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Antenatal pomegranate juice rescues hypoxia-induced fetal growth restriction in pregnant mice while reducing placental cell stress and apoptosis

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ABSTRACT

Introduction: There is a need for prophylaxis to reduce placental-associated intrauterine growth restriction (IUGR). Pomegranate juice (PJ) is replete with phytochemicals having biological effects at non-pharmacological concentrations. We test the hypothesis that exposure of pregnant mice to hypoxia late in gestation induces cellular stress in the placenta, which can be ameliorated by antecedent maternal consumption of PJ.

Materials and methods: We exposed pregnant mice to 12% or 21% oxygen, with food *ad libitum* or restricted, and with consumption of PJ or glucose between 12.5 and 18.5 days post conception (dpc). We examined the outcomes of the nine groups (n = 10) at 18.5 dpc, quantifying fetal and placental weights and placental labyrinthine and junctional zone depths and areas. We assayed cellular stress by expression of Hsp90 and apoptosis by TUNEL staining and expression of cleaved caspase 3.

Results: Maternal exposure to 12% oxygen or food restriction in 21% oxygen, induced IUGR, compared to control. The labyrinth to junctional zone ratio was lower in hypoxic *ad libitum*, compared to normoxic food-restricted, placentas. Antenatal PJ prior to and during hypoxic exposure significantly improved fetal growth, reduced Hsp90 expression, and limited apoptosis in the labyrinth, while enhancing junctional zone apoptosis.

Discussion: Maternal exposure to hypoxia induces IUGR, cell stress, and apoptosis in mouse placentas. The labyrinth and junctional zone of the mouse placenta are differentially sensitive to FiO_2 and to PJ. PJ offers benefits in the prophylaxis of IUGR in the mouse, but PJ effects on the junctional zone require further study.

1. Introduction

Intrauterine growth restriction (IUGR) affects hundreds of thousands of pregnancies annually in the U.S. and the majority of these are associated with placental dysfunction. Clinical trials with vitamin C, E, or both, were studied with the goal of reducing placental oxidative stress and improving outcomes in women at risk for pregnancy complications. These studies ultimately showed no benefit, and a debated small risk for harm [1–7]. Currently, low dose aspirin is endorsed as prophylaxis for preeclampsia and IUGR, yet only one-fourth of the at-risk patient population benefits [8]. There is a pressing need to identify other interventions that enhance placental development and reduce placental damage from exogenous insults and reduce mortality, morbidity, and the high healthcare costs in offspring from to limit the occurrence of IUGR.

Pomegranate juice (PJ) is a dietary supplement with more than 100 phytochemicals, many of which have cellular effects in non-pharmacological concentrations [9]. Although typically cited as a rich source of polyphenol antioxidants [10,11], PJ contains many bioactive compounds including ellagitannins, flavonoids, vitamins, and trace elements, among others [9]. Ingestion of PJ improves the function of endothelial and neuronal cells exposed to oxidative stress [9]. PJ also affects NF-kB signaling pathways to positively regulate inflammation and cell death in both prostate and breast cancer [12]. Antenatal administration of PJ to pregnant mice reduces brain

Injury in their newborns when exposed to hypoxia-ischemia [13,14]. We showed that antenatal PJ reduces labor-induced oxidative stress in human placental villi while also limiting stressor-induced

Abbreviations: ad lib, ad libitum; HPX, hypoxia at 12% oxygen; IUGR, intrauterine growth restriction; JZ, junctional zone; Laby, labyrinthine zone; NOX, normoxia at 21% oxygen; PJ, pomegranate juice; FR, food restriction; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling; dpc, days post conception

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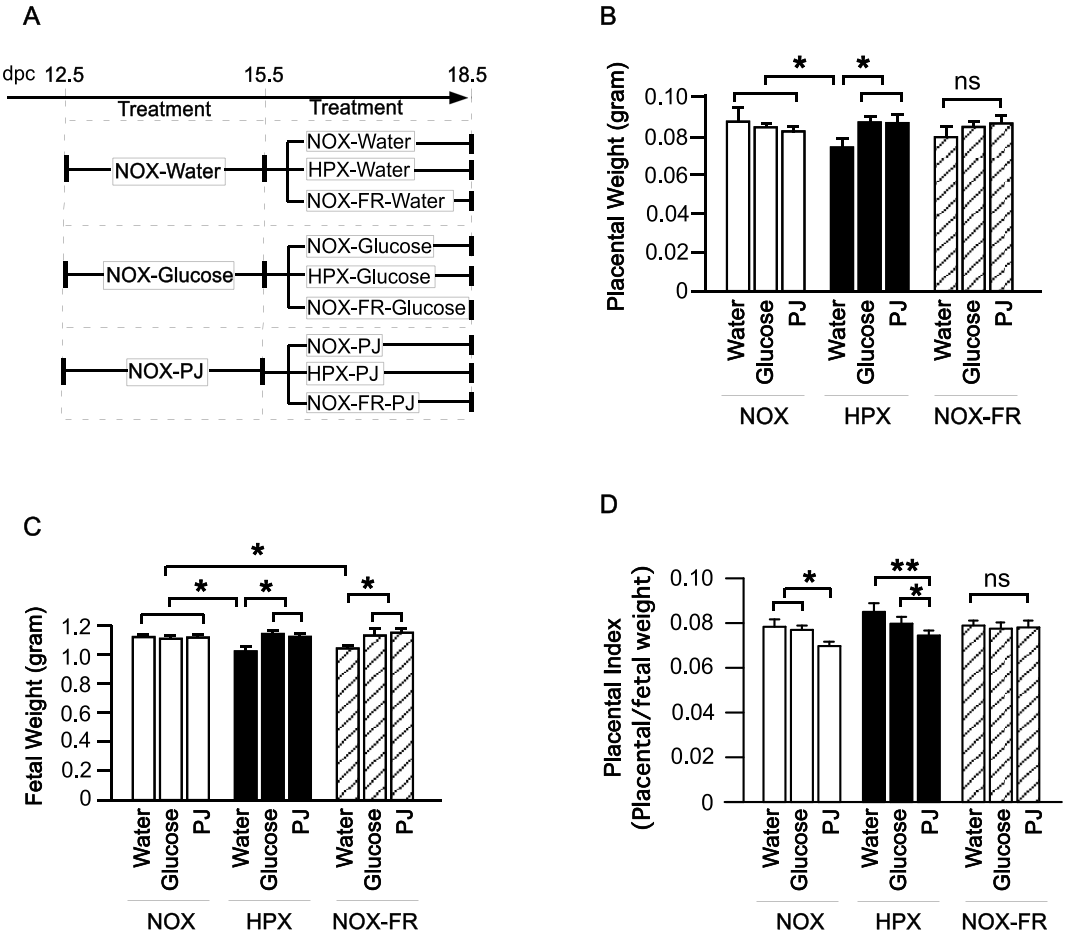

