

James Allen Olson Memorial Lecture

Vitamin A, carotenoids, and inflammation in infancy

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Key messages

- > Vitamin A and specific carotenoids are important immunomodulatory and anti-inflammatory dietary components.
- > Adequate intake of vitamin A, and possibly also of carotenoids, is important for establishing normal pregnancy and resistance to infections in early life.
- > Specific carotenoids promote polarization of T effector cells and macrophages toward anti-inflammatory phenotypes.
- > Xanthophyll carotenoids, including lutein and astaxanthin, suppress neuroinflammation.
- > Lutein inhibits the neuroinflammatory response to hypoxic ischemic brain injury in a perinatal rat model.

Dietary vitamin A and infection in early life

Dietary carotenoids and retinoids play important roles in innate and acquired immunity and in the inflammatory response during pregnancy and development. In particular, vitamin A (VA) deficiency, which affects 190 million children worldwide, increases the likelihood of early childhood mortality due to common infections.^{1,2} In VA-deficient or insufficient states, the increased susceptibility to immune-mediated and inflammatory disorders is related to impaired responses to infection, impaired epithelial barrier function,^{3,4} and immunological defects. In particular, responses to mucosal pathogens are impaired when VA stores are low – in part because VA metabolites promote functional

maturation of innate immune cells.^{3,5,6} In VA-deficient animals, T-cell and occasionally B-cell populations are reduced, and myeloid lineage cells, especially granulocytes, tend to be increased. Infections decrease VA intake as a result of infection-induced anorexia, altered VA intestinal absorption,⁷ and increased urinary excretion.⁸ Effects of systemic inflammation on circulating carotenoid levels, mobilization, or bioavailability have been less studied. Certainly, some carotenoid effects on immunity and inflammation are mediated by provitamin A activity. In addition, immunomodulatory and anti-inflammatory effects of specific xanthophyll carotenoids (including lutein, zeaxanthin, and astaxanthin) appear to be independent of actions on retinoid receptors (RARs, RXRs).

Carotenoids and inflammation in pregnancy and fetal development

The inflammatory response is tightly regulated during reproduction, embryonic and fetal development, and the postnatal transition into infancy. In humans, during implantation, uterine T helper (Th) cells are polarized to a predominant Th1 (pro-inflammatory) over Th2 (anti-inflammatory) cell effector profile and tissue pro-inflammatory cytokines (including IL-6, IL-15, IFN- γ , and TNF- α) promote placental trophoblast invasion into the maternal endometrium, myometrium, and uterine vasculature. This tissue invasion is more extensive than in other mammals, including closely related non-human primates. Placental trophoblast invasion into uterine tissue layers results in recruitment and activation of maternal immune cells. In contrast, after the uteroplacental bed is established during the first trimester, a normal pregnancy state is characterized by immune quiescence – namely, a Th2 cytokine profile and suppression of maternal immunological rejection of the “foreign” fetoplacental unit.⁹ A pro-inflammatory, Th1 cytokine state is reactivated during parturition,¹⁰ resulting in the positive feedback loop that ends with birth and placental expulsion.

