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ORIGINAL CONTRIBUTION

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Vitamin A and β-carotene supply of women with gemini or short birth intervals

A pilot study

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Abstract *Background* An adequate supply of vitamin A during pregnancy and breastfeeding plays an important role for development of foetus and neonate, especially in lung development and function. *Aim of the study* Aim of this pilot study was to analyze vitamin A and β-carotene status and to investigate the contribution of nutrition to the vitamin A and β-carotene supply in mother–infant pairs of gemini or births within short birth intervals. *Methods* Twenty-nine volunteers aged between 21 and 36 years were evaluated for 48 h after delivery. During this time frame a food frequency protocol considering 3 months retrospective was obtained from all participants. In order to establish overall supply retinol and β-carotene levels were determined in maternal plasma, cord blood and colostrum via HPLC analysis. *Results* Regardless of the high to moderate socioeconomic background, 27.6% of participants showed plasma retinol levels below 1.4 μmol/l which can be taken as borderline deficiency. In addition, 46.4% showed retinol intake <66% of RDA and 50.0% did not consume liver at all although liver contributes as a main source for preformed retinol. Despite high total carotenoid intake of 6.9 ± 3.6 mg/d, 20.7% of mothers showed plasma levels <0.5 μmol/l β-carotene. Retinol and β-carotene levels were highly significantly correlated between maternal plasma versus cord blood and colostrum. In addition, significantly lower levels were found in cord blood (31.2 ± 13.0% (retinol), 4.1 ± 1.4% (β-carotene) compared with maternal plasma. *Conclusions* Despite the fact that vitamin A and β-carotene rich food is generally available, risk groups for low vitamin A supply exist in the western world.

Key words vitamin A supply – gemini births – colostrum – β-carotene supply – cord blood

Introduction

During pregnancy and breastfeeding the requirement for vitamin A is increased. On average, vitamin A intake should be 0.3 mg/d higher during pregnancy and 0.7 mg/d during the breastfeeding period compared to non-pregnant or non-breastfeeding female adults (recommended intake: 0.8 mg/d; 1.1 mg/d pregnancy; 1.5 mg/d breastfeeding) (DACH, 2000; FNB). Due to the importance of vitamin A for lung development and maturation, sufficient intake should be particularly ensured during the second and third pregnancy trimesters [1]. The best source of vitamin A is animal liver. The Federal Institute for Consumer

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Protection and Veterinary Medicine (BgVV, 1995), however, advises pregnant women not to consume animal liver. Therefore β-carotene, the major form of provitamin A in the diet, might play an important role as a source of vitamin A.

As the child is dependent on the mother in terms of vitamin A supply before and after delivery (the latter in the case of breastfeeding), great importance is placed on the mother's vitamin A intake during pregnancy. In case of insufficient vitamin A supply the liver store of the child only lasts for a couple of days and can be quickly emptied due to sudden strains or absorption dysfunction [2]. In preterm infants with insufficient vitamin A supply it was shown that the risk of bronchopulmonary dysplasia, a severe complication, is significantly increased [3–5]. In order to explore a potential supply bottleneck during pregnancy and lactation, vitamin A and β-carotene status should be investigated and risk groups identified.

Several studies have been published focusing on vitamin A and carotenoid contents either in cord blood or in colostrum and breast milk in different ethnic groups. However, there are no data on subpopulations from western countries that are considered at high risk for impaired vitamin A status, e.g. gemini births and short birth intervals. Aim of this pilot trial was to analyze vitamin A (retinol) and β-carotene (as provitamin A source) status in maternal blood, cord blood and colostrum to investigate the contribution of nutrition to vitamin A and β-carotene supply in mother–infant pairs of gemini births or births within short birth intervals.

Materials and methods

Subjects and study design

The study was conducted as a single cross-sectional pilot study. Twenty-three women with single conception pregnancy with short birth intervals (defined as second delivery within 24 months) were recruited consecutively at the University Gynaecological Hospital Ulm between September 2003 and August 2004. Additionally six women with gemini were enrolled to the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and the protocol was approved by the ethics committee of the Landersärztekammer Baden-Württemberg and ethics committee of the University of Ulm. All subjects provided informed consent prior inclusion.

The following criteria were met: participants were healthy with absence of chronic disease during pregnancy (gestational diabetics), aged between 20 and 40, normal delivery, full term gestational age (>36 + 0; weeks of pregnancy) and smoking less than five cigarettes/day. Women were eligible to be enrolled to the study if prenatal no supplementation with >2,000 IU/d vitamin A nor with β-carotene >2 mg/d occurred. In total, 58 volunteers were screened. Reasons for exclusion included bad language knowledge and refusal of breast feeding, ambulant delivery, early delivery, intake of β-carotene or vitamin A rich supplements, insulin dependent gestational diabetics and nicotin abusus. In total, 79% of volunteers reported taking micronutrients with focus on folic acid, pregnancy-specific multinutrients, magnesium, iron and iodine. However, most women did not supplement the micronutrients over the whole period of pregnancy but during certain time frames or occasionally.

