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## A comparative study on the role of endothelium in aortic vascular response to phenylephrine in Normal and Diabetic pregnancy

Omeni, AA<sup>1</sup>; Otamere, HO<sup>2</sup>; Adisa WA<sup>2</sup>; Osifo UC<sup>2</sup>; Iyamu OA<sup>3</sup>; Ediangbe AP<sup>4</sup>; Akpamu U<sup>2</sup>; Odiase DE<sup>5</sup>

<sup>1</sup>Department of Zoology, Faculty of Natural Science, Ambrose Alli University, Ekpoma, Nigeria.

<sup>2</sup>Department of Physiology, Faculty of Basic Medical Science, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria.

<sup>3</sup>Department of Medical Biochemistry, Faculty of Basic Medical Science, College of Medicine, Ambrose Alli University, Ekpoma, Nigeria.

<sup>4</sup>Department of Medical Laboratory Science (Chemical Pathology Unit), College of Medicine, Ambrose Alli University, Ekpoma, Nigeria.

<sup>5</sup>Department of Anatomy, College of Medicine, University of Benin, Nigeria.

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### ABSTRACT

Pregnancy is known to be associated with alteration in maternal conditions and functions. Almost all tissues and organs are involved. This study examines the role of endothelium in aortic vascular contractile response to phenylephrine in normal and Diabetic Pregnancy. Wister rats were grouped into two; A and B. Group A were non diabetic Pregnant rats comprising of sub-group A<sub>1</sub> and A<sub>2</sub>. Group B were diabetic pregnant rats also comprising of sub-groups B<sub>1</sub> and B<sub>2</sub>. Intraperitoneal injection of streptozotocin was used to induce Diabetes. Group A<sub>1</sub> and B<sub>1</sub> have their endothelium intact while that of A<sub>2</sub> and B<sub>2</sub> were removed. Using 2mm aortic segment under standard organ bath conditions, tension was measured with Isometric transducer (FT.03) connected to glass polygraph (7D). Contraction to phenylephrine was observed to be concentration dependent. With intact endothelium, the maximal contraction of the rings from diabetic pregnant rats (group B<sub>1</sub>) were significantly ( $P < 0.05$ ) enhanced than those from the normal pregnant rats (group A<sub>1</sub>). The removal of endothelium from both groups (A<sub>2</sub> and B<sub>2</sub>) present significant reduction ( $P < 0.05$ ) in respond to phenylephrine. Diabetes Mellitus alters endothelia function. The enhanced contraction to phenylephrine in aortic blood vessels in pregnancy may be related to endothelial dysfunction.

**Key words:** Aorta, Diabetes, Endothelium, Phenylephrine, Pregnancy,

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### INTRODUCTION

Pregnancy is known to cause profound changes in maternal anatomical [1], physiological and metabolic functions [2]. To this regard, reports have shown pregnancy to be accompanied by increased plasma volume, cardiac output, resting pulse rate [1] as well as decrease systemic vascular resistance [3]. Oxidative stress [4];[5];[2], gestation

diabetes, preeclampsia [6], are some other pathologies that may occur during pregnancy. Human metabolic adaptations of pregnancy tend to provide weight gain, increased fat deposition, insulin resistance, hemodilution and hyperlipidemia [7]. Of interest in this study is the connection of diabetes and pregnancy in relation to vascular function. There is evidence that pregnancy causes the vascular endothelium to release potent vasodilators such as prostacyclins and nitric oxide [8] and interferes with calcium entry into vascular smooth muscle cells [9]. Also, vascular dysfunction in diabetes has been associated with alterations in the function of the endothelium; and endothelial dysfunction itself has been linked to development of atherosclerosis [10];[11]. Although studies reported vascular reactivity to some vasoactive agents is blunted in normal pregnancy [12], decreased endothelium-dependent relaxation has been observed in blood vessels from several models of diabetes in rats and in humans[13];[14]. In contrast, enhanced endothelium-dependent relaxation has also been reported [15];[16]. In line with these observations and conflicting results obtained from several studies on vascular responses in diabetes and pregnancy, this study has been designed to examine the role of endothelium in the contractile response of the aorta in diabetic and normal pregnancy using Wistar rat as a model.

