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# COMBINED INTRAVITREAL RANIBIZUMAB AND ORAL SUPPLEMENTATION WITH DOCOSAHEXAENOIC ACID AND ANTIOXIDANTS FOR DIABETIC MACULAR EDEMA

## Two-Year Randomized Single-Blind Controlled Trial Results

MARÍA LAFUENTE, MD,* LOURDES ORTÍN, PHD,* MARÍA ARGENTE, MD,* JOSÉ L. GUINDO, PHD,* MARÍA D. LÓPEZ-BERNAL, MD,* FRANCISCO J. LÓPEZ-ROMÁN, MD, PHD, MSC,*† MARÍA J. GARCÍA, PHD,* JUAN CARLOS DOMINGO, PHD,‡ JERÓNIMO LAJARA, MD*†

**Purpose:** To assess the 2-year effectiveness of intravitreal ranibizumab combined with a dietary supplement rich in docosahexaenoic acid (DHA) plus antioxidants in 62 patients with diabetic macular edema.

**Methods:** In a randomized single-blind controlled study, 33 subjects (42 eyes) received intravitreal ranibizumab alone and 29 (34 eyes) combined with DHA (1,050 mg/day). Monthly ranibizumab (0.5 mg) was given for the first 4 months followed by on as-needed treatment.

**Results:** At 24 months, the difference between groups in the decrease of central subfield macular thickness was significant in favor of the DHA supplementation group (95% confidence interval of the difference 7.20–97.656; $P = 0.024$), although improvement in best-corrected visual acuity measured in the Early Treatment Diabetic Retinopathy Study letters did not reach statistical significance (95% confidence interval 5.4–11.2, $P < 0.66$). At 24 months, gains of >5 and >10 letters were significantly higher in the DHA supplementation group as compared with controls when the worse and better seeing eyes were considered but other differences at 12 months and 24 months were not found.

**Conclusion:** Intravitreal ranibizumab combined with DHA supplementation reduced central subfield macular thickness after 2 years of follow-up as compared with ranibizumab alone in patients with diabetic macular edema. This anatomical improvement was accompanied by a trend for an amelioration of vision.

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Diabetic macular edema (DME) is one of the main causes of loss of vision in patients with diabetic retinopathy (DR).[1–3] It has been estimated that up to 15% of diabetic patients will develop DME over the course of their lives,[4] affecting the central fovea in 2% to 10% of patients.[5,6] The purpose of current treatment of DME is to improve visual aquity, for which it is essential an early diagnosis and treatment of both DR and DME as well as to manage risk factors adequately. The treatment of DME is rapidly evolving, and the era of laser therapy is being quickly replaced by the era of pharmacotherapy.[7,8] The identification of vascular endothelial growth factor (VEGF) as an important pathophysiological mediator of DME has prompted the development of specific VEGF antagonists. The efficacy of intravitreal inhibition of VEGF with

ranibizumab given monthly for up to 24 months or less frequently using a variety of as-needed regimens results in rapid and sustained improvement of vision and retinal anatomy.[9–13]

Several lines of evidence suggest that oxidative stress and inflammation are involved in the pathogenesis of DME.[14–18] It has been shown that proinflammatory cytokines are elevated in the extracellular matrix, endothelium, vessel walls, and vitreous of eyes in patients with proliferative DR.[19] Also, supplementation with some nutrients present in our diet offers a degree of protection against progression of retinal changes in age-related macular degeneration.[20–24] These are the xanthophylls, carotenoids, lutein, and zeaxanthin, vitamins E and C, minerals such as zinc and copper, and omega-3 long-chain polyunsaturated fatty acids (ω-3 PUFAs), all of them can help to attenuate oxidative stress. Docosahexaenoic acid (DHA) is highly concentrated in the retina and in the retinal vascular endothelial cells, which suggests its role in protecting human retinal pigment epithelial cells from oxidative stress[25,26] and inflammation.[27] Docosahexaenoic acid contributes to maintain vascular integrity while reducing pathological neovascularization,[17,18] an antiangiogenic mechanism, which might be an advantageous adjunctive effect to current intravitreal anti-VEGF treatment in DR.[28] It has been shown that DHA at normal physiological doses attenuates the effect of cytokine-induced inflammatory signaling (tumor necrosis factor–alpha, interleukin 1 beta, and VEGF) by inhibiting translocation of NF-κB in human endothelial retinal cells.[19]

Based on the current experimental and clinical data linking ω-3 PUFAs and the potential beneficial antiangiogenic, antioxidant, and antiinflammatory role in DR,[27,29–31] we hypothesized that oral supplementation with high dose of DHA (1 g) together with eicosapentaenoic acid, a mixture of B vitamins, vitamins C, E, lutein, zeaxanthin, and minerals might contribute to enhance the effect of intravitreal ranibizumab in DME. To this purpose, a randomized, single-blind, controlled study was designed to assess the 2-year effectiveness of intravitreal ranibizumab combined a high-rich DHA oral nutraceutical formulation in patients with DME.