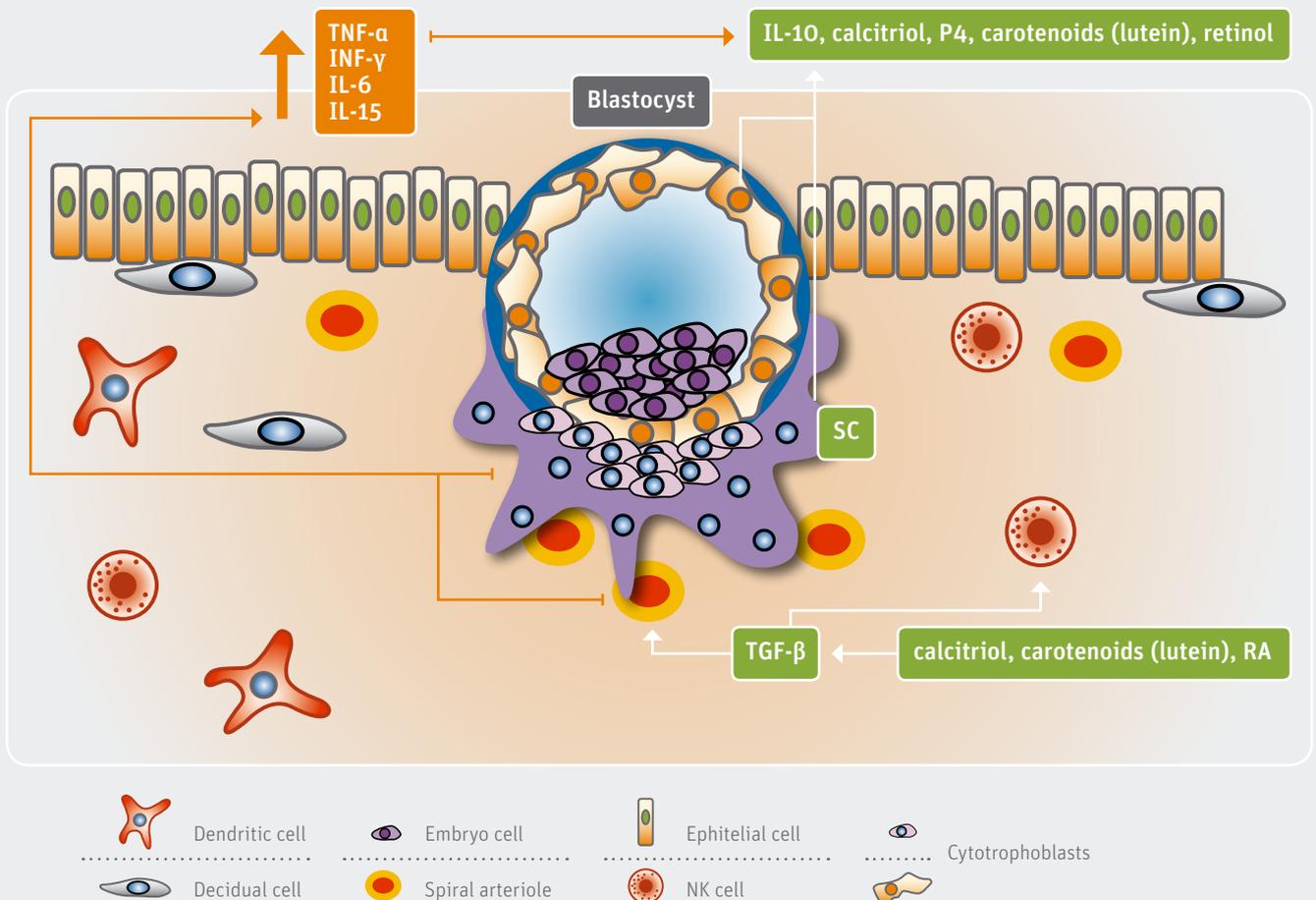
In preeclampsia, a major pro-inflammatory and pro-oxidant disorder of placentation and pregnancy, uteroplacental inflammation and pro-inflammatory Th1 cytokines persist into the second and third trimester. Retinol and certain carotenoids (e.g.,

lutein) may suppress this inflammation by antagonizing the pro-inflammatory cytokine response and by promoting TGF- β activity (Figure 1). TGF- β produced by macrophages interacts with natural killer (NK) cells, facilitating maternal tissue acceptance of trophoblasts while preventing them from killing fetal cells. In order to prevent excessive inflammation that could result in the rejection of the fetal allograft, several systemic factors including retinol and carotenoids, calcitriol, IL-10, progesterone (P4) produced by decidual cells, trophoblasts, and syncytiotrophoblasts act as anti-inflammatory factors modulating this immunosuppressive microenvironment. Retinoic acid (RA) and certain carotenoids are also known to induce TGF- β . It is intriguing that analyses of maternal carotenoid concentrations suggest inverse relationships between plasma lutein, α - and β -carotene, and lycopene with risk or severity of preeclampsia as well as another pro-inflammatory condition in pregnancy, diabetes mellitus.¹¹⁻¹⁴

“Analyses of maternal carotenoid concentrations suggest inverse relationships between plasma lutein, α - and β -carotene, and lycopene”

Immune cell (lymphocyte) proliferative responses to mitogens are also retinoid-dependent.^{15,16} In pregnant mice, supplementation with VA and β -carotene affects immune cell functions during ontogenesis.¹⁷ In these experiments, the dams were provided with a control diet or different retinoid- and carotenoid-enriched (4,500 retinol equivalents/kg) diets beginning at conception. The VA and β -carotene supplementations variously in-

