

Diabetic complications in pregnancy: is resveratrol a solution?

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Abstract

Diabetes is a metabolic disorder that, during pregnancy, may affect fetal development. Fetal outcome depends on the type of diabetes present, the concentration of blood glucose and the extent of fetal exposure to elevated or frequently fluctuating glucose concentrations. The result of some diabetic pregnancies will be embryonic developmental abnormalities, a condition referred to as diabetic embryopathy. Tight glycemic control in type 1 diabetes during pregnancy using insulin therapy together with folic acid supplementation are partially able to prevent diabetic embryopathy; however, the protection is not complete and additional interventions are needed. Resveratrol, a polyphenol found largely in the skins of red grapes, is known to have antidiabetic action and is in clinical trials for the treatment of diabetes, insulin resistance, obesity and metabolic syndrome. Studies of resveratrol in a rodent model of diabetic embryopathy reveal that it significantly improves the embryonic outcome in terms of diminishing developmental abnormalities. Improvements in maternal and embryonic outcomes observed in rodent models may arise from resveratrol's antioxidative potential, antidiabetic action and antidyslipidemic nature. Whether resveratrol will have similar actions in human diabetic pregnancy is unknown. Here, we review the potential therapeutic use of resveratrol in diabetes and diabetic pregnancy.

Keywords: diabetic pregnancy, neural tube defects, resveratrol, hyperglycemia

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Introduction

Diabetes is a metabolic disease in which high blood-glucose concentrations exist either because the body does not produce sufficient insulin (type 1 diabetes) or because cells fail to properly respond to insulin (type 2 diabetes).¹ A third kind of diabetes, known as gestational diabetes, occurs when a pregnant woman develops high blood-glucose levels typically during the second half of pregnancy due to the hormones of pregnancy.² Gestational diabetes can revert to normal following pregnancy, but sometimes precedes the development of type 2 diabetes. Recent data of the National Diabetes Fact Sheet shows that total 25.8 million children and adults in the USA (8.3% of the population) have diabetes (Centers for Disease Control and Prevention, 2011; http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf).³ All forms of diabetes increase the risk of long-term complications. Up to 67% of adults with diabetes have high blood pressure.³ Long-term diabetes damages blood vessels. Diabetics have double the risk of heart attack or stroke. Diabetes causes nerve damage in the heart, which can make a heart attack painless or 'silent,' and affects blood vessel formation in the retina of the eye, leading to visual symptoms and sometimes

blindness (diabetic retinopathy).^{1,3} High blood sugar can lead to scarring of kidney tissues that may result in chronic kidney diseases (diabetic nephropathy). Diabetes also affects the nervous system (diabetic neuropathy), causing numbness, tingling and pain in the feet, and increasing the risk of skin damage because of altered sensation.^{1,3} As diabetes affects every part of the body, it can also have devastating complications to the mother and embryo.

Diabetic pregnancies are a serious concern

The incidence of diabetes mellitus and its sequelae is rising in the general population, and it is an additional risk factor in pregnancy, causing birth defects commonly referred to as diabetic embryopathies. Although most embryonic organs are affected by maternal diabetes, the central nervous system is comparatively more susceptible to high glucose.^{4–13} Among the various malformations in diabetic embryopathy, neural tube defects (NTDs) are one of the most common.^{5,14–16} Although the incidence of NTDs in diabetic pregnancies has been reduced by intensive insulin treatment and glucose monitoring, they are still 2- to

6-fold greater than in normal pregnancies.^{17–19} Folic acid consumption alone or in combination with vitamin E diminishes the diabetes-induced embryonic malformations in diabetic pregnancy.^{20–22} Currently, folic acid supplementation is standard practice to avoid birth defects, but the protection afforded by folic acid supplementation against diabetes-induced birth defects is not enough. Approximately 50% of congenital malformations can be prevented if folic acid is supplemented before and during pregnancy.^{23–25} The folic acid tolerable intake limit is thought to be 1 mg/day; however, a few studies advocate the use of high-dose folic acid (4–5 mg/day) during diabetic pregnancy.²⁶ Folate is needed for DNA synthesis but can also serve as a methyl group donor for DNA methylation, and thereby alter the embryo's epigenome by epigenetic modification of DNA.²⁷ Recently, DNA methylation by folate and the C677T mutation of the enzyme methylenetetrahydrofolate reductase (MTHFR), and thereby impaired MTHFR function have been shown as one of the cause of recurrent fetal loss and potential factor in the etiology of autism.^{28,29} There are currently no drugs or nutritional supplements available for complete protection against embryonic malformation during diabetic pregnancy, and NTDs and other birth defects are still a serious concern in diabetic pregnancies. These gaps in knowledge provide a compelling reason to further investigate alternative options for the prevention of neural tube defects in diabetic pregnancies.

