INCREASE OF PROSTAGLANDIN E2 IN THE REVERSAL OF DUCTAL CONSTRICTION AFTER POLYPHENOL RESTRICTION

Short title: PGE2 and Constriction

Classification: Obstetrics

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Abbreviations:

DA – Ductus Arteriosus

PG – Prostaglandin

PGE2 - Prostaglandin E2

COX – Cyclooxygenase

NSAIDs - Nonsteroidal anti-inflammatory drugs

TP - Total polyphenols

FFQ - Food Frequency Questionnaire

SD - Standard deviation

Plain Language Summary

This controlled clinical trial showed that the maternal restriction of polyphenols consumption increased the systemic levels of prostaglandins E2 in mothers during the third trimester of pregnancy. In addition, the eco-doppler analysis revealed a reversal of ductal constriction of the fetus, preventing congenital heart diseases.

ABSTRACT

Objective: Anti-inflammatory substances that inhibit the synthesis of prostaglandins, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and polyphenol-rich foods, can cause constriction of the fetal ductus arteriosus. This study aimed to test the hypothesis that reversal of fetal ductal constriction after maternal restriction of polyphenol-rich foods, in the third trimester of pregnancy, is accompanied by increased prostaglandin E2(PGE2) plasma levels. **Method:** A controlled clinical trial was designed. The intervention group included women in the third trimester of pregnancy with fetal diagnosis of ductal constriction and not exposed to NSAIDs. The control group consisted of third-trimester normal pregnancies. Both groups answered a food frequency questionnaire, were submitted to Doppler echocardiographic examination and had blood samples collected for the PGE2 level analysis. Intervention group participants received dietary guidance to restrict the intake of polyphenol-rich foods, and repeated the procedure after two weeks. **Results:** 40 mothers were assessed in the control group and

35 in the intervention group. Mean maternal age (26.6 years) and mean body mass index (30.12 kg/m2) were similar between the groups. Intra-group analysis showed that dietary guidance reduced the mean consumption of polyphenols (1234.82 to 21.03 mg/day, $p<0.001$) significantly increasing in plasma concentration of PGE2 (1091.80 to 1136.98 pg/ml, $p<0.05$) in the intervention group. Also, Doppler results showed reversal of ductal constriction. No significant changes were observed in the control group. **Conclusions:** Dietary intervention for maternal restriction of polyphenol-rich foods in the third trimester of pregnancy is accompanied by increase in plasma levels of PGE2 and improves fetal ductal constriction.

INTRODUCTION

Fetal ductal constriction is a clinical condition with high morbidity and potential mortality. Patency the fetal ductus arteriosus (DA) depends on circulating prostaglandin (PG), which is produced by the cyclooxygenase (COX) pathway during inflammatory response[1] and is physiologically released especially during the third trimester of pregnancy[2]. COX-inhibiting substances, such as nonsteroidal anti-inflammatory drugs (NSAIDs), can interfere with PG metabolism and induce constriction of the DA[3-6]. To manage or prevent DA constriction, it is therefore important to reduce fetal exposure to drugs that may interfere with PG biosynthesis, such as anti-inflammatory drugs and polyphenol-rich foods[1,7-13].

Polyphenols, the most abundant antioxidants present in the diet, are widely distributed in vegetable foods. One of the possible mechanisms to explain their activity is inhibition of synthesis and release of inflammatory mediators[14]. However, clinical studies investigating the effect of polyphenols on inflammatory responses are

inconclusive and, in most cases, only evaluate clinical outcomes[15,16].The role of polyphenols on the inflammatory response, and associated modifications of the plasma concentration of PGE2 in pregnant women, has not yet been studied.

Despite the potential benefits of a diet rich in polyphenols[9-13], a high consumption of these substances in the third trimester of pregnancy may reduce plasma levels of PGE_2 and result in DA constriction[17-20], so that a restriction of their ingestion during this period is already recommended[21].Fetal ductal constriction has high prevalence[22] and may result in severe fetal and neonatal complications[23,24].These considerations raised the hypothesis that reversal of fetal ductal constriction after maternal restriction of polyphenol-rich foods is accompanied by increase in PGE2 levels. Demonstration of this effect would represent an advancement in knowledge and might result in changes on dietary guidance during pregnancy and prevention of perinatal complications, with potential impact in terms of public health.

