

CONSTRICTION OF FETAL DUCTUS ARTERIOSUS AND MATERNAL INTAKE OF POLYPHENOL-RICH FOODS



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Abstract

Fetal ductal constriction is a potentially severe functional alteration, often causing right ventricular overload and insufficiency, tricuspid regurgitation and neonatal pulmonary hypertension. Classically, maternal administration of indomethacin and/or other nonsteroidal antiinflammatory drugs interfere in prostaglandin metabolism, leading to ductal constriction. However, many cases of fetal ductal constriction, as well as of persistent neonatal pulmonary artery hypertension, remain without an established etiology, being referred as "idiopathic". In recent years, a growing body of evidences has shown that herbs, fruits, nuts, and a wide diversity of substances commonly used in daily diet, because of their high content of polyphenols, have definitive effects upon the metabolic pathway of inflammation, with consequent inhibition of prostaglandins synthesis. This anti-inflammatory action of polyphenols, when ingested during the third trimester of pregnancy, may interfere with the dynamics of fetal ductus arteriosus flow and cause ductal constriction. This review has the purpose to approach these new evidences, which may influence dietary orientation during pregnancy.

Key words: ductal constriction, polyphenols, pulmonary hypertension, prostaglandins, antiinflammatory substances

The ductus arteriosus originates from the distal portion of the left sixth aortic arch, which connects the left pulmonary artery to dorsal aorta¹. Its histological structure is formed by an internal elastic membrane, a tunica media muscular layer and an external adventitial layer. The muscular layer is predominant, which makes the ductal structure different from aorta and pulmonary artery, and increases with gestational age. It has a circumferential orientation, mainly at the external layers, which facilitates and makes effective ductal constriction².

The ductus arteriosus is positioned between the pulmonary artery, near the emergency of left pulmonary artery, and the aorta, at the zone of the isthmus. At the aortic-pulmonary plan, around 80-85% of the poorly saturated blood (50% oxygen) ejected by the right ventricle into the main pulmonary artery passes through the ductus arteriosus to the descending aorta (around 75 ml/min), and as a result will mix with the blood coming from the left ventricle. Due to the high pulmonary vascular resistance, only 15-20% (near 15 ml/min) are directed to the lungs. Around 40-50% of the descending aorta flow pass through the umbilical arteries and return to the placenta for the hematosis. The remaining blood will nourish the organs and the inferior body half³.

The ductus arteriosus shows a peculiar differentiation program in order to prepare itself for postnatal spontaneous closure¹. There is a relationship between gestational age and the histological maturation of the ductus⁴. The process of fetal intimal thickening starts at the second trimester of pregnancy and is characteristically a continuous process. This mechanism of intimal thickening seems to be linked to prostacyclin syntase (PGI₂ syntase), which has a regulating role on ductal patency⁵. During ductus arteriosus closing there are higher PGI₂ syntase levels in smooth muscle cells at the sites of intimal thickening than in other places. These findings demonstrate the relationship between ductal morphology and the presence of PGI₂ syntase^{6,7}. Vascular remodelling also seems to be associated to dedifferentiation of the smooth muscle cells and to apoptosis present in the areas of tunica media and intimal layers⁸.

Hemodynamic alterations during the immediate neonatal period occur at the moment of cessation of placental blood circulation, lung insuflation, pulmonary vasodilation and foramen ovale closure. The sudden increase in systemic vascular resistance and the decrease in pulmonary vascular resistance generate a reverse flow through the ductus arteriosus and an abrupt increase

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Figure 1. Ductus entering the descending aorta. 3D image in a 32 weeks normal fetus

in pulmonary flow. Some minutes after birth, 90% of the blood ejected by the right ventricle is directed to the pulmonary arteries. With the decrease in pulmonary vascular resistance there is an increase in pulmonary blood flow, which culminates with ductal occlusion^{9,10}. The functional closure of ductus arteriosus is initiated by a mechanism induced by the higher blood oxygen concentration¹¹. This mechanism, albeit mediated by prostaglandins and endothelins, is intrinsic to smooth muscle cells¹². It is a potentially reversible phenomenon which occurs 8-72 hours after birth, secondary to muscular constriction. After this event, there is a remodelling of the vascular wall, with neointimal formation caused by proliferation and migration of smooth muscle cells from the tunica media to the subendothelial layers. This seems to be the final event of a process which initiates at the second trimester of pregnancy, starting with the accumulation of glycosaminoglycans at the subendothelial region⁵. Usually, the ductus arteriosus remains patent for some hours or days in the neonatal period.

Physiological closure of the ductus in the term neonate starts with a phase of functional obliteration secondary to the wall vessel muscular constriction. The closure is gradual and is completed more frequently in 10 to 15 hours after birth¹³. Observations in neonates show that the arterial duct start to close at the pulmonary arterial end, and then the constriction spread to the aorta¹⁴. After completion of ductal occlusion, the arterial ligament is formed¹⁵. If the ductus arteriosus remains patent in

a term neonate, this is considered a pathological condition. The premature baby shows a delay in the remodelling process of the tunica media layer and is less responsive to oxygen, probably as a result of the immaturity of the structures^{16,17}.

