TITLE OF FORM:

“Evaluation of the antioxidant activity of lutein/zeaxanthin early administered to premature newborns”

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Translation from Romanian

Evaluation of the antioxidant activity of lutein/zeaxanthin early administered to premature newborns

TYPE OF STUDY: PILOT STUDY OF 50 (25 CASES + 25 CONTROLS) SUBJECTS RECRUITED

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Title of study	**"Evaluation of the antioxidant activity of lutein/zeaxanthin early administered to premature newborns"**
Study Justification	During late years, there were many studies proving that lutein, a nutrient which is part of the carotenoids family, can be a real and important factor in preventing and protecting against numerous chronical diseases that affect millions of people in the entire world (such as in diabetic retinopathy in adult). The specialized literature, richer in information, is the one containing scientific studies and demonstrations which highlight and confirm the fact that lutein is able to reduce or slow down the risk of appearance of an ophthalmic pathology. Lutein is a polar soluble hydroxylate deriving from xantophylls that belong to the caroteniods family. The carotenoids are linear polyenes, *i.e.* hydrocarbons with conjugated double bonds, containing 40 atoms of charbon representing the highest class of phytochemical substances present in fruits, vegetables, human serum, breast milk, as well as human tissues. Carotenoids and their metabolites were largely studied in human serum and highlighted the physical and chemical characteristics through sophisticated

	methods (HPLC, magnetic resonance spectroscopy, mass spectrometry, UV spectrophotometry, etc.). Through these studies there were identified until present time, 27 carotenoids and 8 metabolites in the human body. Generaly, known carotenoids ingested, absorbed and metabolized by humans and found in serum are in number of 35. Lutein is the most important carotenoid present selectively in certain tissues of the human body, mainly at the level of the retina, macula (hence the name) and lens. In tissues and serum it is found together with a carotenoid dihydroxide, it isomer, zeaxanthin. Lutein, usualy ingested with other nutrients is partly eliminated directly through fecal matters (50-90%) and partially absorbed with the help of food fats. Incorporated in chylomicrons, it enters the blood flow where thanks to its liposolubility, it ties to the lipoprotein molecules (preferable HDL) and so it gets to different organs: liver, breast, colon, cervix, and ocularly, to the lens, iris and retina where, it selectively focuses in its central region (macula). In the cell, it is places in the entire thickness, from the exterior to the interior, in cellular membranes, tiding up to its polar terminal groups by its own membranes. Lutein and zeaxanthin are present at the level of the umbilical cord and pass through the placental barrier; they are also present in the plasmatic ones, in breast milk and especially in the colostrum. Concerning the way of administrating it, the lutein presents, through its specific characteristics, an elevated bioavailability after oral administration. The hematic levels of lutein, after providing nutriments rich in carotenoids, are increased with 67% from the 14% observed at beta-caroten. Published studies proved that the chrono-active form of carotenoids is able to determin a slow release in the intestine offering a better absorption and reducinf the necessity to be assimilated together with food. Through interdisciplinary and coordinated studies, performed both *in vitro* and *in vivo*, there were identified different action mechanisms; particularly, it was demonstrated a defense mechanism of the tissue function by lutein, which is produced through the neutralization (quench) phenomenon of singlet oxygen and reactive oxygen species (ROS). This action provides the molecule with different activities: an antioxidant function, anti-inflammatory properties, properties which promote anti-tumoral effects, induction of detoxification enzymes and positive effect on proteins promoting the communication between joints (up-regulation). Recently, there have appeared experimental and chemical data proving that the oxidative stress and harmful actions determined by ROS can play an important role in the pathogenesis of many neurological diseases as Alzheimer, Parkinson in adult and ROP and NEC in newborns. This is due to the fact that the nervous system is characterized by membranes high in polyunsaturated fats, the first cellular compounds affected by ROS attack being the lipid perioxidation. A similar mechanism can appear to certain ocular tissues (macula, lens, retina) which, containing high amounts of polyunsaturated fat acids, are more vulnerable than other structures with oxidative degradations induced by ROS. Due to the fact that carotenoids are amongst the most powerful antioxidants existing in nature, there are being developed new researches concerning the functional role of these substances in preventing neurodegenerative diseases in newborns. For the first time, few researchers have managed to prove that lutein and zeaxanthin have a protecting role against the photostress of the human macula. This demonstration was possible by isolation, identifying the chemical structure and measuring the concentration of lutein and zeaxanthin and their oxidation products from the eyes of the donners.