The purpose of this study was to test the hypothesis that reversal of fetal ductal constriction in the third trimester of pregnancy, after maternal restriction of polyphenol-rich foods, is accompanied by increase in plasma levels of PGE2.

METHODS

The study was approved by the Research Ethics Committee of the Institute of Cardiology of Rio Grande do Sul. All participants signed an informed consent form after having been fully informed of the purpose of the study. The study was conducted according to guidelines of Resolution 466/12 of the National Council of Health for research with human beings, including anonymity and privacy of participants. A randomized clinical trial was not conducted due to the risk of maintenance fetal ductal

constriction in the intervention group, considering that the conceptual model to obtain a state of equipoise would not be met[25].

Study design

Controlled clinical trial. A group with normal echocardiographic diagnosis was used as control. The predictor variable was nutritional guidance and the outcome variable the modification of the plasma levels of PGE2.

Study population

Women with singleton pregnancies of 28 weeks or more, submitted to fetal echocardiography for any indication, were included. The intervention group included cases with no use of drugs or pharmacological anti-inflammatory agents and with a diagnosis of fetal ductal constriction, as evidenced by echocardiographic findings, namely: DA peak-systolic velocity greater than or equal to 1.40 m/s, maximum diastolic velocity greater than or equal to 0.30 m/s and ductal pulsatility index, determined as (systolic velocity minus diastolic velocity)/mean velocity, equal to or less than 2.2.The control group consisted of normal fetuses, with no diagnosis of fetal ductal constriction.

Exclusion criteria for fetal characteristics were: cardiac malformations (except for ductal constriction) or any other malformations; restricted intrauterine growth; increased nuchal translucency (> 95 percentile); present or suspected chromosomal disorders detected by fetal echocardiography and by obstetrical ultrasound morphological assessment; signs of any type of hydropsfetalis, or heart arrhythmia. Maternal exclusion criteria included disorders such as hypertension, diabetes mellitus, structural or functional cardiac abnormalities, current infections and positive serologies (toxoplasmosis, HIV, cytomegalovirus, hepatitis C); current use of nonsteroidal anti-inflammatory drugs, steroids, antidepressants, illicit drugs, alcohol or smoking; multiple

pregnancy; ongoing labor or premature rupture of membranes; unsuitable echocardiographic window; having received previous nutritional guidance in relation to restricted intake of polyphenol-rich foods; and refusal to participate in the study.

Logistics

In cases with fetal ductal constriction, the mothers received nutritional guidance to avoid polyphenol-rich foods, as explained below. Pregnant women from the control group were also interviewed to assess the amount of total polyphenols (TP) in the diet, but were not advised to avoid polyphenol-rich foods. Blood was collected from all participants, for the evaluation of inflammatory processes. All women were directed to return in two weeks for echocardiographic assessment, blood collection and nutritional evaluation.

Fetal echocardiography was performed with a commercially available ultrasound equipment capable of producing good-resolution images in M mode, two-dimensional, continuous and pulsed Doppler and color flow mapping, devoted exclusively to the project. Electronic transducers were used with second harmonic imaging with frequencies of 1.7 to 5 mHz.

Echocardiographic examinations assessed, by sequential analysis, the atrial situs, heart position, systemic and pulmonary venous drainage, atrioventricular and ventricular-arterial connections, foramen ovale, aortic arch, ductal arch and possible malformations. The DA was evaluated by color-flow Doppler in a longitudinal plane of the ductal arch or a transverse plane at the level of 3 vessels and trachea (3VT). The presence of flow turbulence was registered. The 2-mm pulsed Doppler sample was positioned at the most distal, descending aortic end of the DA, with a maximum angle of 30°, with no angle correction. 100-200 Hz filters, 50 cm/s scanning and 5 spectrum

curves were applied. Clear Doppler and uniform speeds were recorded. Fetal heart rate was recorded. In one of the cycles, the peak systolic, peak diastolic and end diastolic velocities (m/s) were determined. The DA pulsatility index was calculated automatically by the echocardiography system after manual tracing of the spectral curve, using the equation: (peak systolic velocity - diastolic peak velocity)/mean velocity.