## Patients and Methods

This randomized, single-masked controlled and prospective study was performed with the approval of the Ethics Committee of Hospital Universitario Morales Meseguer (Murcia, Spain). The study was conducted in accordance with the principles of the Declaration of Helsinki for the protection of human subjects, and written informed consent was obtained from all participants. The study was registered in the European Clinical Trials Database (EudraCT) (EudraCT trial number 2015-001082-74 for the Sponsor's Protocol code number EN-14/12).

### *Study Design and Patients*

Patients of both sexes, aged <85 years were enrolled during ophthalmologic appointments at the study center, the Hospital Universitario Morales Meseguer in Murcia, Spain, between September and December 2012. All patients diagnosed of type 2 diabetes mellitus with decreased vision due to center-involved DME documented on optical coherence tomography (OCT) were eligible to enroll. The OCT eligibility criterion was central subfield macular thickness (CSMT) ≥290 μm. A decrease in visual acuity was also an eligibility criterion but a specific cutoff point was not established. Visual acuity ranged between 20/32 and 20/400 (78–24 Early Treatment Diabetic Retinopathy Study [ETDRS] letters). To be included, patients had to be able to receive intravitreal treatment with ranibizumab and not having received any treatment 3 months before the onset of the trial. Patients unable to participate in the study according to the criteria of the investigator and those who refused to sign the written consent were excluded from the study as were those using vitamin/mineral or fatty acids (FAs) supplements, and those with hypersensitivity to these compounds.

All patients received four loading doses of ranibizumab (0.5 mg/0.05 mL; Lucentis, Novartis Farmacéutica, Barcelona, Spain) during the first 4 months and then treated on as-needed (pro-re-nata) basis. Improvement was defined as a gain of 5 or more ETDRS letters of best-corrected visual acuity (BCVA) and/or a 100 μm decrease in CSMT measured by OCT as compared with the previous visit. Stability criteria were defined as no change over the last three monthly consecutive visits. Criteria for retreatment were loss of

From the *Department of Ophthalmology, Hospital Universitario Morales Meseguer, Murcia, Spain †Faculty of Health Sciences, San Antonio Catholic University of Murcia, Murcia, Spain; and ‡Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain.

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Reprint requests: María Lafuente, MD, Department of Ophthalmology, Hospital Universitario Morales Meseguer, Avenida Marqués de los Vélez s/n, E-30008 Murcia, Spain; e-mail: marialafuentelh@gmail.com

stability in terms of a difference in BCVA ≤5 ETDRS letters and a difference in CSMT ≥100 μm. All candidates for retreatment received two loading doses of ranibizumab and were further evaluated.

Injection of intravitreal ranibizumab was performed as an outpatient procedure in an operating room, under an operating microscope, using topical anesthesia with 0.1% tetracaine and 0.4% oxybuprocaine (Colircusí Anestésico Doble; Alcon Cusí, S.A., Barcelona, Spain) and strict aseptic techniques. After topical anesthesia, the ocular surface and the lid were disinfected with povidone-iodine. We used a speculum, sterile gloves, and a surgical drape. Intravitreal injection of 0.5 mg ranibizumab in 0.05 mL was performed using a 30-gauge needle at 3.5 mm to 4 mm posterior to the limbus. The injection site was compressed by cotton swab to avoid reflux. After this, the fundus was examined to rule out any complications and to check perfusion of the central retinal artery.

Study patients were randomized to standard intravitreal ranibizumab either with or without DHA oral supplementation (control group) (1,050 mg/day) (Brudyretina 1.5 g; Brudy Lab S.L., Barcelona, Spain). This is a concentrated DHA triglyceride having a high antioxidant activity patented[26] to prevent cellular oxidative damage. Randomization was performed for each individual patient using a table of random numbers. When both eyes were affected, they were included in the same study group. The treatment evaluator (M.L.L.H.) who also evaluated the study variables (visual acuity, etc.) was masked of which subjects were receiving DHA supplementation. An independent safety evaluator (J.L.G.) was not masked of whether or not patients had been assigned to the DHA supplementation group, although this evaluator was unaware of results of the outcome variables.

The composition of the nutraceutical formulation is detailed in Table 1. Patients were instructed to take 3 capsules of Brudyretina 1.5 g, once daily.

### *Outcome and Procedures*

Patients were visited at the outpatient clinic of the Department of Ophthalmology every month. At each visit, BCVA and measurement of CSMT by OCT (Stratus OCT; Carl Zeiss Meditec, Dublin, CA) were performed. Best-corrected visual acuity was assessed using an ETDRS optotype at 2 m distance from the observer. At each visit, the nutraceutical supplement was delivered to the patient for 1-month treatment. Compliance with DHA supplementation was assessed at the study visits and by a telephone call at 15-day intervals. A fluorescein angiography and OCT retinal nerve fiber analysis were performed at the beginning of the study and at 12 months and at 24 months. Outcome variables included the number of ranibizumab intravitreal injections during the study period, BCVA, changes of CSMT (OCT), serum