	During the pregnancy, the percentage of fat acids of pregnant women's plasma increases with approximately 51%. The increase refers manly to the concentration of saturated (+ 57%) and monounsaturated (+ 65 %) fat acids, but also polyunsaturated fat acids increase significantly. Because thse polyunsaturated fat acids are very sensitive to oxidation, the modification of their plasmatic levels influences the state of the antioxidant systems on the mother and subsequently to the fetus. Many studies have proved that the increase of the susceptibility to the peroxidation of polyunsatured fat acids on pregnant women is accompanied by an equivalent increase of the tocopherol plasmatic concentrations which, immediately after birth, decrease sharply. The plasmatic concentrations of antioxidants on newborns, comparative to the mother's were smaller. In the umbilical cord, the levels of tocopherols and carotenoids are significantly smaller that the ones registered in the maternal plasma and the concentration of polyunsaturated fat acids on the newborn is significantly higher and a lot more increased than on the mother. Furthermore, specific studies showed a growing interest towords the oxidative stress and oxygen reactive species which supposedly accumulate after birth. Many practices usualy used in the delivery room, for example the drugs given to pregnant woman to ease her pain, the newborn's extraction methods, the techniques to minimize body temperature decrease, blocking the umbilical cord and especially the use of oxygen to 100 % or a ventilated room (Room Air, RA) for newborns presenting asphyxia signs, do not always prove to be efficient, but they can also compromise the health of the newborn as a result of a significant increase of free radicals. Some specific studies have compared the levels of free radicals, highlighted with particular markers, in the plasma of the umbilical cord of newborns with asphyxia treated 100% with oxygen or 21% with oxygen, comparative to a control group of children without asphyxia. The levels of free radicals were significantly increased immediately after birth in all three groups and grew at the two groups of newborns with asphyxia. In the group treated 21% with oxygen, these values decreased and at 28 days after birth, they have reached the same level of the newborns without asphyxia, whereas at the group treated 100% with oxygen the levels of free radicals still remains very high. Thus, it results that a short exposure of the newborn to 100% oxygen is the cause of an extended oxidative stress state and a consistent increase of free radicals which seem to be involved in different diseases and pathologies during the first months of life, especially in the preterm infant increasing the incidence of ROP, IVH, BPD, NEC and infections significantly. As a result, it is desirable that the newborn can increase the level of antioxidant protection to reestablish the oxido-reductive balance and to prevent the problems occurred from an extended exposure to high levels of free radicals and oxygen reactive species. Oxidative stress, as well as in the case of age diseases, is considered one of the main factors which determine the deterioration of the retina. The attempt to maintain a correct balance between oxidant and antioxidant factors can help to prevent or reduce damage to the eyes or other tissues that occur in the newborn, especially preterm. The premature birth is the most frequent cause of mortality, morbidity and disability. Premature babies have an extremly high risk to develop ocular or neurological lesions. The main complication at visual level that may appear is called retinopathy of prematurity, so called ROP. Oxidative stress is involved in

