

Maternal Restriction of Polyphenols and Fetal Ductal Dynamics in Normal Pregnancy: An Open Clinical Trial

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Abstract

Background: We have recently demonstrated reversal of fetal ductal constriction after dietary maternal restriction of polyphenol-rich foods (PRF), due to its inhibitory action on prostaglandin synthesis.

Objective: To test the hypothesis that normal third trimester fetuses also improve ductus arteriosus dynamics after maternal restriction of polyphenols.

Methods: Open clinical trial with 46 fetuses with gestational age (GA) \geq 28 weeks submitted to 2 Doppler echocardiographic studies with an interval of at least 2 weeks, being the examiners blinded to maternal dietary habits. A validated food frequency questionnaire was applied and a diet based on polyphenol-poor foods ($<30\text{mg}/100\text{mg}$) was recommended. A control group of 26 third trimester fetuses was submitted to the same protocol. Statistics used t test for independent samples.

Results: Mean GA was 33 ± 2 weeks. Mean daily maternal estimated polyphenol intake (DMPI) was 1277mg, decreasing to 126mg after dietary orientation ($p=0.0001$). Significant decreases in systolic (SDV) and diastolic (DDV) ductal velocities, and RV/LV diameters ratio, as well as increase in ductal PI were observed [DSV= $1.2 \pm 0.4\text{m/s}$ (0.7-1.6) to $0.9 \pm 0.3\text{m/s}$ (0.6-1.3) ($p=0.018$); DDV= $0.21 \pm 0.09\text{m/s}$ (0.15-0.32) to $0.18 \pm 0.06\text{m/s}$ (0.11-0.25) ($p=0.016$); RV/LV ratio = 1.3 ± 0.2 (0.9-1.4) to 1.1 ± 0.2 (0.8-1.3) ($p=0.004$); ductal PI= 2.2 ± 0.03 (2.0-2.7) to 2.4 ± 0.4 (2.2-2.9) ($p=0.04$)]. In the control group, with GA of 32 ± 4 weeks, there were no significant differences in DMPI, mean SDV, DDV, PI and RV/LV ratio.

Conclusion: The oriented restriction of third trimester maternal ingestion of polyphenol-rich foods for a period of 2 weeks or more improve fetal ductus arteriosus flow dynamics and right ventricular dimensions. (Arq Bras Cardiol. 2013;101(3):217-225)

Keywords: Prostaglandins; Anti-inflammatory Agents; Constriction; Ductus Arteriosus; Pregnancy; Fetal Heart; Polyphenols / pharmacology.

Introduction

The relationship between maternal consumption of polyphenols and fetal ductal constriction in the third trimester of pregnancy has been demonstrated in several clinical and experimental studies. It is believed that the basic mechanism of this association involves the inhibitory action of polyphenols on the synthesis of prostaglandins, similar to anti-inflammatory drugs as classically described for many decades.

Recently, we demonstrated that ductal constriction occurring in the absence of maternal intake of anti-inflammatory drugs in the third trimester of pregnancy is reversed by dietary restriction of polyphenol-rich foods, such as herbal teas, mate, coffee, dark chocolate, coffee, from grape, orange, tangerine, red fruits, apple and olive oil¹. Other clinical and experimental evidences

support the association of changes in fetal ductus arteriosus flow and maternal consumption of foods with high concentration of natural anti-inflammatory substances such as polyphenols^{2,3}.

A food frequency questionnaire designed to quantify the concentration of polyphenols ingested by pregnant women in the third trimester was recently validated in our setting⁴. It represents an informative and practical method to determine the consumption of polyphenol-rich substances, according to usual dietary habits.

This study tests the hypothesis that dietary maternal restriction of polyphenol-rich foods during two weeks or longer, in the third trimester of pregnancy, also improves ductus arteriosus flow dynamics in normal fetuses, as previously shown in fetuses with ductal constriction.

Methods

Study Design

This open clinical trial was designed to assess the effect of maternal restriction of polyphenol-rich foods on ductus arteriosus flow dynamics in fetuses without cardiac anatomical or functional abnormalities.

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Patients

In November 2005, a structured program for routine evaluation of the ductus arteriosus flow dynamics was implemented at the Fetal Cardiology Unit of the Institute of Cardiology of Rio Grande do Sul. Third-trimester fetuses, with or without risk factors for cardiac abnormalities, were assessed by Doppler echocardiography. The study group was composed of 46 healthy fetuses with normal ductal flow, from women over 28 weeks of gestation who customarily consumed polyphenol-rich foods and consented to participate in the study. A control group of 26 third-trimester healthy fetuses from normal mothers was also evaluated. In this group, used for comparison, no dietary intervention was applied. None of the mothers reported the use of drugs containing nonsteroidal anti-inflammatory drugs (NSAID) and/or steroids, or the use of other legal or illegal drugs during the third trimester of pregnancy, and acute or chronic maternal disease was not present.

