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The Effect of Docosahexaenoic Acid (DHA) Supplementation on the Macular Function in Patients with Best Vitelliform Macular Dystrophy

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the

requirements for the degree of Master of Science

in

Medical Sciences - Ophthalmology

Edmonton, Alberta

Fall 2005

Abstract

Best vitelliform macular dystrophy (BVMD) is a slowly progressive macular dystrophy with typical juvenile onset. Docosahexaenoic acid (DHA) has been shown to provide benefit against the development of macular degeneration. We designed a double-masked, randomized, placebo-controlled, crossover clinical trial of eight patients affected by BVMD. DHA was given to the patients, and primary outcomes were: plasma DHA levels, visual acuity (ETDRS), VF-14 questionnaire, multifocal ERG (mfERG), and electro-oculogram (EOG). Our results showed all eight patients had increased plasma DHA levels during periods of DHA supplementation. Visual acuity, VF-14 scores, and mfERG amplitude benefits were all statistically insignificant. Increase in the EOG Arden ratio in the second crossover was statistically significant for the placebo treatment. A carryover effect of DHA supplementation was a confounding error. Our pilot trial demonstrated the safety of DHA supplementation, and an expanded trial is needed to examine the full effects of DHA supplementation on BVMD.

Acknowledgements

I would like to thank Dr. Ian MacDonald, my supervisor, for his generosity in allowing me to take part this special project. He has been a thoughtful and inspiring mentor for me during my studies, and without his guidance, I would not have been able to complete this research. I am forever indebted to him for the chance he took on me and the opportunity he gave me to accomplish my goals. Ian has shown me how to be a successful clinician scientist, scholar, and a gentleman. I would like to thank my second committee member, Dr. Marc Hébert, who was very kind and generous in providing the help and resources that I needed to be successful. He was always helpful in giving me advice to complete my work. My third committee member, Dr. Naweed Syed, has known me the past nine years and he has always been there for me. He has never said the word 'no' to me. Naweed provided the basic building blocks to make me become a competent and inspired scientist. I would also like to thank Dr. Yves Sauvé who was kind and gracious enough to chair my defense committee. There are others that I would like to thank including Peggy Kaminski and Iris Odynski in the Glaucoma Clinic who helped with conducting the electrophysiology studies; Christina Sereda and Dr. Dean Mah who helped with the mutation analysis; and Jacqueline Jumpsen, Dr. Y.K. Goh, and Dr. Tom Clandinin who helped with the DHA analysis. I would also like to thank Karr-Ming Lee for her support and help in the preparation of my thesis. She has inspired and motivated me to improve myself and the work that I do. Last but not least, I give the biggest thanks to my parents for their unconditional love and support. I owe my success to them. Thank you ever so much.

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Introduction

Docosahexaenoic acid (DHA) is a long-chained polyunsaturated fatty acid (LCPUFA). It is often referred to as an omega-3 fatty acid as it has a 22 carbon chain backbone, and six double bonds starting at the third carbon atom from the methyl end of the molecule (22:6n-3) (Salem Jr., 1999). DHA is considered an essential fatty acid because humans are incapable of producing it with basic building blocks in sufficient amounts. Plants and phytoplankton, however, are able to produce the precursors of DHA such as alpha-linoleic acid (ALA; 18:3n-3). Animals, including humans, are able to convert the alpha-linolenic acid to DHA, but the human diet is still the main source for DHA through the consumption of oily fish such as salmon, tuna, and mackerel.

The second class of polyunsaturated fatty acids is the omega-6 fatty acids. Linoleic acid (LA; 18:2n-6) is an omega-6 fatty acid found in seeds, oils, and animal products such as margarine. Like DHA, animals are able to elongate this precursor to 20 or 22 carbon chains including arachidonic acid (ARA; 20:4n-6). Humans have the enzymes necessary to convert the two classes of PUFA into longer chain molecules, but we cannot convert one class of PUFA to the other. The enzymes required to elongate the carbon chain (elongases) and desaturate the chains to produce double bonds (desaturases) metabolize both omega-3 and omega-6 PUFA, resulting in metabolic competition between the two PUFA classes. The abundance if not excessive supply of omega-6 PUFA in the Western diet may impair the formation of DHA from omega-3 PUFA, which leads to a relative imbalance of tissue lipids with possible metabolic consequences.

For the past two decades, numerous studies have investigated the role of PUFAs in cellular and physiological processes. DHA has been shown to exert its effects on synaptic transmission (Piomelli, 1994) and neurotransmitter function, notably on dopamine and serotonin (Delion et al., 1994; de la Pressa Owens and Innis, 1999; Zimmer et al., 2002). It also has effects on ion channels (Freedman et al., 1999; Leifert et al., 1999), signal transduction pathways (Litman et al., 2001; Mitchell et al., 2003), and gene expression (Kitajka et al., 2002; Rojas et al., 2003). Although these actions have been observed, the molecular and biochemical mechanisms of these effects are still unknown. Researchers have speculated that the fatty acids may influence the microenvironment of the membrane bilayers (Salem Jr. et al., 2001; Innis, 2003), which in turn modulates the activity of the membrane proteins (Litman et al., 2001), receptors (Mata de Urquiza et al., 2000), transport systems, and ion channels (Freedman et al., 1999; Leifert et al., 1999). Phospholipids and their fatty acids may act as precursors for eicosanoids, a group of hormone-like compounds such as prostaglandins, thromboxanes, and leukotrienes that regulate various cellular and physiological functions (Piomelli, 1994; Tapiero et al., 2002; Salem Jr. et al., 2001).

Given the importance that LCPUFAs have on cellular physiology, researchers examined whether these fatty acids have a role to play in physiological functions. Studies examining the health benefits and risks have been performed on both animal models and humans. Omega-3 fatty acids in fish oil are protective against coronary vascular disease (CVD) by lowering serum triglycerides, reducing the occurrence of arrhythmia and acting as an antiatherogenic and antithrombotic agent (Djousse et al.,

2003; Pauletto et al., 1996; Kang and Leaf, 1996). High doses of the fatty acids can reduce blood pressure in mildly hypertensive individuals (Knapp and Fitzgerald, 1989; Engler et al., 2003), and reduce inflammatory disease especially those suffering from autoimmune disorders such as rheumatoid arthritis (Simopoulos, 2002).

The nervous system's ability to persistently retain a high level of DHA suggests that there are functional consequences of neural DHA loss (Salem Jr. et al., 2001). During fetal development, DHA is incorporated into the phospholipids membranes of the retina and brain and continue to accumulate in the first two years of life (Connor et al., 1992; Clandinin et al., 1980). DHA is the major polyunsaturated fatty acid in the outer segments of the retina rods and cones. It constitutes up to 50% of the fatty acids in phosphatidylethanolamine (PE) and phosphatidylserine (PS), and as much as 80% of all polyunsaturated fatty acids in these photoreceptors (Giusto et al., 2000; Sastry, 1985). The phospholipids of brain gray matter contain high proportions of DHA in PE and PS forms as well as high amounts of ARA in the form of phosphatidylinositol (PI). The characteristic lipid composition in the retina and brain suggests that specific mechanisms are present to allow for the accumulation of LCPUFAs, and this accumulation of DHA serves to function in visual and neural processes (Innis, 2003).

Dietary deficiency of LCPUFAs has been examined in animal models. The studies usually involve a two-generation diet restriction in which the mother is raised on a LCPUFA-deficient diet and her offspring are then studied. This regimen causes a decline in brain and retinal DHA, and a decline of 50-80% is associated with a change

in neural function (Salem Jr. et al., 2001). Reduced amplitude in the electroretinogram (ERG) of both the a- and b-wave (Wheeler and Benolken, 1975), decreased brightness discrimination (Yamamoto et al., 1991), increased age of eye opening (Wainwright et al., 1991), and inhibited spatial task acquisition and memory (Moriguchi et al., 2000) have all been shown in rodent models of n-3 LCPUFA deficiency. Primate studies have shown a reduced visual acuity, longer implicit time in the ERG (Conner and Neuringer, 1984), and an impaired recovery of dark-adaptation (Neuringer et al., 1986) associated with LCPUFA deficiency.

In human infants, n-3 LCPUFA deficiency produces similar effects compared to those in animal studies. It has been found that formula-fed infants have decreased brain DHA when compared to those breast-fed (Farquharson et al., 1992; Makrides et al., 1994). Human milk contains more than 150 different fatty acids, and 15-20% of all fatty acids are composed of ALA, LA, ARA, and DHA (Innis, 2003). The amounts of DHA have been shown to vary depending on diet. Human milk from Western diets generally contain 0.1-0.5% DHA (Innis, 2003), whereas Asian diets from China and Japan contain 1-2.8% DHA owing to the higher consumption of seafood in these populations (Connor, 1995; Nakajima, 2000). Visual acuity was found to be significantly related to blood lipid DHA at two and twelve months but not four and six months of age among term breast-fed infants (Innis et al., 2001). In preterm infants, DHA supplementation to infant formula has shown to be beneficial in the ERG threshold and amplitude (Uauy et al., 1990; Birch et al., 1992a) and the visual evoked potential (VEP) (Birch et al., 1992b). Benefits of DHA supplementation in formula are

also extended to full-term infants. The VEP and mental development improved with DHA supplemented formula compared to formula alone (Birch et al., 1998, 2000). As a result, the government has approved infant formula supplementation with DHA.

Outside of the nervous system, DHA has also played a role in patients with cystic fibrosis (CF). The gene responsible for this condition is the cystic fibrosis transmembrane conductance regulator (CFTR), which encodes a protein that is an ATP-gated chloride channel regulated by cAMP-dependent protein kinase phosphorylation (Collins, 1992). Patients with CF have pulmonary insufficiency and recurrent pulmonary infections leading to pulmonary failure and premature death (Wilmott and Fiedler, 1994). Most patients also have pancreatic insufficiency and ileal hypertrophy. CF patients have elevated arachidonic acid (AA) in the phospholipid fraction from bronchial alveolar lavage fluid (Gilljam et al., 1986). The AA is an agonist of inflammatory processes and stimulus of mucus secretion. A mouse model of CF (cftr -/- mice) was shown to have lungs that are primed for inflammation through the increase in AA (Heeckeren et al., 1997). Essential fatty acid abnormalities have been observed in patients with CF and may have a fundamental role in the symptoms and progression of the disease (Strandvik, 1992; Farrell et al., 1985; Freedman et al., 2004). As DHA is a major competitor with AA for the enzymes of LCPUFA metabolism, it causes down-regulation of AA incorporation into the membrane phospholipids (Freedman et al., 1999). Researchers subsequently asked whether DHA supplementation has a role to play in CF. Freedman and researchers (1999) found a lipid imbalance of phospholipid-bound AA and DHA in ileum, pancreas, and lung

from cftr -/- mice. Oral administration of DHA to these mice corrected this lipid imbalance and reversed the observed pathology including blockage of *Pseudomonas* endotoxin-enhanced lung inflammation, and reversal of abnormal pancreatic cell morphology. An eight-month study of DHA supplementation in patients with CF was conducted, and results revealed a significant decrease in arachidonic acid level with a corresponding decrease in two inflammatory marker concentrations, serum immunoglobulin G and alpha-1 antitrypsin (De Vizia et al., 2003). Pulmonary function testing showed a significant improvement in the forced expiratory volume (FEV) and the number of days required to treat lung infections with antibiotics.

Given the positive effects of DHA in animal models and human patients with CF, there may be a role for DHA in other conditions that involve chloride channels or ionic channels in general.

Best vitelliform macular dystrophy is an inherited form of macular degeneration first described by Best (1905). Best disease is an autosomal dominant, slow progressive macular dystrophy with onset usually in early childhood and sometimes teenage years. Patients begin with normal vision and progress to decreased central acuity and metamorphopsia. Both the age of onset and severity of disease are variable (Godel et al., 1986; Fishman et al., 1993; Loewenstein et al., 1993). Patients retain normal peripheral vision and dark adaptation. The gene mutation for this disorder has been mapped to chromosome 11q13 through linkage analysis (Forsman et al., 1992; Stone et al., 1992; Hou et al., 1996). The isolated gene, VMD2 (vitelliform

macular dystrophy 2), encodes a protein that functions as a chloride channel (Sun et al., 2002).

The diagnosis of Best vitelliform macular dystrophy is based on fundus appearance, the electro-oculogram (EOG), and family history. On fundus examination, patients typically have a yellow yolk-like macular lesion. Retinal findings are not generally present at birth and do not typically appear until the age of five to ten years (MacDonald et al., 2003). Lesions are bilateral, but can appear unilaterally. Multiple lesions and lesions outside of the macula can occur in at least 25% of individuals, and non-foveal lesions without foveal involvement have been reported (Deutman, 1971; Mohler and Fine, 1981; Godel et al., 1986). The disease is classified into four clinical stages, but patients may not progress through each of these stages (Deutman, 1971; Miller et al., 1976; Mohler and Fine, 1981; Godel et al., 1986; Fishman et al., 1993; Marano et al., 2000). The genetic and environmental factors that determine the severity of disease are unknown. The stages of disease are classified in Table 1-1.

Table 1-1. Clinical staging of BVMD.

Stage	Characteristic	Fluorescein Angiogram
0	The macula appears normal and the EOG is abnormal	Normal
1	Retinal pigment epithelium (RPE) disruption in the macular region	Window defects
2	Vitelliform lesion – circular, well-circumscribed, yellow-opaque, homogenous yolk-like macular lesion	Marked hypofluorescence in the zone covered by the lesion
2a	Vitelliform contents become less homogenous to develop a "scrambled-egg" appearance	Partial blockage of fluorescence with non-homogeneous hyperfluorescence
3	Pseudohypopyon phase – lesion develops a fluid level of yellow-coloured vitelline substance	Inferior hypofluorescence from the blockage by the vitelline material with superior hyperfluorescent defects
4a	Orange-red lesion with atrophic RPE and visibility of the choroids	Hyperfluorescence without leakage is seen on FA
4b	Fibrous scarring of the macula	Hyperfluorescence without leakage
4c	Choroidal neovascularization with new vessels on the fibrous scar or appearance of subretinal hemorrhage can be seen	Hyperfluorescence from neovascularization and leakage

Patients in stage 0 or 1 will not change in their stage of disease for ten years and three quarters of them will have a visual acuity of 20/20. A large portion of patients with stage 2 or 3 disease will advance in stage within five to ten years, but the majority of patients will have a visual acuity of 20/40 or better. The majority of patients in stage 4 will remain unchanged over a five year period. Ten percent of stage 4a and 16% of 4b will progress to stage 4c. Ten percent of patients will have a visual

acuity of 20/20 and 19% of them will lose two lines or more in visual acuity over eight to ten years. Disease progression is naturally based on age. For patients age 40 or younger, 75% will have vision of 20/40 or better in their better eye and 66% will have vision of less than 20/40 in the worst eye. Patients at the age of 50 or older will have a visual acuity of 20/70 in their best eye, 50% of the time, and vision worse than 20/100 in the worse eye.

Electrophysiological studies can distinguish Best vitelliform macular dystrophy from other retinal diseases. The main diagnostic tool is the electro-oculogram (EOG). The EOG measures indirectly the standing potential of the eye (Arden et al., 1962). The potential is measured by taking the light peak to dark trough ratio or the Arden ratio. Normal subjects have an Arden ratio greater than 1.8, but this ratio is not absolute as it decreases with age after the fourth decade. In Best disease, the EOG is abnormal with a reduced Arden ratio almost always less than 1.5 and usually fall within the range between 1.0 and 1.3 (Cross and Bard, 1974; Blodi and Stone, 1990; Pinckers et al., 1996). The Arden ratio stays constant with age for these patients (Reeser et al., 1970; Pinckers et al., 1996). The full-field electroretinogram (ERG) is relatively normal with a reduced C-wave (Krill et al., 1966; Deutman, 1971; Rover and Bach, 1985; Fishman et al., 1993). Foveal ERG or multifocal ERG (mfERG) reveals reduced amplitudes centrally (Scholl et al., 2002; Palmowski et al., 2003). A new form of mfERG, scanning laser ophthalmoscope-evoked multifocal ERG (SLO-mfERG), is used for the topographic mapping of retinal function and reveals significantly reduced amplitudes in the central concentric region of patients

(Rudolph and Kalpadakis, 2003). Colour vision testing can be performed on Best patients as a significant portion of them has anomalous colour discrimination notably in the protan axis (Godel et al., 1986). Colour vision changes are non-specific and not diagnostic. Another diagnostic modality is optical coherence tomography, which provides a cross-sectional study of retinal anatomy (Pianta et al., 2003). Intermediate clinical stages of disease demonstrate splitting and elevation at the apical surface of the RPE from the photoreceptors with resultant neurosensory detachment of the retina at the macula. Scans of the atrophic clinical stage show thinning of the retina. Histopathologic studies using light and electron microscopy show abnormal accumulation of lipofuscin granules within the RPE throughout the macula and retina (Frangieh et al., 1982; Weingeist et al., 1982; O'Gorman et al., 1988). The accumulation of lipofuscin, waste products of photoreceptor turnover, causes degeneration of RPE cell, and the resultant cell debris and lipofuscin lifts the RPE and the neurosensory retina away from the choriocapillaris causing a deficient exchange of nutrients and wastes.

VMD2 is the only gene isolated to be associated with Best vitelliform macular dystrophy (Forsman et al., 1992 , Stone et al., 1992 , Hou et al., 1996 , Marquardt et al., 1998 , Petrukhin et al., 1998 , Allikmets et al., 1999 , Krämer et al., 2000 , White et al., 2000 , Seddon et al., 2001). Individuals with the disease in whom no mutations in VMD2 could be found have been reported. This result may be a consequence of a failure to detect large deletions and mutations in introns or untranslated 5' and 3' regions of the gene (Petrukhin et al., 1998 , Bakall et al., 1999 , Caldwell et al., 1999 ,

Krämer et al., 2000 , White et al., 2000). It may also be possible that mutations in other genes can cause a similar phenotype. Krämer and colleagues found a 96% mutation detection rate in patients with a positive family history compared to 69% in individuals with no family history of the disease. Mutational analysis for G383C (W93C) is available as it is the most common mutation in individuals who can trace their ancestry to a large Swedish kindred (Nordström and Barkman, 1976; Petrukhin et al., 1998). Best disease shows complete penetrance and variable expressivity. An individual may appear non-penetrant at an early age and then show signs later (Weber et al., 1994a; Weber et al., 1994b). Genotype-phenotype correlations, however, have not been correlated. A recent report by Eksandh and colleagues (2001) described a family with a V89A mutation in the VMD2 gene and a phenotype of late-onset visual failure (age 40-50 years). Patients homozygous for mutations in VMD2 have clinical and electrophysiological findings similar to those of heterozygotes (Nordström, 1980). Currently, there is no cure for Best vitelliform macular dystrophy.

The VMD2 gene is located on chromosome 11q13, and it consists of 11 exons encoding a 585 amino acid (aa) and 68 kDa protein, bestrophin. The hydropathy profile predicts between four and six candidate membrane spanning domains (Tsunenari et al., 2003). Sun and colleagues (2000) showed the existence of a new chloride channel family by using heterologous expression studies to demonstrate that human, *Drosophila*, and *C. elegans* bestrophin homologs form oligomeric chloride channels. By utilizing patch clamp techniques, bestrophin was shown to exhibit a distinct current-voltage relationship, chloride ion sensitivity, and dependence on

intracellular calcium (Sun et al., 2002). Further evidence came from Qu and colleagues (2004) identifying the permeability of bestrophin to anions and a unique amino acid at S79 that plays an important role in anion binding in the pore of the channel. Bestrophin is localized to the basolateral membrane of the RPE, and it has been shown to undergo dephosphorylation by a protein phosphatase (Marmorstein et al., 2000; Marmorstein et al., 2002). This finding suggests that bestrophin participates in a signal transduction pathway related to the modulation of the light peak on the bestrophin.

