See corresponding article on page 931.

Mutations in the gene encoding retinol binding protein and retinol deficiency: is there compensation by retinyl esters and retinoic acid?[1,2]

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Biesalski et al (1) present a novel case of 2 sisters aged 14 and 17 y with very low plasma retinol concentrations (0.19 μmol/L) and plasma concentrations of retinol binding protein (RBP) below the limit of detection in the assay used (<0.6 μmol/L). For comparison, the median plasma retinol concentration of adolescent girls in the United States is ≈1.4 μmol/L (≈40 μg/dL) according to data from the National Health and Nutrition Examination Surveys (2) and a value <0.35 μmol/L (<10 μg/dL) is often interpreted as indicating severe vitamin A deficiency. In their report, Biesalski et al describe 2 point mutations in the *RBP* gene, one on each of the 2 alleles, that result in single amino acid substitutions. The affected sisters came to attention because of ocular manifestations of vitamin A deficiency; however, they are described as being normally developed and lacking other clinical signs of severe vitamin A deficiency. As the authors noted, a Japanese family with low plasma RBP concentrations was reported previously, but the molecular defect was not characterized (3, 4).

The correlation of low plasma holo-RBP and abnormalities of the retina [such as reduced acuity and cellular alterations of the retinal pigment epithelium (RPE)] implies that RBP may be of particular importance in delivering vitamin A to the retina. On the basis of previous biochemical research it was suggested that the basal plasma membrane of RPE cells, which is proximal to capillaries, contains some type of receptor or docking site for RBP, and a candidate protein has been partially characterized (5). Such a receptor could function in the uptake of retinol into RPE cells, where vitamin A is usually stored at relatively high concentrations in cytoplasmic lipid droplets in the form of *all-trans*- and 11-*cis*-retinyl esters (6, 7). Nutritional night blindness is understood to be the result of a chronic undersupply of retinol to the RPE (8), which leads to depletion of the RPE retinyl ester pools, resulting in delayed regeneration of 11-*cis*-retinal after the photobleaching of rhodopsin and a rise in the rod illumination threshold (9). Biesalski et al's observations further support the concept that RBP is of particular importance in maintaining the health and function of the RPE.

Whereas a dietary deficiency of vitamin A reduces plasma retinol and all other forms of the vitamin, the genetic defect in *RBP* observed in these affected siblings did not result in low plasma concentrations of retinyl esters or retinoic acid. The good general health of these sisters implies that other forms of vitamin A can be utilized effectively by most tissues. Future studies in animal models in which plasma RBP is either significantly reduced, as through deletion of the gene for transthyretin (TTR) (10), or specifically deleted through targeted gene disruption will be important in further defining which dietary forms of vitamin A and which retinoid pathways provide functional compensation for a loss of holo-RBP.

What retinoids might these be? Retinyl esters and retinoic acid are 2 prime candidates. The usual plasma concentration of retinyl esters is an order of magnitude lower than the concentrations of retinol and RBP. However, the flux of esterified retinol is quantitatively significant and its potential importance in delivering vitamin A to tissues cannot be inferred from its plasma concentration. When dietary vitamin A is adequate and intestinal absorption is normal, the postabsorptive concentration of plasma chylomicron retinyl esters remains low, typically <5% of the plasma concentration of retinol. Even postprandially after ingestion of usual amounts of vitamin A, chylomicron retinyl esters rise little because clearance is rapid. Given the capacity of chylomicrons to efficiently absorb and transport dietary vitamin A, do organs besides the liver store and utilize chylomicron-derived vitamin A? There is convincing evidence that extrahepatic tissues can take up and metabolize vitamin A carried in chylomicrons (11–13). Moreover, ≈5–10% of the vitamin A in chylomicrons isolated from intestinal lymph is present as unesterified retinol (14), a form that can be readily transferred to other plasma lipoproteins or cell membranes and thus become widely distributed. A small portion of esterified retinol in chylomicrons may exchange with plasma lipoproteins and enter cells through pathways mediated by lipoprotein receptor (15). Although most of the total-body vitamin A in well-nourished humans and animals is stored in hepatic stellate cells as retinyl esters, many extrahepatic tissues contain lower concentrations of stored vitamin A, mostly as esterified retinol, that can be mobilized as needed in an RBP-dependent manner. Additionally, stellate cells have been detected in extrahepatic tissues as well as in liver (16). Thus, an increasing appreciation has developed of the possible importance of extrahepatic tissues in vitamin A storage and metabo-

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lism.

A small proportion of the vitamin A absorbed into enterocytes as retinol or β-carotene leaves the intestine as retinoic acid via the portal circulation (17). Like the chylomicron retinyl esters discussed above, plasma retinoic acid is present in low concentrations, typically 2–3 orders of magnitude less than concentrations of retinol, and turns over rapidly (18). The hormonal activities of vitamin A are mediated by *all-trans*- and 9-*cis*-retinoic acid, the principal ligands for 2 families of nuclear retinoid receptors, and retinoic acid supplied in a retinol-free diet has been shown to support all of the biological activities of vitamin A except for its roles in vision and possibly male reproduction. Other isomers of retinoic acid and several oxygenated retinoids are also present in plasma and these too may have biological activity. Numerous organs besides the intestine can convert retinol to retinoic acid (19, 20), and plasma retinoic acid can equilibrate readily with intracellular retinoic acid. Taken together, these facts imply the presence of an RBP-independent pathway, one in which chylomicrons supply vitamin A to peripheral tissues with the capacity to oxidize retinol to retinoic acid, which in turn may function locally or may be released to plasma and redistributed to other organs.

It is still unknown why the observed single amino acid substitutions in *RBP* result in such low plasma retinol concentrations. Structural alterations in RBP might affect the intracellular folding of the protein or the assembly of holo-RBP before its release from liver into plasma, the stability of its association with retinol or TTR, or the metabolic fate of the retinol-RBP-TTR transport complex in plasma. Because the crystal structure of RBP, a β-barrel protein, is known (21), the newly identified amino acid substitutions can be mapped with respect to the retinol binding site and the RBP surface domains required for interaction with TTR. If a detailed biochemical characterization of the RBP-TTR complex from these interesting siblings is not feasible, it should be possible to recreate the same *RBP* mutations by site-directed mutagenesis of *RBP* complementary DNA and in vitro or in vivo expression. Such detailed studies would likely improve our understanding of the structure-function relations that are important determinants of retinol transport.

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