EDITORIAL (SEE SHIELDS ET AL., P. 2661)

Fetal Programming of Type 2 Diabetes

Is sex important?

Hales and Barker (1) caused a paradigm shift in our thinking about diabetes prevention when they demonstrated that low birth weight (due to growth retardation) predicted type 2 diabetes (the "thrifty phenotype" or "fetal origins" hypothesis). On the other hand, larger babies of diabetic mothers are also at higher risk of diabetes, apart from their genetic susceptibility ("fuel mediated teratogenesis") (2,3). A concept evolved that the intrauterine experiences mold the fetal systems ("programming") and influence future health (4). If the postnatal experiences are at variance with the intrauterine ones, the programmed fetus is susceptible to disease (5). For example, low birth weight babies who put on excess weight in later life are at a higher risk of type 2 diabetes than those who continue to be low weight (6). In the programming hypothesis, the focus is on the role of intrauterine environment and on gene-environment interaction rather than the differences in gene structure, which are the basis of conventional genetics. Genes are clearly important, and their role in intrauterine development and risk of diabetes was highlighted by Hattersley and Tooke (the "fetal insulin hypothesis") (7). The most exciting thought in fetal programming is that intrauterine environment may modify gene expression permanently. A heritable change in gene expression without a change in DNA sequence is called "epigenetic" (8), a term first used by Waddington in developmental biology. Epigenetic changes alter gene function and can be silencing or activating. These changes are inherited mitotically in somatic cells, which could explain long-term effects on gene expression in an organism, contributing to phenotypic diversity. They might also be inherited transgenerationally, affecting the health of future generations. Silencing of one of the two X chromosomes in females is an example of epigenetic change, and clinicians may also be familiar with Prader Willi syndrome, caused by disordered genomic imprinting. During intrauterine life, there are waves of epigenomic modification, intimately associated with growth and development, and opportunities galore for environmental factors to influence these processes. A fetus thus programmed travels a path of limited options.

Today we have only a preliminary knowledge of mechanisms of epigenetic regulation. Various enzymatic modifications affect gene function, including methylation of cytosine residues at CpG dinucleotides in DNA molecule and acetylation of specific lysine residues in the histones that package the DNA, both leading to an alteration in the transcription profile. These patterns of chemical modification are mitotically transmitted and therefore become permanent for the cell line. Methylation is influenced by the availability of 1-C (methyl) donors (vitamin B12, folate, choline, betaine, etc). Animal experiments provided the much-needed molecular proof of nutritional programming: Pregnant viable yellow Agouti mice (a genetically obese mouse) fed a "methylating cocktail" gave birth to offspring who showed a spectrum of coat color and were less obese despite inheriting the mutation. This was a result of increased methylation in the promoter region of the Agouti gene (9,10). In the Pune Maternal Nutrition Study, we found that high maternal homocysteine concentrations predicted fetal growth restriction (11) and that low maternal vitamin B12 and high folate nutrition predicted adiposity and insulin resistance in children at 6 years of age (12), suggesting nutritional programming. Intrauterine life thus provides a window of opportunity to influence the health of an individual. Studies in maternal fetal medicine and cord blood measurements will provide useful clues.

Shields et al. (13) make a welcome contribution to this process by reporting two interesting observations in the Exeter Family Study of Childhood Health. They found that EDTA anticoagulant and refrigeration preserve cord blood insulins for up to 48 h after collection, providing a useful guideline for researchers. The exciting observation is that girls had 15–25% higher concentrations of cord insulin compared with those in boys, independent of the many confounders (maternal size and glycemia in late pregnancy, length of gestation, mode of delivery, and glucose concentration in the cord blood). They claim that girls are "intrinsically" more insulin resistant than boys. The situation is analogous to the finding that cord insulin concentrations are higher in South Asian Indian compared with Caucasian babies (14). It's interesting that in both the situations, the smaller hyperinsulinemic babies have a smaller lean mass but higher body fat (adiposity).

This paper raises important issues: Are females more insulin resistant or only hyperinsulinemic? Is this an intrinsic characteristic, and if so, is it genetic or epigenetic? What is the role of hormones? A sex difference also exists in the cord blood concentrations of growth hormone, IGFs, leptin, and sex hormones.

Insulin has both metabolic and growth-promoting actions. Higher insulin concentrations but similar glucose and smaller body size could indicate higher insulin resistance in girls. This reasoning reflects the well-known principle of feedback regulation in endocrinology exemplified in the homeostatis model assessment (15). The caveats for such an interpretation in cord blood are as follows: the feedback system is not fully mature at birth, and parturition is not a steady-state situation. Thus, the interpretation of insulin resistance should be considered provisional and await direct measurements by clamp studies, which are technically demanding and ethically challenging. Measurement of other markers of insulin resistance (adiponectin, resistin, retinol binding protein 4, etc.) is likely to provide extra information. Another possible explanation for this observation could be that girls may have increased B-cell sensitivity of insulin secretion to circulating glucose and other nutrients (16).

Hyperinsulinemia at birth (unaffected by lifestyle) may suggest that it's intrinsic to female sex. This is the basis of the "sex insulin hypothesis" (17). Does this mean that it is genetic? Females have two copies of the X chromosome, one of which is inactivated soon after fertiliza-

tion (imprinting), though some 15% of genes escape and produce a double-dose effect. Insulin-resistant diabetes is common in both Turner syndrome (monosomy X) (18,19) and in Klinefelter syndrome/variants (polysomy X) (20,21). Intriguingly, in the majority of Turner syndrome cases the deleted X chromosome is paternal, and in Klinefelter syndrome cases the extra X chromosome is mostly maternal, suggesting a possible link between maternal X chromosome, insulin resistance, and diabetes. In addition, some autosomal genes are also imprinted in females, including some that control glucose and fatty acid metabolism (22). On the other hand, cord insulin concentrations are also influenced by paternal insulin resistance (23), and children of diabetic fathers have a lower birth weight, suggesting a paternal influence on fetal insulin resistance (24). Matters are further complicated by hormones; for example, women are more insulin resistant during prepubertal and postmenopausal years, whereas men seem to be more insulin resistant during reproductive years (25).

Most of the recent excitements in genetics of type 2 diabetes are related to B-cell function. Investigations into genetics and epigenetics of sex insulin difference could shed more light on the origins of insulin resistance. Future studies should include maternal metabolic, nutritional, and other factors; a standardized protocol to assess fetal growth; and cord blood and placental tissue collection to investigate genetic and epigenetic factors. Analysis of previous datasets by sex difference and parent-of-origin effect are likely to provide valuable information.

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