

Original Paper

Relationship of Plasma Carotenoids, Retinol and Tocopherols in Mothers and Newborn Infants

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Key words: cord blood, lutein, cryptoxanthin, β -carotene, retinol, tocopherol

Objective: We studied the relationship between maternal and cord plasma concentrations of carotenoids, retinol, and tocopherols in normal mother-baby pairs.

Methods: Healthy pregnant women (n=10) were recruited at a Montréal hospital. Venous blood samples were collected from the mothers at delivery and cord blood was obtained immediately post partum from the umbilical vein after clamping of the cord. All deliveries were full term deliveries and all babies had normal birth weights. Maternal and umbilical cord blood samples were handled identically. Plasma was digested with lipase and plasma carotenoids were extracted and measured using HPLC.

Results: Cord plasma concentration of carotenoids were significantly lower than that of maternal plasma ($p<0.001$). There was a high correlation of lutein ($r=0.889$, $p=0.006$) and cryptoxanthin ($r=0.912$, $p=0.0002$) between maternal plasma concentrations and cord plasma concentrations. The concentrations of the hydrocarbon carotenoids, α -carotene and β -carotene, were also correlated ($r=0.779$, $p=0.0133$, & $r=0.782$, $p=0.0076$, respectively) between maternal plasma and cord plasma. Whereas the plasma concentration of the acyclic carotenoid, lycopene, showed no correlation between the two groups, after adjustment for plasma triglycerides, the lycopene correlation between maternal and cord plasma was the highest ($r=0.975$, $p=0.0001$) of all the carotenoids tested. Cord plasma retinol concentration, which was 50% of that of maternal plasma, was also found to have no correlation with that of maternal plasma. Plasma concentration of α -tocopherol showed no correlation between two groups, whereas there was high correlation between cord and maternal γ -tocopherol concentrations ($r=0.808$, $p=0.0047$).

Conclusion: The nutritional status of mothers affects the nutritional status of their babies for certain fat soluble nutrients.

INTRODUCTION

Newborn fat-soluble nutrient concentrations in blood have been shown to be much lower than maternal values [1]. The reasons for the lower concentrations of plasma fat-soluble nutrients in the newborn are not clear. Even though several epidemiologic studies suggest that dietary carotenoids have biological functions in relation to the prevention of chronic diseases [2–4], the role of carotenoids in the newborn is not known. Thus, it is first necessary to clarify the carotenoid concentrations in the circulation of mothers and their babies, and to delineate the relationship between maternal plasma

carotenoid concentrations and those of the newborn. A few studies have reported concentrations of major plasma carotenoids in the newborn. In the present study, the relationship between maternal plasma and newborn plasma concentrations of carotenoids, retinol and tocopherols are evaluated.

MATERIALS AND METHODS

Subjects

Healthy pregnant women (n=10) were recruited at L'Hôpital Ste-Justine in Montréal. All women underwent uncomplicated pregnancies and gave birth to healthy newborns.

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Abbreviations: Lut = lutein, Zeax = zeaxanthin, Cryp = cryptoxanthin, α -C = α -carotene, β -C = β -carotene, Lyco = lycopene, TG = triglyceride.

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The study protocol was approved by the Hospital's Ethics Committee and informed consent was obtained from each participant. We focused on the association of plasma concentrations of carotenoids, vitamin A and vitamin E between newborn babies and their mothers. Pregnant women were recruited for the study at 24 to 28 weeks of pregnancy.

Plasma Analysis

All-*trans* β -carotene (type IV), α -carotene, lycopene, α -tocopherol, γ -tocopherol, triglyceride hydrolase (*Chromobacterium viscosum*), and cholesterol esterase (*Pseudomonas* species) were purchased from Sigma Chemical Co. (St. Louis, MO). Lutein was purchased from Kemin Industries (Des Moines, IA). Zeaxanthin, cryptoxanthin, 13-*cis* β -carotene, 9-*cis* β -carotene, and echinenone were gifts from Hoffmann-La Roche (Nutley, NJ). Solutions of carotenoids and retinoids were prepared under red light immediately before use. All HPLC solvents were obtained from J.T. Baker Chemical (Phillipsburg, NJ) and were filtered through a 0.45 μ m membrane filter before use.