Resveratrol, a grape antioxidant, and prevention of diabetic embryopathy

Recent studies have demonstrated that resveratrol (*trans*-3,5,4'-trihydroxystilbene), found in red grapes and blueberries, lowers maternal blood sugar, improves maternal lipid profile³⁰ and prevents developmental delays in embryos of rat diabetic dams.³¹ On the basis of these results, resveratrol could be a potential therapeutic agent for the prevention of embryonic malformations including NTDs.³¹ Our understanding of the signaling mechanisms involved in diabetes mellitus has progressed greatly, but very little is known about the mechanism(s) underlying NTDs in diabetic pregnancy. Further studies are needed to determine whether the combined effects of resveratrol and folic acid can completely abolish the birth defects in diabetic pregnancies. Here, we review the literature and provide some new insights to aid further understanding of the signaling mechanisms associated with NTDs and how resveratrol may prevent these defects.

Various plants produce resveratrol to help defend against invading fungi, stress, injury, infection and excessive sunlight. Resveratrol is found in both *cis*- and *trans*-stereoisomeric forms existing as a glucoside (bound to a glucose molecule). The roots of the plant *Polygonum cuspidatum* (KO-jo-kon), mainly cultivated in Asia, provides a rich source of resveratrol from which commercially available *trans*-resveratrol (>98% pure) is isolated by high-speed counter-current chromatography.³² Resveratrol has been reported to possess many salutary effects, including cardioprotection,³³ chemoprevention,^{34,35} and extension of

lifespan in several species.^{36,37} However, the molecular mechanisms underlying the resveratrol functions are different in different diseases. Resveratrol has been in use since ancient times as an herbal formulation termed 'Darakchasava.' The ancient medicinal book of Hindus, *Ayurveda*, more than 4500 years ago described Darakchasava, a heart tonic made from fermented grapes. The formulation is believed to counteract lethargy, weakness and heat exhaustion and stimulate cardiac function. Darakchasava is also thought to have antipyretic, diuretic and diaphoretic effects. Today, Darakchasava contains 1.3–6.0 mg/L of resveratrol, depending on the pharmaceutical company producing it.³⁸

Managing diabetes is one of modern medicine's major challenges, and it remains one of the leading causes of morbidity and mortality among tens of millions of people in the USA alone. Resveratrol has shown promising results in the management of diabetes. In diet-induced obese and diabetic mice, long-term intracerebroventricular infusion of resveratrol corrects hyperglycemia and improves hyperinsulinemia.³⁹ In high-fat-diet-fed mice, it ameliorates insulin resistance, increases the mitochondrial content of cells and prolongs survival.⁴⁰ Resveratrol has beneficial effects on dyslipidemia and obesity. In another study, resveratrol demonstrated significant antihyperglycemic activity, improving insulin levels and oral glucose tolerance tests in *ob/ob* mice.⁴¹ Resveratrol action has been linked to the activation of a histone deacetylase Sirtuin 1 (Sirt1).^{40,41} Administration of resveratrol to diabetic rats results in a significant decrease in blood-glucose concentrations, glycosylated hemoglobin, blood urea and other blood compounds that are usually elevated in type II diabetes and cause damage to body organs.^{39,42} During starvation or limited food, Sirt1 promotes the breakdown of fat stores as a source of energy. Sirt1 is activated through caloric restriction and lengthens rodent lifespan and improves overall health by aiding fat metabolism, increasing immune function, and boosting cardiovascular health.⁴³ Notably, when Sirt1 is activated by resveratrol, diabetes is improved.⁴¹

Resveratrol also activates the 5'-AMP-activated protein kinase (AMPK), which also regulates insulin sensitivity and mitochondrial biogenesis.⁴³ The net effect of AMPK activation is stimulation of hepatic fatty-acid oxidation and ketogenesis; increase of skeletal muscle fatty acid oxidation and muscle glucose uptake; inhibition of cholesterol synthesis, lipogenesis, triglyceride synthesis, adipocyte lipolysis; and modulation of insulin secretion by pancreatic beta-cells. In mice deficient in the catalytic subunit of AMPK ($\alpha 1$ or $\alpha 2$), resveratrol fails to increase insulin sensitivity, glucose tolerance, mitochondrial biogenesis and physical endurance,⁴⁴ indicating the necessity of AMPK in multiple resveratrol-mediated effects.

