Epigenetics and Perinatal Nutrition: A Key Factor of the Life Course Model of Public Health

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Abstract

Preventing disease costs less than treating disease. This discussion explores how an approach to prevent disease must take into account individual and family experiences, as well as genetics, by building on the concepts of personalized medicine and the Life Course Model. A key time to use this approach is early in life, when perinatal nutrition strongly influences future health and disease. Environmental epigenetics provides the mechanism through which to target this approach.

Glossary of Abbreviations

CpG Methylation: A methyl group on the cytosine of CpG dinucleotides
CYP2B6: A hepatic P450 drug metabolizing enzyme
IGF-1: Insulin Growth Factor-1
IGF-1 A: Transcripts that do not include exon 5
IGF-1 B: Transcripts that do include exon 5
IGF-1 P1: Insulin Growth Factor-1 Promoter 1
IGF-1 P2: Insulin Growth Factor-1 Promoter 2
IUGR: Intrauterine Growth Restricted
MicroRNA: Small Non-Coding RNAs

Cost and Health-Care Approach Toward Obesity

Health care costs too much to be sustainable. In the United States, health care costs approximately $2.6 trillion a year. Obesity significantly contributes to this cost. Obesity owns the sobriquet of "public health enemy No. 1" by extracting over $190 billion in health-care expenditures. Obesity also indirectly costs public resources. For example, estimates suggest that obesity in the United States burns an additional $1 billion in gasoline utilization resources.

Unsustainable health-care costs also exist outside the United States. Over the past two years, health-care expenditures increased by approximately 10% worldwide. Surprisingly, health-care expenditures from Latin America and Asia significantly contributed to this increase. The health-care costs in these developing regions track concurrently with an increasing incidence of obesity.

No simple or quick solution exists to eradicate obesity either in the U.S. or worldwide. Moreover, obesity presents through a multifactorial and multidimensional series of pathophysiological events, which further contributes to this dilemma. On one hand, the nidus for obesity lies within individuals based on their and their family's environmental exposures, as well as their individual genetics. This particular nidus, therefore, suggests an individualized approach toward obesity. On the other hand, the impetus for obesity is embedded within domestic, local, regional, and cultural traditions and environments. This impetus suggests a need for a more global approach.

We subsequently need three things to approach a solution to health-care costs, particularly as it relates to a complicated pathophysiology such as obesity. First, we need a paradigm that allows for both an individualized approach toward obesity and an environmental awareness. This paradigm exists through the evolution of personalized medicine into the Life Course Model paradigm.

Second, we need a component of the environment that varies yet exists as an essential common human experience, while remaining accessible to manipulation. The characteristic of variation provides the opportunity to gauge the historical importance of this component of the environment. The characteristic of remaining accessible to manipulation increases the relevance of this component in terms of approaching a solution. Such a component exists in the common human experience of perinatal nutrition.

Third, we need a biological mechanism that allows us to be as specific as possible with our manipulation of the environmental component (perinatal nutrition), thereby allowing for individualization. This biological mechanism must also account for the complexity and variation inherent in human experiences of the environment. This molecular mechanism exists within the field of environmental epigenetics.

First: Personalized Medicine & the Life Course Model

Personalized medicine customizes health care by basing medical decisions, practices and products on individual patient characteristics. Personalized medicine stands on the tenant that individualization of health care reduces costs by minimizing unnecessary medical interventions based on probability. At this time, personalized medicine depends heavily on genetics. Our limited knowledge of how the genome works necessitates that genetics-based personalized medicine focuses on phenotype extremes. Several relevant examples demonstrate the power of this approach despite our present limitations.

From the Purina Companion Animal Nutrition Summit: *Nutrition for Life*; Austin, TX; March 27-29, 2014.