	the etiology of this disease. In fact, premature babies, because of repiratory issues, are oftenly exposed to potentially damaging oxygen concentrations or to phototherapy with high blue light intensity. These therapuetical practices are sources of free radicals. The lutein and zeaxanthin present in the macular pigment, due to their capacity to absorb blue light and their antioxidant action, could have an important role in protecting the eyes of the newborn against damages produced by intense light. On adults, different elements suggest a protective action of lutein against photo-oxidative damage, proved by numerous studies. The studies performed on babies showed that the levels of carotenoids in the first four / six months of life are much reduced. This is due to the fact that the baby's diet is based exclusively on milk, without any solid elements (as vegetables or green leaves), sole sources of this nutrient. Nevertheless, breast fed babies, in average, present high plasmatic lutein levels than babies fed with prepared milk. Different milk formuli for newborns found at present time on the market are not enriched with this type of carotenoids, thus their content of lutein and zeaxanthin is very low, exceting certain formuli which are not traded in Italy prepared using egg mixes. Breast milk, is thus the only source of lutein for the newborn before ablactation, and breast feeding proves to be of considerable importance as primary source of these micronutrients for the newborn, proper development and visual function protection. Taking into consideration the correlation between the lutein in the blood and breast milk and the reduction of its levels, similar to all carotenoids, in milk, after 6 days from birth, there is already an important contribution of nutriments high in lutein during breast feeding. Such diet enriched in lutein is particularly important especially for the mothers of premature babies or babies having a small weight when born. In fact, premature babies and underwight babies need more nutritive substances essential to a fast grow, because they have not benefit from the contribution of highly nutritive and energetic substances transferred from their mothers during the last weeks of pregnancy. Also, the gastrointestinal and renal functions which are not completely developed reduce the absorption and withhold of important micronutrients, amongst which important antioxidants that protect the newborn from the exposure to high level of free radicals produced excessively at birth and several times as a result of the resuscitation techniques used. Breast feeding is important for the antioxidant contribution to the protection of the newborn and definitely the nutritional state of the mother has subsequently an essention part because it influences the nutrition of the newborn, especially concerning certain solvable nutritive elements, such as lutein and zeaxanthin. It should be emphasized that in the literature are already present searches and results with the use of lutein / zeaxanthin in the newborn. The recent Gong work has evaluated the role of lutein / zeaxanthin comparing the data obtained from various studies, including those of Romagnoli, Dani and Manzoni. Furthermore the RCT analysis of Rubin on the subject, it can be concluded that lutein / zeaxanthin are well tolerated and well absorbed from preterm infants also after oral administration. The extremely interesting result that has emerged although not statistically significant (probably due to the small sample) is that supplementation with lutein / zeaxanthin reduced the incidence and severity of ROP. Our protocol is born from the idea that given the interesting results of earlier work is considered important to deepen a dosage of at least 1 ml / kg equal to 0,5 mg of lutein and 0.05 mg of zeaxanthin. It 'important to assess the value of the key markers of oxidative stress biological

	antioxidant potential (BPT) and total hydroperoxide (TH) during and after treatment. Already in a previous work (S. Perrone, M. Longini) has demonstrated a reduction in radicalemia in term infants, during and after administration of lutein / zeaxanthin by determination of the BTP and TH. Preparation based on lutein ans zeaxanthin have never reveled on humans negative or harmful effects after administration, or to the gastrointestinal or sistemis level. In recent studies there were not reported adverse phenomena after administrating 20 mg/day of lutein or zeaxanthin for a period of 6 months, or interactions with other liposovable nutritive elements.
Justifying the non-commercial character of the study	This study is based on the administration of a dietary supplement of lutein/zeaxanthin. It is included in the category of a spontaneous/non-commercial pilot study, because it is born from the need of private healt care of identifying a natural antioxidant product which may reduce the severeness of the diseases related to the prenatal period, limiting the sufferance of newborns and their families, by reducing simultaneously the charges in SSN pregnancy which arise from the standard treatments and short-term and medium to long-term rehabilitation. It was chosen the product *LUTEIN ofta 0,5 gocce,* because Sooft Company proved to be able to supply this drug without any charges, also signing a statement that certifies the fact that the scientific results of the research will remain at the disposal of the scientific community and that the management of the intellectual property protection activity concerning any invention that may derive from the research is made accoding to the law in force and mainly to Art. 65 of the Executive Decree no. 30 from 10 February 2005 – „The Code of Industrial Property". In conclusion the results of the research will remain available for the Scientific Community and shall not be used for commercial purposes or profit.
The registration and trading status of the product participating at the study	The Registers of the Ministry of Health concerning non-pharmacological products **The product is registeres in the Register of dietary supplements on the web site of the Ministry of Health (http://www.ministerosalute.it/alimenti/dietetica) and classified with the following code: 905619452, according to the executive decree no. 111 from 27 January 1992**. These products are submitted to the European Directive concerning food, according to the Executive Decree no. 169 from 21/05/2004, and to the European Directive 2001/20/EC concerning drugs, transposed to Italian level throyh the executive decree 211 from 24/06/2003. Ingredients: solution of 5% Lutein and 2,25% Zeaxanthin with excipients (Corn starch, glucose, potassium sorbate, xanthan gum, citric acid). Description: LUTEIN ofta 0,5 gocce is a dietary supplement based on lutein and zeaxanthin. The lutein and its isomer, zeaxanthin, are two carotenoids highly spreaded in nature, which cannot be synthetized by the human body. They are normaly absorbed through food rich in such carotenoids (vegetables with green leaves, with big leaves as turnips and spinach, and fruits such as apricots and kiwi) and through breast milk. After absorption, the lutein and zeaxanthin are distributed to all tissues, but they are selectively accumulating in the eyes. Mainly, they are focusing in the retina and macula lutea, the latter being the place where clear images are formed, whose development is complete around the 4^{th} or 5^{th} month of life. The lutein and zeaxanthin are the only pigments present at the level of macula lutea and have a protection role. They contribute and maintain the functionality and integrity of the eye's sensitive structures (retina, iris, lens), preventing or reducing the damages caused by free radicals. In specialized literature is largely described their high antioxidant