Focus of the trial was to get information about the retinol and β-carotene status of the described collective with expected special need. For each participant specimens of maternal plasma, cord blood plasma and colostrum were collected within 48 h after delivery for analysis of retinol and β-carotene.

Sampling and preparation

Cord blood samples were obtained directly postpartum after clamping of the cord into EDTA-tubes (5 ml). Samples were protected from light and centrifuged for 10 min at 3,000*g* at 4°C. Within 48 h after birth, venous maternal non-fasting blood (EDTA-blood, 5 ml) was collected and centrifuged (10 min, 3,000*g*, 4°C). Haemodilution factors were estimated to be less than 10% and therefore were not considered for the interpretation of retinol and β-carotene data in maternal plasma.

Colostrum was collected during the first 48 h after delivery. It was performed either by hand expression or electric pump. Some donors provided several samples throughout this period to gain volumes of 4 ml in total, which were pooled before analysis. After preparation all samples were immediately stored at −20°C until analysis.

Analysis

Maternal plasma

For quantification of retinol and β-carotene in maternal plasma (50 µl), samples were extracted using extraction buffer (Chromsystems, Munich, Germany), internal standard in ethanol from Chromsystems and mobile phase (see HPLC analysis) in ratio of 1:2:2:2, respectively. After vigorous shaking and centrifugation, supernatant was used for analysis.

Cord blood plasma

Due to low concentrations in cord blood plasma, sample preparation was optimized in proportion of

volumes. 100 µl plasma was mixed with internal standard in ethanol and extraction buffer from Chromsystems in ratio of 1:1:2, respectively.

β-carotene in colostrum

Ethanol was added to colostrum for protein precipitation. Colostrum was saponified with 50% NaOH for 30 min at room temperature in a dark place. Internal standard from Chromsystems was added and samples were extracted with *n*-hexane/THF (9:1). Upper organic phase was removed, dried under N_2 and reconstituted in mobile phase.

Retinol in colostrum

For protein precipitation 500 µl ethanol including 1% BHT was added to 500 µl colostrum and saponified with 500 µl NaOH 50% for 40 min at 85°C. After incubation, samples were mixed with 500 µl *n*-hexane/toluole 1:1. After phase separation the organic layer was directly used for quantification of retinol using a normal phase HPLC method. Method was modified according to Strobel et al. [6].

HPLC analysis

Maternal plasma and cord blood plasma samples for retinol and β-carotene as well as colostrum samples for β-carotene were analyzed using an isocratic reversed phase HPLC (Waters, Eschborn, Germany) method with a Spherisorb ODS 2, 125 × 4 mm, 3 µm column. The mobile phase consisted of acetonitrile:methanol:tert-butyl methyl ether including 1% BHT, 165:50:18 (v/v/v). Flow rate was adjusted to 1.8 ml/min. For peak detection and spectrum characterization a UV–Vis detector (Waters 996, Eschborn, Germany) was used and operated at 325 nm (retinol) and 484 nm (β-carotene). Standard curves were generated with external standards for retinol and β-carotene (Sigma-Aldrich, Germany).

Retinol determination in colostrum was performed using a normal phase HPLC method with GromSil 80 CN-2PR, 250 × 4 mm, 3 µm (Grom, Herrenberg, Germany). The mobile phase consisted of *n*-hexane/isopropanol 97:3 (v/v) and flow rate was adjusted to 1 ml/min. For peak detection and spectrum characterization a UV–Vis detector (Waters 996, Eschborn, Germany) was used and operated at 325 nm.

Dietary and socio-economic assessment

A food frequency protocol was performed by the participants covering the last 3 months under supervision of a physician. The questionnaire was not validated, however, the results provide information about vitamin A and carotenoid intake. The tool was provided by EBISpro for windows (J. Erhardt, Willstätt-Legelshurst) and optimized to meet the requirements for this study. In detail, 20 different vegetables and 19 different fruits were included. In total, the questionnaire covered 150 different food items with focus on foods high in vitamin A and carotenoid content. For analysis, the software EBISpro was used.

EBISpro is based on the BLS-data base. Different carotenoids are not distinguished and for calculation of retinol equivalents a conversion factor of 1:6 is used for all carotenoids. However, according to present knowledge, conversion factors of 1:12 (β-carotene, fruits) and 1:24 (other vitamin A active carotenoids, 1:26 vegetables) are discussed for provitamin A carotenoids [7–9]. Therefore, the effective contribution to vitamin A supply from carotenoid sources can hardly be calculated with EBISpro. That's why only vitamin A intake calculated as preformed vitamin A (retinol) is considered for data interpretation in this study. However, carotenoid intake and contribution to vitamin A supply calculated with different conversion factors is summarized in Table 2.

To assess further demographic data, a socio-economic questionnaire was conducted.

