

Original Investigation | CLINICAL TRIAL

Treatment With 9-*cis* β -Carotene-Rich Powder in Patients With Retinitis Pigmentosa

A Randomized Crossover Trial

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IMPORTANCE Retinitis pigmentosa (RP) is the leading cause of incurable inherited blindness in the developed world, with an estimated prevalence of 1 in 3500 individuals. Therefore, it is important to develop new treatments for this disease.

OBJECTIVE To determine the effect of oral treatment with 9-*cis* β -carotene on visual function of patients with RP.

DESIGN Randomized, double-masked, placebo-controlled, crossover clinical trial.

SETTING University tertiary medical facility.

PARTICIPANTS Thirty-four patients with RP who were at least 18 years of age. Twenty-nine patients completed the study and were included in the analysis.

INTERVENTIONS Patients were treated daily for 90 days with capsules containing 300 mg of 9-*cis* β -carotene-rich alga *Dunaliella bardawil* (β -carotene, approximately 20 mg) or placebo (starch). Following a 90-day washout period, they were treated for 90 days with the other capsules.

MAIN OUTCOMES AND MEASURES The primary outcome was the change for both eyes from baseline to the end of each treatment in dark-adapted maximal electroretinographic b-wave amplitude. The secondary outcomes were the changes in light-adapted maximal b-wave amplitude, dark- and light-adapted visual field, and best-corrected visual acuity.

RESULTS The mean change in dark-adapted maximal b-wave amplitude relative to initial baseline was +8.4 μ V for 9-*cis* β -carotene vs -5.9 μ V for placebo ($P = .001$). Ten participants (34.5%) had an increase of more than 10 μ V for both eyes (range, 11-42 μ V) after 9-*cis* β -carotene treatment compared with no participants after placebo treatment. The percentage change in light-adapted b-wave response was +17.8% for 9-*cis* β -carotene vs -3.0% for placebo ($P = .01$). No significant differences were found between the groups for visual field and best-corrected visual acuity. No adverse effects were observed.

CONCLUSIONS AND RELEVANCE Treatment with 9-*cis* β -carotene significantly increased retinal function in patients with RP under the tested conditions. The optimal therapeutic regimen will be determined in future, larger clinical trials. 9-*cis* β -Carotene may represent a new therapeutic approach for some patients with RP.

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Retinitis pigmentosa (RP) is a leading cause of incurable blindness with an estimated prevalence of 1 in 3500 individuals.¹ The disease is part of a group of clinically similar phenotypes of varying severity and is associated with more than 150 genetically heterogeneous causes. Retinitis pigmentosa predominantly affects the rod photoreceptor system, resulting in night blindness in the early phase of the disease, with loss of midperipheral vision that progresses to tunnel vision. Later, cone photoreceptors are involved, causing progressive decline of visual acuity. Disease progression can be assessed by electroretinography (ERG), a sensitive objective test that characterizes RP by reduced or absent dark-adapted b-wave responses.

In many patients with RP, the disease is caused by a defect involving enzymes of the retinoid cycle. This cycle is initiated by the light-induced isomerization and splitting of rhodopsin (composed of opsin and 11-*cis*-retinal) into opsin and all-*trans*-retinal. The latter is subsequently reconverted to 11-*cis*-retinal in the retinal pigment epithelium.²

The retinoid 9-*cis*-retinal, which has a light absorption spectrum similar to that of 11-*cis*-retinal, can replace the latter when its availability is limited owing to a retinoid cycle defect.³ Animal models with such defects, among them models of Leber congenital amaurosis (a severe form of RP with early childhood onset), demonstrate significant functional and morphological improvements after treatment with 9-*cis*-retinal.^{2,4-9} Moreover, oral synthetic retinoid improves visual function in patients with Leber congenital amaurosis.¹⁰ A previous study from our laboratory¹¹ showed that, in patients with congenital stationary night blindness (fundus albipunctatus), a retinoid cycle defect caused by *RDH5* mutation, retinal functions are restored after oral treatment with the Food and Drug Administration-approved 9-*cis* β -carotene-rich alga *Dunaliella bardawil* powder.¹² Because 9-*cis* β -carotene is a known precursor of retinal, the results strongly support the potential of this retinoid precursor as a possible treatment for RP.

