Published in final edited form as:
Clin Lab Med. 2013 June ; 33(2): 207–233. doi:10.1016/j.cll.2013.03.017.

New Concepts in Diabetic Embryopathy

Zhiyong Zhao, Ph.D. and
Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, 655 West Baltimore Street, BRB 11-041, Baltimore, Maryland, 21201. Phone (410) 706-8401

E. Albert Reece, M.D., Ph.D, MBA.
Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, 655 West Baltimore Street, BRB 14-029, Baltimore, Maryland, 21201. Phone (410) 706-7410

Zhiyong Zhao: zzhao@fpi.umaryland.edu; E. Albert Reece: deanmed@som.umaryland.edu

Synopsis

Diabetes mellitus is responsible for nearly 10% of fetal anomalies in diabetic pregnancies. Although aggressive perinatal care and glycemic control are available in developed countries, the birth defect rate in diabetic pregnancies remains much higher than that in the general population. Major cellular activities--i.e., proliferation and apoptosis--and intracellular metabolic conditions--i.e., nitrosative, oxidative, and endoplasmic reticulum stress--have been demonstrated to be associated with diabetic embryopathy using animal models. Translating advances made in animal studies into clinical applications in humans will require collaborative efforts across the basic research, preclinical, and clinical communities.

Keywords

Diabetic embryopathy; birth defects; hyperglycemia; apoptosis; cell proliferation; metabolism; intracellular stress; intervention

Introduction

Diabetes mellitus is a metabolic disease, primarily due to high concentrations of glucose in the circulation (1). Hyperglycemia interrupts normal cellular metabolism and signaling and eventually causes organ dysfunction (2, 3). Nearly two centuries after diabetes was first recognized (4), its association with congenital birth defects and fetal mortality in pregnancy was recognized and referred to as diabetic embryopathy (4, 5). Prior to the introduction of insulin, diabetes-associated fetal and maternal mortality rates were nearly 70% and 40%, respectively (6, 7). Since the administration of insulin to control glycemia in pregnant women, these mortality rates have decreased dramatically to nearly 12% (8–15). In addition to the control of glycemia with insulin, aggressive perinatal care and neonatal management also contributed to the decline of maternal and fetal mortality (10, 16–19).

Unfortunately, the present birth defect rate in diabetic pregnancies (about 10%) is still higher than that in the general population (3%) and appears to be on the rise (7, 12, 13, 15, 20–25). The reasons for this increase in birth defect rates are complex. One reason is that

Correspondence to: Zhiyong Zhao, zzhao@fpi.umaryland.edu.

there has been a rapid increase in diabetic patients in the population which includes women of childbearing age (26). It is estimated that approximately 8,000 babies in the United States are born each year with maternal diabetes-associated congenital malformations. The incidence of these malformations also has risen to near-epidemic level in developing countries.

Tackling this issue involves battles at a number of fronts: diagnosing fetal anomalies must be improved; technologies that can recognize developmental malformations as early as the embryogenesis period are needed; and better prenatal and planned pregnancy consultations should be implemented to help reduce diabetes-associated birth defects. These remain ongoing challenges for perinatal care providers.

An important goal in eliminating birth defects is to develop therapeutic interventions that can protect embryos from hyperglycemic insult. This goal can only be achieved by understanding the cellular and molecular mechanisms underlying diabetic embryopathy. Basic research using animal models has contributed a considerable amount of information about the manifestations of fetal abnormalities, but more work is still needed.

Pre-gestational and gestational diabetes

Diabetes mellitus is a chronic disease manifested by hyperglycemia and its associated metabolic factors. This condition is usually diagnosed by measuring the levels of plasma glucose, expressed as mg/dL or mM, and glycosylated hemoglobin A (HbA_{1c}), indexed as percentage of total hemoglobin A (27–29). Manifestation of diabetes can be the result of insulin deficiency (type 1) or insulin resistance (type 2).

- Type 1 diabetes, or insulin-dependent diabetes, is caused by autoimmune destruction of insulin-producing β-cells in the pancreas (30, 31).
- Type 2 diabetes, or non-insulin-dependent diabetes, is caused by failure in insulin signaling to regulate cellular glucose uptake (32–34).

Diabetes mellitus, either type 1 or type 2, diagnosed in women before pregnancy is referred to as pre-gestational diabetes (20, 35–37). When hyperglycemia is detected after the onset of pregnancy, usually in the third trimester (24–28 weeks), the pregnant woman is considered having gestational diabetes mellitus (GDM) (38–42). According to guidelines from the International Association of Diabetes in Pregnancy Study Group (IADPSG), women who have a fasting plasma glucose ≥ 126 mg/dL and $HbA_{1c} \geq 6.5\%$ are diagnosed as having GDM (43).

Congenital birth defects in infants of diabetic mothers have been found to be associated with pre-gestational diabetes that is uncontrolled in the first trimester of pregnancy (44–48) Although a few cases have suggested a link between GDM and fetal birth defects, this association remains unclear and lacks strong supporting evidence (49, 50). One possible reason for the controversy is that some women with early-onset type 2 diabetes are misdiagnosed as having GDM when first screened in the second trimester (39, 51). Nevertheless, it is well established that GDM can lead to many adverse fetal outcomes, including macrosomia, hypoglycemia, hypocalcemia, and hyperbilirubinemia (51–53).

High glucose as a major teratogenic factor

Human studies have demonstrated a strong link between maternal glycemic level, as indicated by the association of plasma glucose and HbA1c levels (54, 55) with the incidence of congenital malformations in offspring (56–61). Other adverse metabolic factors produced in diabetes mellitus, such as ketone bodies, advanced glycation end products, and branched

chain amino acids, may have synergetic effects with glucose on disrupting normal embryonic development (62–66). The putative teratogenic effects of hyperglycemia are supported by studies that demonstrate a reduction in the incidence of birth defects following clinical interventions targeted at achieving euglycemia (67–70). Animal studies also have shown that isolated embryos in culture exposed to high concentrations of glucose develop malformations similar to those seen in human diabetic pregnancies (62, 71–73). These observations indicate that high glucose in either type 1 or type 2 diabetes is a major teratogenic factor that disturbs embryonic development.

The major effort to eliminate adverse outcome in pre-gestational diabetes and GDM complicated pregnancies is to control glycemic levels (20, 74–76). Clinical management of diabetic pregnancy with insulin and oral hypoglycemic medications can achieve the euglycemic standards, recommended by Diabetes Control and Complications Trials and International Federation of Clinical Chemistry and Laboratory Medicine (Table 1) (77–79). However, with respect to pre-gestional diabetes, optimal glycemic control prior to conception appears to be a challenge because:

- most women with diabetes do not seek preconception care,
- most have unplanned pregnancies (80).
- even in women who plan pregnancies and receive preconception counseling, euglycemia is difficult to achieve and maintain in non-clinical settings.

Therefore, alternative approaches to glucose control to prevent birth defects need to be developed and implemented.

Malformations and Diagnosis

Maternal diabetes-associated fetal anomalies can be seen in any organ system but are most common and severe in the central nervous system (CNS), cardiovascular system (CVS), craniofacial region, and caudal structure (Table 2) (11, 12, 25, 44, 55, 81, 82).

Central nervous system

The most common structural defects resulting from diabetic embryopathy occur in the brain region and spinal cord of the CNS () (12, 25, 83–86). Some of these can be recognized as early as in the first trimester using ultrasonography.

- Anencephaly is characterized as absence of the cerebral hemispheres with the brain stem and portions of the midbrain intact. It is readily discernible in the first trimester (Figure 1) (76, 87, 88). In the second trimester, it is more evident as poorly formed cranial bones and symmetric absence of the calvarium (76, 87, 89).
- Holoprosencephaly, the complete or partial absence of the midline echo within the fetal brain, can be diagnosed as early as 14 weeks of gestation, though diagnosis at the 20-week anomaly scan is more common (90–92).
- Excencephaly, the absence of the superior vault and convolution of the brain, can be diagnosed as early as 10 weeks of gestation (Figure 2) (93).
- Ultrasonic diagnosis of CNS defects such as spina bifida usually occurs in conjunction with detection of the second trimester maternal blood biomarker α-fetoprotein (81, 94–97).

Craniofacial structures

In newborns of diabetic mothers the most common anomalies in the craniofacial regions are hemifacial microsomia and microtia in newborns of diabetic mothers (82, 98). Cleft palate and lip also occur in relatively high frequency (99–101). Hearing impairment in children has been found to be associated with diabetic pregnancy (82, 98, 102), likely due to developmental defects in the inner and middle ear (102).

Prenatal diagnose of cleft lip and cleft palate relies a combination of coronal and axial two-dimensional ultrasound scans (103). Cleft lip can be easily recognized from coronal scan images, however, when cleft lip extends into the palate, the axial scan of the maxilla can provide the image of the defects (104). Cases of lateral cleft lip/palate often present a so-called maxillary pseudomass visualized in two-dimensional sonographs (105).

Cardiovascular system

Fetal cardiac defects associated with maternal diabetes have been extensively characterized. Anomalies commonly are present in:

- myocardium-derived structures
  - atria,
  - ventricles
  - interventricular septum.
- endocardium-derived structures
  - conotruncal spetum
  - ventricular septum
  - atrio-ventricular valves (44, 47, 106–108).

Sonographic imaging remains the standard method to diagnosis fetal cardiac abnormalities. Technological advances in imaging, especially in high frequency imaging and improvements in resolution, have aided perinatal care providers in recognizing cardiac defects as early as 10 weeks of gestation.

Most heart anomalies, including double outlet hearts, atrial septal defects, and ventricular septal defects, can be diagnosed using the four-chamber view, a transverse projection through the fetal thorax above the level of the diaphragm (Figure 3) (109). Measurement of the thickness of the ventricular walls in the sonographs can reveal myocardial hypoplasia. Due to the position of the canning plane, the four-chamber view usually misses defects in the structures in the outflow tracts. A so-called base view, with scans superior to the four-chamber view, can reveal abnormalities in the aorta and pulmonary artery. Complex defects such as Tetralogy of Fallot, which involves ventricular septal defects and great vessel defects, may require a combination of four-chambered and base views to diagnose.

Color Doppler provides an added advantage to practitioners because it can detect minor defects in the heart by exhibiting changes in blood flow pattern. The association of increased first trimester nuchal translucency with fetal cardiac defects has been observed; however, more studies are still needed to establish it as a diagnostic marker (110, 111).

Caudal regression

Caudal regression syndrome, also known as caudal dysplasia, is characterized as the absence or hypoplasia of caudal trunk and limbs (112, 113). Caudal regression syndrome can be

diagnosed by noting a shortened spine and abnormal lower limbs. This anomaly can be detected in the fetus as early as second trimester.

Mechanisms of Diabetic Embryopathy

Developmental mechanisms

Central Nervous System—Diabetic embryopathy causes embryonic and fetal abnormalities as a result of a disturbance in normal organogenesis. In the CNS, most anomalies occur because of a failure of early neural tube formation, referred to as neural tube defects (NTDs) (114–116). The formation of the neural tube, or neurulation, occurs in human embryos from weeks 3–6 of gestation (114). Neurulation begins with formation of the neural ectoderm, which develops into the neural folds. The neural folds grow dorsal-laterally and eventually fuse at the dorsal midline along the body axis to form the neural tube (117). In diabetic embryopathy, neurulation is perturbed in young embryos, leading to NTDs such as exencephaly or spina bifida (62, 73, 118, 119). Studies using diabetic rodents with poorly controlled hyperglycemia have recapitulated the same CNS defects observed in human fetuses (Figure 4) (120–122).

Cardiovascular System—The development of the heart is a complex process, involving multiple tissue types. The atria, ventricles, and interventricular septum are derived from the myocardium, whereas the membranous septa in the atrioventricular channel and the outflow tracts and associated valves are derived from the endocardium (123–126).

The most common abnormality of the ventricles is hypoplastic left heart syndrome (44). It is associated with under development of the myocardium during early cardiogenesis (121). Defects in endocardium-derived structures appear to be associated with endocardial cushions (Figure 5) (121, 127), bulbous structures that develop during early embryogenesis at the atrioventricular junction and the bulbous cortis (outflow tract) as a result of the production of the extracellular matrix (128, 129). During development of the endocardial cushions, endocardial (endothelial) cells differentiate into mesenchymal cells. This process is known as the endothelial-mesenchymal transformation (EMT) (128, 130). These mesenchymal cells migrate into the extracellular matrix to fill the acellular space and promote the growth of the cardiac structures (130). The endocardial cushions develop toward each other and eventually fuse to form a continuous septum (125). After fusion, the endocardial tissues undergo dramatic remodeling to connect with the interventricular and primary atrial septum and form valves (130, 131).

In embryos of diabetic pregnancies, early development of the endocardial cushions is inhibited (121). An impact of maternal hyperglycemia on later endocardial cushion remodeling also has been observed (132). Further research is needed to determine the significance of these developmental processes in cardiac malformation in diabetic embryopathy.

Craniofacial—In the craniofacial region, abnormalities in facial structures may be associated with dysmorphogenesis of cartilages (133, 134). These cartilages, such as the Meckel's cartilage in the mandibular arch, are not only involved in early morphogenesis of the facial processes, but also give rise to many types of bony structures in the craniofacial regions such as the auditory ossicles (135, 136).

Cellular mechanisms

The development of organ systems involves cell proliferation, death, migration, and differentiation. Excessive programmed cell death (apoptosis) has been observed in the dorsal

region of the neural tube and is associated with NTDs in diabetic embryopathy (71, 122, 137–142). In the developing heart, cell proliferation at the early stages and apoptosis at the late stages of cardiogenesis appear to be associated with cardiac malformations in the embryos of diabetic pregnancy (121). In addition to cell proliferation, endocardial cell differentiation and migration also are suppressed by maternal hyperglycemia (121, 127).

Development of the cardiac outflow tract requires cell migration from the neural crest in the dorsal region of the neural tube. These neural crest cells contribute significantly to the septation of the outflow segment and the formation of the great vessels (143, 144). Malformations in the outflow segment may be due to impaired neural crest cell migration, which can be caused by increased apoptosis (145, 146).

Craniofacial structure development also requires neural crest cells that migrate from the anterior neural tube during early embryogenesis (147–149). Studies using animal models have shown that maternal diabetes perturbs neural crest cell migration in embryos (133, 150). Increased apoptosis in the mandibular arch has been observed in embryos of diabetic women, though it is uncertain whether those cells are of neural crest-origin (146). The impact of maternal diabetes on the differentiation of the neural crest-origin craniofacial mesenchymal cells remains to be demonstrated.