Given the evidence of the positive influence DHA has on patients with CF, a disease affecting a chloride channel, it is logical to determine whether it has an effect on patients with Best vitelliform macular dystrophy, also a disease affecting a chloride channel. We hypothesize that DHA supplementation will positively affect macular function in patients with BVMD. In order to test this hypothesis, we conducted a double-blinded, randomized, placebo-controlled clinical trial testing the effect of docosahexaenoic acid on the macular function of patients with Best vitelliform macular dystrophy.

Methods

Trial Design

This was a double-masked, randomized, placebo-controlled, crossover designed clinical trial of one cohort with eight patients from three families affected by Best vitelliform macular dystrophy due to mutations in the bestrophin gene. Participants were randomized to receive either oral DHA supplementation (equivalent of 20 mg/kg body weight/day) for a period of two months or placebo. The dose of DHA was tolerated without adverse effect in any of our patients, in the preliminary investigations. DHA has been accepted as "GRAS" (generally regarded as safe) by the US FDA and is freely available over the counter in Canada and marketed on the internet. After two months, subjects crossovered to either placebo or DHA in a traditional crossover design. Again the same cycle occurred following these two months, and by the end of six months, the patient then continued on the same regimen of pills for an additional two months to assess carryover effect. Two-month treatment periods were chosen based on previous studies that showed DHA deficiency could be fully recovered after eight weeks of DHA supplementation in both animal and human studies (Moriguchi et al., 2001, MacDonald et al., 2004). Table 2-1 details the path that two groups of patients undertook in this clinical trial.

Table 2-1. Treatment course over eight months for patients in Groups 1 and 2.

Group \ Month	0 – 2	2 – 4	4 - 6	6 – 8
1	A_1	B_2	A_3	A_4
2	B_1	A_2	B_3	B_4

A = DHA, B = Placebo

Five visits were required for the study. An initial 2 visits on separate days provided a baseline and assurance that the patient could undergo the mfERG) and four subsequent visits at 2, 4, 6 and 8 months. Written informed consent was obtained from participants before any procedures were performed. Clinical examinations occurred at the Regional Eye Centre of the Royal Alexandra Hospital, Edmonton. A comprehensive ophthalmologic examination occurred at each visit.

Sample population

Eight individuals (all male) from three families were recruited. Inclusion criteria included: 1) ability to understand and sign the informed consent (parents if younger than 18), 2) compliance with all the study procedures (age under 9 may preclude some patients), and 3) a genotype and phenotype consistent with Best vitelliform dystrophy. Exclusion criteria included: 1) participant could not satisfy the inclusion criteria, 2) baseline variability of the mfERG exceeded 25%, 3) baseline variability of the EOG exceeded 10%. Note: if the subject could not reliably perform the EOG test, the subject was still included in the study, but only if the mfERG was

shown to be reliable, and 4) pregnant women or lactating women who were planning to get pregnant in the next year.

Randomization and masking

Random numbers were selected to generate a sequence. Each sequential participant was given a number by the clinical trial coordinator. The coordinator assigned the appropriate pre-labelled bottle, which corresponded to the participant number. The masked pre-labelled bottle was provided to a dietician who monitored patient compliance and collect nutrient intake information. All clinic staff and participants were be masked to study treatment assignments. Any patient requesting unmasking was reported to the principal investigator who discussed the circumstances that led to the request and tried to mediate. If unmasking was required, it was reported and considered as an adverse event.

Outcome Variables

Primary:

The primary outcome variable of this study was the effect of DHA on the amplitudes and implicit times of the mfERG and flicker responses, and the change of the Arden ratio as measured by the EOG. The mfERG amplitudes and implicit times were averaged separately over rings 1-2 and rings 3-5 comprising the central five degrees (foveal) and the remaining 15 degrees visual field (parafovea), respectively. Replicate measures were obtained at each visit to ensure an adequate estimate of the variability within participants.

Secondary:

Secondary outcome measures were: 1) the subjective response of participants as measured with the VF14 questionnaire; 2) visual acuity as measured by ETDRS visual acuity testing; 3) plasma DHA during the period of supplementation measuring response to supplementation and an index of compliance.

Evaluation of Macular function

The mfERG and the EOG (Arden ratio) are abnormal in Best vitelliform dystrophy. In our protocol, the mfERG allows the simultaneous recording of 61 focal ERG responses originating from the central 20 degrees of the macula and reflects retinal function. The EOG tests the function of the RPE by examining the standing potential generated by the RPE cell layer. All electrodiagnostic protocols followed the guidelines provided by the International Society for Clinical Electrophysiology of Vision (ISCEV).

At each interval visit, the pupils were dilated and an mfERG (VERIS system) was performed using DTL electrodes to evaluate central cone retinal function. The amplitudes and latency of responses from the central 2 rings (0-5 degrees) of the mfERG was averaged and represented as the foveal responses. The responses from rings 3-5 (5-20 degrees) represented those peripheral to the fovea. The EOG was performed according to standard protocols and LKC UTAS 3000 acquisition software computed the Arden ratio.

DHA and placebo supplementation

Patients received oral supplementation of 20 mg/kg body weight of DHA (DOHASCO Martek BioSciences, Columbia MD). Placebo capsules contained corn

oil flavoured with 1% fish oil. Participants returned bottles with unused capsules at each study visit. MTI, Edmonton stored the drug at room temperature and dispensed DHA or placebo to individual participants.

VF14 questionnaire

Prior to beginning the nutritional supplementation and during the course of treatment, patients' perception of their vision was assessed with a VF14 questionnaire. The VF14 provides an index of 14 vision-dependent activities of daily living and has been validated for patients with macular degeneration (MacKenzie et al., 2002).

Statistical Analysis

The measurements occurred at baseline, 2 (A_1/B_1), 4 (A_2/B_2), 6 (A_3/B_3), and 8 (A_4/B_4) month time points (A = DHA; B = Placebo). With randomization, there were two opportunities to observe the effect of DHA supplementation. The two baseline measurements were used to monitor reliability of the measure in the patient being tested. For the mfERG, a normal intervisit variation of 25% was considered acceptable (represents approximately a 0.10 log unit variation). For the EOG ratio, up to 10% inter-visit variation was considered normal. If reproducibility could not be achieved in a particular patient for a particular test (with no technical explanation), the latter test was not included in the statistical analyses. All baseline measurements were averaged to form a total baseline response. The response from each eye was separated, and the responses from the fovea and the parafovea were separated for the mfERG.

For the first crossover, the outcomes while the patients were on DHA (A_1 and A_2) were compared to the outcomes from placebo (B_1 and B_2), collectively. The

analysis utilized the Wilcoxon Signed Ranks Test, which is a non-parametric test equivalent to the paired T-test. The paired t-test assumes that the (population) distribution of the differences has a normal distribution, but this assumption cannot be made as our trial consists of only eight patients. The second crossover was also analyzed using the Wilcoxon Signed Ranks Test with the data collected from patients on DHA (A_2 and A_3) and placebo (B_2 and B_3). A combined crossover analysis of the first and second crossover were employed the combination of DHA and placebo data of four groups (A_1 and B_1; A_2 and B_2; A_2 and B_3; A_3 and B_2). Again, the Wilcoxon Signed Ranks Test was utilized to analyze the combined crossover.

A correlation analysis was conducted to examine whether the primary outcomes were related to plasma DHA levels. The small number of patients in this clinical trial did not allow the assumption of a normal distribution to be made. Thus, the Pearson correlation could not be used, and the nonparametric method using Spearman's rank correlation was appropriate in this case. The Spearman's rank correlation was used to analyzed all the primary outcomes data with the corresponding plasma DHA data.

In our design of the clinical trial, the fourth treatment period, from month six to month eight, was utilized to examine the carryover effects of DHA, by having the patients continue on the same pill (DHA or placebo) as in the third treatment period. The carryover analysis relied on comparing the periods A_3 and A_4, B_3 and B_4, and B_1 and B_3. Comparing periods before and after DHA treatment allowed us to see carryover effects as well, which gave us the third analysis pairing. Results of the

plasma DHA concentration was used to assess carryover via the Wilcoxon Signed Ranks Test. The outcome variables for the first and second crossover were also assessed for carryover. In the first crossover, patients in Group 1 would have carryover effects in the second period after the DHA treatment in the first, compared to Group 2 who would not have carryover effect by taking placebo prior to DHA. For the second crossover, Group 2 would experience carryover effects, while Group 1 would have potentially decreased effect since the placebo period (month 4) will be influenced by DHA from the first period. The analysis used the Wilcoxon Signed Ranks Test.

Results

Table 3-1. Summary of patients' phenotype and genotype.

Patient Number	Age	Visual Acuity (ETDRS)	Stage Classification	Genotype
1	40	OD -0.10 OS 0.00	OD 2 OS 4a	G383C
2	64	OD -0.10 OS 0.10	OD 4a OS 4b	G383C
3	58	OD 0.80 OS 0.20	OD 4b OS 4a	G383C
4	39	OD -0.10 OS 0.20	OD 3 OS 3	Del901(GAT)
5	38	OD 0.80 OS -0.20	OD 4a OS 2	G383C
6	36	OD -0.20 OS 0.00	OD 2a OS 4a	G383C
7	26	OD -0.10 OS 0.20	OD 2 OS 4a	G383C
8	23	OD -0.30 OS -0.30	OD 2 OS 2	R218C

Individual Analysis

Patient #1

Table 3-2: Pattern of treatment for Patient #1.

Months	Treatment	Plasma DHA Level (μg/mL)
Baseline	-	37.96
Baseline – 2	DHA	23.57
2 – 4	Placebo	16.88
4 – 6	DHA	47.96
6 – 8	DHA	52.31

Measurements were taken at Baseline and at the end of 2, 4, 6, and 8 months.

Figure 3-1 shows the changes in the multifocal electroretinogram (mfERG) amplitudes of the right eye following different trial treatments. Plasma DHA levels were used as a monitor of the treatment type, treatment compliance, and treatment carry-over. In this patient, the average central rings amplitude decreased 22% during

the placebo treatment, and then increased back to baseline in the DHA treatment period. Similarly, during the placebo treatment period, the plasma DHA level decreased by 28% and increased by 184% in the DHA treatment period. For the average peripheral rings amplitude decreased 24% during the placebo treatment, and increased approximately 10% at the end of the trial when plasma DHA levels continue to increase into the second consecutive DHA treatment period.

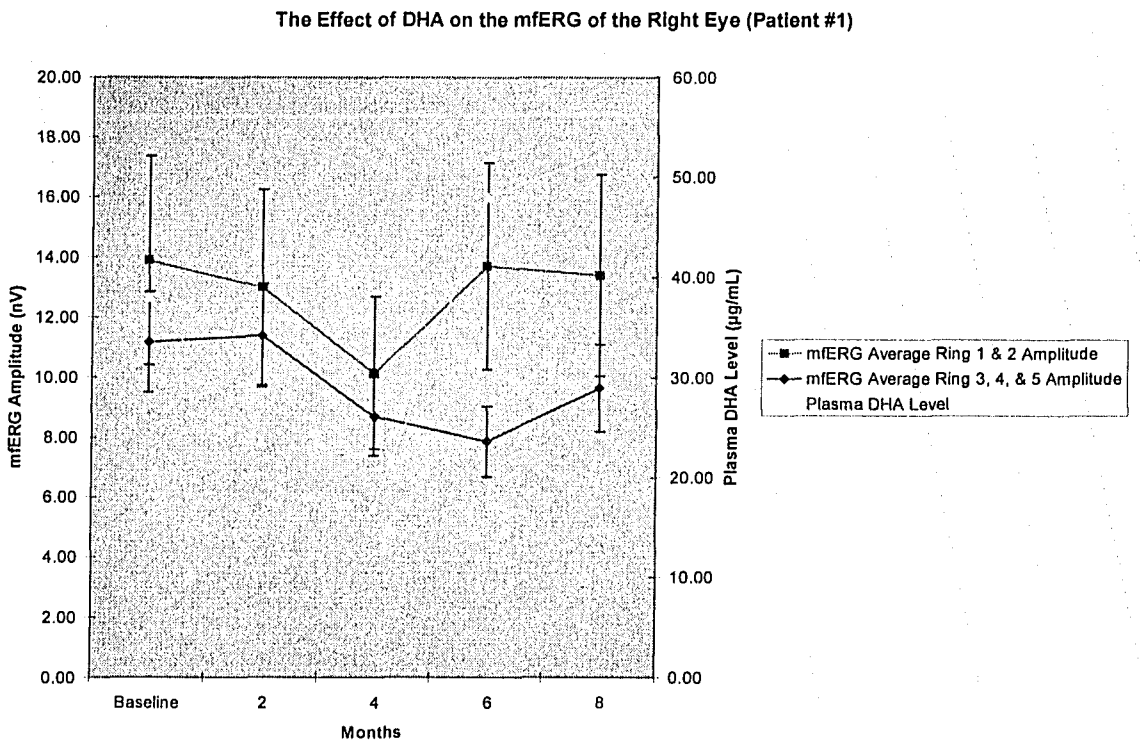

Figure 3-1: The effect of DHA on the mfERG amplitudes of the right eye. The average central rings (Rings 1 & 2) and average peripheral rings (Rings 3, 4, & 5) amplitudes are included.

The effect of DHA on the mfERG amplitudes of the left eye is shown in Figure 3-2. The average central rings amplitude decline steadily over the first three treatment periods with a cumulative 30% decreased before an increase of 52% with the peaking

of plasma DHA levels. The average peripheral rings amplitude declined after the first treatment period into the third treatment period (28% total decrease) before increasing in the last period (26%) with the increase in plasma DHA level.

The third graph shows no appreciable change in the electro-oculogram (EOG) with change in DHA (Right eye: OD; Left Eye: OS) (Figure 3-3). The Arden ratio for both eyes stayed relatively the same (within 10% between treatment periods) throughout the entire trial.

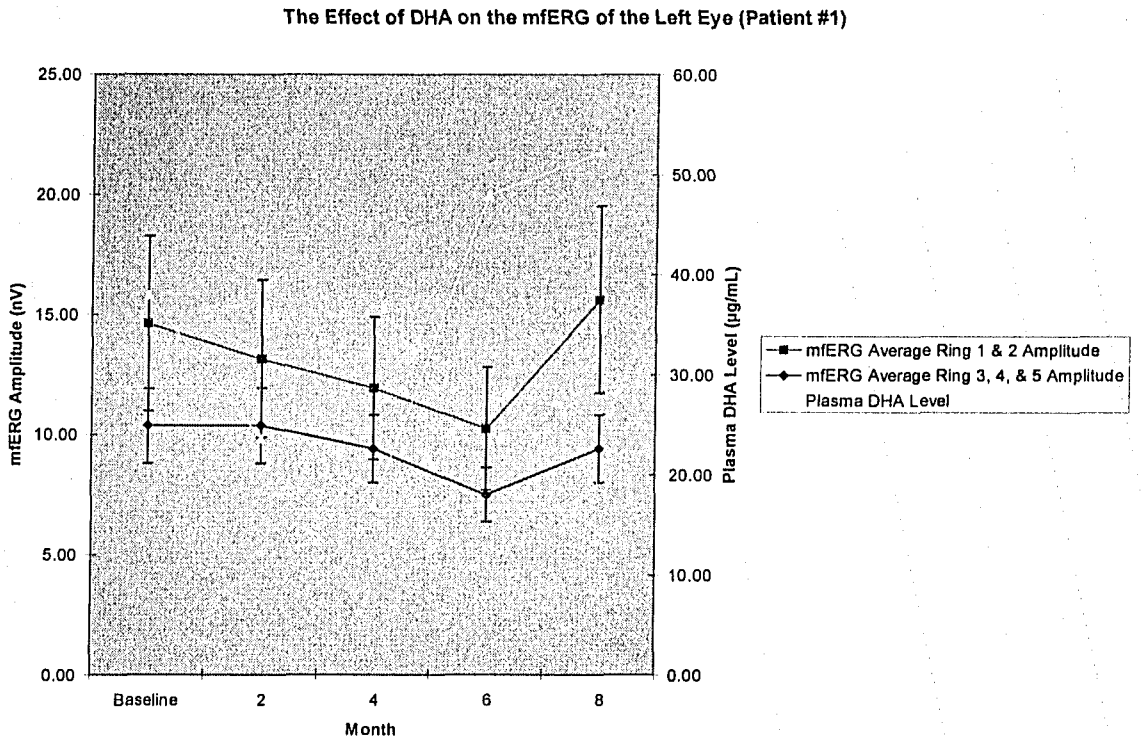

Figure 3-2: The effect of DHA on the mfERG amplitudes of the left eye. The average central rings (Rings 1 & 2) and average peripheral rings (Rings 3, 4, & 5) amplitudes are included.

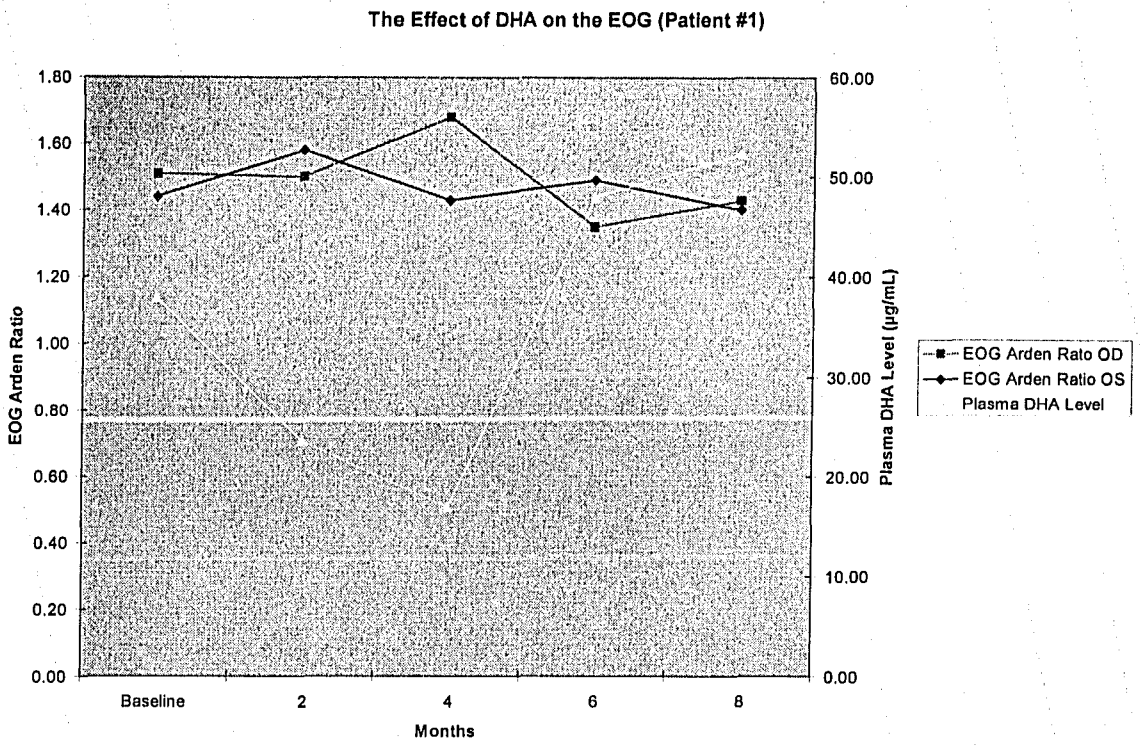

Figure 3-3: The effect of DHA on the electro-oculogram (Arden ratio) of the right and left eye.