Statistics

For statistical evaluation Graph Pad Prism 3.0 was used. Data are described as means ± SD. The significance of differences was determined by unpaired Student's *t*-test, two-tailed. The correlations between specimens of mother–infant pairs were measured by linear regression analysis and for calculation of *P* values Pearson correlation coefficient was used. Statistical significance was set at $P < 0.05$ (*), high significance was considered at $P < 0.01$ (**) and very high significance at $P < 0.0001$ (***).

Results

Maternal and neonatal characteristics are summarized in Table 1. Volunteers were well educated, only one volunteer had no school graduation. According to questionnaire, net income was in a range of 1,500–3,500 €/month indicating a normal to high socio-economic status. Taking body mass index calculated from body weight prior pregnancy, mothers showed a good nutritional status with 25.2 ± 5.7 kg/m^2.

Food frequency protocol

Food frequency protocols were completed by 28 volunteers. Mean energy intake with 12.6 ± 3.7 MJ/d was

Table 1 Demographic and socio-economic data of maternal–infant pairs

Demographic data	
Number of maternal/neonatal pairs	29
Short birth interval (within 24 month) (n = 23)	79.3%
Gemini birth (n = 6)	20.7%
Gestational age	39.14 ± 1.48 weeks
Neonatal birth weight; n = 35	3,140 ± 564 g
Parity mean ± SD	2.17 ± 0.76
1 (n = 2; gemini)	6.9%
2 (n = 23)	79.3%
3 (n = 2)	6.9%
≥4 (n = 2)	6.9%
Maternal age; (n = 29) mean ± SD	28.9 ± 4.0 years
Maternal body mass index prior pregnancy; (n = 27*) mean ± SD	25.2 ± 5.7 kg/m^2
Education years (school years)	11.4 ± 3.2 years
Net income/month	
<2,000 €	39.3%
>2,000 €	60.7%

* Data missing of two mothers

above recommended intake of 10.6–11.1 MJ, taking into account the additional requirement of 1.1 MJ/d during pregnancy (DACH 2000 [10]). Neither vegetarians nor vegans took part in the trial. Mean meat and liver consumption accounted for 2.9 times a week and 3.1 times a year, respectively. 50.0% of volunteers did not consume liver at all. Based on preformed vitamin A sources, 75.0% of volunteers did not reach the recommended intake levels of 1.1 mg vitamin A equivalents (recommended by German Nutrition Society) during pregnancy. Considering carotenoid intake using a conversion factor of 1:12, still 67.8% of the women would not meet the requirements during the breastfeeding period, assuming that there was no change in nutritional habits, see Table 2.

Total carotenoid intake ranged between 1.3 and 16.3 mg/d with a mean intake of 6.9 ± 3.6 mg/d. With the assumption that β-carotene is the predominant carotenoid the intake levels indicate a well-nourished population group. Most participants had normal plasma levels of β-carotene, however 20.7% of the participants showed levels below 0.5 μmol/l, which is the "recommended plasma level" [11]. Between total carotenoid intake and plasma β-carotene levels no correlation could be observed.

Retinol

The frequency distribution (Table 3) of maternal plasma retinol showed that 27.6% of the study population had levels below 1.4 μmol/l which can be taken as a borderline deficiency near to the deficiency range (<1.05 μmol/l) according to WHO, 1998. In one case the retinol level was in a clear vitamin A deficiency range (0.66 μmol/l).

Between retinol intake and maternal plasma retinol a high correlation (P = 0.002) is found. Similarly, a high correlation between retinol levels in maternal plasma and cord blood, respectively, colostrum can be seen (P = 0.0058 and P = 0.015). If only pairs with maternal retinol >1.4 μmol/l were considered the correlation would disappear (Fig. 1 left).

Retinol concentration in cord blood accounted for 31.2 ± 13.0% of the maternal concentration and a high accumulation was seen in colostrum with in average 224.8 ± 161.3% of the maternal plasma level.

β-carotene

Maternal plasma versus cord plasma (r = 0.8366, P < 0.0001) and colostrum (r = 0.8023, P < 0.0001) showed high correlations between the mother and infant pairs (Fig. 1 right).

Compared to maternal plasma significantly lower levels of β-carotene were seen in cord plasma (P < 0.0001) and colostrum (P = 0.0002). On average, only 4.1 ± 1.4% were found in cord plasma and 62.7 ± 38.7% in colostrum compared to maternal plasma (Table 4).

Group differentiation: short birth interval versus gemini

Furthermore, the study group was divided into two subgroups considering mother–infant pairs of gemini births versus short birth intervals.

Figure 2 shows, that in cases of gemini births mean values of retinol and β-carotene were lower. Yet, statistical significance was only present for retinol in samples of maternal plasma (P < 0.05) and colostrum (P < 0.0001). All other samples showed no statistical significance due to small sample size.