Resveratrol is safe, but its bioavailability is poor

Resveratrol use in humans has no pronounced toxicity,⁴⁵ and orally administered *trans*-resveratrol is well-absorbed. A study performed by Walle *et al.*⁴⁶ using

¹⁴C-*trans*-resveratrol (oral dose of 25 mg) in humans showed that 70% of the resveratrol dose was absorbed by the body. Metabolites of resveratrol (conjugated forms) in plasma peaked at 30–60 min postadministration with a plasma half-life of 9.2 h.⁴⁶ In contrast, only small amounts of unmodified resveratrol (<5 ng/mL) were detected in plasma in a similar timeframe. Resveratrol absorbed from the gastrointestinal tract is rapidly metabolized to glucuronide (3-*O*-glucuronide and 4'-*O*-glucuronide), sulfate (resveratrol 3-*O*-sulfate), and hydroxylate forms. The majority of orally dosed resveratrol is found in urine as sulfate- or glucuronic acid-conjugates. Food intake with a single dose of 400 mg *trans*-resveratrol delays absorption.⁴⁷ Thus, orally ingested resveratrol is both rapidly metabolized and excreted, accounting for its low bioavailability. However, a few studies show that the bioavailability of resveratrol can be enhanced by using more potent resveratrol analogs (i.e. SRT501)⁴⁸ and enhanced delivery methods such as liposomal encapsulation,⁴⁹ or combining it with another natural product from black pepper (*Piper spp.*), piperine.⁵⁰ However, further validation of these strategies will be needed before resveratrol can be used in diabetic pregnancy.

Health benefits of resveratrol

According to ClinicalTrials.gov (<http://clinicaltrials.gov/>), there are more than 50 recent studies involving resveratrol. These trials investigate the possible role of resveratrol in different diseases such as diabetes, Alzheimer's disease and cancer. There are 12 clinical trials where resveratrol is specifically being focused for type 2 diabetes, obesity, insulin resistance or metabolic syndrome.^{51,52} One of the human clinical trial is investigating whether resveratrol when given orally to obese and type 2 diabetic subjects, will decrease reactive oxygen species (ROS) generation and prevent the production of the pro-inflammatory transcription factor nuclear factor- κ B.⁵¹ A second trial is investigating the cellular and molecular effects of resveratrol on inflammatory mediators and insulin resistance in obese non-diabetic and type 2 diabetic subjects (<http://clinicaltrials.gov/>). Another clinical trial is being conducted to determine the effect of resveratrol on endothelial function.⁵³ The endothelium participates in the control of blood vessel function, in part by regulating vessel dilation via nitric oxide; however, endothelial function becomes abnormal in diabetic patients, and this abnormality contributes to the development of cardiovascular disease.³³ In diabetic (Lepr^{db}) mice, resveratrol restores endothelial function by interfering with tumor necrosis factor α -mediated activation of NADPH oxidase and thereby preserves nitric oxide availability.⁵⁴ Preclinical observations suggest that resveratrol is safe and has enormous potential in the treatment of obesity and insulin resistance in humans. Current investigations are evaluating whether resveratrol is able to counteract the detrimental effects of obesity. One trial is examining the effect of resveratrol on improving the metabolic profile of adults with insulin resistance (<http://clinicaltrials.gov/>). Resveratrol's antioxidative potential is being

compared with calorie restriction in clinical trials to determine: (i) gene expression and lipid profile changes, (ii) insulin's efficacy to control blood sugar and (iii) changes in other blood and tissue markers of metabolic and cardiovascular health.⁵¹ Another current study with obese subjects is aimed to test resveratrol's ability to improve mitochondrial activity and fatty acid oxidative capacity in skeletal muscle upon a high-fat challenge (<http://clinicaltrials.gov/>).

