Prenatal Programming and Epigenetics in the Genesis of the Cardiorenal Syndrome

Ravi Nistala\textsuperscript{a} Melvin R. Hayden\textsuperscript{a} Vincent G. DeMarco\textsuperscript{a,b} Erik J. Henriksen\textsuperscript{d} Daniel T. Lackland\textsuperscript{e} James R. Sowers\textsuperscript{a–c}

\textsuperscript{a}University of Missouri Diabetes Cardiovascular Center, \textsuperscript{b}Department of Physiology and Pharmacology, and \textsuperscript{c}Harry S. Truman VA Medical Center, Columbia, Mo., \textsuperscript{d}Department of Physiology, University of Arizona College of Medicine, Tucson, Ariz., and \textsuperscript{e}Department of Neurosciences, Medical University of South Carolina, Charleston, S.C., USA

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Abstract
The presence of a group of interacting maladaptive factors, including hypertension, insulin resistance, metabolic dyslipidemia, obesity, and microalbuminuria and/or reduced renal function, collectively constitutes the cardiorenal metabolic syndrome (CRS). Nutritional and other environmental cues during fetal development can permanently affect the composition, homeostatic systems, and functions of multiple organs and systems; this process has been referred to as ‘programming’. Since the original formulation of the notion that low birth weight is a proxy for ‘prenatal programming’ of adult hypertension and cardiovascular disease, evidence has also emerged for programming of kidney disease, insulin resistance, obesity, metabolic dyslipidemia, and other chronic diseases. The programming concept was initially predicated on the notion that in utero growth restriction due to famine was responsible for increased hypertension, and cardiovascular and renal diseases. On the other hand, we are now more commonly exposed to increasing rates of maternal obesity. The current review will discuss the overarching role of maternal overnutrition, as well as fetal undernutrition, in epigenetic programming in relation to the pathogenesis of the CRS in children and adults.

James R. Sowers, MD
D109 HSC Diabetes Center
One Hospital Drive
Columbia, MO 65212 (USA)
Tel. +1 573 882 0999, E-Mail sowersj@health.missouri.edu
Introduction

The fetal/developmental origin of the disease hypothesis originally proposed by Barker and Osmond [1] and Barker [2] presaged numerous clinical and basic science observations implicating fetal undernutrition and/or low birth weight and associated epigenetic influences in the development of common chronic diseases such as hypertension, kidney disease, cardiovascular disease (CVD), liver disease, diabetes, and other metabolic abnormalities (fig. 1; table 1) [1–32]. However, emerging data suggest that persons who are exposed to adverse in utero conditions do not have to manifest low birth weights to develop adult chronic medical conditions [14, 33]. Indeed, recent studies have demonstrated strong associations between adult metabolic disorders and neonatal adiposity, and maternal malnutrition in addition to birth weight [33–36]. Further, since overweight and obesity have grown to pandemic proportions in industrialized countries during the past decade, it is of increasing relevance that children whose mothers are obese are at increased risk for developing metabolic disorders, including insulin resistance, diabetic dyslipidemia, hypertension, and kidney disease, all components of the cardiorenal metabolic syndrome (CRS; table 2) [32, 37–43]. Finally, recent evidence has indicated [44] that female rats can develop a diabetic phenotype due to a high fat content in their father’s diet before they were conceived. It has been suggested that epigenetic modifications of the paternal sperm DNA may underlie this interesting observation. Thus, epigenetic influences can emanate from both parents during the perinatal period.

Epigenetic programming is mediated by molecular factors around DNA that alter gene expression independently of the DNA sequence [33]. Epigenetic modifications include DNA methylation, posttranslational histone modifications, and non-coding RNA-mediated silencing processes. DNA methylation patterns are predominantly affected in, and transmitted through, the germ cell line. DNA methylation involves the covalent addition of a methyl group to the 5' position of cytosines within the CpG dinucleotides. This methyl group, which extends into the DNA, can recruit protein complexes that alter chromatin compaction or displace transcription factors and thus silence involved genomic regions. Posttranslational histone modifications, such as methylation, acetylation, and phosphorylation, covalently modify the N-terminal tail of histones and modulate chromatin condensation. RNA silencing is mediated by small, non-coding 19- to 25-nucleotide-long RNA molecules, termed microRNAs, which target up to one third of mRNAs in humans and negatively modulate gene expression.

The early postconceptional period is a seminal time for establishment of DNA methylation patterns. Indeed, the methylation profile of the genome is reprogrammed during early embryogenesis [45]. After fertilization, rapid demethylation changes in the paternal genome take place, except in paternally imprinted genes, heterochromatin around some centromeres, and some repetitive elements [33]. Differential timing of remethylation during gametogenesis may have implications for the relative paternal versus maternal epigenetic effect on the phenotype of the offspring. Animal models representing epigenetic origins of chronic metabolic disease have generally employed maternal or paternal alterations in nutrition (energy or protein intake) during the pre- and postconceptional period. For example, feeding a low-protein diet or creating utero-placental insufficiency in pregnant rats causes DNA methylation responses, which manifest as endothelial dysfunction, increased AT1R (angiotensin II receptor type 1) expression in the kidney and adrenal gland, hypertension, reduced tissue expression of the membrane transporter GLUT4, and reduced numbers of pancreatic β cells and renal glomeruli in the progeny (table 1) [33, 46, 47].