Table 1. Composition of Brudyretina 1.5 g (Brudy Lab S.L., Barcelona, Spain), per Capsule

| Composition | Per Capsule | % Recommended Daily Amount | Per Three Capsules | % Recommended Daily Amount |
|---|---|---|---|---|
| Concentrated oil in ω-3 FAs | 500 mg | | 1,500 mg | |
| TG-DHA 70% | 350 mg | — | 1,050 mg | — |
| EPA 8.5% | 42.5 mg | — | 127 mg | — |
| DPA 6% | 30 mg | — | 90 mg | — |
| Vitamins | | | | |
| Vitamin B1(thiamine) | 0.37 mg | 33 | 1.1 mg | 100 |
| Vitamin B2 (riboflavin) | 0.47 mg | 33 | 1.4 mg | 100 |
| Vitamin B3 (niacin/niacinamide) | 5.3 mg NE | 33 | 16 mg NE | 100 |
| Vitamin B6 (pyridoxine) | 0.47 mg | 33 | 1.4 mg | 100 |
| Vitamin B9 (folic acid) | 66.7 μg | 33 | 200 μg | 100 |
| Vitamin B12 (cobalamin) | 0.83 μg | 33 | 2.5 μg | 100 |
| Vitamin C (ascorbic acid) | 26.7 mg | 33 | 80 mg | 100 |
| Vitamin E (D-α-tocopherol) | 4 mg α-TE | 33 | 12 mg α-TE | 100 |
| Essential trace elements | | | | |
| Zinc | 1.66 mg | 16.66 | 5 mg | 50 |
| Copper | 0.16 mg | 16.66 | 0.5 mg | 50 |
| Selenium | 9.16 μg | 16.66 | 27.5 μg | 50 |
| Manganese | 0.33 mg | 16.66 | 1 mg | 50 |
| Other components | | | | |
| Lutein | 3 mg | — | 9 mg | — |
| Zeaxanthin | 0.3 mg | — | 0.9 mg | — |
| Glutathione | 2 mg | — | 6 mg | — |

TG-DHA, triglyceride-bound DHA; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; NE, niacin equivalent; TE, tocopherol equivalent.

levels of glycosylated hemoglobin (HbA1c) as an indicator of metabolic control, plasma total antioxidant capacity (TAC) as biochemical marker of oxidative stress, and the FAs profile on the erythrocyte membrane (ω-6 arachidonic acid, ω-3 DHA, and ω-6/ω-3 ratio) as bioavailability of the oral DHA supplementation. Serum HbA1c, plasma TAC and erythrocyte membrane ω-6 arachidonic acid, ω-3 DHA, and ω-6/ω-3 ratio were measured at baseline and at 12 months and 24 months.

Total antioxidant capacity in plasma samples was measured using the OxiSelect Total Capacity Assay kit (STA-360; Cell Biolabs Inc, San Diego, CA) following the manufacturer's protocol. Uric acid equivalent was used to calculate copper-reducing equivalent values (μM copper-reducing equivalent). The composition of FAs was determined using the method described by Lepage and Roy,[32] analyzed by gas chromatography–mass spectrometry, and identified by comparing the elution pattern and relative retention times of FA methyl esters with a reference FA methyl esters mixture (GLC-744 Nu-Check Prep. Inc, Elysian, MN). The results were expressed in relative amounts (% of total FA).

### *Statistical Analysis*

The sample size was calculated for the average difference between groups in CSMT. The significance level was set to 5% and the power to 80%. An expected mean difference of 75 $\mu$m was used assuming an SD of 135 $\mu$m.[33] A sample of 40 evaluable subjects per treatment group was required. Because the primary analysis was based on the per-protocol population, a drop-out rate of 10% was assumed. Therefore, 44 patients per treatment group were required, with a total of 88 patients. In this study, the unit of analysis was the eye for CSMT and BCVA; however, the unit of analysis was the patient when results of BCVA were stratified by ETDRS letter gains, no change, or loss. In this case, for the 14 patients with both eyes affected, the analysis was performed considering 1 eye per patient, both for the eye with the worst outcome and for the eye with the best outcome. Categorical data are expressed as frequencies and percentages and continuous data as mean ± SD and 95% confidence interval (CI). The chi-square ($\chi^2$) test or the Fisher's exact tests were used for the comparison of categorical variables between the study groups. The relationship between the study variables and the BCVA at 24 months was assessed with the Pearson's product–moment correlation coefficient. Mixed linear model analysis was used to assess differences in BCVA, CSMT, and HbA1c levels between the study groups throughout the 24-month study period (covariate: baseline BCVA, CSMT, or HbA1c;