In this randomized, placebo-controlled crossover study of parallel groups, we assessed the effects of oral treatment with capsules of 9-*cis* β -carotene-rich *D bardawil* powder on the visual functions of 29 patients with clinically diagnosed RP.

Methods

Patients

Candidates aged 18 or older, whose ERG responses were compatible with a diagnosis of RP (detailed in the eMethods section in Supplement), were recruited from the patient database at the Sheba Medical Center. Thirty-four eligible patients were enrolled in this double-masked, placebo-controlled, crossover clinical trial between April 1, 2008, and May 31, 2009. All patients gave their informed consent to participate. The study adhered to the Declaration of Helsinki, was approved by the Institutional Review Board Committee at the Sheba Medical Center, and was registered at <http://www.clinicaltrials.gov>. Some epidemiological studies reported increased lung cancer rates after high, long-term, synthetic all-*trans* β -carotene supplementation in smokers.^{13,14} Although other studies did

not find this correlation¹⁵⁻¹⁷ and although we used a natural β -carotene composed of several isomers, we decided to exclude smokers from the current study.

Protocol

Patients were randomly assigned (using computer-generated numbers) to a 90-day regimen of 4 daily capsules (Figure 1), each containing 300 mg of powder of either the 9-*cis* β -carotene-rich alga *D bardawil* (β -carotene, approximately 20 mg) or placebo (starch). The β -carotene consisted of approximately equal quantities of 9-*cis* and all-*trans* isomers.¹² *Dunaliella* capsules were administered to 16 patients (the “*Dunaliella*-first” group), and the other 18 (the “placebo-first” group) received the placebo. These treatments were followed by a 90-day washout period for all subjects and then a crossover treatment period lasting 90 days.

The 90-day time period was chosen on the basis of a previous study in which 7 patients with fundus albipunctatus showed significant improvements in peripheral visual field and rod recovery rates after 90 days of treatment with the algae extract.¹¹ This period of treatment was also used for nonocular indications, including psoriasis and atherosclerosis.^{18,19}

Five patients were withdrawn by the investigators after randomization because of pregnancy (1 patient) or low adherence to treatment (4 patients who forgot to take more than half of the capsules). Three of the 5 patients were receiving treatment and 2 patients were receiving placebo at the time of withdrawal (Figure 1). Because they were excluded after randomization, the analysis was not performed according to a strict intent-to-treat criteria.

The patients, clinicians, outcome assessors, and data analysts were unaware of patient allocation. Allocation was revealed after completion of data collection and all analysis steps.

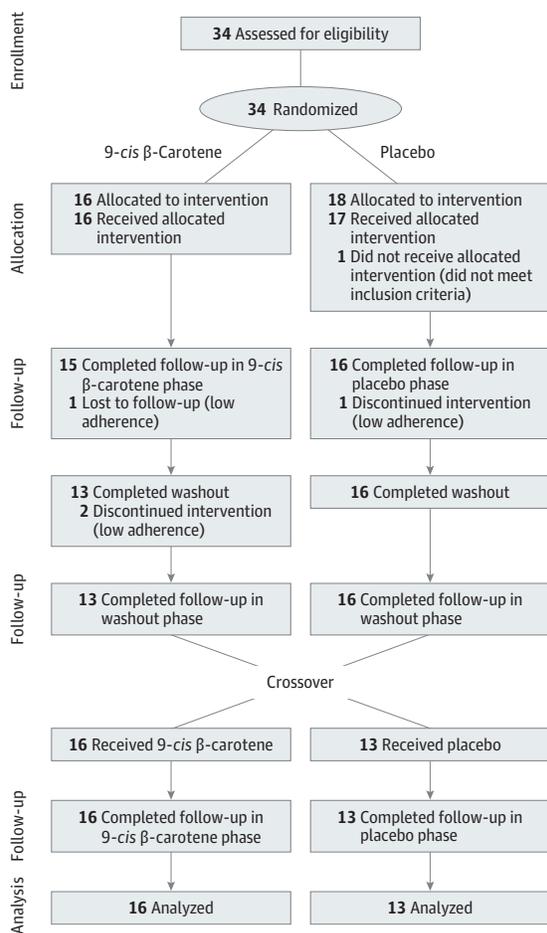
Clinical Evaluation

The tests listed below were all administered 4 times to each patient: at the start of the study (initial baseline) and immediately after each 90-day period, that is, after the first treatment, after washout (postwashout baseline), and after the second treatment.