There are considerable data suggesting that epigenetic changes associated with maternal malnutrition and stress are associated with fetal programming that promotes obesity,
insulin resistance, and diabetes [33]. Intrauterine growth restriction and/or low birth weight followed by accelerated growth and weight gain in infancy and childhood are associated with an increased risk of insulin resistance, obesity, diabetes, and renal and cardiovascular disease (fig. 1) [33, 48–61]. In this regard, the periconceptional period is one of substantial sensitivity to suboptimal nutrition, which can also lead to adverse epigenetic responses without causing any change in birth weight. Maternal and paternal overnutrition can also lead to transgenerational amplification of obesity in animal models and man [33]. In a model particularly relevant to contemporary western societies, a maternal high-fat diet in primates leads to impaired lipid metabolism in the fetus in association with increased histone acetylation and decreased histone deacetylase activity [62]. Moreover, these epigenetic changes may occur independent of maternal quantitative nutrient intake via alterations in cofactors and substrates of DNA methylation [62]. For example, in a study...
in sheep, periconceptional deprivation of maternal folate, vitamin B₁₂, and methionine led to extensive changes in the fetal genome and offspring that, in spite of similar birth weights to controls, became more resistant to insulin, obese, and hypertensive [63]. Further, children whose mothers were vitamin B₁₂ deficient during pregnancy manifested insulin resistance [64]. These data emphasize the importance of maternal micronutrients in fetal programming.

### Table 1. Epigenetic factors involved in the genesis of renal disease, hypertension, and other components of the CRS

<table>
<thead>
<tr>
<th>Timing/site of exposure</th>
<th>Types of exposure</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perinatal</td>
<td>Maternal and paternal malnutrition (both under- and overnutrition), mitochondrial inheritance</td>
<td>Reduced number of nephrons leading to albuminuria, reduced GFR, hypertension, and finally chronic kidney disease. Altered vasoconstrictor and vasodilatory responses of vasculature.</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
<td>Increased apoptosis of myocytes and asymmetric hypertrophy of heart.</td>
</tr>
<tr>
<td></td>
<td>Iron deficiency, ionizing radiation and trace metals, homocysteine, tobacco, alcohol</td>
<td>Increase in coronary vasculature leads to increase in coronary conductance, aberrant DNA methylation of cytosines.</td>
</tr>
<tr>
<td></td>
<td>Yet to be identified factors that modify histone methylation and acetylation</td>
<td>Heart and kidney abnormalities characteristic of CRS.</td>
</tr>
<tr>
<td>Intrauterine</td>
<td>Hormones and methylated compounds (folate, methionine)</td>
<td>Insulin resistance, reduced β-cell mass, impaired glucose tolerance, obesity and diabetes mellitus.</td>
</tr>
<tr>
<td></td>
<td>Hyperglycemia, excessive glucocorticoids, free fatty acids, oxidative stress, inflammation and enhanced renin-angiotensin-aldosterone system activity</td>
<td>Increased AT₁R expression, renal and cardiac structural abnormalities, myocardial and vascular structural changes, and hypertension.</td>
</tr>
<tr>
<td>Placenta</td>
<td>Insufficiency, monozygotic twins</td>
<td>Although direct effects on adult CRS have not been studied well, effects on intrauterine growth and birth weight could have potential effects on CRS as above.</td>
</tr>
</tbody>
</table>

### Table 2. CRS components

1. Obesity, especially central obesity
2. Insulin resistance and increased prevalence of type 2 diabetes
3. Hypertension (increased salt sensitivity, endothelial dysfunction and vascular stiffness)
4. Metabolic dyslipidemia (low HDL, high triglycerides, and increased small dense LDL particles)
5. Albuminuria and/or
6. Reduced GFR (<60 ml/min/1.73 m²)
7. Early onset of metabolic cardiomyopathy characterized by diastolic dysfunction

HDL = High-density lipoprotein; LDL = low-density lipoprotein.
Epigenetic Regulation of Renal Function and Blood Pressure