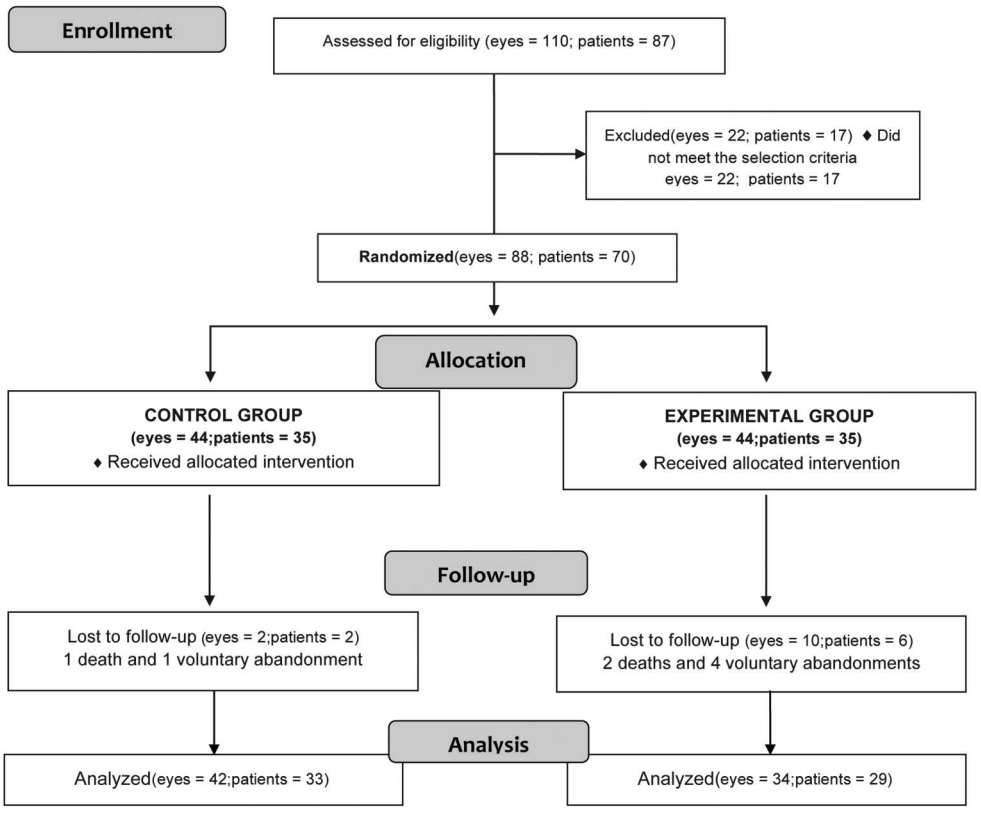


**Fig. 1.** Flowchart of the study patients (and eyes).

random factor: patients). Missing data during evaluation were calculated using the mean between the anterior and posterior measured values. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with the Statistical Package for the Social Sciences, version 11.0 software (SPSS Inc, Chicago, IL).

## Results

A flowchart of the distribution of patients and eyes during the phases of recruitment, randomization, follow-up, and analysis is shown in Figure 1. Of a total of 88 eyes (control group 44, DHA supplementation group 44; 70 patients) initially included in the study, 12 eyes did not finish the 2-year follow-up period. Therefore, 76 eyes (control group 42, DHA supplementation group 34; 62 patients) were finally included and followed over 24 months. Three patients died because of unrelated causes and the remaining 5 were patients being lost during the follow-up or because of voluntary abandonment. There were 41 men and 21 women, with a median age of 67 years (range 52–82 years). Eighty-four percent of patients had diabetes for more than 10 years (range 5–25 years). The characteristics of patients in the two study groups were very similar. Metabolic control HbA1c level of 7.6% (range 6–12.3%) was 7.7% in the DHA supplementation group and 7.5% in the control group.

In the DHA supplementation group, the mean CSMT at baseline of 445 ± 100 $\mu$m (95% CI 428–462) decreased to 302 ± 64 $\mu$m (95% CI 269–335) at 24 months ($P < 0.001$). In controls, the mean CSMT at baseline was 449 ± 109 $\mu$m (95% CI 432–446) and 354 ± 112 $\mu$m (95% CI 323–385) at 24 months ($P < 0.024$). The difference between groups at 24 months was statistically significant (95% CI 7.20–97.656; $P = 0.024$). As shown in Figure 2A, this difference was already evident at the first month after treatment.

At 24 months, the gain of ETDRS letters in the DHA supplementation group was 12.0 ± 5.9 (95% CI 9.0–15.0). The gain of ETDRS letters in the control group was 8.3 ± 9.9 (95% CI 5.4–11.2). At 24 months, the 95% CI of the differences in BCVA measured in ETDRS letters between groups was −0.22 to 7.09 ($P < 0.066$) (Figure 2B).

When the worse seeing eye was considered, there were not significant differences for 5 or 10 EDTRS letters gain at 12 months between the DHA supplementation and control groups (>5 letters 82.7 vs. 71.9%, $P = 0.312$; >10 letters 55.1 vs. 40.6%, $P = 0.255$), although at 24 months significant differences in favor of the DHA supplementation group were