Molecular mechanisms in promoting apoptosis in diabetic embryopathy

Endoplasmic reticulum stress—When exposed to high glucose, embryonic cells take up glucose via glucose transporters (151, 152). An influx of glucose disturbs intracellular metabolic homeostasis and organelle function. Dysfunction of the endoplasmic reticulum (ER) leads to aberrant protein folding and subsequent accumulation of unfolded and misfolded proteins in its lumen (153–155), which cause ER stress (156–159). Under stress conditions, the ER activates a number of molecular cascades, collectively known as the unfolded protein response (UPR), to increase expression of chaperone protein to resolve protein folding crisis, inhibit protein translation, suppress mitosis, and even trigger apoptosis (Figure 6) (160–162). ER stress has been observed in the embryos from diabetic pregnancies (127, 163, 164). The potential causative role of ER stress in embryonic malformation has been demonstrated with experiments using a chemical chaperone to resolve protein folding crisis (127).

Oxidative stress—High glucose also changes the morphology and function of mitochondria (165). Changes in mitochondria interrupt the electron transport chain, leading to generation of reactive oxygen species (ROS) (166, 167). High glucose also reduces the level of intracellular antioxidants, including glutathione (GSH) and thioredoxin (168–171). The imbalance of ROS and antioxidative buffering results in oxidative stress, which perturbs intracellular signaling (Figure 7) (172, 173). Treating diabetic pregnant animals or embryos cultured in high glucose with antioxidants decreases embryonic malformation rates (174–184). Moreover, embryos treated with antioxidants overexpress a transgene of superoxide dismutase resist maternal hyperglycemic insult (185, 186).

Nitrosative stress—Under hyperglycemic conditions, embryonic cells produce high levels of nitric oxide (NO). NO is a second messenger that regulates various intracellular signaling pathways (187, 188) and also can react with ROS to produce more toxic radicals, called reactive nitrogen species (RNS), than NO or ROS alone (189, 190). The high levels of RNS, such as peroxynitrite, generate a condition known as nitrosative stress (Figure 8).

Synthesis of NO is catalyzed by NO synthase (NOS) enzymes. There are three main forms of NOS enzymes: neuronal (nNOS and NOS1); endothelial (eNOS and NOS3); and inducible (iNOS and NOS2)(191–193). Both nNOS and eNOS are constitutively expressed

and do not vigorously respond to extracellular stimulation (194, 195), but iNOS actively responds to extracellular changes with marked upregulation in expression and activity (196–198).

In embryos of diabetic animals, eNOS expression is decreased (199); in contrast, iNOS expression is dramatically increased (200, 201). Experiments using an iNOS knockout model clearly demonstrate that high level of NO is detrimental to the embryo (Figure 8) (120).

Stress response

Phospholipid peroxidation—Cellular stress perturbs intracellular metabolic homeostasis. Phospholipid metabolism is initiated by phospholipases (PLs), including PLA, PLC, and PLD (202, 203). Cytosolic phospholipase A2 ($cPLA_2$) cleaves arachidonic acid from the cell membrane (204–206). In the cytoplasm, arachidonic acid undergoes two major pathways of metabolism: it can be converted into prostaglandin E_2 (PGE_2) by cyclooxygenase-2 (COX-2) (207, 208), or it can be converted into PGE_2-like isoprostanes, such as 8-iso-prostagladin F2 (8-iso-PGF2) and 8-iso-PGF2α, by non-COX-mediated peroxidation involving free radicals (Figure 9) (209, 210).

In embryos of diabetic animals or embryos exposed to high concentrations of glucose *in vitro*, the level of PGE_2 decreases dramatically (211–215). On the other hand, the level of 8-iso-PGF_2 is dramatically elevated in embryos under hyperglycemic conditions as well as in diabetic patients (209, 216). The reduction in PGE_2 in the embryo may be due to a decrease in COX-2 activity and expression (211, 217, 218), suggesting that a shift in arachidonic acid metabolism has occurred from producing PGE_2 to generating isoprostanes. These PGE_2-like isoprostanes have been shown to have damaging effects in animal models of diabetic pregnancy and embryos exposed to high glucose in culture (216, 219), whereas PGE_2 protects embryos from the damages due to hyperglycemic conditions (Figure 9) (211, 218, 220).

Protein kinase C family—Under cellular stress conditions, a number of intracellular signaling systems are affected, including the ones regulated by members of the protein kinase C (PKC) family. The PKC family of serine/threonine protein kinases consists of 12 members, which can be divided into the following three groups, based on their activation mechanisms (221, 222): 1) PKCα, β1, β2, and γ require calcium and diacylglycerol (DAG) for activation; 2) PKCδ, ε, η, ν, and θ require only DAG; and 3) PKCμ, ξ, and ι/λ do not require calcium or DAG, but instead require distinct lipid cofactors (i.e., ceramide and phosphatidylinositol-4-phosphate) (221).

In embryos of diabetic animals, each PKC isoform responds differently to hyperglycemia. For example, PKCα, β2, and δ are phosphorylated and activated under hyperglycemic conditions (141). Consequently, inhibiting PKCα, β2, and δ with isoform-specific inhibitors reduces malformation rates in embryos cultured in a high concentration of glucose (141, 223). Additionally, embryos from diabetic *pkcδ* knockout animal models exhibit significantly lower malformation rate than embryos from diabetic mice having the gene (164). These and other similar studies demonstrate that PKCs play critical roles in diabetic embryopathy.

Mitogen-activated protein kinase family—The mitogen-activated protein kinase (MAPK) family also plays an important role in mediating the effects of maternal hyperglycemia on embryonic development. Members of the MAPK family, including extracellular signal-regulated kinases (ERKs) and c-jun N-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), can usually be activated by oxidative stress. ERKs primarily

regulate cell proliferation and survival, whereas JNKs act on pro-apoptotic pathways (224–226). In embryos of diabetic animals, the levels of phosphorylated ERK1 and 2 are significantly reduced (Figure 8) (227, 228). In contrast, JNKs are activated in embryos of diabetic animals (227–230). Embryos treated with a JNK inhibitor while simultaneously incubated in a high concentration of glucose have a lower malformation rate, compared with those without JNK inhibition (231). More convincing evidence of the role of JNKs in diabetic embryopathy comes from the experiments using *jnk1* and *jnk2* knockout mice. Embryos homozygous for either *jnk1* or *jnk2* deletion show significantly lower malformation rates compared with a wild-type embryos from diabetic animals (231, 232).

Programmed cell death (Apoptosis)

Apoptosis is precisely regulated by a number of factors, including PKCs, JNKs, and members of the Bcl-2 and caspase families (233). Although the mechanisms by which PKCs and JNKs promote apoptosis in diabetic embryopathy remain to be delineated, much is known about the roles that Bcl-2 and caspase family members play in programmed cell death.

In the Bcl-2 family, some members are pro-apoptotic, such as Bax, Bak, and Bid, whereas other members are anti-apoptotic, such as Bcl-2 and Bcl-xL (234). In the caspase family, some members play a role in executing apoptosis. These members, known as effector or executioner caspases, include casapse-3, -6, and -7 (235–237). Effector caspases can be activated by another group of caspases, known as initiator caspases, including caspase-8, -9, and -10 (235–237).

Bax and Bak form a pore in the outer membrane of mitochondria (238, 239), allowing cytochrome C to be release to cytoplasm to induce apoptosis (240–242). During apoptosis, Bax and Bim expression levels increase in the cell (238, 239). Bax is activated by truncated Bid (tBid), which is produced when Bid is cleaved by serine/threonine proteases such as caspase-8.

In diabetic embryopathy, caspase-8 cleaves Bid into tBid, which stimulates the release of cytochrome C (140). Cytochrome C binds to apoptosis protease-activating factor-1 (Apaf-1), and the resulting complex activates Caspase-9 by forming an apoptosome. Activated Caspase-9 then activates effector caspases, including Caspase-3, 6, and 7, which turn on caspase-activated DNase and other factors, leading to DNA fragmentation and cell death (Figure 10) (240, 241).

In animal studies, suppression of apoptosis using caspase inhibitors can reduce embryonic malformations in the embryos cultured in high glucose (140). The results imply potential interventional strategies to block apoptosis in embryos in diabetic pregnancies.

Interventions and Clinical Challenges

Research conducted in animal models of diabetic pregnancy has revealed some of the major molecular changes and subsequent intracellular metabolic conditions that occur in embryos in response to hyperglycemia. This information has guided efforts to explore interventional approaches to reduce diabetes-induced embryonic malformations (122, 174, 183, 184, 227, 243–247).

Targeting lipid metabolism

One anomaly that occurs in diabetic embryopathy is aberrant phospholipid metabolism, especially lipoperoxidation. Dietary supplementation with arachidonic acid or myo-inositol in diabetic pregnant animals has been shown to reduce embryonic malformations due to

lipoperoxidation (183, 184, 244). Treatment of diabetic pregnancy animals with polyunsaturated fatty acids also have been shown to exert similar helpful effects on embryonic development (227, 248).

Antioxidative strategies

Targeting oxidative stress to treat diabetic embryopathy, using lipoic acid, ergothioneine, vitamin C, and vitamin E, has been tested in diabetic pregnant animals and shown to decrease embryonic malformations (174, 181, 183, 184, 243, 249). N-acetylcysteine (NAC), which has been used as an antioxidant in clinical practices to treat acetaminophen poisoning (250, 251), has been observed to reduce malformations in animal embryos in culture exposed to high glucose (252–254); however, its effect in human diabetic pregnancy remains to be demonstrated.

Folic acid, or water-soluble vitamin B9, has long been known to reduce NTDs in humans (255, 256). The effect of folic acid in reducing embryonic malformations associated with maternal diabetes has been explored in animal models. Treating cultured rodent embryos with folic acid can reduce neural tube dysmorphogenesis induced by high concentrations of glucose (246). In *in vivo* experiments, diabetic pregnant rats injected with folic acid also exhibit decreased NTDs in their embryos, compared with diabetic pregnant rats not given the supplement (122, 246, 257).

Combination approaches

The combination of antioxidants and phospholipids that target multiple molecular pathways could have more potent effects in reducing fetal abnormalities compared with monotherapy. Cocktails of vitamin E, arachidonic acid, and myo-inositol have been explored in diabetic animal models and have been shown to decrease embryonic malformations (183, 184). The efficacy of antioxidants to prevent fetal defects in human diabetic pregnancy has not been examined. Antioxidant approaches tested to treat similar diseases, such as preeclampsia, have produced unsatisfactory results (258–262). These results have tampered the enthusiasm in applying similar strategies to treat diabetic embryopathy, but exploration of other strategies is warranted.

Recently, alleviating nitrosative stress via iNOS inhibitors has been tested in diabetic embryopathy (163). Oral treatment of diabetic pregnant animals with an iNOS inhibitor decreased embryonic malformation rates (163). Additional studies are needed to test therapeutic agents or dietary supplements that inhibit iNOS in order to translate these basic research findings into clinical applications for human disease.

Clinical challenges

Diabetic embryopathy involves complex molecular interactions. Extensive understanding of its mechanisms is essential for identifying therapeutic targets and developing effective interventions. Many challenges in developing these approaches lie ahead. First, interventions must be safe for the embryo and mother. The ideal approaches include dietary supplementation of non-toxic agents. Second, because developmental malformations occur early on in the first trimester of pregnancy, the dietary supplements must be easily accessible for women to take before conception or soon thereafter. Third, any therapeutic agents administered via oral treatments or dietary supplements must be able to pass the maternal-fetal barrier to exert potent effects on the developing embryo.

To overcome these challenges, it not only needs scientific research to decipher the molecular mechanisms underlying embryonic malformations and develop prevention strategies, but also requires education of the public to raise awareness about the risk of birth defects in

diabetic pregnancy. Preconception counseling and pregnancy planning have been shown to be correlated with reduction in adverse outcomes of pregnancies (263–266). However, more cooperative efforts between perinatal care providers and patients are needed to achieve the goal of eliminating birth defects.

Concluding remarks

Diabetic embryopathy is a global public health issue. Although pregestational screening for maternal diabetes, perinatal care, and postnatal management in developed countries are available to most pregnant women, the rate of birth defects in infants of diabetic mothers remains high. In developing countries, the rates of birth defects and even mortality are high because of unavailable and inadequate care for pregnant women. With the worldwide increase in obesity and type 2 diabetes, diagnosis and management of diabetic pregnancy is a big challenge for the medical community. The increasing public health burden of diabetes in women of childbearing age makes it important to develop interventional approaches to prevent embryonic malformations.

Further understanding of the mechanisms underlying diabetic embryopathy will provide crucial information for developing effective interventions. Clinical approaches must be safe to administer in early pregnancy and accessible to women prior to and soon after conception to be highly effective because diabetes in early pregnancy often goes undetected and many women have unplanned pregnancies. Major cellular activities--i.e., proliferation and apoptosis--and intracellular metabolic conditions--i.e., nitrosative, ER, and oxidative stress--have been demonstrated to be associated with diabetic embryopathy using animal models. Translating advances made in animal studies into clinical applications in humans will require collaborative efforts across the basic research, preclinical, and clinical communities.