Venous blood samples were collected in a vacutainer containing EDTA from the mothers at delivery. Cord blood was obtained immediately post partum from the umbilical vein after clamping of the cord. Maternal and umbilical cord blood samples were handled identically. Samples were protected from light and centrifuged for 15 minutes (800 \times g, 4°C) within 1 hour of collection. Aliquots of plasma were stored at -70°C until analyzed. Plasma carotenoids, retinol and tocopherols were extracted using a modified enzyme extraction method reported earlier [5]. Echinenone, retinyl acetate and tocol were added as internal standards for the analysis of carotenoids, retinoids and tocopherols, respectively. The extracted sample was analyzed for carotenoids, retinoids and tocopherols using a reverse-phase, gradient HPLC system. The HPLC system consisted of a Series 410 LC pump (Perkin-Elmer, Norwalk, CT), a Waters 717 plus autosampler (Millipore, Milford, MA), a C30 carotenoid column (3 μ m, 150 \times 4.6 mm, YMC, Wilmington, NC), an HPLC Column Temperature Controller (Model 7950 Column Heater/Chiller, Jones Chromatography, Lakewood, CO) and a Waters 840 Digital 350 data station. The Waters 994 programmable photodiode array detector was set at 450 nm for carotenoids and 340 nm for retinoids. A fluorescence detector (Ex:292nm, Em:330nm; Waters 470; Millipore) was connected for tocopherol analysis. The HPLC mobile phase was methanol:methyl-tert-butyl ether:water (83:15:2, v/v/v, with 1.5% ammonium acetate in the water, solvent A) and methanol:methyl-tert-butyl ether:water (8:90:2, v/v/v, with 1% ammonium acetate in the water, solvent B). The gradient procedure at a flow rate of 1 ml/minute (16°C) was as follows: 1) 90% solvent A and 10% solvent B for 5 minutes; 2) a 12-minute linear gradient to 55% solvent A; 3) a 12-minute linear gradient to 95% solvent B; 4) a 5-minute hold at 95% solvent B; and 5) a 2-minute gradient back to 90% solvent A and 10% solvent B.

Using this method, lutein, zeaxanthin, cryptoxanthin, α -carotene, 13-*cis* β -carotene, all-*trans* β -carotene, and lycopene were adequately separated. Typical HPLC chromatograms of a pair of plasma are shown in Fig. 1. Carotenoids, retinol and tocopherols were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. The lower limits of detection were 0.2 pmol for carotenoids, 2.0 pmol for retinol and 2.7 pmol for tocopherols. For analyses of the data, adjustments were made for the differences in maternal and fetal triglyceride and cholesterol levels when comparing maternal and fetal plasma concentrations of the carotenoids, retinol and tocopherols.

Cholesterol was released and oxidized by enzymatic reactions (Roche reagents, Roche Diagnostic Systems, Inc., Branchburg, NJ), and quantitated photometrically (COBAS MIRA systems, Roche Diagnostic Systems, Inc., Branchburg, NJ) [6]. Triglycerides were hydrolyzed, phosphorylated and oxidized by enzymatic reactions (Roche reagents, Roche Diagnostic Systems, Inc., Branchburg, NJ), and measured photometrically (COBAS MIRA systems, Roche Diagnostic Systems, Inc., Branchburg, NJ) [7].

Statistical Analysis

Results are expressed as means \pm SEM, and the significance of differences was determined by Student's *t* test. The correlations between maternal plasma nutrient concentrations and cord plasma nutrient concentrations were measured by