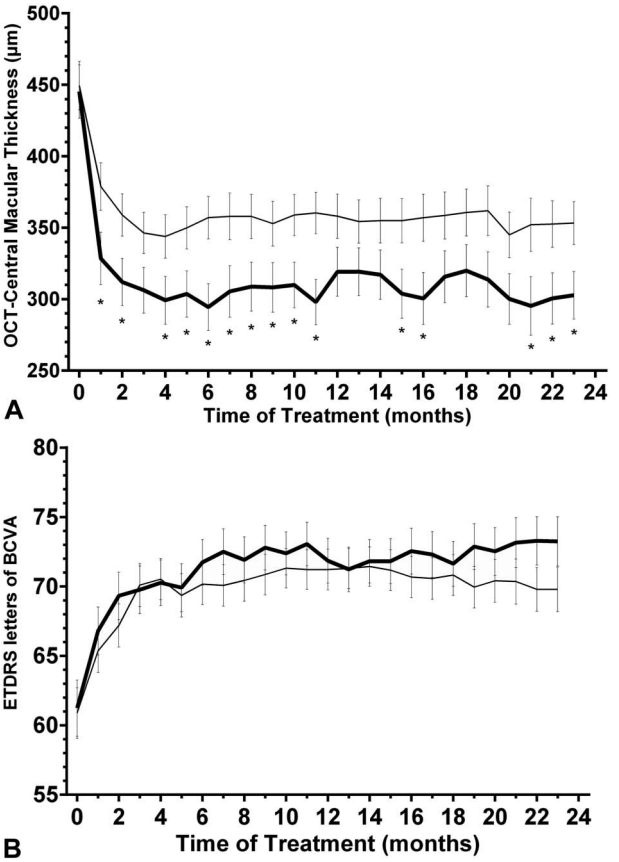


**Fig. 2. A.** Changes of CSMT from baseline to 24 months after treatment with DHA supplementation as compared with controls (asterisk indicates statistically significant differences $P < 0.05$) (DHA supplementation group: Black line; control group: Gray line). **B.** Changes in ETDRS-BCVA letters in the DHA supplementation group and in controls (DHA supplementation group: Black line; control group: Gray line). At all time points, the number of eyes analyzed was 34 (29 patients) in the supplementation group and 42 eyes (33 patients) in the control group.

observed for gain of >5 letters (81.4 vs. 56.7%, $P = 0.044$) but not for gain of >10 letters (55.5 vs. 33.3%, $P = 0.091$) (Table 2). When the better seeing eye was analyzed, similar results were obtained for 5 and 10 EDTRS letters gains at 12 months (>5 letters 89.7% vs. 81.3%, $P = 0.573$). However, at 24 months, the percentage of patients with >10 letters gains was significantly higher in the DHA supplementation group (66.7 vs. 40.0%, $P = 0.044$), although the percentages for 5 letters gains were similar (88.9 vs. 73.3%, $P = 0.137$) (Table 2). Other comparisons of EDTRS letters changes at 12 and 24 months between the study groups were not statistically significant. There was a significant relationship between BCVA at the initiation of treatment and BCVA at 24 months ($r = 0.711$, $P < 0.001$). Also, there was a weak correlation between initial CSMT and final BCVA ($r = 0.26$, $P = 0.027$).

The mean number of intravitreal injections was 6.6 ± 2.1 (95% CI 6.2–7.0) during the first year and

Table 2. Differences in BCVA Between the Study Groups at 12 Months and 24 Months

| ETDRS Letters of BCVA | 12 Months | | | 24 Months | | |
|---|---|---|---|---|---|---|
| | Intervention % (Number), N = 29 | Controls % (Number), N = 32 | *P* | Intervention % (Number), N = 27 | Controls % (Number), N = 30 | *P* |
| Results considering the worse seeing study eye* | | | | | | |
| Gain | | | | | | |
| >5 letters | 82.7 (24) | 71.9 (23) | 0.312 | 81.4 (22) | 56.7 (17) | 0.044 |
| >10 letters | 55.1 (16) | 40.6 (13) | 0.255 | 55.5 (15) | 33.3 (10) | 0.091 |
| >15 letters | 24.1 (7) | 25.6 (5) | 0.403 | 11.1 (3) | 16.7 (5) | 0.546 |
| No change (−5, +5 letters) | 17.2 (5) | 25.0 (8) | 0.459 | 18.5 (5) | 33.3 (10) | 0.204 |
| Loss | | | | | | |
| >5 letters | 0 (0) | 3.1 (1) | 0.96 | 0 (0) | 9.9 (3) | 0.273 |
| >10 letters | 0 (0) | 3.1 (1) | 0.96 | 0 (0) | 6.6 (2) | 0.518 |
| >15 letters | 0 (0) | 3.1 (1) | 0.96 | 0 (0) | 3.1 (1) | 0.957 |
| Results considering the better seeing study eye* | | | | | | |
| Gain | | | | | | |
| >5 letters | 89.7 (26) | 81.3 (26) | 0.573 | 88.9 (24) | 73.3 (22) | 0.137 |
| >10 letters | 58.6 (17) | 56.2 (18) | 0.851 | 66.7 (18) | 40.0 (12) | 0.044 |
| >15 letters | 27.5 (8) | 21.9 (7) | 0.604 | 22.2 (6) | 23.3 (7) | 0.920 |
| No change (−5, +5 letters) | 10.3 (3) | 15.6 (5) | 0.817 | 11.1 | 20.0 (6) | 0.578 |
| Loss | | | | | | |
| >5 letters | 0 (0) | 3.1 (1) | 0.946 | 0 (0) | 6.7 (2) | 0.518 |
| >10 letters | 0 (0) | 0 (0) | 1 | 0 (0) | 3.3 (1) | 0.957 |
| >15 letters | 0 (0) | 0 (0) | 1 | 0 (0) | 0 (0) | 1 |

Intervention: intravitreal ranibizumab plus DHA supplementation; controls: intravitreal ranibizumab alone.
*Worst and best outcome for individual eyes of the study patients including those patients with bilateral DME (n=14).

