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NATIONAL CHILDREN'S STUDY

CORE HYPOTHESIS

Workgroup: Birth Defects

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I. Proposed Core Hypothesis

Impaired glucose metabolism during organogenesis is associated with an increased risk for major structural congenital malformations. Maternal and fetal genetic susceptibilities, and maternal environmental exposures and lifestyle factors modulate the impact of impaired maternal glucose metabolism on the developing fetus.

II. Public Health Significance

Prevalence

Each year in the United States, about 150,000 babies are born with a major birth defect—about 3% of all live births.[1] Impairments in glucose metabolism during pregnancy are associated with an increased risk of birth defects; thus, infants of women with Type I[2], Type II and gestational diabetics have a greater risk of having a birth defect.[3] The risk of birth defects among these infants is estimated at 3-5 times higher than for non-diabetic mothers, and increases as glycemic control worsens. The most common related malformations include cardiac, neural tube defects and musculoskeletal anomalies, which are all induced before the seventh week of gestation.[4;5] Studies have demonstrated that women who are obese, but have not been diagnosed with diabetes, are also at an increased risk for birth defects such as those observed in diabetic embryopathy.[3;6-8] It has been suggested that the underlying mechanism involved in this phenomenon is poor glycemic control, albeit not as diagnosed diabetes.

Over the past four decades the incidence of diabetes among women of childbearing age has increased.[9] In a recent article in *The Lancet*, it was stated that among individuals aged 30-39 years of age, the prevalence of Type II diabetes has increased by 70% and is expected to double by 2010. [10] Based on data from the Third National Health and Nutrition Examination Survey , 1988-1994, the percentage of the women 20 to 39 years of age with physician-diagnosed diabetes is 1.1%. This prevalence is lowest among Caucasians, 0.9% and highest among African-Americans 1.6%.[9] The American Diabetes Association defines undiagnosed diabetes as

a fasting plasma glucose of greater than or equal to 126 mg/dl and impaired fasting glucose as fasting plasma glucose of 110 to <126 mg/dl. [11] Correspondingly, among women 20 to 39 years of age, the prevalence of undiagnosed diabetes is 0.6% and impaired fasting glucose is 1.2%. These rates also vary by ethnic group. The combined prevalence of diagnosed diabetes, undiagnosed diabetes, and impaired fasting glucose is 2.1% among Caucasian women, 5.6% among Mexican-American women, and 5.8% among African-American women. [9]

Prevention

The underlying mechanism by which diabetes leads to birth defects is still being explored. Sufficient evidence exists to conclude that impaired glucose metabolism is a primary biochemical mechanism responsible for diabetic embryopathy. [12-14] The functional bases underlying this mechanism are likely multifactorial, involving complex interactions between genetic susceptibilities, environmental exposures, and lifestyle factors. Some women who have impaired glucose metabolism will have a lower threshold of risk for having a pregnancy affected by a birth defect than others, due to their glycemic control and behavioral choices during pregnancy, and possible genetic predispositions.

Prevention strategies must be instituted prior to conception, to offer dramatic reduction in the numbers of malformations among babies born to diabetic mothers. This is especially true of those with Type I and Type II diabetes, if they are placed under strict metabolic control prior to conception.[15;16] Also, animal models have provided evidence to suggest that antioxidants, including vitamins C and E, can decrease the impact of glucose teratogenicity.[12;17; 14] Because the standard of care in the United States is to begin glucose screening only in the third trimester among women not previously diagnosed with diabetes, existing data on glycemic control at the early stages of organogenesis is minimal. If an association between impaired glucose metabolism and birth defects is demonstrated, the findings might support universal screening of pre-pregnant women of reproductive age, or suggest criteria for selective screening of "high-risk" groups, such as those who are obese.

Importance

We propose the hypothesis detailed in this document as a core hypothesis for the National Children's Study based on several considerations: (1) birth defects associated with poor glycemic control can be lethal, or cause morbidity resulting in significant expenditures of health care resources; (2) preliminary studies suggest that the effects of impaired glucose metabolism can be lessened by readily accessible therapies, providing a base for possible preventive public health recommendations; (3) the prevalence of obesity, and Type II and gestational diabetes in women of childbearing age is increasing steadily; and (4) the longitudinal nature and potential scope of the National Children's Study provide the most robust, and perhaps the only adequate study design to understand the relationship between impaired glucose metabolism and birth defects.

III. Justification for a Large, Prospective, Longitudinal Study

A study such as the National Children's Study can provide new and important information on the relationship between abnormalities of glucose metabolism and birth defects. Researchers will be able to identify overt diabetes, and milder forms of abnormal glucose metabolism, ideally before

conception, and evaluate their effects on the developing fetus. Such comprehensive assessment is important because latent disorders of glucose metabolism are more prevalent than overt diabetes; therefore many birth defect cases could be attributed to small increases in associated teratogenic risk. In addition, because the effects of these metabolic abnormalities are poorly understood relative to those associated with overt insulin-dependent diabetes, the longitudinal study will expand considerably the knowledge base of preventive clinical and public health relevance.

In this study, glucose metabolism can be studied prospectively, using objective measurements. This represents a major improvement over the many retrospective (*e.g.*, case-control) studies in which the presence of disease is based solely on maternal reports. Ideally, measurements should begin before conception, because glucose metabolism can change during pregnancy. Abnormalities of glucose metabolism might have multiple fetal effects that manifest themselves during pregnancy (in fetal death), at birth (as a birth defect) or later (as childhood obesity). The longitudinal component of the study provides a unique opportunity to discover and assess such multiple effects over time.

