Dexamethasone causes defective glucose-6-phosphate dehydrogenase-dependent antioxidant barrier through endoglin in pregnant and non-pregnant rats

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Canadian Journal of Physiology and Pharmacology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>cjpp-2018-0351.R7</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>03-Dec-2019</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Badmus, Olufunto; University of Ilorin, 1HOPE Cardiometabolic Research Team and Department of Physiology, College of Health Sciences; Kwara State University, Public Health Olatunji, Lawrence; University of Ilorin, 1HOPE Cardiometabolic Research Team and Department of Physiology, College of Health Sciences</td>
</tr>
<tr>
<td>Is the invited manuscript for consideration in a Special Issue:</td>
<td>Not applicable (regular submission)</td>
</tr>
<tr>
<td>Keyword:</td>
<td>ADA, endoglin, G6PD, reactive oxygen species, VCAM-1</td>
</tr>
</tbody>
</table>

https://mc06.manuscriptcentral.com/cjpp-pubs
Dexamethasone causes defective glucose-6-phosphate dehydrogenase-dependent antioxidant barrier through endoglin in pregnant and non-pregnant rats

Olufunto O. Badmus¹,², Lawrence A. Olatunji¹*

¹HOPE Cardiometabolic Research Team and Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

²Department of Public Health, Kwara State University, Malete, Nigeria

Running Title: DEX causes increased endoglin and decreased G6PD

*Address correspondence to:
Lawrence A. Olatunji Ph.D.
Department of Physiology,
University of Ilorin,
P.M.B. 1515 Ilorin, 240001, Nigeria.
Tel: +2348035755360
E-mail: tunjilaw@unilorin.edu.ng
Abstract

Glucocorticoid (GC) therapy has been associated with adverse cardiometabolic effects during pregnancy. Inflammation-mediated cardiac dysfunction, an independent risk factor for morbidity and mortality, has been linked to defective glucose-6-phosphate dehydrogenase (G6PD)-dependent antioxidant defenses and increased endoglin expression. We therefore sought to investigate the effects of dexamethasone (DEX) on cardiac endoglin and G6PD-dependent antioxidant defense. Twenty-four rats were randomly assigned to non-pregnant [PRE(-)], DEX-exposed non-pregnant [PRE(-) +DEX], pregnant [PRE(+)] and DEX-exposed pregnant [PRE(+) +DEX] rats, respectively (n = 6/group). PRE(-) and PRE(+) rats received vehicle (po) while PRE(-) +DEX and PRE(+) +DEX groups were administered dexamethasone (0.2 mg/kg; po) between gestational days 14 to 19 respectively. Results showed that DEX caused increased cardiac pro-inflammatory markers (adenosine deaminase (ADA) activity, endoglin, vascular cell adhesion molecule-1 (VCAM-1), tissue injury markers (LDH, GGT, AST, ALT and ALP), metabolic disturbances (elevated fasting plasma glucose, free fatty acid (FFA), lactate, cardiac FFA and lactate) and depressed G6PD-dependent antioxidant defenses (G6PD activity, reduced glutathione/oxidized glutathione ratio and nitric oxide) in pregnant and non-pregnant rats. The present study demonstrates that DEX led to increased cardiac endoglin and VCAM-1 that is accompanied by defective G6PD-dependent antioxidant defenses but not cardiac lipid accumulation in both pregnant and non-pregnant rats.

Key words: reactive oxygen species, ADA, endoglin, G6PD, VCAM-1
Introduction

Maternal physiology is extremely influenced by placental hormones particularly in last trimester of the pregnancy. The variations in hormonal levels usually alter glucose and lipid metabolism and such alterations occur so as to ensure that the foetus receives sufficient supply of nutrients (Homko et al. 1999; Butte 2000). Due to heightened metabolic requirements during pregnancy, the maternal body responds by switching over to fat utilization from that of carbohydrate. Increase in insulin resistance and plasma lipolytic hormonal concentration also facilitates this change (Homko et al. 1999; Butte 2000). Serum total cholesterol and triglyceride concentrations increase markedly during pregnancy (Wiznitzer et al. 2009). Pregnancy-induced elevated triglyceride concentration is due to two factors; increased hepatic lipase activity and reduced lipoprotein lipase activity, leading to enhanced hepatic triglyceride (TG) synthesis and decreased catabolism of adipose tissue respectively (Kershaw and Flier 2004). Elevated triglyceride levels are used to meet maternal metabolic needs while sparing glucose for the foetus.

Normal pregnancy is considered a moderate oxidative stress condition, characterized by an increase in reactive oxygen species (ROS) production, which is partly counterbalanced by an increase in antioxidant mechanisms (Burton and Hung 2003). Oxidative stress is needed during pregnancy for normal cell function, including activation of redox-sensitive transcription factors and activation of protein kinases (Wu et al. 2016; Sultana et al. 2017). However, persistent continual increased oxidative stress will result in diverse disease states such as preeclampsia (Turpin et al. 2015; Ferguson et al. 2017).