1.3 ± 1.5 (95% CI 1.0–1.6) during the second year. However, a total of 50.6% of patients did not require intravitreal ranibizumab treatment during the second year. As shown in Table 3, there were no statistically significant differences in the number of intravitreal injections at 12 months, between 12 months and 24 months, and at 24 months between patients in the DHA supplementation group and controls. At 12 months and 24 months, the 95% CIs for the difference in the mean number of injections were −0.37 to 1.36 and −0.66 to 2.86, respectively. For initial CSMT of <450 $\mu$m compared with >450 $\mu$m, the expected number of injections would be 6.5 vs. 7.5 ($P < 0.004$) at 12 months, 6.9 vs. 9.4 ($P < 0.0001$) at 18 months, and 7.7 vs. 10.6 ($P < 0.003$) at 24 months. However, the mean number of intravitreal injections of ranibizumab was unrelated to either HbA1c levels <7% or >7% (6.74 ± 2.25 vs. 6.54 ± 1.99) or the presence or absence of DHA supplementation (6.91 ± 2.36 vs. 6.74 ± 1.85).

In relation to serum levels of HbA1c, differences between the study groups were not observed, but a trend toward a better metabolic control of the supplemented group was found. However, the magnitude of increases in TAC levels was higher in the DHA supplementation group, although increases in TAC levels at the end of the study as compared with

Table 3. Number of Intravitreal Injections by Treatment Group

| Study Period | Study Groups | | |
|---|---|---|---|
| | Intravitreal Ranibizumab Plus DHA Supplementation | Intravitreal Ranibizumab Alone (Controls) | |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | *P* |
| At 12 months | 6.9 ± 2.4 (6.5–7.3) | 6.5 ± 1.8 (6.2–6.8) | 0.37 |
| Between 12 and 24 months | 2.7 ± 2.7 (2.3–3.1) | 1.8 ± 2.8 (1.4–2.2) | 0.14 |
| At 24 months | 9.6 ± 4.3 (8.9–10.3) | 8.3 ± 4.0 (7.7–8.9) | 0.16 |

pretreatment values were statistically significant in both study groups (Figure 3).

In relation to the FAs profile on the erythrocyte membrane, a significant reduction in the erythrocyte membrane content of ω-6 arachidonic acid at 12 months and 24 months as compared with baseline in the DHA supplementation group, as well as a significant decrease at 24 months as compared with baseline in controls was observed. Between-group differences were statistically significant. In relation to ω-3 DHA (Figure 4A), increases at 12 months and 24 months were only significant in the DHA supplementation group. In this case, between-group differences were also statistically significant. A similar pattern for ω-6/ω-3 ratio was observed as shown in Figure 4B.

The mean (SD) values of all study variables at baseline and at 12 months and 24 months are shown in Table 4.

Intravitreal injections of ranibizumab were well tolerated. Minor adverse events included subconjunctival hemorrhage and cataracts, probably related to the length of the study. One case of endophthalmitis in 1 eye due to *Enterococcus faecalis* was recorded in a bilaterally treated patient. Three patients died for unrelated causes. Other nonocular systemic adverse events were stroke in 4 patients and acute myocardial infarction, dizziness, and gastrointestinal discomfort in 1 patient each.

