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# Hyperglycaemia-induced oxidative stress in gestational diabetes mellitus (GDM)

## Stres oksydacyjny indukowany hiperglikemią w cukrzycy ciążowej (GDM)

### ABSTRACT

**An imbalance between production of reactive oxygen species (ROS) and their clearance by antioxidant defence mechanisms results in the development of oxidative stress. Biological consequences of this state involve oxidative damage of key cellular components such as nucleic acids, lipids, or proteins and, in turn, impairment of cell and tissue function. Evidence from clinical and experimental studies supports the notion that oxidative stress is one of pathologic factors associated with gestational diabetes mellitus (GDM), metabolic disorder defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It has been established that high blood glucose concentrations in diabetic pregnancy induce oxidative stress by several mechanisms, including an enhanced ROS production in mitochondria, the polyol pathway and the hexosamine pathway, as well as protein kinase C (PKC) activation and an advanced glycation end-products (AGEs) formation, and changes in biomarkers of free radical-induced damage and antioxidant defences have been detected in maternal diabetes. Moreover, hyperglycaemia-induced oxidative stress is related with some congenital anomalies in diabetic pregnancy. The current article provides an overview how oxidative stress is related to GDM, with special emphasis on the involvement of the hyperglycaemia-induced mechanisms in ROS overproduction, followed by discussion of indicators of oxidative stress. In addition, the relationship between oxidative stress and congenital malformations in diabetic pregnancy is described. (Diabet. Klin. 2015; 4, 5: 189–198)**

**Key words: biomarker; gestational diabetes mellitus (GDM); oxidative stress; reactive oxygen species (ROS)**

### STRESZCZENIE

**Brak równowagi między produkcją reaktywnych form tlenu (ROS) a ich usunięciem przez antyoksydacyjne mechanizmy obronne prowadzi do rozwoju stresu oksydacyjnego. Biologicznymi konsekwencjami tego stanu są oksydacyjne uszkodzenia kluczowych składników komórkowych, takich jak kwasy nukleinowe, białka lub lipidy, które prowadzą do upośledzenia funkcji komórek i tkanek. Dowody pochodzące z badań klinicznych i eksperymentalnych popierają koncepcję, że stres oksydacyjny jest jednym z patologicznych czynników związanych z cukrzycą ciążową (GDM), definiowaną jako różny stopień zaburzeń tolerancji węglowodanów po raz pierwszy rozpoznany lub rozwijający się podczas ciąży. Ustalono, że wysokie stężenia glukozy we krwi kobiet z GDM indukują stres oksydacyjny poprzez kilka mechanizmów, w tym zwiększoną produkcję ROS w mitochondriach, szlak poliolowy, szlak heksozoaminy, aktywację kinazy białkowej C (PKC) oraz tworzenie końcowych produktów zaawansowanej glikacji (AGEs). Zmiany zarówno w biomarkerach uszkodzeń oksydacyjnych, jak i antyoksydacyjnym systemie obronnym były wykryte u pacjentek z GDM. Ponadto stres oksydacyjny**

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**indukowany hiperglikemią w GDM jest związany z rozwojem wad wrodzonych płodu.**
**Niniejszy artykuł stanowi podsumowanie wiedzy o związku stresu oksydacyjnego z cukrzycą ciążową, ze szczególnym podkreśleniem udziału mechanizmów indukowanych hiperglikemią, które prowadzą do nadprodukcji ROS, oraz omówieniem wskaźników stresu oksydacyjnego. Opisano także związek między stresem oksydacyjnym a występowaniem wad wrodzonych płodu podczas cukrzycy w ciąży. (Diabet. Klin. 2015; 4, 5: 189–198)**

**Słowa kluczowe: biomarker, cukrzyca ciążowa (GDM), stres oksydacyjny, reaktywne formy tlenu (ROS)**

## Introduction

Gestational diabetes mellitus (GDM), defined as hyperglycaemia with onset or first recognition during pregnancy, is one of the most common metabolic disorders occurring during pregnancy that affects from 3% to 34% of all pregnancies, depending on the screening criteria and ethnic group [1, 2]. Although GDM symptoms resolve after delivery, it has negative consequences for both mother (e.g. preeclampsia, preterm delivery, caesarean section, elevated risk of developing type 2 diabetes mellitus and cardiovascular disease after pregnancy) and child (e.g. prematurity, macrosomia, hypoglycaemia, polycythaemia, and hypocalcaemia) [3]. Insulin resistance and $\beta$ cells dysfunction are believed to be two major contributors to the pathogenesis of GDM. Although the precise mechanisms underlying these abnormalities are not fully understood so far, disturbances in the insulin signalling pathway [4, 5] and changes in plasma adipokine levels [6], as well as enhancement in inflammation [7, 8] and oxidative stress [9, 10] have been demonstrated.

Reactive oxygen species (ROS), including either oxygen radicals such as the hydroxyl radical (•OH), peroxyl radical (ROO•), the superoxide anion radical ($O_2^{\bullet-}$), or reactive non-radicals like hydrogen peroxide ($H_2O_2$) or singlet oxygen ($^1O_2$), are highly reactive molecules generated within the cell predominantly as by-products of aerobic respiration and metabolism, which paradoxically can act as a double-edged sword in modulating human physiology. ROS function as second messengers in mammalian cells to regulate signal transduction cascades engaged in cell function, growth, differentiation and death as well as cytokine formation, and angiogenesis [11]. On the other hand, excessive ROS production can attenuate endogenous antioxidant systems leading to oxidative stress, which have detrimental effects on cellular DNA, protein and lipids. Oxidative damage of these biomolecules has been implicated in the pathology and complications of numerous diseases such as cancer, cardiovascular diseases, ischaemic diseases, aging processes and diabetes including GDM.

Pregnancy *per se* is a condition associated with enhanced oxidative stress since the high metabolic demand and elevated requirements of oxygen contributes to increased ROS production in the mitochondria of placenta, thought to be a major source of free radicals at onset of pregnancy and during labour. However, this state is balanced by adequate antioxidant responses. In GDM, when antioxidant defence is impaired then oxidative damage increases. Hyperglycaemia has been directly implicated in the formation of free radicals by the mitochondrial electron-transport chain, enhanced polyol and hexosamine pathway flux, activation of protein kinase C (PKC), and augmented formation of advanced glycation end-products (AGEs) [10]. Moreover, hyperglycaemia-induced oxidative stress has been suggested to play an important role in the aetiology of several congenital anomalies in diabetic pregnancy [12].