Electroretinography was performed under both dark-adapted and light-adapted conditions using an International Society for Clinical Electrophysiology of Vision-compliant protocol (UTAS 3000 system; LKC Technologies).²⁰ Full-field ERG responses were recorded from both eyes. The primary outcome of the study was the dark-adapted ERG maximal b-wave response amplitude, representing rod and cone function objectively.

The 4 secondary outcomes were light-adapted ERG b-wave response amplitudes (objective cone function), dark-adapted chromatic visual field area (subjective rod or cone function),²¹ conventional light-adapted visual field area (subjective cone and rod function), and best-corrected visual acuity, which was measured by a highly qualified optometrist using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart. All patients underwent refraction at each visit, and best-corrected visual acuity scores were calculated according to the number of letters seen.

Figure 1. Study Design



Study design of the 9-cis β -carotene clinical trial.

In 2-color, dark-adapted, chromatic Goldmann visual field testing, which is based on the spectral luminous efficacy function for dark-adapted vision, the rods are more sensitive to shorter wavelengths (approximately 500 nm; blue) than to longer wavelengths (approximately 600 nm; red), and the stimuli were matched for the rods.²¹ Patients were also examined with slitlamp biomicroscopy and ophthalmoscopy. Plasma carotenoids were measured with high-performance liquid chromatography, as described elsewhere.²²

Statistical Analysis

Differences between initial and postwashout baseline values and between baseline and posttreatment values were assessed using a paired *t* test stratified by sequence of treatment. The crossover design exploits the fact that in each time period we have both treatments and each participant receives both treatments, so the comparison of treatments is within participants. Mixed models for crossover design containing fixed effects for treatment, sequence, and period and random effects for patient selection were used to estimate differences between treatments separately for each eye.

The percentage of change from baseline was calculated for each variable studied using the following formula: $[(\text{posttreatment} - \text{baseline})/\text{baseline}] \times 100$. When no ERG response was detected (absolute baseline, 0), we used a baseline of 1 to calculate the percentage of change from baseline; this allowed us to calculate and estimate therapeutic effect regardless of the heterogeneity in baseline among patients.

To estimate the binocular effects of treatment, we first calculated the mean binocular initial baseline, first posttreatment, postwashout baseline, and second posttreatment values. Next, we calculated the change between the initial baseline and the first posttreatment values and the change between the postwashout baseline and the second posttreatment values. The change in the study outcome was used as the dependent variable in each model, and the baseline values were kept in the statistical models as covariates to adjust for the differences observed in baseline values according to sequence.

Results

Clinical and Demographic Data

The 29 participants who completed the study (21 men and 8 women; mean [SD] age, 46.7 [16.9] years; range, 21-74 years) all had reductions in ERG response, which varied in degree. Ages at diagnosis ranged from 5 to 60 years (mean [SD], 30.9 [17.9] years). Two participants were born in Israel, 10 in Europe or America, and 17 in Asia or Africa. Mendelian inheritance studies disclosed 20 autosomal recessive and 9 autosomal dominant patterns. No other genetic information was available.

Effect of *Dunaliella* or Placebo on Plasma Content of All-*Trans* β -Carotene

As expected, treatment with *Dunaliella* triggered a significant increase in the mean plasma content of all-*trans* β -carotene both in the *Dunaliella*-first group ($P = .03$) and in the placebo-first group ($P < .01$; Figure 2). In the *Dunaliella*-first group, all-*trans* β -carotene was decreased after washout, but the baseline remained somewhat higher than the initial level and was not fully restored even after treatment with placebo, pointing to relatively prolonged body storage of β -carotene. As in previous trials, plasma levels of 9-cis β -carotene were below the limits of detection.²²

Comparison of Binocular Initial and Postwashout Baseline Values

In 27 of 29 patients who completed the trial, there was no significant interocular difference in the maximal dark-adapted ERG b-wave responses at initial baseline (eTable 2 in Supplement). Hence, we decided to analyze right and left eyes separately. The placebo-first group demonstrated a higher mean b-wave response at initial baseline (Table 1). The mean dark-adapted ERG b-wave amplitudes at initial baseline did not differ significantly from amplitudes at baseline after washout, although the *Dunaliella*-first patients had higher baseline values after washout than initially (Table 1). No such tendency was

observed in the placebo-first group, suggesting that the wash-out period did not suffice to completely eliminate the *Dunaliella* effects.

