Biochemical but not clinical vitamin A deficiency results from mutations in the gene for retinol binding protein[1,2]

Hans K Biesalski, Jürgen Frank, Susanne C Beck, Felix Heinrich, Beate Illek, Ram Reifen, Harald Gollnick, Mathias W Seeliger, Bernd Wissinger, and Eberhart Zrenner

See corresponding editorial on page 829.

ABSTRACT

Background: Two German sisters aged 14 and 17 y were admitted to the Tübingen eye hospital with a history of night blindness. In both siblings, plasma retinol binding protein (RBP) concentrations were below the limit of detection (<0.6 μmol/L) and plasma retinol concentrations were extremely low (0.19 μmol/L). Interestingly, intestinal absorption of retinyl esters was normal. In addition, other factors associated with low retinol concentrations (eg, low plasma transthyretin or zinc concentrations or mutations in the transthyretin gene) were not present. Neither sibling had a history of systemic disease.

Objective: Our aim was to investigate the cause of the retinol deficiency in these 2 siblings.

Design: The 2 siblings and their mother were examined clinically, including administration of the relative-dose-response test, DNA sequencing of the RBP gene, and routine laboratory testing.

Results: Genomic DNA sequence analysis revealed 2 point mutations in the RBP gene: a T-to-A substitution at nucleotide 1282 of exon 3 and a G-to-A substitution at nucleotide 1549 of exon 4. These mutations resulted in amino acid substitutions of asparagine for isoleucine at position 41 (Ile41→Asn) and of aspartate for glycine at position 74 (Gly74→Asp). Sequence analysis of cloned polymerase chain reaction products spanning exons 3 and 4 showed that these mutations were localized on different alleles. The genetic defect induced severe biochemical vitamin A deficiency but only mild clinical symptoms (night blindness and a modest retinal dystrophy without effects on growth).

Conclusions: We conclude that the cellular supply of vitamin A to target tissues might be bypassed in these siblings via circulating retinyl esters, β-carotene, or retinoic acid, thereby maintaining the health of peripheral tissues. *Am J Clin Nutr* 1999;69:931–6.

KEY WORDS Retinol binding protein, mutation, vitamin A deficiency, transthyretin, retinol, retinyl esters, genomic sequence analysis, night blindness

INTRODUCTION

Retinol binding protein (RBP), a specific plasma transport protein for vitamin A, serves to transport retinol from its storage site in the liver to peripheral target tissues of the body (for reviews, see references 1 and 2). Human RBP is coded by a single gene and is specifically synthesized in the liver (3), where it binds one molecule of retinol. After its secretion into the bloodstream, holo-RBP forms a 1:1 molar complex with another plasma protein, transthyretin (TTR). It has long been recognized that the nutritional status of retinol is one factor regulating RBP secretion from the liver. Studies in rats showed that retinol regulates the rate of RBP secretion without affecting RBP synthesis (4). In cases of vitamin A deficiency, secretion of RBP is blocked, resulting in the accumulation of RBP in the liver and a concomitant decline in RBP concentrations in plasma (5).

Circulating retinol serves as a metabolic precursor of retinal and retinoic acid, and its subsequent cellular uptake is the predominant source of vitamin A for target cells. Many organs, such as the liver, kidney, small intestine, lung, spleen, eye, and testis, depend on a regular supply of vitamin A through retinol from circulating blood. Any condition that interferes with the ingestion, absorption, storage, release, transport, or cellular uptake of vitamin A can lead to deficiencies in target tissues. Therefore, retinol-RBP plasma homeostasis ensures a sufficient and continuous supply of vitamin A (6). Normal concentrations of RBP in human plasma are in the range of 1.5–3.0 μmol/L. Even during low dietary vitamin A intake, the ratio of plasma retinol to RBP remains constant as long as retinyl esters are present in liver stores. The depletion of liver stores as a result of prolonged dietary vitamin A deficiency decreases both retinol and RBP plasma concentrations. According to the World Health Organization classification and numerous clinical studies,

plasma retinol concentrations <0.35 µmol/L indicate the onset of severe vitamin A deficiency that results more or less rapidly in clinical symptoms if not reversed by supplemental intervention (7). Xerophthalmia is a clinical syndrome of vitamin A deficiency that includes night blindness, conjunctival xerosis with and without Bitot spots, and corneal xerosis. Finally, as a result of prolonged vitamin A deficiency, keratomalacia including corneal ulceration and potential subsequent blindness, as well as degeneration of the pigment epithelium, may occur. Retinol concentrations between 0.35 and 0.7 µmol/L are associated with mild vitamin A deficiency, leading to a significantly higher incidence of respiratory and gastrointestinal infections than that observed in vitamin A–replete persons (8, 9).

