

# Potential Effects of Nutrients on Placental Function and Fetal Growth

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## Introduction

Intrauterine nutritional deficits can trigger adaptation mechanisms with modifications that can predispose an individual's later life to various pathologies (cardiovascular, metabolic, endocrine). It is difficult to individualize both the nutrient or nutrients responsible for the damage and the relation between cause and effect quantitatively (nutritional effect entity) and timing (time lag for the start of the pathology). It is therefore not easy to prove that placental function and fetal growth can be deeply influenced by specific nutrients rather than by variation in the intake of calories, that is to say by nutrition as a whole.

Furthermore, every country, except the developing ones, has particular nutritional habits. As a consequence of that, recommendations worked out in some geographical areas with specific nutritional deficit may not be so efficacious as in other areas where this deficit is not as evident (for instance the administration of folic acid in the Mediterranean area).

The results of recently reported studies focus attention on: (1) soluble gas such as nitric oxide (NO) which, as has been demonstrated, can affect both placental function and fetal growth, also taking into consideration that its effects can be mediated both by drugs and nutrients, (2) adequate antioxidant status during pregnancy could prevent and control those mechanisms induced by maternal oxidative stress that could lead to both impaired placental function and fetal growth.

### **Nitric Oxide and *L*-Arginine**

Different noxae are thought to cause fetal growth restriction (FGR). The reduction of placental substrate uptake, in the case of unsuccessful maternal physiological adaptation to pregnancy, may be one of these. In physiological pregnancy, hemodynamic changes and, in particular, uteroplacental perfusion modification occur. It is thought that these changes depend on trophoblastic migration into the walls of the spiral arteries, a process which seems to take place in two steps, the first of which is limited to the decidual portion of the spiral arteries, while the second seems also to involve the myometrial portion. Such a process transforms the spiral arteries in utero-placental vessels, a low resistance, low pressure, and high flow vascular system. In contrast, where such changes are lacking a decrease in fetoplacental unit perfusion occurs. Impaired endothelial function and the consequent decrease in endothelial mediator release, such as nitric oxide (NO), has been proposed as the underlying pathophysiological mechanism. Different study suggests a reduction in the synthesis and/or release of NO in pregnancies complicated by preeclampsia and/or FGR. It would, therefore, appear that the *L*-arginine/NO system modulates the maternal hemodynamic adaptation, and the reduction in NO release may be involved in the development of FGR and/or preeclampsia. A reduction in the blood flow impedance may be considered as a marker of maternal hemodynamic adaptation. At 20–24 weeks of gestation, different studies on flow velocity waveforms have demonstrated an increase in uterine artery impedance in women whose pregnancy may be complicated by preeclampsia and/or FGR. In previous studies, we observed a clear reduction in uterine artery blood flow impedance after sublingual administration of 0.3 mg glyceryl trinitrate, a NO donor, both in normal and FGR and/or preeclampsia complicated pregnancy (table 1). The effect of drug administration was significantly more pronounced in the FGR/preeclampsia-complicated pregnancy. Furthermore, in such cases we observed a reduction in the umbilical artery blood flow impedance. Hence, we have established an FGR treatment protocol based on the use of NO donor drugs, administered by the transdermal route, in order to restore the NO levels which are thought to be insufficient, thus improving placental perfusion (table 2). Since in some FGR cases no significant response was observed in uterine artery dilation after NO donor administration (cases with a worse outcome), we proposed a NO donor test (NO test) to discriminate the FGR cases possibly related to an impaired endothelial function from those with a different origin, thus assisting the decision-making process and allowing a differential treatment approach. We believe that the NO test, carried out at 24 weeks of gestation in women presenting with increased uterine artery blood flow impedance (protodiastolic notch), may allow evaluation of the vascular 'reserve' to dilation, improving the predictive value and sensitivity of Doppler velocimetry in the identification of pregnancies at risk of preeclampsia and/or FGR.