Resveratrol improves maternal glucose and lipid profile during diabetic pregnancy

The ability of resveratrol to lower blood glucose is well documented.^{42,55} In rodent studies, we tested this ability of resveratrol to lower blood glucose in pregnant diabetic dams where diabetes was induced with streptozotocin. Glucose concentrations were measured in dams on every other day of pregnancy and significant decreases at gestation day 9 (22.80%) and 12 (33.32%) were found in the resveratrol-treated diabetic group compared with the diabetic only group.³¹ In addition, significantly increased insulin levels were present in the resveratrol-fed diabetic groups, although it was still much lower than that in the control group.³¹ Diabetes also adversely affects lipid profile, which may indirectly affect embryogenesis and organogenesis.⁵⁶ Furthermore, combined disruption of normal glucose and lipid status may be detrimental to fetal and neonatal growth; some evidence indicates that these effects may be lifelong and contribute to adult obesity. In our studies, excess lipids accumulate in the serum of diabetic dams; however, less lipid accumulation (comparable to that observed in controls) was found in the serum of rat diabetic dams treated with resveratrol.³¹ We further analyzed the lipid profile and found that resveratrol significantly decreased the cholesterol and triglycerides concentrations in the serum of diabetic dams.³¹ Recently, resveratrol was shown to reduce mRNA levels for HMG-CoA reductase (a rate-limiting enzyme for cholesterol synthesis and target for 'statin' drugs) in high-fat-diet-fed hamsters.⁵⁷ Statin molecules are used as drugs to lower the cholesterol and triglycerides in humans, but are not recommended for pregnant or nursing mothers.^{58,59} Therefore, resveratrol could be used as substitute for statins to control excess cholesterol production. Lipid profile is correlated with biologically active estrogen. So, the significant reduction in triglyceride level of diabetic female rat might be due to the ability of resveratrol to act on estrogen receptor signaling.^{60,61} However, whether resveratrol activity is estrogenic or antiestrogenic remains controversial.^{62,63}

Antiteratogenic effect of resveratrol

An antiteratogenic effect of resveratrol has been demonstrated in pregnant mice (C57BL/6J) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Pretreatment with resveratrol by gavage feeding (50 mg/kg body weight [bw]) from gestational day E8 to E13 significantly reduced the incidence and severity of fetal malformations caused