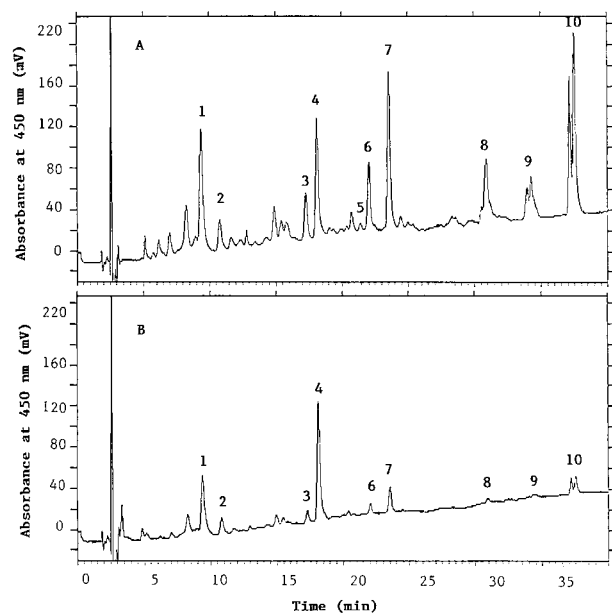


Fig. 1. HPLC chromatograms of the major carotenoids in maternal plasma (A) and cord plasma (B) obtained from a mother-baby pair. The numbered peaks are 1, lutein; 2, zeaxanthin; 3, cryptoxanthin; 4, echinenone (internal standard); 5, 13-*cis*- β -carotene; 6, α -carotene; 7, all-*trans*- β -carotene; 8, and 9, *cis*-lycopene; 10, *trans*-lycopene.

linear-regression analysis using Pearson correlation coefficients using the Statview II program (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Characteristics of the study subjects are shown in Table 1. The mean \pm SEM baseline levels of carotenoids, retinol and tocopherols in the maternal and cord plasma are presented in Table 2. Cord plasma had significantly lower concentrations of carotenoids ($p < 0.001$) compared with those of maternal plasma. The concentration of xanthophylls, such as lutein and zeaxanthin, in cord plasma were 15.5% and 15.1% of maternal plasma concentrations, respectively. However, there was a high correlation of lutein ($r = 0.889$, $p = 0.0006$) and a significant correlation of zeaxanthin ($r = 0.810$, $p = 0.0045$) between maternal plasma concentrations and cord plasma concentrations (Fig. 2). The cord plasma cryptoxanthin, which was 9.8% of maternal plasma concentration, also showed a high correlation ($r = 0.912$, $p = 0.0002$) between the mother-baby pairs (Fig. 2). The concentrations of hydrocarbon carotenoids, α -carotene and β -carotene, in cord plasma were 4.2% and 4.6% of maternal plasma concentrations and similar to lutein and zeaxanthin, there was significant correlation between maternal plasma concentrations and cord plasma concentrations ($r = 0.779$, $p = 0.0133$, $r = 0.782$, $p = 0.0076$; respectively). Unique among the carotenoids, the cord plasma concentration of the acyclic carotenoid, lycopene, which was 3.5% of the maternal plasma concentration, showed no correlation between the two groups.

The concentration of cholesterol in cord plasma was 1/6 of that of maternal plasma, while triglycerides in cord plasma were 1/7 of that of maternal plasma in our study subjects (Table 2). Due to inadequate amounts of sample for two mother-infant pairs, cholesterol and triglyceride were measured in eight pairs of maternal plasma and cord plasma. There was a high correlation between triglyceride concentrations in maternal plasma and cord plasma ($r = 0.896$, $p = 0.0026$). However, no correlation was found between the cholesterol concentration in maternal plasma and cord plasma. When plasma concentrations of carotenoids were adjusted for the triglyceride concentration in

Table 2. Concentrations of Carotenoids, Retinol and Tocopherols in Maternal and Cord Plasma

Nutrients	Plasma		
	Maternal	Cord	
	$\mu\text{mol/L}$ ($n = 10$)		% maternal
Lutein	0.33 ± 0.05	$0.05 \pm 0.01^*$	15.5 ± 1.7
Zeaxanthin	0.08 ± 0.01	$0.01 \pm 0.00^*$	15.1 ± 1.7
Cryptoxanthin	0.59 ± 0.12	$0.06 \pm 0.01^*$	9.8 ± 0.8
α -Carotene	0.19 ± 0.03	$0.01 \pm 0.00^*$	4.2 ± 0.5
all-trans β -Carotene	0.69 ± 0.11	$0.03 \pm 0.01^*$	4.6 ± 0.5
all-trans Lycopene	0.32 ± 0.03	$0.01 \pm 0.00^*$	3.5 ± 0.5
Retinol	1.67 ± 0.13	$0.79 \pm 0.10^*$	50.5 ± 7.3
γ -Tocopherol	3.73 ± 0.49	$0.28 \pm 0.44^*$	7.3 ± 0.7
α -Tocopherol	32.18 ± 2.79	$3.63 \pm 0.52^*$	11.5 ± 1.5
	mmol/L ($n = 8$)		
Cholesterol	2.88 ± 0.18	$0.48 \pm 0.05^*$	16.7 ± 0.02
Triglyceride	3.05 ± 0.35	$0.43 \pm 0.04^*$	14.6 ± 0.01

* Significantly different from maternal plasma values $p < 0.001$.