In this review we will summarize the current perspective on how hyperglycaemia induces oxidative stress in GDM and how hyperglycaemia-induced oxidative stress is connected to congenital malformations in diabetic pregnancy.

## Hyperglycaemia-induced oxidative stress

Several mechanisms underlying hyperglycaemia-induced oxidative stress during GDM have been proposed so far. High glucose can disrupt the electron transport chain in mitochondria, resulting in the $O_2^{\bullet-}$ overproduction. Additionally, ROS can be generated through the hexosamine, polyol and PKC pathways as well as in the process of AGEs formation (Fig. 1) [10].

### The hexosamine pathway

The hyperglycaemia-induced hexosamine pathway begins with the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT) that converts fructose-6-phosphate into glucosamine-6-phosphate (Fig. 1.4). In turn, the glucosamine-6-phosphate inhibits glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the pentose phosphate pathway that generates NADPH. Hence, the enhanced hexosamine pathway lowers NADPH concentration leading to a reduction of glutathione (GSH) level and increased oxidative stress [13]. The end-product of the hexosamine pathway is uridine diphosphate *N*-acetylglucosamine (UDP-Glc-NAc), the substrate for *N*- and *O*-glycosylation of numerous cellular proteins including transcriptional factors [14].

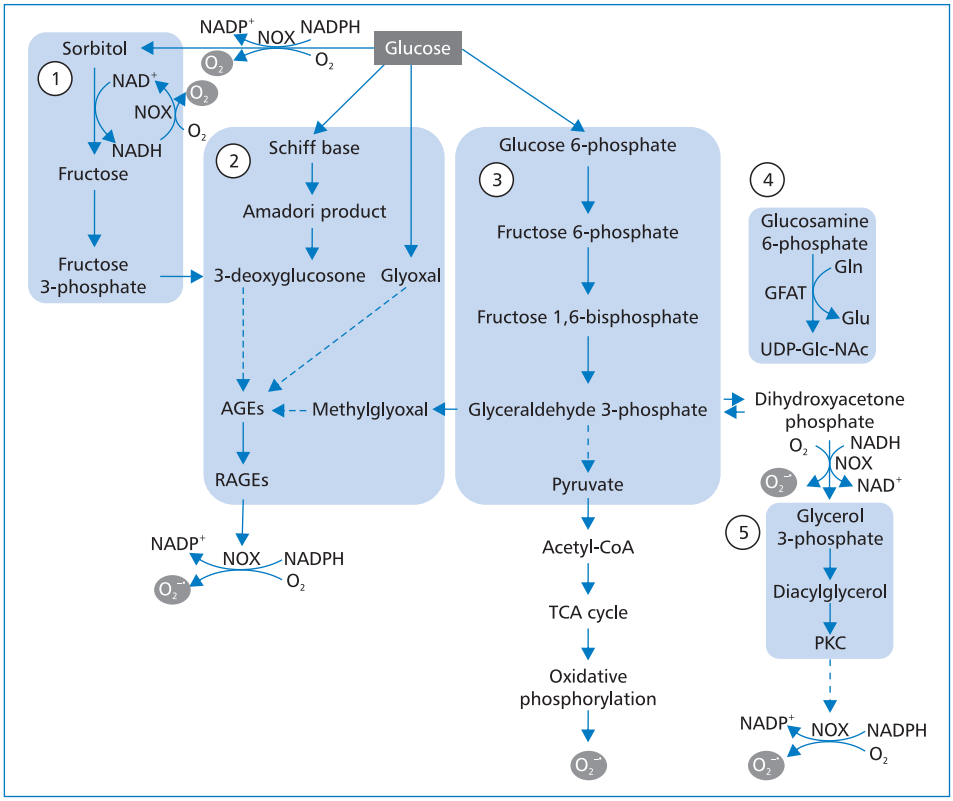


**Figure 1.** The influence of altered carbohydrate metabolism on ROS production in GDM. High glucose concentration in blood activates polyol (1), hexosamine (4) and PKC pathways (5), as well as AGEs production (2). Moreover, glycolysis (3) is increased in the hyperglycaemic state. The enhancement of those pathways leads to higher ROS concentration. GFAT — glutamine:fructose-6-phosphate amidotransferase; Gln — glutamine; Glu — glutamic acid; $NAD^+$/NADH — nicotinamide adenine dinucleotide; NOX — NAD(P)H oxidase; PKC — protein kinase C; TCA cycle — tricarboxylic acid cycle; UDP-Glc-NAc — uridine diphosphate *N*-acetylglucosamine

The relationship of the hexosamine pathway with insulin resistance has been established. Indeed, the *O*-linked *N*-acetylglucosamine modification of insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) has been demonstrated to regulate the post-receptor insulin signalling involved in glucose transport and glycogen storage [15]. Additionally, several studies have revealed that GFAT overexpression in fat and liver causes insulin resistance and deterioration of the $\beta$-cell function [16–18]. Moreover, glucosamine increases the plasminogen activator inhibitor-1 (PAI-1) promoter activity, affecting the development of microvascular diabetic complications such as diabetic nephropathy [14].

## The polyol pathway

Under normal conditions, only approx. 3% of cellular glucose is converted into sorbitol in the polyol pathway, whereas under hyperglycaemic condition approx. 30% of glucose is metabolized in this pathway [19]. The first step of this pathway includes a reduction of glucose to sorbitol by aldose reductase (AR) that utilizes NADPH (Fig. 1.1 and Fig. 2). In the second step, sorbitol dehydrogenase (SDH) catalyses oxidation of sorbitol to fructose with concomitant NADH production. The polyol pathway participates in hyperglycaemia-induced oxidative stress by three mechanisms [20]. First, the AR competes with glutathione reductase (GR) for NADPH as a co-factor, thereby the GSH concentration decreases and the cellular antioxidant capacity impairs. Second, an enhanced SDH activity augments the NADH concentration, the substrate for the NADPH-dependent oxidase (NOX), leading to superoxide anion overproduction. Third, fructose can be converted into fructose-3-phosphate (F-3-P) and 3-deoxyglucosone (3-DG). Since both products are more potent nonenzymatic glycation agents than glucose, they can effectively form AGEs.