Dietary assessment and intervention

After signing an informed consent, and on the same day of the first fetal echocardiographic study, all participants answered a detailed food frequency questionnaire (FFQ) including the total gestation period. In the case of foods that were not consumed during the whole of the pregnancy period, an estimate was made of the daily consumption, by multiplying the reported portion by frequency of use and dividing by the number of days of in the time unit (day, week, month or year). One year was considered as the total days of gestation. The amount of food consumption during the period of investigation was assessed through homemade measures and estimated by pictures⁵.

Total polyphenols in the maternal diet were quantified with the validated FFQ⁴. This instrument presents a list of 52 foods rich in polyphenols, defined as above the 75th percentile, i.e. with at least 30 mg of polyphenol per 100 g of food. This classification followed criteria established by American⁶ and French databases⁷, which present flavonoid content and its subclasses in 385 and 300 foods, respectively. The amount of total polyphenols estimated by the dietary questionnaire was described in milligrams (mg).

Since mate tea (infusion of yerba mate *Ilex paraguariensis*) is not included in the American and French databases, polyphenols this substance were quantified using the Official Methods of Analysis of AOAC International, 18th ed. For this physicochemical test, the yerba mate concentration was 47.4% and the water temperature was 80°C, to reproduce the form of consumption of this beverage in South Brazil⁸.

After application of the FFQ in the first interview, a diet based on polyphenol-poor foods or <30 mg polyphenols/100 mg as listed in the American database⁶, was prescribed to the pregnant women of the intervention group. Two weeks later, at the time of the second fetal echocardiogram, the participants answered again the FFQ, for calculation of the amount of total polyphenols ingested during the period. All pregnant women were then instructed to maintain a restricted diet until the end of pregnancy, and different foods poor in polyphenols, i.e. with a concentration of less than 30 mg of polyphenols

per 100 g of food (below the 25th percentile), were suggested to replace the essential micronutrients that are present in polyphenol-rich food.

The 26 pregnant women from the control group, in which no dietary intervention was conducted, responded to the same questionnaire after completion of the first fetal echocardiography and after two weeks of follow-up, when the control echocardiographic Doppler evaluation was performed.

Assessment of ductal dynamics

A complete fetal Doppler echocardiography and color flow mapping was performed in all patients, with sequential segmental analysis of fetal heart and determination of the situs and atrioventricular and ventriculoarterial connections. Standardized pictures were studied, including aortic and ductal arches. The ductus arteriosus was studied after a suitable image in the sagittal plane was collected. For pulsed Doppler assessment, the ultrasound beam was aligned as parallel as possible to the color flow direction and to the long axis view of the ductus, with an angle smaller than 30°, without using angle correction. The sample volume was positioned in the descending aortic end of the ductus arteriosus.

The right to left ventricular dimensions ratio (RV/LV) was obtained on a four-chamber view in late diastole to assess potential right ventricular pressure changes.

Peak systolic and diastolic ductal flow velocities (SDV and DDV, respectively) were obtained and ductal pulsatility index (PI) obtained by the ratio [(systolic velocity - diastolic velocity) / average velocity] and right to left ventricular diameters ratio (RV/LV) were calculated⁹. Since the PI is not dependent on gestational age, it was used to exclude the diagnosis of ductal constriction¹⁰.

In fetuses of the intervention group, a control fetal echocardiography was performed on the same day of the second nutritional evaluation (after a minimum period of 2 weeks), to assess the effect of maternal dietary intervention on fetal ductus arteriosus flow dynamics and RV/LV ratio.

In the 26 pregnant women from the control group, a fetal echocardiogram was performed at the time of the first nutritional evaluation and after 2 weeks, on the same day of the dietary control evaluation.

For fetal echocardiography, a General Electric Vivid III Expert or Vivid 5S systems, with high-resolution two-dimensional and M-mode imaging, pulsed and continuous Doppler and color flow mapping capability, with multi-frequency convex sector transducers and a frequency range of 4 to 8 MHz, were used. All the analyses were performed by pediatric cardiologists with expertise in fetal echocardiography.

Statistical analysis

Numerical data were presented as mean \pm standard deviation (SD). For comparison of ductal flow velocities, pulsatility indices and the RV/LV ratios before and after the implementation of the dietary maternal restriction of polyphenol-rich foods, the two-tailed Student's t-test for paired samples was used. The Wilcoxon test was applied for comparison of maternal intake of polyphenols in the two

groups. The significance level of 0.05 was used for all statistical tests. The sample size was established considering an alpha-type error of 5% and a beta-type error of 10%.

Results

Maternal consumption of polyphenol-rich foods was documented in all case and control participants. None of the patients included in this study presented a history of consumption of NSAIDS.