Fetal ductal constriction was diagnosed by the following criteria: ductal systolic peak velocity ≥ 1.40 m/s, maximum ductal diastolic velocity ≥ 0.30 m/s and ductal pulsatility index ≤ 2.2. Additional parameters indicative of functional effects of ductal constriction were also evaluated, but were not essential to the diagnosis: ductal flow turbulence at color mapping, right-to-left ventricle diameter ratio ≥ 1.3 (RV/LV ratio), ratio of pulmonary artery to aorta diameters ≥ 1.3 (PA/AO), bulging of the interventricular septum into the left ventricle during the cardiac cycle, and tricuspid valve regurgitation. Pregnant women were included in the study after agreement of at least two researchers, with respect to the diagnostic criteria of fetal ductal constriction.

Two weeks after the first visit, a second Doppler echocardiographic examination was performed, by an investigator blinded for the study, group allocation and the results of the first examination. Normal values of transductal flow velocities and pulsatility index, as well as absence of any other associated abnormalities or complications, were considered as a favorable outcome, and were confirmed by a second examiner.

TP on maternal diet were quantified using a Food Frequency Questionnaire (FFQ) validated for pregnant women[26]. The measurement of TP from the food questionnaire data followed an American database[27], as well as a French database[28].A quantification of food consumption in southern Brazil[29] was also used for these estimates. TP results after analysis of the dietary questionnaires were described in

milligrams per day (mg/day). After answering the FFQ, all pregnant women in the intervention group, with a diagnosis of fetal ductal constriction, were directed to restrict polyphenol-rich foods, thosei.e with more than 30 mg of polyphenol per 100g, according to the American database[27], for the period of two weeks.

After two weeks, participants were submitted to echocardiographic re-evaluation and answered again to the FFQ. The amount of ingested TP during this period was calculated. On reassessment, the participants of the intervention group were oriented to keep the food restriction until the end of pregnancy, and to use alternative foods to replace the essential micronutrients present in polyphenol-rich foods.

Blood was collected before and after intervention for the evaluation of plasma levels of PGE2. Blood samples were centrifuged and the plasma was aliquoted in microtubes and immediately stored at -80° C until the time of analysis. PGE2 was quantified by capture ELISA (eBioscience and Cusabio Biotech kits), in accordance with the manufacturer's instructions. Optical densities were measured in a spectrophotometer (Spectramax M2e, Molecular Devices) at 25°C, with a reduction of background. The measurements were obtained through 4-parameter linear regression (Excel, Microsoft), and data were expressed in picograms of protein per milliliter (pg/mL).

Some precautions were taken for the control of main biases, such as: selection bias, by rigorously following the inclusion and exclusion criteria for the selection of study population; assessment bias, by having all measures performed by observers experienced in fetal echocardiography; and confusion bias,by pairing fetuses with gestational ages within the third trimester in the two groups. Only echocardiographic images of good quality were considered. A Doppler angle less than 30º was used in the

measurements. Data were collected during periods of fetal apnea and with no movement.

Sample size calculation

No report investigating pregnant women for the inflammatory outcome used in this study is available in the literature. Therefore, the sample size was calculated on the basis of the average and standard deviation of PGE2 levels presented in 20 samples (10 from each group) of a pilot of the present study, which was 741±217 pg/ml. An expected difference of 20% in levels of PGE2, based on a convenience sample of 33% of the final sample of this study. A sample of 29 pregnant women in each group was required for a power of 90% and a significance level of 5%.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD) or median and interquartile range interval for continuous variables. Student's t test for independent samples was used for comparison of continuous variables with normal distribution between groups. Pre- and post-treatment diagnostic parameters of fetal ductal constriction in both groups were analyzed by T-test for paired samples. Wilcoxon signed-rank test was used for intra-group analysis of the amount of polyphenols ingested (food questionnaire) and the change of the plasma levels of PGE2 before and after 2-week intervention. A significance level of 5% was considered. The Statistical Package for Social Science Program (S.P.S.S.) version 19.0 was used for data analysis.

