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Vitamin A Supplementation Effects on
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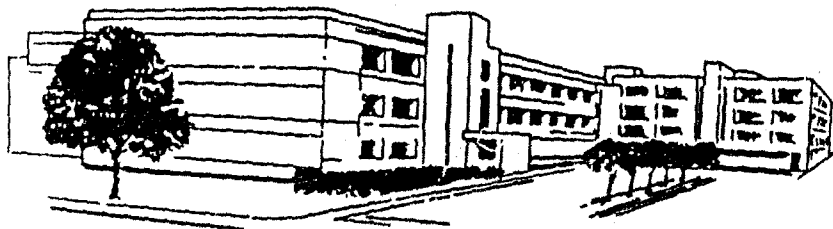
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Betty Burri
and
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Division of Ocular Hazards Research

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Vitamin A Supplementation Effects on Photopic and Scotopic Visual Function and Measures of Vitamin A Status. Harry Zwick, et al

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Abstract

We investigated the effects of vitamin A supplementation in an individual with abnormally low vitamin A-containing transthyretin-bound retinol binding protein (RBP). Measures of spectral dark adaptation and spatial contrast sensitivity suggest a differential return of parafoveal and foveal receptor systems during supplementation. Parafoveal cone systems appear to return more rapidly than foveal cones and rods. Post-supplementation measurements of spectral dark adaptation demonstrated a crossing of spectral dark adaptation functions at 6.5 minutes, close to the appearance of the rod cone break attributed to achromatic measurements of dark adaptation. Contrast sensitivity for the finest spatial frequency showed a delay in its return to near normal levels relative to recovery of mid to low spatial frequencies. While measures of serum retinol, total RBP, and free RBP increased during supplementation, transthyretin-bound RBP failed to show any increase relative to its abnormally low pre-supplementation level. Bodily stores of vitamin A either require more time to return to normal levels than retinol delivered to the retina or this individual possessed a specific genetic deficiency in the bodily mechanism responsible for storing vitamin A.



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Vitamin A Supplementation Effects on Photopic and Scotopic Visual Function and Measures of Vitamin A Status

INTRODUCTION

We have investigated in an otherwise healthy adult male, who had markedly elevated dark adaptation, clinically normal serum retinol, and a pale appearing retina. We measured the effects of vitamin A supplementation on spectral dark adaptation and contrast sensitivity. Three biochemical tests of vitamin A status were utilized: 1) serum retinol; 2) total immunologically active retinol binding protein (RBP) as determined by radial immunodiffusion; and 3) vitamin A-containing free- and transthyretin-bound RBP as determined by high performance liquid chromatography (HPLC).

Spectral measures of dark adaptation utilizing red and green light emitting diodes (LEDs) were used to provide greater separation of rod and cone function during supplementation (1-3). Spatial vision was assessed by contrast sensitivity, so that fine as well as gross spatial mechanisms could be examined during supplementation. Retinal receptor thinning induced by vitamin A deprivation (4) may alter foveal receptor matrix and cause changes in visual acuity and spatial function similar to functional and morphological alterations observed in photic maculopathy (5, 6).

Serum retinol is one of the most commonly used methods for detecting vitamin A deficiency, but it is influenced by protein-calorie malnutrition or alcoholism and is poorly correlated to vitamin A body stores except in cases of true deficiency or toxicity (7). Retinol-binding protein is the major retinol-transporting protein in blood serum (8). Usually RBP circulates in blood in a 1:1 complex with transthyretin (8), but a significant fraction of free RBP (not complexed to transthyretin) is believed to be present in the blood. Total RBP concentration, as measured by radial immunodiffusion, is influenced by the amount of vitamin A stored in the liver and can be used to detect vitamin A deficiencies (9, 10). Unfortunately, total RBP is also influenced by liver disease and protein-calorie malnutrition (11, 12) and is not highly correlated to vitamin A body stores except in deficiency.

Recent investigations have developed techniques for measuring free and transthyretin-bound RBP (13, 14). Measures of free RBP correlate highly to serum retinol and total RBP in humans with adequate or marginal vitamin A status. On the other hand, transthyretin-bound RBP is more highly correlated to liver (and presumably, total body) vitamin A stores measured in marginally deficient or subtoxic rats than free RBP, total RBP, or serum retinol (15). Measures of transthyretin bound RBP were employed to compare normal and human retinal disease (16).