Effects of *Dunaliella* and Placebo Treatments on Maximal Dark-Adapted and Light-Adapted ERG b-Wave Response Amplitudes

The arithmetic mean change in maximal dark-adapted ERG b-wave amplitude (representing objective maximal dark-adapted rod-cone function, the primary outcome of this study) in response to treatment with *Dunaliella* or placebo was analyzed separately in the right and left eyes in all 29 patients. Relative to initial baseline, treatment with *Dunaliella* yielded a significant increase in maximal dark-adapted ERG b-wave amplitude (+8.9 and +7.8 μV for right and left eyes, respectively), whereas placebo treatment resulted in a decrease, which was significant in the right eye (-7.9 and -3.9 μV for right and left eyes, respectively) (Figure 3 and Table 2).

Overall, therefore, *Dunaliella* treatment improved objective rod-cone functions significantly, as reflected by the changes in maximal dark-adapted ERG b-wave amplitude responses (Table 2). The binocular mean change from baseline after treatment with *Dunaliella* (+8.4 μV) was significantly greater than

after placebo treatment (-5.9 μV; *P* = .001). These results were consistent after adjustment for sex, age, and age at diagnosis. Moreover, placebo treatment was followed by some deterioration in the mean binocular maximal dark-adapted ERG b-wave, probably because of insufficient washout in some patients who had received *Dunaliella* before receiving placebo.

Whereas the primary outcome reflects significant improvements in rod and cone functions after 90 days of *Dunaliella* treatment, these objective results are mean values recorded for the entire study group. Not all 29 patients who completed the study showed improvement. In 10 patients (34.5%; 95% CI, 19.0%-52.9%), the dark-adapted maximal b-wave response increased by more than 10 μV in both eyes (range, 11-42 μV) after 9-*cis* β-carotene treatment; 5 of these patients were in the *Dunaliella*-first group and 5 in the placebo-first group. This response was not seen after placebo in any patients.

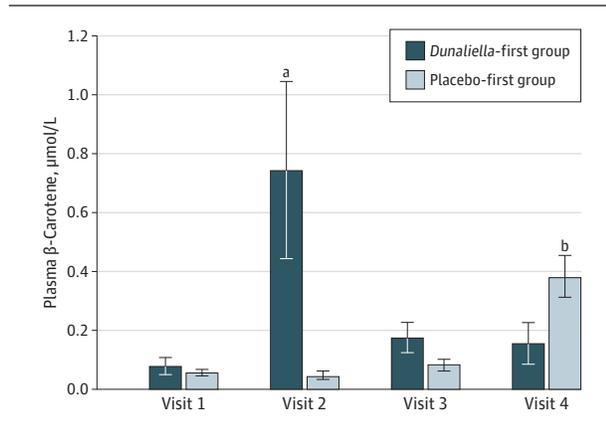
Posttreatment changes in cone function, measured objectively by light-adapted single-flash b-wave amplitudes (a secondary outcome of the study), were also improved by *Dunaliella* treatment. Relative to placebo, treatment with *Dunaliella* yielded a significant improvement. The mean change from baseline to the end of treatment was +17.8% (+3.4 μV) for 9-*cis* β-carotene vs -3.0% (-0.7 μV) for placebo (*P* = .01; *P* = .06 for absolute response) (Table 2 and eTable 3 in Supplement). Thus, both objective measures (rod and cone photoresponse functions) showed significant improvements after 90 days of *Dunaliella* treatment.

Effects of *Dunaliella* and Placebo Treatments on Dark-Adapted and Light-Adapted Visual Field Areas

Treatment with *Dunaliella* also resulted in improved dark-adapted visual field areas. Dark-adapted Goldmann visual field testing showed a significant increase in this factor (night vision) for the whole study group after *Dunaliella* treatment (+8.6 cm²; *P* = .03 relative to baseline) and a nonsignificant increase after treatment with placebo (+3.8 cm²; *P* = .30 relative to baseline) (Table 2 and eTable 4 in Supplement); the increase with placebo can be explained by the subjective nature of the test, which is more susceptible than ERG to placebo effect.