The fourth graph looks at the VF-14 questionnaire score and the change in DHA (Figure 3-4). Patient #1 noticed a subjective change in visual acuity while on the DHA treatment in the third and fourth trial periods.

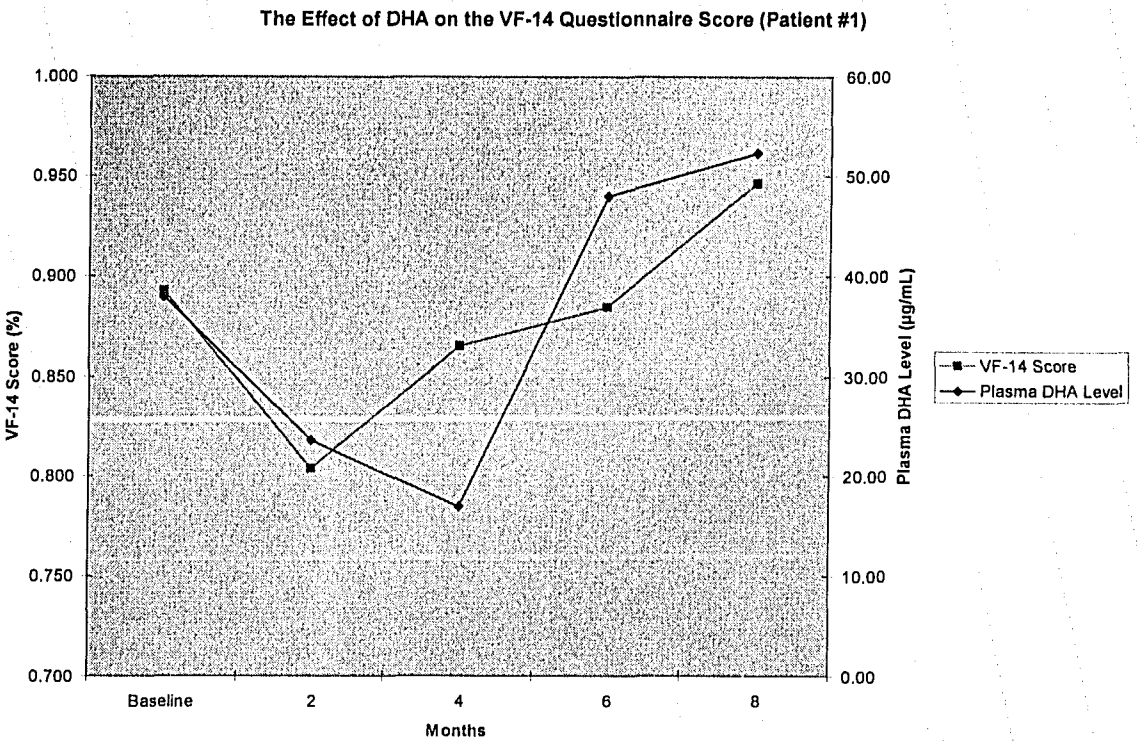

Figure 3-4: The effect of DHA on the VF-14 questionnaire score detailing the subjective measure of the patient's vision.

The last graph (Figure 3-5) for Patient #1 shows the effect of DHA on your visual acuity. A lower score on the ETDRS chart means better visual acuity. At the end of the trial and after the second period of DHA, the right eye had an improvement of two lines of visual acuity. The left eye returned to its baseline vision.

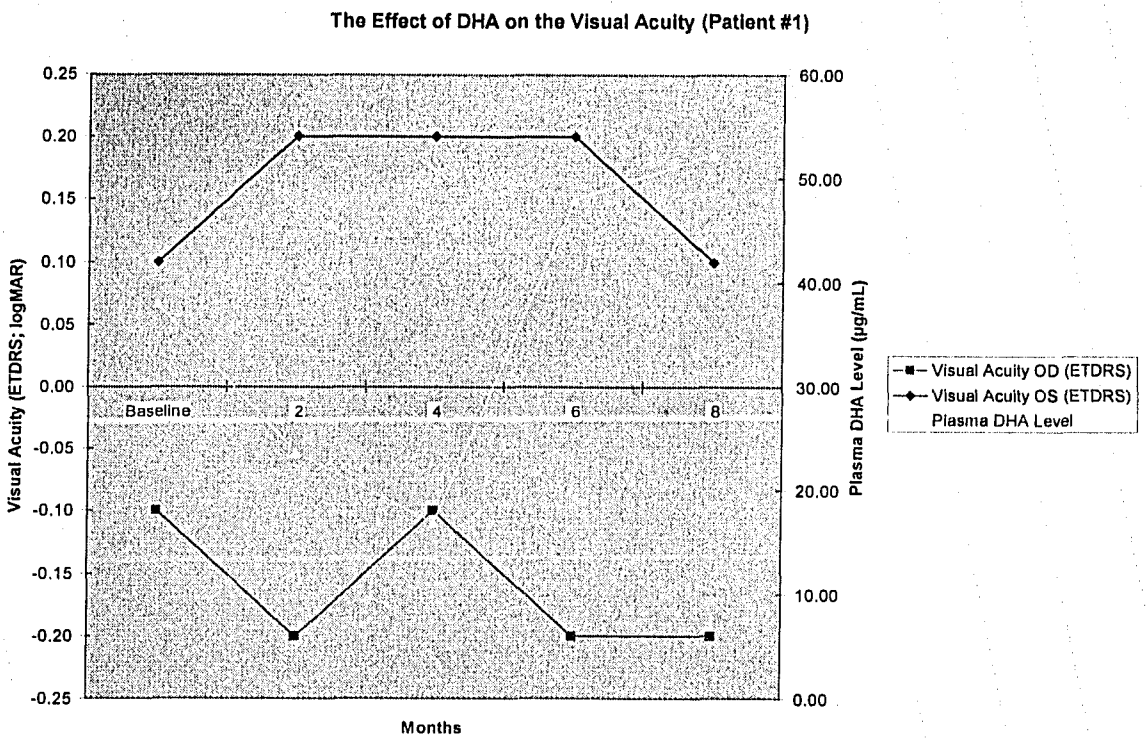

Figure 3-5: The effect of DHA on the visual acuity (ETDRS) of the patient.

Patient #2

Table 3-3. Pattern of treatment for Patient #2.

Months	Treatment	Plasma DHA Level (µg/mL)
Baseline	-	25.74
Baseline – 2	Placebo	13.17
2 – 4	DHA	59.53
4 – 6	Placebo	53.20
6 – 8	Placebo	36.03

Figure 3-6 shows the changes in average central and peripheral rings mfERG amplitudes of the right eye with alterations of plasma DHA levels. The central rings

amplitude decline by approximately 10% from the baseline value at the beginning of the trial, and returned to near baseline at the end of the trial. The amplitude did not increase or decrease in response to rise and fall of plasma DHA. This pattern was similar for the peripheral rings amplitudes.

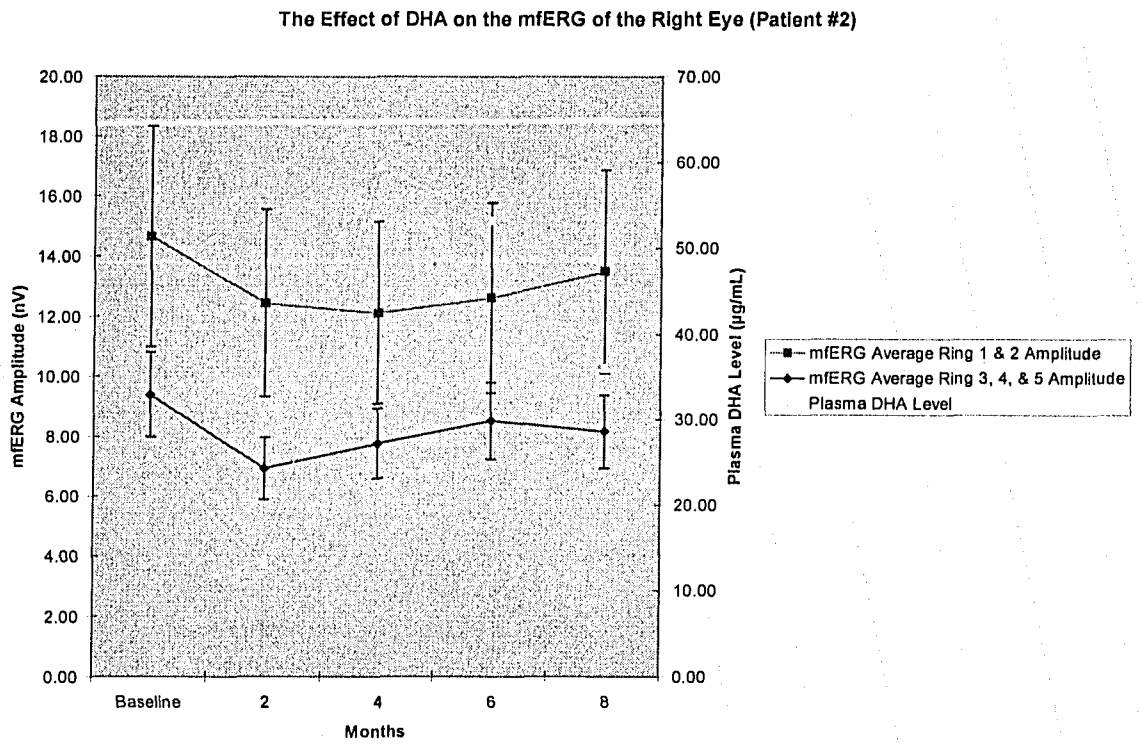

Figure 3-6: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the right eye.

It should be noted that the plasma DHA level detailed in every graph for Patient #2 showed the expected elevation of plasma DHA during the DHA treatment period, but the plasma level continued to be elevated two months later with the switch to the placebo treatment. Also, the plasma DHA level remained elevated from the

baseline value in the four months post DHA treatment. This represented a significant carry-over effect of DHA consumption.

In the left eye, the mfERG amplitudes for both the central and peripheral rings decreased (10 to 20%) in the first and third treatment periods (placebo), and increased (approximately 60% from the low of the placebo period) in the lone DHA treatment period (Figure 3-7). The last treatment period also had a rise in both central and peripheral amplitudes, but with a decrease in plasma DHA level.

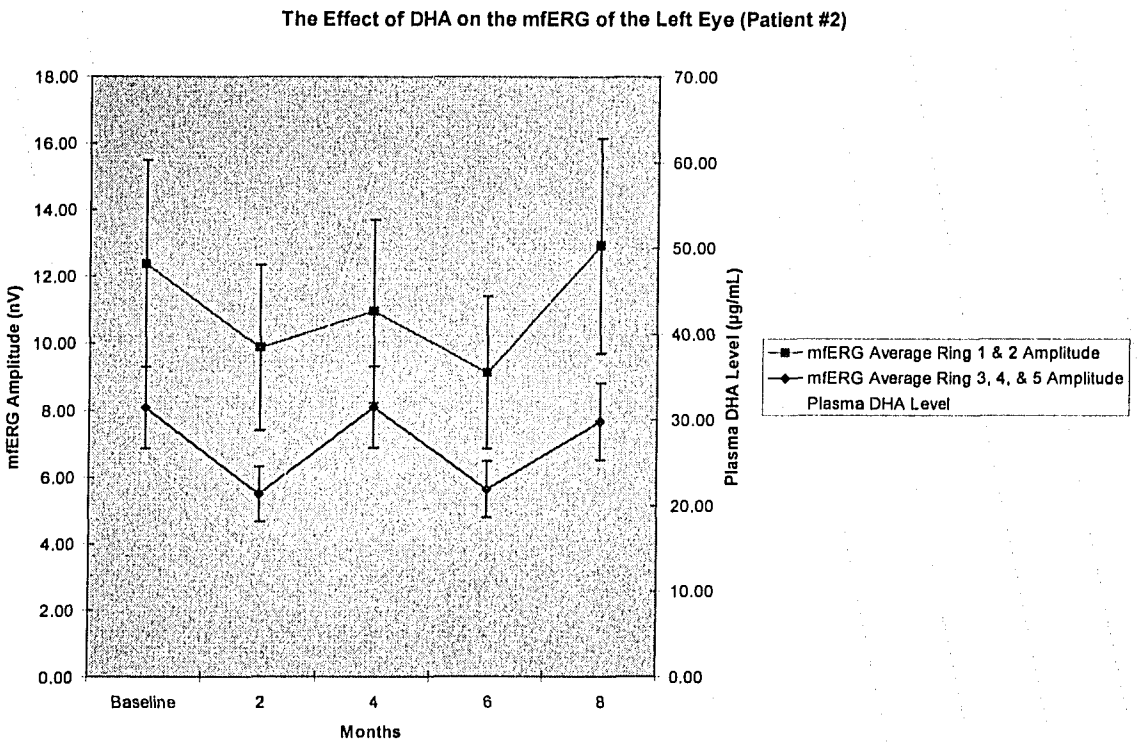

Figure 3-7: The effect of DHA on the average central and peripheral rings of the mfERG of the left eye in Patient #2.

The third graph shows an approximate 20% increase in the electro-oculogram (EOG) of both eyes with the elevation in plasma DHA (Right eye: OD; Left Eye: OS)

(Figure 3-8). The increased in Arden ratio was surprisingly in the placebo treatment period after the DHA treatment.

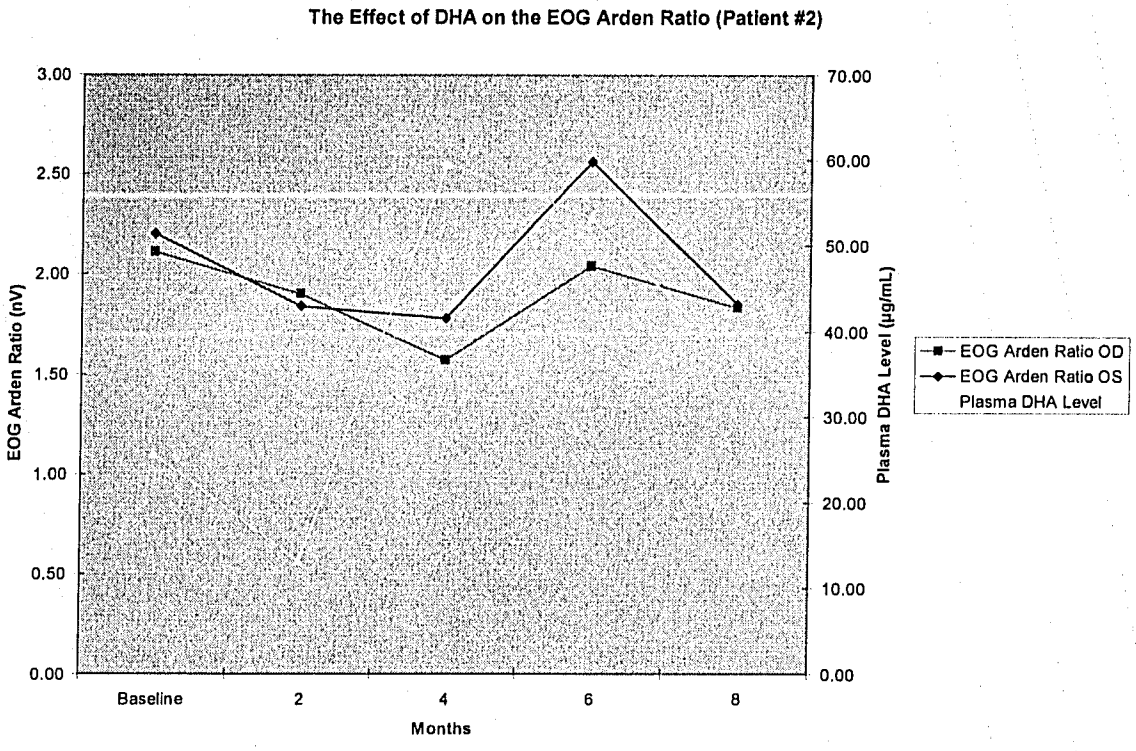

Figure 3-8: The effect of DHA on the Arden Ratio of the electro-oculogram of Patient #2.

Figure 3-9 shows the subjective change in vision during the clinical trail. The patient had marked improvement in subjective vision while on DHA compared to being on placebo. The rise and fall of the VF-14 score followed closely the pattern of the plasma DHA levels.

The effect of DHA on visual acuity can be seen in Figure 3-10. At the end of the trial, the right eye had no improvement in visual acuity. The left eye lost one line

of visual acuity. The change in plasma DHA level did not correspond to a similar change in the vision of the patient.

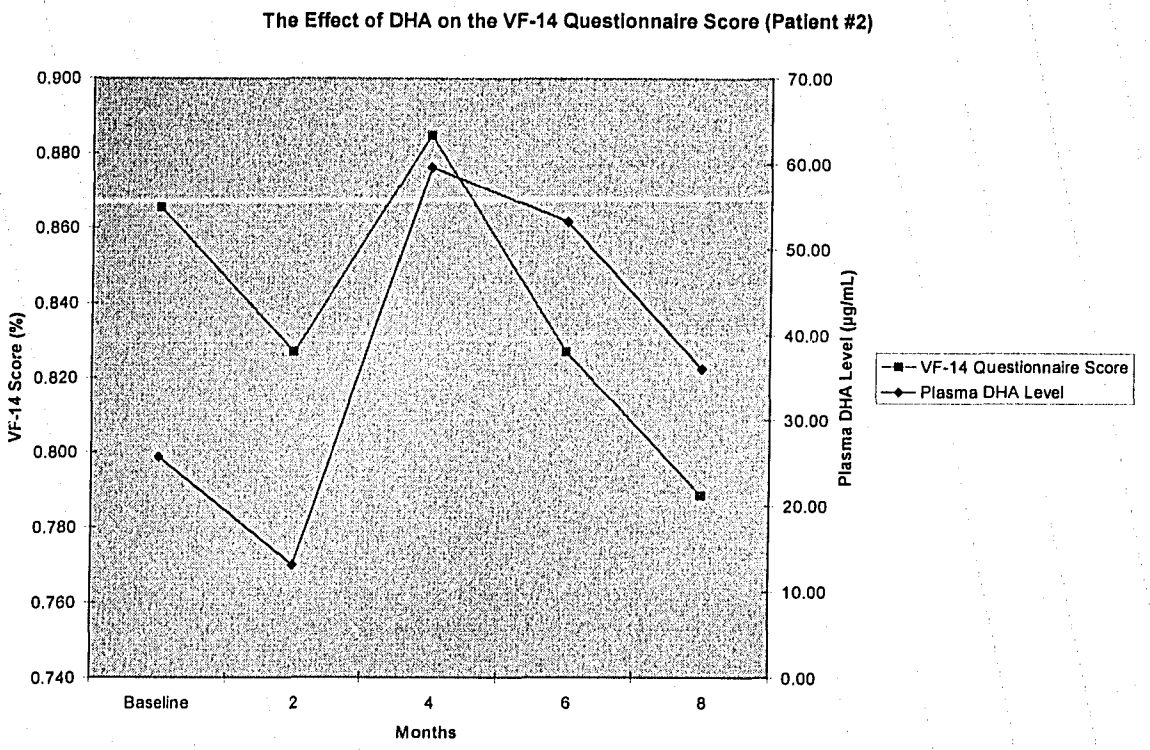

Figure 3-9: The effect of DHA on subjective visual measure using the VF-14 questionnaire.