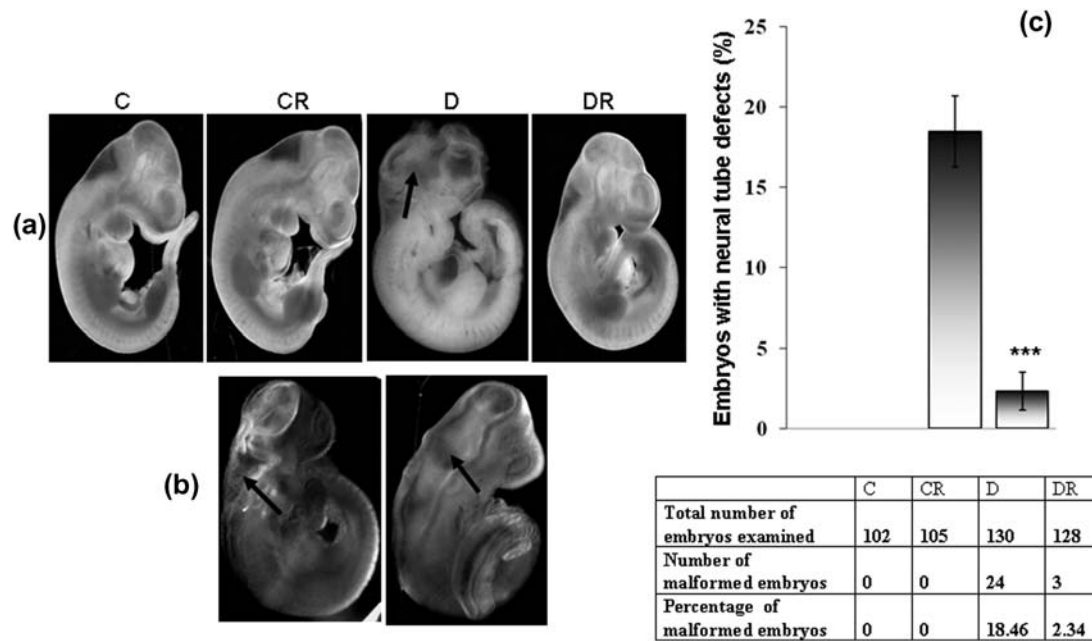


Figure 1 Resveratrol prevents neural tube defects in E12 rat embryos of diabetic dams. (a) Morphological analysis of E12 rat embryos of control (C), control treated with resveratrol (CR), diabetic (D) and diabetic treated with resveratrol (DR) dams; (b) E12 rat embryos of diabetic dams with neural tube defects; (c) Percentage of malformed embryos in C, CR, D and DR groups

by TCDD exposure (oral challenge, 14 $\mu\text{g}/\text{kg}$ bw on E12).⁶⁴ In addition, resveratrol inhibited TCDD-induced immunotoxicity in both the mother and fetus. Resveratrol protects normal non-pregnant mice and pregnant dams and their fetuses from TCDD-induced thymic atrophy and apoptosis, from alterations in the expression of T-cell receptor and co-stimulatory molecules, and from alterations in T-cell differentiation.⁶⁵ In another study, the effect of dietary resveratrol on embryo-fetal survival and development was evaluated in groups of pregnant female Sprague-Dawley rats. Treatment with resveratrol with up to 750 mg/kg bw per day (from gestation day 5 to 20) was well tolerated with no signs of adverse effects on fetal weights, number of implantations, resorptions, live young or pre and post implantation losses. Maternal treatment with resveratrol at dietary doses up to 750 mg/kg bw/day had no effect on the incidence of major or minor fetal abnormalities or the incidence of visceral abnormalities. It was concluded that up to 750 mg/kg bw per day of resveratrol dose represented the no-observed-adverse-effect-level for maternal toxicity and embryo-fetal development.⁶⁶ Recently, in a human clinical trial healthy subjects tolerated a 4.0 g resveratrol dose; however, at this high dose, diarrhea was observed in some participants.⁶⁷ In another study from our lab, we showed that resveratrol exhibits neuroprotective effects in cerebellum by acting at redox regulating proteins in a rodent model of fetal alcohol spectrum disorders.⁶⁸

Resveratrol prevents NTDs in diabetic pregnancies

Diabetic pregnancy can have many complications, especially in terms of embryonic development. Examples

of embryonic impairments include improper or incomplete closure of the neural tube, developmental retardation, impaired rate of neurogenesis and failure to form appropriate neural connections.^{4,7} Furthermore, these developmental issues may be the basis of many future physical, neurological and psychiatric disorders. In humans, diabetic malformations most likely occur during the first trimester.⁶⁹ In embryos of diabetic rat dams, embryonic day 12 is the stage where organogenesis is affected.⁷⁰ We evaluated the outcome of diabetic pregnancy in E12 embryos after gavage feeding of resveratrol (100 mg/kg bw) to diabetic dams. No external malformations were observed in the embryos of control or control treated with resveratrol group (Figure 1a). Approximately 20% of the embryos of diabetic dams were malformed (Figures 1b and c). The malformed embryos were characterized as having defect in neural tube closing. Collectively, these malformed embryos were categorized as having malformations.⁷¹ The number of malformed embryos was significantly reduced when diabetic dams received resveratrol treatment (Figure 1c). Most embryos of diabetic dams, including those that lacked obvious malformations, had reduced weight, crown-rump length and somite number,³¹ and reduced embryo size indicating developmental delay. Resveratrol not only abolishes the diabetes-induced malformation in the embryos but it also rescues embryos from developmental delays.³¹ One way that resveratrol might be acting directly on embryos of diabetic pregnancies is by normalizing hyperglycemia-induced oxidative stress. Apoptosis subsequent to oxidative stress is associated with diabetic embryopathies.¹² In embryos, resveratrol normalizes oxidative stress markers including the increased lipid peroxidation and total thiol concentrations and decreased amounts of reduced glutathione associated

with hyperglycemia.³¹ Attenuation of oxidative stress further reduces the level of activated caspases, thereby reducing apoptosis, and in turn reduces embryonic malformations.³¹

Molecular mechanisms underlying the beneficial effects of resveratrol in diabetic embryopathy

