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Evaluating maternal hyperglycemic exposure and fetal placental arterial dysfunction in a dual cotyledon, dual perfusion model

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ABSTRACT

Background: Gestational diabetes affects almost 1 in 10 pregnancies and is associated with adverse outcomes including fetal demise. Pregnancy complications related to diabetes are attributed to placental vascular dysfunction. With diabetes, maternal hyperglycemia is thought to promote placental vasoconstriction. However, it remains poorly understood if and how hyperglycemia leads to placental vascular dysfunction or if humoral factors related to maternal diabetes are responsible.

Methods and Results: Utilizing a human placenta dual cotyledon, dual perfusion assay we examined the arterial pressure response to the thromboxane mimetic U44619, in cotyledons exposed to normal vs. a hyperglycemic infusion into the intervillous space. Tissues were then analyzed for the activity of key signaling molecules related to vascular tone; eNOS, Akt, PKA and VEGFR2. Results indicate a significant increase in fetal vascular resistance with maternal exposure to hyperglycemia. This response corresponded with a reduction in the phosphorylation of eNOS at Ser1177 and Akt at Thr308. In contrast, VEGFR2 at Tyr1175 and PKA at Thr197 were not different with hyperglycemia.

Conclusion: Reductions of eNOS and Akt phosphorylation at key residues implicated in nitric oxide production suggest that hyperglycemia alters the vasodilatory signaling of placental vessels. In contrast, acute hyperglycemic exposure may not alter vasoconstriction via VEGF and PKA signaling. Altogether our results link hyperglycemic exposure in human placentas to nitric oxide signaling; a mechanisms that may account for the elevations in vascular resistance commonly observed in diabetic pregnancies.

1. Introduction

Gestational diabetes complicates up to 9.2% of all pregnancies and is rising with the increasing incidence of obesity [1,2]. Diabetes in pregnancy is associated with significant risk for developing hypertension and preeclampsia [3–7]. Comorbidities related to placental blood flow dramatically elevate the risk for adverse pregnancy outcomes to include fetal cardiovascular dysfunction and death [8,9]. While perinatal morbidity and mortality have declined over the last two decades, stillbirths among women with diabetes have increased between the years 2005–2011 [3].

Hyperglycemia is associated with endothelial dysfunction in patients with diabetes [4,10]. Cardiovascular disease, stroke, and atherosclerosis have been attributed to endothelial dysfunction in the non-pregnant population [11,12]. This dysfunction is defined as a pathological state in which the endothelium loses its physiologic properties and shifts towards becoming more vasoconstrictive, prothrombotic and proinflammatory [13].

The fetal placental vasculature is modulated by paracrine stimuli, which include local vasoconstrictors and dilators such as nitric oxide (NO), prostacyclins and thromboxane [14]. Nitric oxide, a principle vasodilator, plays a significant role in regulating fetal placental blood flow [15]. Chronic inhibition of NO production leads to fetal growth restriction and increased placental vascular resistance [16]. In animal models and humans, endothelial vasodilation is impaired during acute hyperglycemic conditions both in diabetic and non-diabetics [17–19]. This response can be attributed to the reduced activity of endothelial nitric oxide synthase (eNOS) which is regulated by phosphorylation [20]. Phosphorylation of eNOS at Ser1177 activates NO synthesis and is reported lower in non-pregnant type II diabetic patients [11]. A recent study examined the effects of eliminating eNOS activity in knock-out mice, which resulted in insulin resistance and obesity vs. wild type