Plasma RBP concentrations are tightly regulated and remain constant except during prolonged insufficient dietary vitamin A intake, extreme protein-energy malnutrition (eg, kwashiorkor), disease (eg, measles or kidney and liver diseases), or genetically modified TTR (10–12). In addition, low steady state values for RBP have been reported in a Japanese family (13, 14). The authors suggested that a heterozygous mutation in the RBP gene may be present but this possibility could not be verified by Southern blot analysis.

Here we report 2 unusual cases of a severe and long-lasting biochemical vitamin A deficiency (plasma retinol concentrations <0.19 µmol/L and plasma RBP concentrations <0.6 µmol/L) due to a heterozygous mutation in RBP. The biochemical vitamin A deficiency in these otherwise healthy, well-nourished, and normally developed siblings (aged 14 and 17 y) was associated with no severe clinical symptoms of vitamin A deficiency (15), however, and with normal TTR concentrations.

SUBJECTS AND METHODS

Subjects

Two female siblings aged 17 (A) and 14 (B) y were admitted to the school of optometry clinic in Tübingen with a history of night blindness and slightly reduced visual acuity (15). Ophthalmologic examination revealed atrophy of the posterior segments of the pigment epithelium without changes in the anterior segment. Clinical examinations including ultrasonic examination of internal organs, lung function tests, and measurement of routine laboratory indexes did not show any abnormalities (data not shown).

In both siblings and their mother, dietary intake of vitamin A (eg, from liver-containing meat products) and β–carotene (eg, from carrots and green, leafy vegetables) was evaluated with EBIS nutrition analysis software (E&D Partner, Stuttgart, Germany). In addition, concentrations of retinol, retinyl esters, retinoic acid, RBP, TTR, α-tocopherol, β-carotene, and zinc were measured; the relative-dose-response-test and fat absorption test were administered; and mutations in the RBP and TTR genes were analyzed. Informed consent was obtained from all participating family members after the character and possible consequences of the investigations were explained to them. The research followed the tenets of the Declaration of Helsinki.

Measurement of retinol, retinyl esters, and retinoic acid

Retinol, retinyl esters, and retinoic acid were measured in both siblings and their mother as described in detail elsewhere (16, 17). Briefly, retinol and retinyl esters were measured by conventional HPLC with a model LC-85 ultraviolet detector set at 340 nm, a 650-10S fluorescence detector set at 335 nm excitation and 475 nm emission (slit 10/10 nm), and a pump (series 1), all from Perkin-Elmer (Überlingen, Germany). The chromatographic conditions for separation of retinol were as follows: n-hexane:isopropanol (96:4) as the mobile phase and a flow rate of 3.0 mL/min. The analytic column (250 × 4.6 mm) contained Nucleosil CN (Grom, Herrenberg, Germany) as the stationary phase. The chromatographic conditions for separation of retinyl esters were as follows: n-hexane:diisopropyl ether (98.5:1.5) as the mobile phase and a flow rate of 2.0 mL/min. The analytic column (250 × 4.6 mm internal diameter; 3 µm packing material) and the precolumn (25 × 4.6 mm) contained Spherisorb silica (Bischoff, Leonberg, Germany) as the stationary phase.

Retinoic acid and retinoic acid derivatives were separated on a Kontron HPLC system (Kontron pump 414, Rheodyne injection system 7125, Kontron data system Datapack 450; Kontron, Frankfurt, Germany) under isocratic conditions with a 250 × 4.6–mm reversed-phase column (Ultrasil ODS; Beckman Instruments, Munich, Germany). The mobile phase was acetonitrile, 0.05 mol ammonium acetate/L in water (pH 7.0), and tetrahydrofuran (76:19:5); the flow rate was 2.4 mL/min. Validation and quantitative analysis were performed with standards from the National Institute of Standards and Technology (Gaithersburg, MD).