Discussion

This pilot study was designed to analyze the vitamin A (retinol) and β-carotene status and to relate the contribution of nutrition during pregnancy to the vitamin A and β-carotene supply in mother–infant pairs of gemini births or births within short birth intervals. In 27.6% of women plasma retinol levels were below 1.4 μmol/l which may be considered as a borderline vitamin A deficiency (based on plasma retinol levels). This is a surprisingly high percentage of women at risk, particularly in the light of the moderate to high socio-economic status and good nutritional status in this group of apparently healthy women. Dimenstein et al. [12] examined the influence

Table 2 Food frequency protocol, selected results

Food frequency (n = 28)			
Energy intake (MJ/day) mean ± SD	12.55 ± 3.67		
Meat consumption (frequency) mean ± SD	2.88 ± 1.69		
1–2/week (n = 8)	28.6%		
3–4/week (n = 16)	57.1%		
5–6/week (n = 1)	3.6%		
Daily (n = 3)	10.7%		
Liver consumption per year (frequency) mean ± SD	3.05 ± 5.75		
No consumption (n = 15)	50.0%		
1–2/year (n = 5)	17.9%		
3–4/year (n = 4)	14.3%		
5–6/year (n = 1)	7.1%		
1–2/month (n = 3)	10.7%		
Retinol intake (mg/d) mean ± SD	0.95 ± 0.64		
Retinol intake <0.5 mg/d (n = 5)	17.9%		
Retinol intake 0.5–0.8 mg/d (n = 8)	28.6%		
Retinol intake 0.8–1.1 mg/d (n = 8)	28.6%		
Retinol intake 1.1–1.5 mg/d (n = 3)	10.7%		
Retinol intake >1.5 mg/d (n = 4)	14.3%		
Total carotenoid intake* (mg/d) mean ± SD	6.9 ± 3.6		
Intake <3 mg/d (n = 4)	14.3%		
Intake 3–6 mg/d (n = 9)	32.1%		
Intake 6–9 (n = 10)	35.7%		
Intake >9 mg/d (n = 5)	17.9%		
Total Vitamin A equivalent (RE) (mg/d) mean ± SD	2.11 ± 0.89	1.53 ± 0.71	1.24 ± 0.66
Conversion factor	1:6	1:12	1:24
RE < 0.5 mg/d	–	3.6%	7.1%
RE 0.5–0.8 mg/d	3.6%	7.1%	10.7%
RE 0.8–1.1 mg/d	7.1%	10.7%	35.7%
RE 1.1–1.5 mg/d	21.4%	46.4%	21.4%
RE > 1.5 mg/d	67.9%	32.1%	25.0%

* Calculated on the basis of 20 vegetables and 19 fruits

of nutritional and socio-economic status on retinol in colostrum. In Brazil women, no influence was observed with socio-economic factors but mothers with normal to overweight showed significant higher retinol concentrations than those with rather low weight. Whereas in the trial of Schulpis et al. [13] Albanian mothers showed lower plasma and cord blood retinol concentrations compared to Greek mothers, which was attributed to their low socio-economic and nutritional status. Since in our trial both socio-economic and nutritional status started from optimal conditions, the results are important for western countries and warrant further investigation.

Table 3 Mean concentrations of retinol (μmol/l) in maternal and cord plasma, colostrum

Retinol	
Maternal plasma (n = 29) mean ± SD	1.72 ± 0.49 μmol/l
<1.05 μmol/l (n = 1)	3.4%
1.05–1.4 μmol/l (n = 7)	24.1%
1.41–2.8 μmol/l (n = 20)	69.0%
>2.8 μmol/l (n = 1)	3.4%
Cord blood (n = 35) mean ± SD	0.51 ± 0.27 μmol/l
<0.35 (n = 11)	31.4%
0.35–0.69 (n = 18)	51.4%
0.7–1.049 (n = 5)	14.3%
>1.05 (n = 1)	2.9%
Colostrum (n = 27**) mean ± SD	3.86 ± 2.97 μmol/l
<1.05 μmol/l (n = 5)	18.5%
1.05–2.1 μmol/l (n = 5)	18.5%
2.11–4.2 μmol/l (n = 8)	29.6%
>4.2 μmol/l (n = 9)	33.3%

** In two participants, no colostrum could be obtained within 48 h

The German Nutrition Society (DGE, 2000) recommended a 40% increase in vitamin A intake for pregnant women and a 90% increase for breastfeeding women. The best and major source for vitamin A is liver concerning content and bioavailability. However, it was advised by public authorities (BgVV, 1995) that pregnant women should avoid eating liver due to the high vitamin A concentration in liver and its presumed teratogenic effect. In the studied group, 50% of women followed this recommendation. According to the retinol intake calculated from the food frequency protocol, covering the mean intake of the last 3 months, 75.0% of participants indeed do not reach the recommended values of 1.1 mg retinol equivalent (RE)/d [10]. The observed average intake of retinol (0.95 ± 0.64 mg/d) in these pregnant women showed