Conventional assessment of the light-adapted visual field area, which tests for day and night vision, showed a nonsignificant improvement after treatment with *Dunaliella*, relative to the initial baseline (Table 2 and eTable 5 in Supplement). Assessment of best-corrected visual acuity yielded a mean (SD) ini-

Figure 2. Plasma β-Carotene Levels at Each Visit



Data are presented as means with SEs.

^a*P* = .03 for visit 2 vs visit 1 in the *Dunaliella*-first group.

^b*P* < .01 for visit 3 vs visit 4 in the placebo-first group. All other differences were not significant.

Table 1. Baseline Values of Dark-Adapted Rod-Cone Function^a

Measurement	Dark-Adapted ERG Responses, Mean (SD), μV		
	Placebo-First Group (n = 16)	<i>Dunaliella</i> -First Group (n = 13)	Total
Right eye			
Initial baseline	89 (93)	50 (50)	72 (78)
Postwashout baseline	82 (92)	56 (55)	71 (78)
<i>P</i> value ^b	.30	.09	.80
Left eye			
Initial baseline	88 (86)	52 (50)	72 (73)
Postwashout baseline	85 (80)	55 (51)	71 (69)
<i>P</i> value ^b	.40	.20	.80

Abbreviations: ERG, electroretinographic.

^a Baseline dark-adapted ERG b-wave maximal amplitude responses were measured before the first treatment (initial baseline) and after treatment washout (postwashout baseline).

^b All *P* values are for comparisons between initial and postwashout baseline values.

tial baseline of 58 (25) letters on the ETDRS chart for both eyes. There was no significant change after treatment with either *Dunaliella* (+0.4 letters; $P = .70$) or placebo (+0.15 letters; $P = .90$) and no difference after treatment with *Dunaliella* relative to placebo ($P = .90$) (Table 2 and eTable 6 in Supplement). No adverse treatment effects were observed in any of the patients.

Effect of *Dunaliella* Treatment on Night Vision in 2 Affected Sisters

Findings in 2 sisters with RP were representative of the effects of 90 days of *Dunaliella* treatment on vision. Both patients showed clear improvements in both objective and sub-

jective retinal functions, indicated respectively, by maximal dark-adapted ERG responses (Figure 4A and C) and chromatic (red and blue stimuli) dark-adapted Goldmann visual field measurements (Figure 4B and D).

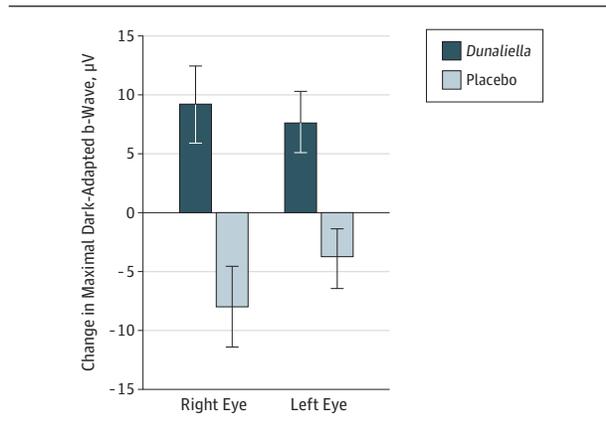
Discussion

In this randomized, double-masked, placebo-controlled crossover study, treatment with 9-cis β-carotene-rich powder of the alga *D bardawil* improved visual functions in patients with RP, as indicated objectively by ERG after 90 days of daily oral treatment with capsules containing either *Dunaliella* or a placebo (Table 2). Subjective testing indicated enlargement of the dark-adapted visual field area after treatment with *Dunaliella* (Table 2). Some benefit was also recorded here with placebo, although this result may be a function of the test's subjective nature or may be due to a learning effect. These results are illustrated by the improvement in both ERG responses and perimetry achieved by 2 sisters after *Dunaliella* treatment (Figure 4).