Enhanced ROS production is strongly associated with cell injury. It may contribute to the initiation and progression of myocardial dysfunction and cardiovascular diseases (CVD) (Sawyer
et al. 2002). Glucose-6-phosphate dehydrogenase (G6PD), a key enzyme in the pentose phosphate pathway, generates nicotinamide adenine dinucleotide phosphate (NADPH) which is required for the regeneration of reduced glutathione (GSH) from its oxidized form (GSSG) (Gupte et al. 2003; Hecker et al. 2013). GSH produced via the glutathione recycling pathway protects the cell by destroying the deleterious ROS. G6PD deficiency has been documented to be the most common enzyme deficiency in the world (Cappellini et al. 2008) and may increase the incidence of CVD by promoting ROS formation. Studies suggest that G6PD is a paradoxical enzyme as too little or too much can severely affect intracellular redox balance, leading to cardiac dysfunction (Jain et al. 2003; Gupte et al. 2007; Assad et al. 2011).

Inflammation is considered an important etiological factor for the development and progression of cardiac dysfunction (Kudo et al. 2009; Maulik et al. 2012). During inflammation, endoglin (CD105) expression is strongly upregulated with concomitant infiltration of inflammatory cells (Torsney et al. 2002). Endoglin has the highest expression on the vascular endothelial cells during pathological conditions, including preeclampsia (Powe et al. 2011). Increased endoglin expression has been suspected to play a critical pathophysiological role in inflammation-mediated cardiovascular events (Lopez-Novoa and Bernabeu, 2010). Endoglin appears to serve as a potentially important diagnostic marker of heart failure (Kapur et al. 2010; Yanavitski and Givertz, 2011). Moreso, enhanced ROS which is associated with oxidative tissue injury seems to be an important factor for enhanced endoglin expression (Valbuena-Diez et al. 2012) and studies have demonstrated that there is a strong correlation between cardiac dysfunction and oxidative stress (Maulik et al. 2012).
Glucocorticoids (GCs) are stress hormones that are synthesized and released by the adrenal cortex in response to activation of the hypothalamic-pituitary-adrenal axis. It acts on almost all cells by regulating several biological processes of the body such as reproduction, growth, metabolic and defense responses (Sapolsky et al. 2000). Due to their well-established and effective anti-inflammatory and immunosuppressive actions, GCs are widely used therapeutically to treat inflammation, autoimmune diseases, and hematological cancers. Nevertheless, GC can exert both positive and negative effects on the heart (Oakley and Cidlowski, 2015). Critically important is the revelation that therapeutic administration of GCs results in adverse effects such as glucose dysmetabolism, cardiac hypertrophy, hypertension, atherosclerosis and osteoporosis (Zecca et al. 2001; de Vries et al. 2002; Schäcke et al. 2002; Ferris and Kahn, 2012). GC also enhances ROS accumulation through decreased glutathione-dependent antioxidant defense mechanism (McIntosh et al. 1998; Patel et al. 2002). In pregnancy, circulating GC rises during late gestation to ensure the development and maturation of fetal organs most importantly the lungs and cardiomyocytes (Kamath-Rayne et al. 2002; Rog-Zielinska et al. 2015). Thus, it is a common practice to administer synthetic GC such as dexamethasone (DEX) to pregnant women in whom preterm delivery is anticipated to reduce the incidence of respiratory distress syndrome in the new-born and prevent neonatal death (Kamath-Rayne et al. 2002). Also, DEX can be used by women during pregnancy to treat fetus that is at risk of congenital adrenal hyperplasia (CAH). In the case of CAH, prenatal treatment of DEX are administered through the pregnant mother in order to suppress oversecretion of fetal adrenal androgen and prevent genital malformations or virilization in the fetus (Lajic et al. 2008)
Since DEX is widely used for various therapeutic reasons, even during pregnancy, it is interesting to note that study evaluating the effects of DEX on endoglin and G6PD in the heart of women is lacking. The current study therefore aimed at investigating the effects of DEX on cardiac endoglin and G6PD-dependent antioxidant defense in pregnant and non-pregnant rats.

**Materials and methods**

**Animals**

The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Ethical Review Committee, University of Ilorin, Ilorin, Nigeria, with protocol identification number: UERC/ASN/2016/486 and every effort was made to minimize both the number of animals used and their suffering. Female Wistar rats weighing 130–150 g were obtained from the Animal House, College of Health Sciences, University of Ilorin, Ilorin, Nigeria and housed in a well-ventilated room maintained under standard environmental conditions with unrestricted access to tap water and standard rat chow.