Values are means \pm SEM.

plasma, the correlations of carotenoids between maternal plasma and cord plasma were all markedly increased (Fig. 3). The effect of adjustment for triglyceride levels was most striking for lycopene, which showed no correlation before this adjustment was made. However, when plasma carotenoid concentrations were adjusted for plasma cholesterol concentrations, the correlations between carotenoid concentrations in maternal plasma and cord plasma did not improve. Mean cord plasma retinol concentration, which was 50% of that of maternal plasma, was found to have no correlation with that of the maternal plasma in the mother-baby pairs. Cord plasma tocopherols were significantly lower ($p < 0.001$) than maternal values. The concentration of α -tocopherol in cord plasma, which was 11.5% of the maternal plasma value, showed no correlation with the maternal value. There was also no correlation between maternal and cord plasma α -tocopherol concentrations after adjustment for triglyceride levels. On the other hand, the concentration of γ -tocopherol in cord plasma, which was 7.3% of that of maternal plasma, showed a high correlation ($r = 0.808$, $p = 0.0047$) with the maternal plasma γ -tocopherol concentration.

DISCUSSION

The nutritional significance of carotenoids in the neonatal period has not been established. However, it is generally accepted that specific dietary carotenoids have their unique biological functions [2,8–11]. In addition to being important vitamin A sources, provitamin A carotenoids, such as α -, β -carotenes and cryptoxanthin, can effectively quench singlet oxygen [12], and trap peroxy and alkoxy radicals [13,14]. Non-provitamin A carotenoids such as lutein and lycopene, other major carotenoids in human plasma, also possess strong

Table 1. Characteristics of Study Subjects¹

Number of maternal/neonatal pairs	10
Maternal age (years)	28.7 ± 1.2
Maternal body mass index (kg/m^2)	25.2 ± 1.8
Gestational age (weeks)	39.7 ± 0.3
APGAR score at 1 minute	9.0 ± 0
5 minutes	9.5 ± 0.2
10 minutes	9.5 ± 0.2
Neonatal birth weight (g)	3667.3 ± 251.4
Neonatal gender (male/female)	7/3
Mothers with smoking habit	4

¹ Values are means \pm SEM.

