hepatocyte nuclear factor β3, regenerating factor-3, etc.) in HC pups probably contributes to the observed altered islet architecture during the period of pancreatic ontogeny, providing the cellular basis for the observed hyperinsulinemia in neonatal HC rats (30; M. Sainivasan, S.G. Laychock, D.J. Hill, M.S. Patel, unpublished observation).

In addition to the changes observed in the islets, there is an increased deposition of glycogen in the liver of HC 12-day-old rats (12). The lipogenic capacity of the liver is also increased in these rats (31). Activities of glucokinase and malic enzyme are 77% and 96%, respectively, of adult levels in the liver of 10-day-old HC rats, suggesting precocious induction of these enzymes by the HC dietary intervention (12).

Consequences in Adult Life

Artificially reared HC neonatal rats weaned onto laboratory chow on Day 24 continue to be hyperinsulinemic into adulthood despite the absence of any dietary intervention (32). In addition, they develop obesity after puberty (both males and females) (33). The growth rate of HC rats is higher than MF rats starting at Day 55, and they are significantly obese on Day 100 (MF male rats at 577 g vs HC male rats at 710 g on Day 100) (33). Although normogly-

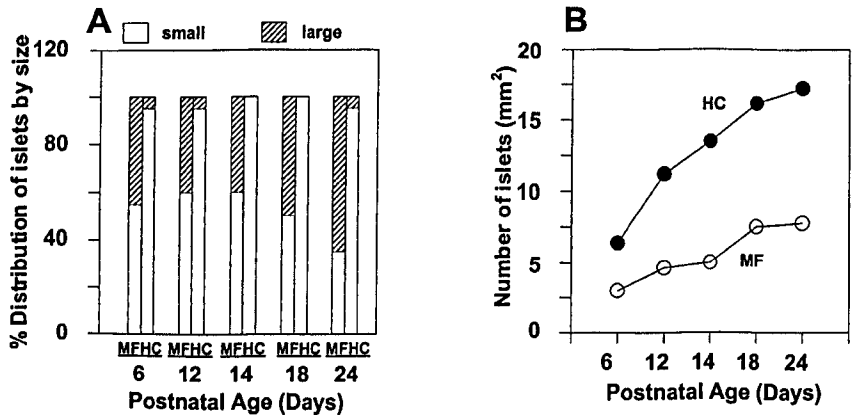

Figure 8. The distribution of islets based on size (A) and the number of islets (B) in pancreas from MF and HC rats from postnatal Days 6 to 24.

cemic, they display an aberrant response to a glucose tolerance test on Days 75 and 270 compared with age-matched MF rats (32).

The 3-fold increase in plasma insulin concentrations of HC (100-day-old) rats compared with age-matched MF rats (Fig. 3A) (34) suggests that the adaptations that occurred in neonatal HC islets are preserved into adulthood and aid in the maintenance of hyperinsulinemia in adulthood. Coincident with the persistence of hyperinsulinemia in adulthood is the marked leftward shift in the insulin secretory response of islets from HC (100-day-old) versus age-matched MF rats (Fig. 4B) (34), and the biochemical correlate of increased low K_m hexokinase activity together with enhanced glucose- and Ca^{2+}-independent insulin release (34). The hyperinsulinemia may also be maintained through the increase in insulin-producing cell mass in the HC pancreas (32). Molecular adaptations in HC islets (100-day-old) include increased preproinsulin gene transcription with concomitant increases in mRNA levels of PDX-1 (34). Gene array analyses in islet mRNA from 100-day-old HC and MF rats suggest significant increases in the expression of several clusters of genes (28). These observations clearly indicate that the early adaptations that occur in islets of neonatal HC rats are programmed and continue to be expressed in adulthood, enabling the persistence of hyperinsulinemia despite the absence of any further nutritional stimulus.

The onset of obesity is aided by the following observations. The organ weights of liver and the epididymal adipose tissue are significantly higher in 100-day-old HC rats (15). The lipogenic capacity of the liver and adipose tissue is significantly increased in HC rats as indicated by increases in the activity of fatty acid synthase and glucose-6-phosphate dehydrogenase (key enzymes in lipogenesis), as well as increased *in vitro* synthesis of lipids (15). In addition, there is a marked increase in the cell size in the epididymal and omental adipose tissue of 100-day-old male HC rats (15).