In the intervention group, the mean gestational age (GA) was 33 ± 2 weeks, and the average maternal consumption of polyphenols was 1,277 mg/day. After dietary orientation to restrict the consumption of polyphenol-rich food, the average consumption of polyphenols was reduced to 126 mg/day ($p=0.0001$) (Figure 1).

The echocardiographic re-evaluation after a minimum period of 2 weeks showed significant decrease in SDV, DDV and in the RV/LV ratio, as well as a significant increase in ductal PI (Figure 2). A reduction in the mean systolic ductal velocity (1.2 ± 0.4 m/s [0.7 - 1.6] to 0.9 ± 0.3 m/s [0.6 - 1.3], $p=0.018$) (Figure 3) and diastolic ductal velocity (0.21 ± 0.09 m/s [0.15 - 0.32] to 0.18 ± 0.06 m/s [0.11 - 0.25], $p=0.016$) (Figure 4) was observed. There was an increase of the mean PI (2.2 ± 0.03 [2.0 - 2.7] to 2.4 ± 0.4 [2.2 - 2.9], $p=0.04$) (Figure 5) and a decrease in the RV/LV ratio (1.3 ± 0.2 [0.9 - 1.4] to 1.1 ± 0.2 [0.8 - 1.3] ($p=0.004$) (Figure 6).

In the control group, the mean GA was 32 ± 4 weeks, and no statistically significant differences were observed in the

parameters re-evaluated after the 2-week period. The mean daily consumption of polyphenol-rich food was 1,192 mg/day at baseline and 1,093 mg/day two weeks later (Figure 1). The average systolic velocity was 1.3 ± 0.3 m/s and 1.4 ± 0.4 m/s in the first and second examination, respectively (Figure 3), whereas for the mean diastolic velocity these values were 0.2 ± 0.1 m/s and 0.22 ± 0.1 m/s (Figure 4), and for ductal PI the results were 2.2 ± 0.2 and 2.3 ± 0.4 , respectively (Figure 5). The mean RV/LV ratio was 1.2 ± 0.3 at baseline and 1.3 ± 0.2 two weeks later (Figure 6).

Discussion

This study demonstrates that, as already reported for fetuses with ductal constriction, dietary intervention for restricting the intake of foods rich in polyphenols by pregnant women in the third trimester improves ductus arteriosus flow dynamics and decreases the right ventricle size in normal fetuses.

The effects of ductal constriction on fetal hemodynamics, usually leading to right ventricular overload, tricuspid regurgitation of varying degrees and especially increasing the risk of neonatal pulmonary hypertension, a potentially severe and sometimes fatal clinical situation, are well known^{11,12}. The best known etiology of constriction of the ductus arteriosus, which was described almost three decades ago, is the maternal consumption of non-steroidal anti-inflammatory drugs in the third trimester of pregnancy. This effect of anti-inflammatory drugs upon