**Table 1.** Feto-maternal hemodynamic changes during sublingual glyceryl trinitrate administration

	Basal	5 min	10 min	20 min
Maternal systolic pressure, mm Hg	147.8 ± 11.94	136.8 ± 8.75	134.0 ± 9.61*	137.4 ± 11.84
Maternal diastolic pressure, mm Hg	92.8 ± 9.23	76.6 ± 5.54*	75.8 ± 6.34*	74.6 ± 4.77*
Maternal heart rate	85.6 ± 10.76	111.0 ± 13.05*	105.4 ± 17.89*	89.6 ± 13.68
Fetal heart rate	142.3 ± 4.61	148.2 ± 4.57	144.2 ± 7.80	151.5 ± 7.76
PI uterine artery	1.70 ± 0.13	0.57 ± 0.03*	0.60 ± 0.08	0.67 ± 0.06
PI umbilical artery	1.45 ± 0.14	1.07 ± 0.20*	1.27 ± 0.21	1.40 ± 0.17
PI middle cerebral artery	1.73 ± 0.61	1.75 ± 0.44	1.68 ± 0.42	1.66 ± 0.66
PI renal artery	2.42 ± 0.63	2.62 ± 0.67	2.55 ± 0.89	2.15 ± 0.49

Values are means ± 2 SD.

\*p < 0.05.

**Table 2.** Results of FGR cases treated with NO donors compared to controls

	FGR-NO (22 cases)	FGR controls (20 cases)
Gestation age at delivery, weeks	33.2	31.5
Birth weight, g	1,150 ± 270	960 ± 220
Mortality	2 (9%)	6 (30%)
IVH 3–4 degrees	1 (4.5%)	5 (25%)
RDS (severe)	5	7
NICU, days	26 ± 7	42 ± 9

By using a different approach, similar effects have been observed with the use of *L*-arginine. Three groups of 9 pregnant women each were infused intravenously with *L*-arginine (30 g/100 ml) for 30 min. One group served as a control, and 2 groups were composed of FGR with or without increased resistances in utero-placental perfusion. A reduction in the PI of the non-placental-sided uterine artery was observed in the FGR group with increased uterine resistances. Such an effect seems very specific since it is evident only in those pregnancies complicated by growth restriction associated with unilaterally increased resistances in utero-placental perfusion. Considering that *L*-arginine is the physiological substrate of NO synthase, we suggest that the unilateral decrease in blood flow resistances observed in the FGR group with increased uterine resistances is sustained through a release in NO, possibly from the vascular endothelium. Furthermore, we suppose that a subpopulation of FGR

fetuses with impaired utero-placental perfusion, a possible cause explaining the arrest of their growth, could benefit of enhancement of the NO pathway.

Facchinetti et al. [1–3] evaluated the biochemical and cardiovascular changes in response to *L*-arginine load in normotensive pregnant women and preeclamptic patients. In such studies, in contrast to the reduced levels of NO byproducts following *L*-arginine infusion (30 g/100 ml), the preeclamptic patients showed blood pressure changes that were similar to but of greater magnitude than those of controls. In particular, diastolic blood pressure was reduced to a greater extent than in normotensive subjects, an effect lasting 30 min after the end of infusion.

The apparent discrepancy between reduced NO production and the increased hypotensive effect of *L*-arginine in patients with preeclampsia could be explained in different ways. During infusion, the *L*-arginine levels attained were 100-fold higher than the physiologic concentrations. In such conditions, it is possible that NO production from unaffected endothelial cells (despite the endothelial dysfunction that has been described in preeclampsia) and/or by other cells in the vessels lumen (i.e., platelets) could explain the hypotensive effect of *L*-arginine load. However, it could not be excluded that hypotension produced by *L*-arginine load is mediated through mechanisms other NO.

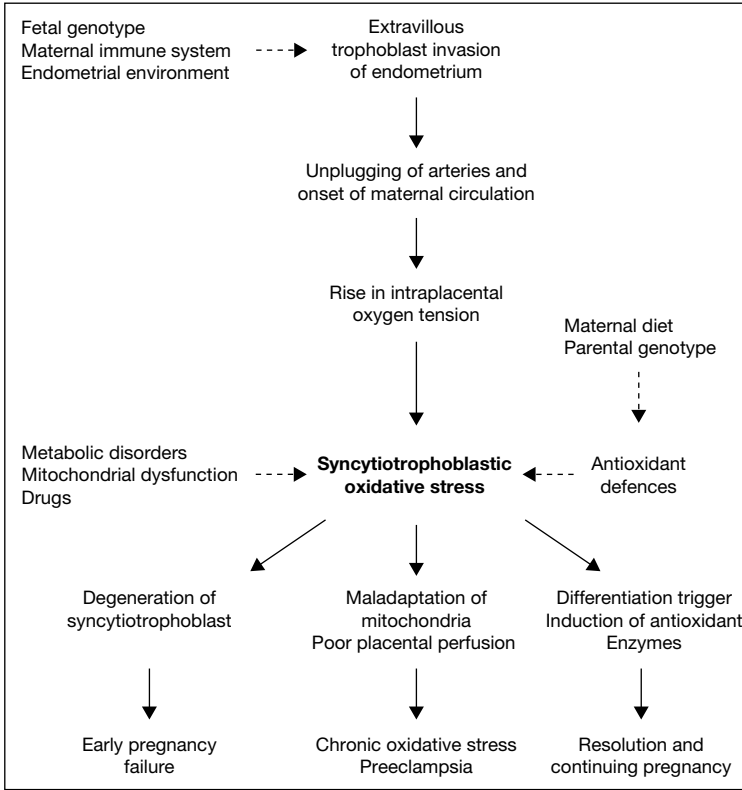
It has also been demonstrated that: (1) a reduction in platelet sensitivity to the antithrombotic effects of the *L*-arginine-NO system takes place as pregnancy progresses, (2) in vivo *L*-arginine administration decreases platelet aggregation in normotensive women, whereas no effects were observed in preeclamptic women.