Since the ductus has a predominant muscular layer, its occlusion is influenced by a number of different constrictor and relaxing factors. Relaxing factors are prostaglandins, nitric oxide and bradykinin, which cause liberation of prostaglandins and nitric oxide. Constrictive factors are oxygen, high doses of bradykinin and autonomic nervous system, both sympathetic and parasympathetic¹⁴. The vasoconstrictive effect is dose-dependent to several neurotransmitters, such as **acetylcholine, histamine, serotonin and catecholamins**. With the increase in gestational age, the ductus becomes less sensitive to the dilating effects and more sensitive to constrictive factor^{14, 18, 19}.

Production of prostaglandins is dependent of two enzymes which act in different states, cyclo-oxygenase-1 (COX-1), expressed endogenously, and cyclo-oxygenase-2 (COX-2), locally induced during inflammatory processes^{7, 20}.

Prostaglandins have been extensively studied, with clear demonstration of its potent vasodilating action upon the ductus arteriosus. However, in the last years new substances with constrictive and dilating effects on the ductus arteriosus have been described. The dilating action of 3- and 5-phosphodiesterase inhibitors upon fetal and neonatal ductus arteriosus in rodents have been reported^{21,22}. This effect was shown to be more potent in fetuses than in neonates, suggesting that these substances could be useful in primary and secondary fetal ductal constriction, especially in preterm fetuses when compared to term fetuses²³. Other substances with dilating effect on the ductus were described, based on the knowledge of the role of endothelin receptors as messengers of postnatal ductal constriction^{9, 24, 25}. It was shown that antagonists of endothelin receptors cause potent in vitro inhibition of the constrictive effect of cyclo-oxygenase inhibitors during fetal life, and of the postnatal physiological ductal constriction induced by oxygen^{26, 27}.

Among the substances with known constrictive effect upon the ductus arteriosus, **indomethacin**, a cyclo-oxygenase inhibitor used in the treatment of premature labor, is one of the most extensively studied 28-30. Fetal ductal constriction may occur after few hours after maternal administration and its action may last for several weeks. For this reason, the absence of signs of ductal constriction after 24 to 72 hours of usage does not exclude the diagnosis^{28, 29, 31-33}. Ductal sensitivity to indomethacin increases with gestational age, occurring in 5-10% of fetuses with less than 27 weeks, but reaching nearly 100% after 34 weeks of gestation^{34, 35}. In addition to indomethacin, it has been shown that several other **nonsteroidal anti-inflammatory drugs (NSAID)**, such

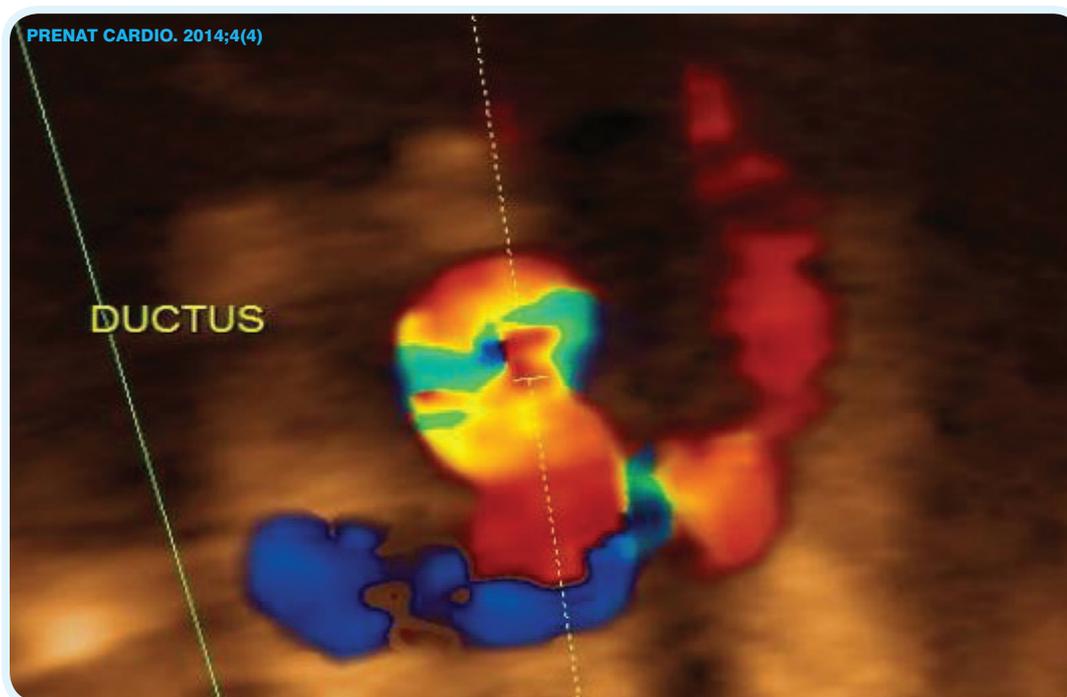


Figure 2. Ductal constriction. Color Doppler in a 33 weeks fetus exposed to a maternal polyphenol-rich diet. Notice the turbulent ductal flow.