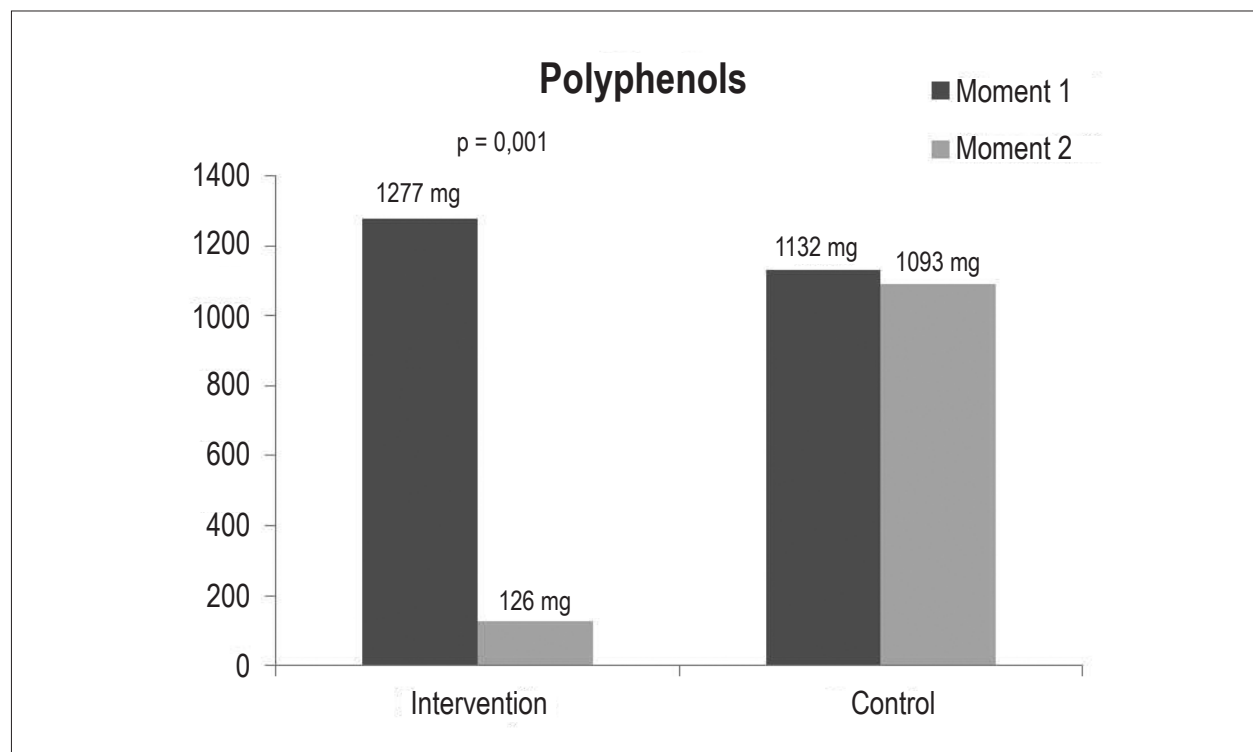


Figure 1 - Assessment of the average maternal consumption of polyphenols through the application of the food frequency questionnaire before (Moment 1) and after (Moment 2) intervention in the intervention and control groups.