Relative-dose-response test

The relative-dose-response test was used to detect marginal vitamin A deficiency according to Loerch et al (18). Dietary vitamin A enters the circulation as long-chain fatty acid esters (retinyl esters) in the chylomicron fraction; these retinyl esters are taken up by the liver and metabolized to retinol. Under normal conditions and with normal vitamin A status, most of the retinol is reesterified and stored in the liver in the form of retinyl esters. However, during vitamin A deficiency when liver stores are depleted, retinol is bound to apo-RBP and released into the bloodstream. Therefore, an increase in plasma retinol of >20% indicates that liver vitamin A stores are depleted A. Briefly, 3.15 µmol vitamin A (3000 IU) was given orally and blood samples were collected before and 5 h after administration. Plasma was immediately frozen at −80°C until analyzed for retinol.

Fat absorption test

To elucidate whether fat absorption was normal, 31.5 µmol retinyl palmitate (corresponding to 30 000 IU vitamin A) was added to a standardized lipid load according to Schrezenmair et al (19). Blood samples were taken before and after administration of the test meal. Plasma was immediately stored at −80°C until analyzed for retinol, retinyl esters, and triacylglycerol.

Measurement of RBP and TTR in human plasma

Total RBP was quantitated by radioimmunodiffusion (Behring Diagnostics, Marburg, Germany). TTR was measured by immunoprecipitation analysis (Incstar Corporation, Stillwater, MN) with a Cobas Mira turbidimetric analyzer (Roche Diagnostics, Grenzach-Wyhlen, Germany).

Identification of mutations in the human RBP and TTR genes

Intronic primers were designed based on the genomic sequences of the human RBP (3) and TTR (20) genes. Oligonucleotide primers for RBP exons 3 and 4 were as follows: forward, 5′-TGT CAT CCT TCT CAC AGT TCT C-3′; reverse, 5′-AAG AAA CCC AGC GAT TTG-3′. Oligonucleotide primers for exon

TABLE 1
Plasma concentrations of retinol, retinyl esters, retinol binding protein (RBP), and transthyretin (TTR) in the 2 affected siblings and their mother

	Sibling A	Sibling B	Mother	Normal range
Retinol (μmol/L)	0.19	0.19	1.38	0.7–1.5
Retinyl esters (μmol/L)	0.14	0.22	0.16	0.05–0.25
RBP (μmol/L)	<0.6	<0.6	1.1	1.5–3.0
TTR (μmol/L)	42	36.9	44.9	32–60
all-trans-Retinoic acid (nmol/L)	2.6	2.7	2.1	2.0–3.3
13-cis-Retinoic acid (nmol/L)	1.8	2.0	2.3	1.3–2.7

TABLE 2
Effects of an oral dose of 31.5 μmol retinyl palmitate/L dispersed in oil on plasma concentrations of retinol, retinyl esters, and triacylglycerols in 2 affected siblings and their mother

	Sibling A		Sibling B		Mother	
	Fasting	5 h	Fasting	5 h	Fasting	5 h
Retinol (μmol/L)	0.19	0.24	0.17	0.22	1.28	1.28
Retinyl esters (μmol/L)	0.16	1.89	0.21	0.67	0.17	1.26
Triacylglycerols						
(mmol/L)	0.96	2.00	0.82	1.80	1.22	2.42
(mg/dL)	84	176	72	158	107	212

3 of TTR were as follows: forward, 5′-TGC CAT GCC ATT TGT TTC-3′; reverse, 5′-ACC AAA ACC AAA ACA ACC C-3′. Genomic DNA isolated from whole blood was used for amplification by the polymerase chain reaction (PCR) with Vent DNA polymerase (Biolabs, Schwalbach, Germany) for 35 cycles at 94.5 °C for 1 min, 60.6 °C (primers for RBP exons 3 and 4) or 55.8 °C (primers for TTR exon 3) for 1 min, and 72 °C for 1 min. PCR products were cloned into the pZerO-1 vector (Invitrogen, NV Leek, Netherlands). Automatic DNA sequencing of ≥4 different clones from each sibling was carried out by 4baselab GmbH (Reutlingen, Germany).