Methods

Figure 1 presents a schema of the apparatus and procedures used to measure spectral dark adaptation. Spectral test stimuli were comprised of red and green LED sources. The spectral output of these sources are shown in the lower left insert of this Figure as the C and E emission spectra. Other emission spectra shown were not employed in the present study. Pulse width modulation of LEDs sources was used to control the apparent intensity of each LED source. Visual threshold LED pulses from 1 microsecond to 1 millisecond depend upon energy. LED amplitude and pulse are reciprocal in determining visual threshold (1-3).

Red and green LED sources were arrayed in individual crosses with a central fixation diode (upper left insert). Measurements of dark adaptation were made at either 16 or 2 degrees from fixation using four LEDs equidistance from the fixation. Red and green crosses were alternated during threshold measurements. The subject's task was to fixate the central LED, to hold his fixation on the central LED until some other source of light appeared in his peripheral view. At this time, he depressed his response button until the light in his peripheral view disappeared while maintaining central fixation. LED threshold measurements were alternately measured in this manner through the course of dark adaptation.

Dark adaptation functions were obtained following 2 minute light adaptation in a 36-inch hemisphere, fitted with chin rest and head support and indirectly illuminated with a tungsten source that produced a uniform illumination of 100 cda/m^2 . In the upper right, a sample dark adaptation function from one individual is presented relative to upper and lower bounds representing two standard deviations above and below the norm for this test (1-

3). The dynamic range of this function and the absolute threshold measured with the green LED are equivalent to that obtained by Sloan (17).

Measurement of contrast sensitivity was made with a video grating contrast sensitivity apparatus (Nicolet) over a spatial frequency range from 0.5 to 22.8 Hz/degree.

Serum retinol was determined by a Series 400 liquid chromatograph equipped with an LC 90 UV-Vis spectrophotometric detector and an LC 100 computing integrator (Perkin-Elmer Corp). Chromatography was on a C18 reverse phase column (18, 19). Total immunologically active retinol-binding protein (RBP) was determined by radial immunodiffusion (Behring Diagnostics) according to manufacturer's procedure.

Vitamin A-containing free- and transthyretin-bound RBP were measured by molecular exclusion HPLC with fluorometric detection as previously described with the exception that Acro LC 13 filters were used instead of Millex filters (14).

Results

In Figures 2a and 2b, spectral dark adaptation functions measured for the red and green LEDs show dramatic changes in the shape of the dark adaptation prior to, during, and post-supplementation periods. The functions for the red LED appeared to return to near normal levels within the first 3 days of supplementation. The function for the green LED shows a similar trend over the 6 or 7 minutes of dark adaptation, with an additional increase in sensitivity that appeared near the end of supplementation and increased to the values shown in the 13-day post-supplementation function.

Prior to supplementation, spectral dark adaptation functions failed to cross. Following supplementation, a crossover occurs between 6 and 7 minutes of dark adaptation (Figure 3).

In Figure 4, log sensitivity is plotted as a function of days prior to, during, and post-supplementation. Changes in sensitivity or contrast sensitivity show a steady improvement during and after supplementation with exception of the 23 Hz/deg grating, which shows a dramatic increase in sensitivity just prior to the termination of

the supplementation period. While full recovery does not occur, sensitivity for all spatial frequencies are within or very close to one standard deviation of the normative sensitivity values for this test of contrast sensitivity.

Vitamin A supplementation increased all biochemical measurements of vitamin A (Figure 5) measured with the exception of transthyretin-bound retinol binding protein which failed to change throughout the supplementation and post-supplementation measurement period.

Clinical measures of visual acuity increased from approximately 20/30 to 20/20 about the same time that contrast sensitivity for the fine spatial frequency, 23 Hz/deg, occurred. An apparent darkening of the macular was observed during the supplementation interval.