References

1. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2012; 35 (Suppl 1):S64–71. [PubMed: 22187472]
2. Porte D Jr, Schwartz MW. Diabetes complications: why is glucose potentially toxic? Science. 1996; 272:699–700. [PubMed: 8614830]
3. King GL. The role of hyperglycaemia and hyperinsulinaemia in causing vascular dysfunction in diabetes. Ann Med. 1996; 28:427–32. [PubMed: 8949974]
4. Barnett, DM.; Krall, LP. The history of Diabetes. In: Kahn, BB.; King, GL.; Moses, AC.; Weir, GC.; Jacobson, AMJSR., editors. Joslin's Diabetes Mellitus. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 1-17.
5. LeCorche E. Du Diabetic dans se rapports avec la vie uterine menstruation et al grusesse. Ann Gynecol. 1885; 24:257.
6. Duncan JM. On puerperal diabetes. Trans Obstet Soc Lond. 1882; 24:256.
7. Joslin, EP. Treatment of Diabetes Mellitus. Philadelphia: Lea and Febiger; 1923. Number of pages
8. Pedersen, J. The Pregnant Diabetic and Her Newborn: Problems and Management. Baltimore: Williams and Wilkins; 1977. Number of pages
9. Olofsson P, Liedholm H, Sartor G, et al. Diabetes and pregnancy. A 21-year Swedish material. Acta Obstet Gynecol Scand Suppl. 1984; 122:3–62. [PubMed: 6388217]
10. Kitzmiller JL, Gavin LA, Gin GD, et al. Preconception care of diabetes. Glycemic control prevents congenital anomalies. Jama. 1991; 265:731–6. [PubMed: 1990188]
11. Kucera J. Rate and type of congenital anomalies among offspring of diabetic women. J Reprod Med. 1971; 7:73–82. [PubMed: 5095696]
12. Soler NG, Walsh CH, Malins JM. Congenital malformations in infants of diabetic mothers. Q J Med. 1976; 45:303–13. [PubMed: 781716]

13. Miodovnik M, Mimouni F, Dignan PS, et al. Major malformations in infants of IDDM women. Vasculopathy and early first-trimester poor glycemic control. Diabetes Care. 1988; 11:713–8. [PubMed: 3224542]
14. Pedersen JF, Molsted-Pedersen L, Mortensen HB. Fetal growth delay and maternal hemoglobin A1c in early diabetic pregnancy. Obstet Gynecol. 1984; 64:351–2. [PubMed: 6462566]
15. Rubin A, Murphy DP. Studies in human reproduction. III. The frequency of congenital malformations in the offspring of nondiabetic and diabetic individuals. J Pediatr. 1958; 53:579–85. [PubMed: 13588508]
16. Damm P, Molsted-Pedersen L. Significant decrease in congenital malformations in newborn infants of an unselected population of diabetic women. Am J Obstet Gynecol. 1989; 161:1163–7. [PubMed: 2686445]
17. Hanson U, Persson B, Thunell S. Relationship between haemoglobin A1C in early type 1 (insulin-dependent) diabetic pregnancy and the occurrence of spontaneous abortion and fetal malformation in Sweden. Diabetologia. 1990; 33:100–4. [PubMed: 2328844]
18. Fuhrmann K, Reiher H, Semmler K, et al. Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. Diabetes Care. 1983; 6:219–23. [PubMed: 6347574]
19. Greene MF, Hare JW, Cloherty JP, et al. First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. Teratology. 1989; 39:225–31. [PubMed: 2727930]
20. Reece EA, Homko CJ. Assessment and management of pregnancies complicated by pregestational and gestational diabetes mellitus. J Assoc Acad Minor Phys. 1994; 5:87–97. [PubMed: 7949826]
21. Kalter H, Warkany J. Medical progress. Congenital malformations: etiologic factors and their role in prevention (first of two parts). N Engl J Med. 1983; 308:424–31. [PubMed: 6337330]
22. Tsang RC, Ballard J, Braun C. The infant of the diabetic mother: today and tomorrow. Clin Obstet Gynecol. 1981; 24:125–47. [PubMed: 7011629]
23. Hadden DR. Diabetes in pregnancy 1985. Diabetologia. 1986; 29:1–9. [PubMed: 3514340]
24. Ballard JL, Holroyde J, Tsang RC, et al. High malformation rates and decreased mortality in infants of diabetic mothers managed after the first trimester of pregnancy (1956–1978). Am J Obstet Gynecol. 1984; 148:1111–8. [PubMed: 6711647]
25. Mills JL. Malformations in infants of diabetic mothers. Teratology. 1982; 25:385–94. [PubMed: 7051398]
26. Harris MI, Flegal KM, Cowie CC, et al. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988–1994. Diabetes Care. 1998; 21:518–24. [PubMed: 9571335]
27. Peacock I. Glycosylated haemoglobin: measurement and clinical use. J Clin Pathol. 1984; 37:841–51. [PubMed: 6381544]
28. Lai LC. Global standardisation of HbA1c. Malays J Pathol. 2008; 30:67–71. [PubMed: 19291914]
29. Consensus C. Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care. 2007; 30:2399–400. [PubMed: 17726190]
30. Eisenbarth, GS. Type 1 Diabetes Mellitus. In: Kahn, CR.; Weir, GC.; King, GL.; Jacobson, AM.; Moses, AC.; Smith, RJ., editors. Diabetes Mellitus. Philladelphia: Lippincott Williams & Wilkins; 2005. p. 399-424.
31. Tisch R, McDevitt H. Insulin-dependent diabetes mellitus. Cell. 1996; 85:291–7. [PubMed: 8616883]
32. Muoio DM, Newgard CB. Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nature reviews. 2008; 9:193–205.
33. Meeuwisse-Pasterkamp SH, van der Klauw MM, Wolffenbuttel BH. Type 2 diabetes mellitus: prevention of macrovascular complications. Expert review of cardiovascular therapy. 2008; 6:323–41. [PubMed: 18327994]
34. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992; 35:595–601. [PubMed: 1644236]

35. Meyer BA, Palmer SM. Pregestational diabetes. Semin Perinatol. 1990; 14:12–23. [PubMed: 2180071]
36. Dunne FP. Pregestational Diabetes Mellitus and Pregnancy. Trends Endocrinol Metab. 1999; 10:179–82. [PubMed: 10370226]
37. Forsbach-Sanchez G, Tamez-Perez HE, Vazquez-Lara J. Diabetes and pregnancy. Arch Med Res. 2005; 36:291–9. [PubMed: 15925019]
38. Ratner, RE.; Passaro, MD. Gestational diabetes mellitus. In: LeRoith, D.; Taylor, SI.; Olefsky, JM., editors. Diabetes Mellitus : A Fundamental and Clinical Text. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 1291-300.
39. Reece EA, Leguizamon G, Wiznitzer A. Gestational diabetes: the need for a common ground. Lancet. 2009; 373:1789–97. [PubMed: 19465234]
40. Ullrich I, Yeater R. Gestational diabetes. W V Med J. 1995; 91:148–51. [PubMed: 7610650]
41. Persson B, Hanson U. Neonatal morbidities in gestational diabetes mellitus. Diabetes Care. 1998; 21 (Suppl 2):B79–84. [PubMed: 9704232]
42. Lucas MJ. Diabetes complicating pregnancy. Obstet Gynecol Clin North Am. 2001; 28:513–36. [PubMed: 11512498]
43. Metzger BE, et al. International Association of D Pregnancy Study Groups Consensus P. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010; 33:676–82. [PubMed: 20190296]
44. Correa A, Gilboa SM, Besser LM, et al. Diabetes mellitus and birth defects. Am J Obstet Gynecol. 2008; 199:237 e1–9. [PubMed: 18674752]
45. Reece EA. Diabetes-induced birth defects: what do we know? What can we do? Curr Diab Rep. 2012; 12:24–32. [PubMed: 22167469]
46. Becerra JE, Khoury MJ, Cordero JF, et al. Diabetes mellitus during pregnancy and the risks for specific birth defects: a population-based case-control study. Pediatrics. 1990; 85:1–9. [PubMed: 2404255]
47. Loffredo CA, Wilson PD, Ferencz C. Maternal diabetes: an independent risk factor for major cardiovascular malformations with increased mortality of affected infants. Teratology. 2001; 64:98–106. [PubMed: 11460261]
48. Ramos-Arroyo MA, Rodriguez-Pinilla E, Cordero JF. Maternal diabetes: the risk for specific birth defects. EurJ Epidemiol. 1992; 8:503–08. [PubMed: 1397216]
49. Farrell T, Neale L, Cundy T. Congenital anomalies in the offspring of women with type 1, type 2 and gestational diabetes. Diabet Med. 2002; 19:322–6. [PubMed: 11943005]
50. Sheffield JS, Butler-Koster EL, Casey BM, et al. Maternal diabetes mellitus and infant malformations. Obstet Gynecol. 2002; 100:925–30. [PubMed: 12423854]
51. Reece EA. The fetal and maternal consequences of gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2010; 23:199–203. [PubMed: 20121460]
52. Garcia Carrapato MR. The offspring of gestational diabetes. J Perinat Med. 2003; 31:5–11. [PubMed: 12661138]
53. Jones CW. Gestational diabetes and its impact on the neonate. Neonatal Netw. 2001; 20:17–23. [PubMed: 12144115]
54. Shields LE, Gan EA, Murphy HF, et al. The prognostic value of hemoglobin A1c in predicting fetal heart disease in diabetic pregnancies. Obstet Gynecol. 1993; 81:954–7. [PubMed: 8497362]
55. Miller E, Hare JW, Cloherty JP, et al. Elevated maternal hemoglobin A1c in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med. 1981; 304:1331–4. [PubMed: 7012627]
56. Greene MF, Hare JW, Cloherty JP, et al. First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. Teratology. 1989; 39:225–31. [PubMed: 2727930]
57. Rose BI, Graff S, Spencer R, et al. Major congenital anomalies in infants and glycosylated hemoglobin levels in insulin-requiring diabetic mothers. J Perinatol. 1988; 8:309–11. [PubMed: 3236099]

58. Lucas MJ, Leveno KJ, Williams ML, et al. Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations. Am J ObstetGynecol. 1989; 161:426–31.
59. Key TC, Giuffrida R, Moore TR. Predictive value of early pregnancy glycohemoglobin in the insulin- treated diabetic patient. Am J ObstetGynecol. 1987; 156:1096–100.
60. Miller E, Hare JW, Cloherty JP, et al. Elevated maternal hemoglobin A1c in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med. 1981; 304:1331–34. [PubMed: 7012627]
61. Ylinen K, Aula P, Stenman UH, et al. Risk of minor and major fetal malformations in diabetics with high haemoglobin A1c values in early pregnancy. BrMed J (Clin ResEd). 1984; 289:345–46.
62. Eriksson UJ, Borg LA, Cederberg J, et al. Pathogenesis of diabetes-induced congenital malformations. Ups J Med Sci. 2000; 105:53–84. [PubMed: 11095105]
63. Horton WE Jr, Sadler TW. Effects of maternal diabetes on early embryogenesis. Alterations in morphogenesis produced by the ketone body, B-hydroxybutyrate. Diabetes. 1983; 32:610–6. [PubMed: 6862109]
64. Hunter ES 3rd, Sadler TW, Wynn RE. A potential mechanism of DL-beta-hydroxybutyrate-induced malformations in mouse embryos. Am J Physiol. 1987; 253:E72–80. [PubMed: 3111272]
65. Hunter ES 3rd, Sadler TW. D-(−)-beta-hydroxybutyrate-induced effects on mouse embryos in vitro. Teratology. 1987; 36:259–64. [PubMed: 3424210]
66. Moore DC, Stanisstreet M, Clarke CA. Morphological and physiological effects of beta-hydroxybutyrate on rat embryos grown in vitro at different stages. Teratology. 1989; 40:237–51. [PubMed: 2595599]
67. Eriksson UJ, Cederberg J, Wentzel P. Congenital malformations in offspring of diabetic mothers--animal and human studies. Rev Endocr Metab Disord. 2003; 4:79–93. [PubMed: 12618562]
68. Persson B. Prevention of fetal malformation with antioxidants in diabetic pregnancy. PediatrRes. 2001; 49:742–43.
69. Fuhrmann K, Reiher H, Semmler K, et al. The effect of intensified conventional insulin therapy before and during pregnancy on the malformation rate in offspring of diabetic mothers. Exp Clin Endocrinol. 1984; 83:173–77. [PubMed: 6373320]
70. Kitzmiller JL, Gavin LA, Gin GD, et al. Preconception care of diabetes. Glycemic control prevents congenital anomalies. JAMA. 1991; 265:731–36. [PubMed: 1990188]
71. Gareskog M, Cederberg J, Eriksson UJ, et al. Maternal diabetes in vivo and high glucose concentration in vitro increases apoptosis in rat embryos. Reprod Toxicol. 2007; 23:63–74. [PubMed: 17034987]
72. Akazawa S. Diabetic embryopathy: studies using a rat embryo culture system and an animal model. Congenit Anom (Kyoto). 2005; 45:73–9. [PubMed: 16131363]
73. Zhao Z, Reece EA. Experimental mechanisms of diabetic embryopathy and strategies for developing therapeutic interventions. J Soc Gynecol Investig. 2005; 12:549–57.
74. Lee-Parritz A. New technologies for the management of pregestational diabetes mellitus. Obstet Gynecol Surv. 2012; 67:167–75. [PubMed: 22901950]
75. Ballas J, Moore TR, Ramos GA. Management of diabetes in pregnancy. Curr Diab Rep. 2012; 12:33–42. [PubMed: 22139557]
76. Reece, EA.; Friedman, AM.; Copel, JA., et al. Prenatal diagnosis and management of deviant fetal growth and congenital malformations. In: Reece, EA.; Coustan, DR., editors. Diabetes Mellitus in Pregnancy. New York: Churchill Livingstone; 1995. p. 219-49.
77. Guideline Development G. Management of diabetes from preconception to the postnatal period: summary of NICE guidance. BMJ. 2008; 336:714–7. [PubMed: 18369227]
78. Ali S, Dornhorst A. Diabetes in pregnancy: health risks and management. Postgrad Med J. 2011; 87:417–27. [PubMed: 21368321]
79. Kitzmiller JL, Block JM, Brown FM, et al. Managing preexisting diabetes for pregnancy: summary of evidence and consensus recommendations for care. Diabetes Care. 2008; 31:1060–79. [PubMed: 18445730]
80. Holing EV, Beyer CS, Brown ZA, et al. Why don't women with diabetes plan their pregnancies? Diabetes Care. 1998; 21:889–95. [PubMed: 9614603]