RESULTS

Measurement of retinol, retinyl esters, retinoic acid, RBP, and TTR

In both siblings, plasma RBP concentrations were below the limit of detection (<0.6 μmol/L) and plasma retinol concentrations were extremely low (**Table 1**). The concentration of retinol in the siblings' mother was low but still within the normal range, whereas maternal RBP was ≈50% of the normal average value. These measurements indicated that a severe biochemical vitamin A deficiency was present in both siblings. As shown in Table 1, plasma concentrations of retinyl esters, TTR, all-trans-retinoic acid, and 13-cis-retinoic acid were within normal ranges in both siblings and their mother. A retrospective nutrition survey (1-mo quantitative recall) showed that vitamin A intake was normal in both siblings and their mother (1.500–2.000 IU/d); the mean calculated intake of β-carotene was 1.5 mg/d in both siblings and their mother.

Relative-dose-response test, fat absorption test, and zinc concentrations

In both siblings and their mother, ingestion of 3.15 μmol retinyl palmitate did not result in any alteration in plasma retinol concentrations (data not shown). These results indicated that liver stores were not depleted of vitamin A; however, these experiments did not exclude the possibility that absorption of fat and retinyl esters was impaired. Therefore, we compared plasma concentrations of retinol, retinyl esters, and triacylglycerol before and after a test meal containing 31.5 μmol retinyl palmitate in a standardized lipid load (**Table 2**). Retinyl esters increased by factors of 11.8 (sibling A) and 3.2 (sibling B). These results suggested that absorption of fat and vitamin A was normal. In contrast, plasma retinol concentrations did not markedly increase, indicating that the release of retinol from liver stores was impaired. Furthermore, baseline concentrations of other fat-soluble vitamins (α-tocopherol and β-carotene) were within normal

ranges (**Table 3**). Because zinc deficiency is frequently associated with low plasma retinol concentrations (21), we measured plasma zinc concentrations. As shown in Table 3, plasma zinc concentrations were normal in both siblings and their mother.

TTR gene analysis

A missense mutation of the TTR gene [transition of amino acid 84 in exon 3 from isoleucine (ATC) to asparagine (AAC) (Ile84→Asn)] has been described (22) and RBP plasma concentrations were reported to be substantially reduced in persons with this mutation. It was therefore conceivable that defects in the formation of the holo-RBP–TTR complex due to mutated TTR may have resulted in the low RBP concentrations observed in our study. We therefore performed genomic analysis of exon 3 of the TTR gene in both siblings and the mother. We compared the DNA sequence of exon 3 with that in a control subject and found no irregularities in nucleotide sequence (**Figure 1**).

RBP gene analysis

Because the analysis of the TTR gene was negative, we investigated the possibility that mutations in the RBP gene induced the extremely low plasma concentrations of RBP and retinol in both affected siblings. Indeed, sequence analysis of the RBP gene of both siblings revealed 2 point mutations (nucleotide numbering is according to reference 3): a T-to-A substitution at nucleotide 1282 (T1282A) in exon 3 and a G-to-A substitution at nucleotide 1549 (G1549A) in exon 4 (**Table 4**). These mutations resulted in an amino acid substitution of asparagine for isoleucine at position 41 (Ile41→Asn) on exon 3 and of aspartate for glycine at position 74 (Gly74→Asp) on exon 4, respectively (23).