FIGURE 1: Model for specific retinoid and carotenoid action on cytokine profiles during implantation. At the fetal/ maternal interface during early pregnancy, a state of controlled inflammation promotes trophoblast invasion of the endometrium, myometrium and uterine spiral arteries. RA and certain carotenoids including lutein, α - and β -carotene, and lycopene may participate in regulating this process and subsequent onset of maternal/placental/fetal immune quiescence. Modified from¹⁰



creased lymphocyte numbers in early and mid-pregnancy and increased the T:B cell ratios.¹⁷ In mice, maternal VA supplementation via intraperitoneal injections have increased serum IgM and Th2-specific IgG1 levels in the offspring.¹⁸ Moreover during postnatal development VA regulates the Th1:Th2 switch and thereby modifies immune and inflammatory responses.^{19,20}

VA, carotenoids, and inflammation in the newborn

Intermittent or sustained systemic inflammation is an important component of many diseases of newborns and infants, and importantly contributes to the pathogenesis of most disorders associated with prematurity, including chronic lung disease (also known as bronchopulmonary dysplasia [BPD]), brain damage, and neurodevelopmental disorders.^{21,22} In a clinical trial among extremely low birth weight (< 1,000 g) preterm infants who have high risk for BPD, VA supplementation (5,000 IU retinyl palmitate, three times weekly for four weeks) significantly decreased the prevalence of BPD.²³ Regarding preventive and therapeutic strategies for hypoxic-ischemic brain injury in both preterm and term infants, interventions increasingly emphasize neurotoxic and neuroinflammatory cascades. A recent focus is to develop strategies for enhancing endogenous neuroprotective mechanisms. Lutein, which is selectively accumulated in retina and brain, has anti-inflammatory activity in retinal diseases and may be a promising neuroprotective agent throughout the lifespan.

“Lutein has anti-inflammatory activity in retinal diseases and may be a promising neuroprotective agent throughout the lifespan”

Both RA²⁴ and lutein²⁵ suppress neuroinflammation mediated by astrocytes and microglia – two cell types important in acute brain injury accompanying preterm and term birth (Figure 2). In addition to lutein, several other xanthophylls, such as astaxanthin, similarly have anti-inflammatory neuroprotective effects. Known mechanisms of xanthophyll (lutein, astaxanthin) mediated neuroprotection include blocking the actions of: NF- κ B signaling on microglial/astrocyte activation, neuronal inflammation, inflammatory cytokine/chemokine release, and neuronal cell death.²⁶

Investigating carotenoid effects on developmental immunity in animal models has been challenging due to pronounced differences in carotenoid absorption, kinetics, and metabolism between primates (humans, macaques) and rodents. Nevertheless, we have shown as proof of principle in a perinatal rat model



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that lutein is transferred from dam to fetuses and via the milk to the pups and suppresses hypoxic ischemic neuroinflammation. In this hypoxic/ischemic (HI) brain injury model, anesthetized neonatal rat pups (day 7) undergo left carotid artery ligation (unilateral brain ischemia) followed by several hours' duration in ambient low-oxygen tension (hypoxia). Pups were sacrificed on day 10 and brain tissue analyzed by histology (hematoxylin/eosin and Nissl staining); immunohistochemistry for markers of hypoxic cell damage, apoptosis/necrosis, and neuroinflammation; gene expression profiling by RT-qPCR; protein expression and activation by western blot; and lutein accumulation by HPLC. Figure 2 illustrates the differences in inflammatory cytokine and receptor whole-brain gene expression between lutein pretreated (y-axis) and untreated (x-axis) animals. The blue lines represent greater than two-fold increased and decreased expression. Lutein upregulated several anti-inflammatory mediators including IL-11, IL-1R, CCR5, and CXCR1; lutein pretreatment decreased expression of multiple pro-inflammatory genes including TNF- α family members, interleukins, and Fas ligand.