Discussion

The sensitivity of vitamin A-containing transthyretin-bound RBP as an indicator of body levels of retinol is demonstrated in this case. Vitamin A supplementation dramatically restored photopic and scotopic measures of visual function. The relative increase in serum retinol, total immunological RBP, and vitamin A-containing free RBP during supplementation demonstrates the ability of these measures to reflect retinol concentrations at the retina.

Transthyretin RBP may not have increased during this time because vitamin A body stores require more time to normalize relative to free RBP. On the other hand, the longer time for restoration of transthyretin-bound RBP may result from either an acquired or genetic deficiency or defect in the storage mechanism of retinol or the RBP complex.

Measurements of spectral dark adaptation during supplementation indicate that cone function returns more rapidly than rod function, as indicated by comparing peripheral measures of spectral dark adaptation for the red and green LED functions (Figures 2 and 3). A definite crossover in spectral dark adaptation does not occur until after supplementation. At this time a crossover occurs between 6 and 7 minutes, which is comparable to the rod cone break measured in achromatic dark adaptation functions. Measurement of contrast sensitivity further suggests that the return of the fine spatial frequency mechanism, which mediates visual acuity and should reflect

foveal cone function, is initially delayed relative to the return of sensitivity of mid to low spatial frequencies.

These differential effects in the recovery of visual sensitivity may reflect morphological differences between parafoveal and foveal cones. The thinner and longer foveal cone outer segments may have more difficulty in absorbing the vitamin A molecule, especially with the tightness of receptor packing in the normal human fovea, requiring more time for restoration of normal retinal kinetic functions. If vitamin A deficiency does produce a thinning of human retinal photoreceptors, as shown by Herron and Riegel (4) for the rat rod receptors, then it is possible that such effects may alter the foveal photoreceptor matrix in the human eye, consequently resulting in a small acuity change associated with vitamin A deficiency. Changes in foveal packing following photic macula damage has been hypothesized to account for recovery in studies of photic foveal damage (5, 6). Vitamin A supplementation may gradually restore the altered foveal receptor matrix, accounting for the delayed increase in fine spatial frequency sensitivity.

We suggest that these structural factors as well as differential kinetics of rod and cone vision account for the hierarchical return of central and peripheral retinal receptor function exhibited by this patient during vitamin A supplementation.

