Challenges in Understanding Diabetic Embryopathy

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Perhaps one of the most devastating diabetes complications is diabetic embryopathy, in which the offspring of a mother with diabetes predating pregnancy develops congenital malformations. These malformations can affect multiple organ systems, including the brain and spinal cord, the heart and major vessel, the kidneys, the gut, and skeletal structures (1,2) and result in pre- or postnatal mortality or disability. As malformations are induced during the earliest stages of organogenesis, coinciding with the first recognition of pregnancy (3), it is important to institute rigorous glycemic control before the onset of pregnancy. Nevertheless, recent studies (4,5) have shown that even in planned pregnancies with optimal prepregnancy care, the incidence of malformations in diabetic pregnancies is still at least twice that in nondiabetic pregnancies. As malformations occur in the offspring of women with either type 1 or type 2 diabetes (or offspring of women who are obese at the beginning of pregnancy and may have undiagnosed type 2 diabetes) (2,6–8), and recent evidence indicates that the incidence of diabetes, particularly type 2 diabetes, predating pregnancy is rapidly increasing (9), the burden of this diabetes complication is likely to increase in coming years unless efforts to prevent diabetic embryopathy are improved. However, unlike other diabetes complications, in which the development of pharmacologic interventions offer the hope of treatment or prevention, prevention of diabetic embryopathy is unlikely to benefit from pharmacologic intervention, because of the risk that drugs that might interfere with diabetic teratogenic pathways might be teratogenic themselves. For example, we have shown that maternal hyperglycemia before organogenesis stimulates production of diacyl glycerol and activity of protein kinase C (PKC) in mouse embryos during formation of the neural tube (10). And yet, while recent findings indicate that use of PKC inhibitors may be efficacious for treating diabetic retinopathy (11,12), it is not feasible to use PKC inhibitors during diabetic pregnancy because they would disrupt angiogenesis, which is necessary for successful implantation, placentation, and organogenesis. Thus, because development of pharmacologic intervention in diabetic pregnancy proves especially challenging, it is all the more important to continue to investigate the mechanisms by which diabetic embryopathy occurs in order to devise feasible approaches to reduce its incidence.

Nevertheless, less appears to be known on a cellular and molecular level how diabetic embryopathy occurs compared with macro- and microvascular complications. The scientific challenge to understanding the pathogenesis of diabetic embryopathy can be partly attributed to the diversity of tissues that can be affected and to the dynamic changing state of differentiation of vulnerable organs during the embryopathy-susceptible period. That is, the cellular damage that occurs in differentiated tissues leading to macro- and microvascular disease may also occur in differentiating embryo tissues, but, in addition, malformation may result because signaling caused by excess glucose metabolism interferes with tissue morphogenesis.

The study by Wentzel, Gäreskog, and Eriksson (13) illustrates some of the difficulties inherent in experimentally investigating diabetic embryopathy and why research in this field often proceeds in only incremental steps. In this study, the authors hypothesized that several candidate genes (such as those involved in antioxidative pathways, apoptosis, or morphogenesis) might be altered as a result of maternal diabetes in a rat model and examined expression of these candidate genes in embryos of nondiabetic rats, as well as in normal and malformed embryos of diabetic rats. They also examined markers of apoptosis in the first visceral arch and the heart, structures that are prone to malformation in the diabetic model. This is not the first study by these authors, or others, on these candidate genes or markers of apoptosis, but this study does offer some new insights. In this study, differences in gene expression and markers of apoptosis were observed between embryos of diabetic and nondiabetic rats, particularly in malformed embryos. However, because the observations were made after structures were formed, it cannot be determined whether the genes whose expression were affected were at all involved in causing the malformations. As the authors state, they can only claim an association with diabetic exposure or malformation. To determine whether genes whose expression differed in embryos from diabetic pregnancies could be involved in causing malformations, investigators would need to confirm that expression is altered in embryos before malformations are apparent. Although, because malformations are not yet apparent, it cannot be determined which embryo(s) will become defective. If altered gene expression is responsible for malformations, it must be detectable (either in the aggregate of individually assayed embryos, or pooled exposed embryos) before malformations are apparent. Then, to show that altered gene expression contributes to malformations, it is necessary to manipulate gene expression to demonstrate that causing the same change in gene expression (increase or decrease) that is affected by diabetes replicates the effects of diabetes on embryos and that blocking the change in gene expression prevents the effects of diabetes on embryos. While embryo gene expression can be manipulated in

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mouse embryos (by using transgenic and knockout technologies), this is not currently possible in the rat. Thus, while it should be possible to investigate whether the gene expression changes noted in embryos after malformations occur can also be detected before manifestation of malformations, it is not currently possible to manipulate expression of these genes in rat embryos; thus, the investigators can only conclude that there is an association with diabetic teratogenesis, as Wentzel, Gäreskog, and Eriksson have carefully done.