81. Reece EA, Hobbins JC. Diabetic embryopathy: pathogenesis, prenatal diagnosis and prevention. Obstet Gynecol Surv. 1986; 41:325–35. [PubMed: 2423939]
82. Ewart-Toland A, Yankowitz J, Winder A, et al. Oculoauriculovertebral abnormalities in children of diabetic mothers. Am J Med Genet. 2000; 90:303–9. [PubMed: 10710228]
83. Zacharias JF, Jenkins JH, Marion JP. The incidence of neural tube defects in the fetus and neonate of the insulin-dependent diabetic woman. Am J Obstet Gynecol. 1984; 150:797–8. [PubMed: 6496607]
84. Barr M Jr, Hanson JW, Currey K, et al. Holoprosencephaly in infants of diabetic mothers. J Pediatr. 1983; 102:565–8. [PubMed: 6834191]
85. Matsunaga E, Shiota K. Holoprosencephaly in human embryos: epidemiologic studies of 150 cases. Teratology. 1977; 16:261–72. [PubMed: 594909]
86. Rusnak SL, Driscoll SG. Congenital Spinal Anomalies In Infants Of Diabetic Mothers. Pediatrics. 1965; 35:989–95. [PubMed: 14296423]
87. Aubry MC, Aubry JP, Dommergues M. Sonographic prenatal diagnosis of central nervous system abnormalities. Childs Nerv Syst. 2003; 19:391–402. [PubMed: 12905003]
88. Cameron M, Moran P. Prenatal screening and diagnosis of neural tube defects. Prenat Diagn. 2009; 29:402–11. [PubMed: 19301349]
89. Drugan A, Weissman A, Evans MI. Screening for neural tube defects. Clin Perinatol. 2001; 28:279–87. vii. [PubMed: 11499052]
90. Turner CD, Silva S, Jeanty P. Prenatal diagnosis of alobar holoprosencephaly at 10 weeks of gestation. Ultrasound Obstet Gynecol. 1999; 13:360–2. [PubMed: 10380303]
91. Peebles DM. Holoprosencephaly. Prenat Diagn. 1998; 18:477–80. [PubMed: 9621381]
92. Wong HS, Tang MH, Yan KW, et al. Histological findings in a case of alobar holoprosencephaly diagnosed at 10 weeks of pregnancy. Prenat Diagn. 1999; 19:859–62. [PubMed: 10521846]
93. Blaas HG, Eik-Nes SH. Sonoembryology and early prenatal diagnosis of neural anomalies. Prenat Diagn. 2009; 29:312–25. [PubMed: 19194866]
94. Cuckle HS. Screening for neural tube defects. Ciba Found Symp. 1994; 181:253–66. discussion 66–9. [PubMed: 7516277]
95. Pergament E. Alpha-fetoprotein and the prenatal diagnosis of neural tube defects. Obstet Gynecol Annu. 1977; 6:173–89. [PubMed: 73159]
96. Haddow JE, Knight GJ, Kloza EM. Alpha-fetoprotein screening in pregnancy-a test whose time has come. Ariz Med. 1982; 39:436–9. [PubMed: 6181763]
97. Haddow JE, Miller WA. Prenatal diagnosis of open neural tube defects. Methods Cell Biol. 1982; 26:67–94. [PubMed: 6182446]
98. Wang R, Martinez-Frias ML, Graham JM Jr. Infants of diabetic mothers are at increased risk for the oculo-auriculo-vertebral sequence: A case-based and case-control approach. J Pediatr. 2002; 141:611–7. [PubMed: 12410187]
99. Aberg A, Westbom L, Kallen B. Congenital malformations among infants whose mothers had gestational diabetes or preexisting diabetes. Early Hum Dev. 2001; 61:85–95. [PubMed: 11223271]
100. Garcia-Patterson A, Erdozain L, Ginovart G, et al. In human gestational diabetes mellitus congenital malformations are related to pre-pregnancy body mass index and to severity of diabetes. Diabetologia. 2004; 47:509–14. [PubMed: 14770278]
101. Spilson SV, Kim HJ, Chung KC. Association between maternal diabetes mellitus and newborn oral cleft. Ann Plast Surg. 2001; 47:477–81. [PubMed: 11716256]
102. Gratz ES, Pollack MA, Zimmerman RD. Congenital facial palsy and ipsilateral deafness: association with maternal diabetes mellitus. Int J Pediatr Otorhinolaryngol. 1981; 3:335–41. [PubMed: 7327849]
103. Pilu G, Reece EA, Romero R, et al. Prenatal diagnosis of craniofacial malformations with ultrasonography. Am J Obstet Gynecol. 1986; 155:45–50. [PubMed: 3524242]
104. Ghi T, Perolo A, Banzi C, et al. Two-dimensional ultrasound is accurate in the diagnosis of fetal craniofacial malformation. Ultrasound Obstet Gynecol. 2002; 19:543–51. [PubMed: 12047531]

105. Nyberg DA, Hegge FN, Kramer D, et al. Premaxillary protrusion: a sonographic clue to bilateral cleft lip and palate. J Ultrasound Med. 1993; 12:331–5. [PubMed: 8515530]
106. Wren C, Birrell G, Hawthorne G. Cardiovascular malformations in infants of diabetic mothers. Heart. 2003; 89:1217–20. [PubMed: 12975424]
107. Ferencz C, Rubin JD, McCarter RJ, et al. Maternal diabetes and cardiovascular malformations: predominance of double outlet right ventricle and truncus arteriosus. Teratology. 1990; 41:319–26. [PubMed: 2326756]
108. Rowland TW, Hubbell JP Jr, Nadas AS. Congenital heart disease in infants of diabetic mothers. J Pediatr. 1973; 83:815–20. [PubMed: 4742573]
109. Rajiah P, Mak C, Dubinksy TJ, et al. Ultrasound of fetal cardiac anomalies. AJR Am J Roentgenol. 2011; 197:W747–60. [PubMed: 21940548]
110. Axt-Fliedner R, Gembruch U. Nuchal translucency and fetal cardiac malformations. Ultraschall in der Medizin. 2010; 31:144–50. [PubMed: 20094976]
111. Clur SA, Ottenkamp J, Bilardo CM. The nuchal translucency and the fetal heart: a literature review. Prenatal diagnosis. 2009; 29:739–48. [PubMed: 19399754]
112. Assemany SR, Muzzo S, Gardner LI. Syndrome of phocomelic diabetic embryopathy (caudal dysplasia). Am J Dis Child. 1972; 123:489–91. [PubMed: 5026223]
113. Welch JP, Aterman K. The syndrome of caudal dysplasia: a review, including etiologic considerations and evidence of heterogeneity. Pediatr Pathol. 1984; 2:313–27. [PubMed: 6393099]
114. Sadler TW. Embryology of neural tube development. American journal of medical genetics. 2005; 135C:2–8. [PubMed: 15806586]
115. Kaufman BA. Neural tube defects. Pediatr Clin North Am. 2004; 51:389–419. [PubMed: 15062676]
116. Hollyday M. Neurogenesis in the vertebrate neural tube. Int J Dev Neurosci. 2001; 19:161–73. [PubMed: 11255030]
117. Altmann CR, Brivanlou AH. Neural patterning in the vertebrate embryo. International review of cytology. 2001; 203:447–82. [PubMed: 11131523]
118. Loeken MR. Current perspectives on the causes of neural tube defects resulting from diabetic pregnancy. Am J Med Genet C Semin Med Genet. 2005; 135C:77–87. [PubMed: 15800853]
119. Chappell JH Jr, Wang XD, Loeken MR. Diabetes and apoptosis: neural crest cells and neural tube. Apoptosis. 2009; 14:1472–83. [PubMed: 19333760]
120. Sugimura Y, Murase T, Oyama K, et al. Prevention of neural tube defects by loss of function of inducible nitric oxide synthase in fetuses of a mouse model of streptozotocin-induced diabetes. Diabetologia. 2009; 52:962–71. [PubMed: 19283362]
121. Zhao Z. Cardiac malformations and alteration of TGFbeta signaling system in diabetic embryopathy. Birth Defects Res B Dev Reprod Toxicol. 2010; 89:97–105. [PubMed: 20127828]
122. Oyama K, Sugimura Y, Murase T, et al. Folic acid prevents congenital malformations in the offspring of diabetic mice. Endocr J. 2009; 56:29–37. [PubMed: 18781038]
123. Wessels A, Markman MW, Vermeulen JL, et al. The development of the atrioventricular junction in the human heart. Circ Res. 1996; 78:110–7. [PubMed: 8603493]
124. Moorman A, Webb S, Brown NA, et al. Development of the heart: (1) formation of the cardiac chambers and arterial trunks. Heart. 2003; 89:806–14. [PubMed: 12807866]
125. Anderson RH, Webb S, Brown NA, et al. Development of the heart: (2) Septation of the atriums and ventricles. Heart. 2003; 89:949–58. [PubMed: 12860885]
126. Anderson RH, Webb S, Brown NA, et al. Development of the heart: (3) formation of the ventricular outflow tracts, arterial valves, and intrapericardial arterial trunks. Heart. 2003; 89:1110–8. [PubMed: 12923046]
127. Zhao Z. Endoplasmic reticulum stress in maternal diabetes-induced cardiac malformations during critical cardiogenesis period. Birth Defects Res B Dev Reprod Toxicol. 2012; 95:1–6.
128. Markwald R, Eisenberg C, Eisenberg L, et al. Epithelial-mesenchymal transformations in early avian heart development. Acta Anat. 1996; 156:173–86. [PubMed: 9124035]

129. Mjaatvedt, GH.; Yamamura, H.; Wessels, A., et al. Mechanisms of segmentation, septation, and remodeling of the tubular heart: endocardial cushion fate and cardiac looping. In: Harvey, RP.; Rosenthal, N., editors. Heart Development. New York: Academic Press; 1999. p. 159-77.
130. Person AD, Klewer SE, Runyan RB. Cell biology of cardiac cushion development. Int Rev Cytol. 2005; 243:287–335. [PubMed: 15797462]
131. Zhao Z, Rivkees SA. Programmed cell death in the developing heart: regulation by BMP4 and FGF2. Dev Dyn. 2000; 217:388–400. [PubMed: 10767083]
132. Kumar SD, Dheen ST, Tay SS. Maternal diabetes induces congenital heart defects in mice by altering the expression of genes involved in cardiovascular development. Cardiovascular diabetology. 2007; 6:34. [PubMed: 17967198]
133. Siman CM, Gittenberger-De Groot AC, Wisse B, et al. Malformations in offspring of diabetic rats: morphometric analysis of neural crest-derived organs and effects of maternal vitamin E treatment. Teratology. 2000; 61:355–67. [PubMed: 10777831]
134. Tanigawa K, Kawaguchi M, Tanaka O, et al. Skeletal malformations in rat offspring. Long-term effect of maternal insulin-induced hypoglycemia during organogenesis. Diabetes. 1991; 40:1115–21. [PubMed: 1936618]
135. Bowden RE. Development of the middle and external ear in man. Proc R Soc Med. 1977; 70:807–15. [PubMed: 341170]
136. Mallo M. Formation of the middle ear: recent progress on the developmental and molecular mechanisms. Dev Biol. 2001; 231:410–9. [PubMed: 11237469]
137. Sun F, Kawasaki E, Akazawa S, et al. Apoptosis and its pathway in early post-implantation embryos of diabetic rats. Diabetes Res Clin Pract. 2005; 67:110–8. [PubMed: 15649569]
138. Phelan SA, Ito M, Loeken MR. Neural tube defects in embryos of diabetic mice: role of the Pax-3 gene and apoptosis. Diabetes. 1997; 46:1189–97. [PubMed: 9200655]
139. Fine EL, Horal M, Chang TI, et al. Evidence that elevated glucose causes altered gene expression, apoptosis, and neural tube defects in a mouse model of diabetic pregnancy. Diabetes. 1999; 48:2454–62. [PubMed: 10580436]
140. Zhao Z, Yang P, Eckert RL, et al. Caspase-8: a key role in the pathogenesis of diabetic embryopathy. Birth Defects Res B Dev Reprod Toxicol. 2009; 86:72–7. [PubMed: 19194987]
141. Zhao Z, Wu Y-K, Reece EA. Demonstration of the essential role of protein kinase C isoforms in hyperglycemia-induced embryonic malformations. Reproductive Sciences. 2008; 15:349–56. [PubMed: 18497343]
142. Singh CK, Kumar A, Hitchcock DB, et al. Resveratrol prevents embryonic oxidative stress and apoptosis associated with diabetic embryopathy and improves glucose and lipid profile of diabetic dam. Mol Nutr Food Res. 2011; 55:1186–96. [PubMed: 21254394]
143. Brown CB, Baldwin HS. Neural crest contribution to the cardiovascular system. Adv Exp Med Biol. 2006; 589:134–54. [PubMed: 17076279]
144. Stoller JZ, Epstein JA. Cardiac neural crest. Semin Cell Dev Biol. 2005; 16:704–15. [PubMed: 16054405]
145. Morgan SC, Relaix F, Sandell LL, et al. Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. Birth Defects Res A Clin Mol Teratol. 2008; 82:453–63. [PubMed: 18435457]
146. Wentzel P, Gareskog M, Eriksson UJ. Decreased cardiac glutathione peroxidase levels and enhanced mandibular apoptosis in malformed embryos of diabetic rats. Diabetes. 2008; 57:3344–52. [PubMed: 18728230]
147. Graham A, Begbie J, McGonnell I. Significance of the cranial neural crest. Dev Dyn. 2004; 229:5–13. [PubMed: 14699573]
148. Chai Y, Maxson RE Jr. Recent advances in craniofacial morphogenesis. Dev Dyn. 2006; 235:2353–75. [PubMed: 16680722]
149. Santagati F, Rijli FM. Cranial neural crest and the building of the vertebrate head. Nature reviews. 2003; 4:806–18.
150. Cederberg J, Picard JJ, Eriksson UJ. Maternal diabetes in the rat impairs the formation of neural-crest derived cranial nerve ganglia in the offspring. Diabetologia. 2003; 46:1245–51. [PubMed: 12830378]

