Scotopic thresholds and plasma retinol in cystic fibrosis

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Patients with cystic fibrosis (CF) often have low plasma concentrations of vitamin A. We have measured dark-adapted scotopic thresholds of 56 patients with CF, ages 4 to 34 years, either with a two-alternative forced-choice procedure or as the final threshold after a full dark-adaptation curve. Fasting plasma vitamin A alcohol (retinol) was measured in 34 of the 56 patients. The average thresholds were higher and retinol values lower in patients than in controls. In two patients with very low (<7 μg/dl) initial retinol levels and elevated thresholds, decreased rhodopsin densities were observed; rhodopsin density and thresholds returned to normal after treatment with oral vitamin A. Rhodopsin density and log sensitivity were linearly related. Only marked decreases in plasma retinol were associated with elevations of dark-adapted threshold and decreases in rhodopsin density, suggesting that the tissues of patients with CF sequester vitamin A to maintain retinal function. (INVEST OPHTHALMOL VIS SCI 23:364-370, 1982.)

Key words: scotopic thresholds, retinal sensitivity, vitamin A, retinol, rhodopsin, cystic fibrosis

Low plasma concentrations of vitamin A are often found in patients with cystic fibrosis (CF). This appears to be secondary to an abnormality in the secretion of vitamin A from the liver and the transport to extra hepatic tissues. If the tissues of patients with CF are indeed vitamin A deficient, reduced scotopic sensitivity may occur. Therefore we set out to determine whether dark-adapted thresholds and dark adaptation are abnormal in patients with CF. In addition, the relation of the dark-adapted threshold to blood retinol level was investigated.

Methods

Patients. The scotopic thresholds of 56 patients with CF were measured. Patients ranged in age from 4 to 34 years (mean 19.5 years) and most had moderate to severe CF. As is characteristic of such patients, two thirds had mild liver disease as indicated by slightly elevated serum levels of glutamate-oxalacetate transaminase, lactic acid dehydrogenase, and alkaline phosphatase. None of these laboratory measures of liver function was correlated with scotopic threshold. Two patients had previously treated strabismus but had good alignment and good vision in each eye at the time of study. All other patients had normal eye examinations and best corrected visual acuity of 20/20 in each eye. The results of Schirmer's tear tests were within the normal range, suggesting that significant
xerophthalmia was not present. None of the patients had any fundus lesions.

**Controls.** Children attending a community health clinic and adult volunteers served as control subjects (n = 36) for the vitamin A measurements. All were in good general health and without visual complaints. They ranged in age from 4 to 40 years (mean = 17.8 years). Eight subjects, ages 4 to 40 years, who had no visual complaints and normal eyes on complete ophthalmic examination, provided normative psychophysical data.

**Vitamin A measurements.** Fasting retinol, retinol-binding protein (RBP), and prealbumin were measured in 34 of the patients. Plasma retinol was determined either fluorometrically after separation on a deactivated alumina column or by high-pressure liquid chromatography. Results from the two methods were similar for control subjects; for nine control subjects, the mean value measured by fluorometry after alumina column separation was 35 µg/dl and 36 µg/dl by high-pressure liquid chromatography. RPB and prealbumin were measured by radial gel diffusion using commercially available kits (Calbiochem-Behring, Somerville, N.J.).

In two patients, hepatic stores of vitamin A were assessed indirectly by a slight modification of the relative dose response (RDR) test described elsewhere. A small, oral challenge dose of 600 µg of retinyl palmitate was used on the patients with CF. In these two patients (see Table I) the RDR test was administered before and within 2 months after daily oral supplementation with 25,000 IU of water-soluble vitamin A.

**Psychophysical tests.** Detection thresholds were measured by a two-alternative forced-choice procedure. This method enabled uniform testing of all patients, including younger children and ill patients who could not be tested in a more conventional manner on an adaptometer. Each patient’s pupils were dilated with cyclopentolate 1% or tropicamide 1%, followed by dark adaptation for 30 min after exposure to room light. Then, seated before a rear-projection screen, the patient was presented with stimuli starting at above-threshold levels. The stimuli were 1 sec duration, 10° diameter, blue (Ilford 621) spots flashed 20° to the right or left of the center of the screen. On each trial, the patient reported stimulus location, right or left. The dark-adapted threshold was determined graphically from the psychometric function as the stimulus intensity at 75% correct detection.