	activity and the advantages of a consumption or supplement. Lutein and zeaxanthin pass through the placental barrier, they are also present in the colostrums and breast milk and it is believed that they take part to the embryogenesis of the retina and to the development of visual functions during the first weeks of life. As a result, the lutein and zeaxanthin are essential to the health of the eye, both on adults and children suring their first hours of life. Thus, the absorption of these substances proves to be necessary in situations of necessity growth or in case of deficient intake, both during the aging period of the ocular structures responsible for the visual function (macula), and during their conservation period. The lutein and zeaxanthin do not present contraindications and can be taken at any age. Use: ***LUTEIN ofta 0,5 gocce*** is indicated in situations of necessity growth or in case of deficient food intake of its components on different age groups. ***LUTEIN ofta 0,5 gocce*** can be useful especially in preventing macular or retina damages.
Study Objectives	***Main objective*** To evaluate if the administration of pharmacology doses of Lutein/Zeaxanthin micronutrients in water (***LUTEIN ofta 0,5 gocce*** – Neoox) is able to reduce the level of radicalemy and at the same time to elevate the antioxidant power of premature newborns (gestational age: ≤ of 32 weeks and / or weight at birth: ≤ 1500 grams).
Study scketch	pilot study, non-commercial, with food supplement, treated vs. control with a ratio 1: 1 double-blind. The identical vials, corresponding to placebo and active product, have an alphanumeric code without meaning for the operator, but that identifies the traty from control, in an anonymous document kept by the Statistical. The document will be placed in a sealed and anonymous envelopeand it will be kept open by the statistician at the time of data analysis.
People participating at the study and their number	***Inclusion criteria*** • Newborns with a weight ≤ 1.500 grams and/or gestational age ≤ 32 weeks; • Male and female newborns; • Newborns whose parents want to sign the information form – informed consent. ***Exclusion criteria*** • Informed consent is not signed • Infants of birth weight ≥ 1.500 gramms and/or gestational age > 32 weeks • infants hospitalized after 36 hours of life • Infants with ophthalmologic disease at randomization • infants with severe malformations ***Number of patients about to be registered*** It is expected to register a total number of 50 newborns divided in two groups: • Experimental group A: 25 newborns treated; • Control group B: 25 newborns treated.
Randomization	Subsequently enrollment patients will be randomly divided into Group A and Group B throught a web system that allows the randomization into three different recruitment centers