151. Li R, Thorens B, Loeken MR. Expression of the gene encoding the high-K (m) glucose transporter 2 by the early postimplantation mouse embryo is essential for neural tube defects associated with diabetic embryopathy. Diabetologia. 2007; 50:682–9. [PubMed: 17235524]
152. Matsumoto K, Akazawa S, Ishibashi M, et al. Abundant expression of GLUT1 and GLUT3 in rat embryo during the early organogenesis period. Biochem Biophys Res Commun. 1995; 209:95–102. [PubMed: 7726869]
153. Stevens FJ, Argon Y. Protein folding in the ER. Semin Cell Dev Biol. 1999; 10:443–54. [PubMed: 10597627]
154. Benyair R, Ron E, Lederkremer GZ. Protein quality control, retention, and degradation at the endoplasmic reticulum. International review of cell and molecular biology. 2011; 292:197–280. [PubMed: 22078962]
155. Groenendyk J, Sreenivasaiah PK, Kim do H, et al. Biology of endoplasmic reticulum stress in the heart. Circ Res. 2010; 107:1185–97. [PubMed: 21071716]
156. Yoshida H. ER stress and diseases. FEBS J. 2007; 274:630–58. [PubMed: 17288551]
157. Banhegyi G, Baumeister P, Benedetti A, et al. Endoplasmic reticulum stress. Ann N Y Acad Sci. 2007; 1113:58–71. [PubMed: 17483206]
158. Fonseca SG, Burcin M, Gromada J, et al. Endoplasmic reticulum stress in beta-cells and development of diabetes. Curr Opin Pharmacol. 2009; 9:763–70. [PubMed: 19665428]
159. Lin JH, Walter P, Yen TS. Endoplasmic reticulum stress in disease pathogenesis. Annu Rev Pathol. 2008; 3:399–425. [PubMed: 18039139]
160. Schroder M. Endoplasmic reticulum stress responses. Cell Mol Life Sci. 2008; 65:862–94. [PubMed: 18038217]
161. Malhotra JD, Kaufman RJ. The endoplasmic reticulum and the unfolded protein response. Semin Cell Dev Biol. 2007; 18:716–31. [PubMed: 18023214]
162. Lai E, Teodoro T, Volchuk A. Endoplasmic reticulum stress: signaling the unfolded protein response. Physiology (Bethesda). 2007; 22:193–201. [PubMed: 17557940]
163. Zhao Z, Eckert RL, Reece EA. Reduction in embryonic malformations and alleviation of endoplasmic reticulum stress by nitric oxide synthase inhibition in diabetic embryopathy. Reprod Sci. 2012; 19:823–31. [PubMed: 22534324]
164. Cao Y, Zhao Z, Eckert RL, et al. The essential role of protein kinase Cdelta in diabetes-induced neural tube defects. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2012; 25:2020–4.
165. Yang X, Borg LA, Eriksson UJ. Altered mitochondrial morphology of rat embryos in diabetic pregnancy. Anat Rec. 1995; 241:255–67. [PubMed: 7710141]
166. Genova ML, Pich MM, Biondi A, et al. Mitochondrial production of oxygen radical species and the role of Coenzyme Q as an antioxidant. Exp Biol Med (Maywood). 2003; 228:506–13. [PubMed: 12709577]
167. Raha S, Robinson BH. Mitochondria, oxygen free radicals, and apoptosis. Am J Med Genet. 2001; 106:62–70. [PubMed: 11579426]
168. Ishibashi M, Akazawa S, Sakamaki H, et al. Oxygen-induced embryopathy and the significance of glutathione-dependent antioxidant system in the rat embryo during early organogenesis. Free Radic Biol Med. 1997; 22:447–54. [PubMed: 8981036]
169. Li R, Chase M, Jung SK, et al. Hypoxic Stress In Diabetic Pregnancy Contributes To Impaired Embryo Gene Expression And Defective Development By Inducing Oxidative Stress. Am J Physiol Endocrinol Metab. 2005
170. Menegola E, Broccia ML, Prati M, et al. Glutathione status in diabetes-induced embryopathies. Biol Neonate. 1996; 69:293–7. [PubMed: 8790907]
171. Yan J, Hales BF. Depletion of Glutathione Induces 4-Hydroxynonenal Protein Adducts and Hydroxyurea Teratogenicity in the Organogenesis Stage Mouse Embryo. Journal of Pharmacology and Experimental Therapeutics. 2006; 319:613–21. [PubMed: 16902051]
172. Djordjevic VB. Free radicals in cell biology. Int Rev Cytol. 2004; 237:57–89. [PubMed: 15380666]

173. Ueda S, Masutani H, Nakamura H, et al. Redox control of cell death. Antioxid Redox Signal. 2002; 4:405–14. [PubMed: 12215208]
174. Sivan E, Reece EA, Wu YK, et al. Dietary vitamin E prophylaxis and diabetic embryopathy: morphologic and biochemical analysis. Am J Obstet Gynecol. 1996; 175:793–9. [PubMed: 8885724]
175. Viana M, Herrera E, Bonet B. Teratogenic effects of diabetes mellitus in the rat. Prevention by vitamin E Diabetologia. 1996; 39:1041–6.
176. Siman CM, Eriksson UJ. Vitamin E decreases the occurrence of malformations in the offspring of diabetic rats. Diabetes. 1997; 46:1054–61. [PubMed: 9166679]
177. Siman CM, Eriksson UJ. Vitamin C supplementation of the maternal diet reduces the rate of malformation in the offspring of diabetic rats. Diabetologia. 1997; 40:1416–24. [PubMed: 9447949]
178. Yang X, Borg LA, Siman CM, et al. Maternal antioxidant treatments prevent diabetes-induced alterations of mitochondrial morphology in rat embryos. Anat Rec. 1998; 251:303–15. [PubMed: 9669757]
179. Viana M, Aruoma OI, Herrera E, et al. Oxidative damage in pregnant diabetic rats and their embryos. Free Radic Biol Med. 2000; 29:1115–21. [PubMed: 11121718]
180. Zaken V, Kohen R, Ornoy A. Vitamins C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. Teratology. 2001; 64:33–44. [PubMed: 11410909]
181. Guijarro MV, Indart A, Aruoma OI, et al. Effects of ergothioneine on diabetic embryopathy in pregnant rats. Food Chem Toxicol. 2002; 40:1751–5. [PubMed: 12419688]
182. Gareskog M, Eriksson UJ, Wentzel P. Combined supplementation of folic acid and vitamin E diminishes diabetes-induced embryotoxicity in rats. Birth defects research Part A, Clinical and molecular teratology. 2006; 76:483–90.
183. Reece EA, Wu YK, Zhao Z, et al. Dietary vitamin and lipid therapy rescues aberrant signaling and apoptosis and prevents hyperglycemia-induced diabetic embryopathy in rats. Am J Obstet Gynecol. 2006; 194:580–5. [PubMed: 16458664]
184. Reece EA, Wu YK. Prevention of diabetic embryopathy in offspring of diabetic rats with use of a cocktail of deficient substrates and an antioxidant. Am J Obstet Gynecol. 1997; 176:790–7. discussion 97–8. [PubMed: 9125602]
185. Li X, Weng H, Reece EA, et al. SOD1 overexpression in vivo blocks hyperglycemia-induced specific PKC isoforms: substrate activation and consequent lipid peroxidation in diabetic embryopathy. Am J Obstet Gynecol. 2011; 205:84 e1–6. [PubMed: 21529760]
186. Hagay ZJ, Weiss Y, Zusman I, et al. Prevention of diabetes-associated embryopathy by overexpression of the free radical scavenger copper zinc superoxide dismutase in transgenic mouse embryos. Am J Obstet Gynecol. 1995; 173:1036–41. [PubMed: 7485290]
187. Jawerbaum A, Gonzalez E. The role of alterations in arachidonic acid metabolism and nitric oxide homeostasis in rat models of diabetes during early pregnancy. Curr Pharm Des. 2005; 11:1327–42. [PubMed: 15853688]
188. Jawerbaum A, Higa R, White V, et al. Peroxynitrites and impaired modulation of nitric oxide concentrations in embryos from diabetic rats during early organogenesis. Reproduction. 2005; 130:695–703. [PubMed: 16264098]
189. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007; 87:315–424. [PubMed: 17237348]
190. Szabo C, Ischiropoulos H, Radi R. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov. 2007; 6:662–80. [PubMed: 17667957]
191. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J. 1994; 298 (Pt 2):249–58. [PubMed: 7510950]
192. Torreilles J. Nitric oxide: one of the more conserved and widespread signaling molecules. Front Biosci. 2001; 6:D1161–72. [PubMed: 11578977]
193. Groves JT, Wang CC. Nitric oxide synthase: models and mechanisms. Curr Opin Chem Biol. 2000; 4:687–95. [PubMed: 11102875]
194. Umar S, van der Laarse A. Nitric oxide and nitric oxide synthase isoforms in the normal, hypertrophic, and failing heart. Mol Cell Biochem. 2010; 333:191–201. [PubMed: 19618122]

195. Zhou L, Zhu DY. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. Nitric Oxide. 2009; 20:223–30. [PubMed: 19298861]
196. Hesslinger C, Strub A, Boer R, et al. Inhibition of inducible nitric oxide synthase in respiratory diseases. Biochem Soc Trans. 2009; 37:886–91. [PubMed: 19614613]
197. Kleinert H, Schwarz PM, Forstermann U. Regulation of the expression of inducible nitric oxide synthase. Biol Chem. 2003; 384:1343–64. [PubMed: 14669979]
198. Kroncke KD, Suschek CV, Kolb-Bachofen V. Implications of inducible nitric oxide synthase expression and enzyme activity. Antioxid Redox Signal. 2000; 2:585–605. [PubMed: 11229370]
199. Kumar SD, Yong S-K, Dheen ST, et al. Cardiac Malformations Are Associated with Altered Expression of Vascular Endothelial Growth Factor and Endothelial Nitric Oxide Synthase Genes in Embryos of Diabetic Mice. Experimental Biology and Medicine. 2008; 233:1421–32. [PubMed: 18824721]
200. Jawerbaum A, Gonzalez ET, Novaro V, et al. Increased prostaglandin E generation and enhanced nitric oxide synthase activity in the non-insulin-dependent diabetic embryo during organogenesis. Reprod Fertil Dev. 1998; 10:191–6. [PubMed: 9801272]
201. Yang P, Cao Y, Li H. Hyperglycemia induces inducible nitric oxide synthase gene expression and consequent nitrosative stress via c-Jun N-terminal kinase activation. Am J Obstet Gynecol. 2010; 203:185 e5–11. [PubMed: 20541731]
202. Waite M. Phospholipases, enzymes that share a substrate class. Advances in experimental medicine and biology. 1990; 279:1–22. [PubMed: 2096693]
203. Kaiser E, Chiba P, Zaky K. Phospholipases in biology and medicine. Clinical biochemistry. 1990; 23:349–70. [PubMed: 2253331]
204. Blobe GC, Khan WA, Hannun YA. Protein kinase C: cellular target of the second messenger arachidonic acid? Prostaglandins Leukot Essent Fatty Acids. 1995; 52:129–35. [PubMed: 7784448]
205. Bonventre JV. Phospholipase A2 and signal transduction. Journal of the American Society of Nephrology : JASN. 1992; 3:128–50. [PubMed: 1391715]
206. Clark JD, Schievella AR, Nalefski EA, et al. Cytosolic phospholipase A2. Journal of lipid mediators and cell signalling. 1995; 12:83–117. [PubMed: 8777586]
207. Liang X, Wu L, Wang Q, et al. Function of COX-2 and prostaglandins in neurological disease. J Mol Neurosci. 2007; 33:94–9. [PubMed: 17901552]
208. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. J Lipid Res. 2009; 50 (Suppl):S29–34. [PubMed: 18952571]
209. Morrow JD, Hill KE, Burk RF, et al. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc NatlAcadSci USA. 1990; 87:9383–87.
210. Morrow JD. The isoprostanes: their quantification as an index of oxidant stress status in vivo. Drug Metab Rev. 2000; 32:377–85. [PubMed: 11139135]
211. Wentzel P, Welsh N, Eriksson UJ. Developmental damage, increased lipid peroxidation, diminished cyclooxygenase-2 gene expression, and lowered prostaglandin E2 levels in rat embryos exposed to a diabetic environment. Diabetes. 1999; 48:813–20. [PubMed: 10102698]
212. Wentzel P, Eriksson UJ. A diabetes-like environment increases malformation rate and diminishes prostaglandin E(2) in rat embryos: Reversal by administration of vitamin E and folic acid. Birth Defects Res A Clin Mol Teratol. 2005
213. Piddington R, Joyce J, Dhanasekaran P, et al. Diabetes mellitus affects prostaglandin E2 levels in mouse embryos during neurulation. Diabetologia. 1996; 39:915–20. [PubMed: 8858213]
214. Jawerbaum A, Gonzalez ET, Sinner D, et al. Diminished PGE2 content, enhanced PGE2 release and defects in 3H-PGE2 transport in embryos from overtly diabetic rats. Reprod Fertil Dev. 2000; 12:141–7. [PubMed: 11302423]
215. Jawerbaum A, Sinner D, White V, et al. Modulation of PGE2 generation in the diabetic embryo: effect of nitric oxide and superoxide dismutase. Prostaglandins Leukot Essent Fatty Acids. 2001; 64:127–33. [PubMed: 11237480]

216. Wentzel P, Eriksson UJ. 8-Iso-PGF(2alpha) administration generates dysmorphogenesis and increased lipid peroxidation in rat embryos in vitro. Teratology. 2002; 66:164–8. [PubMed: 12353212]

217. El-Bassiouni EA, Helmy MH, Abou Rawash N, et al. Embryopathy in experimental diabetic gestation: assessment of PGE2 level, gene expression of cyclooxygenases and apoptosis. Br J Biomed Sci. 2005; 62:161–5. [PubMed: 16411374]

218. Wentzel P, Eriksson UJ. Antioxidants diminish developmental damage induced by high glucose and cyclooxygenase inhibitors in rat embryos in vitro. Diabetes. 1998; 47:677–84. [PubMed: 9568703]

219. Jawerbaum A, Rosello Catafau J, Gonzalez ET, et al. Glucose metabolism, triglyceride and glycogen levels, as well as eicosanoid production in isolated uterine strips and in embryos in a rat model of non-insulin-dependent diabetes mellitus during pregnancy. Prostaglandins. 1994; 47:81–96. [PubMed: 8016386]

220. Goto MP, Goldman AS, Uhing MR. PGE2 prevents anomalies induced by hyperglycemia or diabetic serum in mouse embryos. Diabetes. 1992; 41:1644–50. [PubMed: 1446806]

221. Dempsey EC, Newton AC, Mochly-Rosen D, et al. Protein kinase C isozymes and the regulation of diverse cell responses. Am J Physiol Lung Cell Mol Physiol. 2000; 279:L429–38. [PubMed: 10956616]

222. Shirai Y, Saito N. Activation mechanisms of protein kinase C: maturation, catalytic activation, and targeting. Journal of biochemistry. 2002; 132:663–8. [PubMed: 12417013]

223. Cao Y, Zhao Z, Eckert RL, et al. Protein kinase Cbeta2 inhibition reduces hyperglycemia-induced neural tube defects through suppression of a caspase 8-triggered apoptotic pathway. Am J Obstet Gynecol. 2011; 204:226 e1–5. [PubMed: 21376163]

224. Wada T, Penninger JM. Mitogen-activated protein kinases in apoptosis regulation. Oncogene. 2004; 23:2838–49. [PubMed: 15077147]

225. Lin A, Dibling B. The true face of JNK activation in apoptosis. Aging cell. 2002; 1:112–6. [PubMed: 12882340]

226. Corson LB, Yamanaka Y, Lai KM, et al. Spatial and temporal patterns of ERK signaling during mouse embryogenesis. Development. 2003; 130:4527–37. [PubMed: 12925581]

227. Reece EA, Wu YK, Wiznitzer A, et al. Dietary polyunsaturated fatty acid prevents malformations in offspring of diabetic rats. Am J Obstet Gynecol. 1996; 175:818–23. [PubMed: 8885728]

228. Reece EA, Ma XD, Wu YK, et al. Aberrant patterns of cellular communication in diabetes-induced embryopathy. I. Membrane signalling. J Matern Fetal Neonatal Med. 2002; 11:249–53. [PubMed: 12375679]

229. Yang P, Zhao Z, Reece EA. Activation of oxidative stress signaling that is implicated in apoptosis with a mouse model of diabetic embryopathy. Am J Obstet Gynecol. 2008; 198:130 e1–7. [PubMed: 18166327]

230. Yang P, Zhao Z, Reece EA. Blockade of c-Jun N-terminal kinase activation abrogates hyperglycemia-induced yolk sac vasculopathy in vitro. Am J Obstet Gynecol. 2008; 198:321 e1–7. [PubMed: 18177823]

231. Yang P, Zhao Z, Reece EA. Involvement of c-Jun N-terminal kinases activation in diabetic embryopathy. Biochem Biophys Res Commun. 2007; 357:749–54. [PubMed: 17449011]

232. Li X, Xu C, Yang P. c-Jun NH2-Terminal Kinase 1/2 and Endoplasmic Reticulum Stress as Interdependent and Reciprocal Causation in Diabetic Embryopathy. Diabetes. 2012

233. Ola MS, Nawaz M, Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. Molecular and cellular biochemistry. 2011; 351:41–58. [PubMed: 21210296]

234. Cory S, Huang DC, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene. 2003; 22:8590–607. [PubMed: 14634621]

235. Kumar S. Caspase function in programmed cell death. Cell death and differentiation. 2007; 14:32–43. [PubMed: 17082813]

236. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. Nature reviews. 2008; 9:231–41.