Generational Effects

A novel observation is that HC females spontaneously transmit the HC phenotype (characteristics of chronic hyperinsulinemia and adult-onset obesity) to their progeny (33). Cross-breeding experiments showed that only HC females could transmit this trait to the progeny, indicating no involvement from the paternal side (S. Vadlamudi and M. S. Patel, unpublished observations). HC females are normoglycemic and hyperinsulinemic during pregnancy and lactation (33). Because the macronutrient composition of the milk of lactating HC and MF females is identical and the control treatment of raising second generation HC (2 HC) pups from the time of birth by foster mothers results in the expression of the hyperinsulinemic phenotype, there is clear evidence that rat milk consumed after birth has no effect on the generational effect observed (33). The plasma insulin levels of second generation HC rats are significantly increased (first observed on Day 45), which may be aided by the basal hyperinsulinemia demonstrated by islets isolated from 100-day-old male and female second generation HC rats (35). As observed in the first generation rats, the growth rate of second generation HC rats parallels that of age-matched MF rats up to postnatal Day 55, but there is an increase in the growth rate of these rats from Day 55 onward, with the onset of obesity by Day 100 (33). Adult-onset obesity correlated with a significant increase in the weight of the epididymal adipose tissue on postnatal Day 100 (33). This is associated with an increase in the cell size in the adipose tissue as well as an increase in the activities of the lipogenic enzymes (fatty acid synthase and glucose-6-phosphate dehydrogenase) in both liver and the adipose tissue of 100-day-old second generation HC rats (33). Liver and muscle glycogen content is reduced in 100-day-old second generation male rats and this is associated with decrease in the activity of glycogen synthase (36). The activities of the enzymes comprising the postulated upstream activators of glycogen synthase are also decreased in liver and muscle of 100-day-old second generation HC rats (36). In the epididymal adipose tissue of these rats, glycogen synthase activity is increased, with a concomitant increase in the activities of its presumed upstream activators (37).

Potential Mechanisms of Metabolic Programming

Programming occurs when an early stimulus or insult overlaps with the sensitive window of development of specific organs during early phases of life, resulting in a permanent alteration in the physiology and metabolism of target organs. Thus far, no direct mechanisms for metabolic programming are available. Potential mechanisms have been postulated and discussed by Lucas (3) and Waterland and Garza (38). Structural alterations due to an altered dietary experience have been demonstrated in rats (8). The occurrence of hyperinsulinemia during periods of brain development has been shown to result in disorganization of the hypothalamus causing adult-onset disease conditions (10). The results from the HC rat model show that during the neonatal period, adaptations occur in pancreatic islets (hyperinsulinemia), gut (increased GLP-1 levels), and possibly the hypothalamus in 12-day-old HC rats reared on the HC formula. These organs are potentially important for maintaining glucose levels, suggesting that there is cross-talk between these organs in response to the HC formula. The question also arises as to how these rats remain normoglycemic in the face of such high-circulating insulin levels. The onset of insulin resistance in peripheral tissues may be a possible mechanism. Because hyperinsulinemia is an immediate event (occurs within 24 hr), it appears that islets may be an initial target and the resulting hyperinsulinemia evokes compensatory responses in several other organs, suggesting a multifactorial mechanism for the onset and persistence of hyperinsulinemia in the HC rat. Hyperinsulinemia eventually results in insulin resistance, leading to obesity in adulthood. These conditions finally culminate in the onset of the metabolic syndrome.

The persistence of the HC phenotype in adulthood and its transmission to the next generation may be due, in part, to epigenetic mechanisms. Epigenetic mechanisms can be triggered by changes in the environment and can occur in both somatic and germ cell lineage during development (39). Altered DNA methylation patterns have been reported to be caused by protein and folate deficiency (40). Nutritional alterations early in life may induce altered cell-specific DNA methylation patterns, causing changes in gene expression patterns in specific tissues. Such altered DNA methylation patterns can be transmitted to the daughter cells by replication facilitating immortalization of the initial alterations.

Possible Relevance to the Human Obesity Epidemic

Epidemiological studies suggest that under- or overnourishment during early periods of life via metabolic programming lead to the development of adult-onset diseases (4). The "fetal origins hypothesis" as proposed by Barker (4) indicates the importance of adequate nutrition during fetal development. The results from the HC rat model suggest that the quality of postnatal nutrition is also critical and can cause metabolic programming. It is clear from our results that a mere change in the source of calories (a switch from fats to carbohydrates) without affecting total caloric intake during critical periods of organogenesis can also cause metabolic programming, resulting in pathological conditions in adulthood (14).

At present, the exact mechanisms of the progression of obesity from the time of its beginning are not clearly understood. What are the hormonal and metabolic adaptations that occur in the individual prior to the manifestation of obesity? What are the early critical factors in target tissues that prime the organism for the eventual onset of this condition? Obviously, it is a very difficult proposition to decipher these factors from human studies. In the HC rat model, hyperinsulinemia is the initial event and this event occurs very early in postnatal life, but the visible onset of obesity does not begin until Day 55. Hence, the model provides a unique opportunity to evaluate the underlying mechanisms involving adaptations in target organs and the cross-talk that occurs between them starting from the onset of hyperinsulinemia in the suckling period to the manifestation of full-blown obesity in adult life.

In the light of the results of the HC rat model discussed in this review, it is tempting to speculate that the changes in the weaning practice (formula feeding combined with early introduction of infant foods such as juices, fruits, cereals, etc.) for infants over the past several decades contribute to the observed epidemic of obesity in the United States. This, however, remains to be investigated.

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