ORIGINAL ARTICLE

Fetal ductal constriction caused by maternal ingestion of green tea in late pregnancy: an experimental study

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ABSTRACT

Objectives The aim of this study was to test the hypothesis that experimental maternal intake of green tea in late pregnancy causes fetal ductus arteriosus constriction, probably because of prostaglandin inhibition.

Methods and Results Twelve fetal lambs (pregnancy > 120 days) were assessed before and after maternal administration of green tea ($n = 8$) or water ($n = 4$; controls) as the only source of liquid. After 1 week, echocardiography showed signs of constriction of the ductus arteriosus in all fetuses from mothers ingesting green tea, with increase in mean systolic velocity(from 0.70 ± 0.19 m/s to 0.92 ± 0.15 m/s, 31.4%, $p = 0.001$) and mean diastolic velocity (0.19 ± 0.05 m/s to 0.31 ± 0.01 m/s, 63.1%, $p < 0.001$), decrease of pulsatility index (2.2 ± 0.4 to 1.8 ± 0.3, 22.2%, $p = 0.003$) and increase of mean right ventricular/left ventricular diameter ratio (0.89 ± 0.14 to 1.43 ± 0.23, 60.6%, $p < 0.001$). In the four control fetuses, there were no significant changes. All lambs exposed to green tea also showed at autopsy dilated and hypertrophic right ventricles, which was not present in control fetuses. Histological analysis showed a significantly larger mean thickness of the medial avascular zone of the ductus arteriosus in fetuses exposed to green tea than in controls (747.6 ± 214.6 μm vs 255.3 ± 97.9 μm, $p < 0.001$).

Conclusions This study in fetal lambs shows a cause and effect relationship between experimental maternal exposure of green tea and fetal ductus arteriosus constriction in late pregnancy. © 2012 John Wiley & Sons, Ltd.

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INTRODUCTION

The role of maternal intake of non-steroidal anti-inflammatory drugs (NSAID) in late pregnancy on the genesis of fetal ductal constriction has long been established and the pathophysiological basis to explain this phenomenon is inhibition of circulating prostaglandin biosynthesis.[1–5]

Recent studies suggest that other substances, frequently consumed by pregnant women during gestation, may have anti-inflammatory effects by the same mechanism, thus decreasing prostaglandin levels and being a risk factor for ductal constriction.[2,6–9] Foods and beverages rich in polyphenol compounds, such as herbal teas, grape and orange juice, dark chocolate and many others, fill in that category. We earlier demonstrated that fetal ductal flow dynamics is influenced by the amount of polyphenol maternal intake in human late pregnancy.[10]

Green tea is widely used throughout the world, even during gestation.[11] Its antioxidant and anti-inflammatory effects are well-known and are dependent on their high content of polyphenol components, especially catechins.[12–15] Because these substances have their action mediated by inhibition of prostaglandin biosynthesis, we hypothesized that maternal administration of green tea in late pregnancy in an experimental model of fetal lambs could result in fetal ductal constriction. This study was designed to test this hypothesis.

METHODS

Experimental design

Eight near-term fetal lambs (more than 120 days of gestation) from adult Corriedale sheep carrying only one fetus were assessed using fetal Doppler echocardiography with color flow mapping, before maternal administration of a dilution of green tea in a concentration of 1.7% (33 g of tea for 1.9 L of water) as the only source of liquid. A control fetal Doppler echocardiography was performed 1 week after maternal exposure to green tea. Four control fetuses underwent the same procedure, but the ewes received only water as a liquid

source. The ewes were assigned at random to the experimental or the control group, at a proportion of two cases for each control. There was no need for nasogastric probes to allow the ingestion of green tea or water, thus avoiding sedation or ventilation, and the ewes were allowed to drink at will.