Number of centres	Multicenter pilot study. At the study will participate three Italian experimental centres: Naples, Siena and Padova. The centre from Naples will be the national coordinator and the center from Siena will handle in a centralized way the laboratory results. The center from Padova will help to recruit an adequate number of cases.
Sample size	BAP and TH are two bio-markers with different hypothetical changes clearly different. For this reason we will use the study method proceeds by the following online site http://clincalc.com/stats/samplesize.aspx (Rosner B. Fundamentals of Biostatistics. 7th ed. Boston, MA: Brooks/Cole; 2011). The inserted reference values are for BAP 2361 (±466) μmol/L and for TH 156.75 (±64.0) Ucarr (Perrone S., Longini M. et. Al). The difference for BAP that we hypothesize to find is an increase of at least 500 μmol / L which is approximately 20% of the control data. By setting α= 0.05% and β=0.1% with a power of 90% the number of samples required is 25 + 25 = 50. For the TH assume a decrease of 50 Ucarr which is approximately 30% of the data of the control. By setting α= 0.05% and β=0.2% with a power of '80% also in this case the required number of samples is 25 + 25 = 50. However 25 + 25 is the minimum number. Taking into account any ongoing study waivers (parents may request to discontinue treatment at any time) which go beyond statistical calculations. So in case of necessity we will recruit a number of infants to reach the minimum number according to the design of the protocol (25 + 25).
Treatment and dosage	***Products administered:*** ***LUTEIN ofta 0,5 gocce***, containing a solution of 5% Lutein and 2,25% Zeaxanthin with excipients (Corn starch, glucose, potassium sorbate, xanthan gum, citric acid) (group A) Placebo solution with unique excipients (Demineralised water, potassium sorbate, xanthan gum, citric acid) (group B) ***Experimental Group:*** Group A (25 newborns) will be treated with ***LUTEIN ofta 0,5 gocce***, (1 ml per Kg equal to 0,5 mg of lutein and 0,05 of zeaxantin), divided in two additional administrations during the day from the standard hospital treatment foreseen. The first dose will be given within 36 hours of life, the least to 30th day of life. ***Control Group:*** Group B (25 newborns) treated with Placebo solution (additionaly to the standard hospital treatment foreseen. The first dose will be given within 36 hours of life, the least to 30th day of life.
Way of intake	The product will be administered by adding it in the milk (human or formulae), or in a small quantity of glucose, either orally or by gavage.
Duration of the period of administration	The administration of the product (***LUTEIN ofta 0,5 gocce***) starts within the first 36 hours of life and continues with daily administration for 30 days.
Study duration	***Duration of the observation period of the patient registered*** Since birth to hospital discharge. ***Total duration of the study:*** The study will have a duration of 1 year: the first 6 months will be used to register the newborns in the centers. During this period, there will also be performed the

	laboratory investigations foreseen. The second semester will be used to study and analyse the results achieved.
Procedures	***Methodology:*** When born, premature newborns that are comply with the inclusion parameters will be introduced and separated in two random groups (A and B). All newborns, during the observation period, will be submitted to the blood collection (1 ml) from the umbilical cord and peripheral (at the same time with the routine collections) on which there will be performed blood gas analysis and oxidative stress markers (TH and BAP). All registered newborns will also be submitted to the verification of their history concerning the examination and collection, in view of evaluation the presence or absence of ROP, NEC, IVH, BPD and infections. ***Collection Time*** • T0, basic at birth, before begining the treatment: blood collection from the umbilical vein. • T1, first check, to 15 days after initiation of treatment: peripheral blood collection in view of verifying it before the Lutein/zeaxanthin administration. • T2, second check, at 30th day since the begining of treatment: peripheral blood collection in view of verifying it before the Lutein/zeaxanthin administration. The parents will be informed concerning the experimental procedure and the checks schedules. It will be required the written consent of the parents. The protocol shall be submitted to approval to the local ethics commission. ***Evaluation Parameters:*** **Efficiency:** a) Evaluation of oxidative stress biomarkers in the peripheral blood of the premature newborn before and after the treatment. **Tolerability:** There will be transcribed on the data sheet possible adverse events which may be observed by the doctor. ***Evaluation Scale*** a) Evaluation of the oxidative stress: by deremining the oxidative stress biomarkers and registering them in the data sheet. b) They must be indicated: *Demographic Characteristics* • Sex • Gestational Age • Weight (with percentile) • Mode of delivery • Preeclampsia • Premature rupture of membranes • Using steroids before birth • Using antibiotics before birth • Use of drugs or harmful substance before birth *Clinical features*

	• Apgar score • Use of surfactant • Umbilical catheter • CVC location and duration • Intubation • Mechanical ventilation (procedures and time) • Supplementation of O2 • Incidence of infections • Use and type of antibiotics • Use and type of antifungal • Use and type of post-natal steroids • Use and type inotropic • Use of NPT • Duration of treatment in NICU *Nutritional Characteristics:* • Oral feeding start time • Exclusive oral feeding start time • Fluid volume • Intake calories (protein, carbohydrates and fats) • Use of maternal and /or uman milk ***<u>Other therapies associated and/or permitted</u>*** During the trial period it is not permitted any product administration or antioxidant activities (anti-radicals) or products which contain even a sole component having this result, excepting the protocol.
Basic examination	At birth, as it is foreseen by newborn protocols, the babies will be submitted to several medical examinations.
Control examination	From the Birthing Unit, the newborns will be tranfered to intensive care rooms where they will be submitted to multiple control examinations.
Follow-up examinations	As it is foreseen in neonatal protocols.
Statistical Analysis	The Student t test for independent data is the type of analysis that will be used to assess statistically significant differences between the parameters recorded in the two groups (treated and control). The Student t test is the method of choice for analysis as it allows to evaluate the values of a continuous random variable measured in not very many samples (25 subjects per group). It will be using the statistical package SPSS system.