237. Degterev A, Yuan J. Expansion and evolution of cell death programmes. Nature reviews. 2008; 9:378–90.

238. Willis SN, Adams JM. Life in the balance: how BH3-only proteins induce apoptosis. Curr Opin Cell Biol. 2005; 17:617–25. [PubMed: 16243507]

239. Antignani A, Youle RJ. How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? Curr Opin Cell Biol. 2006; 18:685–9. [PubMed: 17046225]

240. Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene. 2003; 22:8543–67. [PubMed: 14634618]

241. Ferraro E, Corvaro M, Cecconi F. Physiological and pathological roles of Apaf1 and the apoptosome. J Cell Mol Med. 2003; 7:21–34. [PubMed: 12767258]

242. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nature reviews. 2008; 9:47–59.

243. Wiznitzer A, Ayalon N, Hershkovitz R, et al. Lipoic acid prevention of neural tube defects in offspring of rats with streptozocin-induced diabetes. Am J Obstet Gynecol. 1999; 180:188–93. [PubMed: 9914602]

244. Khandelwal M, Reece EA, Wu YK, et al. Dietary myo-inositol therapy in hyperglycemia-induced embryopathy. Teratology. 1998; 57:79–84. [PubMed: 9562680]

245. Reece EA, Khandelwal M, Wu YK, et al. Dietary intake of myo-inositol and neural tube defects in offspring of diabetic rats. Am J Obstet Gynecol. 1997; 176:536–9. [PubMed: 9077602]

246. Wentzel P, Gareskog M, Eriksson UJ. Folic Acid Supplementation Diminishes Diabetes- and Glucose-Induced Dysmorphogenesis in Rat Embryos In Vivo and In Vitro. Diabetes. 2005; 54:546–53. [PubMed: 15677514]

247. Zabihi S, Eriksson UJ, Wentzel P. Folic acid supplementation affects ROS scavenging enzymes, enhances Vegf-A, and diminishes apoptotic state in yolk sacs of embryos of diabetic rats. Reprod Toxicol. 2007; 23:486–98. [PubMed: 17482424]

248. Pinter E, Reece EA, Ogburn PL Jr, et al. Fatty acid content of yolk sac and embryo in hyperglycemia-induced embryopathy and effect of arachidonic acid supplementation. Am J Obstet Gynecol. 1988; 159:1484–90. [PubMed: 3144918]

249. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition. 2001; 17:888–95. [PubMed: 11684397]

250. Zed PJ, Krenzelok EP. Treatment of acetaminophen overdose. Am J Health Syst Pharm. 1999; 56:1081–91. quiz 91–3. [PubMed: 10385455]

251. Kanter MZ. Comparison of oral and i.v. acetylcysteine in the treatment of acetaminophen poisoning. Am J Health Syst Pharm. 2006; 63:1821–7. [PubMed: 16990628]

252. Gareskog M, Wentzel P. N-Acetylcysteine and {alpha}-cyano-4-hydroxycinnamic acid alter protein kinase C (PKC)-{delta} and PKC-{zeta} and diminish dysmorphogenesis in rat embryos cultured with high glucose in vitro. J Endocrinol. 2007; 192:207–14. [PubMed: 17210758]

253. Wentzel P, Ejdesjo A, Eriksson UJ. Maternal diabetes in vivo and high glucose in vitro diminish GAPDH activity in rat embryos. Diabetes. 2003; 52:1222–8. [PubMed: 12716756]

254. Wentzel P, Wentzel CR, Gareskog MB, et al. Induction of embryonic dysmorphogenesis by high glucose concentration, disturbed inositol metabolism, and inhibited protein kinase C activity. Teratology. 2001; 63:193–201. [PubMed: 11320530]

255. Honarbakhsh S, Schachter M. Vitamins and cardiovascular disease. Br J Nutr. 2009; 101:1113–31. [PubMed: 18826726]

256. Van Patten CL, de Boer JG, Tomlinson Guns ES. Diet and dietary supplement intervention trials for the prevention of prostate cancer recurrence: a review of the randomized controlled trial evidence. J Urol. 2008; 180:2314–21. discussion 721–2. [PubMed: 18930254]

257. Higa R, Kurtz M, Mazzucco MB, et al. Folic acid and safflower oil supplementation interacts and protects embryos from maternal diabetes-induced damage. Mol Hum Reprod. 2012; 18:253–64. [PubMed: 22180326]

258. Villar J, Purwar M, Merialdi M, et al. World Health Organisation multicentre randomised trial of supplementation with vitamins C and E among pregnant women at high risk for pre-eclampsia in populations of low nutritional status from developing countries. Br J Obstet Gynecol. 2009; 116:780–8.

259. Robinson I, de Serna DG, Gutierrez A, et al. Vitamin E in humans: an explanation of clinical trial failure. Endocr Pract. 2006; 12:576–82. [PubMed: 17002935]

260. Polyzos NP, Mauri D, Tsappi M, et al. Combined vitamin C and E supplementation during pregnancy for preeclampsia prevention: a systematic review. Obstet Gynecol Surv. 2007; 62:202–6. [PubMed: 17306042]

261. Briasoulis A, Tousoulis D, Antoniades C, et al. The oxidative stress menace to coronary vasculature: any place for antioxidants? Curr Pharm Des. 2009; 15:3078–90. [PubMed: 19754382]

262. Steinhubl SR. Why have antioxidants failed in clinical trials? Am J Cardiol. 2008; 101:14D–19D.

263. Lipscombe LL, McLaughlin HM, Wu W, et al. Pregnancy planning in women with pregestational diabetes. J Matern Fetal Neonatal Med. 2011; 24:1095–101. [PubMed: 21261446]

264. Gabbe SG, Graves CR. Management of diabetes mellitus complicating pregnancy. Obstet Gynecol. 2003; 102:857–68. [PubMed: 14551019]

265. Janz NK, Herman WH, Becker MP, et al. Diabetes and pregnancy. Factors associated with seeking pre-conception care. Diabetes Care. 1995; 18:157–65. [PubMed: 7729291]

266. Willhoite MB, Bennert HW Jr, Palomaki GE, et al. The impact of preconception counseling on pregnancy outcomes. The experience of the Maine Diabetes in Pregnancy Program. Diabetes Care. 1993; 16:450–5. [PubMed: 8432216]

Key points

- Diabetes mellitus in early pregnancy increases the risk of birth defects in infants.
- High glucose induces intracellular stress, increases programmed cell death (apoptosis), and decreases cell proliferation in the embryo.
- Reduction of the risk includes pre-conceptional and early gestational glycemic control.
- Interventions via dietary supplementation remain to be developed.
- Pre-conceptional counseling and planned pregnancy are encouraged for physicians and patients.

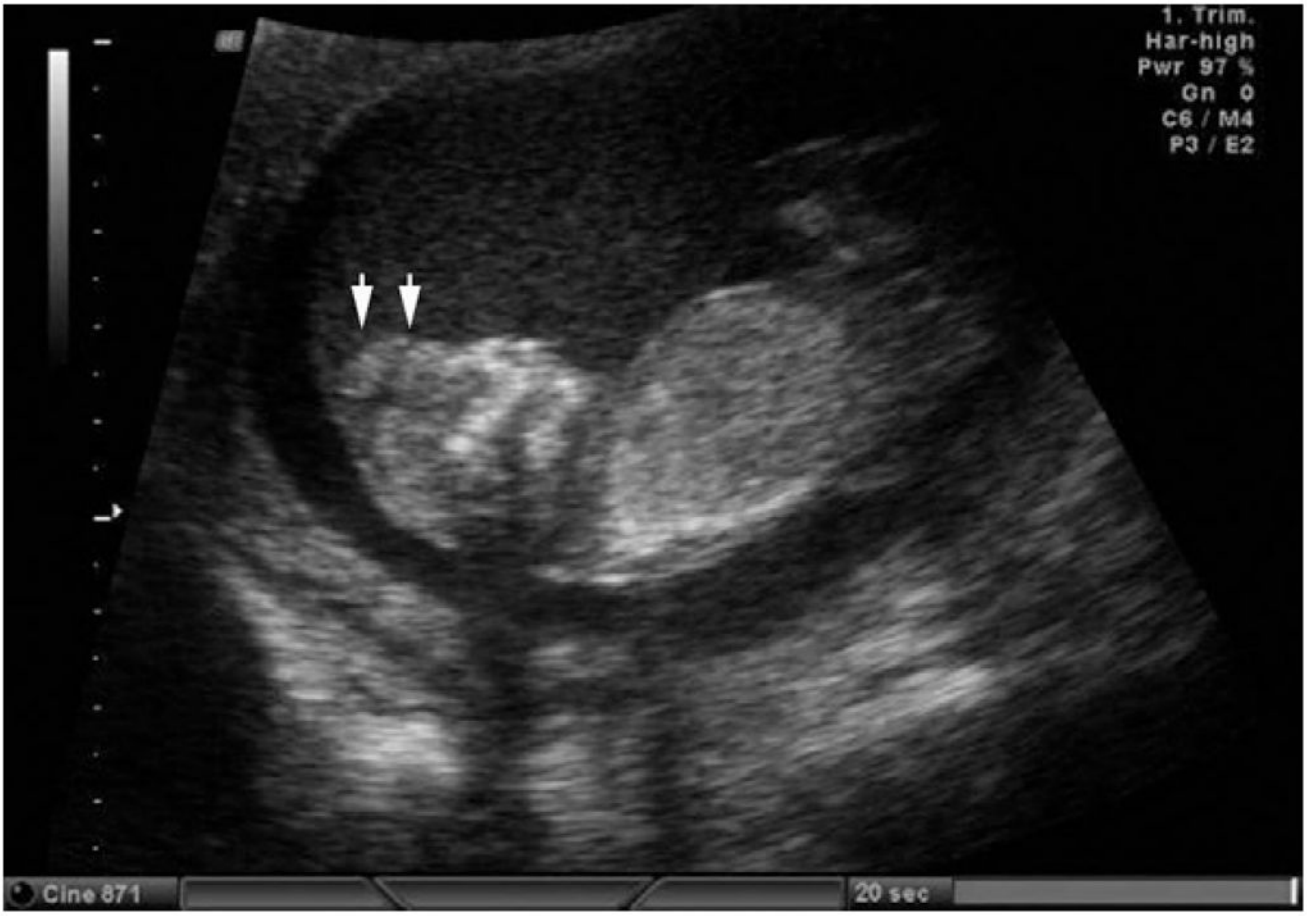

Figure 1.
Two-dimensional ultrasonic scanning of anencephaly in the first trimester. Arrows indicate missing forebrain. Modified with permission from; Cameron M, Moran P. Prenatal screening and diagnosis of neural tube defects. Prenat Diagn 2009;29:402–11.

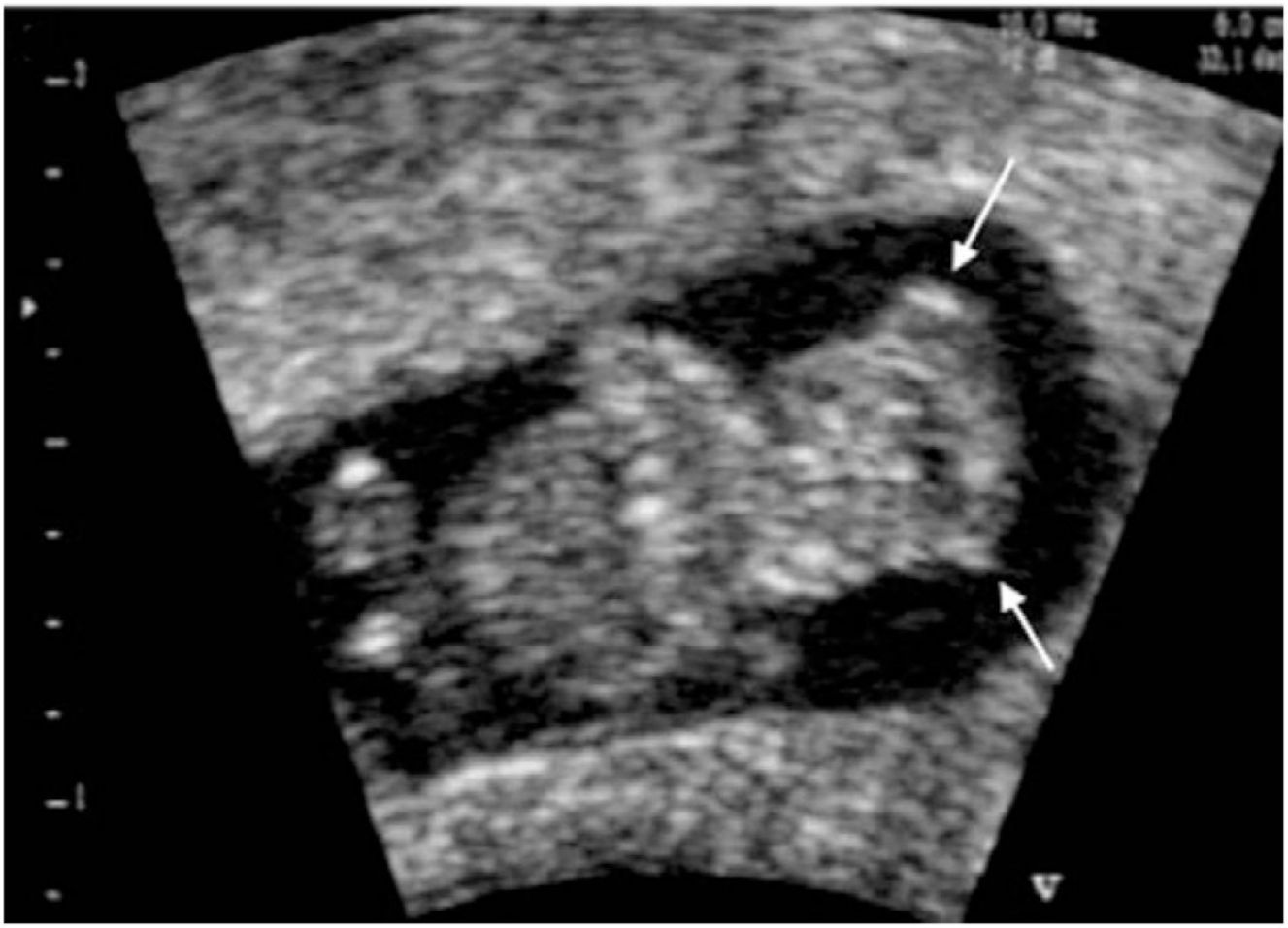

Figure 2.
Two-dimensional ultrasonic scanning of exencephaly in the first trimester. The contours of the brain are irregular (arrows). Modified with permission from; Blaas HG, Eik-Nes SH. Sonoembryology and early prenatal diagnosis of neural anomalies. Prenat Diagn 2009;29:312–25.