**Grouping and Treatment**

Rats were allowed to acclimatize for one week prior to being randomized into four groups. After acclimatization, vaginal smear was done to determine the estrus phases of two groups. These two groups were mated with male Wistar rats at a ratio of three female rats to one male rat during their estrus phases to achieve timed pregnancies (Motta et al. 2018). The presence of spermatozoa and estrus phase (vaginal plug) were taken as gestational day zero (0). Twelve (12) age-matched non-pregnant rats and 12 pregnant rats were randomly assigned to non-pregnant
rats [PRE (-)], dexamethasone-exposed non-pregnant rats [PRE (-) + DEX], untreated pregnant rats [PRE (+)] and dexamethasone-exposed pregnant rats [PRE (+) + DEX] respectively, (n = 6/group). PRE (-) and PRE (+) rats received vehicle (po) daily for 6 days while PRE (-) + DEX and PRE (+) + DEX groups were administered dexamethasone (0.2 mg/kg; po) from gestational day 14 to 19 daily (Gomes et al. 2014) and treatments were discontinued at least 24 h before the end of the experiment.

Sample preparation

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood was collected by cardiac puncture into heparinized bottle and centrifuged at 3000 rpm for 5 minutes. Plasma was stored frozen until needed for biochemical assay. Hearts were excised, blotted and weighed immediately. After weighing, 100 mg of tissue was carefully sectioned and homogenized with a glass homogenizer. The homogenate was used for subsequent biochemical analysis.

Biochemical assay

Plasma and cardiac endoglin, vascular cell adhesion molecule-1 (VCAM-1) were determined by using ELISA kit from Elabscience (Wuhan, China). Fasting plasma and cardiac TG, high-density lipoprotein cholesterol (HDL-C) were measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK) following manufacturer’s instruction. Plasma and cardiac levels of G6PD, oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione peroxidase (GPx), adenosine, adenosine deaminase (ADA) activity, free fatty acid (FFA), lactate, lactate dehydrogenase (LDH), gamma-glutamyl...
transferase (GGT), alanine transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured by standardized enzymatic colorimetric method using assay kit obtained from Fortress diagnostics (Antrim, UK) following manufacturer’s instruction, whereas plasma and cardiac nitric oxide (NO), malondialdehyde (MDA) were measured by non-enzymatic colorimetric assay kit obtained from Oxford Biomedical Research, Inc. (Oxford, MI) following manufacturer’s instruction. TG/HDL-C ratios were estimated as marker of atherogenic lipid indices while reduced to oxidized glutathione ratio (GSH/GSSG) was used as an indicator of oxidative stress. Low density lipoprotein cholesterol (LDL-C) was calculated by Ananandaraja formula (Ananandaraja et al. 2005).

**Histological studies**

Cardiac tissues were fixed in 4% formalin overnight, dehydrated, and embedded in paraffin. The paraffin-embedded samples were sectioned at 3-μm thickness and stained with hematoxylin and eosin (H&E) stains. The slides were examined using light microscopy.

**Statistical Analysis**

Statistical analysis was performed using SPSS software (Version 22; SPSS Inc. IL., USA) and values were expressed as mean ± SD of 6 rats per group. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni’s *post hoc* test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at *p*<0.05.
Results

Effect of dexamethasone on heart, kidney, liver, and uterine weight in pregnant and non-pregnant rats

Heart weight was not comparable among the experimental groups. DEX treatment in non-pregnant did not affect the kidney weight when compared with untreated non pregnant rats. Kidney weight was however reduced in PRE (+) rats when compared with PRE (-) + DEX rats (Table 1). DEX-treated pregnant rats had increased kidney weight when compared with PRE rats. Liver weight was increased in PRE (+) + DEX rats compared with PRE (-) rats. However, liver weight was reduced in PRE (+) rats when compared with PRE (-) and PRE (-) + DEX respectively. PRE (+) + DEX rats on the other hand had increased liver weight when compared with PRE (+) rats (Table 1). Uterine weight was reduced in PRE (-) + DEX rats compared with PRE (-) rats. PRE (+) and PRE (+) + DEX rats had increased uterine weights when compared with PRE (-) and PRE (-) + DEX rats respectively. However, PRE (+) + DEX rats had reduced uterine weight when compared with PRE (+) rats (Table 1).

Effect of dexamethasone on fasting glycemia, lipid metabolism in pregnant and non-pregnant rats

Table 2 depicts the effect of DEX treatment on lipid profiles and fasting glycemia in pregnant and non-pregnant rats. There was increased fasting glycemia across the experimental groups when compared with PRE (-) rats. Also, PRE (-) + DEX and PRE (+) + DEX rats had increased plasma TG levels when compared with PRE (-) but PRE (+) rats had decreased plasma TG level when compared with PRE (-) + DEX rats. Cardiac TG levels showed no changes among the experimental groups. The plasma and cardiac levels of HDL-C were similar across the groups. Plasma LDL-C level was increased in PRE (-) + DEX and PRE (+) + DEX rats when compared
with PRE (-) rats. Cardiac LDL-C was increased across the experimental groups when compared with PRE (-) rats (Table 2). Atherogenic lipid indices; plasma TG/HDL-C ratios were also increased across the experimental groups when compared with PRE (-) (Fig. 1A). However, cardiac TG/HDL-C ratios were similar across the groups (Fig. 1B).