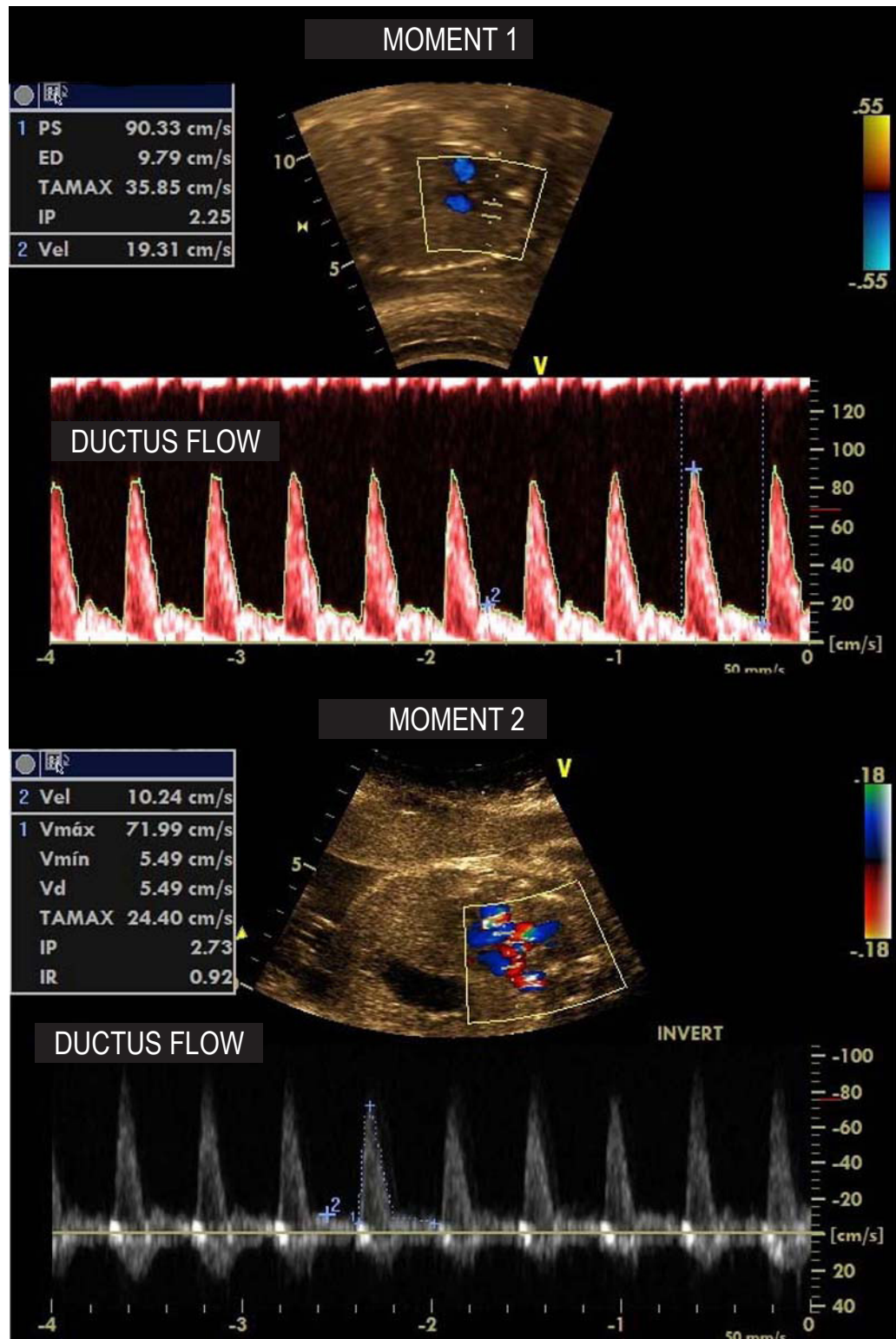


Figure 2 - Doppler echocardiographic findings in a 29-week fetus before and two weeks after dietary intervention for restriction of foods rich in polyphenols. There is decrease of systolic (0.93 m/s to 0.71 m/s) and diastolic (0.19 m/s to 0.10 m/s) velocities, as well as an increase in the ductal pulsatility index (2.25 to 2.73). LV: left ventricle; VD: right ventricle; PA: Pulmonary Artery; Ao: Aorta; DA: Ductus Arteriosus.

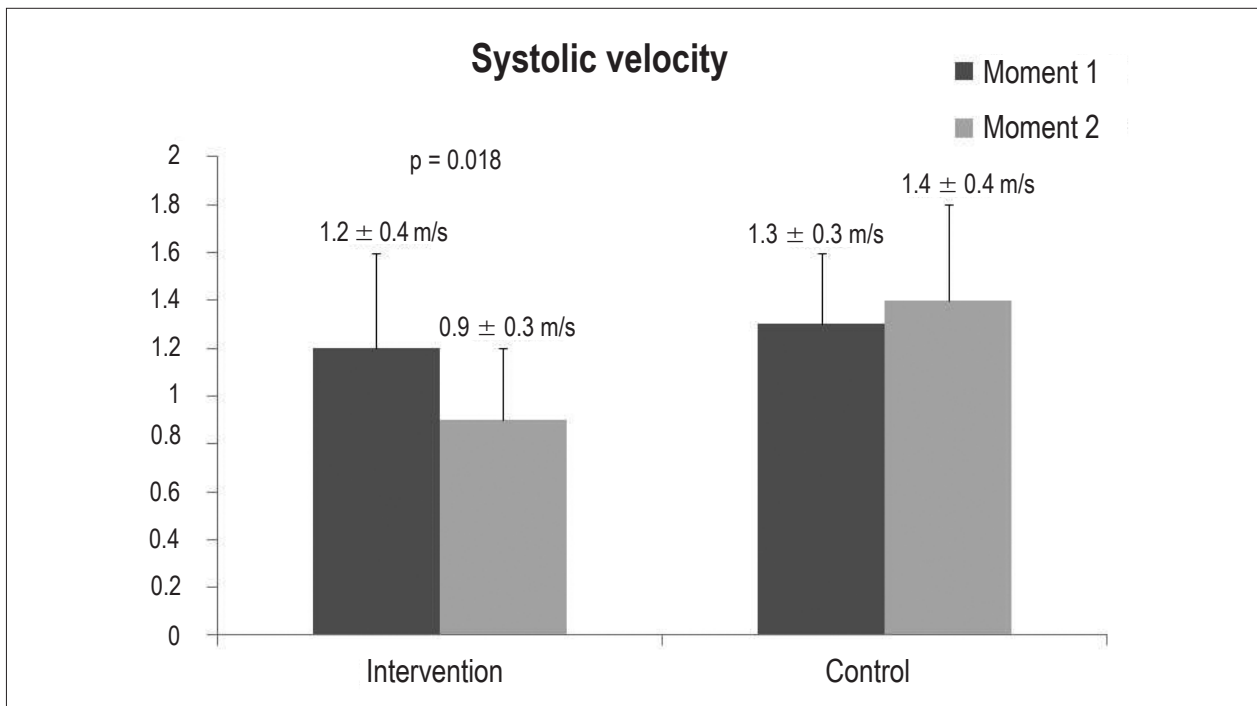


Figure 3 - Ductal systolic velocity before (Moment 1) and after (Moment 2) intervention, in the intervention and control groups.