Maternal hyperglycaemia has been shown to cause a rise in sorbitol concentration in foetal tissue and AR activity in the lenses of diabetic rat foetuses [21, 22]. Alterations in the polyol pathway have also been found in diabetic complications such as cardiomyopathy, neuropathy, nephropathy, and retinopathy [20]. Additionally, a linkage of the AR overexpression to atherosclerosis has been reported in diabetic mice

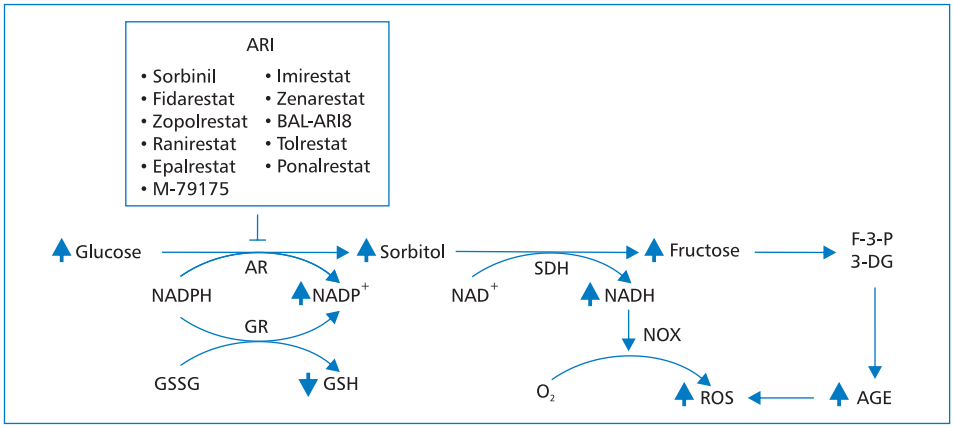


**Figure 2.** Contribution of the polyol pathway to increased oxidative stress during hyperglycaemia. 3-DG — 3-deoxyglucosone; AGEs — advanced glycation end-products; AR — aldose reductase; F-3-P — fructose-3-phosphate; GR — glutathione reductase; GSH — reduced glutathione; GSSG — oxidized glutathione; NOX — NADPH oxidase; ROS — reactive oxygen species; SHD — sorbitol dehydrogenase

[23, 24]. To prevent these abnormalities, AR inhibitors (ARI) such as sorbinil, fidarestat, and imirestat, among others, are currently being intensively studied [20].

## PKC pathway

Due to enhanced glycolysis under hyperglycaemic conditions, glyceraldehyde 3-phosphate (G-3-P) overproduction leads to increased dihydroxyacetone phosphate (DHAP) formation. The reduction of DHAP to glycerol 3-phosphate followed by acylation generates diacylglycerol (DAG) [25]. In turn, an increased DAG concentration activates PKC, predominantly PKC-$\beta$, -$\delta$, and -$\alpha$ isoforms (Fig. 1.5). Hyperglycaemia-induced PKC activation has been detected in heart and superior mesenteric artery of diabetic mice [25, 26]. The relationship between increased PKC pathway and enhanced oxidative stress has been suggested [25]. In fact, it has been shown that PKC-$\beta$ inhibits endothelial NO synthase (eNOS) expression in endothelial cells [27], whereas PKC-$\alpha$ activates NOX5 leading to increased superoxide production [28]. Additionally, NOX-mediated ROS overproduction augments peroxynitrite formation and oxidation of tetrahydrobiopterin ($BH_4$), eNOS co-factor, causing eNOS dysfunction through uncoupling its reductase and oxygenase domains [29].

## Advanced glycosylation end-products (AGEs)

In diabetes, high glucose concentration increases the AGEs formation. The AGEs arise mainly from autoxidation of glucose to glyoxal, but also from decomposition and fragmentation of the Amadori products (Fig. 1.2). First reaction in the production of AGEs is the condensation of the carbonyl group of glucose with a free amino group of a protein that results in the formation of a reversible Schiff base. Subsequently, the Schiff base can be converted into a covalently bound Amadori product followed by degradation to 3-deoxyglucosone and methylglyoxal [30, 31]. These reactive dicarbonyl compounds react directly with amino groups of intracellular and extracellular protein forming AGEs. The AGEs cytotoxicity is a consequence of altered physiological function of specific proteins and cellular ROS production. The latter process is related with the activation of the type I cell surface receptor for AGEs (RAGE), belonging to the immunoglobulin superfamily, with subsequent induction of NOX [32]. The AGE-RAGE interaction also causes stimulation of some members of the mitogen-activated protein kinase (MAPK) family such as extracellular signal-regulated kinases (ERK)-1 and -2, and p38, resulting in activation of nuclear factor $\kappa$B (NF-$\kappa$B) and subsequently induction of the expression of adhesion molecules, cytokines and chemokines [33]. Pro-oxidant and pro-inflammatory functions of the AGE-RAGE axis have been implicated not only in the development and progression of diabetic micro- and macroangiopathy [33], but also in normal human labour accompanied by increased oxidative stress, formation of inflammatory mediators (e.g. cytokines) and uterotonic phospholipid metabolites (e.g. prostaglandins) as well as in adverse pregnancy outcomes. In support of this, AGEs significantly increases *in vitro* release of tumour necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin IL-1$\beta$, IL-6, IL-8, prostaglandin (PG)E2, PGF2$\alpha$ and 8-isoprostane from human placenta and gestational membranes with a concomitant increase in NF-$\kappa$B p65 activation and ERK 1/2 phosphorylation [34], and concentrations of AGE-modified products in umbilical cord blood increase with gestation progression and