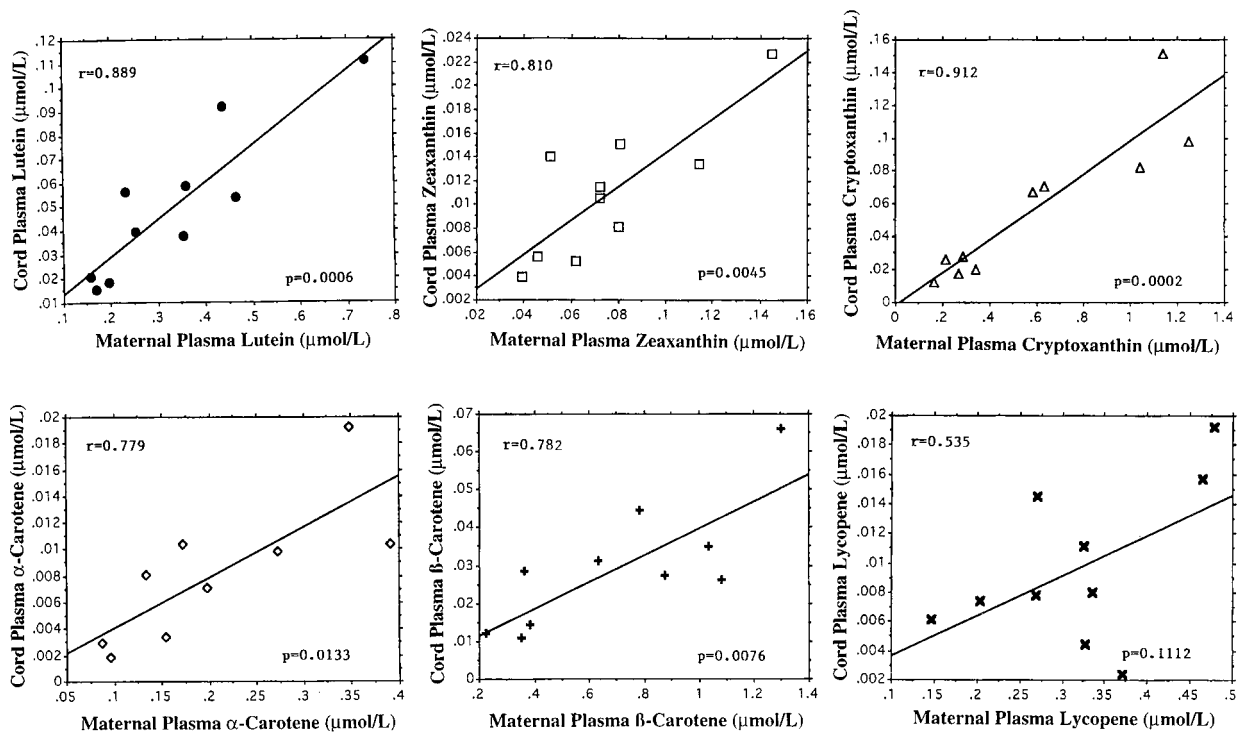


Fig. 2. The correlations of carotenoids between maternal plasma and cord plasma.

antioxidant capability [15]. The intake of foods rich in carotenoids will alter the concentrations of carotenoids in maternal circulation, and thereby modify the carotenoid status of newborns. Cord plasma concentration of carotenoids in our Canadian subjects are comparable with those found in a previous report among Chinese and Japanese subjects [16].

There were higher cord/maternal plasma carotenoid ratios for the more polar carotenoids such as lutein, zeaxanthin and cryptoxanthin (15.5%, 15.1% and 9.8% of maternal values, respectively) than for the non-polar carotenoids such as α -carotene, β -carotene and lycopene (4.2%, 4.6% and 3.5% of maternal values, respectively). Significant correlations were found between maternal plasma and cord plasma carotenoid concentrations except for lycopene. Lycopene concentrations showed no correlation between maternal plasma and cord plasma before adjustment for plasma triglyceride concentrations ($r=0.535$); however the lycopene correlation between maternal and cord plasma was high ($r=0.975$) after this adjustment was made. Furthermore, the correlation of most of the carotenoids was markedly increased between maternal plasma and cord plasma by the adjustment of plasma carotenoid concentrations for the triglyceride concentrations, but not when adjusted for plasma cholesterol concentrations. It has been reported that non-polar plasma carotenoids, such as β -carotene and lycopene, are associated primarily with LDL, whereas polar carotenoids, such as xanthophylls, are distributed equally between LDL and HDL [17,18]. However, Manago et al found that more β -carotene was found in the HDL (55%) fraction

than in the LDL fraction (45%) in cord blood [19]. It has also been shown that cholesterol is the major component of HDL in cord blood, and for the first 7 days after birth [20]. Considering that cholesterol is the major component of LDL and HDL in cord blood and that triglyceride is distributed among all lipoprotein fractions, although in smaller amount, it is probable that circulating carotenoids in maternal plasma are transported to the fetus by both LDL and HDL.

In accordance with others, the mean cord plasma concentration of retinol was found to be about half of that of maternal plasma [21,22]. This is in contrast to the water soluble vitamins which tend to be higher in cord blood than in maternal blood [21,22]. There was no correlation between cord plasma retinol and maternal plasma retinol concentrations, as has been reported earlier [21]. Considering that plasma retinol is transported by retinol binding protein, and is homeostatically controlled [23], it is not surprising to see a lack of relation between the maternal plasma and cord plasma retinol concentration in our healthy mother-baby pairs. Rondo et al found that when moderate maternal vitamin A deficiency occurs, there tends to be a correlation between cord and maternal blood, whereas when maternal vitamin A deficiency is minimal, such a correlation does not occur [21].