**Effect of dexamethasone on plasma and cardiac G6PD-dependent anti-oxidant capacity in pregnant and non-pregnant rats**

Plasma and cardiac G6PD were reduced in PRE (-) + DEX and PRE (+) + DEX rats when compared with PRE (-) and PRE (+) rats. Plasma G6PD was increased in PRE (+) rats whereas cardiac G6PD was not affected when compared with PRE (-) rats. In addition, plasma and cardiac G6PD were increased in PRE (+) rats when compared with PRE (-) + DEX rats (Figs 2A & B). DEX treatment in non-pregnant and pregnant rats led to increased plasma and cardiac oxidized glutathione (GSSG) levels as well as decreased plasma and cardiac reduced glutathione (GSH) level when compared with the PRE (-) and PRE (+) rats respectively (Table 3). Plasma and cardiac glutathione peroxidase (GPx) (Figs. 2C & D) as well as reduced/oxidized glutathione (GSH/GSSG) ratio (Figs. 2E & F) were decreased in DEX-treated pregnant and non-pregnant rats when compared with PRE (+) and PRE (-) respectively.

**Effect of dexamethasone on plasma and cardiac lipid peroxidation and nitric oxide in pregnant and non-pregnant rats**

Plasma and cardiac MDA, a marker of lipid peroxidation was increased in PRE (-) + DEX and PRE (+) + DEX rats when compared with PRE (-) and PRE (+) rats respectively. Plasma MDA level was increased in PRE (+) rats whereas cardiac MDA level in PRE (+) rats was not affected by the treatment when compared with PRE (-) rats (Figs. 3A & B). In addition, plasma and
cardiac NO were decreased in PRE (-) + DEX and PRE (+) + DEX rats when compared with PRE (-) and PRE (+) (Figs. 3C & D).

**Effect of dexamethasone on plasma and cardiac pro-inflammatory markers and cardiac histology in pregnant and non-pregnant rats**

Plasma and cardiac adenosine level was decreased in PRE (+) + DEX rats when compared with PRE (-) and PRE (+) rats respectively. Similarly, cardiac adenosine level was decreased in PRE (-) + DEX rats when compared with PRE (-) rats whereas cardiac adenosine level was increased in PRE (+) rats when compared with PRE (-) + DEX rats (Fig. 4A & B). Plasma and cardiac adenosine deaminase (ADA) activity (Fig. 4C & D) increased in PRE (-) + DEX and PRE (+) + DEX rats when compared with PRE (-) and PRE (+) rats. However, plasma and cardiac ADA activity was not affected in PRE (+) rats when compared with PRE (-). Also, plasma and cardiac endoglin (Fig. 5A & B) and VCAM-1 levels (Fig. 5C & D) were increased in PRE (-) + DEX and PRE (+) + DEX rats when compared with PRE (-) and PRE (+) rats. Plasma and cardiac endoglin and VCAM-1 were not affected in PRE (+) rats when compared with PRE (-) rats (Fig. 5A-D). Cardiomyocytes appear normal in PRE (-) and PRE (+) rats whereas PRE (-) + DEX rats had mild cardiac tissue disruption while PRE (+) + DEX rats had moderate cardiac tissue disruption with cellular infiltration (Fig. 6).

**Effect of dexamethasone on free fatty acid and tissue injury markers (lactate and lactate dehydrogenase) in pregnant and non-pregnant rats**

PRE (-) + DEX and PRE (+) + DEX rats had elevated plasma and cardiac free fatty acid level when compared with PRE (-) and PRE (+) rats (Fig. 7A & B). Also, PRE (-) + DEX and PRE (+)
+ DEX rats had increased plasma and cardiac lactate and LDH when compared to PRE (-) and PRE (+) rats (Fig. 7C -F).

**Effect of dexamethasone on liver function markers in pregnant and non-pregnant rats**

PRE (-) + DEX and PRE (+) + DEX rats had increased plasma AST, ALP, ALT and cardiac AST, ALT, GGT when compared with PRE (-) and PRE (+) rats. In addition, Plasma GGT and cardiac ALP were increased in PRE (+) + DEX rats when compared with PRE (-) and PRE (+) rats respectively. However, plasma and cardiac AST, ALP, ALT and GGT were not altered in PRE (+) rats when compared with PRE (-) rats (Fig 8A-H).

**Discussion**

The results from the present study provide evidence that DEX caused increased cardiac endoglin, VCAM-1, ADA and defective G6PD-dependent antioxidant defenses in both pregnant and non-pregnant rats. These effects induced by DEX were also associated with metabolic disturbances (elevated fasting blood glucose, TG and FFA), oxidative stress (MDA, NO and GSH/GSSG ratio), elevated injury makers (LDH, AST, ALT, ALP and GGT) but not with cardiac dyslipidemia in both pregnant and non-pregnant rats.