Genetics-based personalized medicine powerfully succeeds in oncology, pharmacogenomics and rare catastrophic genetic disease. An example of this success in the oncology field is the present approach toward breast cancer. Many institutes now characterize breast cancer along several molecular axes. These axes include, but are not limited to, expression profiles of human epidermal growth factor receptors, fibroblast growth factor receptors, components of the mTOR pathway, estrogen receptors, and progesterone receptors.[1] An example of success in the field of pharmacogenomics includes CYP2B6.[2] CYP2B6 functions as a hepatic P450 drug metabolizing enzyme. The pharmacogenomics of CYP2B6 include 37 distinct star alleles and greater than 30 SNPs. The relevance of the pharmacogenomics of CYP2B6 lays in the impact on non-nucleoside reverse transcriptase inhibitors, which are a first-line treatment for HIV infection.

Finally, an example of genomic-based personalized medicine succeeding toward catastrophic genetic disease can be seen in a case report by Worthey et al.[3] The child in this case suffered through four years of atypical severe inflammatory bowel disease, extreme failure to thrive, aggressive immunosuppression, and multiple surgeries with progressively diminishing results. Exome sequencing revealed a hemizygous missense mutation in the X-linked inhibitor of apoptosis gene. This finding led to a bone marrow transplant. Within six weeks, the inflammatory bowel disease resolved and the child began to thrive. In these three examples (oncology, pharmacogenomics and catastrophic genetic disease), nothing can diminish the impact of the genetics-based personalized medicine approach on these disease processes.

Unfortunately, many common disease processes, such as obesity, possess characteristics that set up a genetics-based personalized medicine approach for failure. These characteristics include an incremental phenotype and the presence of significant environmental risk factors. Characteristics often found in chronic common diseases present challenges that expose the limitations of a purely genetics-based approach. We place these challenges into two categories: intrinsic and extrinsic.

Intrinsic challenges include the reality that many common chronic diseases result from gender, tissue and molecular interactions. For example, obesity occurs as a result of dysfunction between multiple systems (e.g., endocrine, gastrointestinal, nervous) and tissues (e.g., liver, adipocyte). The singular pathology of obesity results from multiple combinations of dysfunction from these systems and tissues. Another intrinsic challenge includes the robustness of biological networks. This is well-described in a series of articles by Dipple and McCabe.[4-6] In essence, every one of us possesses severe single gene defects that never rise to the level of clinical relevance because of our biological network's robustness. Single gene disease is the exception, not the rule. This robustness occurs because of variation in functional activity thresholds, modifier genes and system dynamics that allow for compensation. The celestial design committee demonstrates wisdom by building into mammals this biological safety net.

A key and often ignored intrinsic challenge exists through our lack of understanding in how our genome functions. Our genomes respond dynamically to our environment. Our lack of understanding can be observed through our present focus on genes that led to functional proteins. This approach enables perceived direct comparisons between expression and disease. However, only 2% of the human genome encodes for protein expression.[7] In another light, 98% of the human genome does not generate proteins. Moreover, we actively transcribe 75% of this non-protein encoding DNA. The fact that the ratio of non-protein encoding DNA to protein-encoding DNA correlates with biological complexity belies the importance of the non-protein encoding DNA. Although all three of these intrinsic challenges will be overcome with time, we are far from approaching an understanding that allows us to meet these challenges.

Extrinsic challenges simply arise from the complexity and variability that compose human experiences. An elegant description of this challenge exists in an analysis by Smith that details the gloomy prospect of randomness.[8] A pivotal point in this review revolves around a study attempting to predict cancer risk based on shared environment and twins. The unexpected findings demonstrate that nonshared environmental influences in twins account for approximately 60 to 80% of cancer risk. In contrast, heritability only accounts for 20 to 40% of cancer risk, and shared environmental influences only account for 20 to 40% of cancer risk. Despite these findings, we believe the message of this review is hopeful. The implicit message is that our present approach contains voids that fail to account for environmental complexity and variability. We believe these voids can be partially filled by the Life Course Model.

The Life Course Model, as we interpret it, hypothesizes that health arises through the interaction of genetics and the accumulation of multifactorial risk factors by individuals and their past lineage.[9] This model, therefore, accounts for the "weathering" that occurs to individuals over the course of a lifetime and potentially accounts for the "weathering" that occurs to families across generations. Historically, this model has been applied to the tragedy of racial disparities in the United States. A benchmark of this disparity is the high infant mortality observed in the U.S. within the African-American community. This disparity occurs, in large part, due to the high incidence of low birth weight (less than 2500 g) in the African-American population.