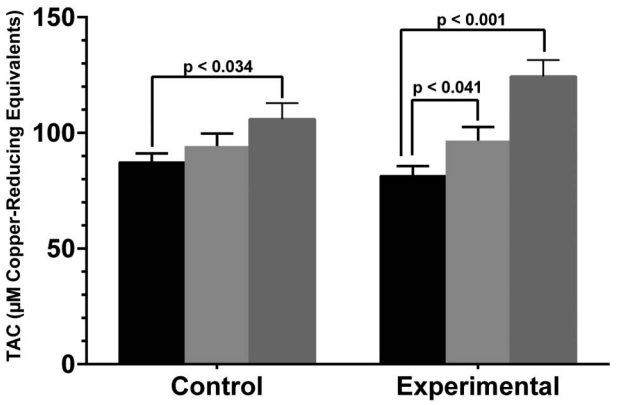


**Fig. 3.** Increases in TAC in the two study groups. (Basal: Black bar; 12 months: Gray bar; 24 months: Dark gray bar).

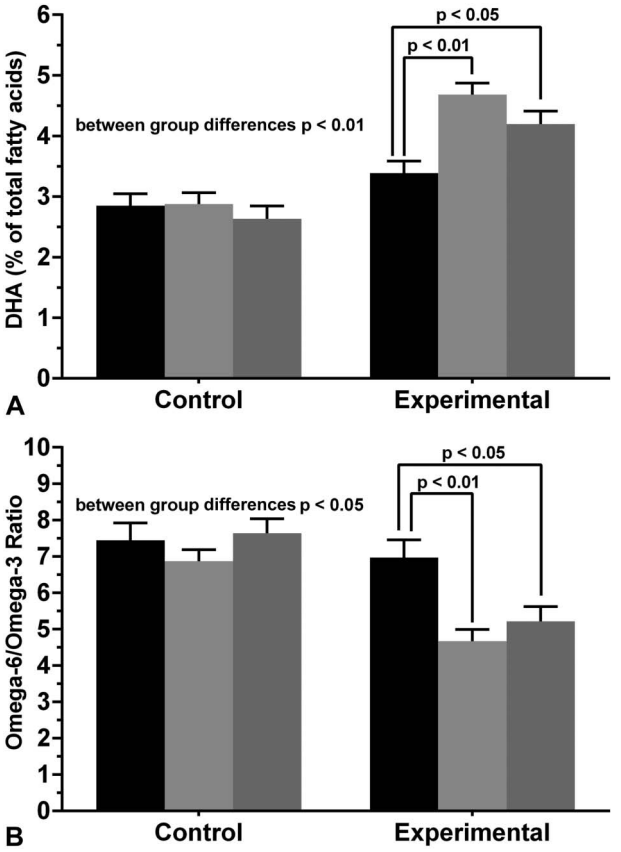


**Fig. 4. A.** Increases at 12 months and 24 months in DHA content on the erythrocyte membrane as compared with baseline in the DHA supplementation group and in controls. **B.** Reduction in the ω-3/ω-6 ratio at 12 months and 24 months as compared with baseline in the two study groups. (Basal: Black bar; 12 months: Gray bar; 24 months: Dark gray bar).

## Discussion

Results of the present randomized single-blind and controlled study performed in routine daily practice shows that oral supplementation with a nutraceutical formulation based on the combination of ω-3 FAs, mainly DHA, vitamins, and trace elements combined with intravitreal ranibizumab was associated with significant anatomical improvement in patients with DME. Reductions of CSMT were maintained over a 24-month follow-up period and were already evident at 1 month, shortly after starting oral nutraceutical formulation intake. Also, there was a trend of amelioration of BCVA of higher magnitude in the supplemented group but differences as compared with controls did not reach statistical significance. It is possible that functional improvement would need more time to become evident, and in this respect, anatomical improvement (reduction of CSMT) may precede visual acuity gains.

Docosahexaenoic acid has an inhibitory effect on the activation of NF-κB, which is responsible for the synthesis of inflammatory cytokines and intracellular and vascular adhesion factors as well as the synthesis of metalloproteinases and VEGF, a crucial proangiogenic factor driving retinal neovascularization. These effects already shown in animal models of type 2 diabetes[14,16,34] would justify DHA supplementation in humans in an attempt to reduce retinal inflammation (edema) and to protect against oxidative stress.[18] As

Table 4. Changes of Study Variables in the Experimental Group (Intravitreal Ranibizumab Plus DHA Supplementation) and in the Control Group (Intravitreal Ranibizumab)

| Variables | Baseline | 12 Months | 24 Months |
|---|---|---|---|
| Visual acuity, EDTRS letters, no. | | | |
| Experimental group | 31.23 (10.47) | 41.85 (8.82) | 43.25 (9.44) |
| Control group | 30.88 (12.08) | 41.22 (9.24) | 39.79 (10.47) |
| CSMT, $\mu$m | | | |
| Experimental group | 445.26 (100.50) | 319.11 (69.83) | 302.72 (64.81) |
| Control group | 449.38 (100.67) | 358.0 (118.10) | 354.20 (112.46) |
| Serum HbA1c | | | |
| Experimental group | 7.67 (1.32) | 7.18 (0.88) | 7.14 (0.80) |
| Control group | 7.56 (1.36) | 7.49 (1.33) | 7.44 (1.08) |
| TAC, $\mu$M CRE | | | |
| Experimental group | 80.64 (20.39) | 97.39 (26.11) | 124.37 (40.17) |
| Control group | 87.49 (22.52) | 96.06 (34.06) | 105.89 (28.78) |
| ω-3 DHA, % total FAs | | | |
| Experimental group | 3.39 (1.01) | 4.68 (1.09) | 4.20 (1.34) |
| Control group | 2.85 (0.94) | 2.88 (0.73) | 2.63 (0.66) |
| ω-6 arachidonic acid, % total FAs | | | |
| Experimental group | 6.97 (2.92) | 4.67 (1.34) | 5.21 (1.83) |
| Control group | 7.44 (2.49) | 6.87 (1.79) | 7.63 (2.11) |

Data expressed as mean and SD in parenthesis.
CRE, copper-reducing equivalent; TAC, total antioxidant capacity; FA, fatty acids.

far as we are aware, the combined effect of oral supplementation with high-rich ω-3 PUFAs and intravitreal ranibizumab in diabetic patients with DME has not been previously assessed. In a previous study, ω-3 supplementation combined with anti-VEGF treatment with bevacizumab was associated with decreased intravitreal VEGF-A levels in patients with exudative age-related macular degeneration.[34]