In this study, the metabolic disturbances observed after DEX treatment in both groups are common features of chronic GC treatment and Cushing syndrome (Macfarlane et al. 2008).

Dyslipidemia characterized by high levels of TG, low-density lipoprotein (LDL-C), free fatty acid and reduced levels of HDL-C enhances the chance of cardiac dysfunction and coronary heart diseases (Carey et al. 2010). Furthermore, TG/HDL-C ratio is recognized as an effective predictor of atherosclerotic CVD (Musso et al. 2011). In this study, DEX treatment resulted in
increased circulating TG but no significant changes in cardiac TG and TG/HDL-C in both pregnant and non-pregnant rats. This is in consonance with the report of previous animal studies in male rats (Dolatabadi and Mahboubi, 2015). Hence, the finding that DEX led to no changes in cardiac TG/HDL-C ratio suggests that DEX-induced metabolic disturbances are independent of cardiac lipotoxicity. Also, elevated plasma and cardiac free fatty acids shown in this study were consistent with previous study that reported that GC increases free fatty acids production in Cushing syndrome (Macfarlane et al. 2008). Free fatty acids exert several injurious effects on the myocardium, thereby contributing to heart failure and sudden cardiac death (Djoussé et al., 2013). Hyperglycemia, a critical factor for CVD was also observed in the current study. Earlier studies have shown that hyperglycemic condition is contributory to cardiac damage (Holland et al., 2007). Thus, our finding that DEX treatment led to elevated circulating and cardiac free fatty acids accompanied by hyperglycemia implies gluelipotoxicity in both pregnant and non-pregnant rats.

Endoglin plays a critical role in maintaining cardiovascular homeostasis. Hence, its upregulated expression is evident during inflammation (Rossi et al. 2013). Reports exist that endoglin level is raised during the early stages of atherosclerosis due to impaired endothelium function (Li et al. 2000). Studies have also shown that increased endoglin regulate cardiac remodeling, and promotes the development of cardiac hypertrophy, fibrosis and morbidity (Kapur et al. 2012). Endoglin is considered an important determinant of heart failure (Kapur et al., 2010; Yanavitski et al. 2011). The finding that DEX treatment resulted in elevated circulating and cardiac endoglin provides evidence for the possible role of endoglin in DEX-induced cardiac dysfunction. It is noteworthy that DEX treatment led to increased endoglin in non-pregnant and pregnant rats. It is
interesting to also note that findings from the current study showed that DEX treatment in pregnant and non-pregnant rats induced elevated plasma and cardiac VCAM-1, ADA activity and caused decreased plasma and cardiac adenosine content. Cardiac inflammation, an independent risk factor for CVD, triggers numerous conditions that damage the heart muscle and cause cardiac remodeling (Marchant et al. 2012). VCAM-1 is a cell-surface adhesion molecule induced on endothelial cells during inflammatory diseases by several signals including ROS and hyperglycemia (Cook-Mills et al. 2011). Reports exist that it is a biomarker of vascular inflammation-mediated atherosclerotic vasculopathy (Cybulsky et al. 2001).

Adenosine has been known to be a strong anti-inflammatory molecule (Bakker et al. 2001; Yang et al. 2006). Adenosine level is regulated by ADA. ADA degrades adenosine by catalysing the deamination of adenosine to inosine activity and earlier study has documented the critical role of elevated ADA activity in inflammatory processes (Röhrborn et al. 2015). Taken together, this study suggests that DEX-induced metabolic disturbances are associated with cardiac pro-inflammatory markers and endoglin could play considerable etiological role.