The 12 ewes from both groups were separated from the general flock and left to feed in the same space, with the same environmental conditions of temperature and humidity, at the Veterinary School of University of Rio Grande do Sul, in Porto Alegre. Both groups were fed with alfalfa. Each sheep received an average of 1.9 L per day, or a total of 15,5 l for the experimental group. As a result, the green tea ewes consumed an average of 4149.7 mg of polyphenol/day/sheep (94.6 mg of polyphenol/kg/day), according to the United States Department of Agriculture (USDA) database for the flavonoid content of selected foods table.[12] The control group received the same amount of water (average of 1.9 L per day). A total of 32 packages of 100 g of green tea (Camellia sinensis) were used. The green tea was prepared the night before administration and stored in hot water at 85° C in a covered recipient for 10 min. Then, the tea leaves were removed and the tea was let in room air temperature for 10 h until administration.

Fetal Doppler echocardiographic studies utilized a Siemens Cypress system or a General Electric Logic 4 system, with 2D pulsed and continuous Doppler and color flow mapping capability. At 2D echocardiography, the ductus arteriosus was imaged in sagittal or longitudinal planes and Doppler velocities were measured by positioning the sample volume in the descending aortic end of the ductus arteriosus, with a maximal insonation angle of 20°. The ratio between right and left ventricular dimensions was obtained on a four-chamber view in late diastole to assess right ventricular repercussion. An increase in mean ductal velocities, a decrease in mean pulsatility index, and an increase in mean right ventricular/left ventricular diameter (RV/LV) ratio greater than 20% after exposure were considered signs of ductal constriction.

All studies were performed by the same observer. The possible technical difficulties were prevented by extensive previous training during a period of several months with pregnant ewes. All the examinations were performed with the help of a veterinarian echocardiographer, who participated in the studies giving his technical insights. Systolic and diastolic ductal velocities were measured, as well as the ductal pulsatility index, obtained by the ratio [(systolic – diastolic velocity)/mean velocity]. The presence of ductal flow turbulence, tricuspid and/or pulmonary regurgitation and leftward interventricular septal bulging were searched.

After the control echocardiographic study, the lambs were delivered by cesarean section. As a premedication, they received meperidin 5% (3 mg/kg intramuscular) and acepromazin 1% (0.01 mg/kg intramuscular). The anesthetic induction was made with propofol (3–4 mg/kg I.V.). Anesthesia was maintained with isofluran, and oxygen was administrated by orotracheal intubation. Intraoperative analgesia was made with fentanyl (0.005 mg/kg I.V.), and for postoperative analgesia, cetoprofen 10% (2 mg/kg/day intramuscular for 5 days) and tramadol (9 mg/kg TID intramuscular for 3 days) were used. Immediately after birth, the lambs were sacrificed with a jugular injection of tiopenthal (25 mg/estimated kg weight until cardiac arrest), according to the guidelines of the Brazilian College of Animal Experimentation, in order to obtain the heart–lung specimens for pathological examination.

Morphological and histological analyses

Morphological and histological analyses of the mediastinum were performed. Macroscopically, the right ventricular free wall was measured at the tip of the tricuspid valve. At the same level, maximal diastolic right ventricular diameter was obtained. Right ventricular hypertrophy and dilation in fetuses from the study group were arbitrarily considered when these measurements were above 2 standard deviations of the mean of the control fetuses.

Aortic and pulmonary artery sections were obtained and stained with Verhoeffer-Van Gieson. The fetal ductus was sectioned transversally and also stained with Verhoeffer-Van Gieson. The avascular zone was considered the region of the ductus wall without vasa vasorum between the endothelial cells lining the ductus lumen and the leading edge of the vasa vasorum entering the muscle media from the adventitia. Taking into account the possible variation of the avascular zone thickness in the same ductus, the maximal measured thickness was considered in every fetal lamb.