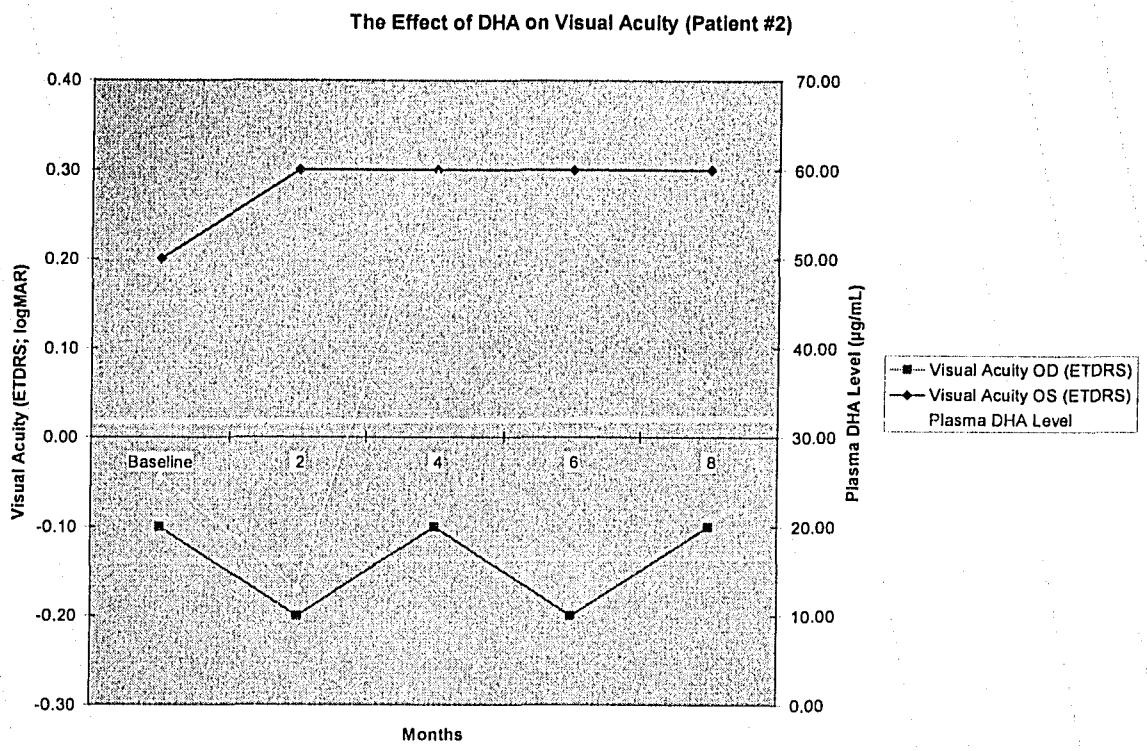

Figure 3-10: The effect of DHA on the visual acuity (ETDRS) of Patient #2.

Patient #3

Table 3-4. Pattern of treatment for Patient #3.

Months	Treatment	Plasma DHA Level (µg/mL)
Baseline	-	23.38
Baseline – 2	DHA	68.39
2 – 4	Placebo	39.57
4 – 6	DHA	72.64
6 – 8	DHA	113.54

Figure 3-11 shows the effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the right eye. The central rings amplitude declined by 28% cumulatively over the eight-month trial, whereas the peripheral rings

amplitude decreased by about 10% at the end of the trial. The rise and fall of plasma DHA did not seem to correlate with the change in amplitudes.

As in Patient #2, the consumption of DHA seemed to have a carry-over effect. The placebo taken in the second trial period did not lower the plasma DHA level back to baseline suggesting retention of DHA in the plasma, and the continued elevation of plasma DHA in the third and fourth treatment period (DHA treatment).

The left eye also showed decline in the average mfERG amplitudes, but not to the extent of the right eye (Figure 3-12). The central and peripheral rings amplitudes decreased by approximately 10% at the end of the trial. An increase in amplitude during the second trial period (placebo) of 10% was seen given the decline in plasma DHA level.

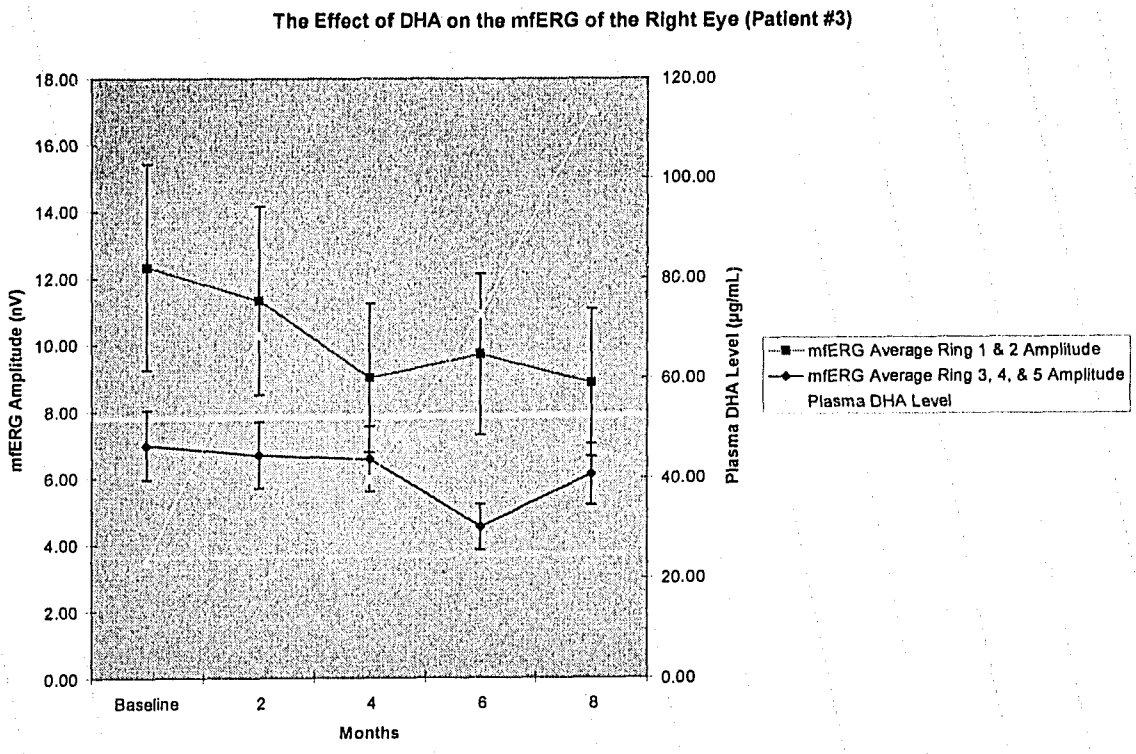

Figure 3-11: The effect of DHA on the average central and peripheral rings of the mfERG of the right eye in Patient #3.

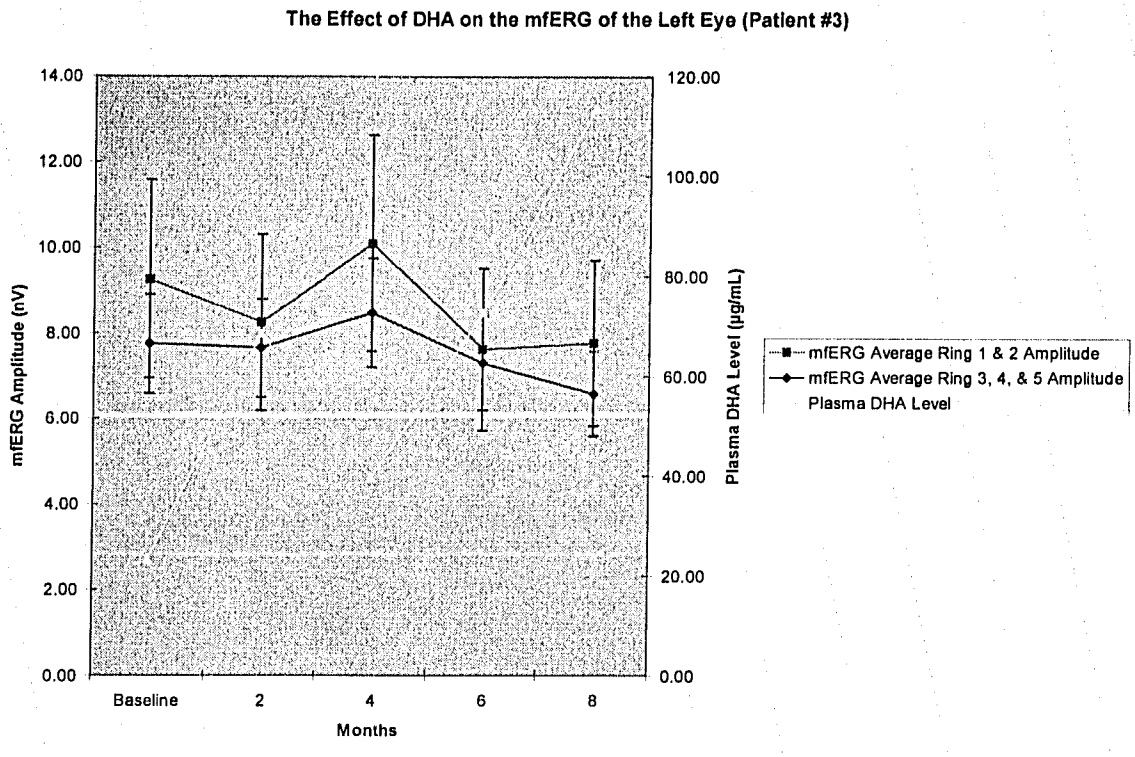

Figure 3-12: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the left eye.

The effect of DHA on the EOG of both eyes (Right eye: OD; Left Eye: OS) (Figure 3-13). The Arden ratio of the right eye stayed relatively the same until the third treatment period (DHA treatment) where it increased by 40%, but it returned near baseline in the consecutive DHA treatment period. For the left eye, the Arden ratio increased during the placebo treatment period, in the second period.

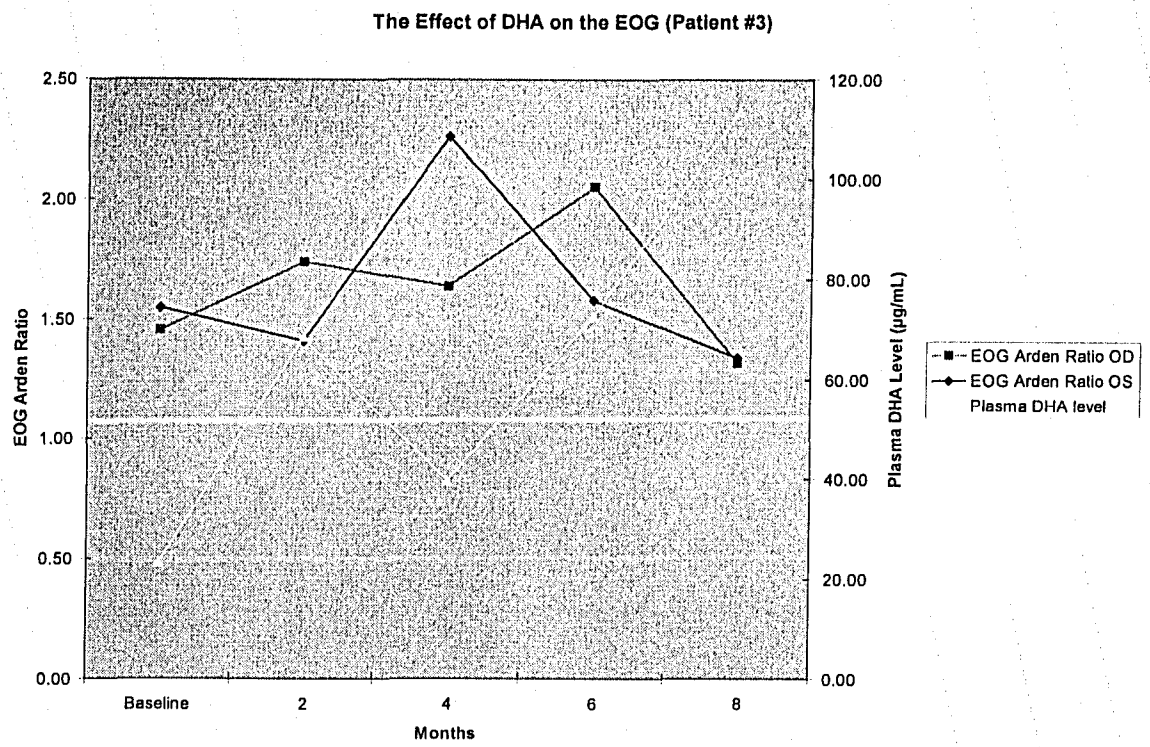

Figure 3-13: The effect of DHA on the electro-oculogram of Patient 3.

The change in the VF-14 questionnaire score and the change in plasma DHA level are seen in Figure 3-14. Patient #3 had a 10% increase in the subjective visual measure in the third treatment period with DHA, only to decline in the last treatment period with increasing plasma DHA level.

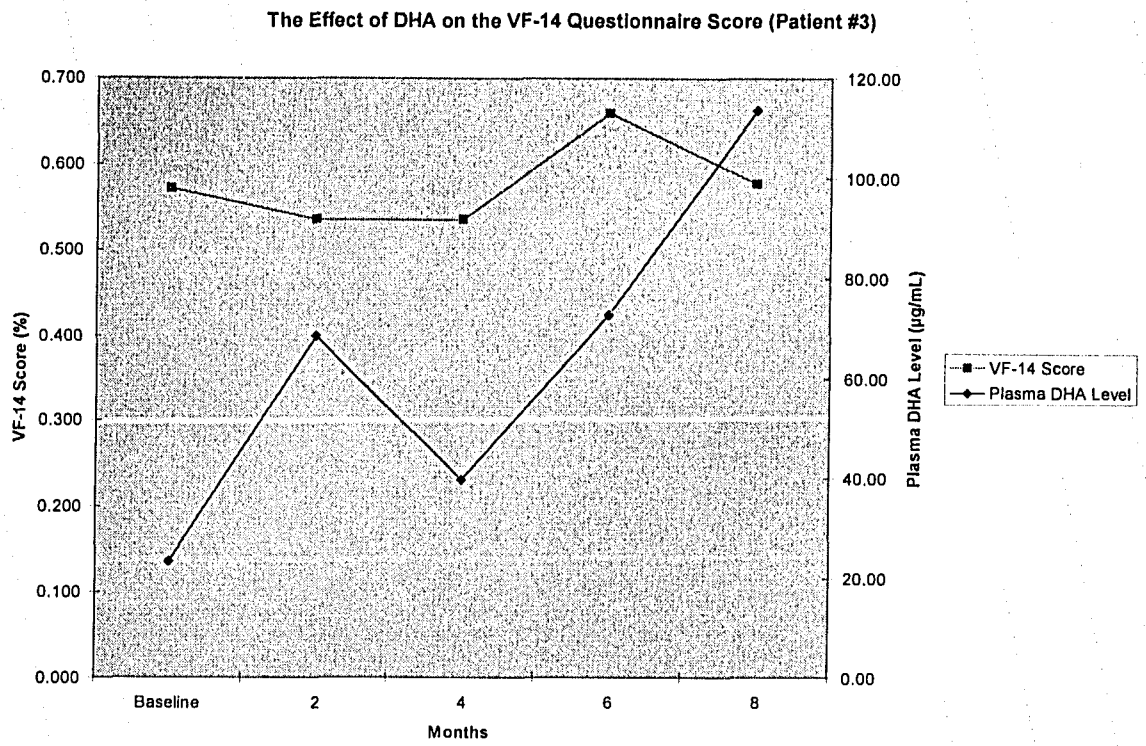

Figure 3-14: The effect of DHA on the subjective vision (VF-14 questionnaire) of Patient #3.

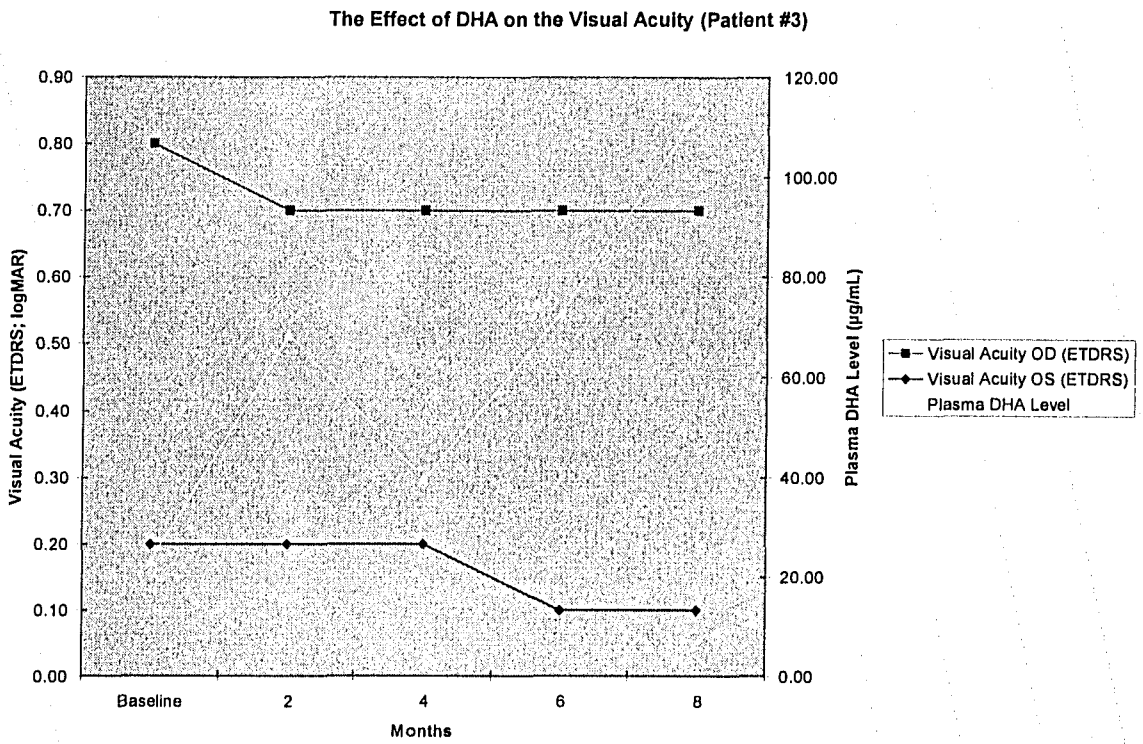

Figure 3-15: The effect of DHA on the visual acuity (ETDRS) of Patient #3.

The visual acuity of the Patient #3 improved by one line in the right and left eye (Figure 3-15). For the right eye, the improvement was evident in the first treatment period (DHA), whereas the left eye improved after the second treatment period (placebo).