Oxidative stress arises from an imbalance between the production of pro-oxidant species, ROS and the body’s antioxidant defense mechanisms (Dhalla et al. 2000). This could be attributed to insufficient scavenging ability of the antioxidants or higher ROS production. Oxidative stress is an important attribute of CVD. G6PD is an enzyme that provides NADPH which further reduces GSSG to generate GSH. GSH is utilized by GPx to reduce hydrogen peroxide and lipid peroxidases to water, thus protecting the cell from oxidative injury. In cardiomyocytes, inhibition of G6PD led to increased intracellular oxidative stress, evidenced by a decrease in GSH, and the
ratio of reduced-to-oxidized glutathione (GSH/GSSG) (Jain et al. 2003). Also, reduced levels of G6PD have been linked to ROS-mediated cardiac hypertrophy (MacCarthy et al. 2001). In the current study, we observed reduced plasma and heart G6PD, GPx and GSH/GSSG ratio during DEX treatment in both pregnant and non-pregnant rats. However, these remarkable findings from the present study suggest that DEX-induced metabolic disturbances and cardiac inflammation could be through enhanced ROS promotion via the defective G6PD-antioxidant pathway. The observed DEX-induced reduced G6PD activity in the study is in agreement with a study that reported the inhibitory effect of DEX on erythrocyte G6PD activity in rats (Ozmen 2005). Therefore, defective G6PD-dependent antioxidant pathway play contributory role in cardiac inflammation induced by DEX treatment in pregnant and non-pregnant rats. The elevated fasting plasma glucose observed in this study might also be due to depressed activity of G6PD-dependent antioxidant defense, since reports have it that deficiency of G6PD causes the buildup of glucose (Heymann et al. 2012). The defective G6PD-dependent antioxidant defenses observed in the heart of rats exposed to DEX suggests that cardiac inflammation induced by DEX is at least in part, due to enhanced oxidative stress. This finding is also evident by the observation that DEX treatment caused reduced NO and increased MDA levels in the heart. Decreased NO and elevated MDA levels observed in the present study is in consonance with report of previous studies in mouse treated with GC (Schäfer et al. 2005). In addition, decreased G6PD reduces the amount of NADPH, a requirement for the formation of NO (Leopold et al. 2001) and impaired NO biosynthesis is associated with enhanced endoglin (Li et al. 2000). More so, previous study from our laboratory showed that inhibition of NO resulted in cardiac hypertrophy in female rats receiving female sex steroids (Olatunji et al. 2017).
The free radical NO is an important mediator of the placentation process. Under physiologic conditions, endothelial release of NO in the placental circulation dilates the fetal placental vascular bed, ensuring feto-maternal exchange. In an oxidative environment, the lack of NOS-stabilizing factors results in NOS-uncoupling. NOS-coupling causes a shift from NO production to superoxide production which maintains oxidative stress (Hodzic et al. 2017). It have been documented that GC decreased endothelial NO production by decreasing eNOS gene transcription which in turn impair NO bioactivity due to increased generation of ROS (Liu et al. 2008). Also, GC cause impaired NO bioavailability through increased ROS production via xanthine oxidase (Iuchi et al., 2003). DEX-induced oxidative stress was previously reported from our laboratory to be associated with reduced NO levels (Badmus and Olatunji 2018) in non-pregnant rats, also GC treatment has been shown to downregulate systemic NO synthesis (Soriano et al. 2013). Similarly, Tain and coworkers reported reduced NO bioavailability in DEX-treated pregnant rats (Tain et al. 2014). This further corroborates the finding in the present study that DEX treatment induced oxidative stress in pregnant and non-pregnant rats since reduced NO bioavailability has been associated with ROS production, endothelial dysfunction and cardiac dysfunction.

Liver dysfunction has been strongly linked with the prognosis of patients with heart failure (Batin et al. 1995) and previous studies have demonstrated that elevated serum LDH, lactate, AST, ALP and GGT levels are linked to increased risk of heart failure and mortality (Pasupathi et al. 2009; Jiang et al. 2013). LDH, an enzyme found in almost all cells including heart muscles converts pyruvate to lactate during oxygen deficit. LDH activity is known to increase during tissue injury. However, earlier studies have reported it to be an early predictor of tissue damage.
and can be regarded as cardiac marker enzyme (Pasupathi et al. 2009; Matsushita et al. 2013). Acute myocardial infarction has been shown to result in increased serum LDH with significantly altered GSH and MDA (Khan et al. 2013). Hence, the findings that DEX treatment led to elevated cardiac pro-inflammatory markers and defective anti-oxidative defense are confirmed by elevated (LDH, AST, ALP, ALT and GGT) in the hearts of pregnant and non-pregnant rats. Also, hyperlactatemia suggest low resting oxidative capacity which has been associated with heart failure.

In conclusion, the present study demonstrates that GC treatment induced inflammation-mediated cardiac dysfunction via defective G6PD antioxidant defense pathway along with increased cardiac endoglin, VCAM-1 and metabolic disturbances in both pregnant and non-pregnant rats. Therefore, DEX-induced elevated ROS production implicated the involvement of defective G6PD-dependent antioxidant defense in DEX-induced cardiovascular damage in pregnant and non-pregnant rats. In light of the finding of the present study, the choice of GC-based therapy in the clinic should be made with caution and benefit must be weighed against damage that can be caused by GC treatment in women especially during pregnancy.

**Conflicts of interest**

The authors have no conflicts of interest to declare.

**Acknowledgements**

The authors appreciate the support of Association of African Universities (AAU; 2017/2018) and International Society of Hypertension (ISH) grant for mentors (2017)
References


Motta, K., Gomes, P.R.L., Sulis, P.M., Bordin, S., Rafacho, A. 2018. Dexamethasone administration during late gestation has no major impact on lipid metabolism, but reduces newborn survival rate in Wistar rats. Front Physiol. 9, 783. doi: 10.3389/fphys.2018.00783.


