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MINIREVIEWS

Birth defects in pregestational diabetes: Defect range, glycemic threshold and pathogenesis

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Abstract

Currently, 60 million women of reproductive age (18-44 years old) worldwide, and approximately 3 million American women have diabetes mellitus, and it has been estimated that this number will double by 2030. Pregestational diabetes mellitus (PGD) is a significant public health problem that increases the risk for structural birth defects affecting both maternal and neonatal pregnancy outcome. The most common types of human structural birth defects associated with PGD are congenital heart defects and central nervous system defects. However, diabetes can induce birth defects in any other fetal organ. In general, the rate of birth defects increases linearly with the degree of maternal hyperglycemia, which is the major factor that mediates teratogenicity of PGD. Stringent prenatal care and glycemic control are effective means to reduce birth defects in PGD pregnancies, but cannot reduce the incidence of birth defects to the rate of that is seen in the nondiabetic population. Studies in animal models have revealed that PGD induces oxidative stress, which activates cellular stress signalling leading to dysregulation of gene expression and excess apoptosis in the target organs, including the neural tube and embryonic heart. Activation of the apoptosis signal-regulating kinase 1 (ASK1)-forkhead transcription factor 3a (FoxO3a)-caspase 8 pathway causes apoptosis in the developing neural tube leading to neural tube defects (NTDs). ASK1 activates the c-Jun-N-Terminal kinase 1/2 (JNK1/2), which leads to activation of the unfolded protein response and endoplasmic reticulum (ER) stress. Deletion of the *ASK1* gene, the *JNK1* gene, or the *JNK2* gene, or inhibition of ER stress by 4-Phenylbutyric acid abrogates diabetes-induced apoptosis and reduces the formation of NTDs. Antioxidants, such as thioredoxin, which inhibits the ASK1-FoxO3a-caspase 8 pathway or ER stress inhibitors, may prevent PGD-induced birth defects.

Key words: Pregestational diabetes; Birth defects; Glycemic threshold; Diabetic embryopathy; Range of defects

Core tip: Pregestational diabetes is a rising problem with gravid impact on adverse pregnancy outcomes. This review concentrates on diabetes-induced birth defects and the underlying mechanism of diabetic embryopathy derived from animal studies. The main defects associated with pregestational diabetes are in the cardiovascular and central nervous systems, and are linearly related to maternal glycemic control. Animal studies reveal oxidative stress and stress kinase signalling-induced apoptosis as key factors in pathogenesis. However, many questions remain unanswered, and the rate of congenital defects in human diabetic pregnancies is still high. The cause of diabetic embryopathy warrants further investigations.

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INTRODUCTION

Major structural or genetic birth defects affect approximately 3% of births[1], and are the leading cause of infant mortality in the United States[2]. The pathogenesis of most birth defects is unknown[3,4]. Pregestational diabetes (PGD) is one of the leading known causes with up to a nine-fold increase in birth defects, compared with the rate seen in nondiabetic pregnancies[5]. Although the risk for birth defects in PGD pregnancies can be significantly reduced by appropriate pregestational counselling and meticulous control of maternal glycemic levels[6], it cannot be equalized to the background risk partially because at least 40% of diabetic pregnancies are usually unplanned[6,7].

The pathophysiology of maternal diabetes induced birth defects is complex, however, clearly relates to maternal glucose levels. The mechanism is not entirely understood, but animal studies have shown it to be associated with decreased cell proliferation and increased cell apoptosis due to high oxidative stress. The second major change seen in animal models of PGD is altered gene expression causing deviation from the normal developmental process.

PGD can affect almost any organ. However, most congenital defects associated with diabetes occur in the cardiovascular, central nervous and musculoskeletal systems[8-10].

In this review we will discuss how PGD increases the risk of birth defects, the range of defects seen in diabetic pregnancies and the relationship of these defects to maternal glucose control. As the risk of birth defects is well established in type 1 and type 2 diabetes mellitus, while it is still controversial in gestational diabetes[11], we will focus this review on PGD only.

RANGE OF DEFECTS

PGD is associated with a wide range of anomalies in almost any fetal organ. Based on available literature, women with PGD have 2- to 9-fold higher risk for having babies with birth defects, with a prevalence of birth defects of 2.7%-18.6%, compared with the healthy population[5,12-17], having a prevalence of birth defects of 2%-3%.