as nimesulide³⁶, diclofenac³⁷, aspirin, metamizole, ibuprofen³⁸ and many others also have the potential to cause constriction of ductus arteriosus. Selective cyclo-oxygenase-2 inhibitors, such as rofecoxib^{20,38,39}, have also shown a constrictive ductal effect on rat and sheep fetuses⁴⁰⁻⁴². **Sulindac**, another prostaglandin inhibitor drug utilized in premature labor, was demonstrated to have a milder and more transient constrictive effect on fetal ductus than indomethacin³³. Glucocorticoids also show effects upon ductus arteriosus patency^{43,44}, but the pathophysiologic mechanism involved in the alteration of ductal tonus does not seem to be the same. There is apparent reduction in the ductal sensitivity to prostaglandin E₂, which may be consequent to inhibition of the enzymatic liberation of arachidonic acid from phospholipids, a step in prostaglandin synthesis preceding cyclo-oxygenase⁴⁵. Similar to other anti-inflammatory drugs, the ductal effect of glucocorticoid is dose-dependent⁴⁶. Moreover, if associated to selective or non selective NSAID, glucocorticoid have a synergistic action which increase frequency and severity of ductal constriction; its incidence may double, possibly due to glucocorticoid ability to decrease the ductal sensitivity to prostaglandin^{20,30}. Other experimentally tested substances with proven constrictive action on rat fetuses are retinoic acid^{20,30,34,47}, antagonists of prostanoid EP₄ receptors^{21, 48, 49} and inhibitors of nitric oxide synthesis (L-name)⁵⁰⁻⁵³, the latter with proven effects in humans. Recently, a novel mechanism for sustaining postnatal ductal constriction induced by oxygen has been described, based on activation of the enzyme Rho-kinase⁵⁴.

The action of NSAID result from inhibition of prostaglandin synthesis, by inactivation of cyclo-oxygenases. COX-1 and COX-2 are enzymes involved in prostaglandin, prostacyclin

and thromboxane biosynthesis⁷. This inhibitory mechanism interferes with the synthesis of prostaglandin G₂, which is a precursor of prostaglandin E₂eF₂^{28,29}. The use of anti-inflammatory drugs during pregnancy for the treatment of premature labor, pre-eclampsia or intrauterine growth restriction through prostaglandin biosynthesis inhibition has allowed the study of the relation between ductal constriction and cyclo-oxygenase inhibitors.

Indomethacin is the drug with prostaglandin inhibiting action most widely reported in the literature. Its inhibitor effect on cyclo-oxygenase is reversible, persisting until the drug is excreted^{32,55}. The drug passage through the placental barrier occurs freely during the second half of pregnancy, being minimal in early gestation⁵⁶. The response to indomethacin is individual in each fetus, and even in a twin pregnancy only one fetus could be affected, which suggests differences in ductus maturation⁵⁷. The ductus arteriosus becomes more sensitive to indomethacin as gestational age increases, ductal constriction occurring in 5-10% in fetuses with less than 27 weeks, 15-20% in fetuses between 27 and 31 weeks, 50% at the 32nd week and near 100% above 34 weeks. The occurrence of ductal constriction before the 27th week is uncommon, but there are reports of cases with 22 weeks⁵⁸.

As already mentioned, many other nosteroidal anti-inflammatory compounds beside indomethacin are potentially involved in ductal constriction, such as nimesulide³⁶, diclofenac³⁷, aspirin, metamizole, o ibuprofen³⁸, and others⁵⁹. An experimental study in rats has suggested a gradation in the magnitude of the action of NSAID upon the fetal ductus, being the constrictive effect dose-dependent⁶⁰.

Glucocorticoids are synthetic hormones which mimic endogenous cortisol actions, a hormone produced by the glomerular zone of the adrenal gland. Glucocorticoids also act on ductal patency. As occurs with the majority of other anti-inflammatory substances, this effect is dose-dependent⁴⁶. There is enzymatic liberation of arachidonic acid, blocking prostaglandin synthesis⁴⁵, and apparent reduction in sensitivity of the ductus to prostaglandin E₂. Despite the tendency to premature closure of ductus



Figure 3. Ductal constriction. 3D image obtained with STIC of a tortuous and constricted ductus in a 29 weeks fetus.

arteriosus, the mechanism of action seems to be related to a primary alteration of the vessel, decreasing vascular reactivity to the relaxing effects of prostaglandin E_2 , without altering its synthesis^{40, 61}. The association of corticosteroids with indomethacin has shown a synergistic effect⁶², and the incidence of ductal constriction doubles when these drugs are taken together, even though other studies have shown that the incidence of ductal constriction with glucocorticoid in isolation was similar to that of a control group³⁰.

Premature constriction of ductus arteriosus is followed by fetal hemodynamic repercussion. The higher resistance in the ductus generates blood flow turbulence, with increase in systolic and diastolic velocities and decrease in ductal pulsatility index. As a result, there is dilation of the pulmonary artery, right atrium and right ventricle, right to left bulging of the interventricular septum, tricuspid and pulmonary insufficiency and systolic and diastolic ventricular dysfunction^{36, 63}.