Sequence analysis of cloned PCR products spanning exons 3 and 4 revealed that these mutations were present on different alleles (**Table 5**). DNA sequence analysis of the RBP gene of the healthy mother identified the point mutation in exon 3 (T1282A), whereas no mutation was present in exon 4. The RBP gene of the mother had a mosaic structure, ie, only one allele was affected with the mutation in exon 3 and the other allele showed no mutation. Although the paternal RBP gene was not available

TABLE 3
Plasma concentrations of the fat-soluble vitamins α-tocopherol and β-carotene and the trace element zinc in 2 affected siblings and their mother

	Sibling A	Sibling B	Mother	Normal range
α-Tocopherol (μmol/L)	37	32	28	20–40
β-Carotene (μmol/L)	0.35	0.33	0.27	0.2–0.6
Zinc (μmol/L)	12.8	15.9	17.1	12–23

```
TTR exon 3  GAAAACCAGT GAGTCTGGAG AGCTGCATGG GCTCACAACT GAGGAGGAAT
Control     GAAAACCAGT GAGTCTGGAG AGCTGCATGG GCTCACAACT GAGGAGGAAT
Siblings    GAAAACCAGT GAGTCTGGAG AGCTGCATGG GCTCACAACT GAGGAGGAAT
Mother      GAAAACCAGT GAGTCTGGAG AGCTGCATGG GCTCACAACT GAGGAGGAAT

TTR exon 3  TTGTAGAAGG GATATACAAA GTGGAAATAG ACACCAAATC TTACTGGAAG
Control     TTGTAGAAGG GATATACAAA GTGGAAATAG ACACCAAATC TTACTGGAAG
Siblings    TTGTAGAAGG GATATACAAA GTGGAAATAG ACACCAAATC TTACTGGAAG
Mother      TTGTAGAAGG GATATACAAA GTGGAAATAG ACACCAAATC TTACTGGAAG

TTR exon 3  GCACTTGGCA TCTCCCCATT CCATGAGCAT GCAGAG
Control     GCACTTGGCA TCTCCCCATT CCATGAGCAT GCAGAG
Siblings    GCACTTGGCA TCTCCCCATT CCATGAGCAT GCAGAG
Mother      GCACTTGGCA TCTCCCCATT CCATGAGCAT GCAGAG
```

FIGURE 1. DNA sequencing of exon 3 of the gene encoding transthyretin (TTR) in a control individual, 2 siblings with severe biochemical vitamin A deficiency but only mild clinical symptoms, and the siblings' healthy mother. No irregularities in the nucleotide sequence of exon 3 of the TTR gene were present. Additionally, the missense mutation described by Waits et al (22) (amino acid substitution of asparagine for isoleucine at position 84) was not present, indicating that TTR function was normal in both affected siblings and their mother. The codon for the amino acid at position 84 of the TTR gene is indicated in bold.

for genomic analysis, it is conceivable that both siblings inherited the mutation in exon 4 from their father. The presence of 2 mutations in both affected siblings resulted in complete loss of RBP function; as a result, plasma concentrations of retinol and RBP were extremely low. The single mutation in exon 3 (Ile41→Asn) of the maternal RBP gene or likely exon 4 (Gly74→Asp) of the paternal RBP gene did not result in severely decreased RBP concentrations because of the normal DNA sequence of the corresponding allele.

DISCUSSION

We identified and characterized the genetic basis of a naturally occurring mutation in RBP in 2 German sisters and their mother and its resulting effects on plasma concentrations of retinol and RBP. Both siblings had 2 mutations in different exons of the RBP gene, whereas the mother had only 1. The genetic defect in both teenage siblings induced night blindness and a modest retinal dystrophy but no pronounced effects on growth or other physiologic functions that are normally affected during vitamin A deficiency. Our study indicated a limited requirement for holo-RBP for maintaining the health of peripheral tissues. Therefore, it is conceivable that an alternative route exists that supplies vitamin A to target tissues. Our data point toward a role of chylomicron retinyl esters in supplying vitamin A to most extrahepatic tissues, although we cannot rule out a role for holo-RBP when it is normally present.

A previous study in a Japanese family (a mother and 2 siblings) showed plasma concentrations of RBP that were ≈50% of the normal range (1.0–1.1 μmol/L) (13, 14). One of the children, aged 19 mo, developed keratomalacia, a typical sign of vitamin A deficiency, during a measles infection. The low plasma RBP concentration of 1.0 μmol/L may have predisposed this child to developing keratomalacia because circulating vitamin A concentrations are transiently reduced during measles infection (24). The authors suggested that the affected Japanese family might be heterozygous for a mutation. Southern blot analysis of gene restriction patterns, however, revealed no major defect in the RBP gene of the affected family members (14). The underlying genetic defect was not studied further by genomic DNA sequence analysis.