An Olympus BX40F4 (Olympus, Japan) binocular microscope was utilized for histological measurements, with computer-assisted analysis. Approximations of 100, 200, and 400 X were used. Considering the already demonstrated correlation between the medial avascular zone (without vasa vasorum) thickness of the ductus arteriosus and ductal constriction in fetal lambs, this parameter was measured and compared in cases and control fetuses, according to techniques already described.[16]

Statistical analysis

Data are expressed as mean ± standard deviation. Mean ductal medial avascular zone thickness in cases and controls were compared by Student *t*-test. Wilcoxon paired test was used to compare ductal flow parameters and RV/LV ratios before and after exposition of fetal lambs to green tea. Student *t*-test was also utilized to compare mean ductal velocities and pulsatility index, as well as RV/LV ratios, in cases and controls. Alpha level was set at 0.05 for all statistical tests.

Reproducibility of the measurements was tested. Intra-observer variability for each variable was assessed in all fetuses by repeating offline the measurements on two occasions (2 days apart) under the same basal conditions. Inter-observer variability was performed with offline measurements the same day by a second observer who was unaware of the results of the first examination. Intra-class correlation coefficients were calculated to measure the strength of the agreement between the two sets of measurements for each variable.

Ethical considerations

This study protocol UP 3888/06 was approved by the Research Ethics Committee of the Institute of Cardiology of Rio Grande do Sul, Porto Alegre. Animals were treated according to the guidelines for the use and care of laboratory animals of the Brazilian College of Animal Experimentation (http://www.cobea.org.br), affiliated to International Council of Laboratory Animal Science (ICLAS).

RESULTS

Twelve near-term fetal lambs were examined 1 week after maternal administration of green tea to eight and water to the remaining four (control group) as the only source of liquid based on its habitual daily fluid intake. Comparison of the fetal Doppler echocardiographic assessment obtained before maternal exposure to green tea to a second examination performed 1 week after exposure demonstrated evidences of constriction of ductus arteriosus. Fetuses exposed to maternal ingestion of green tea showed increase in mean ductal peak systolic velocities (0.70 ± 0.19 m/s to 0.91 ± 0.15 m/s, 31.4%, $p = 0.001$) and mean peak diastolic velocities (0.19 ± 0.05 m/s to 0.31 ± 0.01 m/s, 63.1%, $p < 0.001$), decrease of pulsatility index (2.2 ± 0.4 to 1.8 ± 0.3, 22.2%, $p = 0.003$) and increase of mean RV/LV (0.89 ± 0.14 to 1.43 ± 0.23, 60.6%, $p < 0.001$) (Figures 1 and 2), as well as ductal flow turbulence, leftward ventricular septal bulging, and tricuspid regurgitation in all. Pulmonary regurgitation was present in two fetuses. In the four control fetuses receiving only water, there was no significant difference between pre-exposure and post-exposure in all Doppler echocardiographic parameters (peak systolic velocities: 0.64 ± 0.18 m/s and 0.60 ± 0.17 m/s, $p = 0.086$; peak diastolic velocities: 0.18 ± 0.04 m/s and 0.12 ± 0.06 m/s, $p = 0.06$, pulsatility index: 2.2 ± 0.3 and 2.4 ± 0.4, $p = 0.12$, RV/LV: 0.91 ± 0.15 and 0.80 ± 0.18, $p = 0.06$). There was no difference between basal ductal mean velocities, pulsatility index, and RV/LV ratio in fetuses exposed to green tea and contols, but 1 week after exposure, ductal mean velocities and RV/LV ratio were higher and mean pulsatility index was lower than in fetuses which received only water. All fetuses, both from the study group and the control group, remained is sinus rhythm, with no significant changes in heart rate, before and after exposition to green tea or water.

At autopsy, all fetal specimens exposed to maternal intake of green tea showed grossly dilated and hypertrophic right ventricles, not present in the control fetuses exposed only to

Figure 1 Doppler echocardiographic images of a fetal lamb before and after maternal ingestion of green tea for 1 week. (A) Doppler tracing of the fetal ductus arteriosus before maternal exposure to green tea, showing low systolic and diastolic velocities; (B) two-dimensional echocardiographic image of the fetal ductus 1 week after exposure, with color flow mapping showing ductal turbulence; (C) pulsed Doppler spectrum of the ductal flow 1 week after maternal exposure to green tea, showing increased systolic and diastolic velocities and decreased pulsatility index when compared with basal value