REFERENCES

Lutein: description, pharmacokinetics activity and characteristics, mechanism of action, safety and tolerability

1. Shao, A. and Hathcock, JN. Risk assessment for the carotenoids lutein and lycopene. Regul Toxicol Pharmacol. 2006 Aug;45(3):289-98.
2. Khachik F, London E, de Moura FF, Johnson M, Steidl S, Detolla L, Shipley S, Sanchez R, Chen XQ, Flaws J, Lutty G, McLeod S, Fowler B. Chronic Ingestion of (3R,3'R,6'R)-Lutein and (3R,3'R)- Zeaxanthin in the Female Rhesus Macaque. Invest Ophthalmol Vis Sci. 2006 Dec;47(12):5476-86.
3. Khachik F, de Moura FF, Chew EY, Douglass LW, Ferris FL 3rd, Kim J, Thompson DJ. The effect of lutein and zeaxanthin supplementation on metabolites of these carotenoids in the serum of persons aged 60 or older. Invest Ophthalmol Vis Sci. 2006 Dec;47(12):5234-42.
4. Trevithick-Sutton CC, Foote CS, Collins M, Trevithick JR. The retinal carotenoids zeaxanthin and lutein scavenge superoxide and hydroxyl radicals: a chemiluminescence and ESR study. Mol Vis. 2006 Sep 30;12:1127-35.
5. Thürmann PA, Schalch W, Aebischer JC, Tenter U, Cohn W. Plasma kinetics of lutein, zeaxanthin, and 3-dehydro-lutein after multiple oral doses of a lutein supplement. Am J Clin Nutr. 2005 Jul;82(1):88-97.
6. Rajendran V, Pu YS, Chen BH. An improved HPLC method for determination of carotenoids in human serum. J Chromatogr B Analyt Technol Biomed Life Sci. 2005 Sep 25;824(1-2):99-106.
7. During A, Dawson HD, Harrison EH. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe. J Nutr. 2005 Oct;135(10):2305-12
8. Reboul E, Abou L, Mikail C, Ghiringhelli O, André M, Portugal H, Jourdheuil-Rahmani D, Amiot MJ, Lairon D, Borel P. Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). Biochem J. 2005 Apr 15;387(Pt 2):455-61.
9. Lutein and zeaxanthin. Monograph. Altern Med Rev. 2005 Jun;10(2):128-35
10. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr. 2003;23:171-201. Epub 2003 Feb 27. Review.
11. Tyssandier V, Reboul E, Dumas JF, Bouteloup-Demange C, Armand M, Marcand J, Sallas M, Borel P. Processing of vegetable-borne carotenoids in the human stomach and duodenum. Am J Physiol Gastrointest Liver Physiol. 2003 Jun;284(6):G913-23. Epub 2003 Jan 10
12. Cardinault N, Gorrand JM, Tyssandier V, Grolier P, Rock E, Borel P. Short-term supplementation with lutein affects biomarkers of lutein status similarly in young and elderly subjects. Exp Gerontol. 2003 May;38(5):573-82.
13. Krinsky NI. Possible biologic mechanisms for a protective role of xanthophylls. J Nutr. 2002 Mar;132(3):540S-542S. Review.
14. Granado F, Olmedilla B, Blanco I. Nutritional and clinical relevance of lutein in human health. Br J Nutr. 2003 Sep;90(3):487-502. Review.