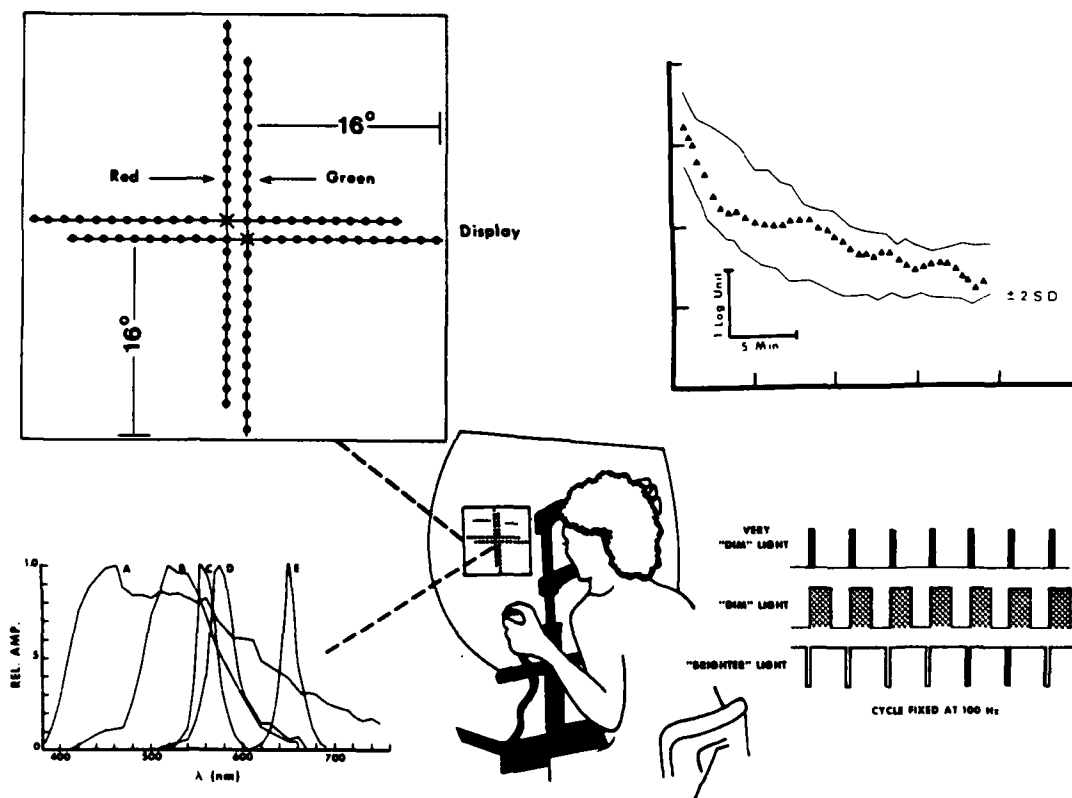


Figure 1. Schematic illustration of LED dark adaptation. Upper right: sample dark-adaptation function showing how the threshold pulse width decreases in dark adaptation. Lower right shows the correlation of pulse width with apparent intensity. The relative spectral transmission curves of the LED sources available to this apparatus are shown in the lower left corner. The C and E diodes were employed in this experiment. The LED light sources were arranged in a cross pattern (upper right). They were equated in peak luminance at approximately $(12 \times 10^{-6} \text{ lm/cm}^2 \text{ sr})$ by using radiometric measurement from an EG&G 580 Radiometer. All LEDs in both horizontal and vertical meridians were adjusted to meet this luminance output.

Supplementation Effects on Red LED

Dark Adaptation

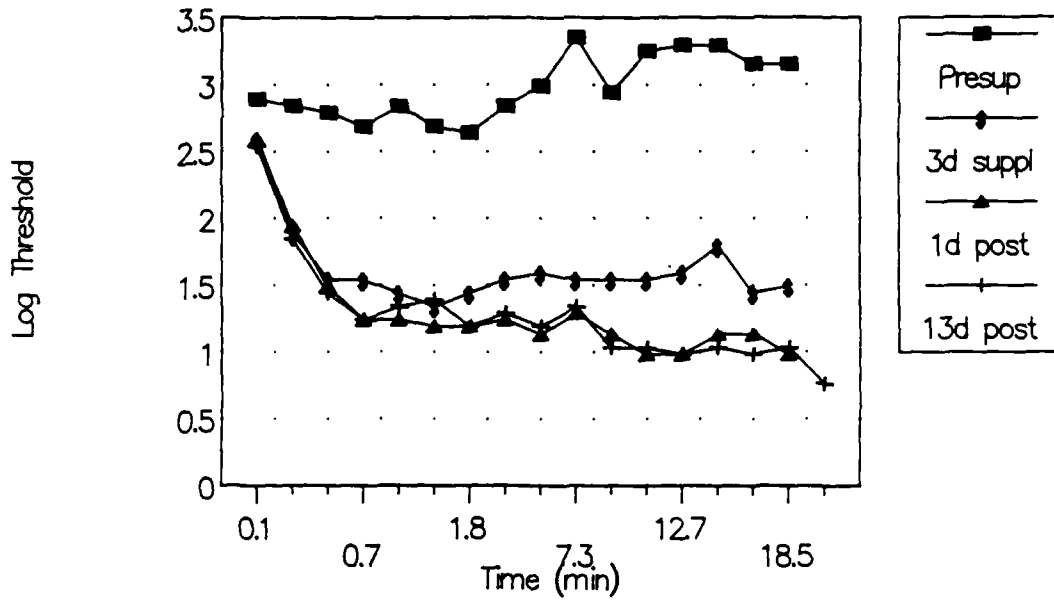


Figure 2a. Spectral dark adaptation for the red LED measured prior to, during, and postvitamin A supplementation.