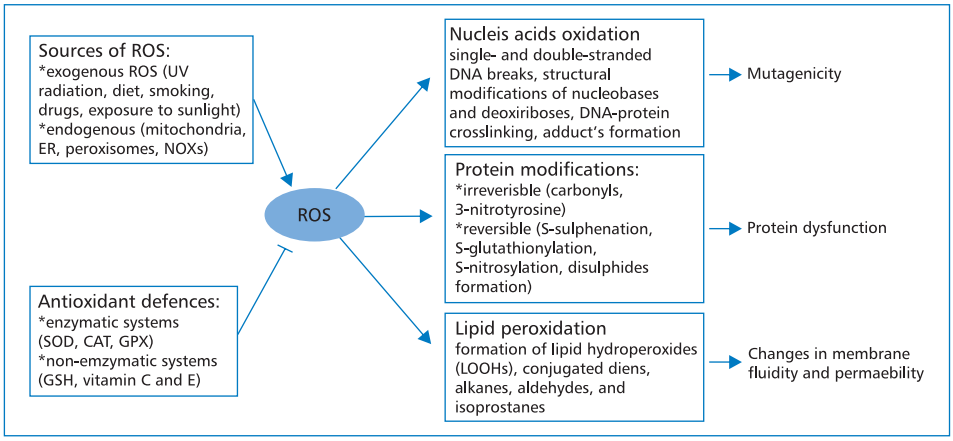


**Figure 3.** Sources of ROS, antioxidant systems, and biological consequences of high ROS concentration

in preeclampsia [35]. In regard to a relationship of AGEs with GDM, significantly higher serum AGE levels have been found in diabetic patients [36]. Moreover, increased AGE concentrations have been detected in GDM patients with foetal distress, foetal malformation and stillbirth compared with diabetic patients with normal foetal outcome, suggesting that AGEs may be an important risk factor for abnormal outcome in infants of GDMs [37].

## Indicators of oxidative stress in GDM

Oxidative stress can trigger oxidative damage of essential cellular biomolecules such as DNA, proteins, and lipids (Fig. 3). Among lipid molecules present in cellular membrane, polyunsaturated fatty acids (PUFAs) are particularly ROS-sensitive since they contain double bonds between two carbon atoms that weaken neighbouring C-H bonds, thus facilitating a hydrogen atom removal during the attack of ROS [38]. Lipid peroxidation proceeds as a free radical mediated chain reaction resulting in the formation of several products such as lipid hydroperoxides (LOOHs), conjugated dienes, alkanes, aldehydes, and isoprostanes (Fig. 3). An increased LOOH level is a common indicator of oxidative stress [39]. The reactive end-products of lipid peroxidation such as malonyl dialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) can readily react with cellular proteins and DNA, leading to the formation of stable adducts [40]. MDA can react with thiobarbituric acid yielding thiobarbituric acid reactive substances (TBARS), which are quantified to monitor lipid peroxidation. However, MDA is not specific for oxygen radical-induced lipid peroxidation since it can be also produced by cyclooxygenase in enzymatic lipid peroxidation. Additionally, free-radical-catalysed peroxidation of arachidonic acid independent of cyclooxygenase results in formation of PGF2-like compounds, namely F2-isoprostanes. Among them, 8-iso-prostaglandin F2$\alpha$ (8-iso-PGF2$\alpha$) is an important marker of endogenous lipid peroxidation [41]. The functional consequence of lipid peroxidation is an increase in membrane fluidity and permeability [38].

Among protein oxidative modifications, the irreversible products are protein carbonyls (PCO) and 3-nitrotyrosine (3-NT) (Fig. 3). Protein carbonyls are formed on several amino acids residues such as arginine, histidine, lysine, proline, threonine and cysteine. Carbonylation may lead to AGEs formation, starting from condensation of amino group, predominantly at lysine and arginine side chains, and carbonyl generating *N*-glycoside that is transformed into the Schiff's base and then the Amadori product [42]. The 3-NT is formed between reactive nitrogen species (RNS) and a protein's tyrosine residue. 3-NT is thought to be a highly selective modification since not all tyrosine residues on a target protein can be nitrated [43]. The reversible protein oxidation includes cysteine modification products such as sulphenic acids, nitrosothiols, and s-glutathione. Cysteine is usually oxidized to sulphenic acid, which in turn can undergo S-nitrosylation (-SNO), S-glutathionylation (protein-SSG), S-sulphenation (-SOH) or can be converted into disulphide (S-S-) [43]. These cysteine oxidative modifications are involved in redox regulation of protein functions through ROS and RNS.

## Biomarkers of oxidative damage

In GDM, lipid peroxidation products are usually measured as indicators of oxidative stress. Numerous studies have revealed increased MDA and TBARS levels in maternal and cord blood as well as in placenta from pregnancies complicated by GDM (Tab. 1) [44–57]. Additionally, MDA level has been found to positively correlate

**Table 1. Biomarkers of oxidative damage of lipids and proteins in GDM women**

| Biomolecule | Biomarker | References |
|---|---|---|
| Lipid | ↑TBARS | [44–47] |
| | ↑MDA | [48–57] |
| | ↑LOOH | [59–61] |
| | ↓LOOH | [58] |
| | ↑8-isoprostanes | [9, 26] |
| Protein | ↑AOPP | [51, 62] |
| | ↑POOH | [62] |
| | ↑PCO | [9, 62] |
| | ↑3-NT | [62] |
| | ↓3-NT | [58] |

↑increased; ↓decreased; 8-oxo-dG — 8-hydroxydeoxyguanosine; AOPP — advanced oxidation protein products; LOOH — lipid hydroperoxides; MDA — melanodialdehyde; 4-HNE — 4-hydroxy-2-nonenal; 8-iso-PGF2$\alpha$ — 8-iso-prostaglandin F2$\alpha$; POOH — protein hydroperoxides; PCO — protein carbonyl; 3-NT — 3-nitrotyrosine

with blood glucose concentration [40]. Besides MDA and TBARS, increased serum LOOHs concentration has also been detected in women with GDM (Tab. 1) [59–61].