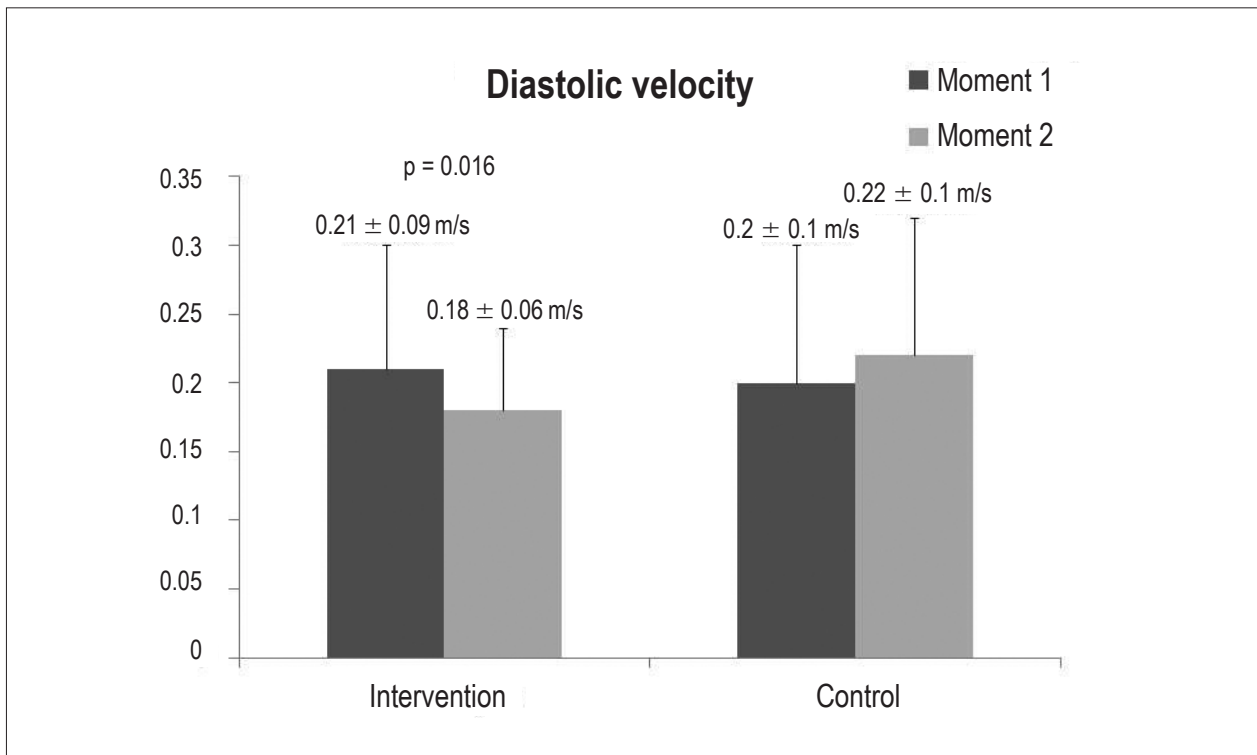


Figure 4 - Ductal diastolic velocity before (Moment 1) and after (Moment 2) intervention, in the intervention and control groups.

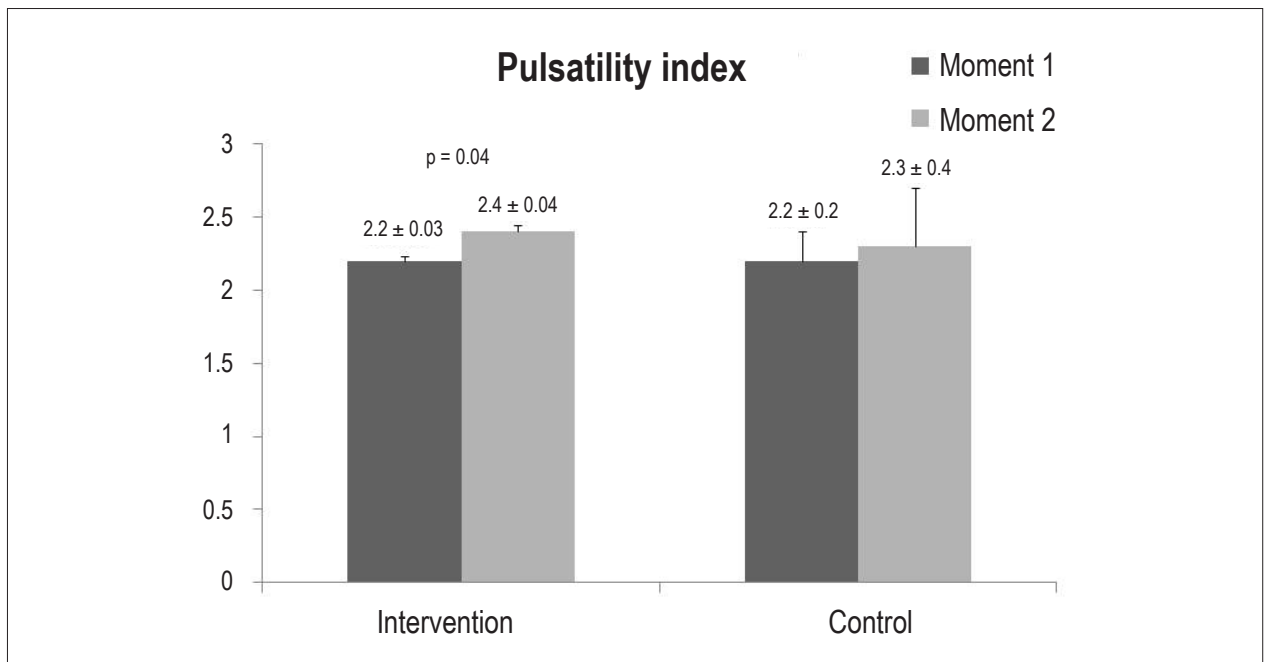


Figure 5 - Assessment of the pulsatility index (PI) before (Moment 1) and after (Moment 2) intervention, in the intervention and control groups.