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15. Giuseppe Buonocore, Monica Tei, Serafina Perrone. Lutein as protective agent against neonatal oxidative stress. Journal of Pediatric and Neonatal Individualized Medicine 2014;3(2):e030244.
16. Perrone S, Tei M, Longini M, Santacroce A, Turrisi G, Proietti F, Felici C, Picardi A, Bazzini F, Vasarri P, Buonocore G. Lipid and protein oxidation in newborn infants after lutein administration. Oxid Med Cell Longev. 2014;2014:781454. doi: 10.1155/2014/781454. Epub 2014 Apr 30.
17. Perrone S, Longini M, Marzocchi B, Picardi A, Bellieni CV, Proietti F, Rodriguez A, Turrisi G, Buonocore G. Effects of lutein on oxidative stress in the term newborn: a pilot study. Neonatology. 2010;97(1):36-40. doi: 10.1159/000227291. Epub 2009 Jul 7.
18. Shoji H, Koletzko B. Oxidative stress and antioxidant protection in the perinatal period. Curr Opin Clin Nutr Metab Care. 2007 May;10(3):324-8. Review.
19. Fokkelman K, Haase E, Stevens J, Idikio H, Korbutt G, Bigam D, Cheung PY. Tissue-specific changes in glutathione content of hypoxic newborn pigs reoxygenated with 21% or 100% oxygen. Eur J Pharmacol. 2007 May 7;562(1-2):132-7. Epub 2007 Feb 8.
20. Franco MC, Akamine EH, Rebouças N, Carvalho MH, Tostes RC, Nigro D, Fortes ZB. Long-term effects of intrauterine malnutrition on vascular function in female offspring: implications of oxidative stress. Life Sci. 2007 Jan 30;80(8):709-15. Epub 2006 Nov 11.
21. Mercer JS, Erickson-Owens DA, Graves B, Haley MM. Evidence-based practices for the fetal to newborn transition. J Midwifery Womens Health. 2007 May-Jun;52(3):262-72. Review.
22. Kopsell DA, Lefsrud MG, Kopsell DE, Wenzel AJ, Gerweck C, Curran-Celentano J. Spinach cultigen variation for tissue carotenoid concentrations influences human serum carotenoid levels and macular pigment optical density following a 12-week dietary intervention. J Agric Food Chem. 2006 Oct 18;54(21):7998-8005
23. Fanaris, Bel BO, Guidettic E et al. Ruolo della Luteina nella prevenzione delle patologie oculari nel neonato. Rivista Italiana di Medicina Pediatrica 2006;numero speciale:51-53.
24. Kvansakul J, Rodriguez-Carmona M, Edgar DF, Barker FM, Köpcke W, Schalch W, Barbur JL. Supplementation with the carotenoids lutein or zeaxanthin improves human visual performance. Ophthalmic Physiol Opt. 2006 Jul;26(4):362-71.
25. Rodriguez-Carmona M, Kvansakul J, Harlow JA, Köpcke W, Schalch W, Barbur JL. The effects of supplementation with lutein and/or zeaxanthin on human macular pigment density and colour vision. Ophthalmic Physiol Opt. 2006 Mar;26(2):137-47
26. Provis JM, Penfold PL, Cornish EE, Sandercoe TM, Madigan MC. Anatomy and development of the macula: specialisation and the vulnerability to macular degeneration. Clin Exp Optom. 2005 Sep;88(5):269-81. Review.
27. Yanoff M and Duker i.S (2005) “Ophthalmology” Edizione italiana ed 2003 ristampa 2005, Antonio Delfino Editore medicina-scienze, volume 1, cap 1.3
28. Santosa S, Jones PJ. Oxidative stress in ocular disease: does lutein play a protective role? CMAJ. 2005 Oct 11;173(8):861-2.
29. van Leeuwen R, Boekhoorn S, Vingerling JR, Witteman JC, Klaver CC, Hofman A, de Jong PT. Dietary intake of antioxidants and risk of age-related macular degeneration. JAMA. 2005 Dec 28;294(24):3101-7.
30. Schweigert FJ, Bathe K, Chen F, Büscher U, Dudenhausen JW. Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. Eur J Nutr. 2004 Feb;43(1):39-44. Epub 2004 Jan 6
31. Jewell VC, Mayes CB, Tubman TR, Northrop-Clewes CA, Thurnham DI. A comparison of lutein and zeaxanthin concentrations in formula and human milk samples from Northern Ireland mothers. Eur J Clin Nutr. 2004 Jan;58(1):90-7.
32. Nolan J, O'Donovan O, Kavanagh H, Stack J, Harrison M, Muldoon A, Mellerio J, Beatty S. Macular pigment and percentage of body fat. Invest Ophthalmol Vis Sci. 2004 Nov;45(11):3940-50.