### MATERIALS AND METHODS

This study was carried out between June and September 2010 at the Physiology Laboratory of the College of Medicine, Ambrose Alli University, Ekpoma, Nigeria.

**Animals:** Wistar rats (250-300gm) of 12 – 14 weeks of age were used for this study and were procured from the Animal House of Ambrose Alli University, Ekpoma. They were housed in a stainless steel cage (50 × 40 × 20 cm) with plastic bottom grid and a wire screen top in Physiology Laboratory, Ambrose Alli University, Ekpoma, Nigeria. They were fed ad libitum with tap water and pelleted feeds purchased from Bendel feeds and flour meal Ewu, Nigeria Limited and allowed to acclimatize for 2 weeks. The animals were assigned into two groups A and B, further divided into 2 sub-groups with 5 animals each (designated A1 & A2; and B1& B2; n = 5 rats each). Two male Wistar rats were introduced to each group to allow for mating. The animals were allowed to mate for 3 days after which the male animals were removed from the cage. Pregnancy was confirmed by palpation [17] and vaginal smear microscopy method [18];[19]. The group A comprises of pregnant rats which received citrate buffer only, while the group B are pregnant rats made diabetic by intraperitoneal injection of streptozotocin (60 mg/kg) in citrate buffer at PH 7.4 on the 7<sup>th</sup> day after the males had been withdrawn. They were then monitored daily for the development of glycosuria, using Uritrix strips. Streptozotocin and Uritrix were obtained from Ames Division Miles Laboratories, England. The experiments were carried out according to the Guide for the Care and Use of Laboratory Animals, National Academy of Sciences.

**Organ Bath studies:** Both groups (A and B) were sacrificed by stunning on the 11<sup>th</sup> day (corresponding to 18±3 day gestation). The descending thoracic aortae of each rat were carefully and quickly isolated, free of connective tissues and put into beakers containing pre-warmed physiological salt solution (PSS). The solution was continually bubbled with a gas mixture of 95% oxygen and 0.5% carbon dioxide and temperature was thermostatically maintained at 37°C. Each of the aortae was cut into 2mm ring segments, and used under standard organ bath conditions. Tension in the blood vessel preparations were measured using Isometric force recording Transducers (FT.03) which was coupled to glass (7D) polygraph. The rings from both the groups (A and B) labeled 'A2 and B2' had their endothelium removed mechanically by gently rubbing the inner surface of the blood vessel rings with a roughened glass rod as described by [20]. The effectiveness of the procedure was confirmed by absent of 10<sup>7</sup>m acetylcholine to relax the rings. The tissues were then allowed to equilibrate for 90 minutes in the organ bath under an optimal resting tension of 1gm. The resting tension being that at which the tissue generated the greatest contraction to 10<sup>7</sup>m noradrenalin [20].

**Experimental protocol; Concentration response test to phenylephrine:** Aortic rings, with endothelium (A1, B1) and without endothelium (A2, B2) from pregnant (group A) and diabetic pregnant (group B) rats were exposed to cumulatively increasing concentrations of phenylephrine (10<sup>-9</sup> to 10<sup>-4</sup> ML<sup>-1</sup>). A higher concentration was applied to the bath when the effect of the previous application was stable.

**Data analysis:** The mean ± standard deviation (X ± SD) and one-way ANOVA (LSD) statistical test was performed using SPSS version 17 software with the significance level set at p<0.05.