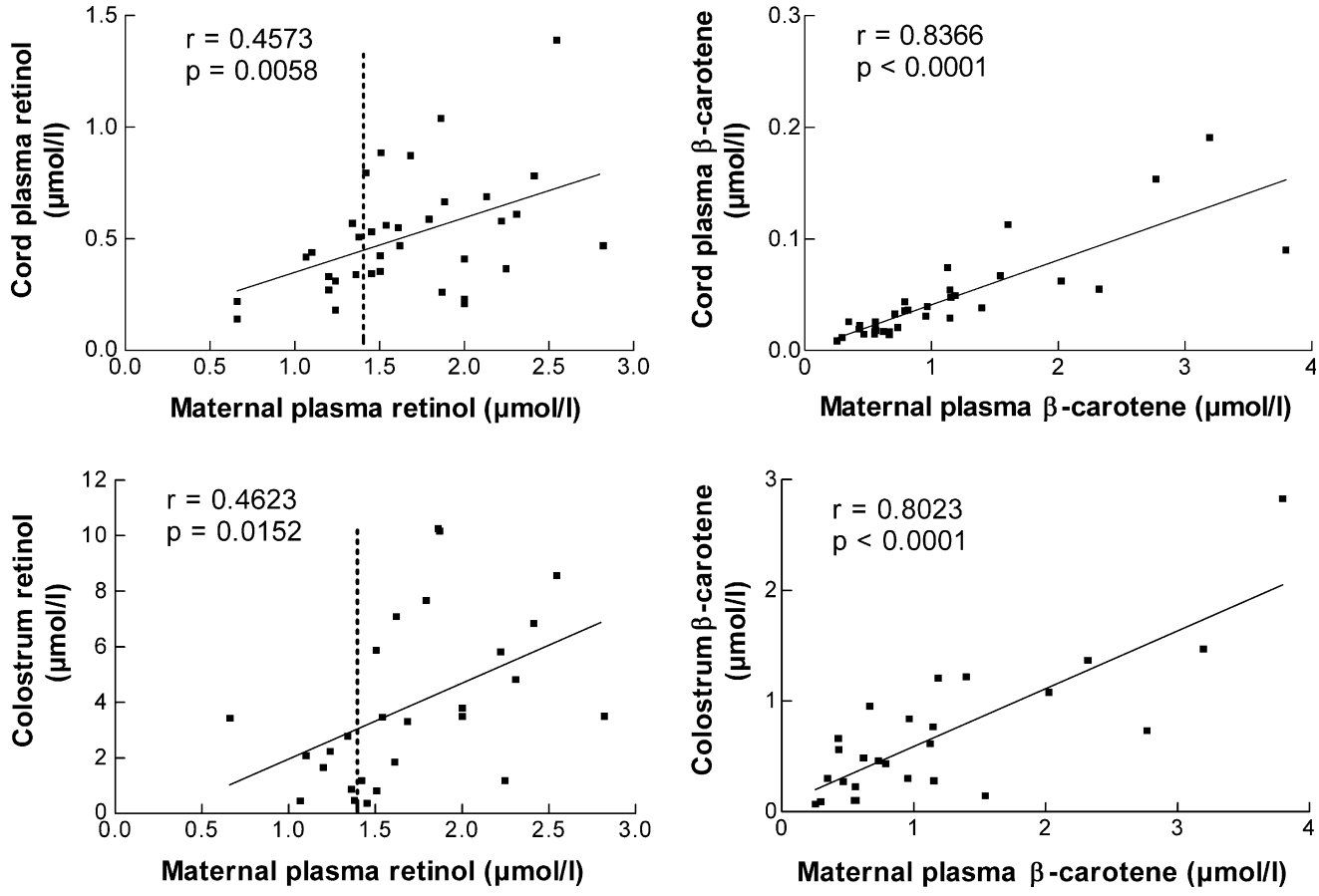

Fig. 1 Correlations of retinol (left) and β-carotene (right) maternal plasma versus cord plasma and colostrum; Pearson correlation coefficient.
- - - - Threshold 1.4 µmol/l plasma retinol

Table 4 Mean concentrations of β-carotene (µmol/l) in maternal and cord plasma, colostrum

β-carotene	
Maternal plasma (n = 29) mean ± SD	1.15 ± 0.89 µmol/l
<0.5 µmol/l (n = 6)	20.7%
0.51–1.0 µmol/l (n = 11)	37.9%
1.1–1.5 µmol/l (n = 5)	17.2%
1.51–2.0 µmol/l (n = 2)	6.9%
>2.0 µmol/l (n = 5)	17.2%
Cord blood (n = 35) mean ± SD	0.044 ± 0.040 µmol/l
<0.025 µmol/l (n = 14)	40%
0.025–0.05 µmol/l (n = 12)	34.3%
0.051–0.1 µmol/l (n = 6)	17.1%
>0.1 µmol/l (n = 3)	8.57%
Colostrum (n = 26***) mean ± SD	0.68 ± 0.61 µmol/l
<0.3 µmol/l (n = 8)	30.7%
0.3–0.6 (n = 6)	23.1%
0.61–0.9 (n = 5)	19.2%
0.91–1.2 (n = 2)	7.7%
>1.2 (n = 5)	19.2%

*** In two participants, no colostrum could be obtained within 48 h. Additionally, one sample volume was too small for β-carotene detection

levels below average intake in industrialized countries (1.54 mg RE/d) [14, 15]. Total energy intake, which was relatively high compared to recommended values suggests that food assessment was overestimated indicating even lower retinol intakes than presented.