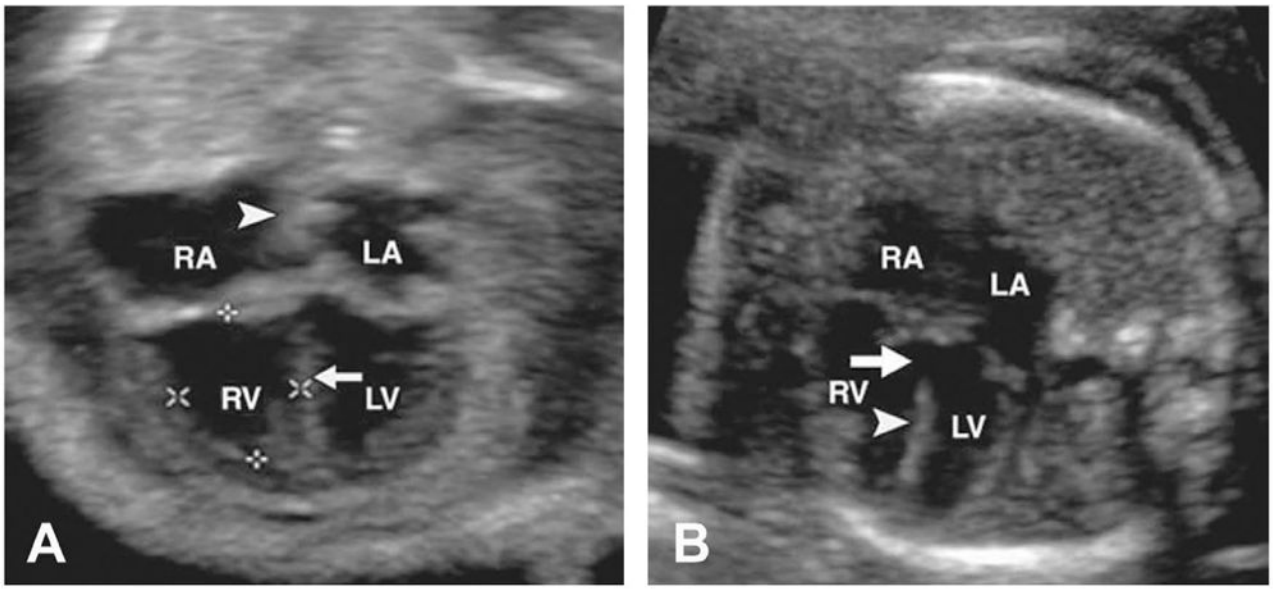

Figure 3.
Four-chamber view of fetal hearts. (A) Normal heart. Arrow indicates interventricular septum. Arrowhead indicates interatrial septum. (B) Heart with VSD (arrow) in inlet portion of interventricular septum (arrowhead). LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Modified with permission from; Rajiah P, Mak C, Dubinksy TJ, et al. Ultrasound of fetal cardiac anomalies. AJR Am J Roentgenol 2011;197:W747–60.

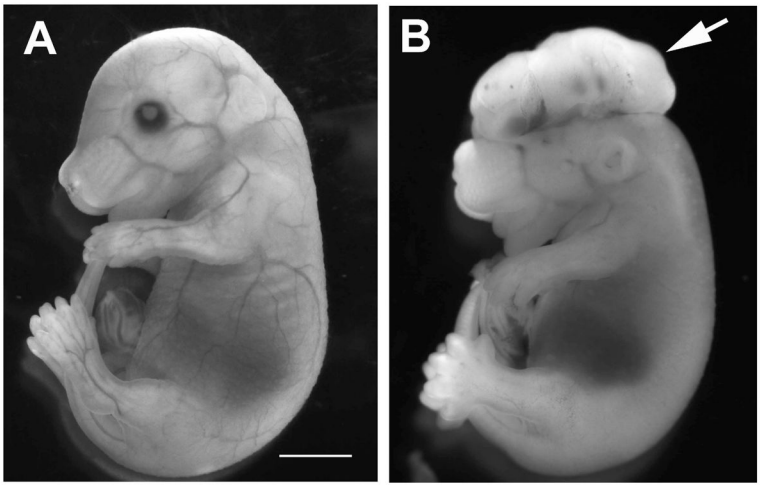

Figure 4.
Exencephaly in mouse E15.5 fetus of diabetic pregnancy. (A) Nondiabetic control. (B) Diabetes. Arrow indicates exencephaly. Scale bar=3 mm.

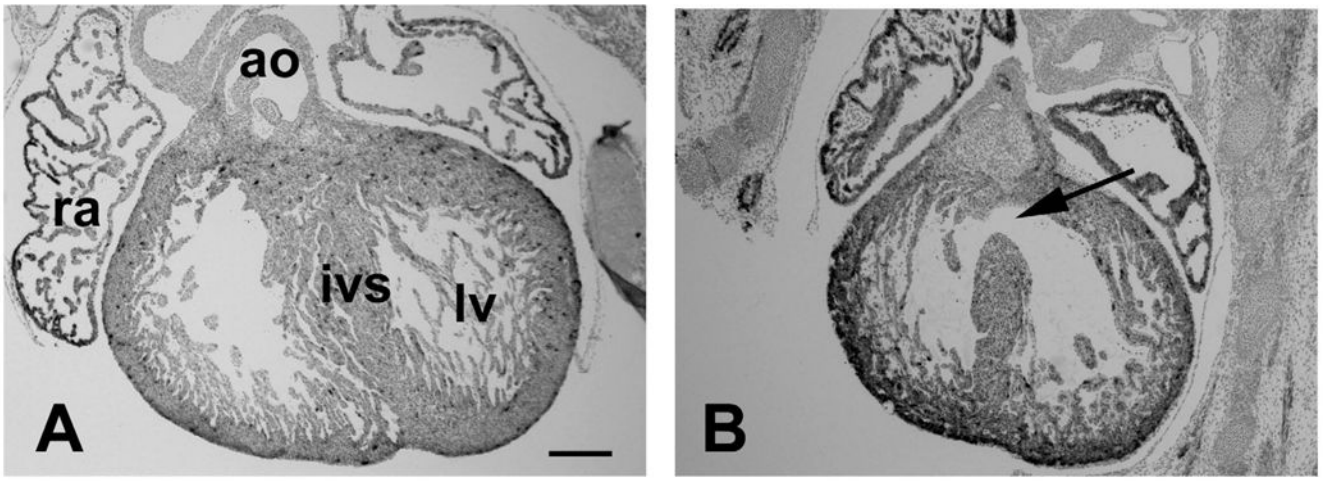

Figure 5.
AVSD in mouse E15.5 fetus of diabetic pregnancy. (A) Heart of nondiabetic control. (B) Heart of diabetic group with VSD (arrow). ao, aorta; ivs, interventricular septum; lv, left ventricle; ra, right atrium. Scale bar=200 μm.

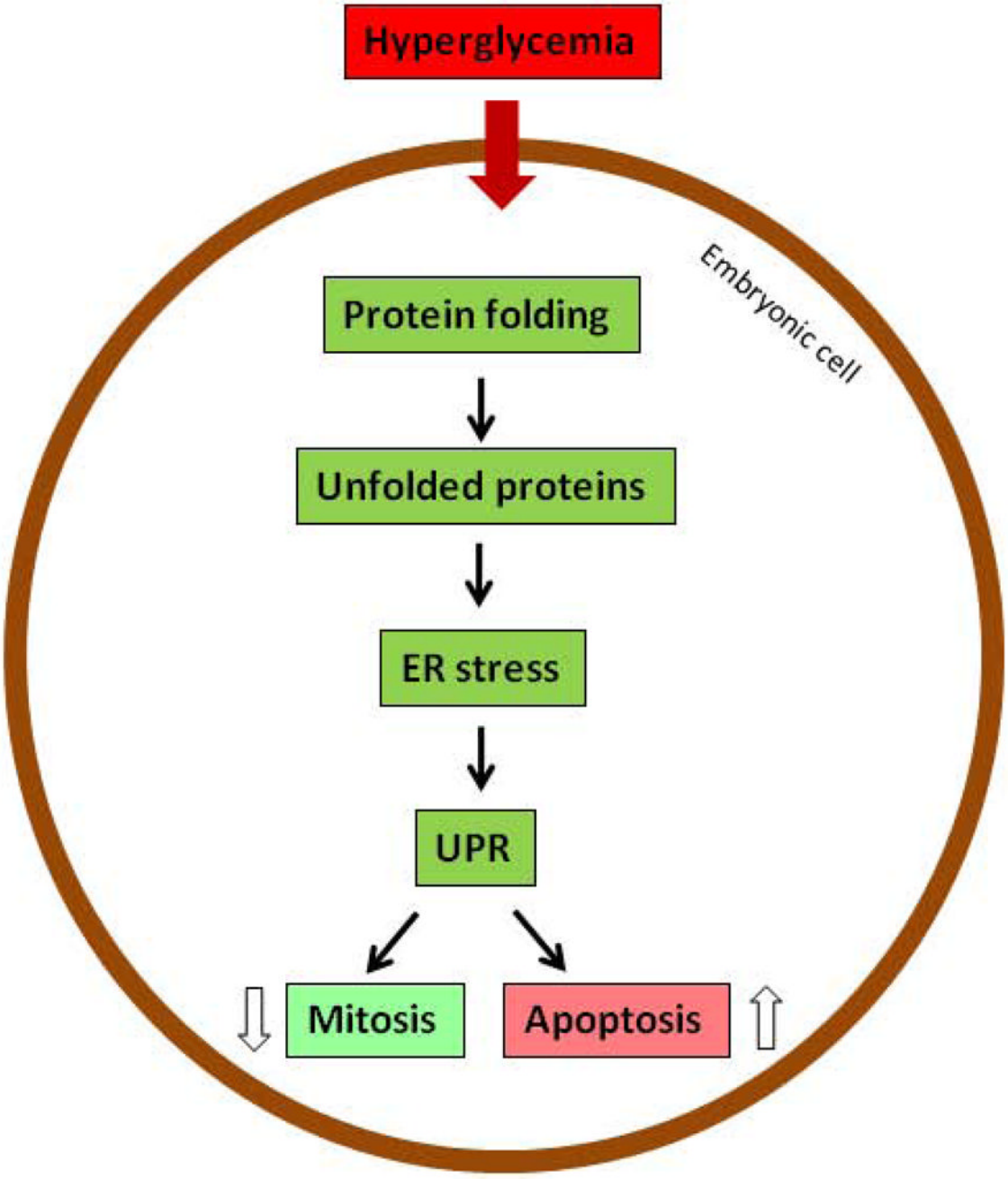

Figure 6.
ER stress in diabetic embryopathy. ER, endoplasmic reticulum; UPR, unfolded protein response.

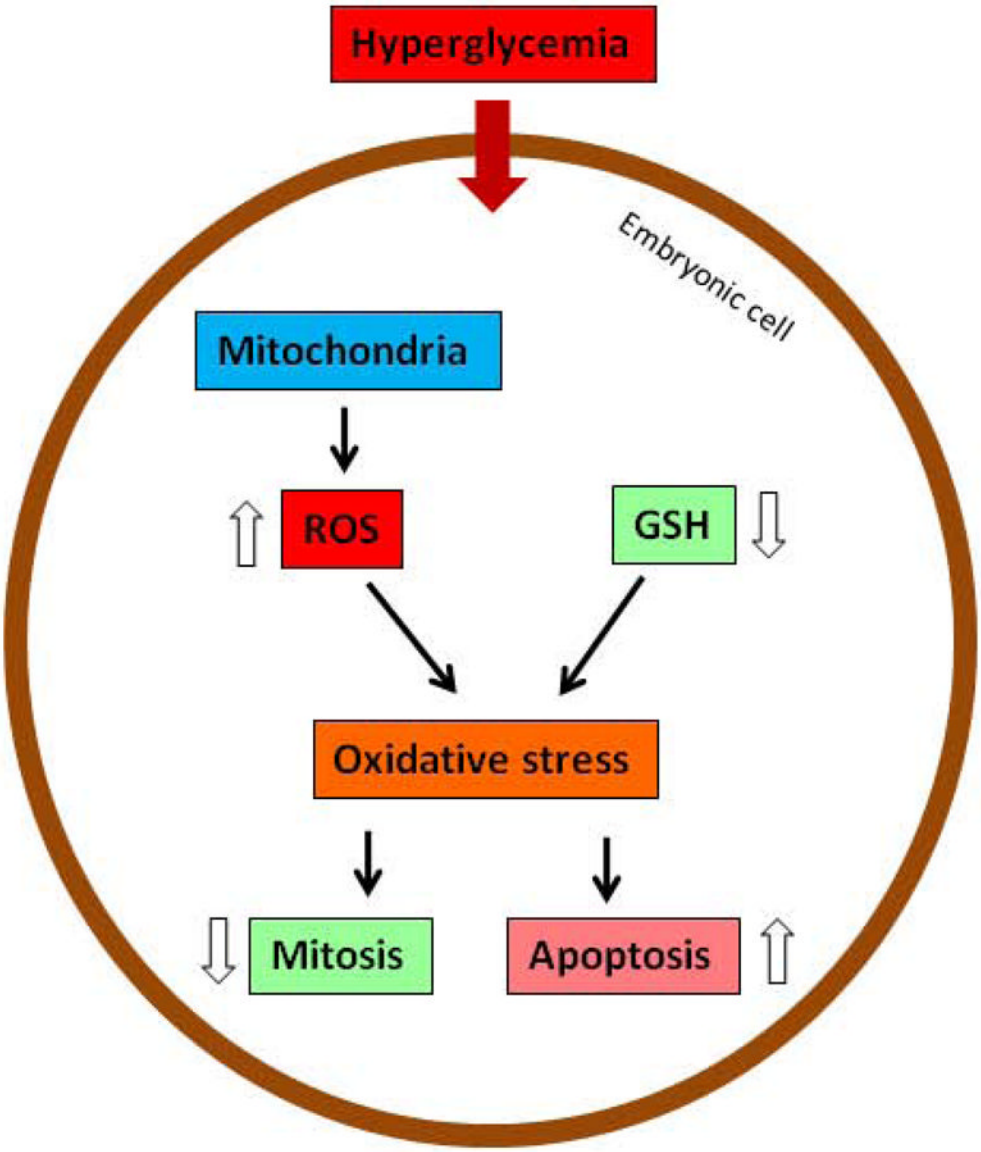

Figure 7.
Oxidative stress in diabetic embryopathy. GSH, glutathione; ROS, reactive oxygen species.