Although any birth defect can be associated with PGD, more anomalies are seen in the cardiovascular, central nervous and skeletal systems[9,10,18]. Rare abnormalities almost exclusively associated with diabetes include caudal regression syndrome with femoral shortening and sacral agenesis. Caudal regression syndrome consists of a spectrum of congenital anomalies of the lower spine and hips, is associated with genitourinary and lower limb defects, and is one of the few syndromes in which the presence of maternal diabetes has always to be considered as the cause, if not confirmed[19]. Although very rare, some studies found this syndrome to be 200-600 times more prevalent in infants of diabetic mothers compared with nondiabetic mothers[16]. Be that as it may, it can still be seen also in non-diabetic mothers as described in a case report by Versiani *et al*[20] who reported 2 cases of caudal dysplasia sequence - one woman had PGD while the other had gestational diabetes.

Previous studies assessed the prevalence of congenital anomalies in the diabetic population. Eidem *et al*[21] Studied the risk of congenital anomalies related to type 1 diabetes. They compared major congenital anomalies (excluding minor anomalies as defined by the EUROCAT system) between women with pregestational type 1 diabetes and controls. All women registered at the Norway national delivery registry from 1999 to 2004 were included. Anomalies were registered in 5.7% (91/1583) offspring of women with type 1 diabetes, compared with 2.9% in the background population. The risk for cardiovascular anomalies was 3 fold higher in the diabetic group (3.2% *vs* 0.94%, respectively) with ventricular septal defect (VSD) (12/1583) and patent ductus arteriosus (10/1583) being the most prevalent birth defects in the cardiovascular system and the most common compared with all other birth defects.

Garne *et al*[22] used the same EUROCAT classification to describe birth defects in 18 population-based EUROCAT registries of congenital anomalies, which included births between 1990 to 2005, and compared 669 diabetes cases with 92976 non-diabetes cases. The authors showed significantly increased odds ratios for neural tube defects (anencephaly and encephalocele, but not spina bifida) and several subgroups of congenital heart defects. Other birth defects found in increased odds ratios in

infants of pregnancies complicated by pregestational diabetes were anotia, omphalocele and bilateral renal agenesis. Multiple congenital anomalies were present in 13.6% of the diabetes cases and 6.1% of nondiabetes cases. The odds ratio for caudal regression sequence was very high (26.4, 95%CI: 8.98-77.64), but only 17% of all caudal regression cases occurred in pregnancies complicated by PGD.

Most recently, Correa *et al*[23] reviewed the association of type 1 and type 2 PGD with 39 birth defects using the National Birth Defects Prevention Study. The authors examined databases from 10 birth defects surveillance systems in the United States looking for isolated (one or more major defects in same organ system or with known sequence) or multiple birth defects (2 or more major, unrelated defects) from births between 1997 to 2003. They found an association between PGD and 11 cardiac defects and 7 non cardiac defects.

Galindo *et al*[12] prospectively followed 126 women with pregestational diabetes in a single tertiary centre in Spain. They reported 17 offspring with congenital anomalies (13.5%). Although chromosomal abnormalities are not generally associated with PGD, one fetus had trisomy 21 and atriventricular septal defects. Eight fetuses were listed with major birth defects (6.3%), 35.3% of fetuses had cardiovascular defects, and genitourinary defects accounted for another 23.5%. They noted positive correlation between high levels of haemoglobin A1c (HgA1c) > 7% at early pregnancy and major congenital anomalies. Despite this study including only small number of women, the prospective nature of it allows strength in addressing its results.

Wender-Ozegowska *et al*[16] studied a group of 198 diabetic women and found 17 pregnancies that resulted in infants with birth defects (8.6%). The most common defects were in the cardiovascular system (5.5%), with 7/198 (3.5%) of cases being atrioventricular septal defects (3 cases of VSD and 3 cases of atrial septal defect). Wren *et al*[17] focused their research on cardiovascular birth defects. They followed 609 diabetic pregnancies and found that 3.6% of the women delivered infants with cardiovascular defects, the most common being transposition of great arteries, truncus arteriosus and tricuspid atresia. Janssen *et al*[18] studied 1511 PGD cases, with congenital birth defects prevalence of 7.2%. The most common defects occurred in the cardiovascular system, NTDs, cleft lip/palate and skeletal defects.