33. Turgut M, Basaran O, Cekmen M, Karatas E, Kurt A, Aygun AD. Oxidant and antioxidant Ievels in preterm newborns with idiopathic hyperbilirubinaemia. J Paediatr Child Health. 2004 Nov;40(11):633-7.
34. Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, Pei K, Tsipursky M, Nyland J. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). Optometry. 2004 Apr;75(4):216-30.
35. Vento M, Asensi M, Sastrei, LioretA, Garcia-Sala F, Vina (2003) i. Oxidative stress in asphyxiated term infants resuscitated with 100% oxygen. J Pediatr 2003;142:240-6.
36. Broekmans WM, Berendschot TT, Klöpping-Ketelaars IA, de Vries AJ, Goldbohm RA, Tijburg LB, Kardinaal AF, van Poppel G. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. Am J Clin Nutr. 2002 Sep;76(3):595-603.
37. Gossage CP et al (2002) " Carotenoid composition of milk during the first month postpartum and the response to b-carotene supplementation" Am J Clin Nutr 76; 193-7.
38. Jewell VC, Northrop-Clewes CA, Tubman R, Thurnham DI. Nutritional factors and visual function in premature infants. Proc Nutr Soc. 2001 May;60(2):171-8. Review.
39. Vento M, Asensi M, Sastre J, García-Sala F, Pallardó FV, Viña J. Resuscitation with room air instead of 100% oxygen prevents oxidative stress in moderately asphyxiated term neonates. Pediatrics. 2001 Apr;107(4):642-7.
40. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001 Oct;119(10):1417-36. Erratum in: Arch Ophthalmol. 2008 Sep;126(9):1251.
41. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M. Carotenoid supply in breast-fed and formula-fed neonates. Eur J Pediatr. 2000 Jan-Feb;159(1-2):86-90.
42. Grantham-McGregor SM, Walker SP, Chang S. Nutritional deficiencies and later behavioural development. Proc Nutr Soc. 2000 Feb;59(1):47-54. Review
43. Kiely M, Cogan PF, Kearney PJ, Morrissey PA. Concentrations of tocopherols and carotenoids in maternal and cord blood plasma. Eur J Clin Nutr. 1999 Sep;53(9):711-5.
44. Yeum K.J et al (1998) "Relationship of plasma carotenoids, retinol and tocopherols in Mothers and Newborn infants". J of American College of Nutrition 17(5): 442-7.
45. Oostenbrug G.S. et al (1998) "Maternal and neonatal plasma antiossidant levels in normal pregnancy, and the relationship with fatty acid unsaturation". Br. J of Nutrition 80: 67-73.
46. Bonn D. Keeping the stork at bay until the time is right. Lancet. 1998 Feb 21;351(9102):576
47. Sommerburg O, Keunen JE, Bird AC, van Kuijk FJ. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br J Ophthalmol. 1998 Aug;82(8):907-10
48. Jackson JG, Eric L, Lien A, Sharon J, White B, Nicholas J, Bruns C, Charles F, Kuhlman A. Major carotenoids in mature human milk: longitudinal and diurnal patterns. The Journal of Nutritional Biochemistry 1998 Jan;9(1):2-7.
49. Khachik F, Spangler CJ, Smith JC Jr, Canfield LM, Steck A, Pfander H. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. Anal Chem. 1997 May 15;69(10):1873-81
50. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp Eye Res. 1997 Jul;65(1):57-62.

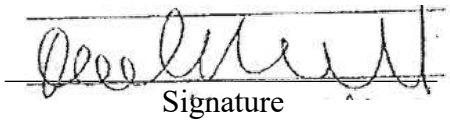
51. Leung AK, Siu TO, Chiu AS, Robson WL, Larsen TE. Serum carotene concentrations in normal infants and children. Clin Pediatr (Phila). 1990 Oct;29(10):575-8; discussion 579-80.
52. Nakamura H, Lee Y, Uetani Y, Kitsunezuka Y, Shimabuku R, Matsuo T. Effects of phototherapy on serum unbound bilirubin i icteric newborn infants. Biol Neonate. 1981;39(5-6):295-9.

SIGNATURES FOR APPROVAL

Signature

9-6-2017

Date



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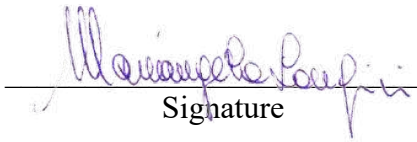

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