It has been demonstrated in a study in fetuses from 28 to 32 weeks of gestation after indomethacin administration that the drug shows a reversible constrictive effect on the ductus with significant reduction of pulsatility index and association with secondary disturbances, mainly in the right ventricle, as a result of the increase in afterload, observed after about 4 hours, and normalization 24 hours after withdrawal of the substance. It was suggested that the hemodynamic alterations secondary to ductal constriction are right ventricular dilation and signs of heart failure, followed by concentric hypertrophy, decrease

in right ventricular chamber caused by mass increase and left ventricular compromise. These ventricular repercussions were more prominent in the presence of tricuspid regurgitation^{64, 65}. Flow redirected through the foramen ovale results in left chambers volumetric overload⁶⁵⁻⁶⁷. The aortic isthmus shows a rapid increase in its sectional area in response to local increased flow⁶⁸. This redistribution keeps peripheral perfusion and may explain the findings from clinical experience that show that severe ductal constriction is well tolerated for some days in the human fetus.

Past experimental studies used to speculate that constriction of ductus arteriosus resulted in increase of the tunica media layer of pulmonary arteries and generates a secondary increase in intrauterine vascular pulmonary resistance⁶⁹. The hemodynamic alterations in ductal constriction may be related to pulmonary vascular alterations. In the vast majority of studies directed to increase the knowledge about fetal and neonatal pulmonary arterial hypertension, fetal ductal constriction is the experimental model of choice. In a classical study, administration of indomethacin to fetal lambs was followed by fetal ductal constriction and pulmonary hypertension⁷⁰. Blocking of prostaglandin biosynthesis has probably a direct effect in pulmonary arterioles in mammalian fetuses^{71, 72}. The sustained increase in right ventricular afterload is capable to produce morphological, functional and hemodynamic modifications, with chronic histological and degenerative alterations of the right ventricular myocardium⁷³. Severe ductal constriction may interfere in placental flow and myocardial performance, and may lead to fetal death. If ductal constriction is less severe or chronic, fetal pulmonary arterial hypertension may be a consequence of excessive development of the arteriolar smooth muscular layer and constriction of the pulmonary arterioles. Predominance of the increased thickness of the muscular layer and of the aerial pathway mass is a feature less described in neonatal pulmonary hypertension consequent to other causes^{74, 75}. In cases related to maternal drugs usage, ventricular dysfunction may reverse after its suspension. However, if this picture is not treated, it may be followed by endocardial ischemia and right ventricular papillary muscles dysfunction, and later on by heart failure, hydrops and potentially death⁵⁸. Intrauterine ductal constriction may cause transient or permanent tricuspid regurgitation and neonatal myocardial ischemia^{69, 76}.

In clinical practice, in cases with severe ductal constriction after prostaglandin inhibitory drugs, the suspension of its usage may result in decrease of ductal velocities and increase in pulsatility index within 24 hours, with posterior normalization of hemodynamic consequences⁵⁵. Mild cases may be approached with just

a decrease in the administered substance concentration, but in every fetus serial echocardiographic follow-up is recommended²⁸.

The evidence of fetal cardiac dysfunction was described as a characteristic feature of fetal closure of ductus arteriosus⁷⁷. In severe cases, interruption of pregnancy may be indicated, with neonatal cardiopulmonary resuscitation. The clinical course after birth depends on the severity of intrauterine right ventricular cardiac insufficiency and to the response to elevation in pulmonary vascular resistance⁷⁸.

Long term prognosis is still uncertain, but when early evolution is favorable, there are usually no late complications. After the occurrence of fetal heart failure, right ventricular functional abnormalities may persist throughout the neonatal period, even in those patients with a benign outcome.

Utilization of echocardiographic and Doppler techniques has allowed that the diagnosis of fetal ductal constriction, formerly possible only in necropsy, could be made in prenatal life⁷⁹. Fetuses at risk for development of premature constriction of ductus arteriosus could be monitored and submitted to early intervention when necessary.

Echocardiographic diagnosis of fetal ductal constriction is based on the presence, at color Doppler, of turbulent flow in the ductus with increased systolic velocity SV (higher than 1.4 m/s), increased diastolic velocity DV (higher than 0.30 m/s) and decreased pulsatility index PI (below 2.2). In the first publications, the cutoff point for the pulsatility index was described as 1.9⁸⁰, but more recent studies have considered a somewhat higher limit^{81, 82}. The PI

is independent of the ultrasound angle and is useful in the differential diagnosis when there is increased ductal flow without concomitant constriction. This situation may occur when the increased SV is caused by an increase in right ventricular output. The PI does not change with gestational age and should be used to define the diagnosis⁸³ (FIGURE 4). When there is total occlusion of the ductus arteriosus, absence of transductal flow is considered diagnostic. With the increase in afterload secondary to ductal constriction, the fetal heart shows initially proliferative growing and, at later stages, hyperplasia is substituted to apoptosis and hypertrophic response⁸⁴. There is characteristically increase in right to left diameters ratio, an increase in pulmonary artery to aorta ratio and interventricular septum bulging toward the left ventricle³³.