Although many studies discuss diabetes associated congenital anomalies, the leading type of birth defects and the exact rate of diabetes associated birth defects are difficult to determine. The literature varies widely in what is considered the "leading" type of anomaly, as well as the rate of anomalies, because of suboptimal coding of the diabetic pregnancies according to White's classification, differences in anomaly coding and reporting bias. Moreover, the recognition of a birth defect may not always occur in the immediate neonatal period, thus, may not be entered into a registry. On the other hand, over documentation of diabetes related birth defects, compared with birth defects in the nondiabetic population, may occur because of the known association of defects to diabetes, or the relatively high transfer of infants of diabetic pregnancies to neonatal intensive care units, both leading to a more thorough neonatal examination.

Based upon the available data, it seems that the major organ systems affected by PGD are the cardiovascular and the central nervous systems, which could be related to their embryonic origin in the neural crest. However, PGD can increase the rate of any other birth defects described above. Therefore, all pregnant women with PGD are advised to undergo a detailed anatomical scan and full fetal echocardiography to screen for possible birth defects, regardless of their having type 1 or type 2 diabetes mellitus or glycemic control at the time of pregnancy.

GLYCEMIC THRESHOLD FOR BIRTH DEFECTS

Periconceptional HgA1c is used as a surrogate marker for glycemic control, and is almost linearly related to PGD-induced birth defects. Previous studies have shown that stringent glucose control prior to or at early pregnancy, during organogenesis, can significantly reduce the incidence of birth defects[12,24-29]. However, there are still questions to be answered. Hanson *et al*[25] studied 532 type 1 PGD women and compared their malformation rate to 222 nondiabetic women. The rate of malformations did not differ significantly between the diabetic and the control groups (4.3% *vs* 2.4%) although significant different was find in levels of first trimester HgA1c. The median value of HgA1c was 7.7% in the diabetic and 5.3% in the control group ($P < 0.001$). However, when higher levels of HgA1c were evaluated (HgA1c greater than 10.1% - equal to 8 SD above the normal mean control value), there was statistically significant higher occurrence rate of congenital malformation ($P < 0.01$).

Wender-Ozegowska *et al*[16] tried to determine the cut-off for first trimester glycemic levels for prediction of congenital anomalies. They used whole day glycemic profile as well as HgA1c to assess glycemic control. Their cohort included 198 diabetic pregnancies and 4700 nondiabetic pregnancies. The rates of malformation were 8.6% and 3.6%, respectively. They determined the cut-off of value of HgA1c that could prediction congenital malformations as 9.3% (measured up to 16 wk).

Other studies demonstrated the connection between poor glycemic control before or early at pregnancy: Ylinen *et al*[29] found that the mean HgA1c for women whose infants had congenital malformations was higher than that measured for women with healthy infants prior to 15 gestational weeks (9.5% *vs* 8.0%, respectively). Mironiuk *et al*[27] compared an occurrence rate of congenital malformations in newborn to mothers with

type 1 diabetes (*n* = 170), newborns of healthy mothers (*n* = 26368) and mothers with GDM (*n* = 56). They demonstrated that type 1 diabetes was associated with congenital malformations (11.2%, 1.8% and 2.2%, respectively) and that the risk of major birth defects was directly proportional to the level of maternal blood glucose control during the first trimester. These studies all support the idea that lack of glycemic control leads to congenital malformations, but do not indicate what HgA1c value should be maintained to reduce the risk for infant anomalies. Moreover, Shields *et al*[30] tried to find cut-off value associated with congenital anomalies but failed to do so, and Lucas *et al*[31] proved the association of PGD with congenital anomalies only when HgA1c was combined with other factors such as diabetic classification (White's classification) and maternal vascular complications. The Atlantic-Diabetes in Pregnancy (DIP) trial compared PGD pregnancy outcomes in the same population after a change in pre-pregnancy care policy and improved glycemic control throughout pregnancy. In the Atlantic DIP study, the first trial conducted from 2005-2007, included 104 diabetic pregnancies (87% type 1 PGD). The authors reported two pregnancies with birth defects that had HgA1c values of 6.6% and 5.4% at early pregnancy. The second trial, conducted from 2008-2010, included 168 pregnancies, with more women affected by type 2 PGD (81/168, 48%) and lower HgA1c values (7.3% compared to 6.9%); however, despite of presumed better prenatal care and the lower HgA1c - rate of malformation reported was not changed between the trials. The authors did not report the type of birth defects seen in either of the two studies[32,33].