Our finding that significant improvement in response to *Dunaliella* treatment (as defined above) occurred in only some of our patients (34.5%) was not unexpected. Because the pathogenesis of RP varies among patients, it can be assumed that while certain specific mechanisms are potentially amenable to treatment, others are not.

The smaller mean improvement found in the light-adapted than in the dark-adapted ERG b-wave response amplitude is probably attributable to the fact that the cones are less strongly affected than the rods in RP. This finding is consistent with recent reports of significant functional and structural improvements after treatment with 9-cis-retinal in mouse

Figure 3. Change in Maximal Dark-Adapted Electroretinographic b-Wave Response



The main outcome was the change in maximal dark-adapted electroretinographic b-wave response. The change from baseline to end point in maximal dark-adapted b-wave response was analyzed separately in each eye through paired t test analysis in 29 patients. Data are presented as means and SEs.

Table 2. Treatment Outcomes in 29 Patients: Changes From Baseline to End Point^a

Outcome ^b	<i>Dunaliella</i> Treatment	Placebo Treatment	P Value ^c
Dark-adapted ERG response (primary outcome)			
Right eye			
Change in absolute response, mean (SE) μV [P value]	+8.9(3.4)[.01]	-7.9 (3.4) [.03]	.001
Change from baseline, % [P value]	+89.7[.007]	-16.5 [.60]	.02
Left eye			
Change in absolute response, mean (SE), μV [P value]	+7.8(2.6)[.004]	-3.9 (2.6) [.15]	.003
Change from baseline, % [P value]	+20.7[.03]	-16.6 [.08]	.006
Both eyes			
Change in absolute response, mean (SE), μV [P value]	+8.4(2.8)[.004]	-5.9 (2.8) [.04]	.001
Change from baseline, % [P value]	+39.8[.002]	-15.9 [.20]	.002
Light adapted ERG response ^d			
Change in absolute response, mean (SE), μV [P value]	+3.4(1.5)[.03]	-0.7 (1.5) [.60]	.06
Change from baseline, % [P value]	+17.8[.003]	-3.0 [.60]	.01
Dark-adapted visual field area ^d			
Change in absolute response, mean (SE), cm ² [P value]	+8.6(3.8)[.03]	+3.8 (3.8) [.30]	.40
Light-adapted visual field area ^d			
Change in absolute response, mean (SE), cm ² [P value]	+7.8(4.5)[.09]	-0.5 (4.5) [.90]	.19
Best-corrected visual acuity ^d			
Change in absolute response, mean (SE), No. of letters [P value]	+0.4(0.9)[.70]	+0.15 (0.9) [.90]	.90

Abbreviation: ERG, electroretinographic.