RESULTS

Table 1 and fig 1 shows the mean concentration and distribution response respectively of aortic vascular rings to phenylephrine in normal pregnant (group A) and diabetic pregnant (group B) rats with endothelium either intact (A1, B1) or removed (A2, B2). There was a steady increase in contraction in response to a rising concentration of phenylephrine. However, at concentration of  $10^{-5}$ , a fluctuating response pattern was noticed for sub-group A1 and A2. Increase in concentrations of phenylephrine brought about increase in aortic vascular reactivity irrespective of the presence or absence of endothelium. In normal pregnancy (group A), absence of endothelium (A2) brought about significant increase ( $p < 0.05$ ) in aortic vascular response to phenylephrine. EC50 value was also significantly higher in sub-group A2 ( $4.9 \times 10^{-8}$ ) compared to sub-group A1 ( $2.47 \pm 0.39 \times 10^{-7}$ ) (Fig 2). In diabetic pregnancy (group B), absent of endothelium (B2) were observed to caused a significant decrease ( $p < 0.05$ ) in aortic vascular response to phenylephrine compared to the corresponding treatments with endothelium intact (B1). However, there was decrease in the value of EC50 from  $3.5 \pm 0.41 \times 10^{-7}$  in B1 to  $3.2 \pm 0.32 \times 10^{-7}$  in B2, this decreased was not statistically significant (Fig 2).

Table 1: Dose response of aortic rings to phenylephrine

Concentration	Group A (Pregnant rats)		Group B (Diabetic pregnant rats)	
	A1	A2	B1	B2
$10^{-9}$	4.0±2.7 <sup>a</sup>	45±19.7 <sup>b</sup>	83.3±53.7 <sup>c</sup>	60±24.3 <sup>bc</sup>
$10^{-8}$	56.0±16.2 <sup>b</sup>	512±106.5 <sup>c</sup>	250±68.7 <sup>d</sup>	223.3±42.5 <sup>d</sup>
$10^{-7}$	394±110.6 <sup>c</sup>	1530±137.8 <sup>d</sup>	888.3±123.7 <sup>e</sup>	713.3±94.1 <sup>f</sup>
$10^{-6}$	1007±143.8 <sup>e</sup>	2040±144.7 <sup>e</sup>	1276.6±222.6 <sup>f</sup>	1040±172.3 <sup>e</sup>
$10^{-5}$	1139±103.4 <sup>e</sup>	2181±145.4 <sup>f</sup>	1500±243.4 <sup>e</sup>	1200±199.3 <sup>h</sup>
$10^{-4}$	1019±170.5 <sup>e</sup>	2112±126.0 <sup>f</sup>	1556.6±249.1 <sup>e</sup>	1250±212.1 <sup>h</sup>
EC50 value	$2.47 \pm 0.39 \times 10^{-7}$	$4.9 \times 10^{-8}$	$3.5 \pm 0.41 \times 10^{-7}$	$3.2 \pm 0.32 \times 10^{-7}$

Values are X±SD; A1 and B1= with endothelium intact; A2 and B2= with endothelium removed; A= normal pregnant rats; B= diabetic pregnant rats. Values in a row and in each column having different superscripts are significantly different ( $p < 0.05$ ).

