Early Life Nutrition and Epigenetic Markers

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Epigenetic processes are fundamental to development because they permit a range of phenotypes to be formed from a genotype. Across many phyla such epigenetic processes confer Darwinian fitness because the changes are induced in part in response to external signals that indicate to the developing organism critical aspects of the environment in which it will live after birth. Environmental features such as abundance and quality of food or the number of predators and related stress levels may be critical in determining the chance of the offspring surviving to reproduce, given that from an evolutionary point of view this is more important than longevity or health. Epigenetic processes are increasingly recognized to be complex[1] and include DNA methylation, changes in structure of the histone proteins around which genomic DNA is wrapped, and noncoding RNAs (Fig 1).[2]

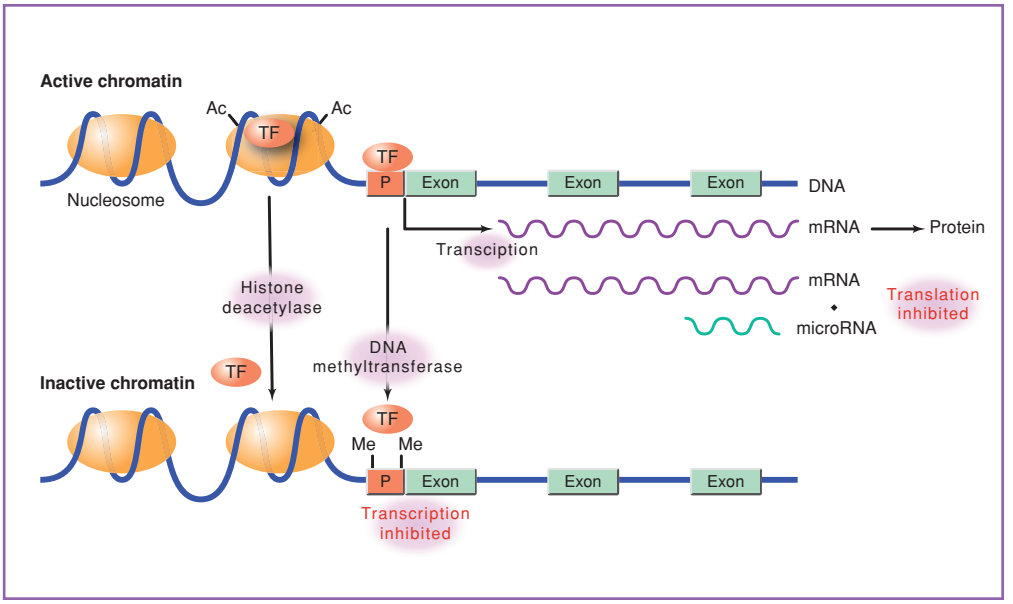

Fig 1. Complexity of epigenetic processes.[2]

Source: Gluckman PD et al. Mechanisms of disease: Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med*. 2008;359:61-73. Reprinted by permission of the Massachusetts Medical Society.

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When epigenetic changes affect the promoter regions of genes they can alter the access of transcription factors to these regions and thus produce effects on gene transcription. Nutritional signals during development have been shown extensively to induce such epigenetic effects in animal models. Because such effects alter metabolic control systems, they can influence an individual's response to later challenges such as an obesigenic lifestyle and thus the risk of obesity and noncommunicable disease including cardiovascular disease and diabetes.[3,4]

The evolution of such epigenetic processes probably did not equip humans for the modern world, especially that now experienced in developed societies.[5] An unbalanced diet can lead to malnutrition even in those who have access to plentiful food. In developed societies many women consume poor-quality diets, resulting in nutritional deficiencies on one hand, or overweight and obesity on the other. While maternal undernutrition remains a major problem in developing societies, maternal overweight, excessive weight gain in pregnancy, and gestational diabetes are growing concerns. Thus unbalanced nutrition has effects on development across a broad dietary spectrum.