Supplementation Effects on Green LED

Dark Adaptation

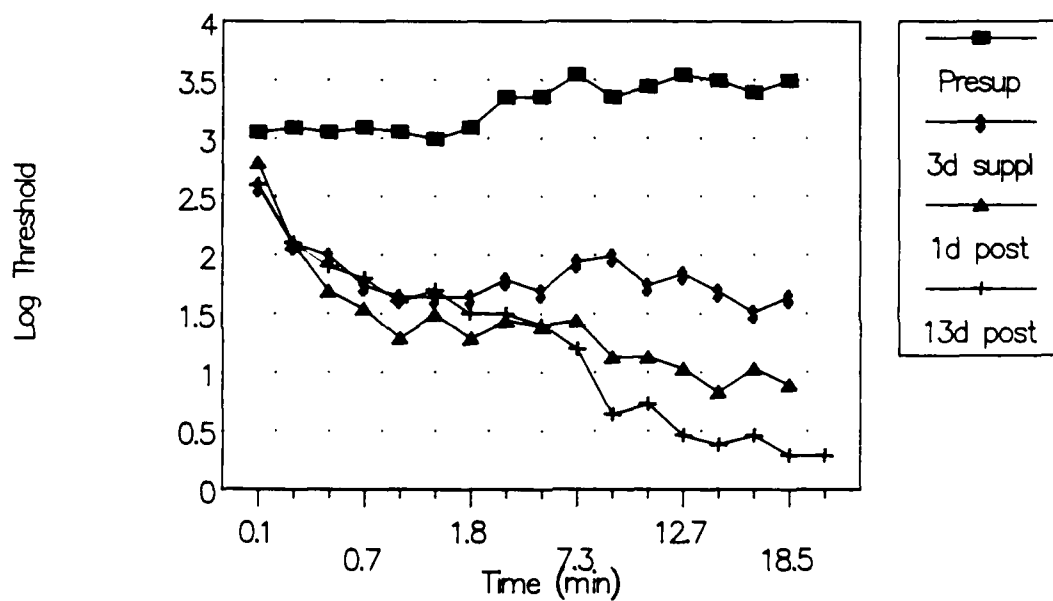


Figure 2b. Spectral dark adaptation for the green LED measured prior to, during, and postvitamin A supplementation.

Comparison of Spectral Dark Adaptation Following Vitamin A Supplementation

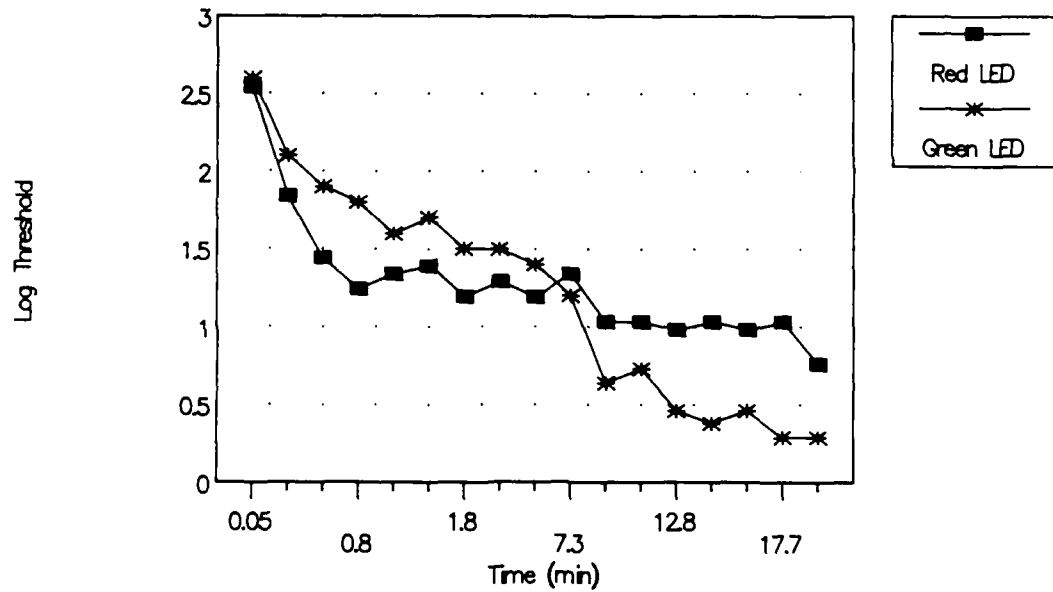


Figure 3. Postsupplementation red and green dark adaptation functions showing crossover between 6 and 7 minutes.