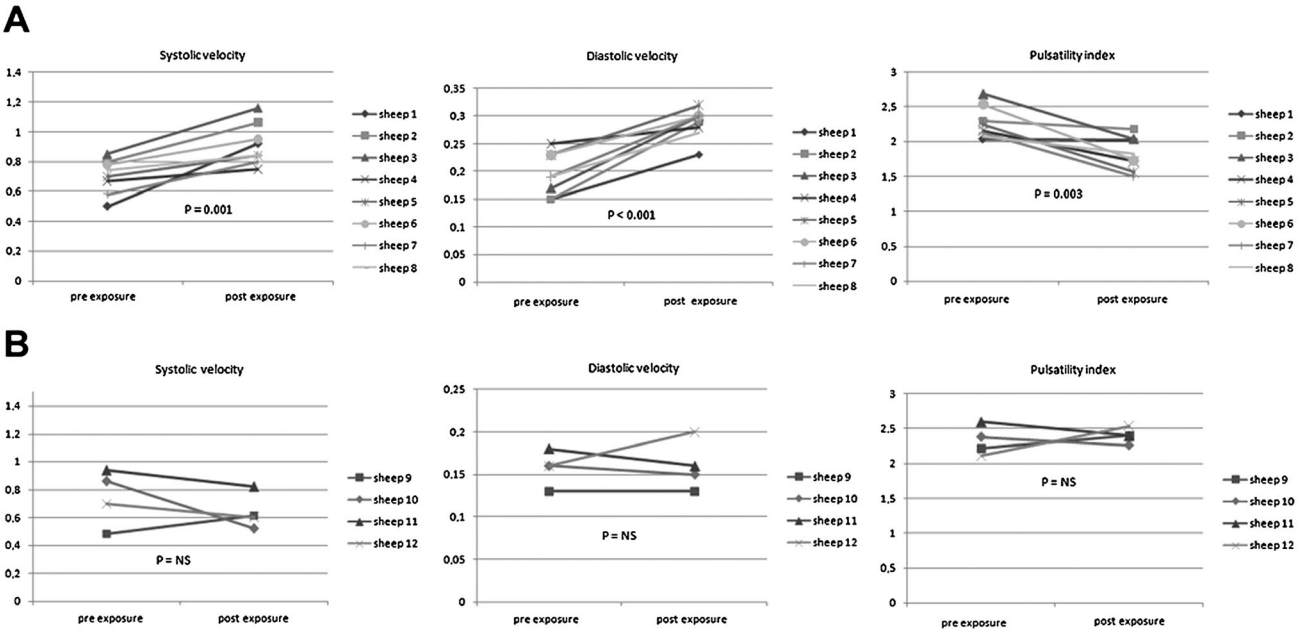

Figure 2 (A) Graphic demonstration of fetal ductal flow velocities and pulsatility index before and after maternal exposure to green tea for 1 week; (B) ductal flow velocity and pulsatility index in control fetuses exposed only to water