Lutein: breast milk levels and dietary supplementation

The fetus acquires carotenoids via transplacental transfer from the mother. Hence, fetal and cord blood carotenoids levels are, in part, dependent on maternal dietary intake. In newborns and infants, breast milk is the intended complete diet. As in the case for transplacental carotenoid transfer, breast milk carotenoid levels also rely on maternal diet. Among the various carotenoids in human milk, lutein is usually predominant.²⁷ In term newborns, lutein supplementation suppresses measures of systemic oxidant stress.²⁸

glycation end-product formation; suppressing scavenger receptor expression; suppressing lymphocyte and macrophage activation;³³ and modulating T cell polarization, e.g., increasing T regulatory (Treg) and decreasing Th17 cell expansion.⁵ Recent investigations point to important roles in T cell polarization for several other, presumably non-provitamin A xanthophylls. One example is fucoxanthin, found in brown algae. Fucoxanthin-mediated Treg induction and Th17 inhibition lead to suppressed inflammation and autoimmunity.³⁴ Recent data indicate xanthophylls are also regulators of macrophage-dependent immune responses. The dietary xanthophyll astaxanthin, which is found in pink-orange fish and crustaceans, drives IL-10 production in (anti-inflammatory) M2 macrophages. Similarly, lutein and astaxanthin both support monocyte polarization away from the 'killer' M1 macrophage phenotype to M2 macrophages (Rubin LP, unpublished data).

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“Retinoids and carotenoids are important mediators of the immune system and inflammatory balance during development and early life”

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Conclusions

Retinoids and carotenoids are important mediators of the immune system and inflammatory balance during development and early life. The effects of vitamin A on embryonic and fetal development, immunity, epithelial integrity, and protection from infection are well recognized. More recent evidence also highlights retinoid (e.g., retinoic acid, retinaldehyde, pro-vitamin A apocarotenoids) effects on immune cell differentiation and polarization. Carotenoids, particularly xanthophylls, also appear to play important and, perhaps, critical roles as anti-inflammatory and immunoregulatory regulators. Lutein, in particular, in experimental animal studies and clinical studies in infants, suppresses systemic inflammation and mitigates the inflammatory response to retina and brain injury. Finally, recent findings point to a wider function of various human dietary xanthophylls in differentiation and polarization of immune cells including effector T cells, macrophages, and dendritic cells.

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References

01. WHO. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. Geneva: World Health Organization, 2009.
02. Cediel G, Olivares M, Brito A et al. Interpretation of serum retinol data from Latin America and the Caribbean. *Food Nutr Bull* 2015;36(2):S98–108.
03. Stephensen CB. Vitamin A, infection, and immune function. *Annu Rev Nutr* 2001;21:167–92.
04. Biesalski HK, Nohr D. New aspects in vitamin A metabolism: the role of retinyl esters as systemic and local sources for retinol in mucous epithelia. *J Nutr* 2004;134:S3453–7.
05. Gong X, Rubin LP. Carotenoids in early life (Chapter 10). In: Tanumihardjo SA, ed. *Carotenoids in human health*. New York: Springer Science and Business Media, 2013. pp. 167–79.
06. Guo Y, Brown C, Ortiz C et al. Leukocyte homing, fate, and function are controlled by retinoic acid. *Physiol Rev* 2015;95:125–48.
07. Rahman MM, Mahalanabis D, Alvarez JO et al. Acute respiratory infections prevent improvement of vitamin A status in young infants supplemented with vitamin A. *J Nutr* 1996;126:628–33.
08. Mitra AK, Alvarez JO, Stephensen CB. Increased urinary retinol loss in children with severe infections. *Lancet* 1998;351:1033–4.
09. Chaouat G. Reconsidering the Medawar paradigm placental viviparity existed for eons, even in vertebrates; without a “problem”: Why are Tregs important for preeclampsia in great apes? *J Reprod Immunol* 2016;114:48–57.
10. Barrera D, Díaz L, Noyola-Martínez N et al. Vitamin D and inflammatory cytokines in healthy and preeclamptic pregnancies. *Nutrients* 2015;7:6465–90.
11. Cohen JM, Beddaoui M, Kramer MS et al. Maternal Antioxidant Levels in Pregnancy and Risk of Preeclampsia and Small for Gestational Age Birth: A Systematic Review and Meta-Analysis. *PLOS ONE* 2015;10:e0135192.
12. Azar M, Basu A, Jenkins AJ, Nankervis AJ et al. Serum carotenoids and fat-soluble vitamins in women with type 1 diabetes and preeclampsia: a longitudinal study. *Diabetes Care* 2011;34:1258–64.
13. Williams MA, Woelk GB, King IB et al. Plasma carotenoids, retinol, tocopherols, and lipoproteins in preeclamptic and normotensive pregnant Zimbabwean women. *Am J Hypertens* 2003;16:665–72.
14. Palan PR, Mikhail MS, Romney SL. Placental and serum levels of carotenoids in preeclampsia. *Obstet Gynecol* 2001;98:459–62.
15. Wang W, Ballow M. The effects of retinoic acid on in vitro immunoglobulin synthesis by cord blood and adult peripheral blood mononuclear cells. *Cell Immunol* 1993;148:291–300.

