Metabolic programming by nutrition during early development

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Incidence of obesity and diabetes is increasing at an alarming rate not only among the populations of the affluent nations but also amongst the populations of the developing nations. Understanding the mechanisms that cause the onset of these pathological conditions is a requisite to effectively tackling this problem. In this context the role of early nutritional experiences as a causative factor is being extensively investigated. This article briefly reviews the field of metabolic programming vis-a-vis an altered nutritional milieu during perinatal period and consequent adaptive metabolic patterning and metabolic imprinting in adult and/or consequent offspring.

Pioneering studies in rats by McCance and Widdowson and subsequent other studies in animals established that dietary experiences during the early phases of life have permanent effects on growth, body composition, brain function and metabolism in the adult. In addition to the emphasis on meeting nutritional requirements the effect of nutrients on health including adult degenerative diseases, cancer and cognitive function are being extensively examined in humans. Vulnerable periods exist during the early phases of life (fetal, infancy and childhood) when the quality as well as the quantity of specific nutrients influence cellular and metabolic processes. These influences have implications for long-term health consequences even in humans.

With the recognition that both animals and humans experience long-term effects of early life nutritional experiences, an attempt has been made in this review to discuss briefly about metabolic adaptations during development in model systems subjected to an altered nutritional milieu during prenatal and/or immediate postnatal life and possible mechanisms responsible for programming these modifications into adult life and into the next generation.

Adaptations to nutritional alterations induced during normal early development—Fetal and neonatal growth regulation is a complex process controlled by the interactions amongst genetic, nutritional and environmental factors. The most influential factor during fetal and neonatal life limiting growth potential is probably substrate supply. Figure 1A depicts the appearance of enzymatic clusters that appear during fetal, suckling and weaning periods in the rat liver in parallel with the changes in the composition of the diet that the animal receives during each of these periods. During pregnancy the nutrition of the fetus is dictated by the supply of nutrients via placenta and correlates, for example with the synthesis of glycogen and lipogenesis in the rat liver (Fig.1A). At birth, placental supply of nutrients ceases abruptly and the mode, composition and frequency of the nutrient supply are drastically modified as breast milk becomes the sole source of diet during the early suckling period. In most species, this milk is a high-fat, low-carbohydrate diet. This change necessitates rapid metabolic adaptations in carbohydrate metabolism (gluconeogenesis) to maintain glucose homeostasis in the newborn (Fig. 1A). Upon weaning, the young ones are switched from maternal milk to a diet high in carbohydrate-derived calories resulting in the expression of several enzymes in cluster III (Fig. 1A). In most mammals, the prenatal-postnatal transition and the suckling-weaning transition require important metabolic adaptations in carbohydrate metabolism due to changes in altered nutritional sources (Fig. 1A). Figure 1B indicates the adaptive changes in rat liver when the natural program of rat milk is replaced by a high-carbohydrate milk formula during the suckling period. In this case, the cluster III enzymes appear prematurely and cluster II enzymes are significantly reduced (Fig. 1B).

Altered nutritional experience during the prenatal period—Development of fetus is vulnerable to alterations in the intrauterine nutritional environment. Hales and Barker have reported that poor maternal and fetal nutrition affect development of structure and function of a variety of organs. Consequently,
metabolism in various organs is permanently changed or programmed. Chronic degenerative diseases such as ischemic heart disease, diabetes and hypertension are associated with derangements in fetal development. Both men and women with low birth weights are prone to diabetes and hypertension in adulthood. Children with syndromes of protein malnutrition such as Kwashiorkor exhibit glucose intolerance and reduced insulin secretory responses during adulthood.

Protein restriction during pregnancy and lactation in rats has been studied extensively to analyze the metabolic programming effects of early malnourishment. The following observations on protein deprivation during pregnancy indicate that this treatment has several implications for the pancreas of the offspring—(i) morphological alterations detrimental to development of β cells leading to permanent insulin deficiency and functional changes in islets of offspring with consequences in adulthood; (ii) alterations in vascularization of islets; and (iii) changes in β cell balance between replication and apoptosis, contributing to impaired insulin release in adult life. Offspring born to mothers on a low-protein diet and nursed by these mothers had decreased glucokinase activity in their pancreas at 6 weeks and even at 3 months of age.