Although there are limited data on protein oxidation in GDM, several studies have demonstrated increased levels of protein damage markers, including PCO, 3-NT, protein hydroperoxides (POOHs), and advanced oxidation protein products (AOPP), in plasma, serum, leukocytes, and placenta in GDM patients (Tab. 1) [9, 51, 62]. Moreover, the positive correlation between POOHs and glycated haemoglobin $A_{1c}$ ($HbA_{1c}$) has been found in GDM women [62]. By contrast, decreased plasma 3-NT and LOOHs levels in GDM women at 28 weeks of gestation has also been reported [58]. The reasons for that difference are uncertain, but may stem from a relatively small number of patients participating in this study.

## Endogenous antioxidants

Endogenous enzymatic antioxidants such as superoxide dismutase (SOD) that generates hydrogen peroxide from superoxide radicals and catalase (CAT) along with glutathione peroxidase (GPX) which decompose hydrogen peroxide are believed to be the first line of defence against ROS propagation, oxidative stress and tissue damage in the organism. Therefore, these enzymes have been studied most frequently of the antioxidant systems during diabetic pregnancy so far (Fig. 3). It has been demonstrated that CAT activity is reduced in maternal cord plasma as well as in placenta of GDM women (Tab. 2) [53]. The SOD activity has been found to be generally decreased in GDM patients [44, 56, 59, 63, 64], although there are also studies showing its increased activity in cord blood and placenta of diabetic women (Tab. 2) [9, 53]. GPX activity has been reported to be decreased in cord blood [53] and unaltered in placenta of GDM women [9, 53]. It is important to note that the most significant differences in GPX activity have been seen in maternal blood. Discrepancies among studies may arise from the variety of biological material analysed (*i.e.* whole blood, plasma, or erythrocytes), the different diagnostic GDM criteria and/or sample size used.

Human studies on the importance of non-enzymatic antioxidants, *i.e.* tocopherol (vitamin E), ascorbate (vitamin C), and carotenoids (vitamin A), in GDM are both contradictory and confusing [44, 50, 54, 55, 63–69]. The vitamin E concentration has been found to be increased or decreased or unchanged by diabetes (Tab. 2). These inconsistencies may be partially explained by different diagnostic GDM criteria and small sample sizes. In the case of vitamin C, the majority of research has demonstrated its diminished plasma level in GDM women, but there is one study showing its increased serum level in GDM patients (Tab. 2) [44]. The vitamin A concentration has been generally found to be unchanged in GDM patients, however, Suhail *et al.* [50] have revealed its reduced plasma concentration (Tab. 2).

## Oxidative stress and adverse outcomes in diabetic pregnancy

The increased rate of foetal malformations in diabetic pregnancy is a major clinical issue, but the precise mechanisms underlying these changes currently remain poorly understood. Oxidative stress is considered as an important pathway by which diabetes may affect the normal development of the embryos. Early embryonic development is characterized by relative immaturity of antioxidant defence mechanisms, thus, predisposing the embryo to increased risk of ROS-induced oxidative damage of proteins, DNA, and lipid. In support of the significance of ROS in the embryonic maldevelopment of diabetic pregnancy, the addition of SOD to "diabetic culture medium" containing high levels of glucose and ketone bodies has been shown to reduce hyperglycaemia-induced development anomalies in 10.5-day-old rat embryos in culture [70]. Other antioxidant enzymes or low-molecular-weight antioxidants added to the diet or *in vitro* have been also reported to diminish embryonic dysmorphogenesis in pregnant diabetic rats [71–73].

Among diabetic pregnancy-induced malformations, defects in neural tube and heart are the most commonly occurring [12, 74]. Studies on the role of genetic factors involved in the development of neural tube defects (NTDs) in a mouse model of diabetic em-

**Table 2. Antioxidant enzyme activities and vitamin concentrations in GDM**

| GDM/NGT | GDM criteria | Sample type | CAT | SOD | GPX | vit C | vit A | vit E | Ref |
|---|---|---|---|---|---|---|---|---|---|
| **Mother** | | | | | | | | | |
| 16/27 | Carpenter | Erythrocytes | | ⇔ | ↓ | | ⇔ | ⇔ | [54] |
| 20/20 | O'Sullivan | Erythrocytes | ⇔ | ↓ | | | | | [56] |
| 59/60 | WHO | Erythrocytes/serum | | ↓ | | ↑ | ⇔ | ↓ | [44] |
| 18/18 | ACOG | Erythrocytes/plasma | | ↓ | | ⇔ | | ⇔ | [64] |
| 23/23 | ADA | Plasma | | | | ↓ | ↓ | ↓ | [50] |
| 13/13 | ADA | Plasma | ↓ | ⇔ | ⇔ | | | | [53] |
| 53/25 | NDDG | Plasma | ↓ | ↓ | ↓ | | | | [59] |
| 25/25 | WHO | Plasma | | | | ↓ | | ↓ | [65] |
| 20/20 | O'Sullivan | Plasma | | | | ↓ | | ↓ | [66] |
| 27/40 | NDDG | Plasma | | | | | | ↑ | [67] |
| 35/45 | ADA | Plasma | | | | | | ⇔ | [69] |
| 20/20 | O'Sullivan | Plasma/serum | | ↓ | | | | ⇔ | [63] |
| 30/30 | ACOG | Serum | | | | | | ↑ | [55] |
| 10/12 | Carpenter | Serum | | | | | ⇔ | | [68] |
| **Cord & placenta** | | | | | | | | | |
| 24/25 | ADIPS | Placenta | | ↑ | ⇔ | | | | [9] |
| 13/13 | ADA | Plasma | ↓ | ↑ | ↓ | | | | [53] |
| | | Placenta | ↓ | ⇔ | ⇔ | | | | |
| 19/13 | WHO | Plasma | | ↓ | | | | | [57] |
| | | Placenta | | ↓ | | | | | |
| | | Placenta | | ↓ | | | | | |
| 27/40 | NDDG | Plasma | | | | | | ↓ | [67] |