To estimate the risk of vitamin A deficiency, categories of low, moderate and high risks were set to be >100% RDA, 67–99% RDA and <66% RDA based on Pedro et al. [16]. With these categories, based on preformed vitamin A intake (retinol) 46.4% showed a high risk and 28.6% had a moderate risk for vitamin A deficiency. Assuming that nutritional habits remained largely the same after giving birth, only four women out of the 29 women investigated met the recommended intake levels for vitamin A (retinol) during breastfeeding.

In agreement with other reports [17–21], in the present study cord plasma retinol values of about 31.2 ± 13.0% showed significantly lower (P < 0.0001) levels than in maternal plasma. Godel et al. [22] suggested 50–60% of maternal values may represent a normal range for newborn infants [22, 23]. Taking

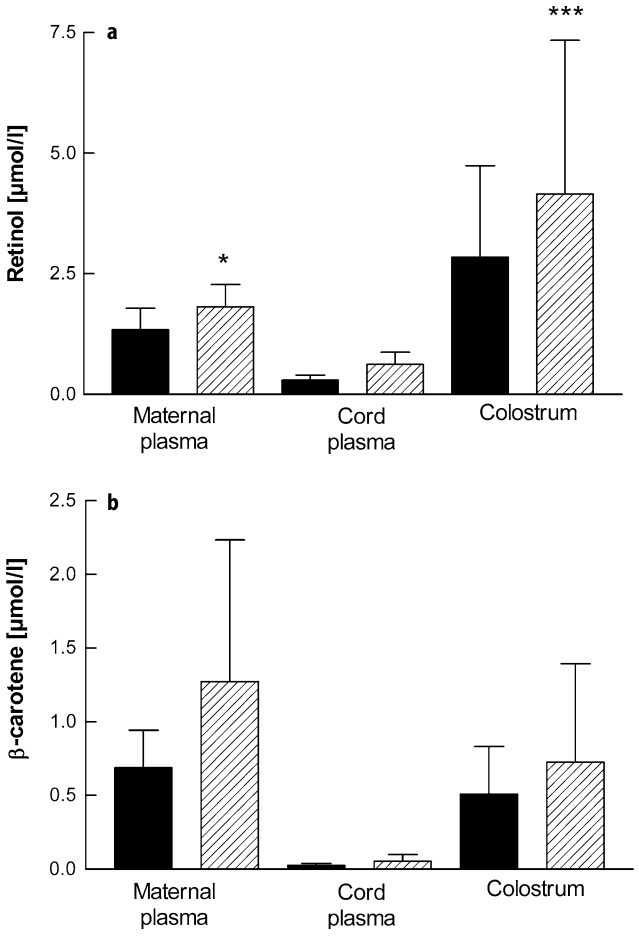

Fig. 2 Retinol (a) and β-carotene content (b) in maternal plasma, cord plasma and colostrum separated in groups. Values are expressed as mean ± SD. * $P = 0.0464$; *** $P < 0.0001$. ■ Specimen of gemini mother–infant pairs $n = 6$. ▨ Specimen of short birth intervals mother–infant pairs $n = 23$

this range into account, our ratio (cord: maternal plasma) seems rather low.

The plasma retinol level is homeostatically regulated. In literature no correlation between cord plasma retinol concentrations and maternal plasma has been reported [17, 24]. However, according to our study, when low maternal plasma concentration occurs a correlation arises. This observation supports the previous assumption by Rondo et al. [24] that in borderline maternal vitamin A deficiency homeostatic control is no longer operating.

There is no clear consensus on the "cut-off" concentration for vitamin A deficiency in cord blood. Levels below 0.35 and 0.7 µmol/l are discussed [21]. Taking 0.35 µmol/l as cut off level, in the present study 31.4% of the newborns showed levels below.

The World Health organization [25] recommends a daily minimum intake of 0.63 µmol retinol/d for the nursling, in order to meet basic requirements. The colostrum plays a major role in the early delivery of vitamin A to the newborn and to top up its liver stores. For the development of liver stores, however, values between 1.2 and 1.3 µmol/d during the first year are recommended. During the average intake of milk of the nursling of about 750 ml/d, the mother's milk needs to show a concentration of 1.6 µmol/l in order to satisfy the recommended intake requirements [26, 27]. Taking 1.6 µmol/l as threshold value, in the present trial 25.9% of colostrum retinol levels showed lower levels. It has to be considered that the milk retinol concentration decreases significantly over time. According to Macias and Schweigert [28] the mean vitamin A concentration is 3.56 µmol/l in colostrum, 1.76 µmol/l in transitory, and 1.1 µmol/l in mature milk. Based on these data mothers with 1.6 µmol/l retinol in colostral milk will not be able to ensure sufficient storage of vitamin A in the liver of the newborn with transitory or mature milk. Indeed, fetal liver stores are clearly related to the plasma levels of the mother during pregnancy [2]. Consequently low storage of vitamin A in fetal liver might not be compensated in the newborn.