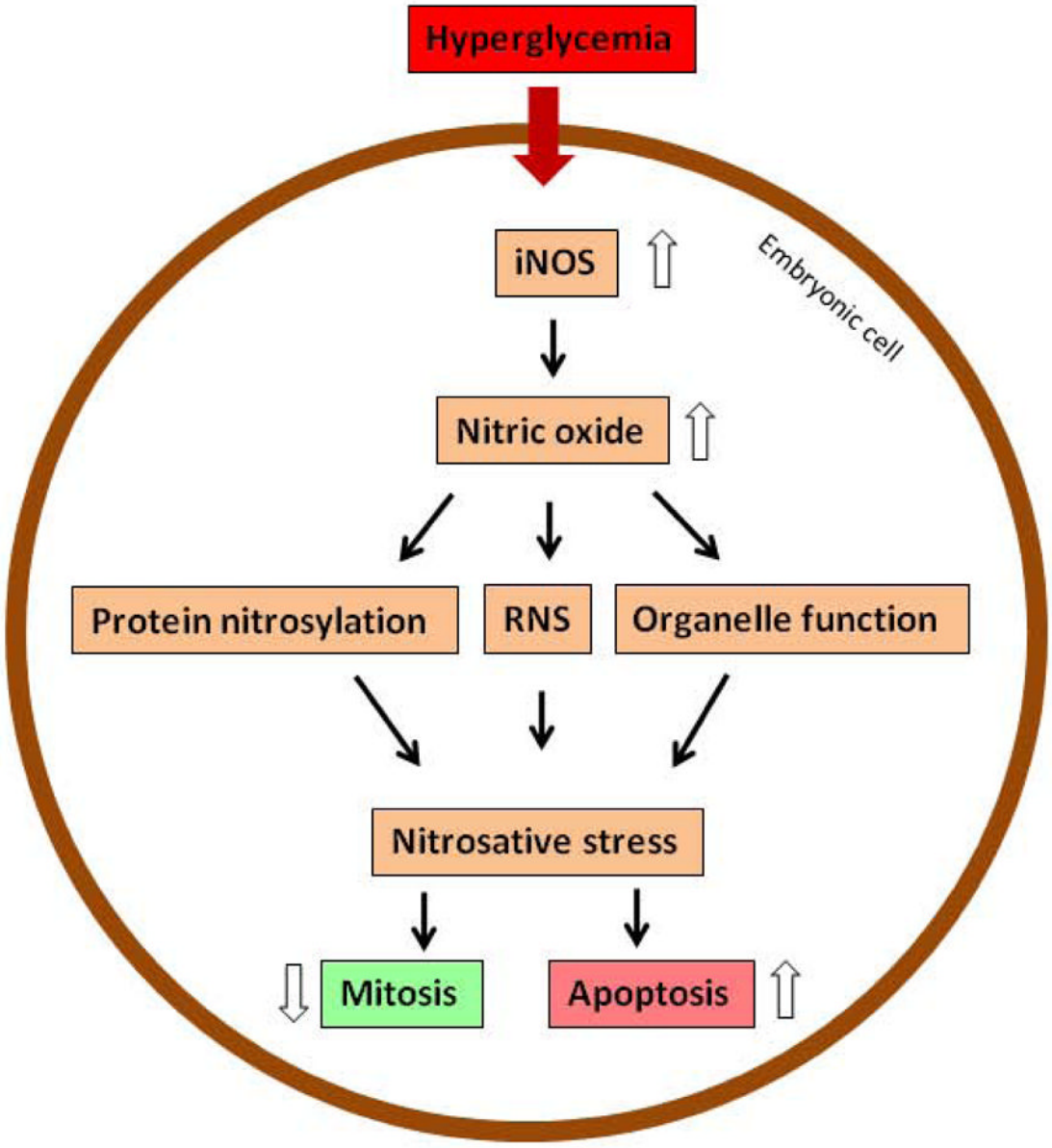

Figure 8.
Nitrosative stress in diabetic embryopathy. iNOS, inducible nitric oxide synthase; RNS, reactive nitrogen species.

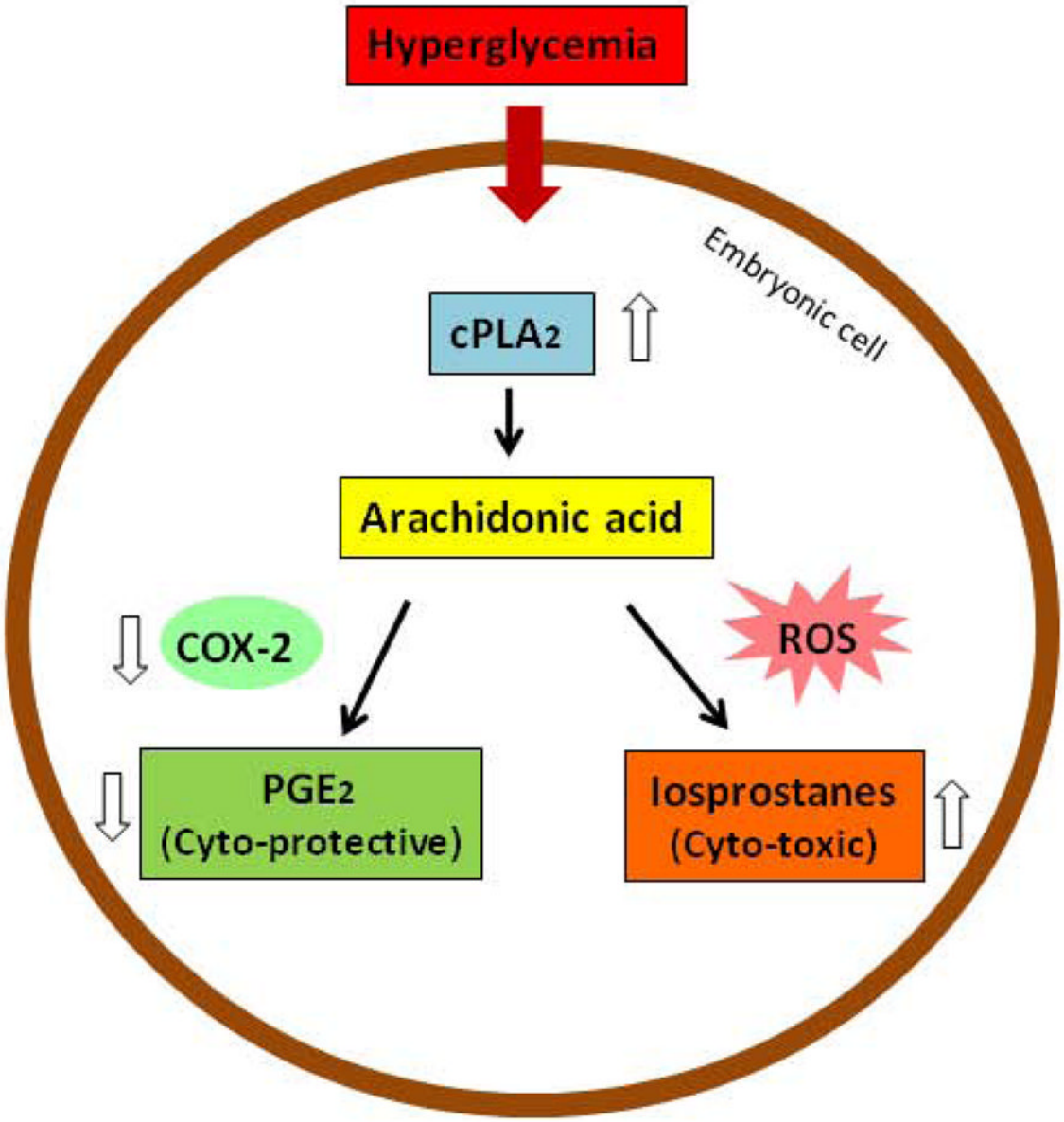

Figure 9.
Lipoperoxidation in diabetic embryopathy. COX-2, cyclooxygenase-2; cPLA2, cytosolic phospholipase A2; PGE2, prostaglandin E2; ROS, reactive oxygen species.

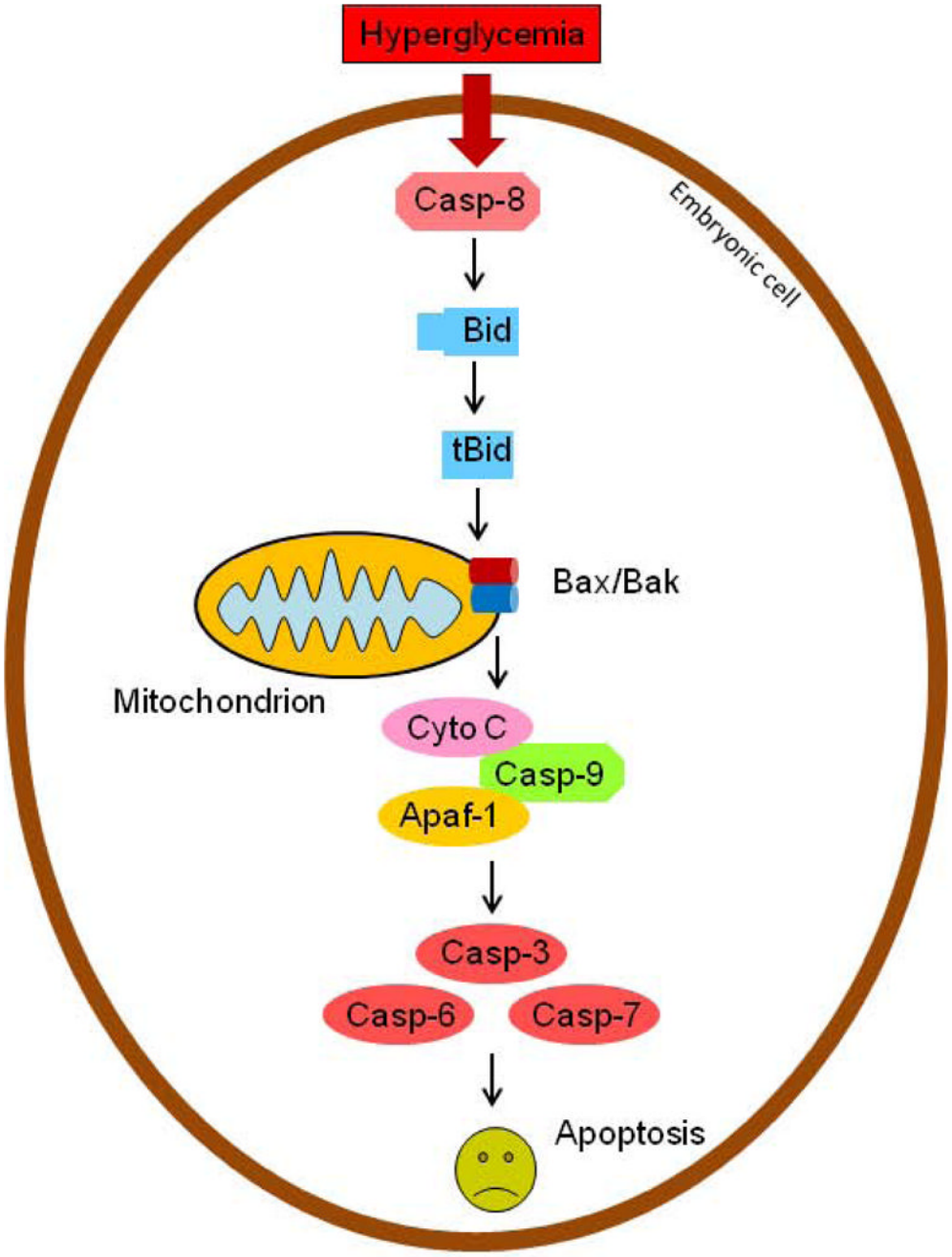

Figure 10.
Caspase-8-regulated apoptotic pathway in diabetic embryopathy. Apaf-1, apoptotic protease-activating factors-1; Casp, caspase; Cyto, cytochrome;

Table 1

Recommendations for pre-conception HbA1c targets

	USA	UK
DCCT	<7.0%	<6.1%
IFCC	<53 mmol/mol	<43 mmol/mol

DCCT, Diabetes Control and Complications Trials; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine.

Table 2

Developmental anomalies in major organ systems in diabetic embryopathy

Central nervous	**Craniofacial**	**Cardiovascular**	**Skeletal**
Anencephaly	Hemifacial	Conus arteriosus defects	Sacral agenesis
Encephalocele	microsomia	Transposition of great vessels	Sacral hypoplasia
Exencephaly	Macrostomia	Tetralogy of Fallot	Limb defects
Microcephaly	Cleft palate	Ventricular septal defects	Vertebral defects
Hydrocephaly	Cleft lip	Pulmonary vavle defects	Caudal regression
Holoprosencephaly	Microtia	Patent ductus arteriosus	
Spina bifida	Micrognathia	Hypoplastic left heart syndrome	
	Craniosynostosis	Coarctation of the aorta	
	Anotia/Microtia	Right ventricular septal defects	
	Eye defects	Atrial septal defects	
		Heterotaxia	