↑ increased; ↓ decreased; ⇔ unchanged; ACOG — American College of Obstetricians and Gynaecologist; ADA — The American Diabetes Association; ADIPS — The Australian Diabetes in Pregnancy Society; Carpenter — Carpenter and Coustan criteria; NDDG — The National Diabetes Data Group; NGT — normal glucose tolerant women; O'Sullivan — O'Sullivan and Mahan criteria; WHO — World Health Organization

bryopathy have revealed that NTDs are associated with deficient expression of *Pax-3*, the gene encoding a transcription factor that is required for neural tube closure and that is expressed in neuroepithelium, neural crest, and somitic mesoderm [75]. Decreased *Pax-3* expression appears to be involved in enhanced apoptosis, the central mechanism in the induction of diabetic embryopathy, since apoptotic cells have been found at sites of neural tube defects in embryos carrying null mutation of the *Pax-3* gene [75]. These findings indicate that the *Pax-3* gene contributes to NTDs. Further studies in this field have disclosed that maternal hyperglycaemia increases the hypoxic state of the embryo, leading to inhibition of *Pax-3* expression and increase of NTDs and this process is associated with increased oxidative stress [76]. Hence, diabetes-induced oxidative stress may also change embryonic gene expression, contributing to the embryonic damage.

Lately, new data have emerged connecting hyperglycaemia-induced oxidative stress to inducible NOS (iNOS) up-regulation and nitrosative stress in diabetic NTDs. In this regard, it has been presented that suppression of oxidative stress with the use of both *in vitro* recombinant human soluble SOD (SOD1) treatment and *in vivo* SOD1 overexpression inhibited hyperglycaemia-increased iNOS expression and consequent nitrosative stress along with apoptosis in diabetic embryopathy [77].

Congenital heart anomalies, including endocardial cushion (EC) defects, persistent truncus arteriosus (PTA) and ventricular septal defects (VSD), are one of the most common malformations in offspring of diabetic mothers, but their molecular basis remains obscure [74]. Recently, maternal diabetes-induced congenital heart defects have been demonstrated to associate with the ultrastructural changes, *i.e.* the presence of swollen mitochondria and disorganized myofilaments with poorly developed adherence junctions, in cardiomyocytes of the ventricular wall as well as with the down-regulation of several genes, *i.e. Bmp4*, *Msx1*, and *Pax3*, involved in development of cardiac neural crest [78]. The role of oxidative stress in the cardiac defects in embryos of diabetic pregnancy has been proposed by Wang et al. [79] who have found that SOD1 overexpression in transgenic mice attenuates oxidative stress and

reverses maternal hyperglycaemia-impaired signalling of transforming growth factor-$\beta$ (TGF-$\beta$), which is essential for cardiogenesis.

## Conclusions

GDM is a problem of great clinical importance underlying the development of numerous adverse maternal and perinatal outcomes. It is now generally accepted that an increased oxidative stress contributes to the pathogenesis of GDM and hyperglycaemia is a crucial factor that triggers this process through several mechanisms such as the polyol pathway, the hexosamine pathway, PKC activation, AGEs formation, and the mitochondrial electron transport chain. Although alterations in biomarkers of oxidative stress have been found in GDM women, more experimental evidence is required to help resolve some controversies regarding levels of both antioxidant enzymes and non-enzymatic antioxidants in maternal and cord plasma, as well as in placenta. Moreover, there is still an urgent need to determine the precise molecular mechanisms underlying the relationship between oxidative stress and major congenital malformations such as defects in neural tube and heart in diabetic pregnancy.

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## Conflict of interest

The authors report no competing interests.

## REFERENCES

1. Coustan DR, Lowe LP, Metzger BE. The hyperglycemia and adverse pregnancy outcome (HAPO) study: can we use the results as a basis for change? J Matern Fetal Neonatal Med 2010; 23: 204–209.
2. Murphy N. First evidence-based criteria for diagnosis of gestational diabetes. The IHS Provider 2010; 35: 215–219.
3. Forsbach-Sánchez G, Tamez-Peréz HE, Vazquez-Lara J. Diabetes and pregnancy. Arch Med Res 2005; 36: 291–299.
4. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care 2007; 30: S112–S119.
5. Colomiere M, Permezel M, Lappas M. Diabetes and obesity during pregnancy alter insulin signalling and glucose transporter expression in maternal skeletal muscle and subcutaneous adipose tissue. J Mol Endocrinol 2010; 44: 213—223.
6. Wojcik M, Chmielewska-Kassassir M, Grzywnowicz K, Wozniak L, Cypryk K. The relationship between adipose tissue-derived hormones and gestational diabetes mellitus (GDM). Endokrynol Pol 2014; 65: 134–142.
7. Kuzmicki M, Telejko B, Zonenberg A et al. Circulating pro- and anti-inflammatory cytokines in Polish women with gestational diabetes. Horm Metab Res 2008; 40: 556–560.
8. Kuzmicki M, Telejko B, Szamatowicz J et al. High resistin and interleukin-6 levels are associated with gestational diabetes mellitus. Gynecol Endocrinol 2009; 25: 258–263.
9. Coughlan MT, Vervaart PP, Permezel M, Georgiou HM, Rice GE. Altered placental oxidative stress status in gestational diabetes mellitus. Placenta 2004; 25: 78–84.
10. Lappas M, Hiden U, Desoye G, Froehlich J, Hauguel-de Mouzon S, Jawerbaum A. The role of oxidative stress in the pathophysiology of gestational diabetes mellitus. Antioxid Redox Signal 2011; 15: 3061–3100.
11. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44–84.
12. Gabbay-Benziv R, Reece EA, Wang F, Yang P. Birth defects in pregestational diabetes: Defect range, glycemic threshold and pathogenesis. World J Diabetes 2015; 6: 481–488.
13. Zhang DX, Gutterman DD. Mitochondrial reactive oxygen species--mediated signaling in endothelial cells. Am J Physiol Heart Circ Physiol 2007; 292: H2023–H2031.
14. Goldberg HJ, Whiteside CI, Fantus IG. The hexosamine pathway regulates the plasminogen activator inhibitor-1 gene promoter and Sp1 transcriptional activation through protein kinase C-beta I and -delta. J Biol Chem 2002; 277: 33833–33841.
15. Patti ME, Virkamäki A, Landaker EJ, Kahn CR, Yki-Järvinen H. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. Diabetes 1999; 48: 1562–1571.
16. Hebert LF Jr, Daniels MC, Zhou J et al. Overexpression of glutamine:fructose-6-phosphate amidotransferase in transgenic mice leads to insulin resistance. J Clin Invest 1996; 98: 930–936.
17. Veerababu G, Tang J, Hoffman RT et al. Overexpression of glutamine: fructose-6-phosphate amidotransferase in the liver of transgenic mice results in enhanced glycogen storage, hyperlipidemia, obesity, and impaired glucose tolerance. Diabetes 2000; 49: 2070–2078.
18. Kaneto H, Xu G, Song KH et al. Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell function through the induction of oxidative stress. J Biol Chem 2001; 276: 31099–31104.
19. Barnett PA, González RG, Chylack LT Jr, Cheng HM. The effect of oxidation on sorbitol pathway kinetics. Diabetes 1986; 35: 426–432.
20. Tang WH, Martin KA, Hwa J. Aldose reductase, oxidative stress, and diabetic mellitus. Front Pharmacol 2012; 3: 87.
21. Sussman I, Matschinsky FM. Diabetes affects sorbitol and myo--inositol levels of neuroectodermal tissue during embryogenesis in rat. Diabetes 1988; 37: 974–981.
22. Simán CM, Naeser P, Eriksson UJ. Increased lenticular aldose reductase activity and high incidence of congenital cataract in the offspring of diabetic rats. Acta Ophthalmol (Copenh) 1993; 71: 629–636.
23. Vikramadithyan RK, Hu Y, Noh HL et al. Human aldose reductase expression accelerates diabetic atherosclerosis in transgenic mice. J Clin Invest 2005; 115: 2434–2443.
24. Vedantham S, Noh H, Ananthakrishnan R et al. Human aldose reductase expression accelerates atherosclerosis in diabetic apolipoprotein E-/- mice. Arterioscler Thromb Vasc Biol 2011; 31: 1805–1813.
25. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414: 813–820.
26. Lappas M, Permezel M, Ho PW, Moseley JM, Wlodek ME, Rice GE. Effect of nuclear factor-kappa B inhibitors and peroxisome proliferator-activated receptor-gamma ligands on PTHrP release from human fetal membranes. Placenta 2004; 25: 699–704.
27. Kuboki K, Jiang ZY, Takahara N et al. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. Circulation 2000; 101: 676–681.
28. Chen F, Yu Y, Haigh S et al. Regulation of NADPH oxidase 5 by protein kinase C isoforms. PLoS One 2014; 9: e88405.
29. Martini SR, Kent TA. Hyperglycemia in acute ischemic stroke: a vascular perspective. J Cereb Blood Flow Metab 2007; 27: 435–451.