It has been observed that NO production is enhanced in severe preeclampsia, possibly as a compensatory phenomenon (although it does not necessarily represent an improvement in the clinical condition) for the increased synthesis and release of vasoconstrictors and platelet-aggregating agents.

In this regard adequate availability of n-3 fatty acids for the fetus, especially eicosapentaenoic acid and docosaexanoic acid, are highly important.

### **Antioxidants**

Increased oxidative stress is associated with pregnancy and may be related to some pathologies such as pregnancy-induced hypertension, preeclampsia and even FGR (fig. 1). Vitamins C and E,  $\beta$ -carotene and other food components with marked antioxidant properties may play an essential role in creating the antioxidant defense system, protecting against damaging reactive species in healthy pregnancy (table 3, 4). A low antioxidant intake has been reported in pregnant women and a lower total antioxidant capacity has been found in the cord blood of newborns of smoking mothers. Pregnant women may be considered at risk for oxidative damage if their diet does not supply adequate



**Fig. 1.** Role of oxidative stress in implantation and pregnancy maintenance.

intake of antioxidants. In a previous study we assessed the antioxidant total plasma capacity of women from early pregnancy to delivery, and of their newborns, and related the values obtained to the dietary intake of the same women during pregnancy. A reliable and specific method, namely the oxygen radical absorbance assay (ORAC), was used. We found that ORAC values decreased progressively during pregnancy, reaching the lowest value at delivery (fig. 2). However, the mothers' dietary habits remained unchanged during pregnancy. This suggests that a transient imbalance between antioxidant requirements and intake occurred gradually and progressively during pregnancy. The ORAC values of the newborns' cord blood were highly correlated with the mothers' values observed in the third trimester and at delivery. However, newborns' ORAC values were lower than those observed in their mothers' during the first and second trimesters of pregnancy, thus indicating a close time relationship between the mothers' and newborns' ORAC values. In the pregnant women of this study, (pro)-vitamin antioxidant

**Table 3.** Some maternal antioxidants present in the human body

Name	Acts	Present in
Superoxide dismutase (SOD)	Super oxide	Cytosol mitochondria
Catalase	H <sub>2</sub> O <sub>2</sub>	Blood, bone marrow, mucus, membrane, kidney, liver
Glutathione peroxidase (GOP)	H <sub>2</sub> O <sub>2</sub> , lipid peroxidation	Membranes of lipids, hemoglobin and erythrocytes

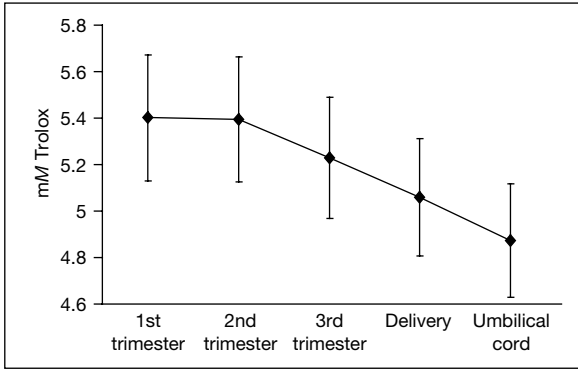
**Table 4.** In vivo antioxidant sources

In the form of drugs
Vitamins A, C and E
Cystine, glutathione, methionine
Bioflavones, Se, Zn
Food sources
Green and yellow vegetables
Herbs: tumeric, garlic, grapes, tea, berries, carrots, spinach, broccoli
Red meat, kidneys, liver and lipoic acid

intake was satisfying, whereas the intake of fruit and vegetables, which are rich not only in antioxidant vitamins but also in other antioxidant compounds, was rather low. Since the increased antioxidant requirements were not met by adequate consumption of fruit and vegetables, it can be speculated that the decrease in total antioxidant capacity in these women may have been related to their diet which was too low in antioxidant compounds. Therefore, the content of foods with antioxidants should be adequate in the diet of pregnant women. If this is not possible, supplement of vitamins C and E and  $\beta$ -carotene should be encouraged.