Other studies have demonstrated a linear relationship between HgA1c and major congenital defects. Greene *et al*[34] showed an increase in PGD-induced birth defects correlating to the level HgA1c. Their risk for major malformation was 3.0% when HgA1c taken at first trimester was less than or equal to 9.3% and 40% with HgA1c was greater than 14.4% (RR = 13.2; 95%CI: 4.3-40.4). Todorova *et al*[35] studied 124 pregnancies complicated by pregestational diabetes. The mean values of HgA1c were significantly higher in pregnancies complicated by fetal malformations (*n* = 19/15.3%) than those values measured in pregnancies without fetal malformations [9.01% (SD ± 1.53) *vs* 8.06% (SD ± 1.64) *P* = 0.022, respectively].

In conclusion, it is clear that glycemic control is associated with a reduced risk of congenital anomalies. However, the recommended threshold of HgA1c for pregestational diabetic women planning pregnancy is still not known. Reaching a consensus as to what HgA1c level a diabetic pregnant woman should strive for has remained elusive for many reasons. First, differing definitions for major and minor anomalies, and taking into account the various forms of diabetes, limit the ability to derive conclusions from the published data. Second, PGD pregnancies can have the same spectrum of anomalies as nondiabetic pregnancies which makes it difficult to differentiate PGD-induced anomalies from others encountered in nondiabetic pregnancies. Third, most studies use HgA1c to reflect the level of glycemic control, but HgA1c only measures a woman's average glycemic control over a 3-mo period. Therefore, it does not necessarily reflect a woman's level of glycemic control during organogenesis and embryogenesis. Moreover, because it is an average measurement, the same level of HgA1c may reflect completely different glycemic patterns, one being constant around the average, and the other with larger fluctuations lower and above from the mean HgA1c value[35]. The effects of such fluctuations (*i.e.*, episodes of acute hyperglycemia and hypoglycemia with "normal" HgA1c) are yet to be determined. Fourth, given the complicated metabolic nature of diabetes, coupled with metabolic changes that are normal during pregnancy, other factors may influence the developing embryo. Finally, lower prenatal detection rates of fetal anomalies (due to obesity, lack of prenatal care, *etc.*) in diabetic women may lead to statistic skewing when studying infants with congenital anomalies born to PGD mothers[36,37].

DOES DIABETES TYPE AFFECT THE RATES OF CONGENITAL ANOMALIES?

Diabetes can be classified by the mechanism (type 1, type 2 or gestational diabetes) or alternatively, by White's classification[38]. Rates of diabetes, both type 1 and types 2 are increasing all over the world[39,40] causing an increase in the incidence of maternal diabetes in pregnancy[41]. Although hyperglycemia is a common mechanism for teratogenicity, differences in disease characteristics, such as age of onset, ethnicity, obesity and duration of disease, may affect the disease impact on the perinatal outcome and the rate of congenital anomalies. Current literature does not specify the risk for anomalies for any type of diabetes separately, as some studies include only type 1 diabetes[25,42], others only type 2 diabetes[32] and only a few compare the outcomes between them[43,44].

Gonzalez-Gonzalez *et al*[44] retrospectively compared the outcomes of type 1 diabetes (*n* = 904), type 2 diabetes (*n* = 516), gestational diabetes (*n* = 3188) and control (*n* = 115996) deliveries in Ontario, Canada. Congenital anomalies were most frequently observed in women with type 1 diabetes and gestational diabetes, with risk approaching 1.5- to 2-times that of controls. They found a total of 44 anomalies (6.1%) among all women with diabetes, most commonly in the cardiovascular system. Adjusted OR for anomalies was 3.5, 1.7, 2.5 and 1.9 for type 1, type 2, gestational diabetes and control deliveries, respectively. Peticca *et al*[45] found similar results in a multicentre study in Italy. They prospectively compared 504 type 1 PGD to 164 type 2 PGD pregnancies. The rate of birth defects was significantly higher in women with type 1 diabetes (5.9%), compared with in women with type 2 diabetes