In our study, low plasma RBP and retinol concentrations may have been related to 2 mutations in the RBP gene. In both affected siblings, we identified 2 unique mutations within the RBP gene that were localized on different alleles. The first mutation (on exon 3) resulted in an amino acid substitution at position 41 in which the nonpolar amino acid isoleucine was replaced by the uncharged polar amino acid asparagine. The second mutation (on exon 4) resulted in an amino acid substitution at position 74 in which the nonpolar amino acid glycine was replaced by the acidic amino acid aspartate. Because both parents were healthy we concluded that a single mutation of the RBP gene in either exon 3 or exon 4 does not result in pathologic symptoms. However, the maternal plasma RBP concentration (1.1 μmol/L) was only 50% of the normal average value, a finding that might be explained by the mosaic structure of the RBP gene.

It is well documented that defects in the formation of the RBP-TTR complex result in defective release of retinol from the liver. A lack of TTR protein in mice as a result of disruption of the TTR gene (ie, in knockout mice) was linked to retinol concentrations that were only 6% of normal values and RBP concentrations <5%

TABLE 4
Genomic analysis of exons 3 and 4 of the gene encoding retinol binding protein[1]

	Exon 3, nucleotides 1278–1286	Exon 4, nucleotides 1544–1552
Control subject		
Nucleotide sequence	AAC ATC GTC	GGT GGG CAG
Amino acid	Ile[2]	Gly[3]
Sibling A		
Nucleotide sequence	AAC AAC GTC	GGT GGA CAG
Amino acid	Asn	Asp
Sibling B		
Nucleotide sequence	AAC AAC GTC	GGT GGA CAG
Amino acid	Asn	Asp
Mother		
Nucleotide sequence	AAC AAC GTC	GGT GGG CAG
Amino acid	Asn	Gly

[1] Shown is the T-to-A substitution at nucleotide 1282 in exon 3 for the siblings and the mother and the G-to-A substitution at nucleotide 1549 in exon 4 for the siblings only. The nucleotide numbering is according to reference 3.
[2] Amino acid position 41.
[3] Amino acid position 74.

TABLE 5
Mutations in the gene encoding retinol binding protein (RBP)[1]

	Affected siblings		Healthy mother		Healthy father (proposed)	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Point mutation, exon 3	−	+	−	+	−	−
Point mutation, exon 4	+	−	−	−	+	−

[1] Point mutations are shown in Table 4. Both affected siblings were heterozygous for the mutations in exons 3 and 4. The mother was heterozygous for the mutation in exon 3 but had no mutation in exon 4. The paternal RBP gene was not available for genomic DNA analysis, but is proposed to be heterozygous for the mutation in exon 4 and negative for the mutation in exon 3. The described sequences are available in the EMBL database under accession no. AF 025334 for exon 3 and accession no. AF 025335 for exon 4.

of normal values (25). In another case, plasma RBP was markedly reduced in kindreds with familial amyloidotic neuropathy (FAP). Interestingly, RBP concentrations were decreased only in clinically affected family members with correspondingly low plasma TTR concentrations (10–12). Another case of low plasma RBP concentrations linked to defective TTR was also described (22). Individuals from a kindred with an amino acid substitution of serine for isoleucine at position 84 (Ile84→Ser) showed substantial reductions in plasma RBP concentrations. Indeed, RBP affinity to the TTR molecule is lower for recombinant Ile84→Ser TTR than for normal recombinant TTR (26). In the present study, we concluded that the low RBP concentrations were not due to FAP or mutated TTR because there were no clinical signs of FAP, TTR mutations were absent, and, importantly, TTR plasma concentrations were normal. Our results indicated that the secretion of retinol from the liver was defective because of the Ile41→Asn and Gly74→Asp mutations in RBP.