In addition, a range of studies now shows that such epigenetic processes can be passed beyond the subsequent generation, raising questions about the importance of nongenomic inheritance in demographic and secular changes in disease risk. Accumulating evidence also shows that epigenetic effects can be passed via the paternal line, presumably because not all epigenetic marks are erased in the packing of DNA into the sperm and because even the small amount of sperm cytoplasm contains some small noncoding RNAs, which can have effects on the oocyte or zygote. Indeed, a range of studies now indicates that very early embryonic development is sculpted by epigenetic processes. These extend beyond merely imprinted genes (where the expression of the allele depends on the parent of origin). Environmental factors such as nutrition can have graded epigenetic effects on a much wider panel of genes throughout development. These perceptions are important because they suggest that nutritionally induced epigenetic processes may start to operate even before a couple know that they have conceived.

Animal studies reveal how maternal nutrition affects the phenotype of the developing offspring, via epigenetic changes in endocrine, cardiovascular, and metabolic control that prepare the offspring for a predicted postnatal environment. Where developmental cues (nutritional, endocrine, etc) turn out to be inappropriate, or the environment changes between generations, the phenotype is mismatched.

Under these conditions experimental animals exhibit many of the features and risk factors equivalent to human noncommunicable disease, such as obesity, reduced muscle mass and bone density, fatty liver, high blood pressure and vascular endothelial dysfunction, and insulin and leptin resistance. Such "mismatched" offspring also show altered appetite, hyperphagia and changes in food preference, altered stress responses and anxiety, reduced learning, and changes in the timing of puberty. Some data indicate possibly similar effects in mismatched human subjects.[5] It is important to note that such effects are not pathological in themselves, but are manifestations of altered responses to the postnatal environment, including nutrition. This is not to say that pathological changes in phenotype are not induced by more severe undernutrition or overnutrition, but these occur at the extremes of the range and are more equivalent to teratogenic effects.

To pursue epigenetic concepts in the human, we have utilized prospective cohorts of children who had undergone detailed phenotyping and for whom we had previously measured aspects of the mother's diet in pregnancy in detail. We focused on possible epigenetic effects at the promoters of candidate genes, selected on the basis of animal data and the previous literature, using an array-based discovery procedure followed by replication in separate cohorts. We extracted DNA from umbilical cord tissue stored at birth to gain information about the effects of prenatal nutrition on epigenetic processes. Such tissues may be invaluable where the epigenetic changes occurred during early development and so may be expected to be manifest in all somatic tissues. For the retinoid X receptor A (RXRA), which is involved in responses to fatty acids, metabolic control, and tissue differentiation, we found a highly significant correlation between mother's carbohydrate intake in early pregnancy and methylation at a specific cytosine-phosphate-guanine (CpG) dinucleotide in the RXRA gene, and also between this methylation and the child's adiposity at 6 or 9 years of age (Fig 2).[6] The effects were replicated in two separate cohorts.

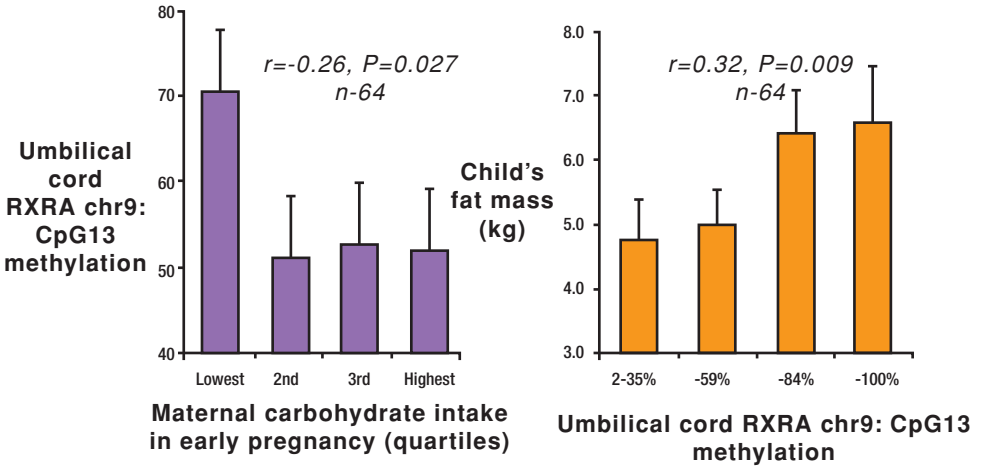

Fig 2. Data from Southampton prospective cohort showing strong correlation between level of DNA methylation at one CpG in the RXRA gene and mother's carbohydrate intake in early pregnancy (left) and child's fat mass at age 9 years (right). These effects were independent of mother's BMI or child's birthweight.[6] CpG = cytosine-phosphate-guanine (dinucleotide), RXRA = retinoid X receptor A, BMI = body mass index