(2.0%). Difficulties in glycemic control, increased disease severity and frequent episodes of ketoacidosis and hypoglycaemia were postulated as explanations for this difference. Other studies have found the opposite results[46,47]. Clausen *et al*[47] compared 389 type 1 and 146 type 2 diabetics delivering in the United Kingdom. Pregnancies affected by type 2 diabetes had worse perinatal outcomes with congenital abnormalities (12.3% in type 2 *vs* 4.4% in type 1; $P = 0.002$) accounting for most of this difference. In 2005, Clausen *et al*[47] showed similar results with almost doubled number of congenital anomalies among pregnant women with type 2 diabetes (6.6%) compared to type 1 diabetes (2.9%)[46]. Both studies cited a delay in antenatal care, suboptimal glycemic control prior to conception, inadequate folate supplementation and maternal obesity, as possible reasons to explain the worse outcomes in pregnancies complicated by type 2 diabetes. Another explanation could be the tendency toward oral glycemic agents in type 2 diabetes, which may lead to less than optimal glycemic control compared to the use of insulin only in type 1 diabetes. Conversely, Roland *et al*[48], showed a high rate of congenital anomalies in women with type 1 diabetes and type 2 diabetes, compared with the nondiabetic population, with no significant difference between them. Jensen *et al*[43] found similar results.

Over the past years, there has been a great improvement in perinatal outcomes for women with type 1 diabetes. However, with rising incidence of obesity and type 2 diabetes, it seems that type 2 diabetes has become a more prominent concern. Women with type 2 diabetes tend to be under less stringent control of their glucose values, either because they use oral glycemic agents, have lower compliance with management strategies or have a lower prevalence of pre-pregnancy counselling, as sometimes type 2 diabetes is regarded as a "lesser" problem compared with type 1 diabetes. Moreover, lack of folate supplementation, obesity with lower ultrasound detection rates and other demographic differences between the women with type 1 or type 2 diabetes may account for the differences showed by different studies comparing the types of diabetes. There is no doubt that pre-pregnancy care and good glycemic control is equally important in type 2 and type 1 diabetes.

PATHOGENESIS OF DIABETES INDUCED BIRTH DEFECTS

Most data regarding the pathophysiology of diabetes associated birth defects originates from animal studies. Our research group, as well as others, have shown that maternal diabetes triggers multiple cellular stress responses and subsequent aberrant signal transduction pathways[49-51]. All these may contribute to gene dysregulation and apoptosis in the affected organs of the developing embryo leading to structural birth defects. Studies from animal models have shown that maternal diabetes increases the production of cellular reactive oxygen species and simultaneously impairs endogenous cellular antioxidant capacity, leading to an overall oxidative stress in the embryo[50,52-55]. Maternal diabetes also appears to increase the expression of inducible nitric oxide synthase (iNOS)[56], whose enzymatic activity catalyzes the reaction of superoxide with nitric oxide to produce reactive nitrogen species. Work in animal models indicates that reactive nitrogen species create a severe form of oxidative stress, nitrosative stress, which is responsible for the activation of cellular stress signalling. Our laboratory has shown that over expression of an antioxidant enzyme, superoxide dismutase 1 (SOD1), in SOD1 transgenic mice, mitigates maternal diabetes-induced oxidative stress and reduces embryonic malformations in diabetic pregnancies[57-59]. Likewise, studies indicate that eliminating the *iNOS* gene in iNOS knockout mice reduces the incidence of embryonic malformations caused by maternal diabetes[60]. Therefore, our work and that of others has shown that oxidative and nitrosative stress mediates the teratogenicity of maternal diabetes in the developing embryo.

In our studies, maternal diabetes-induced oxidative stress activates the c-Jun-N-terminal kinase 1/2 (JNK1/2)[49,50,61-63]. Deletion of either *JNK1* or *JNK2* gene ameliorates maternal diabetes-induced NTDs formation[49,50], supporting the hypothesis that activation of the cellular stress kinases, JNK1/2, mediates the adverse effect of maternal diabetes on neural tube closure. In the absence of JNK1 or JNK2, maternal diabetes-induced apoptosis in the neuroepithelial cells are blunted[49,50]. Thus, JNK1/2 activation induced by maternal diabetes transmits the pro-apoptotic signal emanating from oxidative stress under diabetic conditions[49,50].