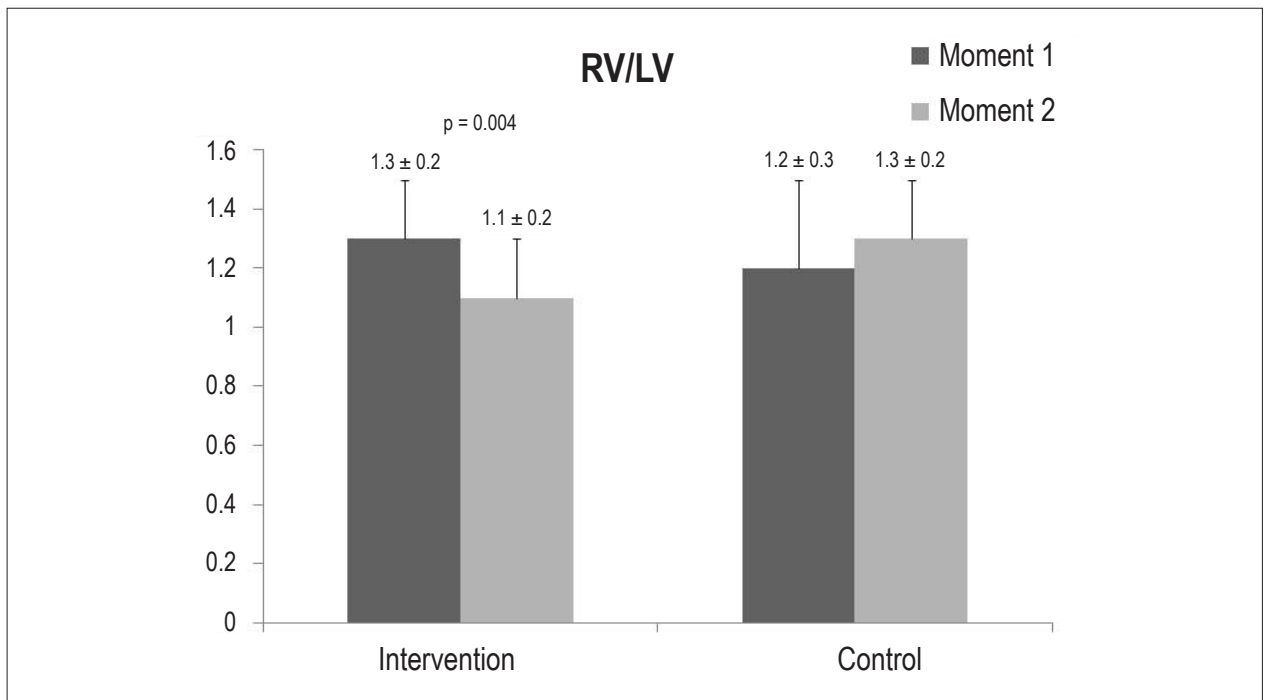


Figure 6 - Follow-up of the ratios between the diameters of the right and left ventricles (RV/LV) before (Moment 1) and after (Moment 2) intervention, in the intervention and control groups.

the ductus is secondary to inhibition of the metabolic route of prostaglandin, especially of cyclooxygenase-2, preventing the transformation of arachidonic acid into prostaglandin¹³⁻¹⁵.

The literature reports on the mechanism of antioxidant and anti-inflammatory action of polyphenols, which are beneficial to a large portion of the population, and the scientific evidence of their ethnomedicinal effect, show that a large number of molecules derived from functional foods and plants have been isolated and even introduced successfully in the international pharmaceutical industry^{16,17}. It has been demonstrated unambiguously that the polyphenols decrease oxidative stress (including in pregnancy)¹⁸, plasma triglyceride and cholesterol levels¹⁹, blood pressure²⁰⁻²², the consequences of gastric hypersecretion²³, the development of some neoplasms²⁴⁻²⁷ and atherosclerosis^{28,29}, the manifestations of aging³⁰ and Alzheimer's disease^{31,32}, and various other health problems. Polyphenols such as quercetin and kaempferol, among many others, are present in many foods and their anti-inflammatory and antinociceptive activities have been shown to be as or more powerful than those of indomethacin³³⁻³⁵. These effects are dependent on the inhibition of modulation of the arachidonic acid and the synthesis of prostaglandins, especially E-2, which is responsible for fetal ductus arteriosus patency. So, it seems clear that this same mechanism is responsible for the only known harmful effect of these substances and foods in which they are present in higher concentration, the observed ductal constriction after maternal intake of foods rich in polyphenols in the third trimester of pregnancy. Foods rich in polyphenolic compounds include mate, black and green tea, other homemade teas, black chocolate, black coffee, grape, orange, tangerine, red fruits, olive oil and other foods which are also usually consumed⁶. Several experimental studies and clinical trials have demonstrated the causal relationship between polyphenols and ductal flow changes, as well as increase in ductal flow velocities and in the right ventricular diameter in normal fetuses exposed to diets rich in polyphenols^{2,3,36,37}.