It is interesting to notice the following results emerged from two recent studies. (1) During labor in healthy women at term, uterine contractile activity may generate reactive oxygen species (ROS) through the process of repetitive ischemia and reperfusion. With the significant depletion of vitamin C during labor, the administration of water-soluble vitamin C may scavenge ROS in the aqueous phase and recycle lipid-soluble vitamin E to combat ROS-induced tissue damage.

(2) Preterm premature rupture of the membranes has been correlated with maternal vitamin C and E status during pregnancy. This hypothesis, if confirmed, should stimulate initiation of therapeutic trials to test the efficacy of enhanced supplementation with vitamin C and E, or other nutrients with antioxidant properties, during pregnancy, so as to prevent preterm premature rupture of the membranes.



**Fig. 2.** Total antioxidant capacity of healthy maternal and umbilical cord blood during pregnancy [5].

Since diet and supplement use are modifiable behaviors, corroboration of these findings would suggest a possible intervention strategy.

## Conclusions

As stated above, the involvement of the *L*-arginine/NO pathway in the regulation of endothelial activity as well as in platelet function suggests a possible implication in the pathogenesis of FGR sustained by placental insufficiency (hemodynamic failure – an alteration in vascular compliance). Thus the enhancement of such a system by substrate administration and/or the use of NO-donor drugs could play a role in complicated pregnancy, characterized by an alteration in vascular compliance. Obviously, substrate treatment must be practical (i.e. oral route versus intravenous). Recently two studies addressed this question. The first [1] demonstrate that *L*-arginine, initially administrated by the intravenous route then orally, reduces blood pressure without an effect on fetal growth, and prolongs pregnancy in patients with pregnancy-induced hypertension with or without proteinuria. The second [4] concluded that oral *L*-arginine supplementation did not reduce the mean diastolic blood pressure after 2 days of treatment compared with placebo in preeclamptic patients with gestational age varying from 28 to 36 weeks. Whether *L*-arginine treatment could be clinically beneficial for the mother or the fetus if started earlier in the disease process remains to be seen.

Thus, it would appear that NO-donor drugs by the transdermal route represent a more simple and efficacious form of administration in clinical practice.

As far as antioxidants are concerned, a prudent diet during pregnancy could prevent pathological events in mothers, guarantee the best intrauterine

antioxidant milieu and allow a desirable total antioxidant status of newborns, so as to meet their antioxidant needs and let them start their lives in optimal conditions. In fact, newborns are brought into an environment that is hyperoxic compared to the uterus and need increased protection against peroxidation.

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### Discussion

*Dr. Pencharz:* I want to comment on your arginine supplementation. This is something we have been looking at in piglets. We have been trying to look at the arginine requirements and the 3 components for arginine, protein synthesis, urea cycle activity and nitric oxide synthesis. Quantitatively nitric oxide synthesis is by far the smallest component and we couldn't really define the requirement as we could for the urea cycle and for protein synthesis.

*Dr. Di Renzo:* These experiments are in piglets, although in humans we did not obtain similar results for the nitric oxide donors like glyceryl trinitrate. But with arginine at a very, very high amount these results can be achieved, even though with a low compliance because there are a lot of side effects if arginine is given intravenously to the mothers. So we think that we have to find a way to improve nitric oxide turnover, but not just giving arginine.

*Dr. Rosenquist:* You showed a list of compounds and said that they released free radicals and, also well-know teratogens. Are you suggesting that the teratogenic effect is because they generate free radicals?

*Dr. Di Renzo:* There are some suggestions about that, especially in diabetic embryopathy concerning a possible involvement of free radicals. Some experiments in rat models increasing the different compounds of the glucose cascade, for instance ketone and so on, decreased antioxidant or increased oxidative stress. It has been found, at least in this model, that an increase in oxidative stress, possibly related to the altered glucose metabolism at the beginning of pregnancy, causes a development of fetal malformations. This also applies to some of the drugs that impair endothelial function, for instance cocaine or drugs like thalidomide, that may have an effect through an increase in free radicals. In the crucial time between 5 and 11–12 weeks of gestation, the increase in free radicals may lead to a teratogenic effect [1].