Source: Godfrey KM et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes*. 2011;60:1528-1534. Copyright 2011 American Diabetes Association. Reprinted with permission from the American Diabetes Association.

Further studies are required to determine the timing of a critical window after birth during which it may be possible to restore epigenetic changes in offspring exposed to a suboptimal environment before birth, although again animal studies provide proof of principle that either endocrine interventions in the neonate[7] or dietary interventions after weaning[8] produce effects on epigenetic processes induced prenatally, although the underlying mechanisms are not known.

In summary, the results of a range of animal studies, and of the first few investigations in humans, give hope that epigenetic changes measurable in early life may serve as valuable biomarkers of later risk of disease. It is not known whether biomarkers that have been measured to date lie on the causal pathway to greater risk of disease. Nonetheless, they can serve as better measures of prenatal environment than, for example, birth weight, which can be affected by a complex interaction between genetic and environmental factors and which is not necessarily affected by unbalanced nutrition. These biomarkers may be helpful in devising new interventions in early life aimed at reducing later risk of noncommunicable disease, and possibly in monitoring the efficacy of such interventions. This will be important

because a “one-size-fits-all” intervention is unlikely to be efficacious and may increase risk in certain individuals.[9,10] In view of the importance of meeting the challenge of noncommunicable disease globally,[11] the development of such biomarkers is a priority.

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Q & A

Q: Dr Hanson, you demonstrated that low protein leads to hypomethylation of peroxisome proliferators-activated receptor alpha (PPARα), while low carbohydrate leads to hypermethylation of retinoid X receptor A (RXRA). How do heterodimers PPARα and RXRA work if you have two variants with low protein or carbohydrate?

Dr Hanson: Do not forget that this is the low-protein, isocaloric model, so there is actually increased carbohydrate. So we should expect that the results in the animal study would be opposite of the human study, and the data show that. I do not want to duck the question, but we have to be careful when extrapolating from animal data to humans, from one species to another, because they all have different adaptive strategies, different metabolism, and different levels of maturity at birth and during their early postnatal life. This is proof of principle, however, and we see in the rat a metabolic phenotype in the offspring that we can correct. We have to do the human studies to see whether such changes actually relate to disease risk.

Q: How do you envision the functional interpretation of epigenetic biomarkers? How might the RXRA hypermethylation in the umbilical cord risen? From stem cells?

Dr Hanson: It looks hopeful. Some data suggest that changes in RXRA in the direction that we see are associated with changes in adipocyte number and differentiation. Of course, we are still not sure whether what we are seeing in the umbilical cord is just the shadow of things in other parts of the body or whether we might only see it in that particular tissue. It is correlation, not causation, at this point.

Q: I am interested in the temporal relationship between the protein restriction in your animals' fathers and conception. How long do these males have to be on low protein to have the effect that you saw?

Dr Hanson: These are not our data, but I recall that it was a few weeks [Carone et al. *Cell.* 2010;143:1084-1096]. Presumably the diet is affecting either the small RNAs or indeed the DNA packaging into protamine at the time the sperm are formed, because we know that even though textbooks say epigenetic changes are erased in the sperm during those processes, we know they are not. There could be enough of a signal to the transcriptional machinery in the zygote toward developing a phenotype even from that early point in life. That is what these data seem to show. Much stronger data have come from Skinner's group using endocrine disrupters

[Anway et al. *Science*. 2005;308:1466-1469]. They show changes in the male lineage through four or five generations. I think it is interesting that this dietary challenge produces such an effect.