Pre and Post Changes in Contrast Sensitivity for Spatial Frequency

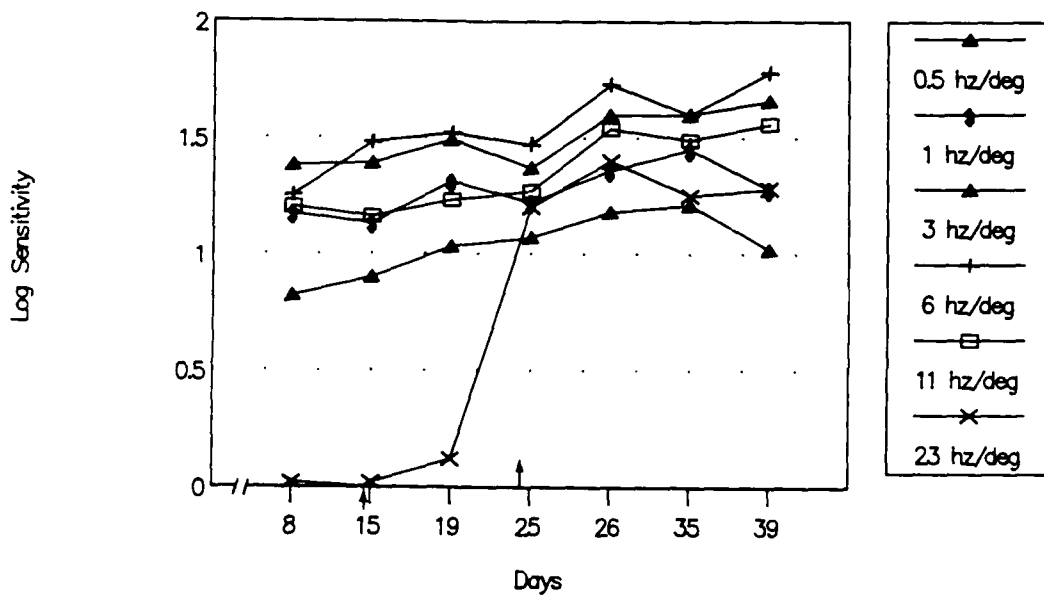


Figure 4. Log contrast sensitivity vs. days prior to, during, and postvitamin A supplementation for different spatial frequencies. Vertical arrows on the abscissa represent the beginning and end of the supplementation period. The points at the right represent the normal contrast sensitivity function for these spatial frequencies.