Table 1: Effects of dexamethasone on organ weights in pregnant and non-pregnant rats

<table>
<thead>
<tr>
<th></th>
<th>PRE (-)</th>
<th>PRE (-) + DEX</th>
<th>PRE (+)</th>
<th>PRE (+) + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight</td>
<td>3.6 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>3.1 ± 0.4</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>(g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney weight</td>
<td>8.1 ± 0.4</td>
<td>9.0 ± 0.6</td>
<td>6.1 ± 0.2#</td>
<td>8.9 ± 0.4&amp;</td>
</tr>
<tr>
<td>(g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver weight</td>
<td>34.1 ± 2.6</td>
<td>44.1 ± 2.4*</td>
<td>26.8 ± 1.3##</td>
<td>40.9 ± 2.6&amp;</td>
</tr>
<tr>
<td>(g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterine weight</td>
<td>5.5 ± 1.5</td>
<td>2.2 ± 0.7*</td>
<td>11.8 ± 0.2##</td>
<td>8.1 ± 0.2##&amp;</td>
</tr>
<tr>
<td>(g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.
Table 2: Effects of dexamethasone on fasting blood glucose, plasma and cardiac lipid profile in pregnant and non pregnant rats

<table>
<thead>
<tr>
<th></th>
<th>PRE (-)</th>
<th>PRE (-) + DEX</th>
<th>PRE (+)</th>
<th>PRE (+) + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose</td>
<td>3.1 ± 0.1</td>
<td>5.4 ± 0.1*</td>
<td>4.0 ± 0.3*#</td>
<td>5.0 ± 0.3*&amp;</td>
</tr>
<tr>
<td>(mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (mg/dl)</td>
<td>67.0 ± 8.8</td>
<td>110.7 ± 1.3*</td>
<td>75.8 ± 8.3#</td>
<td>116.5 ± 2.5*&amp;</td>
</tr>
<tr>
<td>Heart (mg/100mg tissue)</td>
<td>65.8 ± 29.1</td>
<td>68.9 ± 5.8</td>
<td>67.4 ± 2.2</td>
<td>60.1 ± 1.5</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (mg/dl)</td>
<td>24.4 ± 0.4</td>
<td>24.7 ± 0.5</td>
<td>26.5 ± 0.7</td>
<td>25.3 ± 0.8</td>
</tr>
<tr>
<td>Heart (mg/100mg tissue)</td>
<td>26.1 ± 0.8</td>
<td>25.5 ± 0.5</td>
<td>25.7 ± 1.9</td>
<td>25.3 ± 1.0</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (mg/dl)</td>
<td>16.5 ± 0.3</td>
<td>25.7 ± 0.4*</td>
<td>16.2 ± 0.9#</td>
<td>27.2 ± 0.6*&amp;</td>
</tr>
<tr>
<td>Heart (mg/100mg tissue)</td>
<td>19.9 ± 1.0</td>
<td>37.7 ± 1.2*</td>
<td>25.4 ± 0.8*#</td>
<td>39.1 ± 0.2*&amp;</td>
</tr>
</tbody>
</table>

Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX ; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.
Table 3: Effects of dexamethasone on plasma and cardiac GSSG and GSH in pregnant and non pregnant rats

<table>
<thead>
<tr>
<th></th>
<th>PRE (-)</th>
<th>PRE (-) + DEX</th>
<th>PRE (+)</th>
<th>PRE (+) + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidized glutathione</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (mg/dl)</td>
<td>0.2 ± 0.1</td>
<td>0.9 ± 0.3*</td>
<td>0.2 ± 0.1#</td>
<td>1.5 ± 0.5*##&amp;</td>
</tr>
<tr>
<td>Heart (mg/100mg tissue)</td>
<td>0.2 ± 0.0</td>
<td>1.9 ± 0.3*</td>
<td>0.3 ± 0.1#</td>
<td>1.2 ± 0.7*##&amp;</td>
</tr>
<tr>
<td><strong>Reduced glutathione</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (mg/dl)</td>
<td>7.0 ± 0.8</td>
<td>3.1 ± 0.3*</td>
<td>7.3 ± 0.4#</td>
<td>2.6 ± 0.1*##&amp;</td>
</tr>
<tr>
<td>Heart (mg/100mg tissue)</td>
<td>5.7 ± 0.6</td>
<td>1.8 ± 0.4*</td>
<td>7.6 ± 1.1#</td>
<td>2.2 ± 0.2*##&amp;</td>
</tr>
</tbody>
</table>

Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group. [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.

**FIGURE LEGENDS**

Figure 1. Effect of dexamethasone (DEX) treatment on plasma and cardiac triglyceride/high density lipoprotein-cholesterol (TG/HDL-C; A & B) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. Cardiac TG/HDL-C was comparable across the treatment groups whereas plasma TG/HDL-C was increased across the treatment groups when compared to PRE (-) rats. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.
**Figure 2.** Effect of dexamethasone (DEX) treatment on plasma and cardiac glucose-6-phosphate dehydrogenase (G6PD; A & B), glutathione peroxidase (GPx; C & D), reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio (GSH/GSSG; E & F) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. DEX led to defective G6PD-dependent antioxidant capacity when compared to untreated groups. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.