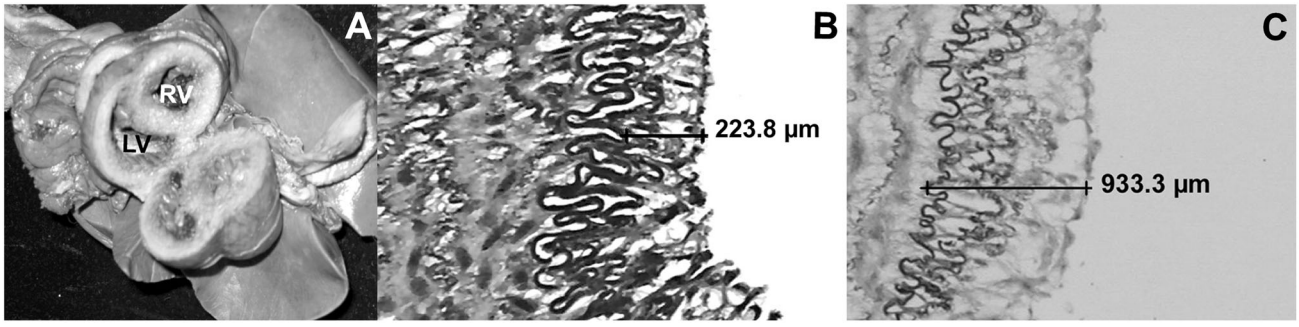

Figure 3 Anatomical and histological features of fetal ductal constriction after maternal exposure to green tea. (A) anatomical specimen of a fetal lamb exposed to green tea: heart showing right ventricular dilation and hypertrophy; (B) histological view (200×) of the ductus arteriosus of a control fetus whose mother received only water, with normal avascular zone thickness (223.8 µm); (C) histological view (200×) of a constricted ductus with marked increase of medial avascular zone thickness (933.3 µm) stained by Verhoeff-Van Gieson. There is immature fibrosis with increase in elastic fibers

water. Histological analysis showed a significantly larger mean thickness of the medial avascular zone of the ductus arteriosus in fetuses exposed to maternal ingestion of green tea than in controls ($747.6 \pm 214.6\,\mu m$ vs $255.3 \pm 97.9\,\mu m$, $p < 0.001$) (Figure 3)

Inter-observer variability of the Doppler measurements was less than 10% of the average and intra-observer variability was less than 20% of the average in all cases for each variable.

DISCUSSION

This study shows that maternal exposure to green tea in the final third of pregnancy caused constriction of fetal ductus arteriosus in an experimental model of fetal lambs. It was demonstrated that the group exposed to concentrated green tea had a significant increase in the ductus arteriosus medial avascular zone thickness when compared with the control group, which showed normal avascular zone thickness. The muscle media of all arteries have an avascular zone adjacent to the lumen, which lacks vasa vasorum.

Previous studies have already shown that pharmacological constriction of fetal ductus arteriosus, induced by indomethacin administration to pregnant ewes, have lead to alterations in the pulmonary vasculature (increase in the medial width/external diameter ratio, by hypertrophy of the vascular smooth muscle) and caused fetal pulmonary hypertension.[17] Another study also reported that indomethacin administered to fetal lambs caused ductal constriction, with an increase in thickness of the medial avascular zone of the fetal ductus arteriosus, which is particularly vulnerable to changes in oxygen supply.[16] There is hypoxia and remodeling of the ductus arteriosus after constriction, with a greater expression of vascular endothelial growth factor and a consequent increase in the avascular zone thickness. In addition, at autopsy, all fetal specimens in the present study exposed to maternal intake of green tea showed dilated and hypertrophic right ventricles, not present in the control fetuses. Even though in humans a higher flow volume than in fetal lambs enters the lung from the pulmonary artery, there is still a huge amount of blood (about 80% of the combined output) which is shunted through the ductus and, for this reason, even a small ductal constriction may interfere with the right ventricular function and with the pulmonary artery pressure. The pulmonary vascular endothelium has been shown to be altered in experimental ductal constriction, with an increased medial layer and a decreased external diameter.[17] An alternative method to assess the influence of green tea on ductal patency would be the measurement of the isometric tension of isolated ductal rings in a preparation of Krebs solution using vasoactive agents with or without adjunction of green tea in the medium.

The ewes were not monitored after administration of green tea in this 'chronic' exposure study, with the ewes being isolated from the general flock and allowed to drink the substance at libitum.

Very few reported negative maternal effects to green tea, and these are mainly related to its caffeine content, such as central nervous system and muscle stimulation. Some concerns about the possible decrease of first trimester maternal folate levels in human pregnancies exposed to green tea have been raised. None of these effects were expected to interfere with the present experiment.

In accordance to autopsy findings, fetal ductal systolic and diastolic velocities obtained using Doppler echocardiography increased significantly and the ductal pulsatility index significantly decreased 1 week after a concentrated solution of green tea has been daily administrated to the pregnant ewes. Even though mean systolic velocity was not as high as that described in humans, an evident alteration of ductal dynamics could be inferred. More importantly, the increase in mean diastolic peak velocity and the decrease of ductal pulsatility index observed in this study were considered relevant diagnostic evidences of fetal ductal constriction.