As vitamin A is homeostatically regulated in plasma, monitoring vitamin A in breast milk is used as an indicator for vitamin A status [29]. In the present study, the retinol concentration in colostrum was significantly correlated with the β-carotene concentration ($r = 0.5502$, $P = 0.0036$). These findings were previously reported by Canfield et al. [30] where besides β-carotene, provitamin A carotenoids even showed stronger correlations suggesting that carotenoids are a predictor for vitamin A supply of mother–infant pairs. The enzyme 15,15′-mono-oxygenase, responsible for the initial step in the conversion of dietary provitamin A to retinol was shown to be expressed in mammary tissue [31]. This indicates, that high β-carotene supply may contribute to the enrichment of vitamin A in colostrum (225% compared to plasma). But there is still uncertainty about the conversion efficiency of carotenoids in breast milk and further research in this field is needed [14].

In Germany total carotenoid intake was reported as 5.3 mg/d with 1.1–1.4 mg/d accounts for β-carotene [32, 33]. These data are consistent with our data showing a high mean intake of total carotenoids of 6.9 mg/d. Nevertheless, 20% of mothers showed plasma concentrations of β-carotene below 0.5 µmol/l.

β-carotene seems to pass the placenta barrier only to a very small extent. In the study, the concentration in cord blood with 4.1 ± 1.4% of maternal plasma levels is highly significantly lower ($P < 0.0001$) comparable with other reports [17]. The two compartments maternal plasma and cord blood are highly correlated ($r = 0.8366$, $P < 0.0001$). A good maternal supply is important for neonate supply. At birth

newborn plasma concentrations are 10-fold smaller than those in 1-year-old infants [29].

During the first months of life breast milk or infant formulas are the only sources of diet for the newborn. A high correlation is seen between β-carotene levels in maternal plasma and in colostrum ($P < 0.0001$) with colostrum levels of about 63% of maternal plasma levels. Infants who received breast milk showed higher plasma β-carotene levels than formula fed infants [29]. Noteworthy, β-carotene concentrations in colostrum are up to five times higher than those in mature breast milk [29]. The nutritional significance of carotenoids in the neonatal period has not been clarified so far but may play an important role as a source of provitamin A, as an antioxidant and immune modulator.

Conclusion

The trial clearly indicates that despite substantial food availability in the western world, vitamin A and β-carotene supply in mother–infant pairs of mothers with gemini births or short birth intervals may not be optimal. A marginal supply of the mother results in a marginal supply of the newborn. To minimize possible complications [1] adequate vitamin A supply during pregnancy and breastfeeding is important. Further studies are needed to elucidate whether these findings can be confirmed in larger population groups with defined socio-economic and nutritional backgrounds. If confirmed, specific nutritional recommendations for these groups at risk would be warranted to guarantee adequate vitamin A supply.