30. Basta G, Schmidt AM, De Caterina R. Advanced glycation end-products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004; 63: 582–592.
31. Grossin N, Wautier MP, Wautier JL. Red blood cell adhesion in diabetes mellitus is mediated by advanced glycation endproduct receptor and is modulated by nitric oxide. Biorheology 2009; 46: 63–72.
32. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol Endocrinol Metab 2001; 280: E685–E694.
33. Ramasamy R, Vannucci SJ, Yan SSD, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology 2005; 15: 16R–28R.
34. Lappas M, Permezel M, Rice GE. Advanced glycation end-products mediate pro-inflammatory actions in human gestational tissues via nuclear factor-kappa B and extracellular signal-regulated kinase1/2. J Endocrinol 2007; 193: 269–277.
35. Tsukahara H, Ohta N, Sato S et al. Concentrations of pentosidine, an advanced glycation end-product, in umbilical cord blood. Free Radic Res 2004; 38: 691–695.
36. Buongioruo AM, Moreui S, Sagratella E, Castaldo P, Di Virgilio A, Maroccia E. Levels of advanced glycosylation end-products (AGE) in sera of pregnant diabetic women: comparison between type1, type2 and gestational diabetes mellitus. Ann 1st Super Sanita 1997; 33: 375–378.
37. Guosheng L1, Hongmei S, Chuan N, Haiying L, Xiaopeng Z, Xianqiong L. The relationship of serum AGE levels in diabetic mothers with adverse fetal outcome. J Perinatol 2009; 29: 483–488.
38. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41: 1819–1828.
39. de Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radic Biol Med 1999; 26: 202–226.
40. Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev 2014; 2014: 360438.
41. Chen X, Scholl TO. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. Curr Diab Rep 2005; 5: 282–288.
42. Höhn A, König J, Grune T. Protein oxidation in aging and the removal of oxidized proteins. J Proteomics 2013; 92: 132–159.
43. Cai Z, Yan LJ. Protein Oxidative Modifications: Beneficial Roles in Disease and Health. J Biochem Pharmacol Res 2013; 1: 15–26.
44. Grissa O, Ategbo JM, Yessoufou A et al. Antioxidant status and circulating lipids are altered in human gestational diabetes and macrosomia. Transl Res 2007; 150: 164–171.
45. Rajdl D, Racek J, Steinerová A et al. Markers of oxidative stress in diabetic mothers and their infants during delivery. Physiol Res 2005; 54: 429–436.
46. Mazzanti L, Nanetti L, Vignini A et al. Gestational diabetes affects platelet behaviour through modified oxidative radical metabolism. Diabet Med 2004; 21: 68–72.
47. Orhan H, Onderoglu L, Yücel A, Sahin G. Circulating biomarkers of oxidative stress in complicated pregnancies. Arch Gynecol Obstet 2003; 267: 189–195.
48. Lewandowski KC, Stojanovic N, Press M, Tuck S, Lewiński A, Karbownik-Lewińska M. Raised concentrations of lipid peroxidation products (LPO) in pregnant women with impaired glucose tolerance. Ann Agric Environ Med 2014; 21: 429–434.
49. Xin G, DU J, Wang YT, Liang TT. Effect of oxidative stress on heme oxygenase-1 expression in patients with gestational diabetes mellitus. Exp Ther Med 2014; 7: 478–482.
50. Suhail M, Patil S, Khan S, Siddiqui S. Antioxidant Vitamins and Lipoperoxidation in Non-pregnant, Pregnant, and Gestational Diabetic Women: Erythrocytes Osmotic Fragility Profiles. J Clin Med Res 2010; 2: 266–273.
51. Karacay O, Sepici-Dincel A, Karcaaltincaba D et al. A quantitative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24-36 weeks of gestation. Diabetes Res Clin Pract 2010; 89: 231–238.
52. Madazli R, Tuten A, Calay Z, Uzun H, Uludag S, Ocak V. The incidence of placental abnormalities, maternal and cord plasma malondialdehyde and vascular endothelial growth factor levels in women with gestational diabetes mellitus and nondiabetic controls. Gynecol Obstet Invest 2008, 65: 227–232.
53. Biri A, Onana A, Devrimb E, Babacanc F, Kavutcua M, Durakb I. Oxidant Status in Maternal and Cord Plasma and Placentral Tissue in Gestational Diabetes. Placenta 2006, 27: 327–332.
54. Peuchant E, Brun JL, Rigalleau V, et al. Oxidative and antioxidative status in pregnant women with either gestational or type 1 diabetes. Clin Biochem 2004, 37: 293–298.
55. Santra D, Sawhney H, Aggarwal N, Majumdar S, Vasishta K. Lipid peroxidation and vitamin E status in gestational diabetes mellitus. J Obstet Gynaecol Res 2003; 29: 300–304.
56. Chaudhari L, Tandon OP, Vaney N, Agrawal N. Lipid peroxidation and antioxidant enzymes in gestational diabetes. Indian J Physiol Pharmacol 2003; 47: 441–446.
57. Kinalski M, Sledziewski A, Telejko B et al. Lipid peroxidation, antioxidant defence and acid-base status in cord blood at birth: the influence of diabetes. Horm Metab Res 2001; 33: 227–231.
58. Georgiou HM, Lappas M, Georgiou GM et al. Screening for biomarkers predictive of gestational diabetes mellitus. Acta Diabetol 2008; 45: 157–165.
59. López-Tinoco C, Roca M, García-Valero A et al. Oxidative stress and antioxidant status in patients with late-onset gestational diabetes mellitus. Acta Diabetol 2013; 50: 201–208.
60. Vural M, Camuzcuoglu H, Toy H et al. Evaluation of the future atherosclerotic heart disease with oxidative stress and carotid artery intima media thickness in gestational diabetes mellitus. Endocr Res 2012; 37: 145–153.
61. Camuzcuoglu H, Toy H, Cakir H, Celik H, Erel O. Decreased paraoxonase and arylesterase activities in the pathogenesis of future atherosclerotic heart disease in women with gestational diabetes mellitus. J Womens Health 2009; 18: 1435–1439.
62. Gelisgen R, Genc H, Kayali R et al. Protein oxidation markers in women with and without gestational diabetes mellitus: a possible relation with paraoxonase activity. Diabetes Res Clin Pract 2011; 94: 404–409.
63. Kharb S. Activity of extracellular superoxide dismutase in gestational diabetes. Res J Obstet Gynecol 2010; 3: 1–4.
64. Dey P, Gupta P, Acharya NK et al. Antioxidants and lipid peroxidation in gestational diabetes--a preliminary study. Indian J Physiol Pharmacol 2008; 52: 149–156.
65. Kharb S. Total radical-trapping antioxidant potential in gestational diabetes. Int J Gynaecol Obstet 2008; 103: 257–258.
66. Surapaneni KM. Oxidant-antioxidant status in gestational diabetes. J Clin Diagn Res 2007; 4: 235–238.
67. Sobki SH, Al-Senaidy AM, Al-Shammari TA, Inam SS, Al-Gwiser AA, Bukhari SA. Impact of gestational diabetes on lipid profiling and indices of oxidative stress in maternal and cord plasma. Saudi Med J 2004; 25: 876–880.
68. Tepper BJ, Kim YK, Shete V, Shabrova E, Quadro L. Serum retinol-binding protein 4 (RBP4) and retinol in a cohort of borderline obese women with and without gestational diabetes. Clin Biochem 2010; 43: 320–323.
69. Sánchez-Vera I, Bonet B, Viana M et al. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. Metabolism 2007; 56: 1527–1533.
70. Eriksson UJ, Borg LAH. Diabetes and embryonic malformations. Role of substrate-induced free oxygen radical production for dysmorphogenesis in cultured rat embryos. Diabetes 1993; 42: 411–419.
71. Cederberg J, Eriksson UJ. Antioxidative treatment of pregnant diabetic rats diminishes embryonic dysmorphogenesis. Birth Defects Res A Clin Mol Teratol 2005; 73: 498–505.