RESULTS

Samples were collected between May 2014 and August 2016. 41 pregnant women were recruited for the control group and 37 for the intervention group, but we

lost 1 mother in the control group and 2 in the intervention group due to unexpected pregnancy loss. The characteristics of the sample, presented in Table I, were similar for the two groups, exception of gestational age which, even showing differences, was in all cases within the third trimester of pregnancy.

Table II presents the reported and FFQ-evaluated consumption of polyphenols, as well as the plasma levels of PGE2 observed in the two points of the study. Initial evaluation showed lower median consumption of polyphenols (904.30 mg/day) and levels of plasma PGE2 (584.16 pg/mL) in the control group than in the intervention group (1,234.82 mg/day and 1,091.80 pg/mL, respectively), but the difference was not statistically significant.

Intra-group analysis showed a significant reduction in the consumption of polyphenols ($p<0.001$) in the intervention group, accompanied by increase in plasma levels of PGE2 ($p<0.05$), after the two-week intervention. No significant differences were observed in the control group for consumption of polyphenols ($p=0.455$) or plasma levels of PGE2 ($p=0.150$).

Figures 1, 2 and 3 present results for the diagnostic evidences of ductal constriction before and after treatment, for the control and intervention groups. After the 15-day dietary guidance, the intervention group showed reduction of systolic velocity (1.92±0.29 m/s vs 1.34±0.32 m/s, $p<0.001$) as well as of diastolic velocity values (0.45±0.20 m/s vs 0.22±0.06 m/s, $p<0.001$), and increased pulsatility index (1.96±0.17 m/s vs 2.53±0.20 m/s, $p<0.001$), reflecting reversal of ductal constriction. No differences were observed in the control group for these parameters 15 days after the first echocardiographic assessment, with similar results for systolic velocity (0.86±0.25 m/s vs 0.68±0.47 m/s, $p=0.193$), diastolic velocity (0.14±0.025 m/s vs 0.16±0.024 m/s,

p=0.415) and pulsatility index (2.54±0.28 m/s vs 2.81±0.23 m/s ,p=0.075).

DISCUSSION

This is the first study to evaluate the relationship between consumption of polyphenols in pregnant women with premature fetal ductal constriction and the possible association with plasma levels of PGE2. The results showed that after nutritional guidance and reduction of the consumption of polyphenols in the intervention group, plasma levels of PGE2 increased and fetal ductal constriction was reversed. No modifications were observed in the control group during the period of the study. These findings suggest that maternal restriction of polyphenol-rich foods in the third gestational trimester reverses fetal ductal constriction through increase in plasma levels of PGE2. We are not aware of any other comparative orientation for reversal of fetal ductal constriction unrelated to pharmacological agents.

Several studies have demonstrated a reduction of PGE2 after polyphenol administration[30-35]. Our results, showing increase in plasma levels of PGE2 after restriction of polyphenols in the third trimester, reinforce this knowledge. The reversal of fetal ductal constriction observed in the intervention group by intra-group analysis, associated with an increase in plasma levels of PGE2, is attributed to the maternal restriction of polyphenol-rich foods during 15 days, showing that this period of time is sufficient for this effect. The modification of inflammatory indicators by introduction or removal of polyphenols depends on the individual variability in absorption and excretion, bioavailability of the substance, period of exposure or restriction to the food and the amount of polyphenols present, which varies depending on the food source[9,10,15,26]. Among prostaglandins, PGE2 is the main mediator of the inflammation

in the cardiovascular system[13].Studies with patients presenting inflammatory conditions show important changes in the levels of PGE2 after intervention with dietary polyphenols, as is the case of the present study in which women in the final period of gestation, considered a pro-inflammatory period, were investigated[15].