For older, cooperative patients, full dark-adaptation curves were also obtained with a Maxwellian-view adaptometer. After exposure to a white light (6.2 log scotopic trolands for 2 min) that bleached over 99% of the pigments in a 5° diameter patch of retina 16° temporal to the left fovea, the method of limits was used to determine thresholds. The stimulus was a 20 msec, 2° diameter spot that was concentric with the bleached patch. The time courses of recovery after the full bleach were assumed to be exponential for both cones and rods. The exponential curves were fit by eye to the data and the time constant was determined. Measured at the retina, the average normal threshold was 4.36 log equivalent quanta (503 nm) sec⁻¹ cm⁻² on the adaptometer and 4.29 log equivalent quanta (503 nm) sec⁻¹ cm⁻² by the two-alternative method. * 

**Reflection retinal densitometry.** Measurements of rhodopsin density at 560 nm in a 5° patch of retina, 16° temporal to the left fovea, were made by a previously described method. The rhodopsin density at 500 nm was calculated from the transmissivity difference equation following the method described by Alpern and Pugh. It was assumed that the densitometer stray light was negligible and constant.

**Results**

The distributions of fasting plasma retinol values for patients and for control subjects are shown in Fig. 1. Retinol values were lower in patients with CF than in controls. The mean

*A calibrated photodiode (UDT) was used to measure the µwatts/cm² that the stimulus lights delivered when attenuated only by a narrow band interference filter, peak transmission at 503 nm. In both the Maxwellian-view adaptometer and the forced-choice apparatus, the relative intensities of steady 503 nm light and blue (Ilford 621) light that caused a 1 log unit elevation of normal observers’ (pupils dilated and paralyzed pharmacologically) dark-adapted threshold for seeing the test stimuli were determined. Thus the value of the blue light could be estimated as log equivalent quanta (503 nm) sec⁻¹ cm⁻². *
Fig. 1. Distribution of fasting plasma retinol values of patients with CF and control subjects. Data for 34 patients and 36 controls are shown. The retinol values of patients were significantly lower than those of controls \( (t = 5.64, \, df = 68, \, p < 0.001) \). The mean age of the patients was 19.5 years and of the controls 17.8 years.

The distributions of retinol values of patients was 21 \( \mu g/dl \) and 32 \( \mu g/dl \) for controls \( (t = 5.64, \, df = 68, \, p < 0.001) \). None of the controls had retinol values under 15 \( \mu g/dl \), but seven of the 34 (21%) patients did.

Fig. 2. Distribution of log relative thresholds of dark-adapted subjects using the two-alternative forced-choice method. Circles, Results for 56 patients with CF; solid line, mean log relative threshold of eight control subjects; dashed lines, \( \pm 2 \) S.D. of the normal mean. Measured at the retina the mean log relative threshold (represented by 0 on the graph) of the control subjects was at 4.29 log equivalent quanta \( (503 \, nm) \) sec\(^{-1}\) cm\(^{-2}\) \( (S.D. = 0.18) \). Although the average threshold of patients with CF was significantly higher than that of controls \( (t = 2.9, \, df = 62, \, p < 0.05) \), there was much overlap of the results obtained for the two groups.

Fig. 3. Full dark-adaptation curves were obtained from 15 patients with CF and seven control subjects (Fig. 3). Although the rates of cone recovery for patients and controls did not differ, the rates for rods were significantly different \( (t = 2.82, \, df = 20, \, p < 0.01) \). There was a significant correlation between time constants of the rod branch of the full dark-adaptation curve and fasting plasma retinol levels \( (r = 0.76, \, p < 0.05) \) for patients but not for controls. However, the time constants of the cone branch for both patients and controls were not significantly correlated with retinol levels.
Fig. 3. Mean dark-adaptation curves for patients and control subjects. Dashed curve, Mean for control subjects; solid curve, mean for patients. Both the rod and cone branches of these curves obtained after a full bleach were assumed to be exponential. The mean time constants of patients were 131.7 sec (S.D. = 37.3) for cones and 450.8 sec (S.D. = 56.9) for rods. The mean time constants of controls were 116.7 sec (S.D. = 30.0) for cones and 380.7 sec (S.D. = 30.0) for rods. The patients' rates of rod recovery are different than those of controls (t = 2.82, df = 20, p < 0.01) and are correlated with plasma retinol levels (r = 0.76, p < 0.05). The time of the rod-cone break and the final threshold did not differ significantly between controls and patients.

The mean final dark-adapted threshold, determined from full dark-adaptation curves, was 4.31 log equivalent quanta (503 nm) sec\(^{-1}\) cm\(^{-2}\) for patients and 4.36 log equivalent quanta (503 nm) sec\(^{-1}\) cm\(^{-2}\) (S.D. = 0.18) for controls. Final thresholds on the adaptometer agreed well with those obtained by the two-alternative forced-choice method. There was a significant correlation with the thresholds obtained by the two procedures both for patients (r = 0.68, p < 0.05) and for control subjects (r = 0.810, p < 0.05).