Patient #4

Table 3-5. Pattern of treatment for Patient #4.

Months	Treatment	Plasma DHA Level (μg/mL)
Baseline	-	19.67
Baseline – 2	DHA	58.14
2 – 4	Placebo	14.07
4 – 6	DHA	50.80
6 – 8	DHA	47.69

Figure 3-16 shows the effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the right eye. The average central rings amplitude decreased by 45% in the first treatment period while on DHA supplementation. The peripheral rings amplitude decreased by 32% in the first treatment period. Following the drop, the central rings amplitude increased by approximately 40% during the next two periods including a placebo and DHA treatment period. The peripheral rings amplitude stayed relatively the same after the initial drop. The change in plasma DHA did not seem to correspond to changes in the mfERG amplitude.

The left eye had an initial increase of 34% in the central rings amplitude and less than 10% decrease in the peripheral rings amplitude for the first treatment period (Figure 3-17). Thereafter, the central rings amplitude declined by 42% in the next two

treatment periods before returning to near baseline at the end of the trial. The peripheral rings stayed relatively the same through to the end of the trial.

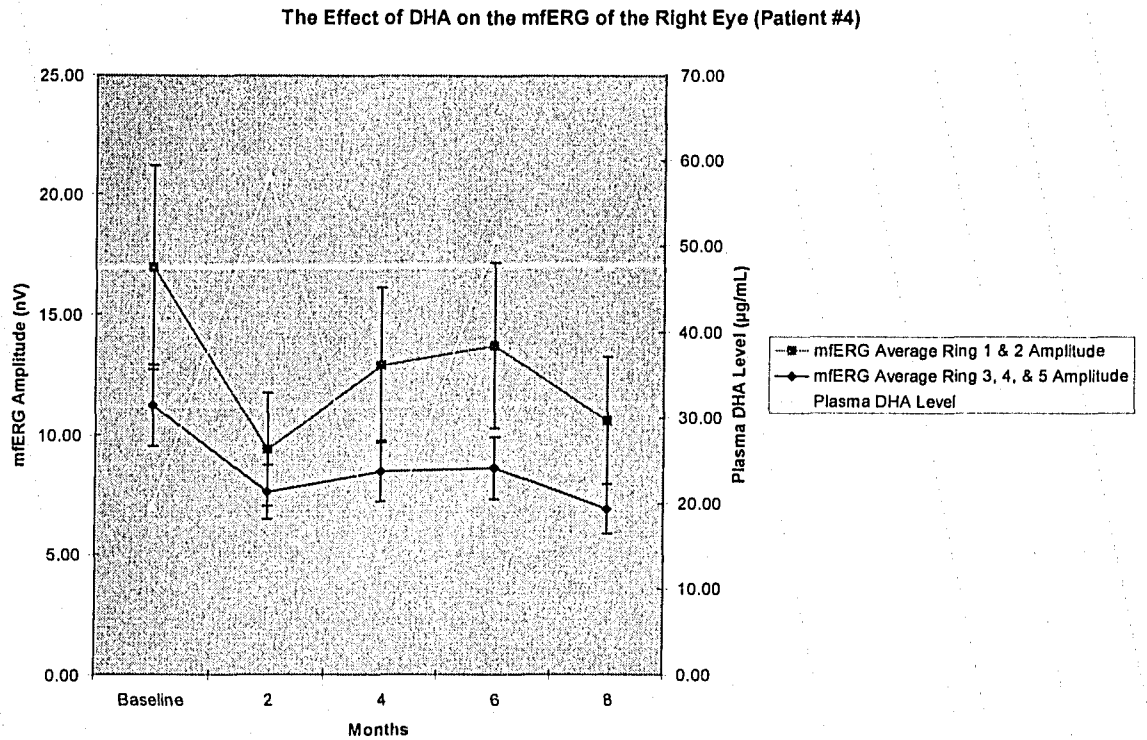

Figure 3-16: The effect of DHA on the average central and peripheral rings amplitude of the mfERG of the right eye in Patient #4.

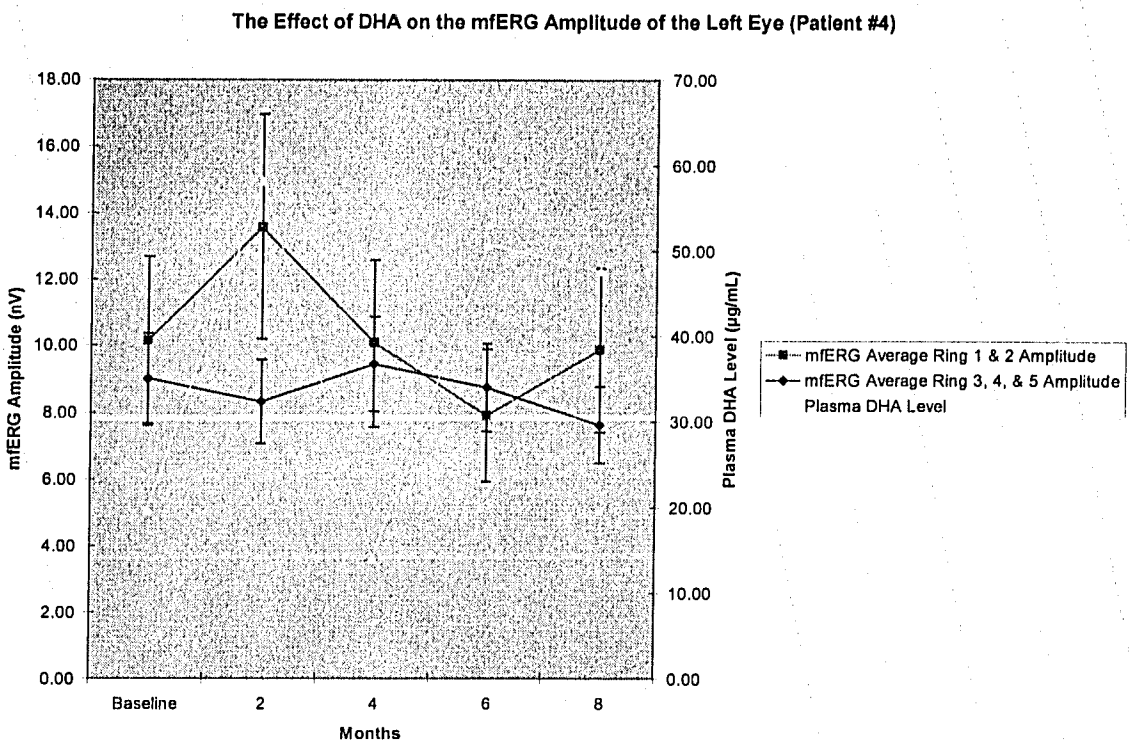

Figure 3-17: The effect of DHA on the average central and peripheral rings amplitude of the mfERG of the left eye in Patient #4.

The effect of DHA on the electro-oculogram (EOG) of both eyes is shown in Figure 3-18. The Arden ratio increased by 10% in the right eye and over 60% in the left eye by the end of the trial after two consecutive DHA treatment periods. The change in plasma DHA did not reflect changes in the EOG Arden ratio for Patient #4.

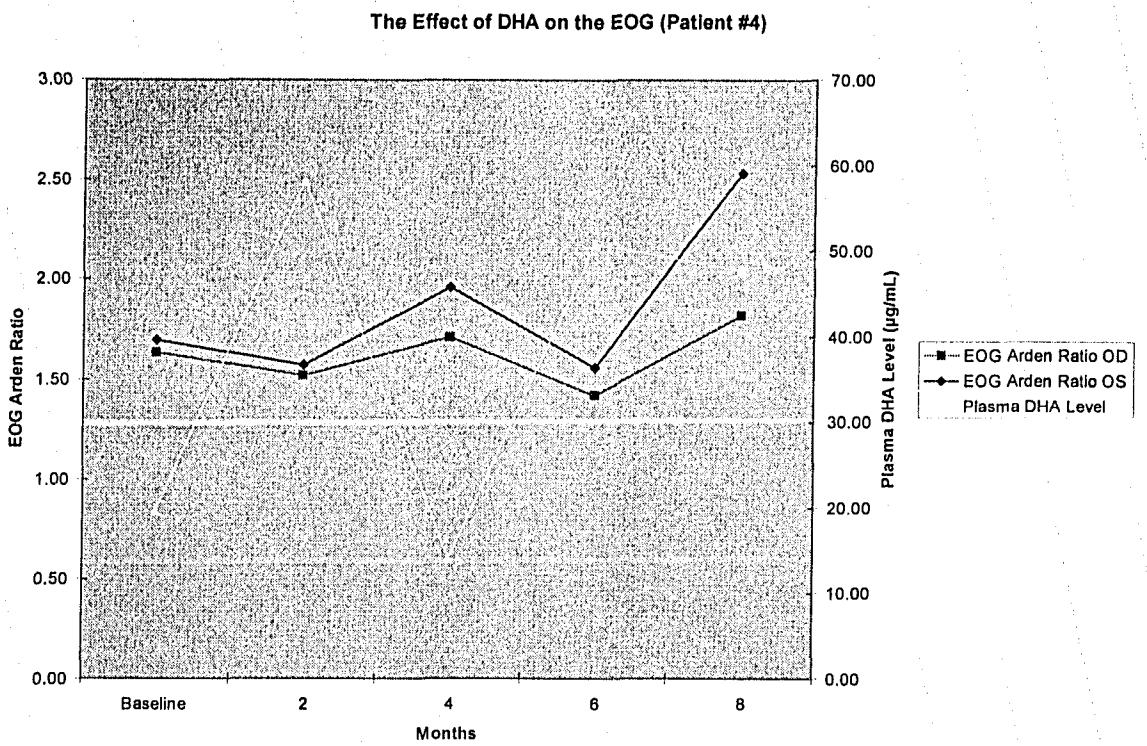

Figure 3-18: The effect of DHA on the electro-oculogram Arden ratio for Patient #4.

The VF-14 questionnaire score and the change in DHA can be seen in Figure 3-19. Patient #4 noticed a subjective improvement in his vision in treatment periods 2, 3, and 5, and a worsening of vision during period 4. The improvement noticed in period 3 and worsening in period 4 did not correspond to changes in the plasma DHA level.

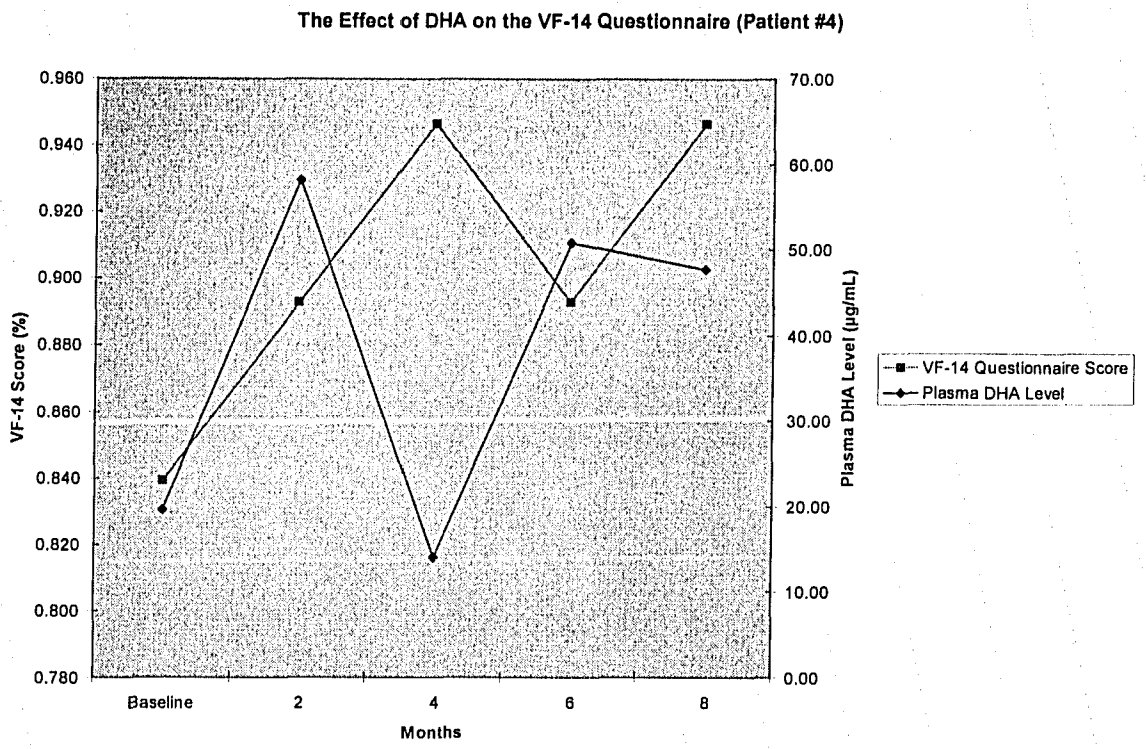

Figure 3-19: The effect of DHA on the subjective visual measure, VF-14 questionnaire.

The effect of DHA on the patient's visual acuity is shown on Figure 3-20. The right eye had the same acuity until the third treatment period when it gained one line improvement in visual acuity only to lose it in the last treatment period. The left eye gained one line of vision initially in the first treatment period and lost it in the third treatment period while on DHA. At the end of the trial, the left eye gained one line of visual acuity while the right eye became worse and lost one line of visual acuity.

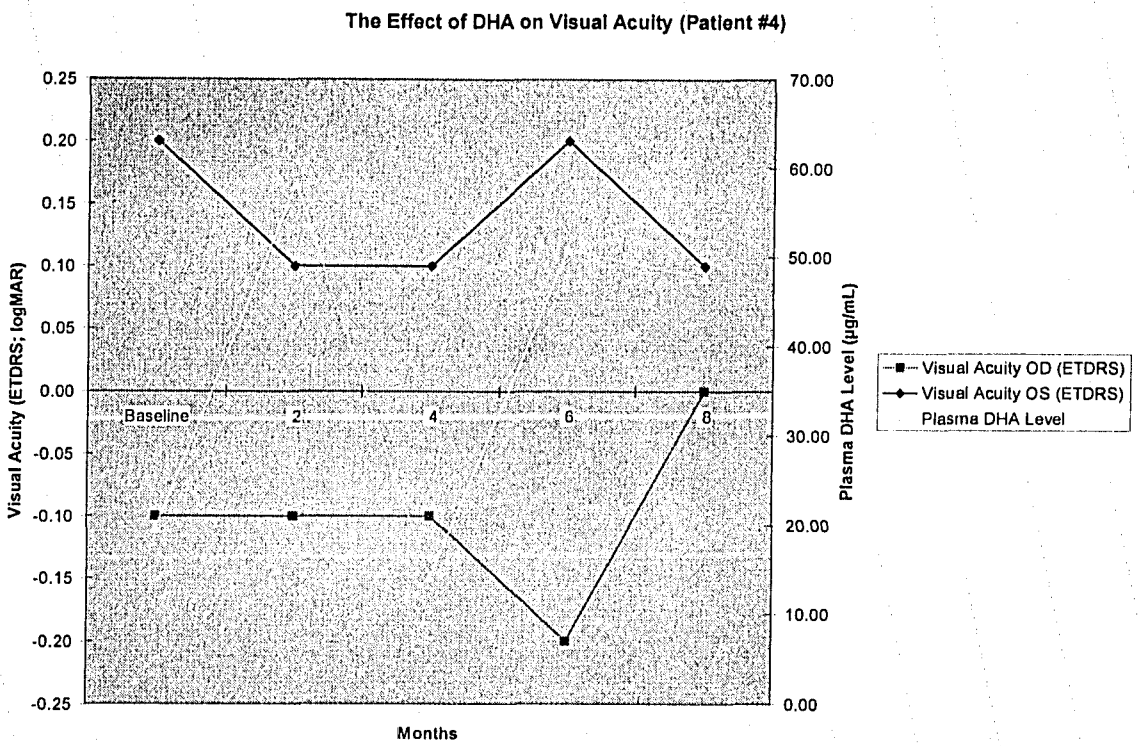

Figure 3-20: The effect of DHA on the visual acuity (ETDRS) of Patient #4.

Patient #5

Table 3-6: Pattern of treatment for Patient #5.

Months	Treatment	Plasma DHA Level (µg/mL)
Baseline	-	15.87
Baseline – 2	DHA	38.36
2 – 4	Placebo	15.13
4 – 6	DHA	54.10
6 – 8	DHA	35.98

The effect of DHA on the mfERG of the right eye is shown in Figure 3-21. The average central rings amplitude declined by 30% in the first treatment period (DHA

supplementation) before increasing approximately 10 to 20% in the latter periods of treatment. The peripheral rings amplitude decreased by 32% in the first treatment period, before staying relatively stable until the last treatment period with DHA supplementation showing a decrease of 18%.

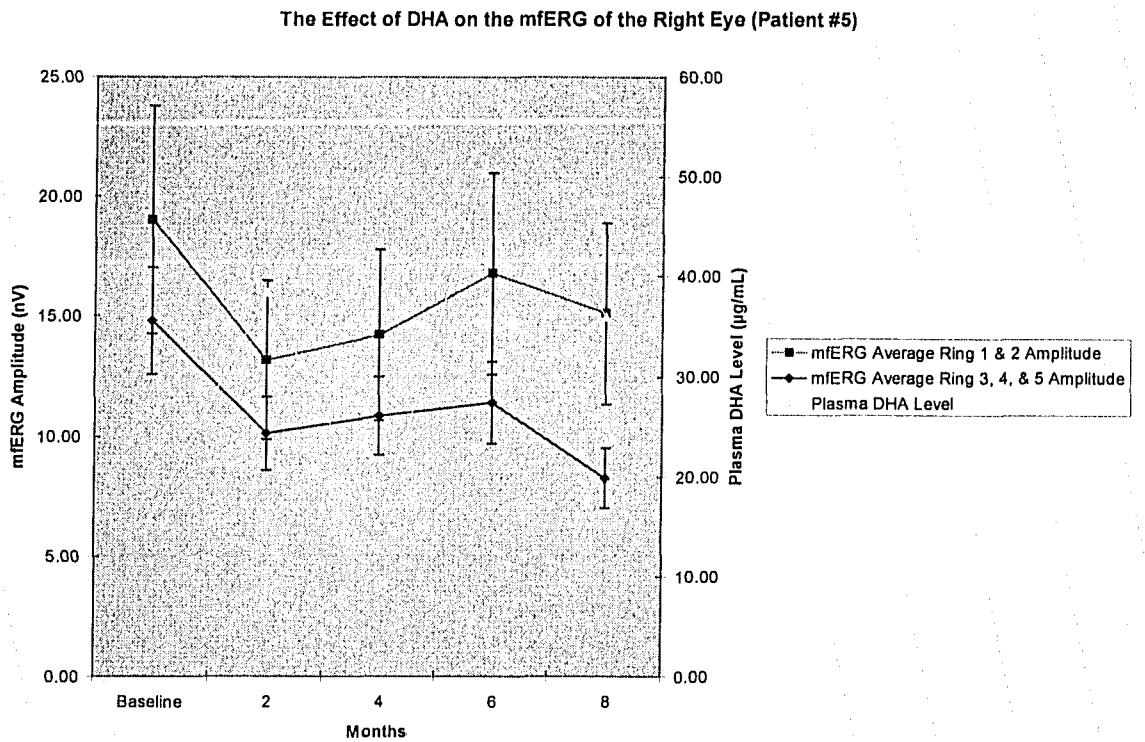

Figure 3-21: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the right eye in Patient #5.