JNK1/2 belongs to the mitogen-activated protein (MAP) kinase family, whose activation follows a three-tier cascade: MAP three kinases activate MAP kinase kinases, which in turn trigger JNK1/2 phosphorylation. Subsequent studies have revealed the upstream kinases in diabetic embryopathy, including apoptosis signal-regulating kinase 1 (ASK1)[64]. ASK1 is a three kinase that leads to JNK1/2 activation. We have observed that *ASK1* gene deletion abolishes maternal diabetes-induced JNK1/2 activation, as well as activation of four major transcription factors downstream of JNK1/2. Similar to our findings in the *JNK1* and *JNK2* gene deletion studies, *ASK1* gene deletion blocks maternal diabetes-induced apoptosis in the developing neural tube, and consequently reduces the number of embryos with NTDs[64]. Thus, the ASK1-JNK1/2 pathway, which is activated by oxidative stress, appears to play a causal role in the induction of diabetic embryopathy.

Recently, we have worked to understand how cellular ASK1-JNK1/2 kinase signalling relays its pro-apoptotic signals to the nucleus[64]. We have observed that ASK1 activation increases the activity of Forkhead transcription factor 3a (FoxO3a), and that FoxO3a

induces TNFR1 associated death domain (TRADD)[64]. TRADD up-regulation triggers caspase 8 activation, which, in turn, activates the caspase cascade and leads to apoptosis[64]. Both germline deletion and conditional deletion of the *FoxO3a* gene significantly reduce maternal diabetes-induced apoptosis and NTDs formation, underscoring the potential importance of FoxO3a in the induction of diabetic embryopathy[64]. We have successfully inhibited the whole stress pathway, ASK1-JNK1/2-FoxO3a-TRADD-caspase 8, using an endogenous ASK1 inhibitor, thioredoxin. Thioredoxin treatment ameliorates NTDs formation in embryos of diabetic dams and cultured embryos[64]. These studies reveal the ASK1 initiated stress signalling with the activity of a transcription factor and pro-apoptotic gene expression.

We and others have shown that JNK1/2 activation leads to endoplasmic reticulum (ER) stress[50]. Under ER stress, the unfolded protein response (UPR) is activated, and prolonged UPR activation induces apoptosis[65,66]. Maternal diabetes *in vivo* and high glucose *in vitro* induce ER stress and activate the UPR pathway[50]. Treatment with an ER chemical inhibitor, 4-Phenylbutyric acid, reduces high glucose-induced JNK1/2 activation, neuroepithelial cell apoptosis and NTD formation[50]. Deletion of either the *JNK1* or the *JNK2* gene abrogates maternal diabetes-induced UPR and ER stress[50]. These findings support a reciprocal causation between JNK1/2 and ER stress in diabetic embryopathy. Other studies have found that activation of the inositol-requiring enzyme 1 alpha, one of the major UPR arms, activates ASK1 and subsequently leads to JNK1/2 activation and apoptosis[67,68], which support our hypothesis that ASK1 plays a major role in diabetic embryopathy.

CONCLUSION

While the risk for major congenital defects has been well established in women with pregestational diabetes, many questions remained unanswered. For example, it is still unknown why some organs display vulnerability to glucose teratological effects more than others, or why meticulous control of maternal blood glucose does not reduce the rate of birth defects to the background risk of non-diabetic population, or why some women with pregestational diabetes will have normal pregnancies even when their levels of HgA1c are way above the accepted threshold for birth defects.

The key to understanding the true relationship between PGD and birth defects lies in a basic understanding of the pathogenesis of congenital defects. Based on our research, the oxidative stress-triggered ASK1 pathway mediates the pro-apoptotic effect of maternal diabetes and high glucose *in vitro* by inducing pro-apoptotic gene expression and causing UPR and ER stress. The endogenous ASK1 inhibitor, thioredoxin, and the ER stress inhibitor, a Food and Drug Administration approved drug, may be potential therapeutics against maternal diabetes-induced structural birth defects.

We believe further research to evaluate glucose teratological effects on different embryonic organs, *via* animal models that can encompass the diversity seen in human diabetes, is needed. Research should utilize type 1 and type 2 models, as well as models of chronic and acute glycemia and how these two conditions may affect organogenesis. Our hope is that, by combining all known clinical data with current and future basic and translational science studies, we will be able to establish better ways to care for the diabetic mother and prevent the mal-effects of maternal diabetes on the developing embryo.

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