Right ventricular systolic and diastolic function are impaired in fetuses with ductal constriction, assessed by different methods^{73, 79, 85}. The hemodynamic compromise is considered mild when there is mild or no tricuspid and/or pulmonary regurgitation, with normal chambers diameters; moderate in the presence of tricuspid regurgitation with right ventricular dilation without hypertrophy and/or impaired contractility and severe when the tricuspid and/or pulmonary insufficiency is important or there is functional pulmonary atresia, right ventricular dilation with ventricular parietal hypertrophy and alteration in right ventricular contractile function. The compromise is also considered severe when there is total ductal occlusion or, alternatively, in the presence of a PI lower than 1.0, associated to any degree of hemodynamic repercussion^{23, 79}. Since the constrictive effect upon the ductus arteriosus is predominantly dose-dependent⁶⁰, it is usual the resolution of hemodynamic alterations after

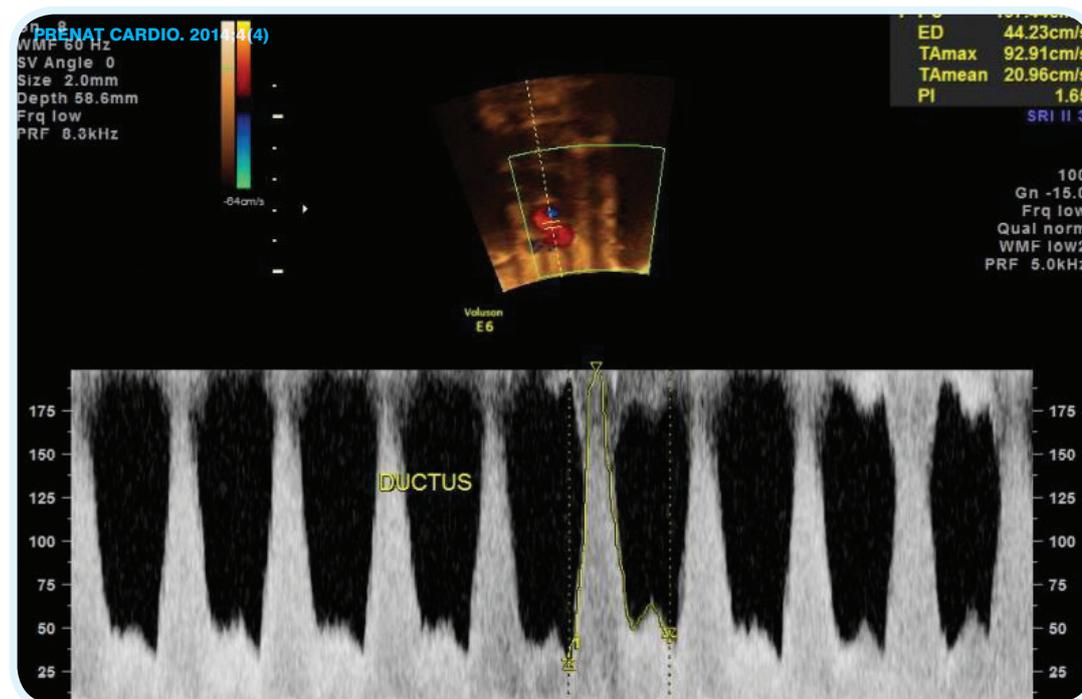


Figure 4. Pulsed Doppler tracing of a ductal flow in a 31 weeks fetus with constriction of DA caused by maternal ingestion of dark chocolate and grape juice. There is high systolic and diastolic peak velocities, as well as a decrease pulsatility index.

suspension of the causing substances without development of fetal or neonatal cardiac dysfunction^{33, 34, 86-88}. Even in the presence of a severe ductal constriction after maternal utilization of drugs with prostaglandin inhibiting effect, withdrawal of their use may show reversal of the increased SV and DV within 24 hours, with improvement of the hemodynamic alterations³³. In some more severe cases, interruption of pregnancy may be necessary, sometimes with

immediate cardiopulmonary neonatal resuscitation. Despite not having been established the association between duration of fetal ductal constriction and the prevalence and severity of neonatal pulmonary hypertension¹⁹, it is obviously important to remove the cause as soon as possible, in order to allow early recovery.

The decision to interrupt pregnancy should take in account fetal pulmonary maturity, the severity of clinical and echocardiographic manifestations of ductal constriction and the presence or not of a progressive pattern. In the immediate neonatal period, the physiologic ductal closure associated to hemodynamic changes usual to this period allow normalization of the cardio-circulatory alterations secondary to the increased right ventricular overload. However, as already mentioned, the prolonged increase in right ventricular pressure, when transmitted to the lungs, may cause a reactive pulmonary arteriolar vasoconstriction with secondary pulmonary artery hypertension, which will need intensive treatment⁸⁹. Since persistent pulmonary hypertension of the neonate without cardiac abnormalities occurs in approximately 1/1000 liveborns, and around 23% of the cases do not have a known definitive etiology, very probably many of these cases are secondary to undetected fetal premature constriction of ductus arteriosus⁹⁰. Thus, ductal constriction should always be considered an etiological possibility in "idiopathic" neonatal persistent pulmonary hypertension. This disorder carries a bad prognosis and is characterized by postnatal persistence of increased pulmonary vascular resistance, cyanosis due to right-to-left shunts through the foramen ovale and ductus, decreased pulmonary blood flow and severe hypoxemia⁹¹.⁹² Persistent pulmonary hypertension of the newborn has been associated to antenatal exposure to NSAID^{19, 93-102}, even though a recent case-control study could not confirm this risk¹⁰³. In fetal lambs, mechanical occlusion of the fetal ductus arteriosus reproduces the hemodynamic and structural features of persistent pulmonary hypertension of the newborn. Experimental prenatal exposition to NSAID has demonstrated alterations similar to those found in ductal constriction, with increased thickness of the smooth muscle layer of the pulmonary arterial vasculature^{29, 104-106}.