Likewise, similar to autopsy results, fetal RV/LV dimension ratios also significantly increased after maternal ingestion of green tea for 1 week, accounting for the higher right ventricular pressure as a result of increased afterload. The control group, in which no fluid other than pure water was given to the sheep, did not show changes in fetal ductus arteriosus velocities and pulsatility index, nor in RV/LV dimension ratios after 1 week. In addition, other signs of right ventricular overload were present in all the exposed fetuses, such as leftward bulging of the interventricular septum and tricuspid regurgitation.

Serial echocardiographic studies to determine the early stages of ductal patency alterations were not performed,

because the purpose of the study was to establish a cause and effect relationship between high maternal exposure to green tea and fetal ductal constriction. The experimental design did not include maternal and fetal blood sampling, being the endpoints the Doppler and histological data.

During the third trimester of human pregnancy, patency of fetal ductus arteriosus is dependent on the presence of circulating prostaglandins.[18–20] As a consequence, substances that inhibit prostaglandin biosynthesis may have a constrictive action upon the ductus. NSAID inhibits the prostaglandins pathway and for this reason may cause ductal constriction, a potentially severe clinical condition that may lead to neonatal pulmonary hypertension and its potentially severe consequences.[2–4,17,21,22]

Anti-inflammatory effects of flavonoids or polyphenols present in commonly consumed foods and beverages are widely reported in the literature.[13,23–27] They act based on the inhibition of COX-2 mediated transformation of arachidonic acid in prostaglandins and are analogous to that of NSAID. Green tea is made up by non-fermented and non-oxidated leaves of Camellia sinensis, which contain large amounts of polyphenolic compounds, especially flavonoids. Among the predominant flavonoids are catechins: epicatechin, gallocatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG), which is the most abundant catechin in green tea, with a concentration of 7.0 g per 100 g of dry leaves.[12] Flavonoids and catechins present a series of biological activities, such as antioxidant, chemoprotective, anti-inflammatory, and anticarcinogenic effects. EGCG has potent anti-inflammatory action, inhibiting *in vitro* the activation of nuclear factor-beta transcription factor at the same time that it inhibits degradation of IKB-alpha induced by cellular activation mediated by tumoral necrosis factor-alpha.[14,23,28] The anti-inflammatory action of EGCG seems to be associated to a decrease in activation of inhibitor of kappa B kinase protein, involved in IK-beta fosforilation. EGCG being the most pharmacologically active and anti-inflammatory compound, it is believed that it is the green tea substance which most interferes in the process of fetal ductus arteriosus constriction, by attenuation of cyclooxygenase-2 and inducible nitric oxide synthetase.[14,15,23,28,29]

A previous study demonstrated that fetal ductal constriction is more frequently observed in mothers consuming polyphenol-rich foods, with known antioxidant and anti-inflammatory effects.[8] Amelioration or complete reversion of the constrictive effect upon the ductus after interruption of NSAID usage has already been established.[30] The same effect was also observed following maternal discontinuation of polyphenol-rich beverages in more than 90% of controlled exposed fetuses.[31]

We have recently shown that normal fetuses have higher ductal flow velocities and right ventricular size when exposed to maternal ingestion of a polyphenol-rich diet (above the 75th percentile for the local population) than fetuses exposed to a polyphenol-poor maternal diet (below the 25th percentile), in the absence of ductal constriction.[10] These changes are probably related to the anti-inflammatory effects of these substances on ductus arteriosus dynamics. The observations for us represent a *cause and effect* relationship, which was the sole purpose of the experiment. Because there was a large amount of polyphenol substances administered, the period of 1 week to assess the results was considered sufficient. We are of course aware of the intrinsic methodological restrictions of the animal experimentation and of the difficulty for translation of the animal experimental findings to a clinical situation.