The Life Course Model explains this adverse outcome of low birth weight through the high prevalence of multifactorial risk factors that accumulate from conception to death in the African-American population. Many proponents of the Life Course Model also point out the historical accumulation of risk factors on many African-American families over the past 300 to 400 years in the United States. An observation reinforcing this model is that the time span spent homeless over a woman's lifetime predicts the risk of low birth weight better than the occurrence of the homelessness during pregnancy.[10] Another observation reinforcing this model is that a maternal grandmother's exposure to neigh-

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borhood poverty during pregnancy predicts the risk of low birth weight in her grandchildren.[11] Regression analysis reveals that this risk exists independent of the mother's exposure. Indeed, 25% of low birth weight infants delivered to mothers not born with a low birth weight can be statistically attributed to generational residence in low-income neighborhoods. The Life Course Model also can be applied to important epidemiological events that educate us about the importance of perinatal nutrition as well as provide insight into possible current and relevant interventions, particularly in terms of the current issue of obesity.

Second: Perinatal Nutrition

Perinatal nutrition plays a pivotal role in lifelong health. This truth is a core tenant of the Developmental Origins of Disease hypothesis. This truth became concrete within the scientific community's consciousness through many diverse studies that included multiple countries, cultures and races. Three key characteristics exist within the most rigorous of these diverse studies, including the Nurse's Health Study and the Dutch famine of 1944 to 1945. The first characteristic involves a differentiating environmental event, the most common being famine, followed by a nondifferentiating environment. The second characteristic involves the appearance of a significant morbidity that occurs temporally distant from the initial differentiating environmental event. The third characteristic involves a comparison cohort not afflicted with the morbidity that is genetically comparable. The above-mentioned studies embed rigor into their observations by controlling for confounding factors, such as gestational age, maternal smoking, socioeconomic status, ethnicity, parental lifestyle, medical history, maternal diabetes, and physical activity, among other things. The incontrovertible conclusion of these cohort studies is that poor perinatal nutrition leads to multiple adult diseases, not the least of which is obesity.[12-14]

Obesity becomes a relevant worldwide issue when a relatively recent and currently relevant famine is taken into account, such as the Chinese famine. The Chinese famine, which occurred from 1959 to 1961, is among the largest in human history.[15,16] The Chinese famine differs from the Dutch famine through spanning a longer period of time, impacting a population already struggling from chronic undernutrition, varying across regions, and disproportionately affecting rural regions.

The Chinese famine also differs from the Dutch famine in terms of the impact on subsequent generations. In contrast to the Dutch famine, first-generation adults who were *in utero* during the Chinese famine suffered from decreased adult height and neurodevelopmental outcome. These adults were, of course, initially characterized by low birth weight secondary to poor perinatal nutrition. In the second generation, in contrast, recent evidence suggests an unanticipated and cross-generational effect. Specifically, grandchildren of the women impacted by the Chinese famine demonstrate a predisposition to greater-than-normal birth weight. In other words, these children are large for gestational age, which predisposes them toward adult insulin resistance and obesity.

Key Concepts

Developmental Origins of Disease: Early life events predict later life diseases such as obesity.
Food Desert Areas: Communities that lack access to affordable healthy foods usually as a result of poverty and not abundance.
Life Course Model: The accumulation of a family's and individual's experiences significantly influences the health of the individual.

The impact of this famine, therefore, potentially reverberates over subsequent generations because insulin resistance and obesity during pregnancy predispose toward large-for-gestational-age infants. As alluded to in the introduction, the social and financial costs to China and the rest of the world will strain resources, considering the millions of individuals potentially affected across generations. Unfortunately, the rest of the world fails to learn from this experience.