In relation to the effectiveness of intravitreal administration of ranibizumab in patients with DME, our results in routine clinical practice are consistent with data reported in clinical trials.[9,10,33,35] In the RESTORE extension study,[36] in which long-term efficacy and safety profile during 3 years of individualized ranibizumab treatment in patients with DME were evaluated, ranibizumab was effective in improving and maintaining BCVA and central retinal subfield thickness outcomes, with a progressive declining number of injections. The mean gain letters was 8.0 in the previous ranibizumab (0.5 mg) group, 6.7 in the previous ranibizumab (0.5 mg) plus laser, and 6.0 in the previous laser group. In all cases, BCVA was lower than the mean gain of 10.31 ETDRS letters found at 24 months in our study. Although our results were somewhat better, statistically significant differences were not found. At 36 months, the percentages of patients in the RESTORE extension study with BCVA gains of ≥5 letters were 66.3% and 65.1% in the previous ranibizumab 0.5 mg group and the previous ranibizumab 0.5 mg group plus laser, respectively. The corresponding figures for BCVA gains of ≥ 10 letters were 47% and 44.6%, and of ≥15 letters 27.7% and 30.1%, respectively. These data are similar to those found among controls in our study, with higher percentages for the gains of >5 and >10 letters in the DHA supplementation group (81.4 and 55.5% for the eyes with the worst outcome, and 88.9 and 66.7% for the eyes with the best outcome). Our results of reduction of CSMT in the DHA supplementation group at 24 months (145 m$\mu$) are similar to 142.1 m$\mu$ and 145.9 m$\mu$ in the ranibizumab groups at 36 months reported in the RESTORE study.[36] In relation to the number of injections,[34] patients treated with ranibizumab received a mean of 3.7 injections between Months 12 and 23 and a mean of 2.7 injections between Months 24 and 35, which is somewhat higher than a mean of 1.3 (SD 1.5) injections during the second year in our study. This difference may be explained by less strict criteria for retreatment in relation to central macular thickness used in our population as compared with the RESTORE study.[36] The safety profile reported in the RESTORE extension study[36] is similar to our findings, with eye pain and cataract as the most frequent ocular adverse events. However, no cases of endophthalmitis were observed.[32]

In the RISE and RIDE studies,[37] the percentages of patients randomized to ranibizumab 0.3 mg and 0.5 mg with a gain of ≥15 ETDRS letters from baseline at 24 months were 44.8% and 39.2%, respectively, in the RISE study, and 33.6% and 45.7%, respectively

in the RIDE study. These percentages are higher than those found in our study and may be explained by a higher number of injections received by the patients of these trials (median 24 injections). However, we also observed an early improvement of visual acuity during the first month after starting treatment with ranibizumab. In these studies, statistically significant changes versus sham were observed as early as 7 days after the first injection. These strong gains in visual acuity achieved with ranibizumab at Month 24 were sustained through Month 36.[12]

The present results should be interpreted taking into account some limitations of the study, especially the single-blind design and the relatively few patients included in the study groups. Although OCT measurements are objective, vision is an important outcome measure in DME especially because CSMT is not correlated with visual function. In our study, visual acuity was not the primary outcome but was included as one of the outcomes together with CSMT, HbA1c, TAC, and erythrocyte membrane ω-6 arachidonic acid, ω-3 DHA, and ω-6/ω-3 ratio. However, the results obtained were comparable with those reported in clinical trials[9,36,37] and, therefore, the use of intravitreal ranibizumab in patients with DME is extensively applicable to daily clinical practice. Although in the protocol of ETDRS optotypes include to sum 30 letters to all patients who see the 9 letters at 2 m or 4 m, results recorded in the case report form were obtained directly from the optotypes, without adding the 30 letters established by the protocol. Accordingly, our visual acuity is similar to that of other studies and not as low as that resulting from this confusion. In this respect, the baseline visual acuity was 60.8 ETDRS letters in the control group and 61.2 ETDRS letters in the DHA supplementation group, which corresponded to 20/63 in the Snellen chart and, therefore, similar to visual acuities of other randomized studies.[9] Although compliance with the nutraceutical formulation was checked at each monthly study visits by asking the patient to bring the empty box and by telephone calls twice a month made by a member of the manufacturer company of DHA to recall about the importance of an adequate daily dosing, the lack of control over dietary intake of the subjects was among the limitations of the study. However, the significant increase in the erythrocyte membrane content of DHA shown in the study is a reliable indirect indicator of good adherence in the supplemented group.

In conclusion, in patients with DME treated with intravitreal ranibizumab, the addition of a dietary supplement rich in DHA plus antioxidant vitamins, minerals, and xanthophylls reduced CSMT after 2 years of follow-up as compared with intravitreal ranibizumab alone. This anatomical improvement was accompanied by a trend for an amelioration of BCVA. A double-masked randomized trial of larger sample size powered for vision should be conducted to assess the influence of DHA supplementation associated with anti-VEGF therapy on functional visual outcomes in DME.

**Key words:** diabetic macular edema, docosahexaenoic acid, essential fatty acids, intravitreal ranibizumab, omega-3 polyunsaturated fatty acids, nutraceutics, antioxidative treatment.

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