Tocopherol concentrations in our Canadian maternal and cord plasma samples were relatively lower than earlier reported values [16,24,25]. However, there is a wide range of vitamin E values depending on the analytical assay used and the population being studied. In accordance with a previous study [24],

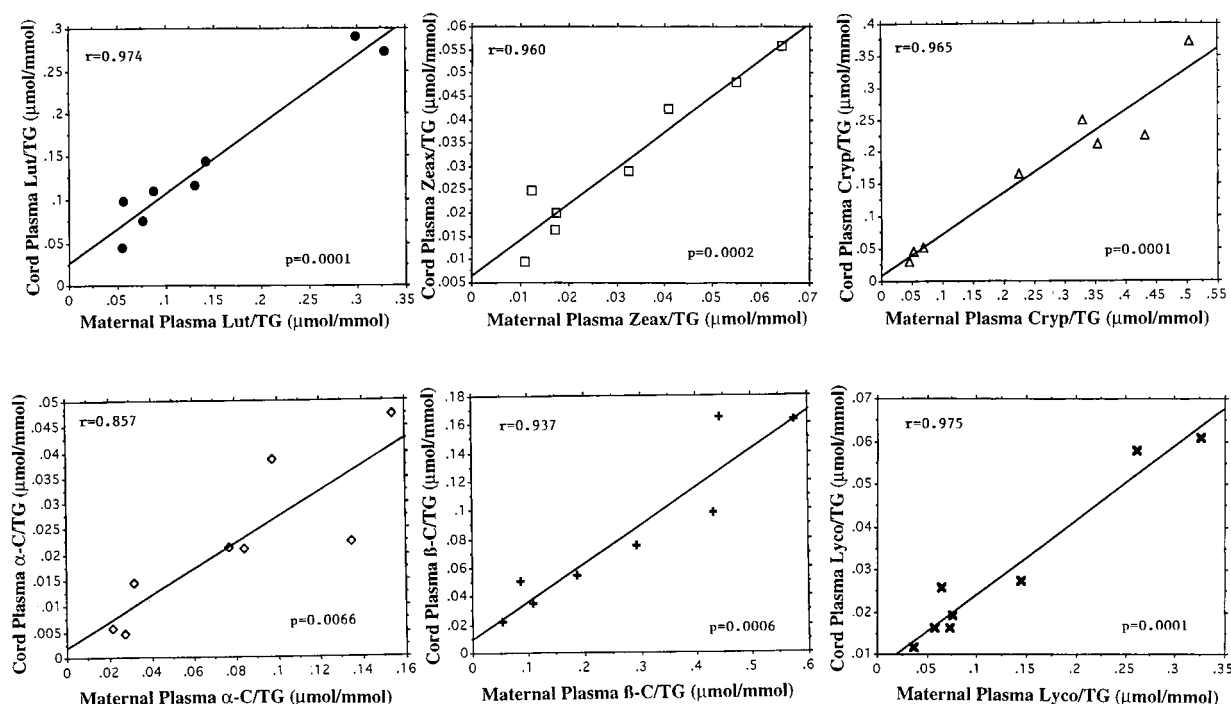


Fig. 3. The correlations of carotenoids/triglyceride between maternal plasma and cord plasma.

tocopherol concentrations of maternal plasma are significantly higher than those of cord plasma ($p < 0.001$). The difference in the concentrations of tocopherols between maternal plasma and cord plasma may be due to lower tocopherol transport capacity in newborns as compared with mothers [16,24,26]. It has been reported that the lower transport capacity in newborns is a reflection of the low cord levels of LDL and of lipids [26]. In contrast to other studies, no correlation was found between maternal and cord plasma α -tocopherol concentrations, either before or after adjustment for differences in lipid levels [1,24,27,28]. These different findings might due to differences in the study populations. However, the cord plasma concentration of γ -tocopherol (which was less than one-tenth that of maternal plasma level) showed a high correlation with maternal plasma γ -tocopherol level.

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