In the U.S., food desert areas represent a lack of insight on our part and another risk factor for the racial disparities discussed above. Poor access to healthy and affordable food characterizes food desert areas. Although the phrase "food desert area" suggests a literal absence of retail food in a defined area, the most common meaning in the U.S. is differential accessibility to healthy and affordable food between social economically advantaged and disadvantaged areas. For example, areas with a high proportion of African-Americans have fewer supermarkets or chain stores per capita as well as fewer midsized stores.[17] Furthermore, the most severe food deserts in New York City are within east and central Harlem and north and central Brooklyn, which have the highest proportion of African-American residents.[18] The most favorable areas within New York are on the Upper East Side, which is predominantly a middle- and upper-income area. Evidence exists that better access to supermarkets reduces the risk for obesity, whereas greater access to convenience stores increases the risk for obesity. The field requires further work to focus on the impact of food deserts relative to pregnancy and long-term health outcomes within the context of the Developmental Origins of Disease. However, the presence of food deserts provides a possible intervention as we look for solutions against obesity. To target individuals (a long-term goal) and to measure the impact of an intervention, we need a biological mechanism. We believe environmental epigenetics represents one such mechanism.

Third: Environmental Epigenetics

Epigenetics regulate planned or programmed gene expression in eukaryotes. Epigenetics determine accessibility of DNA to transcription factor machinery to multiple varied mechanisms. A cell cannot express a gene without invoking epigenetics. We divide epigenetics into two fields. Developmental epigenetics allow for cells to maintain distinct rigid programs of gene expression. This includes programs of gene expression that occur during development or that maintain tissue specificity.

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Nobody needs a hepatocyte to become a neuron by accident. Environmental epigenetics allow for cells to adapt and respond to the environment. We believe this is what occurs within the Developmental Origins of Disease hypothesis. Broadly speaking, we believe that your phenotype is a product of your genetics, your family's exposures and your exposures.

Epigenetics invoke multiple mechanisms. DNA CpG methylation is the most well-studied but not necessarily best-understood. DNA CpG methylation involves putting a methyl group on the cytosine of CpG dinucleotides. CpG dinucleotides occur disproportionately in CpG islands. Two-thirds of human promoters are located within CpG islands. Most promoters located within these islands stay unmethylated. Although DNA CpG methylation associates with gene silencing, initiation of silencing does not require this event. More likely, DNA CpG methylation functions to maintain a repressed state of transcription.

DNA CpG methylation studies lend themselves to human epidemiological studies because they require little sample and appear easy to interpret. Studies demonstrate that DNA CpG methylation varies with maternal macro- and micro-nutrient intake, the mode of conception, the mode of delivery, maternal smoking, paternal habits, and maternal emotional status. Unfortunately, we color the interpretation of the studies with our natural human tendency toward naïve reductionism. A capability to take into multiple individual and familial characteristics to predict a DNA methylation status, let alone a predisposition toward obesity based on DNA methylation status, does not yet exist. This is partly due to our inability to account for the effects of methylation across a whole genome as well as the binomial nature of DNA methylation. You are either methylated or unmethylated. The complexity and variability of the human condition rarely fits within a simple binomial equation.

In contrast, histone code inherently allows for that complexity and variability, but as a community, we struggle to interpret its meaning. Histone proteins make up the nucleosome core that DNA wraps around. Histone proteins have N-terminal tails that extend from the nucleosome core and are subject to covalent modifications. These covalent modifications make up the histone code. The histone code performs three basic functions. Some histone codes regulate the charge of histone lysine residues and thereby moderate charge dependent interactions between nucleosomes and DNA. A covalent modification that often leads to this function is histone acetylation. Other histone codes regulate the physical accessibility of chromatin regulating and transcription complexes to DNA. A covalent modification that often leads to this function is histone methylation. Finally, histone codes also can regulate charge and affinity simultaneously. A covalent modification that often leads to this function is histone phosphorylation.

The histone code within a single cell contains over 4×10 the 30th possible permutations. Furthermore, 51 distinct chromatin states exist based on varying combinations of histone covalent modifications. These states affect transcription and expression based on the specific complex(es) interacting with the chromatin. Subsequently, the histone code contains the capacity to act as a dynamic storage system that records significant interactions between your chromatin and the environment. Unfortunately, we are still in our infancy of understanding how to interpret the histone code, but we will get there.