Considering the diagnostic parameters of fetal ductal constriction, the intervention group showed a decrease in systolic and diastolic velocity values, and an increase in pulsatility index, after 15 days of dietary guidance, with reversion of ductal constriction. No differences were observed in the control group. These results corroborate a previous study aimed at demonstrating this effect[18].

Polyphenols have similar effects than NSAIDs in inhibiting COX, this decreasing prostaglandins synthesis[11]. In a recent study, supplementation with polyphenol capsules to women of childbearing age inhibited the increase in markers of inflammation and oxidative stress[36]. Experimental studies showed that maternal consumption of polyphenols in the third gestational trimester changes dynamics of fetal DA, which may result in ductal constriction[19]. Clinical studies have shown that maternal consumption of higher amounts of polyphenol-rich foods can be associated with changes in DA dynamics[17], and that maternal restriction of these substances can reverse ductal constriction[18].

The main limitation of the present study was that was not randomized. However, this design was defined by considerations on the existing evidence on the relationship between consumption of polyphenols and ductal constriction and the lack of information on the safe consumption of polyphenols in late gestation. This ethical decision prevented a possible worsening of fetal ductal constriction, which shows no spontaneous reversal, when a polyphenol-rich diet is maintained[18]. In other words, there

was no equipoise to justify randomization of this study[25].

To control for selection, assessment and confusion biases, inclusion and exclusion criteria were strictly followed for the selection of study population, and all measurements were performed by observers with experience in fetal echocardiography. Echocardiographic images of poor quality were not considered.

In conclusion, the increased level of the inflammation marker observed in this study is possibly related to the restricted consumption of polyphenol-rich foods, and no modifications were observed in the control group that did not receive nutritional guidance. The results of this nutritional guidance for maternal restriction of polyphenol-rich foods at the end of pregnancy showed that the observed pro-inflammatory effect is probably associated to increase of plasma levels of PGE2, which may explain the reversal of fetal ductal constriction.

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FIGURE LEGENDS

Figure 1. Systolic velocity assessed in the first fetal echocardiographic examination and 15 days later, in the intervention and control groups.

Figure 2. Diastolic velocity assessed in the first fetal echocardiographic examination and 15 days later, in the intervention and control groups.

Figure 3. Pulsatility index assessed in the first fetal echocardiographic examination and 15 days later, in the intervention and control groups.

TABLES

Table 1. Characteristics of the sample

Characteristic	Control group (n=40)	Intervention group (n=35)	P
Age (years)	25.38 ± 6.74	28.17 ± 8.12	0.10
Gestational age (weeks)	29.48 ± 2.56	31.63 ± 2.37	<0.001
Current BMI* (kg/m²)	30.04 ± 5.38	30.22 ± 4.91	0.87

Results presented as mean ± standard deviation.

* Body mass index determined in the first consultation.

Comparison between groups performed using Student's t-test for independent data ($p<0.05$).

Table 2. Consumption of total polyphenols and levels of prostaglandin E2.

Parameter	Control group (n=40)	Intervention group (n=35)
TP (mg/day)		
First consultation	904.30 (266.83 - 6343.44)	1234.82 (289.65 - 5522.94)
Return	885.78 (312.79 - 3045.76)	21.03 (8.58 - 58.09)
p (pre / post-intervention)*	0.455	**<0.001**
PGE2 (pg/mL)		
First consultation	584.16 (342.51 - 962.73)	1091.80 (910.67 - 1320.15)
Return	864.32 (382.39 - 1232.74)	1136.98 (940.67 - 1481.89)
p (pre / post-intervention)*	0.150	**0.038**

Results presented as median (P25 – P75).

TP, Total Polyphenols; PGE2, Prostaglandin E2.

*Comparison between groups performed using Wilcoxon signed-rank test ($p<0.05$).