**Figure 3.** Effect of dexamethasone (DEX) treatment on plasma and cardiac malondialdehyde (MDA; A & B), nitric oxide (NO; C & D) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. DEX led to increased plasma and cardiac MDA levels and decreased NO when compared to untreated groups. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.

**Figure 4.** Effect of dexamethasone (DEX) treatment on plasma and cardiac adenosine (A & B), adenosine deaminase activity (ADA; C & D) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. DEX led to decreased plasma and cardiac adenosine levels but increased ADA activity when compared to untreated groups. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs
PRE (-); \#p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.

**Figure 5.** Effect of dexamethasone (DEX) treatment on plasma and cardiac endoglin (A & B), plasma and cardiac vascular cell adhesion molecule-1 (VCAM-1; C & D) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. Plasma and cardiac endoglin, VCAM-1 were significantly elevated in DEX-treated non-pregnant and pregnant rats when compared with untreated groups. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [\*p<0.05 vs PRE (-); \#p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.

**Figure 6.** Effect of dexamethasone (DEX) treatment on cardiomyocyte morphology in non-pregnant: [PRE (-) & PRE (-) + DEX] and pregnant rats [PRE (+) & PRE (+) + DEX]. Cardiomyocytes appear normal in PRE (-) & PRE (+) rats. DEX treatment led to mild disruption of cardiac tissue in PRE (-) + DEX rats. Also, there are moderate disruptions of cardiac tissue with cellular infiltration in PRE (+) + DEX rats. (H &E paraffin stain; ×100, Longitudinal section).

**Figure 7.** Effect of dexamethasone (DEX) treatment on plasma and cardiac free fatty acid (FFA; A & B), lactate (C&D) and lactate dehydrogenase (LDH; E & F) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. DEX led to increased plasma and cardiac FFA, lactate and LDH when compared to untreated groups. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [\*p<0.05 vs PRE (-);
Figure 8. Effect of dexamethasone (DEX) treatment on plasma and cardiac aspartate aminotransferase (AST; A & B), alkaline phosphatase (ALP; C & D), alanine transaminase (ALT; E & F) and gamma-glutamyl transferase (GGT; G & H) in non-pregnant [PRE (-)] and pregnant [PRE (+)] rats. Dexamethasone treatment led to elevated tissue injury markers when compared to untreated groups. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SD of six rats per group [*p<0.05 vs PRE (-); #p<0.05 vs PRE (-) + DEX; &p<0.05 vs PRE (+)]. PRE (-); non-pregnant rats, DEX; dexamethasone, PRE (+); pregnancy.
Fig. 1

A

Plasma TG/HDL

| PRE | - | - | + | + |
| DEX | - | + | - | + |

B

Cardiac TG/HDL

| PRE | - | - | + | + |
| DEX | - | + | - | + |
**Fig. 2**

**Panel A**
Plasma G6PD (U/L)
- PRE: -
- DEX: +
- PRE + DEX: +

**Panel B**
Cardiac G6PD (U/g protein)
- PRE: -
- DEX: +
- PRE + DEX: +

**Panel C**
Plasma GPx (U/L)
- PRE: -
- DEX: +
- PRE + DEX: +

**Panel D**
Cardiac GPx (U/g protein)
- PRE: -
- DEX: +
- PRE + DEX: +

**Panel E**
Plasma GSH/GSSG ratio
- PRE: -
- DEX: +
- PRE + DEX: +

**Panel F**
Cardiac GSH/GSSG ratio
- PRE: -
- DEX: +
- PRE + DEX: +
Fig. 4

A. Plasma Adenosine (mM)

B. Cardiac Adenosine (mM/100mg tissue)

C. Plasma ADA (U/L)

D. Cardiac ADA (U/g protein)
**Fig. 5**

A. Plasma Endoglin (ng/ml)

B. Cardiac Endoglin (ng/100mg tissue)

C. Plasma Vcam-1 (pg/ml)

D. Cardiac VCAM-1 (pg/100mg tissue)

PRE - - + + +
DEX - + - + +

* * &
#

PRE - - + + +
DEX - + - + +

* * &
#
**Fig. 7**

A. Plasma FFA (mg/dl)

B. Cardiac FFA (mg/100mg tissue)

C. Plasma Lactate (mg/dl)

D. Cardiac Lactate (mg/100mg tissue)

E. Plasma LDH (U/L)

F. Cardiac LDH (U/g protein)

PRE and DEX treatments are indicated as follows:
- PRE: -
- DEX: +

Significance levels:
- *: p < 0.05 compared to PRE
- #: p < 0.05 compared to DEX
- &: p < 0.05 compared to PRE vs. DEX