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Carotenoid supply in breast-fed and formula-fed neonates

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Abstract Carotenoids have various biological functions including their role as antioxidants. For humans fruits and vegetables are the only source of carotenoids. In the first months breast milk and/or formula preparations are the only nutrition for infants. To study the influence of nutrition on the plasma carotenoid profile in newborns, breast milk, different formula preparations, and the plasma of breast-fed (BF) and formula-fed (FF) newborns were analyzed by high-performance liquid chromatography. The method used allowed β-carotene, α-carotene, lycopene, and β-cryptoxanthine to be detected and all four were found in breast milk. In colostrum carotenoids were up to five times higher than in mature breast milk ($P < 0.05$). In contrast, not all carotenoids could be found in formula preparations. β-Carotene was detected in four out of eight, and β-cryptoxanthine in three out of eight formula preparations. Lycopene and α-carotene were not detectable in any of the formula preparations. Four formula preparations did not contain any carotenoids. FF infants had different plasma carotenoid profiles compared to BF infants. β-Carotene was significantly lower in FF infants [14 (0–32) μg/l, median and interquartile ranges] than in infants after birth [24 (19–310) μg/l, $P < 0.05$], and BF infants [32 (22–63) μg/l, $P < 0.05$]. While newborns after birth had measurable plasma concentrations of lycopene (16 [14–18] μg/l) and of α-carotene [5 (0–8) μg/l), these carotenoids were no longer detectable in FF infants after day 14.

Conclusion FF and BF infants show significant biochemical differences in plasma carotenoid concentrations.

Key words Carotenoids · Nutrition · Breast milk · Formula preparation · Newborns

Abbreviations *BF* breastfed · *FF* formula fed · *BHT* butylated hydroxytoluene

Introduction

In recent years carotenoids have received attention as a group of micronutrients with a wide spectrum of biological functions. Some of them function as provitamin A, but they also serve as antioxidants. Carotenoid action in vitro is associated with lowered DNA damage, and in epidemiological studies with a lowered incidence of certain types of cancer, cataract, or of ischemic heart disease [2, 8, 17, 19]. Carotenoids are of importance in the process of aging and play an important role in immuno-enhancement [1, 11]. Data from a number of epidemiological studies also suggest that a high intake of these substances can be associated with a decreased risk of ophthalmological disorders such as cataracts and age-related macular degeneration [16]. Since mammals are not able to synthesize carotenoids they have to obtain them from food. Fruit and vegetables have been shown to be sufficient sources of carotenoids for humans [10].

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Few data are available on carotenoid supply and plasma concentrations in newborns [4, 15, 21]. For newborns breast milk and/or formula preparations are the only way to obtain carotenoids. To the best of our knowledge, no data are available on the carotenoid profile in breast-fed (BF) newborns compared with that in formula-fed (FF) newborns. Therefore, the aim of our study was to evaluate the concentrations of four major carotenoids (α-carotene, β-carotene, lycopene, and cryptoxanthine) in breast milk and in several commercially available formula preparations. Furthermore, plasma concentrations of these carotenoids were determined in newborns at the time of birth and 2 to 6 weeks after birth. To investigate whether formula preparations provide the same supply of carotenoids as mother's milk does, these results were related to the kind of nutrition received.

Patients and methods

Patients

A total of 83 blood samples from different preterm and term-born infants fed breast milk or formula preparations and not having hyperbilirubinaemia, liver disease, or any chronic disease were collected in the Department of Neonatology of the University Children's Hospital of Heidelberg, FRG. Blood samples were taken from the infants only when routine hematological examinations were necessary and when informed consent was given by the parents. The remaining blood from the routine examinations was centrifuged (10 min, $500 \times g$) and the plasma was stabilized with 0.03% butylated hydroxytoluene (BHT) and frozen immediately at −80°C. In addition, 24 breast milk samples (6 colostrum samples, 18 samples of mature milk) from 14 mothers were collected. To investigate the carotenoid profile in formula nutrition, eight different brands for term and preterm babies, including two hypoallergenic formula preparations (Beba 0, Humana 0, Beba Pre, Aptamil Pre, Pre Aletemil, PreAponti, Humana HA, Aletemil HA), were analyzed. Three samples of each formula preparation were extracted and analyzed for carotenoids; the values given are means of the three samples.

To study the influence of nutrition in BF and FF infants the profile of plasma carotenoids in infants after birth (n = 23) was compared to the profile in infants aged 2 to 6 weeks. Therefore, three groups of newborns were studied as well as the group of newborns on the day of birth. The first group (n = 18) consisted of infants fed only breast milk (BF), the second group of infants (n = 22) were fed breast milk and formula nutrition (BF/FF), and the third group of infants (n = 20) were fed only formula preparations (FF). Infants fed formula preparations were chosen only if they were older than 2 weeks, so that the influence of postnatal feeding could be evaluated.