The results of the present study corroborate the conceptual hypothesis, demonstrating the effect of maternal exposure of green tea on the fetal ductus arteriosus in late pregnancy in an experimental model of fetal lambs. Other beverages and foods rich in polyphenol substances, commonly used during human gestation, may have similar actions and should be tested in other studies.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Maternal intake of non-steroidal anti-inflammatory drugs in late pregnancy may cause fetal ductal constriction as a result of inhibition of prostaglandin biosynthesis.
- Foods rich in polyphenol compounds, such as herbal teas, may have anti-inflammatory effects by the same mechanism, thus decreasing prostaglandin levels and being a risk factor for ductal constriction.
  We have demonstrated that fetal ductal flow dynamics is influenced by the amount of polyphenol maternal intake in human late pregnancy.

WHAT DOES THIS STUDY ADD?

- This study shows that maternal exposure to green tea in the final third of pregnancy caused constriction of fetal ductus arteriosus in an experimental model of fetal lambs.

REFERENCES

1. Takami T, Momma K, Imamura S. Increased constriction of the ductus by dexamethasone, indomethacin, and rofecoxib in fetal rats. Circ J 2005;69:354–8.
2. Luchese S, Manica JL, Zielinsky P. Intrauterine ductus arteriosus constriction: analysis of a historic cohort of 20 cases. Arq Bras Cardiol, 2003;81:405–10, 399–404.
3. Momma K, Hagiwara H, Konishi T. Constriction of fetal ductus arteriosus by non-steroidal anti-inflammatory drugs:study of additional 34 drugs. Prostaglandins 1984;28:527–36.
4. Koren G, Florescu A, Costei AM, Boskovic R, Moretti ME. Nonsteroidal antiinflammatory drugs during third trimester and the risk of premature closure of the ductus arteriosus: a meta-analysis. Ann Pharmacother 2006;40:824–9.
5. Toyoshima K, Takeda A, Imamura S, Nakanishi T, Momma K. Constriction of the ductus arteriosus by selective inhibition of cyclooxygenase-1 and-2 in near-term and preterm fetal rats. Prostaglandins Other Lipid Mediat 2006;79:34–42.