Maternal Undernutrition

A number of animal and human studies have shown an important relationship between maternal malnutrition, low birth weight of infants, and the development of kidney disease and hypertension in offspring (table 1) [43]. During the development of the kidney, various genetic and epigenetic factors program the number of nephrons, termed the ‘nephron endowment’ [65, 66]. In this regard, the number of nephrons per kidney varies considerably between individuals (ranging between 800,000 and 1,800,000) [43]. It has been reported that a lower number of nephrons is a predictor for the development of hypertension [65–69]. An important autopsy study [70] reported that the number of glomeruli was considerably lower in hypertensive than in normotensive persons that died in traffic accidents. In that study, the estimated filtration surface was considerably higher in hypertensive persons with low nephron numbers than in normotensive persons. In another histomorphometric study, researchers observed fewer glomeruli in babies with low birth weight than in those with normal birth weight [71]. In other animal models, investigators have noted increased abnormal glomeruli in offspring with low birth weights [72]. Low birth weight is associated with increased risk for elevated levels of albuminuria, decreased glomerular filtration rates (GFRs), and increased rates of renal disease progression and end-stage renal disease [43]. In the Dutch study [14] where mothers had been exposed to low caloric or low protein intake during pregnancy, urinary albumin excretion was higher in the offspring of these women, especially when they were exposed to malnutrition during mid-trimester [73]. In another large registry analysis, significant relationships were detected between low birth weight and reduced renal function [74], chronic kidney disease [75], and end-stage renal disease [76]. Further, low birth weight has been shown to accelerate primary kidney disease [77, 78]. This finding is consistent with animal studies which demonstrated that reduced nephron numbers at birth predispose to progression of renal disease related to primary immunological, hypertensive, and metabolic kidney diseases [79–82].

The results of a number of animal and human studies indicate that a low nephron number in the perinatal period is related to the risk of developing hypertension [30, 43, 66–70, 83–105]. Evidence in humans comes from observations in monozygotic and dizygotic female twins with a low birth weight (<2,500 g) [83]. In a follow-up study, 24-hour blood pressure in adults was substantially higher in twins with a birth weight <2,500 g. This observation was subsequently confirmed in another large cohort [106]. Results from experimental studies suggest that reduced nephron number in the perinatal period is related to the risk of development of hypertension in offspring [81–83]. The hypertension that develops in small-birth-weight offspring is often characterized by increased salt sensitivity (table 1) [83]. There is experimental evidence that this is related, in part, to increased AT1R-mediated sodium/hydrogen exchanger 3 activity in the proximal tubules [84–87], increased mineralocorticoid-mediated distal tubule/collecting duct sodium reabsorption [88, 89], and increased renal sympathetic activity [90]. Moreover, the hypertension seen in these individuals is accompanied by endothelial dysfunction [98], vascular stiffness [99], and microvascular disease [102–104], as well as by the comorbid conditions of obesity and diabetes [30, 100].

Maternal Overnutrition

While in utero nutrient restriction has been shown to promote hypertension, CVD, and chronic kidney disease in offspring, we are more commonly faced with maternal overnutrition. In this regard, high birth weight is associated with increased propensity for the development of CRS (table 2) [33–42, 106–127]. In westernized industrialized societies, up to 25% of mothers are obese, and in relatively recent studies >40% of women gained excessive weight with their pregnancies [128, 129]. Excessive weight gain in the mother is associated with ex-
cessive obesity and metabolic disease in offspring [130–133]. For example, children from Brazil with high birth weight (as well as those with a low birth weight) had high blood pressure levels [134]. In a recent study, high maternal pre-pregnancy body mass index and a high birth weight were the most powerful predictors of a higher blood pressure in childhood [42]. Factors which may predispose to the development of CRS in offspring of overweight mothers include both circulating maternal biochemical factors and local placental factors. In this respect, higher relative weights in Pima Indian offspring at the age of 5–24 years [135], and increased risk of childhood obesity and diabetes in a broad-based US population [41, 136, 137] were observed across increasing levels of maternal glucose concentration.

The impact of placental exposure to excessive glucocorticoids on offspring has been termed ‘glucocorticoid programming’ [138–147]. Indeed, epigenetic changes associated with stress can cause indelible changes in the transcriptome. These epigenetic changes are associated with subsequent abnormal physiological function in cardiomyocytes [148, 149], an abnormality seen in CRS (table 2) [106–108]. Indeed, the exposure to excess endogenous or exogenous glucocorticoids during the perinatal period may program the kidney and other organs of the offspring for hypertension, glucose intolerance, and other CRS components in adulthood (table 1). Because of reduced activity of the 11β-hydroxysteroid dehydrogenase type 2 enzyme in maternal diseases such as preeclampsia, placental cortisol availability may be endogenously increased [144]. It is quite likely that other hormones such as aldosterone, angiotensin II, and catecholamines will have important roles in regulating placental growth, function, and blood supply, and thus exert important roles in fetal programming [146, 147]. Future investigation in this area should parcel out the respective roles of different nutritional and hormonal factors in the epigenetic regulation of the various CRS components.

Conclusion

Epigenetic modulation of adult CRS is an active area of investigation. Traditionally, maternal undernutrition was considered to underlie fetal programming. However, evidence is emerging that overnutrition in both mothers and fathers may predispose the fetus and future adult to CRS. The epigenetic mechanisms underlying fetal programming are only beginning to be understood. Future studies will define the molecular pathways leading up to this phenomenon. Importantly, little is understood what effects nutritional and hormonal factors exert on the fetal genome. Specifically, studies should include the assessment of the different mechanisms in conjunction with one another. Further, the studies should include diverse populations with long-term follow-up. Prevention/intervention strategies in fetal and early life should be developed.

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