Another challenge that confronts this kind of research is the relatively small differences in expression of candidate genes that can lead to disparate findings when the same genes are studied in different labs or even in the same lab using different methods. Wentzel, Gäreskog, and Eriksson (13) discuss how they and others have previously investigated expression of some of the same candidate genes studied in this report and propose why some of the results are at variance with previously published results. Nevertheless, because metabolic and signaling networks operate in concert, small changes in gene expression or enzymatic activity may be difficult to detect consistently, but the biological consequences may be amplified if several participants in a signal transduction cascade are similarly affected. Given the difficulty in determining whether or not expression of candidate genes or activity of candidate enzymes is consistently altered in different experimental settings, it seems that the efforts of research into the mechanisms responsible for diabetic embryopathy should focus on asking the questions: *1*) Are these changes in gene expression or enzymatic activity functionally significant in causing malformations in diabetic pregnancy? and *2*) If these genes/enzymes are not responsible for diabetic embryopathy, how do they occur?

REFERENCES

1. Becerra JE, Khoury MJ, Cordero JF, Erickson JD: Diabetes mellitus during pregnancy and the risks for the specific birth defects: a population-based case-control study. *Pediatrics* 85:1–9, 1990
2. Correa A, Gilboa SM, Besser LM, Botto LD, Moore CA, Hobbs CA, Cleves MA, Riehle-Colarusso TJ, Waller DK, Reece EA: Diabetes mellitus and birth defects. *Am J Obstet Gynecol* 199:237.e1–237.e9, 2008
3. Mills JL, Baker L, Goldman AS: Malformations in infants of diabetic mothers occur before the seventh gestational week: implications for treatment. *Diabetes* 28:292–293, 1979
4. Evers IM, de Valk HW, Visser GH: Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ* 328:915–919, 2004
5. Jonasson JM, Brismar K, Sparen P, Lambe M, Nyren O, Ostenson CG, Ye W: Fertility in women with type 1 diabetes: a population-based cohort study in Sweden. *Diabetes Care* 30:2271–2276, 2007
6. Schaefer-Graf UM, Buchanan TA, Xiang A, Songster G, Montoro M, Kjos SL: Patterns of congenital anomalies and relationship to initial maternal fasting glucose levels in pregnancies complicated by type 2 and gestational diabetes. *Am J Obstet Gynecol* 182:313–320, 2000
7. Farrell T, Neale L, Cundy T: Congenital anomalies in the offspring of women with type 1, type 2 and gestational diabetes. *Diabet Med* 19:322–326, 2002
8. Watkins ML, Rasmussen SA, Honein MA, Botto LD, Moore CA: Maternal obesity and risk for birth defects. *Pediatrics* 111:1152–1158, 2003
9. Lawrence JM, Contreras R, Chen W, Sacks DA: Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. *Diabetes Care* 31:899–904, 2008
10. Hiramatsu Y, Sekiguchi N, Hayashi M, Isshiki K, Yokota T, King G, Loeken MR: Diacylglycerol production and protein kinase C activity are increased in a mouse model of diabetic embryopathy. *Diabetes* 51:2804–2810, 2002
11. Aiello LP, Davis MD, Girach A, Kles KA, Milton RC, Sheetz MJ, Vignati L, Zhi XE: Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. *Ophthalmology* 113:2221–2230, 2006
12. Shen LQ, Child A, Weber GM, Folkman J, Aiello LP: Rosiglitazone and delayed onset of proliferative diabetic retinopathy. *Arch Ophthalmol* 126:793–799, 2008
13. Wentzel P, Gäreskog M, Eriksson UJ: Decreased cardiac peroxidase levels and enhanced mandibular aptosis in malformed embryos of diabetic rats. *Diabetes* 57:3344–3352, 2008