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References

1. Biesalski HK, Nohr D (2003) Importance of vitamin-A for lung function and development. Mol Aspects Med 24:431–440
2. Shah RS, Rajalakshmi R, Bhatt RV, Hazra MN, Patel BC, Swamy NB, Patel TV (1987) Liver stores of vitamin A in human fetuses in relation to gestational age, fetal size and maternal nutritional status. Br J Nutr 58:181–189
3. Chytil F (1996) Retinoids in lung development. FASEB J 10:986–992
4. Atkinson SA (2001) Special nutritional needs of infants for prevention of and recovery from bronchopulmonary dysplasia. J Nutr 131:942–946
5. Verma RP, McCulloch KM, Worrell L, Vidyasagar D (1996) Vitamin A deficiency and severe bronchopulmonary dysplasia in very low birthweight infants. Am J Perinatol 13:389–393
6. Strobel M, Heinrich F, Biesalski HK (2000) Improved method for rapid determination of vitamin A in small samples of breast milk by high-performance liquid chromatography. J Chromatogr A 898:179–183
7. IOM, Institute of Medicine, Food, Nutrition Board (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington, DC
8. De Pee S, West CE, Permaesih D, Martuti, Muhilal, Hautvast JG (1998) Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and b-carotene in schoolchildren in Indonesia. Am J Clin Nutr 68:1058–1067
9. van Lieshout M, West CE, van Breemen RB (2003) Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly beta-carotene, in humans: a review. Am J Clin Nutr 77:12–28
10. Deutsche Gesellschaft für Ernährung (DGE) (2000) Referenzwerte für die Nährstoffzufuhr. Umschau Braus GmbH, Frankfurt am Main, pp 69–77
11. Biesalski HK, Fürst P (1999) Ernährungsmedizin. Georg Thieme Verlag, Stuttgart New York, pp 111–122
12. Dimenstein R, Simplicio JL, Ribeiro KD, Melo IL (2003) Retinol levels in human colostrum: influence of child, maternal and socioeconomic variables. J Pediatr (Rio J) 79:513–518
13. Schulpis KH, Michalakakou K, Gavrili S, Karikas GA, Lazaropoulou C, Vlachos G, Bakoula C, Papassotiriou I (2004) Maternal-neonatal retinol and alpha-tocopherol serum concentrations in Greeks and Albanians. Acta Paediatr 93:1075–1080
14. Ross JS, Harvey PW (2003) Contribution of breastfeeding to vitamin A nutrition of infants: a simulation model. Bull World Health Organ 81:80–86
15. Huffman S, Rasmusson E, Newman V, O'Gara C (1993) Breastfeeding and the environment. A policy brief from Wellstart International. Birth Gaz 10:17–18
16. Pedro RA, Cadelaria LV, Bacos FF, Ungson B, Lanot E (1996) A simplified dietary assessment to identify groups at risk for dietary vitamin A deficiency. Asia Pac J Clin Nutr 5:164–169
17. Yeum KJ, Ferland G, Patry J, Russell RM (1998) Relationship of plasma carotenoids, retinol and tocopherols in mothers and newborn infants. J Am Coll Nutr 17:442–447
18. Shirali GS, Oelberg DG, Mehta KP (1989) Maternal-neonatal serum vitamin A concentrations. J Pediatr Gastroenterol Nutr 9:62–66
19. Gazala E, Sarov B, Hershkovitz E, Edvardson S, Sklan D, Katz M, Friger M, Gorodischer R (2003) Retinol concentration in maternal and cord serum: its relation to birth weight in healthy mother-infant pairs. Early Hum Dev 71:19–28

20. Dejmek J, Ginter E, Solansky I, Podrazilova K, Stavkova Z, Benes I, Sram RJ (2002) Vitamin C, E and A levels in maternal and fetal blood for Czech and Gypsy ethnic groups in the Czech Republic. Int J Vitam Nutr Res 72:183–190
21. Dallaire F, Dewailly E, Shademani R, Laliberte C, Bruneau S, Rhainds M, Blanchet C, Lefebvre M, Ayotte P (2003) Vitamin A concentration in umbilical cord blood of infants from three separate regions of the province of Quebec (Canada). Can J Public Health 94:386–390
22. Godel JC, Basu TK, Pabst HF, Hodges RS, Hodges PE, Ng ML (1996) Perinatal vitamin A (retinol) status of northern Canadian mothers and their infants. Biol Neonate 69:133–139
23. Kiely M, Cogan PF, Kearney PJ, Morrissey PA (1999) Concentrations of tocopherols and carotenoids in maternal and cord blood plasma. Eur J Clin Nutr 53:711–715
24. Rondo PH, Abbott R, Rodrigues LC, Tomkins AM (1995) Vitamin A, folate, and iron concentrations in cord and maternal blood of intra-uterine growth retarded and appropriate birth weight babies. Eur J Clin Nutr 49:391–399
25. Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements (1998) Vitamin and mineral requirements in human nutrition: report of a joint FAO/WHO expert consultation. Bangkok, Thailand, 21–30 September 1998
26. Allen LH (2005) Multiple micronutrients in pregnancy and lactation: an overview. Am J Clin Nutr 81:1206S–1212S
27. Institute of Medicine FaNB (2000) Dietary reference intakes for vitamin C, vitamin E, selenium und carotinoids. National Academy Press, Washington DC
28. Macias C, Schweigert FJ (2001) Changes in the concentration of carotenoids, vitamin A, alpha-tocopherol and total lipids in human milk throughout early lactation. Ann Nutr Metab 45:82–85
29. Sommerburg O, Meissner K, Nelle M, Lenhartz H, Leichsenring M (2000) Carotenoid supply in breast-fed and formula-fed neonates. Eur J Pediatr 159:86–90
30. Canfield LM, Clandinin MT, Davies DP, Fernandez MC, Jackson J, Hawkes J, Goldman WJ, Pramuk K, Reyes H, Sablan B, Sonobe T, Bo X (2003) Multinational study of major breast milk carotenoids of healthy mothers. Eur J Nutr 42:133–141
31. Lindqvist A, Andersson S (2004) Cell type-specific expression of beta-carotene 15,15′-mono-oxygenase in human tissues. J Histochem Cytochem 52:491–499
32. Müller H (1996) Die tägliche Aufnahme von Carotinoiden (Carotine und Xanthophylle) aus Gesamtnahrungsproben und die Carotinoidgehalte ausgewählter Gemüse und Obstarten. Z Ernährungswiss 35:45–50
33. Pelz R, Schmidt-Faber B, Heseker H (1998) Carotenoid intake in the German National Food Consumption Survey. Z Ernährungswiss 37:319–327