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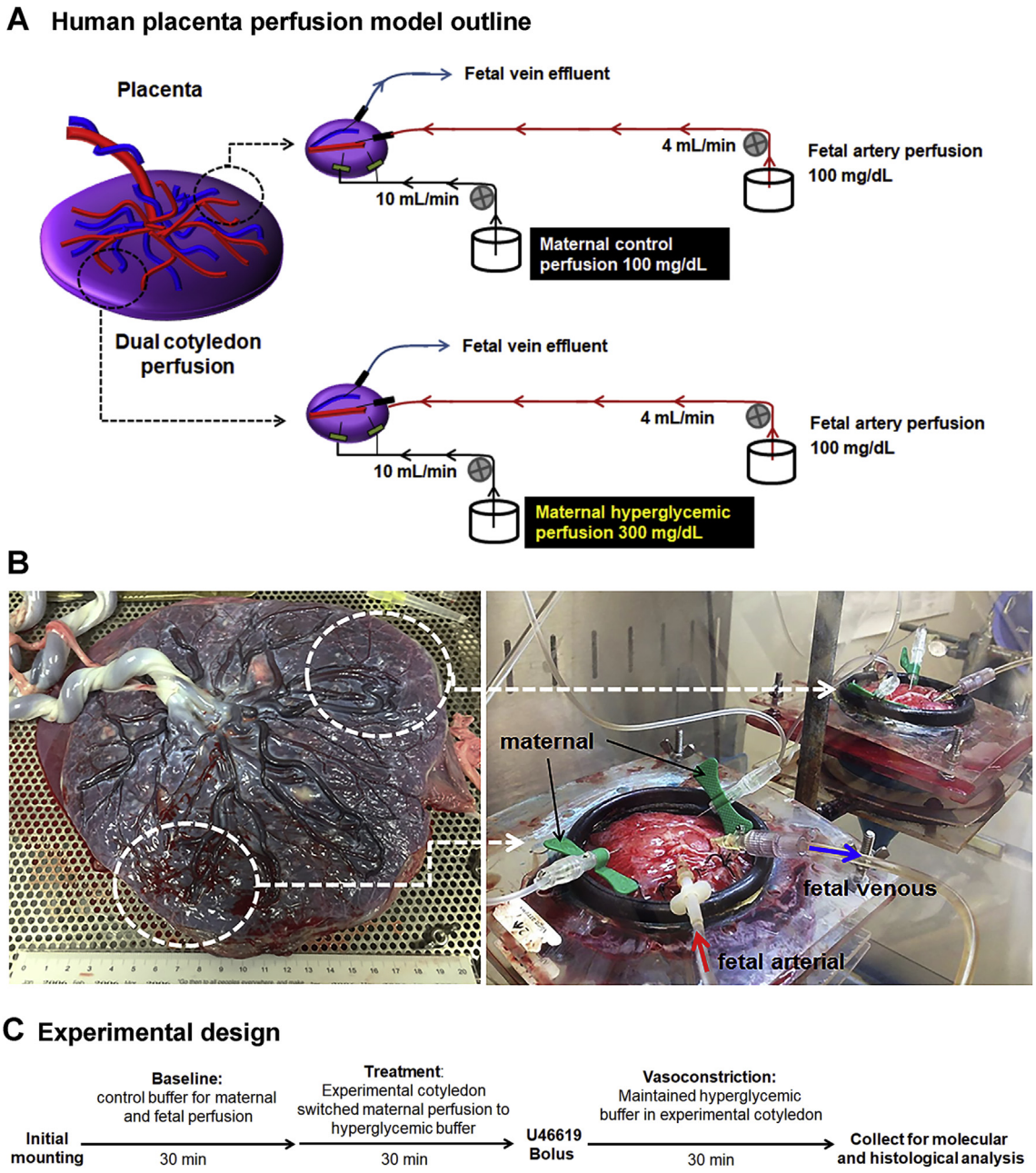

Fig. 1. A. Representation of the dual cotyledon, dual perfusion assay. From each placenta, two cotyledons are excised and their primary artery and vein are cannulated to perfuse the fetal placental vasculature. Butterfly needles inserted under the chorionic plate, simulate maternal perfusion into the intervillous space. From each placenta, one cotyledon received normal buffer (100 mg/dL) and the second hyperglycemic buffer (300 mg/dL) through maternal perfusion. **B.** Visual representation of the cotyledon selection and perfusion into the fetal and maternal circuits. **C.** Outline of the experimental design and timeline of perfusion experiments. Each experiment totals 120 min, split between three 30 min increments beginning with acclimation (baseline), exposure to hyperglycemia (treatment) and vasoconstriction with a bolus of the thromboxane mimetic, U46619.

controls [21]. Endothelial NOS is regulated by a variety of mechanisms including Akt dependent phosphorylation, which is also altered in rodent diabetic models [22–24]. Regulation of NO is also impaired in pregnancies complicated by diabetes, yet the mechanisms of dysfunction remain ill characterized [25].

It remains unclear if placental vascular dysfunction, associated with hyperglycemia in pregnancy, is due to the impairment of eNOS activity [26]. We hypothesized that maternal hyperglycemia is related to fetal placental arterial vasoconstriction and the dysregulation of eNOS. Our objective was to examine if acute maternal hyperglycemia promotes an exaggerated fetal placental arterial vasoconstriction, that is associated with reduced eNOS activity in a human placental dual cotyledon, dual perfusion model (Fig. 1A–C).

2. Materials and methods

Approval to conduct this study was granted by the local Institutional Review Board. Human placentas were exclusively obtained only from scheduled cesarean sections of uncomplicated, non-labored pregnancies. Placentas were excluded if any of the following conditions were present: maternal diabetes, hypertension, tobacco use, or other significant medical complications. General clinical characteristics are presented in Fig. 2A, in accordance with journal recommendations [27].