Polyphenols are chemical structures present in all the superior vegetal organisms. More than 8000 structures are known, and they act on pigmentation, growing, reproduction and resistance of plants against diseases¹⁰⁷. There are flavonoid and non-flavonoid polyphenols.

Flavonoids represent the major family and are the basic structures of tannins or proanthocyanidins. Tannins and its flavonoids are the best known polyphenols in alimentation, because of their presence in **beer and wine**, but a wide variety of polyphenols are present in a great number of foods and beverages. Many studies have investigated the kinetic and extension of absorption of polyphenols by mensuration of plasma concentration and urinary or plasmatic excretion¹⁰⁸.

Flavonoids are the most abundant polyphenols in the human diet and its consumption has triggered the interest of consumers and food industries for many reasons, but mainly because of their biological activity in systems relevant to human health¹⁰⁹. This biological activity is related to anti-inflammatory and antioxidant effects¹¹⁰, based on its interference in the inflammatory cascade, with inhibition of prostaglandin synthesis and mediation of nitric oxide synthetase^{111, 112}.

Polyphenols with greater importance and literature references are catechins mainly in **green and black tea**, resveratrol in **wine and black grape**, chlorogenic acid in **coffee and teas** and flavonoids present in **fruits and vegetables**¹¹³.

The principal alimentary sources with higher concentration in polyphenols are **herbal teas, mate tea, dark chocolate, fruits, natural juices, vegetables, olive and soy oils and red wine**. Among fruits, the highest concentration of flavonoids is orange, red and purple grape, strawberry and other berries, black prune and its derivatives. Vegetables with higher polyphenol concentration are purple onion, green spices, tomato and derivatives. These foods show a concentration above 30 mg of flavonoids per 100g of food, representing an amount above the 75th percentile of the USDA database¹¹³.

In recent years, many investigational studies have been trying to ascertain the real therapeutic effect of substances found in nature and commonly used by general population. Several of these substances have nowadays their anti-inflammatory and antioxidant effects^{114, 115} scientifically and unequivocally demonstrated upon the chain of production of oxidative stress related to inflammatory mediators such as COX-2 and prostaglandin E2, metalproteinases and others. Substances rich in polyphenols are among the most widely used for a variety of reasons, even during pregnancy. The anti-inflammatory and antioxidant effects of these substances are secondary to inhibition of the metabolic route of prostaglandin, especially of cyclooxygenase-2, preventing the transformation of arachidonic acid into prostaglandin^{20, 39, 59}. 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 industry¹¹⁶.

It has been demonstrated unambiguously that the polyphenols decrease oxidative stress (including in pregnancy)¹¹⁷, plasma triglycerids and cholesterol levels¹¹⁸, blood pressure^{119, 120, 121}, the consequences of gastric hypersecretion¹²², the development of some neoplasms¹²³⁻¹²⁵ and atherosclerosis^{126, 127}, the manifestations of aging¹²⁸ and Alzheimer's disease¹²⁹ 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 indomethacin¹²⁹⁻¹³¹.

Green tea, for example, is a compound of young leaves from the plant *Camellia sinensis*¹³². Approximately 30-40% of the leaves' solid extract is composed of polyphenols, mainly catechins. Among the most important catechins present in green tea are epicatechin, gallate-3-epicatechin, epigallocatechin and, predominantly, gallate-3-epigallocatechin, with contains 7g per 100g of dry leaves. Several *in vitro* studies, both in animals and in humans, have demonstrated their antioxidant, anticarcinogenic, anti-inflammatory, probiotic and antimicrobial actions secondary to inhibition of endogenous inflammatory response, dependent on the interference on the prostaglandin synthesis pathway¹³³⁻¹³⁶. Black tea has also showed to be rich in catechins, and the tea compound involving theaflavin has been shown to act on nitric oxide and on the liberation of arachidonic acid. It has already been clearly demonstrated that tea drinkers could benefit from the protective cardiovascular effects exerted by this polyphenol-rich substance^{137, 138}.

Resveratrol, a polyphenol compound found in grape rind, grape juice and red wine, is known by its antioxidant, antithrombotic anti-inflammatory and anticarcinogenic actions¹³⁹. Several studies have demonstrated the effect of resveratrol upon the nervous system, as well as on the liver and the cardiovascular system. One of the possible mechanisms that explain its biological activity is related to a decrease in liberation of arachidonic acid, thus affecting induction of COX-2, with a consequent reduction in prostaglandin synthesis^{140, 141}.

Mate tea, a typical regional beverage very rich in polyphenols, widely consumed in South America, mainly Paraguay, Brazil, Argentina and Uruguay, is obtained from the dried and minced leaves of *Ilex paraguariensis*. Many studies have demonstrated its potent antineoplastic, anti-inflammatory and antioxidant effects, due to the action of its polyphenolic compounds¹⁴².

Orange juice has been shown to have important antioxidant activity as a result of a high content of flavonoids, especially quercetin, and the ability of the phytochemical substance to interact with biomembranes. It was speculated that the daily consumption of orange juice might be useful in providing additional protection against cellular oxidation *in vivo*¹⁴³.