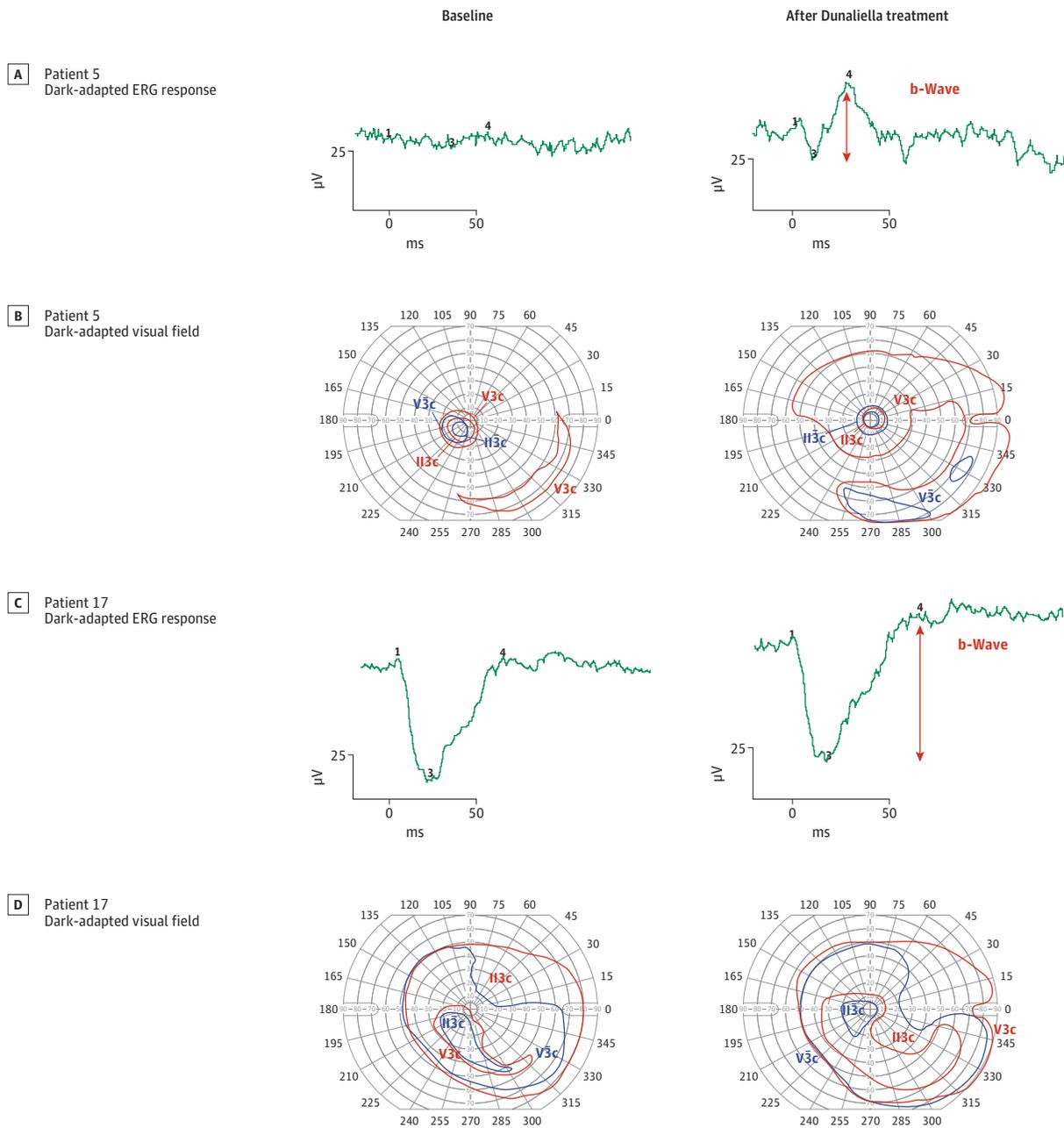
^a Based on a mixed model for the crossover design containing fixed effects for treatment, sequence, and period and random effects for patient selection.

^b Values for change in absolute response represent arithmetic mean (SE) change from baseline values. P values in brackets represent comparison with initial baseline values.

^c P values for comparison between *Dunaliella* and placebo treatments.

^d Mean values for both eyes.

Figure 4. Effect of *Dunaliella* Treatment on Night Vision



Representative results of the effect of *Dunaliella* treatment on night vision of 2 sisters. Arrows mark maximal dark-adapted b-wave. Goldmann kinetic visual fields are shown for large (V3c) and small (II3c) red stimuli (red line) and blue stimuli (blue line).

models of defects in the retinoid cycle, such as *Lrat*^{-/-} and *Rpe65*^{-/-} mouse models of Leber congenital amaurosis and mice with *RDH* mutations.^{2,4-9} In those studies, 9-*cis*-retinal induced the production of twice the amount of isorhodopsin (9-*cis*-retinal and opsin) and doubled the amplitudes of dark-adapted ERG responses.^{4,5,9}

Although the mechanism of the beneficial effect of oral treatment with this alga is not yet known, it seems likely (as also inferred from animal studies^{2,4-9}) that it results, at least

in part, from the high 9-*cis* β -carotene content rather than from other components of *Dunaliella*, such as the equally available all-*trans* form. A recent study showed that 9-*cis* β -carotene (1 mg, injected intraperitoneally 5 times daily for 5 days) failed to improve retinal function in animal models with a retinoid cycle defect.²³ The failure might have been due to the short study duration and the mode of administration, because our patients noticed a treatment effect only after at least 2 months. A recent study from our laboratory of 7 patients with fundus

albipunctatus, a disease caused by an inborn error in the retinoid cycle, demonstrated objective and subjective improvements in visual function after 90 days of treatment with the 9-*cis* β -carotene-rich powder of *Dunaliella*.¹¹ Similar results were reported in an earlier study of successful 9-*cis*-retinal treatment in an animal model bearing the same mutation.⁶