Methods

All reagents used, obtained from Merck (Darmstadt, FRG), Roth (Karlsruhe, FRG), or Sigma (St.Louis, Missouri, USA), were of the highest analytical grade. Plasma tocopherols and carotenoids were determined according to the method of Hess et al. [7]. Carotenoids in mother's milk and formula preparations were detected using the extraction procedure described by Guiliano et al. [5]. The concentration of each parameter was calculated by the external standard method. The concentrations of the standards were measured spectrophotometrically using the respective molar absorbance coefficients.

For high-performance liquid chromatography (HPLC) we used a Beckman System Gold Programmable Solvent Module 126 (Beckman Instruments Inc., Palo Alto, CA), a Beckman UV/Vis-Detector Module System Gold 167, and a Sparc Promis II injector with a 100 µl injection loop. The UV/Vis-Detector was set at wavelength 450 nm from 0 to 9 min, at 470 nm from 9 to 14 min, and at 450 nm from 14 to 25 min to detect the different carotenoids. A reversed phase 5 µm C_{18} column, 250 × 4.6 mm (Beckman 3UE4631) was used. 500 ml of the mobile phase consisted of 342 ml acetonitrile, 110 ml tetrahydrofurane, 34 ml methanol, and 14 ml 1% ammonium acetate. The initial flow rate was 0.5 ml/min.

Statistics

To compare the concentrations of the single groups the non-parametric U-test of Mann, Whitney, and Wilcoxon was used. The data are presented as median, interquartile ranges, minimum, and maximum. Differences between the two groups were considered significant if $P < 0.05$.

Results

Comparing colostrum with mature breast milk we found that colostrum contains up to five times more carotenoids than mature breast milk (Fig. 1). In colostrum we found 75 (66–142) µg/l (median and interquartile ranges) of cryptoxanthine compared with 18 (14–27) µg/l in mature breast milk, 121 (104–141) µg/l of lycopene compared with 32 (30–41) µg/l, 61 (42–78) µg/l of α-carotene compared with 17 (0–26) µg/l, and 254 (176–351) µg/l of β-carotene compared with 58 (46–74) µg/l. All differences were highly significant ($P < 0.01$).

In the different brands of formula preparations only two of the four measured carotenoids could be detected.

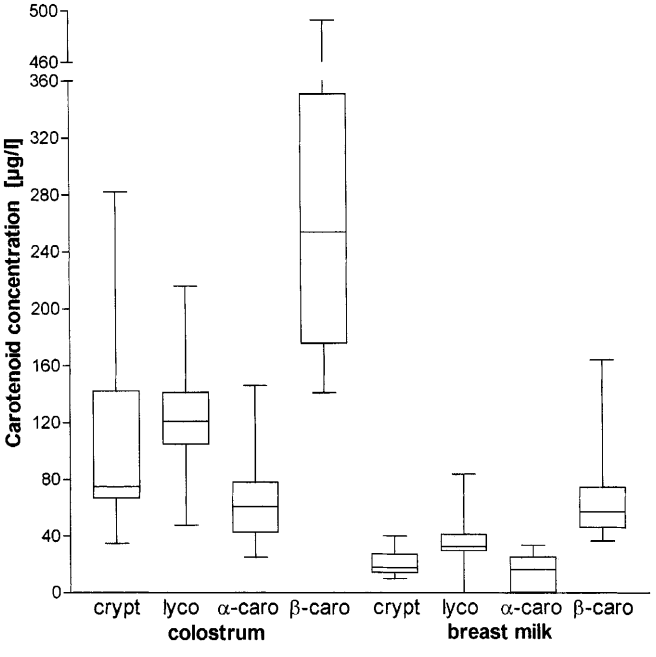

Fig. 1 Concentrations of different carotenoids in colostrum and mature breast milk. Values are given in µg/l as median, interquartile ranges, minimum, and maximum. *Crypt* cryptoxanthine, *lyco* lycopene, α-*caro* α-carotene, β-*caro* β-carotene

β-Carotene was found in four out of eight, and cryptoxanthine was detected in three out of eight preparations. Lycopene and α-carotene were not measurable in any of the formulas studied. Four of the preparations did not contain any of the measured carotenoids. The carotenoid concentrations of the formula preparations compared to the concentrations found in mature breast milk can be seen in Fig. 2.