Resveratrol crosses the blood-brain barrier and exerts protective effects against cerebral ischemic injury⁷² and also exerts potent antioxidant features in the brain of healthy rats.⁷³ There have been no reports, however, on whether resveratrol crosses the placental barrier. Because resveratrol is beneficial against diabetes-induced embryonic malformation, we might cautiously assume that it does cross the placental barrier. Resveratrol works positively in different ways on mother and embryo in diabetic pregnancy to normalize the disease condition.³¹ Improvement in embryonic outcome might be because of improved maternal environment, direct effects on the embryos (as evident from normalized embryonic oxidative stress level), or both.³¹ Diabetes-dependent increases in oxidative stress and its fluctuation, and individual fetal antioxidative capacity determine the severity of embryonic deformity. Diabetic embryopathy studies revealed that the outcome of embryonic development depends on the glycemic, cholesterol and triglyceride levels and on the level of oxidative stress.^{12,31}

We found that administration of resveratrol, a potent antioxidant⁷⁴ in diabetic pregnancy works via three different ways by: (i) reducing excess maternal cholesterol and triglyceride, (ii) reducing maternal blood sugar level and (iii) modulating embryonic oxidative stress and apoptosis.³¹ Resveratrol is a free radical-scavenger and a potent antioxidant and can promote the activities of several antioxidative enzymes.⁷⁵ Reactive oxygen species (ROS) are important intermediates in cellular signal transduction and transcriptional control but excess ROS generation leads to harmful oxidative modification of cellular lipids, proteins and nucleic acids. Indeed, diabetes-induced complications are associated with the increased amounts of ROS. In diabetic dams, with resveratrol treatment there were no changes in expression or activity of the antioxidative enzyme superoxide dismutase, but resveratrol reduced oxidative stress by normalizing the level of reduced glutathione, total thiol, lipid peroxidation and 4-hydroxy-2-non-enal (HNE) accumulation. Excess level of oxidative stress induces the formation of electrophilic aldehydes, such as HNE, as by-products of peroxidation of lipid membranes.⁷⁶ HNE exerts its effects by alterations in cell proliferation, cell-cycle progression and apoptosis in various cell types.^{76,77}

Diabetes impairs Rho GTPase signaling. Could resveratrol reverse impairments?

Rho GTPases (RhoA and Rac1), small-molecular weight G proteins of the Ras superfamily, have been implicated in

cellular processes such as cytoskeletal rearrangement and cell-cycle control.⁷⁸⁻⁸² Active (GTP-bound) and inactive (GDP-bound) Rho conformations function as controls in a myriad of intracellular processes. Conformational cycling is regulated by three main classes of proteins: (i) those catalyzing exchange of GDP for GTP (e.g. guanosine nucleotide exchange factors);⁸³ (ii) those stimulating hydrolysis of GTP to GDP (e.g. GTPase activating proteins [GAPs]);⁸⁴ and (iii) those inhibiting the dissociation of GDP from GTPase (e.g. GDP dissociation inhibitors [GDIs]).⁸⁵ Rho GTPases are also involved in neural tube closing⁸⁶⁻⁸⁹ whereby RhoA accumulates at the apical region of neural plate to promote folding.^{87,90} Enrichment of RhoA at the apical region of the plate induces the constriction of the cells via Rho kinase. The resulting cytoskeletal rearrangement promotes the bending of the plate.⁹⁰ G protein-coupled receptors such as Par1 and Par2 participate in neural tube closing. Genetic deletion of Par1/Par2 yields NTDs.⁹¹ These receptors function by coupling to heterotrimeric G proteins such as Go, Gi and Gz. Pertussis toxin, which inactivates these G proteins, reproduces the NTD-forming effects of Par1 and Par2 genetic. More importantly, Rac1 genetic deletion also produces exencephaly and spina bifida in mouse embryos.⁹¹