In the left eye, the average central and peripheral rings decreased by 20 and 32%, respectively, in the first treatment period (Figure 3-22). The central rings amplitude increased over the next three treatment periods by 60%. The peripheral rings amplitude increased in the next two treatment periods (71%) before decreasing in the last treatment period (20%). The change in plasma DHA did not correspond to changes in the central or peripheral mfERG amplitudes.

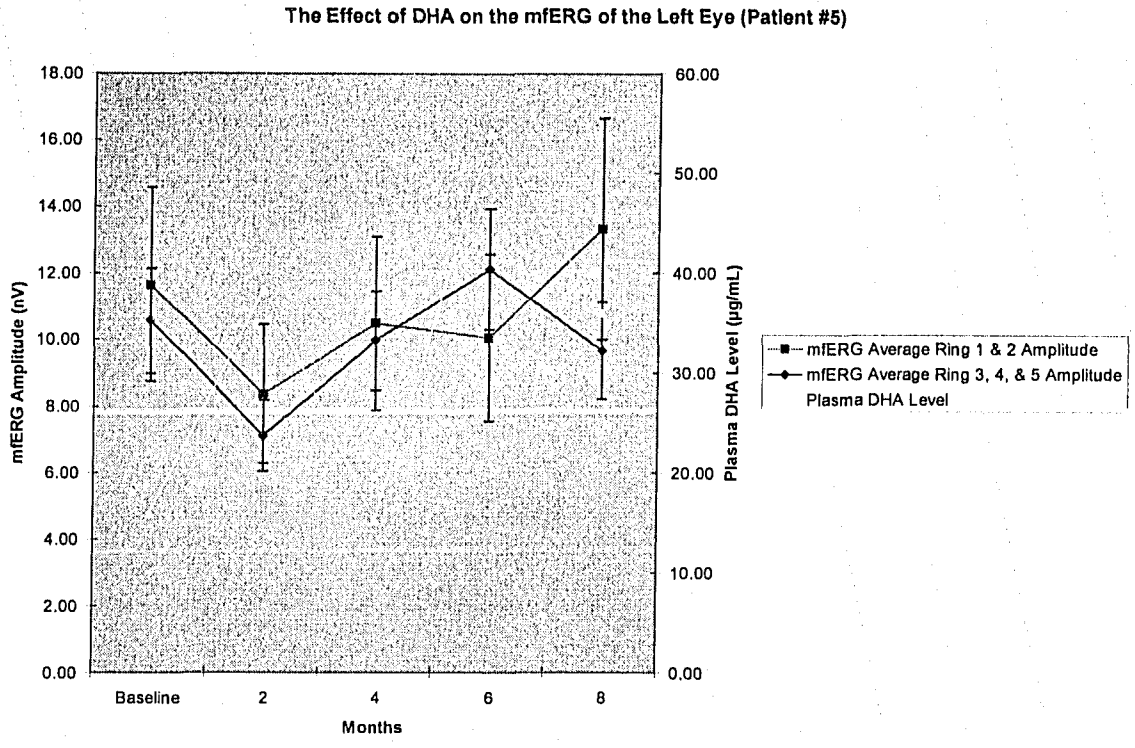

Figure 3-22: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the left eye.

The effect of DHA on the EOG is shown on Figure 3-23. The Arden ratio decreased by 22% in the right eye for the first treatment period (DHA supplementation). The ratio stayed relatively the same thereafter. For the left eye, the Arden ratio increased 12% at the end of the trial. The change in plasma DHA level did not correspond to the changes of the EOG Arden ratio for both eyes.

The effect of DHA on Patient #5's subjective vision is shown on Figure 3-24. The patient had subjective visual improvement in the first and last period of treatment when he was on DHA. The patient had a lower visual measure in the second and third

period of treatment when he was on placebo and DHA, respectively, which then increased to the final measure at month 8 possibly reflect carryover effect.

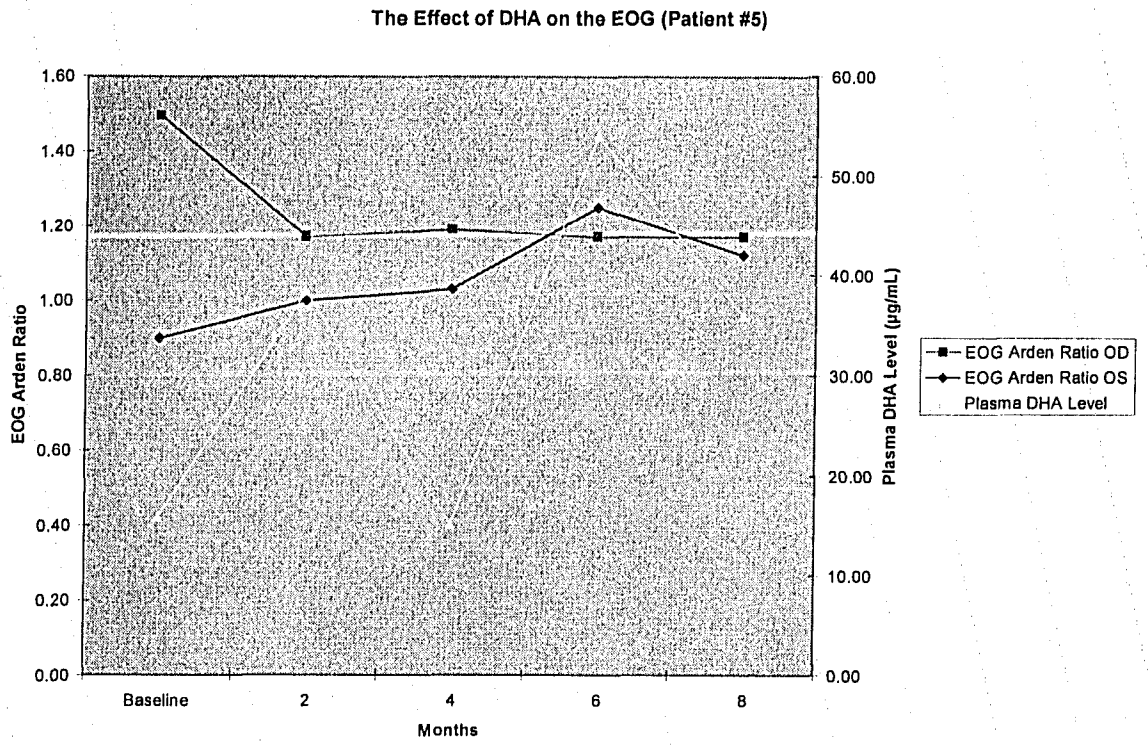

Figure 3-23: The effect of DHA on the EOG of both eyes in Patient #5.

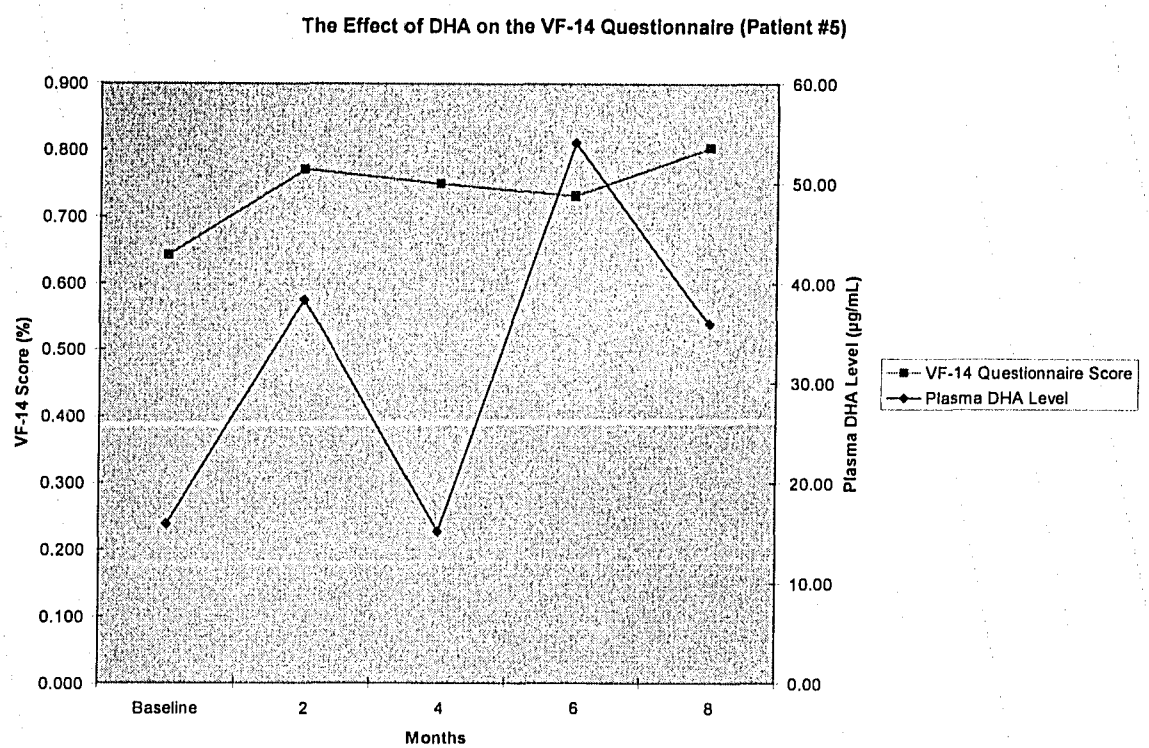

Figure 3-24: The effect of DHA on Patient #5 subjective vision based on the VF-14 questionnaire.

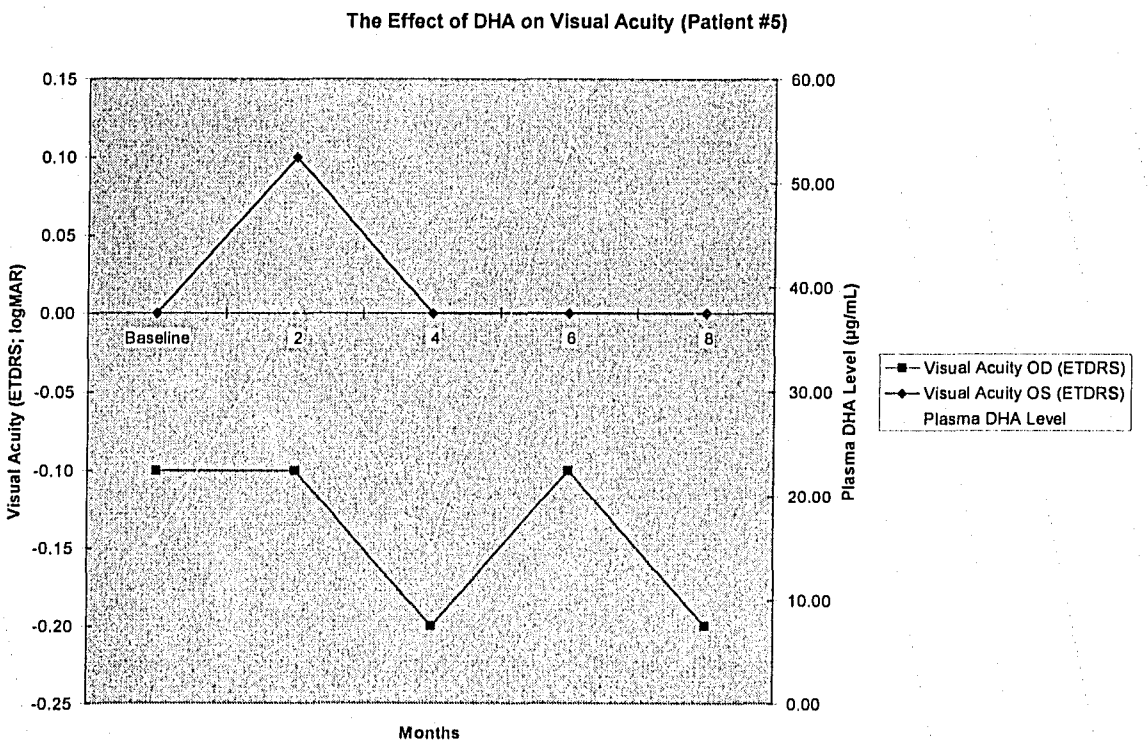

Figure 3-25: The effect of DHA on the visual acuity (ETDRS) of Patient #5.

The effect of DHA on the visual acuity is shown in Figure 3-25. The patient gained one line of visual acuity in the right eye at the end of the trial. The patient experienced improvement in his vision during the second and fourth treatment periods where the patient was on placebo and DHA, respectively. The left eye lost one line of visual acuity during the first treatment period when he was on DHA supplementation, but regained this line of vision during the next treatment period until the end of the trial.

Patient #6

Table 3-7. Pattern of treatment for Patient #6.

Months	Treatment	Plasma DHA Level (μg/mL)
Baseline	-	16.38
Baseline – 2	Placebo	9.06
2 – 4	DHA	52.28
4 – 6	Placebo	22.19
6 – 8	Placebo	19.33

Figure 3-26 shows the average central and peripheral rings amplitudes of the mfERG of the right eye. In the first treatment period with placebo, the central and peripheral rings amplitude decreased by 35 and 28%, respectively. The second treatment period with DHA supplementation demonstrated an increase of 20% for the central rings amplitude and 32% for the peripheral rings amplitude. After the DHA treatment and into the placebo treatment periods, the amplitudes decreased by 13 and 23% for the central and peripheral rings, respectively.

In the left eye, the average central rings amplitude declined in the first and second treatment periods from baseline by 17% (Figure 3-27). The amplitude increased by 26% in the placebo period (third treatment period) after the DHA supplementation (second treatment period). The central rings amplitude declined in the consecutive placebo treatment period, ending the trial with a 16% decrease. The peripheral rings amplitude decreased initially in the first treatment period with placebo by 17%, but it rebounded in the next period with DHA supplementation by 14%. The third and fourth treatment periods with placebo saw a decrease of 14% in the amplitude at the end of the trial.

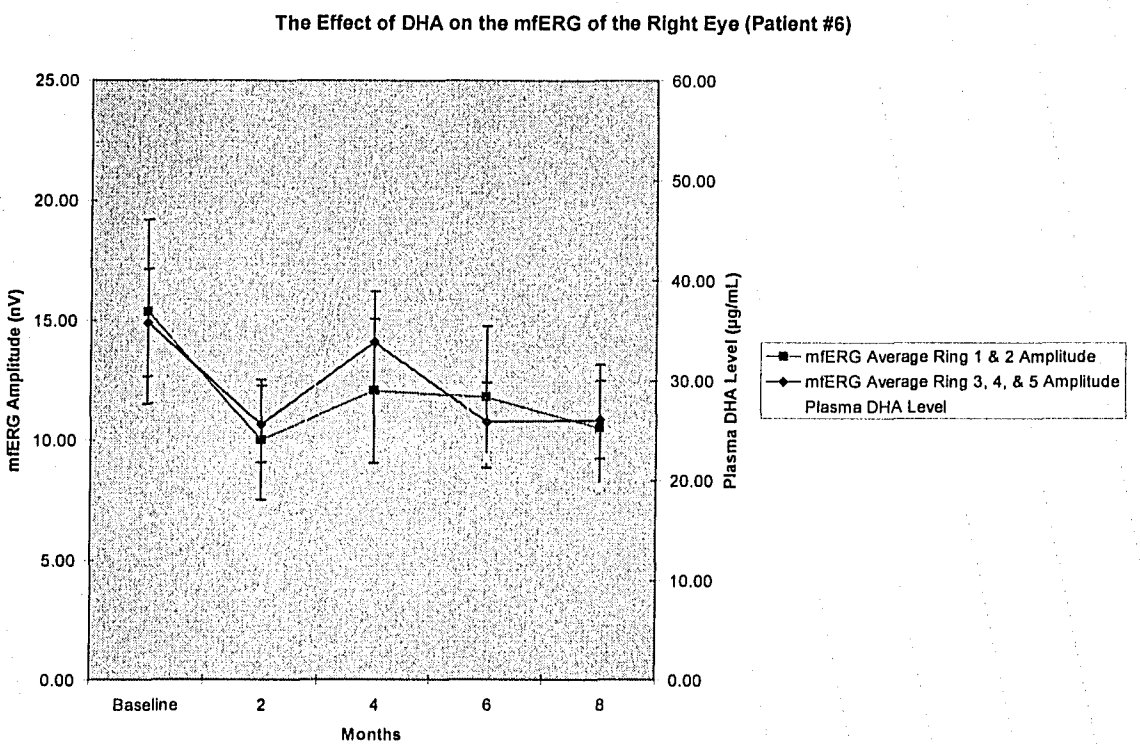

Figure 3-26: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG in the right eye of Patient #6.

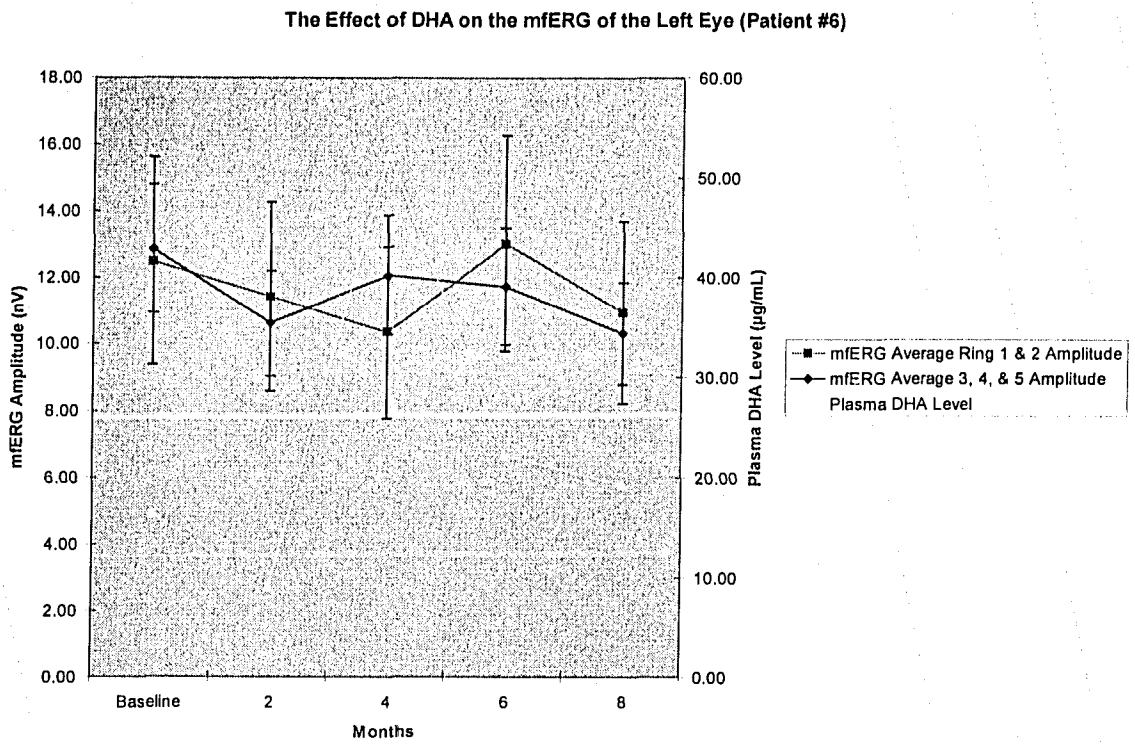

Figure 3-27: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG in the left eye of Patient #6.