Plasma concentrations of cryptoxanthine, lycopene, α-carotene, and β-carotene of infants at birth and postnatally in BF, BF/FF, and FF infants are shown in Fig. 3. At birth the plasma concentrations were: cryptoxanthine 11 (8–12) μg/l, lycopene 16 (14–18) μg/l, α-carotene 5 (0–8) μg/l, and β-carotene 24 (19–31) μg/l (median and interquartile ranges). In BF infants carotenoids were higher than at birth: cryptoxanthine 18 (12–40) μg/l, lycopene 19 (16–31) μg/l, α-carotene 8 (0–12) μg/l, and β-carotene 32 (22–63) μg/l. The differences in concentration of cryptoxanthine, lycopene, and β-carotene were statistically significant ($P < 0.05$). In the group of BF/FF infants the concentration of cryptoxanthine was 13 (10–34) μg/l and that of β-carotene was 20 (14–27) μg/l. The concentrations of lycopene [0 (0–12) μg/l] and of α-carotene [0 (0–3) μg/l] were lower than those at birth. The values of lycopene in the BF/FF group were significantly different from those in infants at birth ($P < 0.005$) and from those in the BF group ($P < 0.005$). In the group of FF infants the cryptoxanthine concentration was 12 (0–22) μg/l and the β-carotene concentration was 14 (0–32) μg/l. The values of β-carotene were significantly lower than those measured in infants at birth ($P < 0.05$) and those in BF babies ($P < 0.01$). α-Carotene was undetectable in all FF infants aged 2 weeks or more. Lycopene too could not be detected in FF infants apart from one term infant at day 20 who had a lycopene concentration of 28 μg/l in plasma, which was very high compared to that in all the other infants in our study. The measured concentration was checked twice. A control sample, taken at the postnatal age of 47 days, showed a plasma lycopene concentration of 16 μg/l. This control sample was not considered in the further statistical analysis of this study.

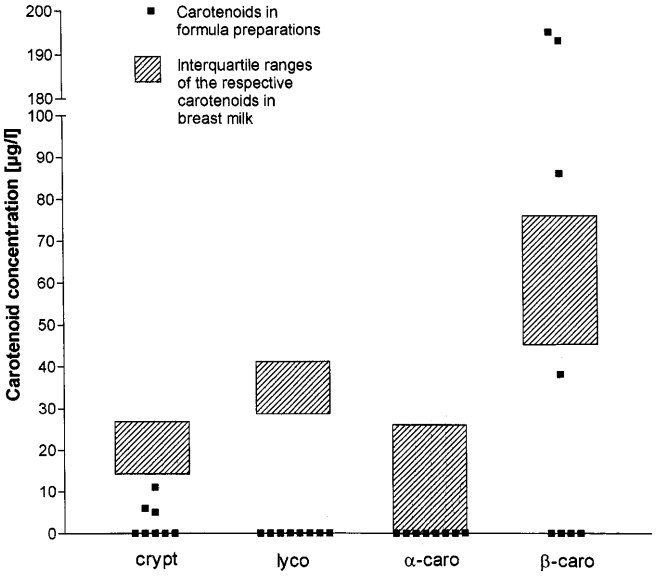

Fig. 2 Carotenoid contents in different brands of formula preparation in comparison to mature breast milk. Concentrations of formula preparations are given in μg/l as means of three measurements. Values of breast milk are given in μg/l as interquartile ranges. *Crypt* cryptoxanthine, *lyco* lycopene, *α-caro* α-carotene, *β-caro* β-carotene