The relation between fasting plasma retinol and log threshold of patients with CF is shown in Fig. 4. For retinol levels greater than 15 \(\mu\)g/dl, log threshold and retinol were significantly correlated (r = 0.57, p < 0.01).

The two patients with the lowest plasma retinol levels had elevations of thresholds that fell to normal levels after treatment daily with 25,000 IU of water-miscible vitamin A by mouth (Table I). The second of these patients had been found 15 years previously to have low vitamin A level and abnormal retinal adaptation that responded to vitamin A therapy. Both of the patients had clinical evidence of liver disease. Before, but not after, treatment with vitamin A both of these patients responded positively to the RDR test (14% and 32%, Table I). An elevation of this test is proposed as an indirect measure of critically depleted liver stores of vitamin A. Results of the RDR test were normal (0% response) after at least 2 months of daily vitamin A supplementation. Thus, during the course
Table I. Summary of vitamin A studies, thresholds, and rhodopsin density of two patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of vitamin A supplementation</th>
<th>Retinol</th>
<th>RBP*</th>
<th>Pre-albumin*</th>
<th>% RDR</th>
<th>Elevation of log relative thresholds</th>
<th>Rhodopsin densityc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>5.5</td>
<td>0.6</td>
<td>9.5</td>
<td>31.2</td>
<td>2.40</td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td>1.5 weeks</td>
<td>8.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.30</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>13.1</td>
<td>2.1</td>
<td>10.3</td>
<td>0</td>
<td>0</td>
<td>0.350</td>
</tr>
<tr>
<td></td>
<td>14.5 weeks</td>
<td>24.2</td>
<td>1.6</td>
<td>11.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1 year</td>
<td>10</td>
<td>1.9</td>
<td>12.6</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7</td>
<td>0.9</td>
<td>10.5</td>
<td>14</td>
<td>0.9</td>
<td>0.310</td>
</tr>
<tr>
<td></td>
<td>1.5 weeks</td>
<td>13</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>20.5</td>
<td>2.5</td>
<td>11.5</td>
<td>0</td>
<td>0</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>1 year</td>
<td>37</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

*Mean RBP for 31 patients with CF was 3.4 mg/dl (S.D. = 1.9) and 4.6 mg/dl (S.D. = 1.1) for 12 controls.

Mean prealbumin for 28 patients with CF was 20 mg/dl (S.D. = 7.4) and 29.4 mg/dl (S.D. = 5.9) for 11 controls.

c Using the transmissivity difference equation, Alpern and Push found normal rhodopsin density, measured in conditions similar to ours to be $D_{500nm} = 0.350$. In our laboratory, measurements on unpracticed observers ($n = 4$) gave $D_{500nm} = 0.337$ (S.D. = 0.009).

of supplementation, liver stores appeared to return to normal, fasting plasma retinol levels increased, thresholds decreased, and rhodopsin density increased as measured by reflection retinal densitometry. Results of the RDR test were normal in two other young adult patients having plasma retinol values of 21 and 22 μg/dl, no clinical evidence of liver disease, and normal dark-adapted thresholds.

Fasting plasma vitamin A was determined as part of a follow-up evaluation 1 year after the final RDR test was performed. Although both patients were instructed to continue the high-dose vitamin A capsule, compliance was admittedly inconsistent. In one patient, fasting plasma retinol declined from 24 to 10 μg/dl and in the other retinol increased from 20 to 37 μg/dl (Table I).

The relation of log thresholds and rhodopsin during the course of vitamin A treatment is shown in Fig. 5. Small decreases in rhodopsin are associated with large elevations of threshold. As previously shown for vitamin A-deficient rats, alterations in rhodopsin content due to vitamin A deficiency are related linearly to log threshold. This relation also applies when rhodopsin is decreased by bleaching, light damage, or some retinal degenerations. Rhodopsin density was also measured in three other patients with CF who had normal thresholds. The mean value of these densities was 0.324 and all were within normal range (for normal, unpracticed observers $D_{500nm} = 0.337$, S.D. = 0.009, n = 4).

Discussion

As a group, patients with CF have elevated thresholds (Fig. 2) and depressed plasma retinol levels (Fig. 1). If fasting retinol values are under 15 μg/dl, the correlation with threshold is significant. The demarcation level of 15 μg/dl, chosen arbitrarily, was based on the observation that none of the control subjects had retinol values below 15 μg/dl.