The effect of DHA on the electro-oculogram (EOG) of both eyes is shown in Figure 3-28. In the right eye, the Arden ratio increased in the first and third treatment periods with placebo treatment compared to baseline. The Arden ratio for the DHA supplementation period decreased by 13% from the placebo treatment period. The last treatment period with placebo showed a decrease of 26% in the Arden ratio from the previous placebo treatment period. For the left eye, the Arden ratio decreased from baseline by 15 and 10% in the first and third treatment period with placebo, respectively. The ratio increased in the third treatment period with DHA supplementation by 15 and 34% compared to baseline and the previous placebo

treatment period, respectively. The ratio in the last treatment period increased compared to the previous treatment period despite placebo treatment.

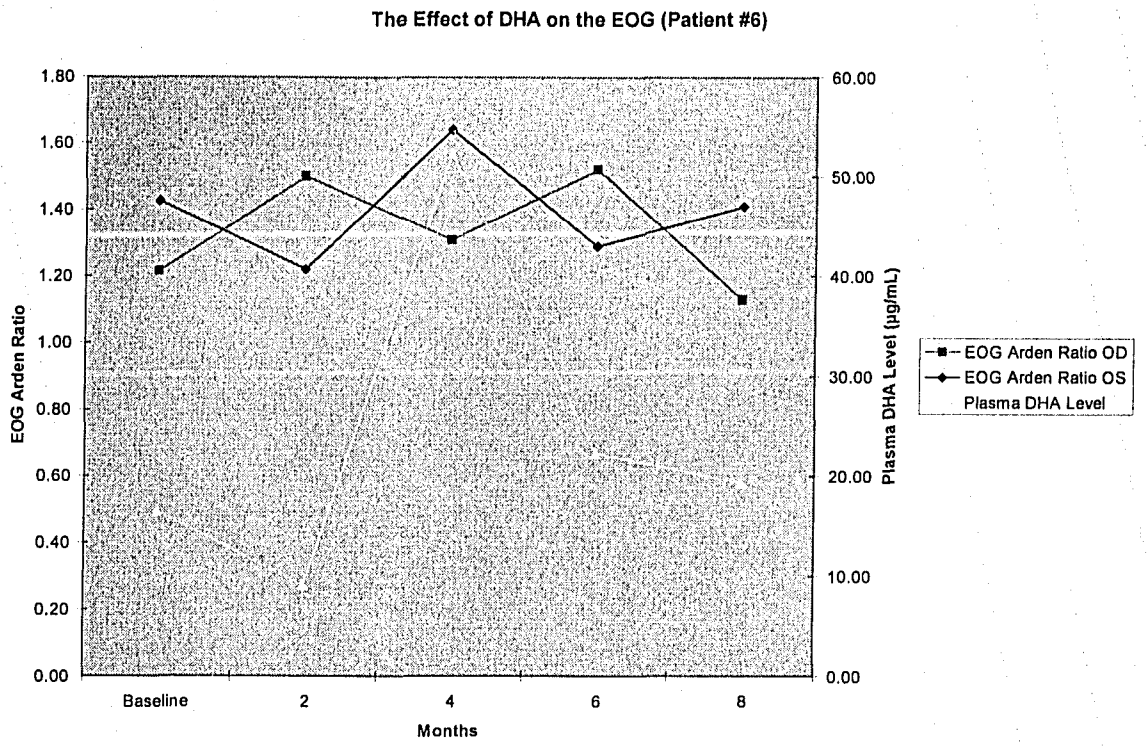

Figure 3-28: The effect of DHA on the EOG Arden ratio of both eyes in Patient #6.

The effect of DHA on the VF-14 questionnaire is shown in Figure 3-29. Patient #6 experienced subjective improvement in vision during the first, second, and fourth treatment periods. The patient had DHA supplementation in the second treatment period only, and their perception of visual improvement was greatest during this period.

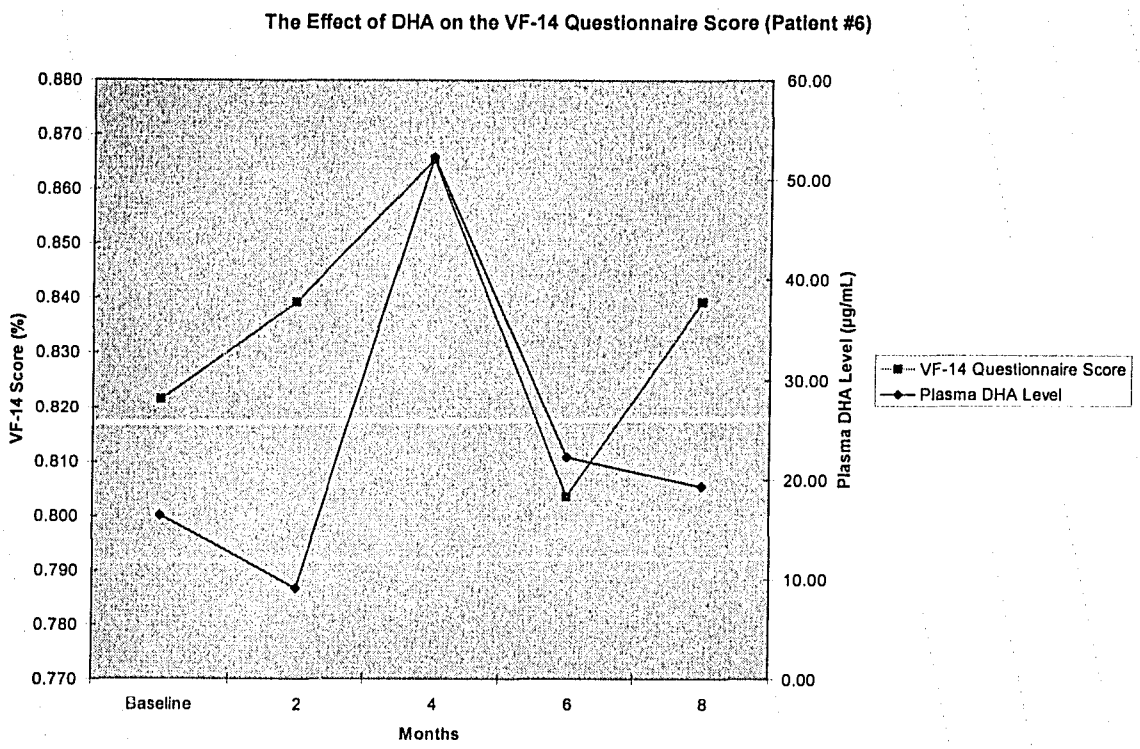

Figure 3-29: The effect of DHA on the VF-14 questionnaire examining the subjective visual perception of Patient #6.

Figure 3-30 shows the effect of DHA on the visual acuity of the patient. The right eye decreased by one line of acuity in the first treatment period and stayed the same through to the third treatment period. The vision in the right eye returned to baseline at the end of the trial. The left eye improved one line of vision with DHA supplementation in the second treatment period. The left eye lost the same line of vision in the next treatment period only to regain it at the end of the trial.

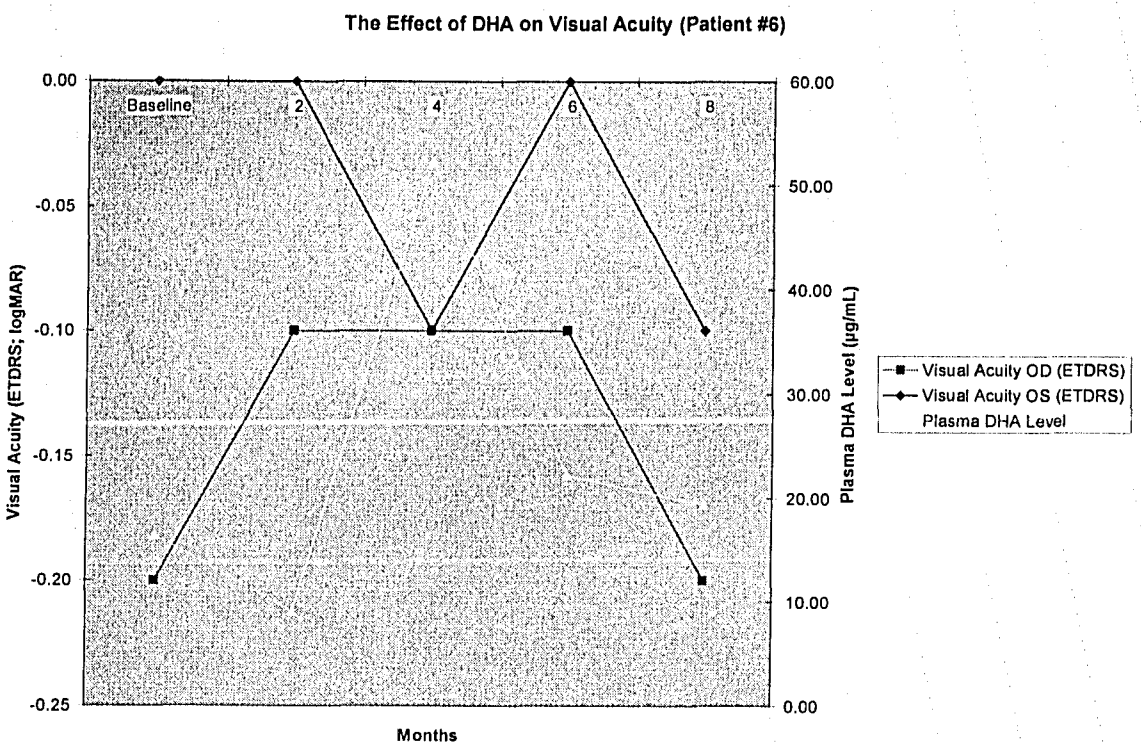

Figure 3-30: The effect of DHA on the visual acuity (ETDRS) of both eyes.

Patient #7

Table 3-8. Pattern of treatment for Patient #7.

Months	Treatment	Plasma DHA Level (µg/mL)
Baseline	-	20.09
Baseline – 2	Placebo	14.28
2 – 4	DHA	47.02
4 – 6	Placebo	41.99
6 – 8	Placebo	10.82

Figure 3-31 shows the effect of DHA on the average and central rings mfERG of the right eye. The central rings amplitude decreased into the second treatment period from baseline for a decrease of 17%. The central rings amplitude increased

from this low to end the trial with a 20% increase and a return to baseline. The peripheral rings amplitude decreased 17% in the first treatment period and maintained this amplitude to the end of the trial.

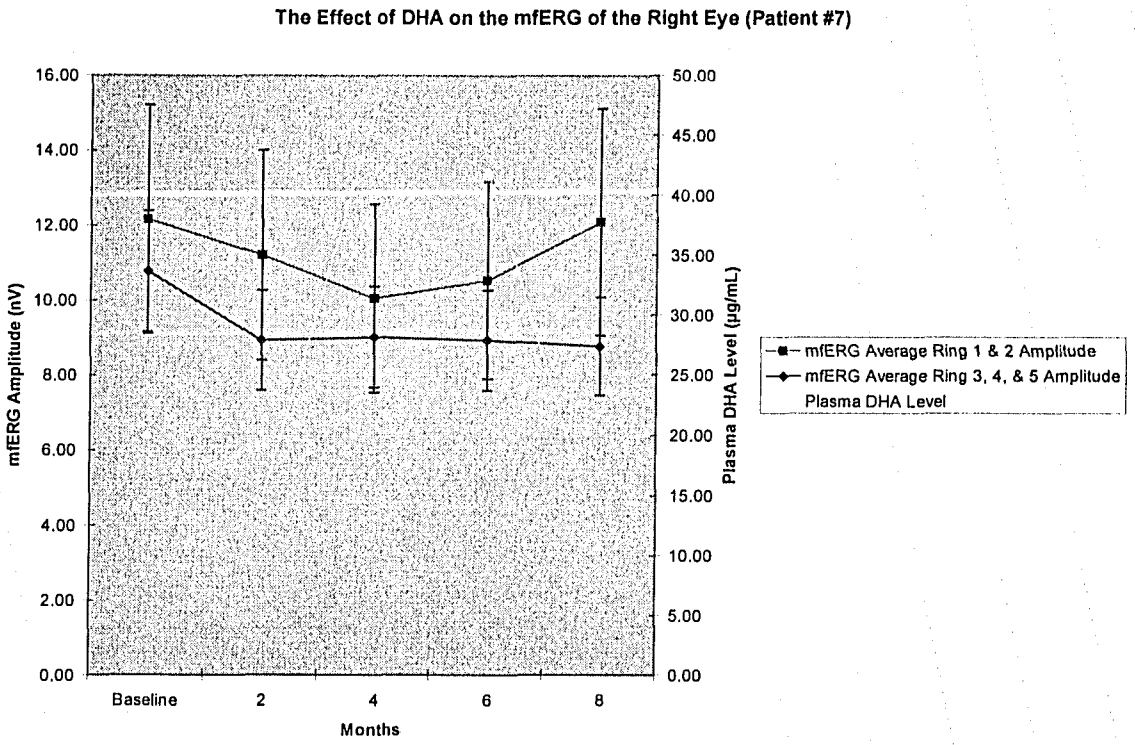

Figure 3-31: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the right eye in Patient #7.

The left eye average central and peripheral rings amplitude is shown on Figure 3-32. The central rings amplitude decreased steadily throughout the trial with a cumulative decrease of 37%. The peripheral rings amplitude followed the same pattern of decrease with a cumulative decline of 36% at the end of the trial. The changes in plasma DHA level did not correspond to changes in the mfERG amplitudes for both eyes. Also, it is important to note that the plasma DHA remained at a high level in the placebo treatment period following DHA treatment.

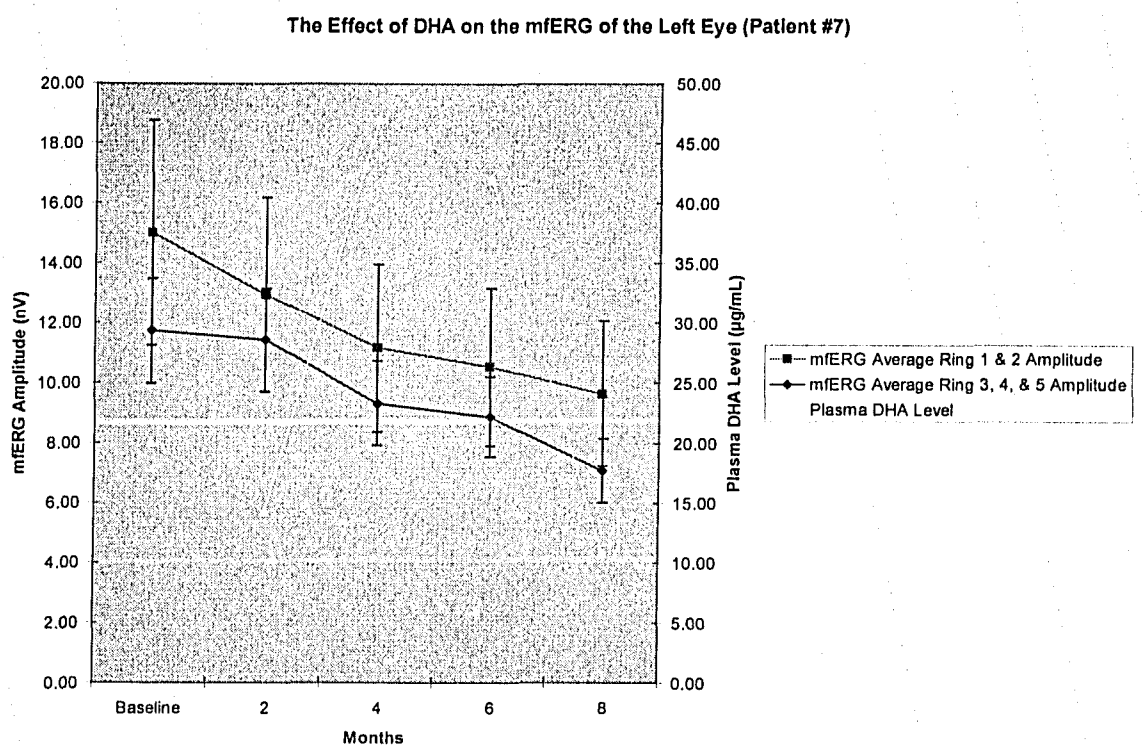

Figure 3-32: The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the left eye in Patient #7.

The effect of DHA on the EOG of both eyes is shown on Figure 3-33. The Arden ratio of the right eye declined steadily into the third treatment period with a cumulative decrease of 34%. The ratio increased at the end of the trial by 20%. For the left eye, the Arden ratio decreased by 34% into the third treatment period before increasing 30% from the low at the end of the trial. The rise and fall of the Arden ratio of both eyes did not correspond to changes in the plasma DHA level.

Figure 3-34 shows the effect of DHA on the VF-14 questionnaire score. Patient #7 subjective perception of vision declined during the second and third treatment periods when the patient was on DHA supplementation followed by placebo treatment. The decrease in subjective vision also coincided with increased plasma

DHA level. The patient experienced an improvement in vision in the first and last treatment period during placebo treatment.

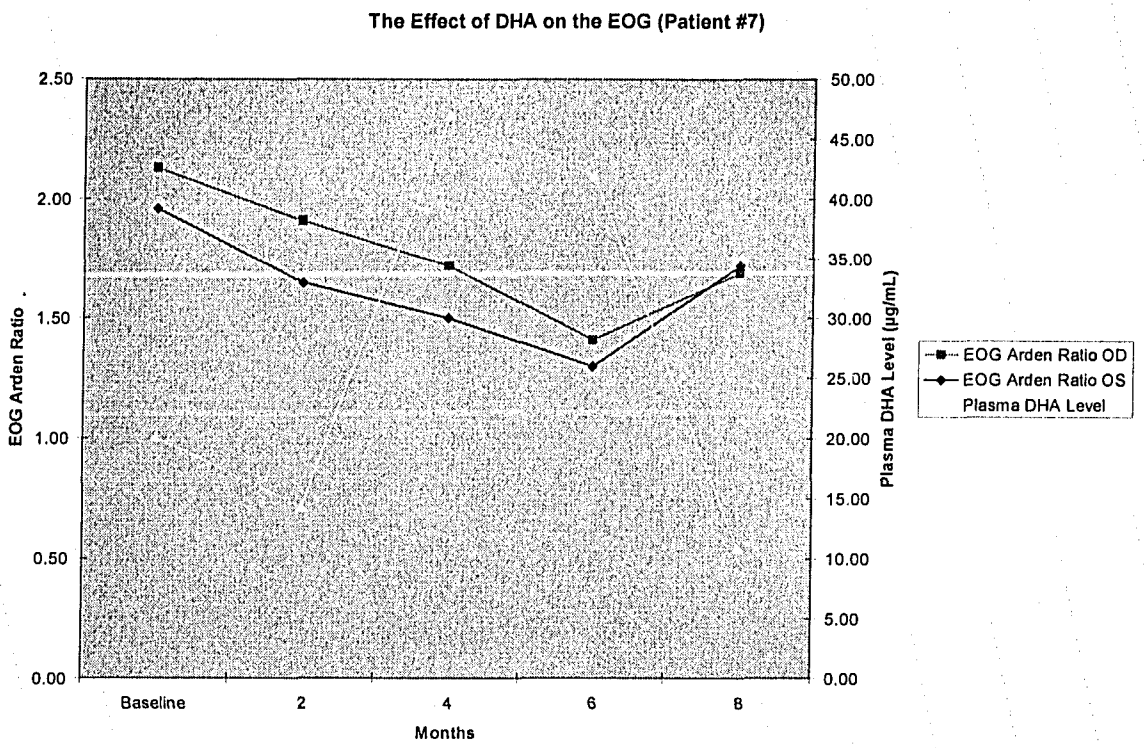

Figure 3-33: The effect of DHA on the EOG of Patient #7.