72. Sivan CM, Eriksson UJ. Vitamin C supplementation of the maternal diet reduces the rate of malformation in the offspring of diabetic rats. Diabetologia 1997; 40: 1416–1424.
73. 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.
74. Wender-Ozegowska E, Wroblewska K, Zawiejska A, Pietryga M, Szczapa J, Biczysko R. Threshold values of maternal blood glucose in early diabetic pregnancy — prediction of fetal malformations. Acta Obstet Gynecol Scand 2005; 84: 17–25.
75. 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: 1189–1197.
76. Li R, Chase M, Jung SK, Smith PJS, Locken MR. Hypoxic stress in diabetic pregnancy contributes to impaired embryo gene expression and defective development by inducing oxidative stress. Am J Physiol Endocrinol Metab 2005; 289: E591–E599.
77. Weng H, Li X, Reece EA, Yang P. SOD1 suppresses maternal hyperglycemia-increased iNOS expression and consequent nitrosative stress in diabetic embryopathy. Am J Obstet Gynecol 2012; 206: 448.e1–448.e7.
78. Kumar SD, Dheen ST, Tay SS. Maternal diabetes induces congenital heart defects in mice by altering the expression of genes involved in cardiovascular development. Cardiovasc Diabetol 2007; 6: 34.
79. Wang F, Reece EA, Yang P. Oxidative stress is responsible for maternal diabetes-impaired transforming growth factor beta signaling in the developing mouse heart. Am J Obstet Gynecol 2015; 212: 650.e1–650.e11.