Dark chocolate shows high concentration of flavonoids and has anti-inflammatory properties. It has been demonstrated to have an inverse association with C-reactive protein, in amounts as low as 20g every 3 days, suggesting that the regular intake of dark chocolate may reduce inflammatory processes¹⁴⁴. Since flavonoids modify the production of pro-inflammatory cytokines, the synthesis of eicosanoids, the activation of platelets and nitric oxide-mediated mechanisms, a growing body

of evidences are available to support a potent action of cocoa flavonoids in inflammation¹⁴⁵.

Many other substances rich in polyphenols present in nature commonly used in daily routine by the general population have also shown definite anti-inflammatory effects secondary to inhibition of the prostaglandin synthesis pathway. Examples are boldine, with anti-inflammatory and antithermic activities¹⁴⁶, propolis, with anti-inflammatory action in asthmatic patients¹⁴⁷, passion fruit, with cytotoxic, anti-inflammatory and scar-promoting effects¹⁴⁷⁻¹⁴⁹, tomato and ginseng, also with anti-inflammatory action on COX-2¹⁵⁰ salvia, with anti-inflammatory effects on acute and chronic processes^{151,152}, chamomile, with moderate antioxidant and antimicrobial activity and significant *in vitro* antiplatelet actions¹⁵³⁻¹⁵⁵, and very many others, with variable concentrations of polyphenol substances presenting anti-inflammatory and antioxidant effects, all of them by interfering with prostaglandin synthesis.

A number of food and beverages such as herbal teas, grape and derivatives, orange, chocolate, fruits and many others, with high concentrations of polyphenols, are freely consumed throughout gestation. Despite the positive effects of polyphenols in general health, as discussed in the previous sections, other studies from our group and others point toward the indication that maternal consumption of polyphenol-rich foods in late pregnancy, specifically in the third trimester, may be harmful to fetal health, as a result of the anti-inflammatory and antioxidant effects of these substances upon the ductus arteriosus, due to the inhibition of prostaglandin synthesis^{81, 156-162}.

Experimental studies to assess the fetal ductal effects of maternal consumption of polyphenol-rich foods utilized ewes in the last third of pregnancy (more than 120 days), corresponding to the third trimester of human gestation.

The first study has shown that fetuses of ewes submitted to an **experimental diet of mate tea or green tea** as the only source of liquid for one week developed ductal constriction, with unequivocal histological signs: right ventricular enlargement, right ventricular hypertrophy and increased avascular zone thickness at the ductal wall¹⁶³.

The second study demonstrated that maternal exposure of green tea for one week was followed by fetal constriction, with increase in mean systolic velocity and mean diastolic velocity, decrease of pulsatility index and increase of mean right ventricular/left ventricular diameter ratio. Morphological repercussion was shown by dilated and hypertrophic right ventricles and increased ductal lumen avascular zone in the group exposed to green tea, but not in those of the control group, whose mothers have received only water¹⁵⁹.

A third experimental study, recently published¹⁶⁴, tested the hypothesis that maternal exposure to a diet with high content of polyphenols is followed by fetal ductal constriction and by alteration of endogenous inflammatory

and oxidant mediators. It was shown that an elevated experimental maternal polyphenol consumption in ewes induced fetal ductal constriction with increased urinary excretion of total polyphenols and alterations in biomarkers of oxidative stress, characterizing the antioxidant and anti-inflammatory actions of polyphenols^{164, 165}.

All clinical studies performed to evaluate the effects of maternal intake polyphenol-rich foods after the third trimester of pregnancy on fetal ductus arteriosus depend on the adequate assessment of its concentration, and there were no validated instruments to quantify total polyphenols in pregnant women. We showed the reproducibility and validity of a food frequency questionnaire (FFQ) with 52 items, to assess the intake of polyphenol-rich foods in pregnant women in Brazil¹⁶⁰.

In another study, we hypothesized that polyphenols or flavonoids present in food and beverages commonly consumed by pregnant women could influence ductal flow dynamics, probably by inhibition of prostaglandin synthesis, and thus be a risk factor for ductal constriction. With that in mind, we compared ductal flow behavior and right ventricular size in third-trimester fetuses exposed and not exposed to polyphenol-rich foods and beverages via maternal consumption. In a prospective analysis, Doppler ductal velocities and right-to-left ventricular dimensions ratio of normal fetuses exposed to polyphenol-rich foods (maternal consumption above the 75th percentile) were compared with normal unexposed fetuses (polyphenol ingestion below the 25th percentile). It was demonstrated that polyphenol-rich foods intake in late gestation triggers alterations in fetal ductal dynamics, since the exposed fetuses had significantly higher ductal flow velocities and increased right ventricles than not exposed fetuses¹⁵⁷.