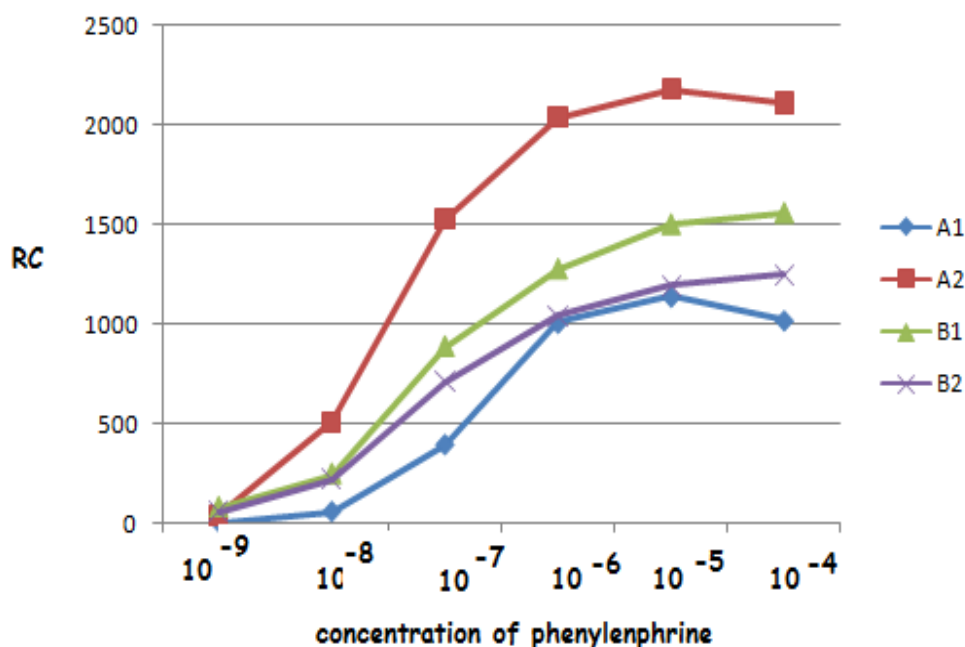
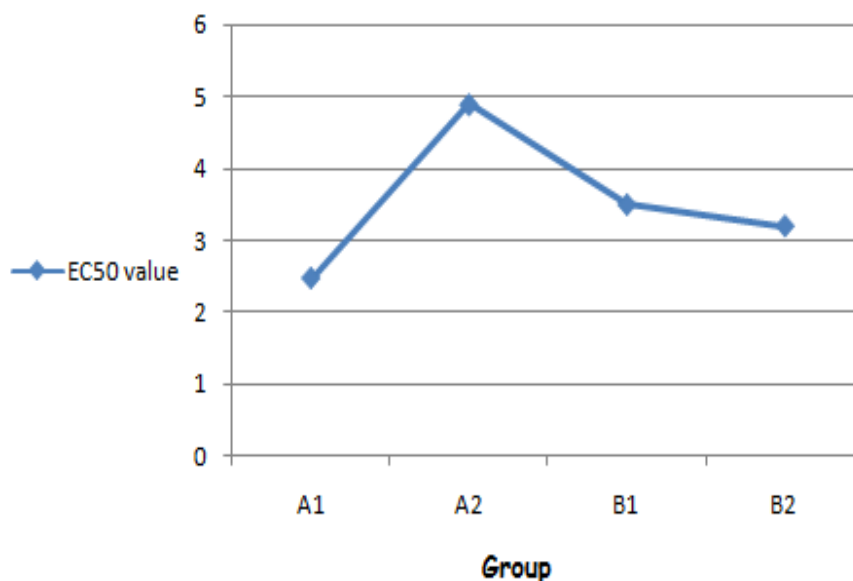


Fig 1: Concentration-response curves for phenylephrine-induced responsiveness in aortic rings of pregnant and diabetic pregnant rats (key: RC= response curves; A1= pregnant rat with endothelium intact; A2= pregnant rat with endothelium removed; B1= diabetic pregnant rat with endothelium intact; B2= diabetic pregnant rat with endothelium removed)



**Fig 2: EC50 response curves for phenylephrine-induced responsiveness in aortic rings of pregnant and diabetic pregnant rats.**  
 (key: RC=response curves; A1= pregnant rat with endothelium intact; A2= pregnant rat with endothelium removed; B1= diabetic pregnant rat with endothelium intact; B2= diabetic pregnant rat with endothelium removed)

In the presence of endothelium (A1 and B1), the concentration response values (curve) in response to phenylephrine were significantly ( $P < 0.005$ ) increased by diabetes in pregnancy. Also, in the absent of endothelium (A2 and B2), concentration response values (curve) were observed to be significantly reduced ( $p < 0.05$ ) which is more favoured with increased concentration in diabetic pregnancy group (B2) compared to corresponding normal pregnant group (A2).