16. Ballow M, Wang W, Xiang S. Modulation of B-cell immunoglobulin synthesis by retinoic acid. *Clin Immunol Immunopathol* 1996;80:S73–81.
17. Garcia AL, Ruhl R, Herz U et al. Retinoid- and carotenoid-enriched diets influence the ontogenesis of the immune system in mice. *Immunology* 2003;110:180–7.
18. Guzman JJ, Caren LD. Effects of prenatal and postnatal exposure to vitamin A on the development of the murine immune system. *Life Sci* 1991;49:1455–62.
19. Gossage C, Deyhim M, Moser-Veillon PB et al. Effect of β -carotene supplementation and lactation on carotenoid metabolism and mitogenic T lymphocyte proliferation. *Am J Clin Nutr* 2000;71:950–5.
20. Ruhl R, Hanel A, Garcia AL et al. Role of vitamin A elimination or supplementation diets during postnatal development on the allergic sensitisation in mice. *Mol Nutr Food Res* 2007;51:1173–81.
21. Dammann O, Leviton A. Intermittent or sustained systemic inflammation and the preterm brain. *Pediatr Res* 2014;75:376–80.
22. Leviton A, Allred EN, Fichorova RN et al, for the ELGAN study investigators. Systemic inflammation on postnatal days 21 and 28 and indicators of brain dysfunction 2 years later among children born before the 28th week of gestation. *Early Hum Devel* 2016;93:25–32.
23. Tyson JE, Wright LL, Oh W et al. Vitamin A supplementation for extremely-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. *N Engl J Med* 1999;340:1962–8.
24. van Neerven S, Nemes A, Imholz P et al. Inflammatory cytokine release of astrocytes *in vitro* is reduced by all-trans retinoic acid. *J Neuroimmunol* 2010;229:169–79.
25. Gong X, Sambalingam D, Rubin LP. Lutein suppresses effects of brain injury in a neonatal rat model: implications for nutritional neuroscience. Presented at the Carotenoids Gordon Research Conference, Lucca, Italy, 2016.
26. Zhou X, Zhang F, Hu X et al. Inhibition of inflammation by astaxanthin alleviates cognition deficits in diabetic mice. *Physiol Behav* 2015;151:412–20.
27. Lipkie TE, Morrow AL, Jouni ZE et al. Longitudinal Survey of Carotenoids in Human Milk from Urban Cohorts in China, Mexico, and the USA. *PLOS ONE* 2015;10:e0127729.
28. Perrone S, Longini M, Marzocchi B et al. Effects of lutein on oxidative stress in the term newborn: a pilot study. *Neonatology* 2010;97:36–40.
29. Rubin LP, Chan GM, Barrett-Reis BM et al. Effect of carotenoid supplementation on plasma carotenoids, inflammation and visual development in preterm infants. *J Perinatol* 2012;32:418–24.
30. Gong X, Rubin LP. Role of macular xanthophylls in prevention of common neovascular retinopathies: retinopathy of prematurity and diabetic retinopathy. *Arch Biochem Biophys* 2015;572:40–8.
31. Yeh PT, Huang HW, Yang CM et al. Astaxanthin inhibits expression of retinal oxidative stress and inflammatory mediators in streptozotocin-induced diabetic rats. *PLOS ONE* 2016;11:e0146438.
32. Tang L, Zhang Y, Jiang Y et al. Dietary wolfberry ameliorates retinal structure abnormalities in db/db mice at the early stage of diabetes. *Exp Biol Med* 2011;236:1051–63.
33. Lo HM, Chen CL, Yang PH et al. The carotenoid lutein enhances matrix metalloproteinase-9 production and phagocytosis through intracellular ROS generation and ERK1/2, p38 MAPK, and RAR β activation in murine macrophages. *J Leukoc Biol* 2013;93:723–35.
34. Kawashima T. A marine carotenoid, fucoxanthin, induces regulatory T cells and inhibits Th17 cell differentiation *in vitro*. *Biosci Biotechnol Biochem* 2011;75:2066–9.