Protein deprivation during gestation, lactation and weaning has variable effects on pancreatic protein and DNA content. These reports indicate that early development of pancreas is sensitive to availability of amino acids and that alterations in availability of these amino acids (for example, by a low-protein diet during gestation) influence functional capacity of pancreas in adult life.

In addition to inducing changes in pancreas,
maternal low protein diet profoundly affects glucose metabolism in liver of offspring. For example, the activity of glucokinase is decreased by ~50% and the activity of phosphoenolpyruvate carboxykinase is increased by ~100% in these rats. Glucokinase is localized in the perivenous zone while phosphoenolpyruvate carboxykinase is localized in periporal zone of the liver. Differences in the activities of these two enzymes indicate that differential replication of perivenous and periporal activities of these two enzymes indicate that cell are induced by altered nutritional milieu to which the offsprings have been exposed during gestation.

Other adaptive changes in the progeny of mothers on a low-protein diet during pregnancy are—(i) low birth weight; (ii) reduction in the number of renal glomeruli which are not normalized even when the pups are subsequently raised on normal diet after birth; (iii) alterations in brain functions in adulthood of these offspring due to maldevelopment of vascularization of cerebral cortex; (iv) lower levels of insulin and IGF-1 in such fetuses which may regulate blood vessel formation, an increase in the placental passage of glucocorticoids, which are potent inhibitors of angiogenesis, and a programmed reduction in plasma triglycerides, HDL and LDL cholesterol in adults of such offsprings.

Abnormal intrauterine environment of the diabetic mother has serious consequences on development of the fetal endocrine pancreas as well as the entire fetal metabolism. Increased fetal insulin secretion in response to maternally derived metabolic fuels in diabetic pregnancy causes fetal macrosomia. Hyperglycemia especially in the third trimester of pregnancy significantly alters the development of the endocrine pancreas. Vulnerability of an animal to gestational diabetes is associated with its own exposure to an abnormal intrauterine environment during early development. There is an increased risk for the onset of non-insulin-dependent diabetes in the offspring when the mother has non-insulin-dependent diabetes. Adult offsprings of mothers, which experience a hyperglycemic condition by continuous glucose infusion during last week of pregnancy, have significantly increased basal glucose levels and normal basal insulin levels compared with control. Furthermore, basal hyperglycemia and impaired glucose-induced insulin secretion persist in these rats at age 8 months. Clinical and experimental studies indicate that hyperinsulinemia is a frequent phenomenon in fetuses and neonates of mothers with gestational diabetes even of a mild degree. Perinatal hyperinsulinism has been reported to be a predisposing factor for adult onset diabetes mellitus, obesity and cardiovascular diseases. Malorganization of hypothalamic-pancreatic axis due to persistent hyperinsulinemia during fetal/neonatal periods has been suggested to contribute to life long susceptibility to diabetes.

Altered dietary experience in early postnatal period—Although it seems improbable that early dietary experiences could predispose to conditions that have their onset mainly in adulthood, several studies have shown that both pre- and post-natal nutrition influence adult outcomes. Critical window for intervention depends on the outcome being studied. Examples cited herein indicate the consequences of early postnatal dietary experiences on adult metabolic programming. McCance has demonstrated that reduction of litter size in rats (implying increased availability of milk during the suckling period) permanently altered growth trajectory of these rats thereby indicating that the suckling period is a critical window for growth programming. It has been shown in rats that nutrition at a critical period of brain development permanently affects brain size, brain cell number, behaviour, learning and memory. Nutritional alterations in baboons during infancy result in adult-onset manifestations such as obesity and alterations in lipid metabolism. Undernutrition in early life may program accelerated aging and predispose to a variety of age-related diseases. Overfed neonatal rats exhibit increased insulin and cholesterol levels in adulthood. Obesity present in childhood increases the likelihood of adult morbidity and mortality.

Pup-in-the-cup model: A new experimental approach—Experimental difficulties limit implementation of alterations in the composition of macronutrients of rat milk during suckling period. The pup-in-the-cup model provides a unique opportunity to artificially raise rats on a tailor-made milk formula during suckling period. This technique provides an opportunity to study the effect of specific nutrient, qualitatively and/or quantitatively. By introduction of cannulas through gastrostomy, the pups can be reared artificially on desired milk formula, away from their natural mothers. This technique has been successfully used in our laboratory to investigate the effects of a modified milk formula on metabolic adaptations in neonatal rats. In this formula the composition of macronutrients is changed...
during that did not undergo any nutritional intervention.