Night blindness is a typical and early sign of vitamin A deficiency (27) and was present in both siblings. However, Bitot spots, corneal changes, and growth retardation, which are usually manifest during advanced stages of vitamin A deficiency, were absent (15). From this we concluded that cellular uptake of vitamin A may have been bypassed by another mechanism or mechanisms. Previously, circulating retinyl esters bound to chylomicrons were proposed to be an alternative mechanism for supplying target tissues with vitamin A (28, 29). Retinyl esters are present at different concentrations in extrahepatic tissues (eg, lung, respiratory mucosa, kidney, testicle, tongue, and inner ear) (16, 28) and may serve as local stores of retinol (17). It is generally accepted that the primary source of vitamin A for extrahepatic tissues is retinol, which, after cellular uptake, may be esterified for intracellular storage. Experiments with parenteral application of unphysiologic retinyl esters (eg, retinyl margarinate) in rats clearly showed that these retinyl esters were taken up by the cells and subsequently metabolized to retinol and reesterified with palmitic acid to form retinyl palmitate (30). These results suggest that circulating retinyl esters may serve as cellular sources of retinol. Indeed, uptake of retinyl esters from chylomicrons and chylomicron remnants (29, 31) and the existence of extrahepatic storage sites have been described (32).

Our study indicated that circulating retinyl esters (either bound to chylomicrons or low-density lipoproteins) were metabolized and used for cellular supply of retinol. Because typical signs of vitamin A deficiency were absent and plasma concentrations of circulating retinyl esters and retinoic acid or β-carotene were normal in all family members, we conclude that the target tissues were adequately supplied with vitamin A. This may explain the night blindness observed in both siblings because retinol and subsequently retinal could not be formed from retinoic acid in these cells. β-Carotene as a source of vitamin A depends on cleavage via the enzyme β-carotene 15,15′dioxygenase. This enzyme is present only in small intestine and liver. Consequently, circulating β-carotene may not function as a vitamin A source for all vitamin A–dependent target tissues.

Our findings in these siblings elucidate new aspects of the role of RBP and other pathways in the cellular supply of vitamin A to extrahepatic target tissues. Knockout animal models will become important in future studies to investigate the cellular supply of vitamin A under normal and pathologic conditions (eg, reduced RBP synthesis and defective release of RBP because of protein-energy malnutrition or liver diseases). Consequently, it will be important to develop new pharmacologic strategies for vitamin A supplementation in persons with altered synthesis and release of RBP.

REFERENCES

1. Blaner WS. Retinol-binding protein: the serum transport protein for vitamin A. Endocr Rev 1989;10:308–16.
2. Goodman DS. Plasma retinol-binding protein. Ann N Y Acad Sci 1980;348:378–90.
3. D'Onofrio C, Colantuoni V, Cortese R. Structure and cell-specific expression of a cloned human retinol-binding protein gene: the 5′-flanking region contains hepatoma specific transcriptional signals. EMBO J 1985;4:1981–9.
4. Soprano DR, Smith JE, Goodman DS. Effect of retinol status on retinol-binding protein biosynthesis rate and translatable messenger RNA level in rat liver. J Biol Chem 1982;257:7693–7.
5. Muto Y, Smith JE, Milch PO, Goodman DS. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. J Biol Chem 1979;247:2542–50.
6. Olson JA. Evaluation of vitamin A status in children. World Rev Nutr Diet 1978;31:130–4.
7. Underwood BA. Methods for assessment of vitamin A status. J Nutr 1990;120(suppl):1459–63.
8. Chandra RK. Increased bacterial binding to respiratory epithelial cells in vitamin A deficiency. BMJ 1988;297:834–5.
9. Sommer A, West KP Jr. Vitamin A and childhood morbidity. Lancet 1992;339:1302–3.
10. Benson MD, Dwulet FE. Prealbumin and retinol-binding protein serum concentrations in the Indiana type hereditary amyloidosis. Arthritis Rheum 1983;26:1493–8.
11. Westermark P, Pitkanen P, Benson L, Vahlquist A, Olofsson BO, Cornwell GG. Serum prealbumin and retinol-binding protein in the prealbumin-related senile and familial forms of systemic amyloidosis. Lab Invest 1985;52:314–8.
12. Shoji S, Nakagawa S. Serum prealbumin and retinol-binding protein concentrations in Japanese-type familial amyloid polyneuropathy. Eur Neurol 1988;28:191–3.