Fig. 1. The influence of FiO_2, food intake, and supplements on fetal and placental weights. (A) Schematic of experimental design. Food available is *ad libitum* if not listed as FR (food restriction). Water, sugar water and PJ were available at all times. (B) Mean ± SD for placenta weight from pooled sites 1- 4 among the nine paradigms with n = 10 dams. Placentas from the right horn were labeled as site 1- 4. Site 1 refers to the placenta that is adjacent to the ovary, and site 4 refers to the placenta that is adjacent to cervix, and sites 2-3 refers to the placentas between site 1 and site 4. (C) Mean ± SD for pooled fetal weights from sites 1- 4 among the nine paradigms with n = 10 damns. (D) Placental index as expressed by the ratio of placental weight divided by fetal weight. *$P < 0.05$, **$P < 0.01$, ANOVA with Bonferroni correction; NOX = FiO_2 of 21%; HPX = FiO_2 of 12%; PJ = pomegranate juice diluted 1:20 in water; glucose = 13% glucose (w/v) in water diluted 1:20 in water; FR = food restriction.

apoptotic death of cultured primary human trophoblasts [15]. Collectively, these observations suggest that PJ has the potential as an over the counter supplement with a therapeutic benefit in women at risk for placental injury and sub-optimal function.

The mature mouse placenta is composed of the labyrinth as a major site of maternal-fetal exchange and a junctional zone, which secretes hormones and growth factors [16,17]. We described that pregnant mice exposed to a FiO_2 of 12% oxygen (HPX) between days 15.5–18.5 dpc evolve IUGR, compared to mice exposed to a FiO_2 of 21% (NOX) [18]. We use this model of IUGR to extend our understanding of effects from maternal exposure to HPX in the mouse placenta, and test the hypothesis that antecedent maternal consumption of PJ ameliorates cell stress.

2. Materials and methods

2.1. Animals

This study was approved by the Animal Studies Committee at Washington University SOM and conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Female C57BL/6 mice (≥6 weeks old) were purchased from Jackson Labs (Bar Harbor, ME). Timed matings occurred for one night, with the morning after mating designated as 0.5 dpc. Pregnancy diagnosis was made in a mouse with a vaginal plug at 0.5 dpc and 3 g heavier on 10.5 dpc.

We conducted a pilot study that built on our previous observations that pregnant mice exposed to 12% oxygen from 15.5 to 18.5 dpc consumed less food, compared to mice exposed to 21% oxygen [18]. We randomized pregnant animals to four groups:

(a) FiO_2 = 12% between 15.5 and 18.5 dpc (HPX, n = 3),
(b) FiO_2 = 21% between 15.5 and 18.5 with food restriction (NOX-FR, n = 3),
(c) Gavaged daily with 250 µl of PJ (POM Wonderful©, Los Angeles, CA) from 12.5 to 18.5, with 12.5–15.5 dpc in FiO_2 = 21% and 15.5–18.5 dpc in FiO_2 = 12% (HPX-PJ, n = 6),
(d) Gavage daily with 250 µl of 13% glucose (w/v) in water from 12.5 to 18.5 dpc, with 12.5–15.5 dpc in FiO_2 = 21% and 15.5–18.5 dpc in FiO_2 = 12% (HPX-glucose, n = 6).

The 13% glucose control reflected the sugar content of the equivalent PJ administered. The food intake was recorded daily in the HPX groups and the average amount of food that the HPX mice consumed were provided to the NOX-FR group between 15.5 and 18.5 dpc.

As described in Results, we observed different placental phenotypes among the HPX groups. We thus extended our study to pregnant mice randomized to one of nine groups (n = 10 for each group; Fig. 1 A), to