Changes in Biochemical Measures of Retinol with Supplementation

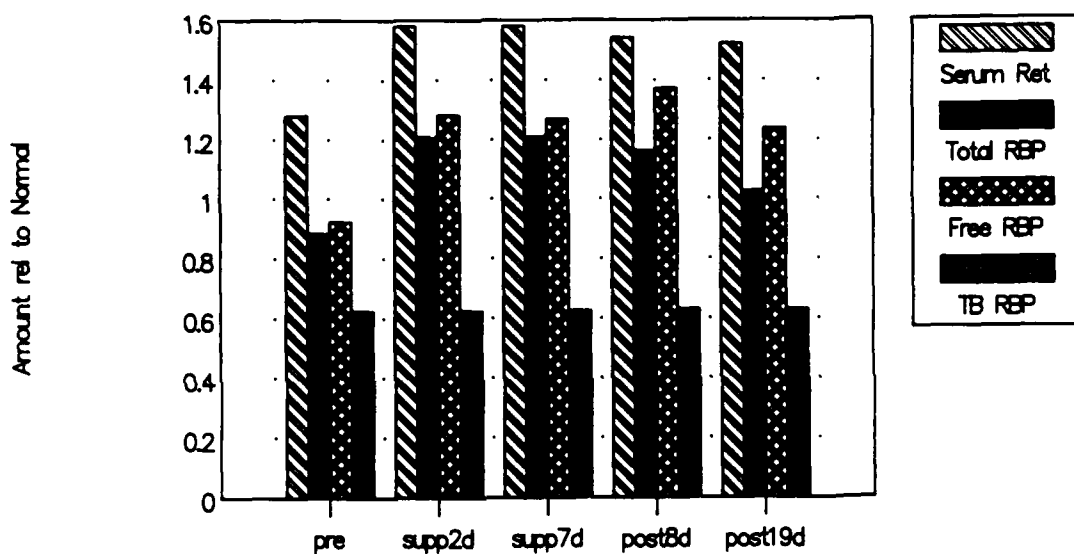


Figure 5. Relative changes in biochemical measures of retinol-Serum Retinol (Serum Ret), Total RBP, Free RBP, and Transthyretin bound retinol binding protein (TB RBP) are shown prior to, during, and postvitamin A supplementation.

REFERENCES

1. Zwick H, O'Mara PA, Beatrice ES, Biggs SL, Van Sice CW. A solid state dark adaptometer - The Letterman Army Institute of Research dark adaptometer. In: Proceedings of 37th AGARD Meeting. Toronto, Canada, 1980.
2. O'Mara PA, Zwick H, Van Sice CW. A microcomputer-controlled solid state dark adaptometer. Behav Res Meth and Instrument 1981;13:747-752.
3. O'Mara PA, Zwick H, Beatrice ES, Lund DJ. Microprocessor controlled light-emitting diode dark adaptometer. Med Biol Eng Comp 1982;20:70-76.
4. Herron WL, Riegel BW. Vitamin A deficiency-induced "rod thinning" to permanently decrease the production of rod outer segment material. Invest Ophthalmol 1974;13:54-59.
5. Zwick H, Bedell RB, Bloom KR. Spectral and visual deficits associated with laser irradiation. Mod Prob Ophthalmol 1974;13:298-306.
6. T'so M. Photic maculopathy in rhesus monkey. Invest Ophthalmol 1973;12:17-34.
7. Rodriguez MS, Irwin MI. A conspectus of research on vitamin A requirements of man. J Nutr 1972;102:909-968.
8. Goodman DS. Plasma retinol-binding protein. In: Sporn MB, Roberts AB, Goodman DS, eds. The Retinoids. Orlando, FL: Academic Press, 1984:41-88.
9. Muto Y, Smith JE, Milch PO, Goodman DS. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. J Biol Chem 1972;247:2542-2550.
10. Mallia AK, Smith JE, Goodman DS. Metabolism of retinol-binding protein and vitamin A during hypervitaminosis in the rat. J Lipid Res 1975;16:180-188.

11. Smith FR, Goodman DS. The effects of diseases of the liver, thyroid, and kidneys on the transport of vitamin A in human plasma. *J Clin Invest* 1971;50:2426-2436.
12. Ingenbleek Y, Van den Schrieck HG, De Nayer P, De Visscher M. The role of retinol-binding protein in protein-calorie malnutrition. *Metabolism* 1975;24:633-641.
13. Fex G, Felding P. Factors affecting the concentration of free holo retinol-binding protein in human plasma. *Eur J Clin Invest* 1984;14:146-149.
14. Burri BJ, Kutnink MA. Liquid-chromatographic assay for free and transthyretin-bound retinol-binding protein in serum from normal humans. *Clin Chem* 1989;35:582-86.
15. Burri BJ, Kutnink MA. Effect of liver vitamin A concentrations on plasma free- and transthyretin-bound retinol-binding protein. *FASEB J* 1988;2:a844-a844.
16. Mariani G. The vitamin A transporting protein complex in human hereditary pigmentous retinal dystrophy. *J Invest Ophthalmol* 1974;13:288-290.
17. Sloan LL. Rate of dark adaptation and regional threshold gradient of the dark-adapted eye: Physiologic and clinical studies. *Am J Ophthalmol* 1947;30:705-715.
18. Chow FI, Omaye ST. Use of antioxidants in the analysis of vitamins A and E in mammalian plasma by high performance liquid chromatography. *Lipids* 1983;18:837-41.
19. Catignani GL, Bieri JG. Simultaneous determination of retinol and alpha tocopherol in serum or plasma by liquid chromatography. *Clin Chem* 1983;29:708-14.

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