13. Matsuo T, Matsuo N, Shiraga F, Koide N. Familial retinol-binding protein deficiency. Lancet 1987;2:402–3 (letter).

14. Matsuo T, Noji S, Taniguchi S, Matsuo N. No major defect detected in the gene of familial hypo-retinol-binding proteinemia. Jpn J Ophthalmol 1990;34:320–4.

15. Seeliger MW, Biesalski HK, Wissinger B, et al. Phenotype in retinol deficiency due to a hereditary defect in retinol-binding protein (RBP) synthesis. Invest Ophthalmol Vis Sci 1999;40:3–11.

16. Biesalski HK, Ehrenthal W, Gross M, Hafner G, Harth O. Rapid determination of retinol (vitamin A) in serum by high pressure liquid chromatography (HPLC). Int J Vitam Nutr Res 1983;53:130–7.

17. Biesalski HK. Separation of retinyl esters and their geometric isomers by isocratic adsorption high-performance liquid chromatography. Methods Enzymol 1990;189:181–9.

18. Loerch JD, Underwood BA, Lewis KC. Response of plasma levels of vitamin A to a dose of vitamin A as an indicator of hepatic vitamin A reserves in rats. J Nutr 1979;109:778–86.

19. Schrezenmeir J, Weber J, Biesalski HK. Postprandial pattern of triglyceride-rich lipoprotein in normal-weight humans after an oral lipid load. Ann Nutr Metab 1992;36:186–96.

20. Sasaki H, Yoshioka N, Takagi Y, Sakaki Y. Structure of the chromosomal gene for human serum prealbumin. Gene 1985;37:191–7.

21. Ahn J, Choi I, Koo S. Effect of zinc deficiency on intestinal absorption, plasma clearance, and urinary excretion of ^{3}H-vitamin A in rats. J Trace Elem Exp Med 1995;7:153–66.

22. Waits RP, Yamada T, Uemichi T, Benson MD. Low plasma concentrations of retinol-binding protein in individuals with mutations affecting position 84 of the transthyretin molecule. Clin Chem 1995;41:1288–91.

23. Wissinger B, Seeliger MW, Frank J, Broghammer M, Biesalski HK, Zrenner E. Mutations in the gene for serum retinol-binding protein in a family with retinal dystrophy. Am J Hum Genet 1997;61(suppl):A27 (abstr).

24. Kjolhede C, Rosales F, Caballero B, Gadomski A. Measles and low serum vitamin A values. J Pediatr 1993;122:499–500.

25. Episkopou V, Maeda S, Nishiguchi S, et al. Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. Proc Natl Acad Sci U S A 1993;90:2375–9.

26. Berni R, Malpeli G, Folli C, Murrell JR, Liepnieks JJ, Benson MD. The Ile-84→Ser amino acid substitution in transthyretin interferes with the interaction with plasma retinol-binding protein. J Biol Chem 1994;269:23395–8.

27. Goetghebuer T, Brasseur D, Dramaix M, et al. Significance of very low retinol levels during severe protein-energy malnutrition. J Trop Pediatr 1996;42:158–61.

28. Biesalski HK, Weiser H. Microdetermination of retinyl esters in guinea pig tissues under different vitamin-A-status conditions. J Micronutr Anal 1990;7:97–116.

29. Blomhoff R, Skrede B, Norum KR. Uptake of chylomicron remnant retinyl ester via the low density lipoprotein receptor: implications for the role of vitamin A as a possible preventive for some forms of cancer. J Intern Med 1990;228:207–10.

30. Gerlach T, Biesalski HK, Weiser H, Haeussermann B, Baessler KH. Vitamin A in parenteral nutrition: uptake and distribution of retinyl esters after intravenous application. Am J Clin Nutr 1989;50:1029–38.

31. Blaner WS, Wei S, Kurlandsky SB, Episkopou V. Retinoid transport in rodents. In: Livrea MA, Vidali G, eds. Retinoids: from basic science to clinical applications. Basel, Switzerland: Birkhäuser-Verlag, 1994:53–78.

32. Nagy NE, Holven KB, Roos N, et al. Storage of vitamin A in extra-hepatic stellate cells in normal rats. J Lipid Res 1997;38:645–58.