Circulating insulin levels are significantly higher in HC rats compared to rat milk formula, there is an immediate onset of hyperinsulinemia which persists throughout the suckling period (postnatal days 4-24); Figure 2A. When four day old pups are artificially raised on a high-carbohydrate (HC) milk formula, there is an immediate onset of hyperinsulinemia which persists throughout the suckling period (postnatal days 4-24); Figure 2A. Because the suckling period is a critical phase in the development of the endocrine pancreas and because the dietary modulation in HC rat overlaps this ontogenic period, adaptive modifications in the development and function of endocrine pancreas in these rats can be expected. Morphometric and immunohistochemical analyses indicate an increase in the insulin-producing mass and prevalence of a significantly increased number of islets in HC rats compared to age-matched mother-fed control (Fig. 2A). Islets isolated from 12 day old HC rats indicate significant alterations in insulin secretory capacity. In HC islets, increased glucose transport and metabolism are indicated by significant increase in contents of GLUT-2 protein and activity of glycolytic enzymes. HC islets from 12 day old rats secrete insulin in the absence of stimuli and under stringent Ca$^{2+}$-depleted conditions, indicating that components of insulin secretory mechanism support basal hyperinsulinemia in these rats. Despite of these metabolic adaptations, HC pups remain normoglycemic and grow normally during this period and continue to grow normally until day 55 (Fig. 2A).

Metabolic patterning in post-weaning period as a consequence of high-carbohydrate (HC) diet during suckling period—In HC rats hyperinsulinemia persists into adulthood despite weaning onto lab chow on day 24 (Ref. 44). Plasma glucose levels continue to be normal although an abnormal oral glucose tolerance test is seen around day 75 (Fig. 2A). There is an increase in growth rate from day 55 onward with a gradual increase in food consumption and distinct obesity by day 100 (25% heavier than age-matched MF rats); (Fig. 2A). Lipogenic capacities of liver and adipose tissue increase in 100 day old HC rats. Islets isolated from 100 day old HC rats exhibit an increase in insulin secretory capacity at a lower glucose concentration indicating that adaptive changes seen in glucose-mediated insulin secretion in islets from 12 day old HC rats persist into adulthood.

Second generation animals (Metabolic imprinting)—Figure 2B is a summary of changes observed in the progeny of females that received high-carbohydrate milk formula during suckling period. It has been observed that the females raised on HC formula during suckling period spontaneously transmitted hyperinsulinemic phenotype to their progeny (Fig. 2B). By selective breeding experiments it has been demonstrated that only females can transmit these characteristics (unpublished observations). The plasma hormonal profiles of the second-generation HC pups resemble those of their age-matched controls during suckling period, but within 48 hr after weaning on day 24 the second generation HC rats exhibit hyperinsulinemia (unpublished observations). The second-generation adult rats exhibit characteristics that nearly match the features of

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**Fig. 2**—The pup-in-the-cup model—(A) immediate and long-term metabolic adaptations to a high-carbohydrate milk formula given only during the suckling period in the rat (effects seen in the same generation receiving the nutritional intervention); (B) metabolic and cellular effects transmitted to successive generations by female rats which received a high-carbohydrate milk formula during their suckling period (effects seen in the next generation that did not undergo any nutritional intervention).
PATEL et al.: METABOLIC PROGRAMMING BY NUTRITION

Metabolic programming by nutrition and adaptive responses.

#### ADAPTIVE RESPONSES

- Nutritional stimulus
  - (prenatal and immediate postnatal periods)
- Signaling molecules
  - (metabolites, hormones, growth factors, neurotransmitters, etc.)
- Transcription factors
- Specific kinases
- Gene transcription
- Modifications in cellular growth and metabolic processes in target organs during critical ontogenic windows during their development.

#### METABOLIC PATTERNING

- Metabolic patterning in adulthood occurs due to persistence of early adaptive responses by the process of transmission of information to daughter cells.
- Metabolic patterning in association with adult-onset factors, for example, sex hormones, leads to further changes in cellular and metabolic processes culminating in diseased states such as obesity, diabetes, hypertension, cardiovascular diseases, etc.