The conceptual hypothesis of this study was that the systolic and diastolic ductal flow velocities would decrease and the ductal pulsatility index would increase, while the ratio between the right and left ventricular diameters would be reduced, in normal fetuses from mothers with a dietary restriction of polyphenol-rich foods, to levels below the 25 percentile considering the mean consumption of the general population previously described⁴, and in comparison with a control group with no dietary intervention. As mentioned above, this hypothesis was based on knowledge obtained in the past years, and particularly with the demonstration of reversal of the ductal constrictive effects following maternal restriction of polyphenol-rich foods¹. Ductal constriction is a non-categorical, "yes or no" phenomenon, but rather a continuous spectrum with increasing severity related to the clinical manifestations of right ventricular overload, tricuspid and/or pulmonary regurgitation and Doppler-echocardiographic findings of increased systolic and

mainly diastolic ductal flow velocities, as well as decrease of the ductal pulsatility index. Therefore, it seems logical to consider that the initial changes, even though still not filling in the classic criteria of constriction, can develop into more serious forms, exceeding the established diagnostic cutoff points. The sample assessed in this study was composed of normal fetuses, with exclusion of those who already had a diagnosis of ductal constriction, in order to demonstrate how the nutritional guidance can decrease the potential risk for development of the disease.

The study was designed as an open clinical trial, in which participants of the control and intervention groups were included at random and in a sequential and not intentional manner, and the examiners were blind to the nutritional information. The results obtained strongly support the conceptual hypothesis, since all the outcomes originally expected were observed.

The food frequency questionnaire is considered the most practical, informative and the most used method to measure the previous diet, allowing the classification of individuals according to their usual eating patterns. It is also an instrument of easy applicability and low cost, so that it can be used in population studies³⁸. In 1973, the FFQ was recommended by the American Public Health Association as one of the methods for dietary assessment³⁹.

The adherence of the pregnant women who participated in this study to the prescribed diet based on polyphenol-poor foods⁶ shows the feasibility of establishing dietary habits with low consumption of these substances. Of course, it is very difficult to remove completely the polyphenols from the diet, but the initial objective of improving the ductal flow dynamics was achieved with the orientation of the prescribed diet. Since many substitute foods with small amounts of polyphenols are widely available, the nutritional needs of the gestational period can be maintained.

Other studies in this subject are underway, including a multicenter international registry, an investigation of the association between neonatal pulmonary hypertension with maternal consumption of polyphenols in the third trimester, a study investigating the association between prostaglandin levels in maternal circulation with the consumption and excretion of polyphenols by pregnant women, and experimental studies with rat and sheep fetuses to explore the mechanisms of ductal constriction and their relationships with the inflammatory cascade, oxidative stress and the levels of polyphenols.

The number of evidences already available recommends, at least, a note of caution with regard to the consumption by pregnant women of foods with high concentrations of polyphenols.

Conclusion

In conclusion, this study demonstrates that the intervention on maternal diet on the third trimester of pregnancy, restricting the intake of polyphenol-rich food for a period greater than or equal to two weeks, results in improved fetal ductal flow dynamics and reduces the dimensions of the right ventricle. This new data can influence obstetric monitoring and guidance of the eating habits of pregnant women at late pregnancy.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Zielinsky P, Piccoli Jr. AL, Vian I, Naujorks AA, Nicoloso LH; Acquisition of data: Zielinsky P, Piccoli Jr. AL, Vian I, Zílio AM, Nicoloso LH, Barbisan CW, Busato S, Lopes M, Klein C; Statistical analysis: Zielinsky P, Naujorks AA; Obtaining funding: Zielinsky P; Writing of the manuscript: Zielinsky P, Piccoli Jr. AL, Vian I, Zílio AM, Barbisan CW, Busato S, Lopes M, Klein C; Critical revision of the manuscript for intellectual content: Zielinsky P, Piccoli Jr. AL, Vian I, Naujorks AA, Nicoloso LH, Lopes M.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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