Oral 9-*cis* β -carotene can access the retina, where it can be converted to 9-*cis*-retinal and combine with opsin to form isorhodopsin.²⁴⁻²⁶ Oral 9-*cis*-retinal also restores ERG activity in experimental animals, which suggests that it may correct the subcellular mislocalization of cone opsin resulting from chromophore loss and associated with cone cell death.^{4,5,27,28} Another possible explanation for the beneficial effect of *Dunaliella* treatment is that 9-*cis*-retinal induces an increase in the endogenous production of 11-*cis*-retinal, as observed in mice with a different inborn error in the retinoid cycle.⁵ This elevated production of 11-*cis*-retinal might be a result of interaction between 9-*cis*-retinal and the retinoid X receptor, a type of nuclear receptor.²⁹ Furthermore, the 9-*cis* β -carotene or its metabolites may reduce inflammation and serve as an antioxidant.³⁰⁻³²

Patients with relatively well-preserved retinal anatomy might be promising candidates for retinoid replacement therapy. Treatment with enhanced *Natronomonas pharaonis* halorhodopsin was recently shown to reactivate light-insensitive human photoreceptors.³³ The mechanism of action for this therapeutic approach was attributed to the fact that cone cell bodies remain present longer than rods in both humans and animals.³⁴ The increase in dark-adapted visual fields in our 2 representative affected sisters was greater with the red than with the blue stimulus (Figure 4), indicating that the improvement was indeed in the cones. Combination therapy might be advisable to optimize other treatments, such as gene therapy, as well as to speed up the therapeutic effect.

No adverse effects were reported when treatment with 9-*cis* β -carotene was continued for more than 3 years after study completion in patients with fundus albipunctatus or for more than 1 year in our patients with RP who continued treatment after the present study.¹¹ The over-the-counter *Dunaliella* capsules we used are approved by the US Food and Drug Administration and have been used for more than 20 years with no known significant adverse effects. This apparent lack of toxicity is consistent with findings in mouse models of inborn errors of metabolism, in which neither acute nor prolonged treat-

ment with 9-*cis* retinoid affected the retinoid cycle during restoration of rod structure and rod retinal function and toxic agents were detected only in small quantities,² probably because of the increase in rhodopsin stability.³⁵

One limitation of this study is the lack of genotyping in patients. This initial trial was designed to examine the efficacy of *Dunaliella* treatment in patients with RP of differing severity. To objectively estimate the therapeutic effect, we included mostly (>90%) patients with recordable ERG responses in both eyes. Because more than 45 genes are associated with RP and hundreds of mutations were identified that account for only 60% of patients with RP,¹ we would need to screen thousands of patients to find genotypically characterized patients with measurable ERG responses. Furthermore, it is statistically unlikely that the genetic defect of the patients solely accounts for their improvement after treatment; other mechanisms are probably involved. Nevertheless, the genetic backgrounds of the responsive and nonresponsive patients would be of interest for our future studies.

Another limitation of this initial study is the small population size. Because we included patients with differing severity of disease and randomized them before initial baseline testing, 5 patients with maximal dark-adapted b-wave responses higher than 100 μ V were randomly included in the placebo-first group compared with only 1 patient in the *Dunaliella*-first group (eTable 2 in Supplement). Hence, higher baseline values were recorded in the placebo-first group (Table 1). Further investigations with a much larger group of patients and block randomization are planned for future trials. Because baseline plasma levels of β -carotene were not achieved after the 90-day washout period, a longer washout period is planned for future clinical trials that will be designed to confirm the results reported here, establishing optimal dosages and therapeutic regimens.

Pending more radical therapeutic methods, including gene and stem cell therapies, 9-*cis* β -carotene treatment might help some patients with RP. It might also be helpful as an adjuvant in future gene therapy, as described for a mouse model of Leber congenital amaurosis.² Some findings suggest that such therapy could protect against age-related retinal dystrophies.³⁵ It seems likely that future treatments will include 9-*cis* β -carotene-based restoration in combination with other approaches that increase the survival of altered photoreceptors.

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