#### METABOLIC IMPRINTING

- Metabolic imprinting is the process by which the early adaptive responses and adult onset conditions in affected animals (first generation) are spontaneously transmitted to the progeny without themselves having to undergo any nutritional intervention in their postnatal life. The intrauterine environment may be responsible for alterations in cell-specific patterns of gene regulatory proteins, chromatin structure, DNA methylation patterns and epigenetic mechanisms which have been implicated as being causative factors for this transmission to succeeding generations.

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**Fig. 3**—A summary of the potential mechanisms that may account for metabolic programming of a dietary experience in early life.

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The first generation adult HC rats such as persistent post-weaning hyperinsulinemia and the development of obesity starting around day 55 (Ref. 47). Tissue-specific alterations in signaling cascade involved in the activation of glycogen synthase in liver, muscle and adipose tissue of 100 day old second-generation rats have also been observed.

**Mechanisms responsible for metabolic patterning and imprinting.**—The question arises how early life experiences are memorized and expressed later in adult life after the withdrawal of the initial nutritional stimulus/insult. Several hypotheses have been proposed.

Because metabolic differentiation represents the process whereby individual cells develop stable quantitative patterns of basal and inducible gene expression, it becomes an important target for adaptations due to an altered dietary environment during early phases of life. Hormones and/or growth factors are potent mediators of the response to an altered nutritional status (Fig. 3). Effects occur at many levels including secretion, binding proteins, cognate receptors, responses to specific stimuli and tissue sensitivity and these changes contribute to adult onset manifestations. For example, increased energy availability increases hepatic growth hormone receptor mRNA abundance, which increases IGF-I levels and growth. Under the conditions of undernourishment, growth hormone receptor mRNA levels increase in muscle, thereby increasing fatty acid oxidation and limiting glucose utilization.

Nutrition can influence expression of those hormone receptors, (e.g. glucocorticoid and thyroid hormone receptors) which act as nuclear transcription factors. They have the potential to influence the expression of a vast array of genes involved in growth, development and differentiation (Fig. 3). These developmentally programmed events are tissue- and cell-specific. For example, growth hormone receptor is more abundant in muscle than in liver at a very early stage in development and hence a given circulating concentration of growth hormone may evoke different responses in these tissues during fetal and postnatal life. Specific nutrients can influence gene expression directly. For example, long-chain polyunsaturated fatty acids are potent inhibitors of the expression of lipogenic enzyme genes. Role of glucose in transcriptional regulation of several genes in liver and β cells is well known.

In addition to cellular effects, changes in organ structure are also be responsible for metabolic programming and imprinting. These changes include gross morphological alterations such as vascularization, innervation, etc. or changes in cell
number (hypertrophy and/or hyperplasia). Alterations in nutrition during early life and consequent effects on brain differentiation may contribute to persistent metabolic disturbances seen in the adult. For example, modifications of set points in the hypothalamus and subsequent alterations in the regulation of appetite, growth, etc. can be initiated by early nutritional interventions.

Lucas has proposed that nutritional changes in early life can affect several aspects singly or in combination and contribute to metabolic programming. These aspects include—(i) altered growth process due to reduced or increased availability of specific nutrients during critical ontogenic periods, (ii) permanently altered gene expression in target tissues, (iii) altered clonal selection (e.g. if the nutritional environment is deficient in structural fatty acids, cells with a slightly more efficient or more active lipogenic pathway can disproportionately populate a tissue), and (iv) changes in cell number. Waterland and Garza have suggested that alterations in metabolic differentiation can be transmitted into adulthood and progeny by (i) autoregulatory patterning of DNA-binding proteins, (ii) modulations in chromatin structure, and (iii) alterations in DNA methylation patterns (Fig. 3).

Concluding remarks

Adaptation to an altered nutritional milieu is a natural mammalian response to an adverse situation to aid survival. But it appears that these changes may prove detrimental when the organism is subsequently exposed to a normal or supranormal nutrition. Both pre- and post-natal nutrition influence adult outcomes in animals as well as in humans. The adaptations are both tissue- and cell-specific; the overlap of the nutritional stimulus or insult with critical windows of development determines the outcome. These effects may contribute to chronic diseases of adult. Knowledge of mechanisms underlying these effects (memory of early life experiences and its expression at a later period in life) is likely to provide clues to causes and ultimate long-range prevention of several adult-onset pathological conditions. In the context of increasing incidence of diseases like obesity and diabetes in developed and developing nations, the present subject could prove to be a promising topic for future research in this newly emerging area of metabolic programming.

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