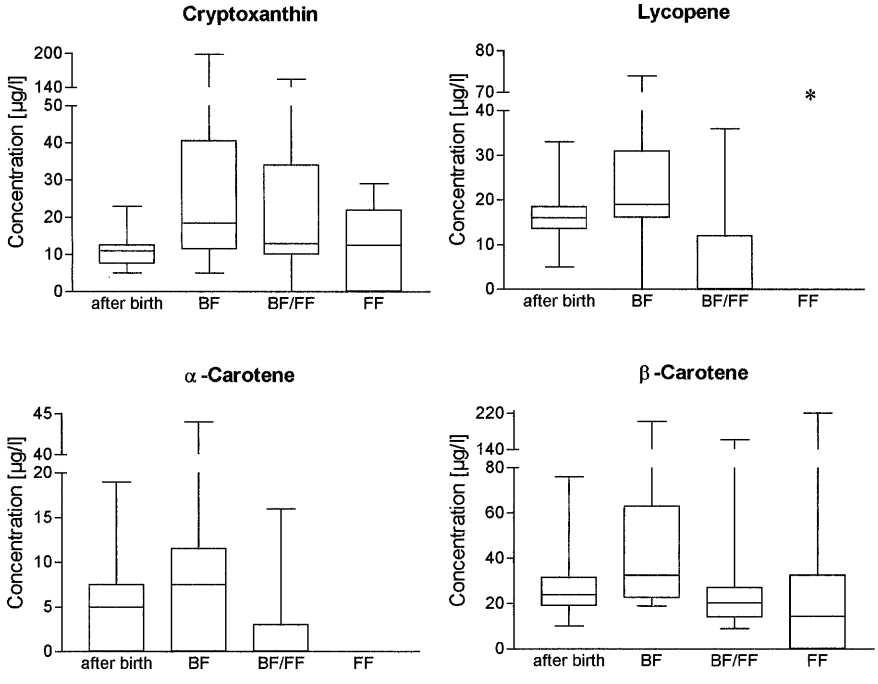

Fig. 3 Plasma carotenoid concentrations of differently fed children and of children on the day of birth. Values are given in μg/l as median, interquartile ranges, minimum, and maximum. *BF* fed with breast milk, *BF/FF* fed with breast milk and formula preparations, *FF* fed only with formula preparations, * value in a single infant

Discussion

To our knowledge little is known about carotenoids in the plasma of newborns [4, 15]. In prenatal life carotenoids are exchanged via the placenta between mother and fetus. The plasma concentration of β-carotene in newborns on the day of birth is reported to be one-eighth of the plasma concentration of the mother [15]. Postnatally, plasma β-carotene increases in BF infants [15].

In the present study we provide the first data on different plasma carotenoids in BF and FF newborns. As these micronutrients can be obtained only from food we compared the carotenoid concentrations in breast milk and formula preparations. Our results show that the carotenoid profile in breast milk is quite different from that in formula preparations. In breast milk all measured carotenoids are detectable. However, like other micronutrients, carotenoids are significantly higher in colostrum than in mature breast milk. In contrast, formula preparations have a totally different carotenoid profile. Four of the preparations analyzed contained β-carotene; three also contained β-cryptoxanthine. However, in four formula preparations none of the measured carotenoids were detectable.

The plasma carotenoid profile of FF newborns is different from that of newborns on the day of birth and from that of BF infants. Lycopene and α-carotene, which were not found in any of the formula preparations, were also not detectable in the plasma of FF infants aged 2 weeks or more. In BF/FF infants lycopene and α-carotene were also detected at much lower levels than in BF infants. These results clearly demonstrate that these carotenoids are consumed and disappear from plasma if not sufficiently provided by nutrition. Since lycopene is considered to be one of the most potent singlet oxygen scavengers [3, 18], this might well affect the antioxidant capacity of these infants. Plasma lycopene could be detected in only one FF infant, in whom the concentration was the highest of all newborns in this study.

The plasma cryptoxanthine concentration in FF newborns was also lower (though not significantly so) than in newborns of the other groups. The cause might be the lower concentrations or absence of cryptoxanthine in formula preparations.

Four out of eight formula preparations contained β-carotene. Two of them had levels close to the concentrations measured in mature mother's milk; two others had β-carotene concentrations which were about four times as high as in mature breast milk. On average, though, the group of FF newborns showed significantly lower β-carotene levels than newborns after birth and BF newborns. This effect might be explained by a reduced antioxidant capacity caused by a lower content of other carotenoids. However, some FF newborns had β-carotene levels up to four times higher than those in BF infants, possibly due to feeding with preparations containing very high β-carotene concentrations. It remains to be discussed if a high-dose supplementation of a single carotenoid is desirable. According to the results of the β-carotene and retinol efficiency trial (CARET) such supplementation might even be harmful under certain conditions [14, 20].

Little is known about the physiological function of carotenoids in the first weeks of human life. It is well established that a number of them serve as precursors of vitamin A [13]. Some carotenoids, notably β-carotene and lycopene, as well as the oxycarotenoids zeaxanthin and lutein, which were not measured in this study, exert antioxidant effects in lipid phases by free-radical or $^1O_2$ quenching [16]. In preterm and term infants carotenoids may function as antioxidants and so contribute to the total free radical trapping ability of the plasma [12]. In in vitro experiments it could also be shown that some carotenoids are important regulators of the immunoresponse [9]. The individual functions of the different carotenoids indicate that the altered carotenoid profile in FF infants may have consequences for the antioxidant function in general and for the individual development of the infants. A normal or above-normal plasma level of β-carotene may possibly compensate for the decreased antioxidant capacity caused by the absence of other carotenoids. However, β-carotene cannot be expected to supply all the individual functions of the depleted carotenoids.

Furthermore, it must be remembered that carotenoids other than those measured in this study may be present in human plasma. Lutein and zeaxanthin, for instance, are the only carotenoids protecting the retina from being damaged by ultraviolet and blue light [6]. Supply of these carotenoids may be important for the development of visual function. Whether lutein, zeaxanthin, and other carotenoids are depleted in FF children, and whether this might have significant consequences for the individual development of the newborn, remains to be investigated.

In conclusion, this study reports biochemical differences in plasma carotenoids between FF and BF infants. Whether these differences have physiological or clinical consequences remains an open question.

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