Rho GTPases play key roles in cytoskeletal rearrangement and neuronal differentiation. During normal development, activation of RhoA leads to formation of stress fibers and focal adhesion complexes.⁷⁹ On the other hand, Rac1 activation participates in membrane ruffling and lamellipodia formation.⁸¹ Studies conducted in primary neurons suggest that Rho GTPases help to regulate the growth of axons and other neuronal processes. In general, Rac1 has a positive effect on neurite extension, whereas RhoA has a positive effect on neurite retraction.⁹²⁻⁹⁴ Experimental evidence indicates that formation of synaptic connections in the neuronal network and their rearrangement are governed by the neuronal actin cytoskeleton.^{95,96} Actin assembly and polymerization as well as actomyosin contraction are chiefly regulated by Rho GTPases.^{97,98} Thus, by controlling neuronal morphology, Rho GTPases might also be essential for learning and memory. Indeed, activation of Rho GTPases by CNF1 toxin induces cerebral actin cytoskeletal re-arrangement and improves learning and memory, as judged by various behavioral tests.⁹⁹ Although many aspects of Rho signaling in the central nervous system are still unclear, the emerging details reveal that alterations or impairments in Rho GTPase signaling result in abnormal neuronal connectivity and deficient cognitive functioning in humans.¹⁰⁰ Rho GTPases participate in retinoic acid (RA)-induced neuronal differentiation. Whereas activation of RhoA is involved in cytoskeletal rearrangement, Rac1 regulates induction of neurite outgrowth.^{80,82} After activation, Rho GTPases bind to their kinase effectors (ROCK-2 for RhoA and PAK1 for Rac1) and translocate to the plasma membrane.^{79,101} Oxidative stress activates RhoA; the ROS produced during oxidative stress react with the redox-sensitive cysteine residues in the phosphoryl-binding loop of RhoA, promoting its activation. This activation does not require the participation of exchange factors or other regulating proteins.¹⁰²

In another study, ROS promoted the activation of RhoA by dissociating Rho GDI. The removal of ROS by the scavenger Tiron prevented the activation of RhoA *in vivo*.¹⁰³ Whether hyperglycemia also activates RhoA by increasing oxidative stress and whether resveratrol prevents oxidative stress to neutralize activation of RhoA are not known.

Diabetes-induced impairments in the activation of Rho GTPase signaling may contribute to neuronal malformations in diabetic embryopathy. Resveratrol corrects the diabetes-induced impairment of retinoic acid receptor (RAR), retinoid X receptor (RXR) and mitogen-activated protein kinase (MAPKs) expression. All these signals play crucial roles during embryonic development. In addition, RARs (RAR $\alpha/\beta/\gamma$) and RXRs (RXR $\alpha/\beta/\gamma$) function as transcription factors controlling RA-directed gene expression.¹⁰⁴ RA (a physiological active metabolite of vitamin A) is necessary for proper embryonic development and rostro-caudal patterning of the nervous system.¹⁰⁵ As heterodimers, RARs and RXRs bind to RA-response elements in DNA of promoters of target genes to regulate their transcription.¹⁰⁴ Vitamin A deficiency results in suppression of RAR activity and contributes to embryonic skeleton hypoplasia.¹⁰⁶ The curly tail-mouse mutant (a model for NTD) displays NTD because of downregulated RAR β and RAR γ expression.¹⁰⁷ Activation of RARs and RXRs has an anti-diabetic effect and helps to activate insulin-sensitizing genes.¹⁰⁸ Suppressed expression of RAR and RXR receptors in E12 embryo of diabetic dams was improved with resveratrol treatment.¹⁰⁹ MAPKs also contribute to growth, differentiation and developmental processes; their activity determines the fate of embryonic organogenesis when the embryo faces teratogenic conditions.¹¹⁰ Hyperglycemia increases ROS, which modulate MAPK signaling.¹¹¹ In the embryo, oxidative stress results in DNA, protein and lipid modifications that disrupt normal development. Resveratrol can also selectively inhibit stress activated MAPK signaling.¹¹² Increased phospho-ERK1/2 and decreased phospho-JNK1/2 and phospho-p38 occurs in the embryos of resveratrol-treated diabetic dams compared with those not exposed to resveratrol.¹⁰⁹

After reviewing the literature on resveratrol, and on the basis of our own studies, we conclude that resveratrol possesses significant blood sugar-lowering activity and anti-oxidative capacity without any observable side-effects^{31,45} and prevents embryonic oxidative stress and apoptosis associated with diabetic embryopathy. Thus, resveratrol may be suitable alone or in combination with insulin and folic acid for use during diabetic pregnancies and for nursing mothers. Animal studies to test whether resveratrol supplementation in combination with folic acid alone and/or with insulin can prevent all embryonic malformations and developmental delays are needed to provide data to support clinical trials in humans.

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