We designed a study with the purpose to test the hypothesis that fetuses with constriction of ductus arteriosus and no history of maternal ingestion of NSAID, but whose mothers have used PRF in the third trimester, show reversal of the ductal constrictive effect and its hemodynamics consequences after maternal dietary intervention aimed at restriction of these substances. A controlled clinical trial of third trimester fetuses with ductal constriction with no history of NSAID intake was performed. All mothers were submitted to a food frequency questionnaire and were oriented to withdraw polyphenol-rich foods, being reassessed after 3 weeks. Doppler parameters were assessed before and after



Figure 5. 2D three-vessels view of a 30 weeks fetus with ductal constriction. The pulmonary artery is larger than the aorta.

discontinuation of these substances. A control group of third trimester normal fetuses, with no ductus arteriosus constriction, in which no dietary intervention was offered, was reviewed after 3 weeks. Ninety-six percent of the fetuses showed complete reversal of ductal constriction. In the control group, there was no significant differences in daily maternal consumption of PRF, ductal velocities, pulsatility index and right ventricular size. This behaviour is similar to that already widely reported upon the withdrawal of NSAID in fetuses with constriction of ductus arteriosus caused by those pharmacological agents, when there is habitual regression of the disorder. The original conclusion of this controlled clinical trial was that reduction of maternal polyphenol intake during pregnancy, especially in the third trimester, is followed by complete reversal of ductal constriction, which may reduce the risk of neonatal hypertension and influence maternal dietary habits in late pregnancy⁸¹.

In a controlled clinical trial, we also demonstrated that, as already reported for fetuses with ductal constriction, dietary intervention for restricting for a period of two weeks or more the intake of foods rich in polyphenols by pregnant women in the third trimester also improves ductus arteriosus flow dynamics and decreases the right ventricle size in normal fetuses¹⁵⁶.

Other studies in the international literature have also discussed the relationship between idiopathic prenatal ductal constriction and maternal consumption of polyphenol-rich foods in late pregnancy, in the absence of exposure to nonsteroidal antiinflammatory drugs^{167,168}. Case reports have shown association of severe ductal constriction with hemodynamic repercussion (hydrops, enlarged right atrium and right ventricle) to maternal ingestion of polyphenols, such as violet vegetable juice, prune berry¹⁶⁹ and camomile herbal tea¹⁶². Kapadia, V., et al reported a case of fetal ductal cons-

triction related to maternal consumption of a juice blend containing the cyclooxygenase and nitric oxide synthase inhibitors anthocyanins and proanthocyanidins for one week in late pregnancy. After birth, the newborn developed persistent neonatal pulmonary hypertension, needing oxygen for a prolonged period¹⁶¹.

When the level of evidences regarding the recommendation of avoidance of polyphenol-rich substances by pregnant women, at the third trimester, is critically analyzed, an important question naturally arises: why not to have performed or to perform a randomized clinical trial to obtain the strongest possible evidence of this action, at the apex of the pyramid? Such a study, in simple terms, would try to resolve the research problem of the value of nutritional intervention (restriction of polyphenols in maternal diet) against no intervention, in the presence of fetal ductal constriction without history of prenatal exposure to NSAID. In this hypothetical trial, the study factor would be the restriction of maternal intake of polyphenol-rich foods in fetuses with ductal constriction in late pregnancy and the outcome the improvement of fetal echocardiographic signs of ductal constriction - decrease in systolic and diastolic ductal velocities, increase in pulsatility index, decrease in right to left ventricular diameters ratio and pulmonary artery to aorta dimensions ratio, of flow turbulence, septal bulging and tricuspid regurgitation. Would there be equipoise in a randomized clinical trial with the proposed intervention and outcomes¹⁷⁰⁻¹⁷⁴? In other words, would it be ethical to perform a randomized clinical trial to assess the benefit of polyphenol restriction in maternal diet at the third trimester in the presence of ductal constriction? The answer to that question, considering the conceptual model to obtain a state of equipoise, is "no"! Such a state of equipoise needs the triangular interrelationship of the 3 points: 1) definite benefit of the study to society; 2) doubt of the investigator about the intervention effectivity; 3) safety of all the subjects in the two arms of the clinical trial^{172,175-178}. Even though there is a clear benefit polyphenols in late pregnancy improves fetal ductal constriction, the two remaining points of the triangle of the conceptual model of equipoise are not fulfilled¹⁷⁹. The uncertainty about the effectivity of maternal restriction of polyphenols is no longer present, based on the previous published studies herein disclosed and, most importantly, safety in the two arms of a randomized clinical trial in which one of them would not receive a clearly effective intervention can not be established. The deleterious effects of ductal constriction upon fetal hemodynamics and the risk of pulmonary arterial hypertension secondary to this functional disorder are well known. There are no reports in the literature of spontaneous reversal of ductal constriction, without removal of the causal factor. How to submit the control group to the risk of keeping the ductus constricted, with all its potential complications? In summary, a randomized clinical trial to assess the effect of maternal dietary intervention in fetuses with ductal constriction, with the level of evidences today

accumulated, do not fulfill the equipoise principles, and for this reason can not be considered ethical.

The number of evidences already available recommend (Class IIa, level B) a note of caution with regard to the consumption by women in the third trimester of pregnancy of foods with high concentrations of polyphenols, as well as nonsteroidal antiinflammatory drugs, in order to avoid triggering constriction of ductus arteriosus, with its potential harmful consequences, such as fetal and neonatal heart failure and pulmonary arterial hypertension of the newborn.

All the information disclosed in this editorial has been discussed in a recently published review article¹⁸⁰.

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Conflict of interest

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