IV. Scientific Merit

Animal studies of impaired glucose metabolism and congenital malformations

Although mechanisms responsible for the birth defects associated with impaired glucose metabolism have not been definitively determined, animal studies suggest that multiple factors contribute to the embryopathies.[18] Ketones, hyperglycemia, hypoglycemia, and somatomedin inhibitors have all been shown to cause defects (NTDs and cardiac) in vitro (mouse and rat models). [19, 20-23] As a potential teratogen, glucose has received the most intense scientific scrutiny. Hyperglycemia can induce an increase in reactive oxygen species (ROS), albeit by poorly understood mechanisms [17;24], and can disrupt inositol metabolism leading to inhibition of protein kinase C and, ultimately, prostaglandin E2.[13] These effects are most pronounced in animal strains known to have an increased susceptibility to the development of diabetes, suggesting a genetic component to the induction of abnormalities.[25;26] The potential role of ROS offers a possible avenue for prevention through clinical antioxidant therapy.[27;28] Indeed, animal studies show that vitamins E and C decrease oxidative stress and reduce malformation rates in diabetic rat models.[29, 30] Furthermore, the genetically diabetes-susceptible strains inherently possess lower levels of antioxidants than diabetes-resistant strains of rats.[31;32]

Hypoglycemia has also been shown to be teratogenic in animal models in which embryos are particularly sensitive during neural tube development and early stages of heart morphogenesis. [14;33] The structure of the developing heart is adversely affected even by short periods of exposure to as little as a 33% decrease in blood glucose. This situation is increasingly relevant in cases of aggressive insulin therapy to regulate metabolism in the diabetic; such therapies can increase the severity and duration of hypoglycemic episodes.

While the exact mechanisms remain to be elucidated, the cumulative animal study evidence strongly supports the teratogenicity of altered glucose homeostasis. Translating this evidence into successful preventive strategies requires further investigation into the critical determinants and measurements of glucose alterations very early in human pregnancy as proposed in this core hypothesis.

V. Potential considerations for measurement frequency

For ideal testing of this hypothesis in the National Children's Study, repeated measurements of serum glucose and other biomarkers of disturbed maternal glucose metabolism would be made prior to conception and throughout pregnancy. Data and biological samples on factors that may modulate the impact of impaired glucose metabolism, such as maternal environment and lifestyle, and maternal, paternal and fetal genotype would need to be collected. The frequency and nature of evaluations of fetal well being and neonatal growth and dysmorphology would be carefully considered. Table I provides one possible schedule for repeated measurements of individual factors prior to, during, and after pregnancy.

Table I. Possible schedule for collecting measures of impaired glucose metabolism and modulating factors

	Weeks of gestation									
Tests	**Pre-conception**	**0**	**6**	**12**	**18**	**24**	**30**	**36**	**40**	**Post Natal**
Maternal Lifestyle factors	X	X	X	X	X	X	X	X	X	X
Random fasting glucose	X									X
Glucose Tolerance	X						X			X
Maternal HgA_1C	X	X	X	X	X	X	X	X	X	X
24 hr glucose monitor	X	X	X	X	X	X	X	X	X	X
Maternal Fat Cell	X			X					X	
<u>DNA</u>										
Maternal	X									
Paternal	X									
Fetal or Infant	X									X
Ultrasound - Prenatal				X		X		X		

VI. Power Analysis

With 100,000 pregnancies, the study should have sufficient power to study major birth defect outcomes under reasonable scenarios (see Table II). For example, the study will have greater than 90% power to detect small increases in risk (relative risk = 2) for all birth defects combined, even for rare exposures (1%, estimate of overt diabetes among women of childbearing age). For more common glucose abnormalities (5%—10% of women) it should be possible to identify increases in risk for common subgroups of birth defects.

Table II. Power to detect teratogenic effects in a cohort of 100,000 pregnancies, by exposure frequency, relative risk of teratogen, and baseline frequency of birth defects.

		Birth Defect Frequency (among unexposed)			
		1 in 33 (all birth defects combined)	1 in 500 (eg, some heart defects, some groupings)	1 in 1,000 (eg, neural tube defects)	1 in 2,000 (eg, tetralogy of Fallot)
Exposure Rate		**Relative Risk**			
1% (1,000 pregnancies)	2	0.995	0.345	0.245	0.185
	3	0.999	0.686	0.500	0.37
	5	0.999	0.949	0.810	0.643
	10	0.999	0.999	0.986	0.914
3% (3,000 pregnancies)	2	0.999	0.619	0.420	0.288
	3	0.999	0.950	0.792	0.591
	5	0.999	0.999	0.984	0.894
	10	0.999	0.999	0.999	0.997
5% (5,000 pregnancies)	2	0.999	0.780	0.549	0.369
	3	0.999	0.992	0.912	0.727
	5	0.999	0.999	0.999	0.967
	10	0.999	0.999	0.999	0.999
10% (10,000 pregnancies)	2	0.999	0.944	0.754	0.519
	3	0.999	0.999	0.989	0.895
	5	0.999	0.999	0.999	0.998
	10	0.999	0.999	0.999	0.999

Note: shaded areas denote power in excess of 80 percent (alpha error is fixed at 0.05).Power calculations were done using SABER software (L. James, CDC). [34]

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