6. Zielinsky P, Manica JL, Piccoli A, *et al.* Ductal flow dynamics and right ventricular size are influenced by maternal ingestion of polyphenol-rich common beverages in normal pregnances. Ultrasound Obstet Gynecol 2007a;30:397.
7. Zielinsky P, Manica JL, Piccoli A, *et al.* Experimental study of the role of maternal consumption of green tea, mate tea and grape juice on fetal ductal constriction. Ultrasound Obstet Gynecol 2007b;30:515.
8. Zielinsky P, Manica JL, Piccoli A, *et al.* Fetal ductal constriction triggered by maternal igestion of polyphenol-rich common beverages: a clinical approach. J Am Coll Cardiol 2007c;49:258A.
9. Zielinsky P, Manica JL, Piccoli A, *et al.* Green tea and mate tea cause fetal ductal constriction: an experimental study. J Am Coll Cardiol 2007d;49:259A.
10. Zielinsky P, Piccoli AL, Jr., Manica JL, *et al.* Maternal consumption of polyphenol-rich foods in late pregnancy and fetal ductus arteriosus flow dynamics. J Perinatol 2010;30:17–21.
11. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Prev Med 1992;21:334–50.
12. USDA. USDA Database for the Flavonoid Content of Selected Foods Release, 2007. Available at: http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02-1.
13. Geronikaki AA, Gavalas AM. Antioxidants and inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. Comb Chem High Throughput Screen 2006;9:425–42.
14. Hussain T, Gupta S, Adhami VM, Mukhtar H. Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. Int J Cancer 2005;113:660–9.
15. Lee JS, Oh TY, Kim YK, Baik JH, So S, Hahm KB, Surh YJ. Protective effects of green tea polyphenol extracts against ethanol-induced gastric mucosal damages in rats: stress-responsive transcription factors and MAP kinases as potential targets. Mutat Res 2005;579:214–24.
16. Clyman RI, Chen YQ, Chemtob S, Mauray F, Kohl T, Varma DR, Roman C. In utero remodeling of the fetal lamb ductus arteriosus: the role of antenatal indomethacin and avascular zone thickness on vasa vasorum proliferation, neointima formation, and cell death. Circulation 2001;103:1806–12.
17. Levin DL, Mills LJ, Weinberg AG. Hemodynamic, pulmonary vascular, and myocardial abnormalities secondary to pharmacologic constriction of the fetal ductus arteriosus. A possible mechanism for persistent pulmonary hypertension and transient tricuspid insufficiency in the newborn infant. Circulation 1979;60:360–4.
18. Michelakis ED, Thebaud B, Weir EK, Archer SL. Hypoxic pulmonary vasoconstriction: redox regulation of O2-sensitive K + channels by a mitochondrial O2-sensor in resistance artery smooth muscle cells. J Mol Cell Cardiol 2004;37:1119–36.
19. Slomp J, Gittenberger-de Groot AC, Glukhova MA, Conny van Munsteren J, Kockx MM, Schwartz SM, Koteliansky VE. Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. Arterioscler Thromb Vasc Biol 1997;17:1003–9.
20. de Reeder EG, Gittenberger-de Groot AC, van Munsteren JC, Poelmann RE, Patterson DF, Keirse MJ. Distribution of prostacyclin synthase, 6-keto-prostaglandin F1 alpha, and 15-hydroxy-prostaglandin dehydrogenase in the normal and persistent ductus arteriosus of the dog. Am J Pathol 1989;135:881–7.
21. Momma K, Konishi T, Hagiwara H. Characteristic morphology of the constricted fetal ductus arteriosus following maternal administration of indomethacin. Pediatr Res 1985;19:493–500.
22. van Marter LJ, Leviton A, Allred EN, Pagano M, Sullivan KF, Cohen A, Epstein MF. Persistent pulmonary hypertension of the newborn and smoking and aspirin and nonsteroidal antiinflammatory drug consumption during pregnancy. Pediatrics 1996;97:658–63.
23. Kumar N, Shibata D, Helm J, Coppola D, Malafa M. Green tea polyphenols in the prevention of colon cancer. Front Biosci 2007;12:2309–15.
24. Schinella G, Fantinelli JC, Mosca SM. Cardioprotective effects of Ilex paraguariensis extract: evidence for a nitric oxide-dependent mechanism. Clin Nutr 2005;24:360–6.
25. de la Lastra CA, Villegas I. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. Mol Nutr Food Res 2005;49:405–30.
26. Bixby M, Spieler L, Menini T, Gugliucci A. Ilex paraguariensis extracts are potent inhibitors of nitrosative stress: a comparative study with green tea and wines using a protein nitration model and mammalian cell cytotoxicity. Life Sci 2005;77:345–58.
27. Fantinelli JC, Schinella G, Cingolani HE, Mosca SM. Effects of different fractions of a red wine non-alcoholic extract on ischemia331 reperfusion injury. Life Sci 2005;76:2721–33.
28. Mandel S, Weinreb O, Amit T, Youdim MB. Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (−)-epigallo-catechin-3-gallate: implications for neurodegenerative diseases. J Neurochem 2004;88:1555–69.
29. Wheeler DS, Catravas JD, Odoms K, Denenberg A, Malhotra V, Wong HR. Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. J Nutr 2004;134:1039–44.
30. Vermillion ST, Scardo JA, Lashus AG, Wiles HB. The effect of indomethacin tocolysis on fetal ductus arteriosus constriction with advancing gestational age. Am J Obstet Gynecol 1997;177:256–9; discussion 259–61.
31. Zielinsky P, Piccoli AL, Jr., Manica JL, *et al.* Reversal of fetal ductal constriction after maternal restriction of polyphenol-rich foods: an open clinical trial. J Perinatol 2011, advance online publication, 3 Nov 2011, DOI:10-1038/jp.2011.153