## DISCUSSION

The present study provides information on the role of endothelium on vascular responses to phenylephrine during pregnancy and pregnancy accompanied by diabetes. Although contradictory reports have been the bases of available literature, this study shows enhance contractile responses to phenylephrine in aortic vascular smooth muscle during pregnancy. This finding disagrees with the study of Aloamaka et al [3], who reported consistently significantly decreased contractions of pregnant rat aortic rings to phenylephrine compared to those of the non-pregnant rats. Although reduced reactivity were observed in guinea-pig uterine arteries [21], rat aorta [12] and no difference in the contractions of carotid arteries from pregnant and non-pregnant guinea-pigs [21], interestingly, report in response to phenylephrine shows that there is enhanced vascular reactivity in the hind limb of pregnant ewes [22]. In this regard, Aloamaka et al. [3] concluded that species and/or regional vascular variation in the effect of pregnancy on the responses of blood vessels to phenylephrine exist. The response of vascular smooth muscle to a variety of vasoactive agents is altered during pregnancy [23]. Pregnancy is characterized by a blunted pressor and vasocontractile response to vasoactive substances in women [24];[25] and in other mammals, such as the rat [26];[27]. Furthermore, this study reveal that in the absent of endothelium, aortic vascular response to phenylephrine in normal pregnancy is significantly enhanced compared to when the endothelium is intact. This finding proposes the vascular protective role of endothelium in response to vaso-active agents like phenylephrine. The involvement of the vascular endothelium in the relaxation of arterial smooth muscle caused by acetylcholine has been demonstrated [20];[28]. Furthermore, it has been shown that different endothelium derived relaxing substances such as nitric oxide (NO), endothelium derived hyperpolarizing factor (EDHF) and prostanoids could also be involved in the mediation of the Ach-induced vasorelaxation, depending on the vessel being studied [29];[30];[31];[32];[33];[34]. Different endothelial derived relaxing substances can be said to be the reason for the response observed in this study. Perhaps therefore, in endothelium dysfunction, the formation or activities of these different endothelial derived relaxing substances are impaired or non functional. The study also shows enhanced vascular system response to phenylephrine in diabetic pregnancy (group B) compared to normal pregnancy (group A1). This finding is in

accordance with the reports of Poston and Taylor [35] and Sobrevia and Mann [36] who reported endothelial dysfunction in diabetes. This may explain the cause of hypertension in pregnancy as well as diabetic pregnancy considering the enhance activity of aortic vascular system to phenylephrine observed. Oxidative stress is an important factor in the pathogenesis of many of the chronic complications of diabetes [37];[38];[39];[40]. Also, diabetes is known to lead to endothelial dysfunction. It is therefore worthy to suspect that pregnancy accompanied with diabetes is a condition of multifunctional alterations affecting the vascular system, vascular regulatory mechanism and factors amongst others. Maternal conditions, including diabetes has been reported to produce an adverse environment for the developing fetus, resulting in increased risk of obesity, hypertension, insulin resistance and dyslipidemia [41];[42];[43]. There is however paucity of knowledge of the mechanisms that underlie the adverse long-term metabolic and cardiovascular programming that occurs after exposure to maternal diabetes [44].

This study reveals that diabetic pregnancy (group B1 and B2) causes increased vascular response to phenylephrine compared to the normal pregnancy (see *Fig 1*). This effect appears to be directly proportional to dosage. Interestingly, when the endothelium was removed in diabetic pregnant rats (group B2), reductions in the response of the aortic vascular system were observed. It was also noticed that endothelia removal in normal pregnancy (group A2) brought about the most reactive response of aortic vascular system to phenylephrine. This comparative effect in endothelium dysfunction in normal pregnancy (A2) to the diabetic pregnancy (B2) may be explained by the report of Omer *et al.*[15]. He observed diabetes to lead to an increase in total nitric oxide synthase (NOS) activity in the heart, aorta and uterus. Could it be that the remover of the already malfunction endothelium in diabetic re-engine the physiological regulatory defense mechanism in aortic vascular system? Could the remover of the already malfunction endothelium trigger the different endothelium derived relaxing substances in aortic vascular system? This finding suggests a re-consideration of endothelia state in the treatment of diabetes and thus the call for further studies in this regard. Clinical data suggest that the role of NO is not uniform on different blood vessels and is influenced by the presence of different diabetic complications [45];[46]; a variability and heterogeneity in endothelial NO functions in different blood vessels is also observed in experimental diabetes [47]. Diabetic complications are aggravated by pregnancy [48] but the contribution of endothelium during such condition has not, to our knowledge, been reported. Here we find that removal of endothelium in diabetic pregnancy might relax aortic vascular response to phenylephrine. However, the mechanism to this effect is unclear and hence the need for further research.

### CONCLUSION

Endothelia dysfunction is associated with diabetes mellitus and this may have implication for diabetes complicated with hypertension in pregnancy.

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