Other epigenetic mechanisms exist. This includes the 98% of the genome that does not encode proteins. Other epigenetic mechanisms include microRNA. These are small non-coding RNAs that mediate post-transcriptional regulation of gene expression. MicroRNAs regulate up to 60% of expression of our genome. Another epigenetic mechanism includes long non-coding RNA. This is a diverse class of transcripts that lack an open reading frame. These transcripts appear to regulate gene expression through both direct and indirect mechanisms. Long non-coding RNAs also may function as an interface between the transcriptome and the proteosome.

Animal models provide the best evidence of a relationship between perinatal nutrition and environmental epigenetics. More specifically, animal models demonstrate that suboptimal perinatal nutrition affects the epigenetic programming of important physiological genes and that targeted perinatal nutritional supplementation moderates the effects of suboptimal perinatal nutrition. A common cause of suboptimal perinatal nutrition in developed countries involves uteroplacental insufficiency, often caused by diseases of pregnancy-induced hypertension, such as preeclampsia.

Uteroplacental insufficiency decreases the transfer of nutrients from the mother to the baby. The suboptimal transfer causes the baby to be intrauterine growth restricted (IUGR). Human IUGR offspring suffer from increased risk toward multiple postnatal/adult morbidities including obesity. We use a well-characterized model of bilateral uterine artery ligation in the rat to mimic the human condition. This model results in rat pups that are 25% smaller than the norm and that develop postnatal obesity.

We use this model to study the effect of suboptimal perinatal nutrition on the epigenetic programming of genes implicated in the development of obesity, such as insulin growth factor 1 (IGF-1).[19] Serum levels of IGF-1 play a role in regulating multiple processes, such as adipocyte homeostasis, as well as insulin resistance. Hepatic production of IGF-1 determines serum levels of this protein. Our interest in this gene was further piqued by the observation that the IGF-1 gene generates multiple transcribed products based on differential exon usage. This variation requires epigenetic manipulation and, as such, suggests vulnerability to environmental epigenetic manipulation. Moreover, variation like this stands as a key characteristic of mammalian gene expression that allows us to carry fewer genes than simpler organisms, such as the worm.

The hepatic IGF-1 gene generates multiple transcribed products. IGF-1 promoter 1 (IGF-1 P1) may initiate transcription from multiple start sites. IGF-1 P1 appears to predominate early in life and in the basal levels of IGF-1 production. IGF-1 promoter 2 (IGF-I P2) also contains multiple start sites as well as growth

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hormone response elements. IGF-1 P2 becomes more active after birth and responds to diet as well as growth hormone stimulation. The hepatic IGF-1 transcript may not include the presence of exon 5, which may affect endoplasmic reticulum processing. Transcripts that do not include exon 5 are designated IGF-1 A transcripts. Transcripts that include exon 5 are designated IGF-1 B transcripts. The length of the IGF-1 poly A tail also varies significantly from tissue to tissue. In general, tissues, such as the liver, that produce relatively large amounts of hepatic IGF-1 transcript use short poly A tails. Tissues, such as the lung, that tightly control paracrine levels of IGF-1 often use long poly A tails.

In humans, uteroplacental insufficiency decreases fetal and early postnatal serum IGF-1 levels. As adults, IUGR humans also appear to suffer from dysregulation of IGF-1 homeostasis.[20] The exact characteristic of this dysregulation appears to vary with the extent of postnatal catch-up growth. Uteroplacental insufficiency-induced IUGR in the rat similarly decreases serum IGF-1 levels in the fetus and through weaning. Moreover, decreased levels of hepatic IGF-1 transcripts characterize the IUGR liver.[19] The timing of the impact of IUGR on specific hepatic IGF transcripts varies. For example, IUGR decreases IGF-1 P1 and IGF-1 A transcript levels only at day of life 0. In contrast, IUGR decreases IGF-1 P2 and IGF-1 B transcript levels at both day of life 0 and day of life 21 (weaning). The impact of IUGR upon hepatic IGF-1 transcripts corresponds with changes in the epigenetic characteristics of the rat hepatic IGF-1 gene. For example, IUGR enriches hepatic DNA CpG methylation at IGF-1 P2 at day of life 21. The impact of IUGR on IGF-1 P2 DNA CpG methylation appears more robust in males. Gender-specific epigenetic responses are common.