Despite the correlation of low plasma retinol (<15 μg/dl), dark-adapted threshold, and the suggestion from the data of Fig. 4 that a function relating retinol and log threshold saturates (i.e., above some level, further increases in retinol will not further decrease log threshold), we cannot give a limiting value of plasma retinol below which the dark-adapted threshold would certainly be elevated. For one thing, our data do not allow us to specify completely the contribution of hepatic and retinal pigment epithelial functions (that are interposed between the plasma and neural retina) to retinal sensitivity and rhodopsin content in these patients.

Furthermore, the dark-adapted threshold may not be the most sensitive index of low plasma retinol; slow recovery of the rod portion of the dark-adaptation curve may indicate deranged retinal function (Fig. 3) even if plasma retinol were not extremely low. Cone adaptation appeared to be less affected by low retinol levels. This appears to be consistent with the finding in rats with diet-induced vitamin A deficiency; rods show more exten-
sive morphologic changes than cones.\textsuperscript{19} In our pediatric and ill population we have not evaluated the rate of adaptation as fully as the dark-adapted threshold; only 27% (15/56) of the patients had full dark-adaptation curves obtained. Such testing may be more suitable for other populations that are at risk for vitamin A deficiency.

Four patients with low retinol values of 8 to 13 μg/dl had dark-adapted thresholds within the normal range. Each of the four patients had severe CF,\textsuperscript{3, 4} clinical evidence of liver disease, and levels of both plasma vitamin A carrier protein, RBP, and prealbumin that were less than half those of controls. Hence, in these four patients, even though plasma retinol was low and intravascular transport may have been less robust than normal, retinal function was adequate to permit normal dark-adapted sensitivity. Such patients may have intraretinal mechanisms, not yet fully specified,\textsuperscript{20} that protect retinal function from hypovitaminosis.

The two patients who initially had very low plasma retinol, evidence of inadequate liver vitamin A stores, low rhodopsin content, and low retinal sensitivity recovered normal sensitivity when high-dose supplements of oral vitamin A were provided. A daily high-dose supplement was necessary to sustain elevated plasma retinol levels. Initial rhodopsin densities that were only 12% to 20% below normal were associated with large elevations of threshold; during recovery from vitamin A deficiency, small increases in rhodopsin content of the dark-adapted retina decreased the log threshold (Fig. 5). All points in Fig. 5 lie above the dashed curves that would apply if the lowering of threshold were due to an increase in the probability of photon absorption as the patients recovered from vitamin A deficiency. In fact, the points for the patients are close to the line that Rushton\textsuperscript{16} found to describe the relation of rhodopsin and log threshold of a dark-adapting rod monochromat.

The present results are consistent with a previous report\textsuperscript{21} that, in a patient with vitamin A deficiency secondary to malabsorption after a small bowel by-pass, a moderate decrease in rhodopsin content was associated

\begin{equation}
I_\ell = \frac{(1 - e^{-2.3D})(1 - e^{-2.3D})}{(1 - e^{-2.3D})}
\end{equation}

where, $I_\ell$ is the threshold, $D$ is the axial density of an outer segment, and $f$ is the fraction of normal rhodopsin present. The dashed curves represent relations that would hold if there were an inverse relation of threshold and rhodopsin. The upper curve is for very low axial density, i.e., $D$ approaching 0. The lower curve is for $D = 1$, which is among the highest densities reported for photopigment in outer segments.
with a large elevation of threshold. The threshold of that patient was above the cone threshold; thus, the relation of rhodopsin and scotopic thresholds for that patient could not be evaluated directly. For our two patients, the relation of rhodopsin and log threshold may be fit by a log-linear relation similar to that which describes the effects of vitamin A deficiency on rat rhodopsin and b-wave sensitivity.14

In summary, low plasma retinol levels (<15 µg/dl) are correlated with elevations of the dark-adapted threshold in patients with CF, but retinols of all values are correlated with the rate of rod recovery after a full bleach. The average rate of recovery of patients with CF is less than that of control subjects. Supplementation with high oral doses of water-miscible vitamin A resulted in increased scotopic sensitivity and rhodopsin density in two patients who initially had very low (<7 µg/dl) plasma vitamin A. As previously shown in vitamin A-deficient rats, there is a linear relation between log threshold and reduced rhodopsin levels in these vitamin A-deficient patients.

We appreciate the technical support of D. Bankson for determinations of plasma retinol, RBP, and prealbumin. We are grateful to Prof. M. Alpern and B. N. Baker for comments on an earlier version of the manuscript. We thank D. Clark for her patience in typing the manuscript.

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