*Dr. Patel:* I am interested in the number of molecules listed for antioxidants like vitamin C, vitamin A, glutathione and various other compounds. In the recent literature there is an additional compound listed as lipoic acid and its effects in diabetic conditions [2]. Do you have any experience with lipoic acid or is there literature indicating that it may be beneficial during pregnancy?

*Dr. Di Renzo:* I did not indicate all the drugs, but you are correct, lipoic acid is another antioxidant. I don't know if there are experiences that can be useful for pregnancy. I did not mention all the different molecules that can help maintain a normal oxidative status in pregnancy. But it is clear that for instance the study that we did may not be a definitive way to look at this now. We have some data about pathological pregnancies. The data showed pertain to fully normal pregnancies, at least according to the diet that we have in the central part of Italy. There is a decrease in the antioxidant power at least on the blood circulation of the mothers, and this is important because it is related to the cord blood concentration. There is a ratio which is practically very similar and we are demonstrating the same in the preterm babies. Now if you consider that the term baby has less oxidative defenses and is brought into a very high oxygen environment, it is clear that probably he or she needs more antioxidants, which is not apparently normally given to the mother at least with the diet that we supply. As I said the diet can change the antioxidant power a lot, but this can be due to the fact

that probably in the preterm baby we need to supply more antioxidants because the level of antioxidants, especially in the term newborn, is low, and it also decreases in the mother, especially in the second and the third trimester compared to the first.

*Dr. Luo:* There is actually more and more evidence suggesting that free radicals are very good physiological molecules which are involved in many physiological pathways. Especially for developing fetuses and even neonates, if we give too much antioxidants they might have an effect on the cell proliferation. Could you comment on that?

*Dr. Di Renzo:* I am not so familiar with this because I am not a neonatologist. In the study we performed looking at different milk formulas, there are more antioxidants than in the normal milk of breast-feeding mothers in normal situation, that is twice more. This can be good probably in preterm newborns, but I don't know how good it is if we feed newborns with this kind of formula having such an antioxidant power. I cannot predict that. However, there is a sharp difference between what is given by nature and what is not given by a formula which is evidently not so similar to human milk, at least in this respect.

*Dr. Duan:* Dr. Rosenquist, can you comment on that because you have done a lot of research in embryology.

*Dr. Rosenquist:* Not really, I don't feel confident to comment.

*Dr. Korzhynskyy:* There were observations in premature neonates in which it was attempted to give them antioxidants as high-dose medication, vitamin E for instance, and it caused an increased rate of necrotizing enterocolitis and sepsis. Probably we must be very careful to give medication to pregnant women as well. The effect was not the one desired, and it will probably be safer to modify the diet, something like grape juice, because medication can produce undesired side effects.

*Dr. Lam:* I have some comments on antioxidants. I am involved in the treatment of difficult patients: newborns several weeks old or a few months old, with major chronic problems which I felt were due to heavy metal overload. I went through all the available antioxidants that we can supply over the counter. The problem with vitamin C is that it is just water-soluble. When it is taken it is not bioavailable to clear anything across the lipid membrane. Vitamin E, although it is lipid-soluble, its bioavailability is so low, literally only a low percentage will be absorbed. However, there is an antioxidant called  $\alpha$ -lipoic acid and more than 100 articles have been published on this antioxidant.  $\alpha$ -Lipoic acid is a unique, universal antioxidant. It has got a very high availability when it is taken orally; I tried it on my patients. I read the literature and although it is such a useful antioxidant, it has never been applied in obstetrics and never been applied in pediatrics. But the patients are responding very well and I will give a very short presentation this afternoon about this aspect. I would suggest that those of you in the audience doing research apply this drug because it has a very beneficial effect on hypoxia hyperemia and the process of hypoxia hyperemia will generate a lot of free radicals, and this  $\alpha$ -lipoic acid acts very well. Right now it is very strange that it is an over-the-counter drug in the United States and it only serves as an anti-agent, but literally there are practically no side effects. A professor from Ukraine said that it is not doing the work of a plain antioxidant. I wonder whether he used  $\alpha$ -lipoic acid at all, because another antioxidant may not have this potency, and it is not really having the effect of the antioxidant.

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