Uteroplacental insufficiency-induced IUGR in the rat also affects the hepatic IGF-1 histone code. Our initial findings confused us. We studied the hepatic IGF 1 promoters and found enrichment of histone code that usually corresponds with increased transcription. Examples of this include increased lysine 14 acetylation on histone 3 as well as increased lysine 4 trimethylation on histone 3. Subsequent introspection led us to study multiple histone codes across the whole length of the gene. We found that IUGR significantly decreased lysine 36 trimethylation on histone 3 across the whole gene, and the impact was quite robust toward the 3' region of the gene. *In vitro* studies postulate that lysine 36 trimethylation on histone 3 facilitates RNA polymerase II elongation of the transcript. The studies taught us that to understand epigenetic regulation of a gene by focusing on the promoter and/or 5' region of the gene leads to a very restricted understanding.

A frustrating challenge in the epigenetic field includes our present inability to make tissue and gene specific changes in individual epigenetic characteristics, such as histone code. This limits our ability to test Koch's postulates. Our approach to temporally moderate this challenge involves collaborating through other models and determining if specific epigenetic events appear more vulnerable to suboptimal perinatal nutrition. In collaboration with colleagues at the University of Iowa, we found that maternal diabetes in the rat decreased hepatic IGF-1 transcript levels at day of life 21.[21] This decrease in hepatic IGF-1 transcript levels corresponded well with decreased lysine 36 trimethylation on histone 3 similar to the uteroplacental insufficiency-induced IUGR rat findings. The pathways regulating lysine 36 trimethylation on histone 3 represent the target of our present research program.

Another target of our research program involves demonstrating that perinatal nutritional supplementation moderates the effects of suboptimal perinatal nutrition. A wonderful example of this includes the project of Dr. Aagaard-Tillery during her reproductive scientist development program fellowship. Dr. Aagaard–Tillery presently serves as an associate professor at the Baylor College of Medicine. This project encompassed two hypotheses. The first hypothesis postulates that second-generation rats whose parents suffered from uteroplacental insufficiency-induced IUGR develop obesity and other characteristics of the metabolic syndrome in adulthood. The second hypothesis postulates that feeding a diet enriched to facilitate methyl donation dampens the impact of IUGR on the second generation, essentially normalizing the phenotype. To test these hypotheses, the proposal uses every possible combination of control versus IUGR cross concurrently while comparing control versus enriched diets.

Uteroplacental insufficiency-induced IUGR, indeed, leads to obesity and other markers of the metabolic syndrome in second-generation rats, lending new meaning to the phrase "fat rat." Moreover, decreased hepatic IGF-1 transcript levels characterize the second-generation fat rats. Our findings became most robust in males with two IUGR parents. In contrast, the enriched diet significantly minimizes the transmission of the fat rat phenotype and increases hepatic IGF-1 transcript levels in the second generation. The increase in IGF-1 cash of levels corresponded to a decrease in IGF-1 P2 DNA CpG methylation enrichment. We are often asked if the enriched diet should be used clinically, particularly because of the slimming effects. We strongly advise against it for two reasons. First, such an epigenetically "active" diet may lead to unforeseen complications in the future. Second, the enriched diet caused the rats to smell so badly that our institution required us to essentially quarantine the animals.

Taken as a whole, studies from us and multiple groups prove that environmental epigenetics demonstrate the following: 1) allow for tissue and gender specificity; 2) account for the robustness of biological systems; 3) account for non-protein encoding DNA; 4) allow for the variability and complexity of the human condition; and 5) explain the observations associated with the Life Course Model.

Epigenetics stands as the mechanism that regulates interactions between our genome and the environment. Perinatal nutrition exists as one of the most impactful common components of our environment and influencer of population health. Perinatal nutri-

tion also exists as one of the most accessible components of our environment. If we are going to improve a population's health and sensibly reduce the cost of health care, we must learn to prevent disease rather than treat disease. In the big picture, to paraphrase Winston Churchill, healthy citizens are the greatest assets the world can have. In a more granular context, to paraphrase my children, you cannot choose your parents, but you can choose your environmental epigenetics.

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