The effect of DHA on the visual acuity is shown in Figure 3-35. The change in plasma DHA did not change the visual acuity of either eye. The vision remained the same throughout the trial.

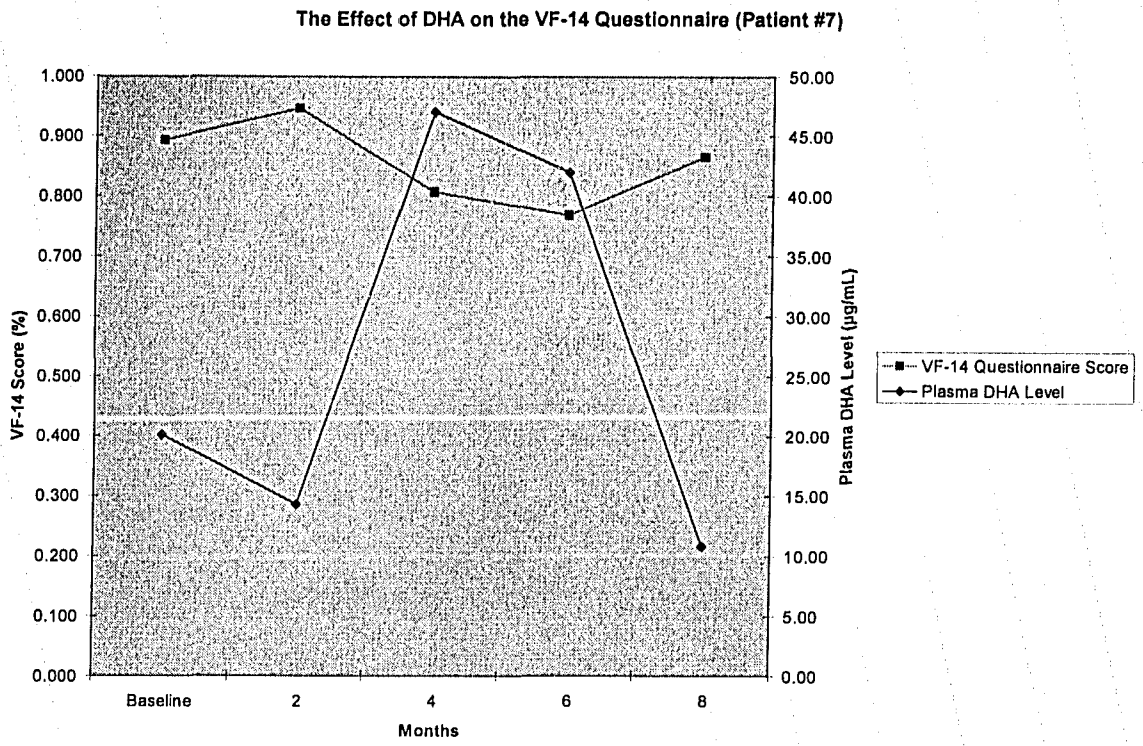

Figure 3-34: The effect of DHA on the VF-14 questionnaire examining the subjective visual perception in Patient #7.

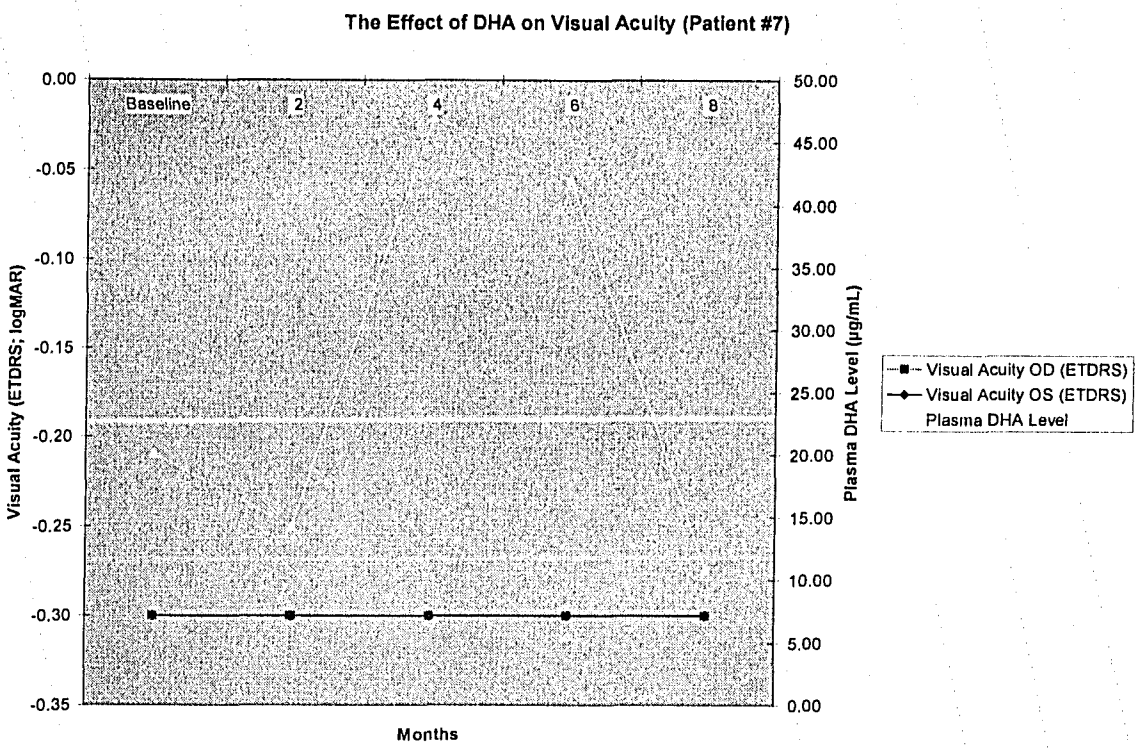

Figure 3-35: The effect of DHA on the visual acuity of Patient #7.

Patient #8

Table 3-9. Pattern of treatment for Patient #8.

Months	Treatment	Plasma DHA Level (µg/mL)
Baseline	-	12.78
Baseline – 2	Placebo	12.43
2 – 4	DHA	19.31
4 – 6	Placebo	22.77
6 – 8	Placebo	14.35

The effect of DHA on the average central and peripheral rings amplitudes of the mfERG of the right eye is shown on Figure 3-36. The central rings amplitude

increased initially in the first treatment period by 7% followed by a steady decrease of 16% to the end of the trial. The peripheral rings amplitude declined in every treatment except for the second treatment period when the patient was on DHA, but the increase was minimal (3%). The changes in plasma DHA did not correspond to changes in mfERG amplitudes. The plasma DHA was elevated in the second and third treatment periods with the patient taking DHA only during the second period. The rise in plasma DHA level was greater in the third treatment period when the patient was on placebo.

For the left eye, the average central and peripheral rings amplitude is shown on Figure 3-37. The central rings amplitude increased during the first and last treatment periods when the patient was on placebo treatment and had the low plasma DHA levels. The increase was minimal at approximately 10%. The second and third periods had high plasma DHA levels, and they had decreases of 27 and 13%, respectively, in amplitude. The peripheral rings amplitude decreased throughout the entire trial ending with a decrease of 35%.

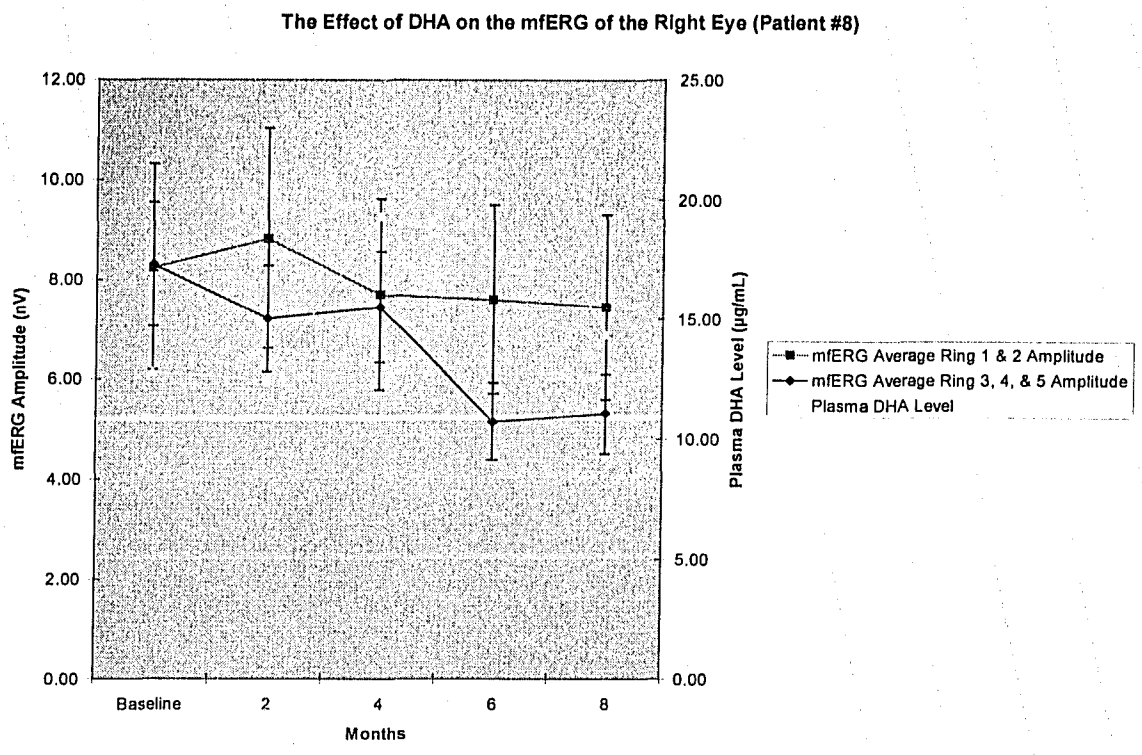

Figure 3-36: The effect of DHA on the average central and peripheral rings amplitude of the mfERG of the right eye in Patient #8.

The effect of DHA on the EOG is shown on Figure 3-38. The Arden ratio of the right eye gradually increased by 10% and peaked in the second treatment period with DHA. The ratio then gradually declined towards the end of the trial to near the baseline level. For the left eye, the Arden ratio decreased initially by 10% with the placebo treatment before peaking in the next period with DHA supplementation. The increase of 26% in the Arden ratio was followed by a gradual decrease to near baseline towards the end of the trial.

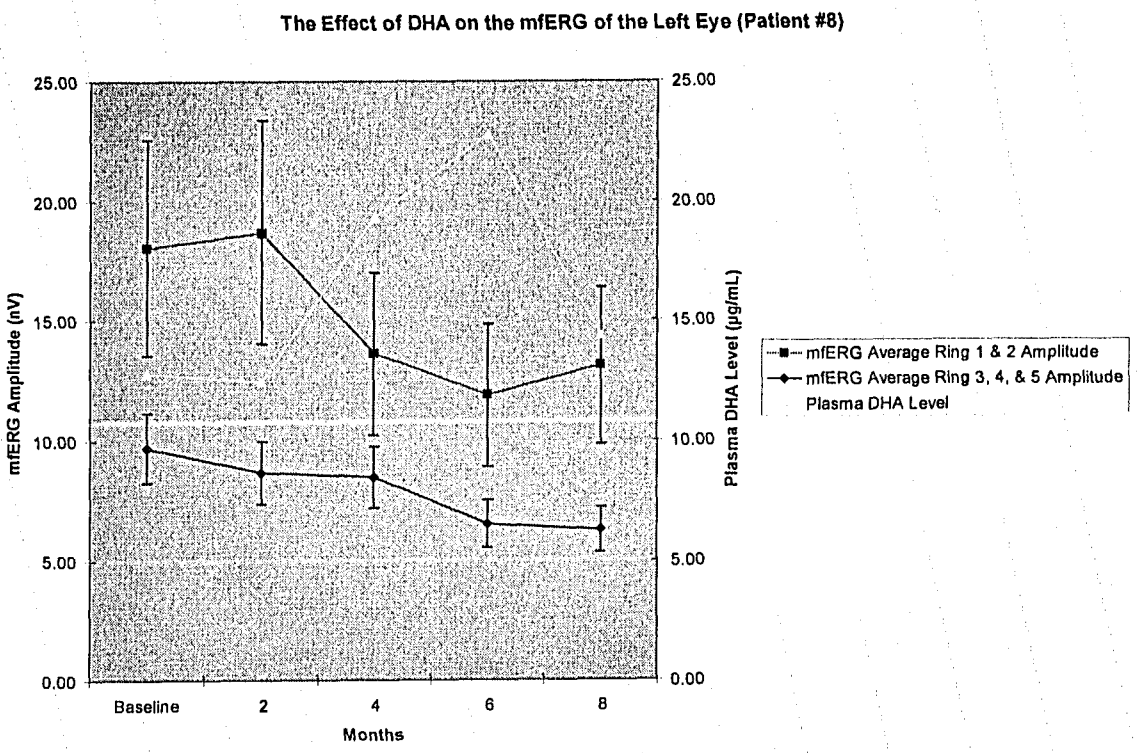

Figure 3-37: The effect of DHA on the average central and peripheral rings amplitude of the mfERG of the left eye.

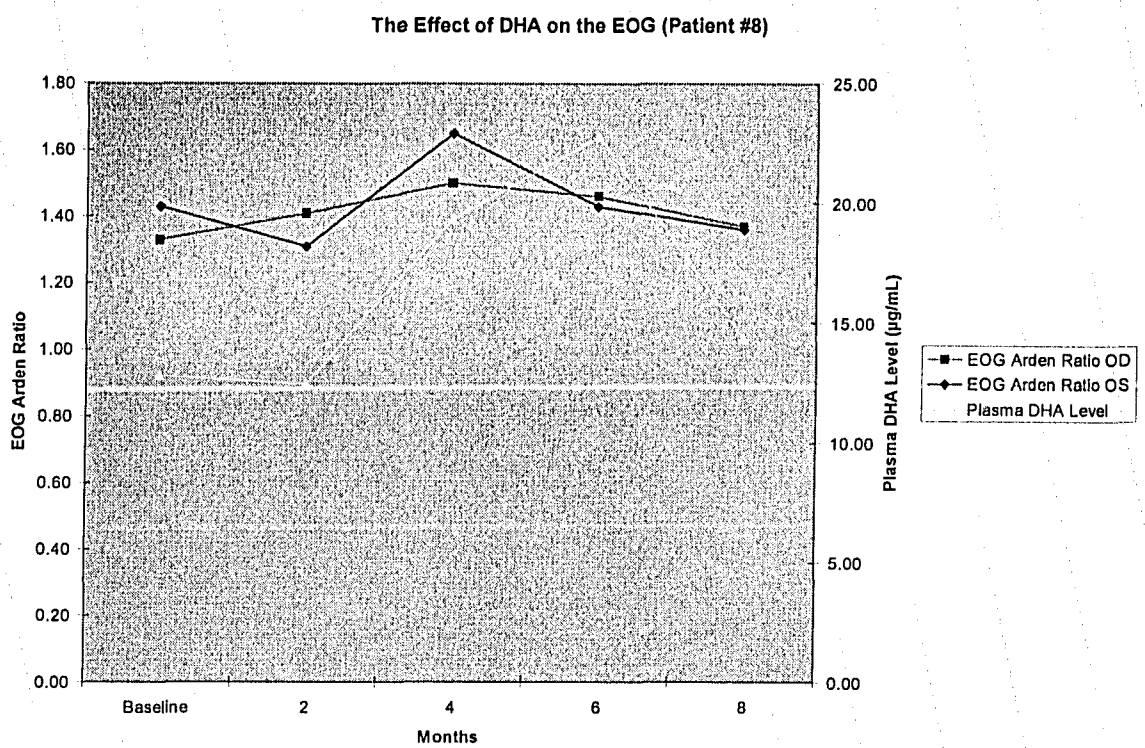

Figure 3-38: The effect of DHA on the EOG of Patient #8.

Patient #8's subjective visual change is shown on Figure 3-39. The VF-14 Questionnaire score increased in the initial treatment period with placebo by 21% and did not change substantially throughout the trial.

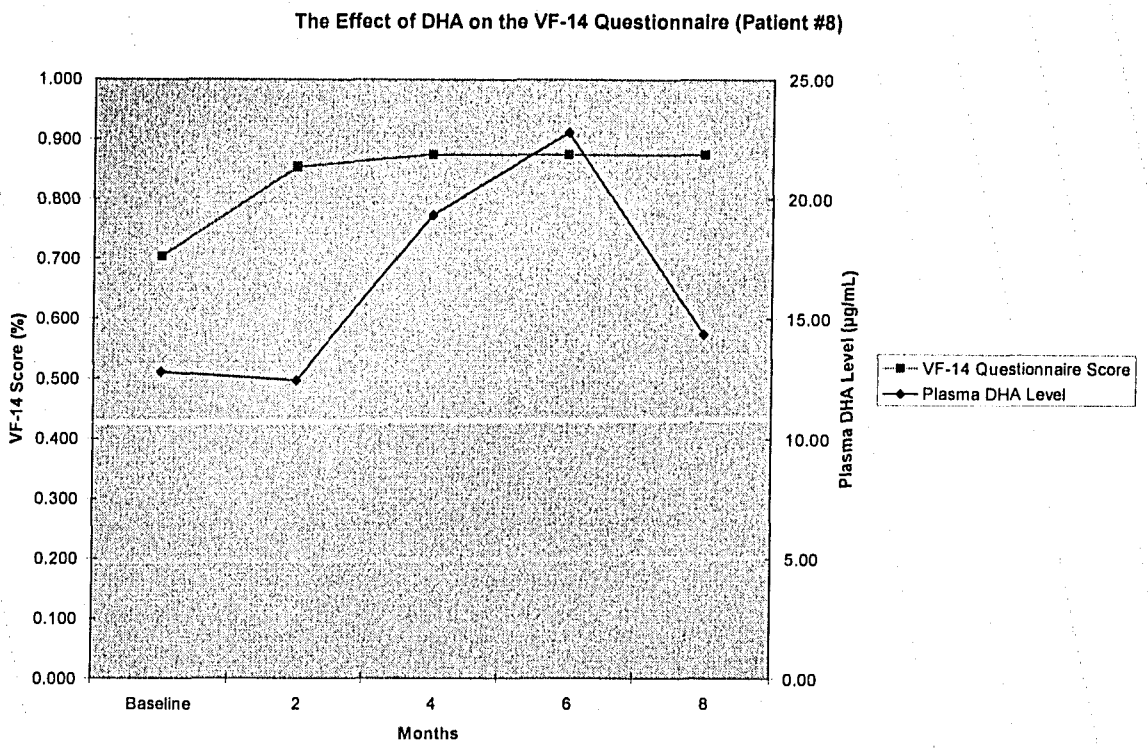

Figure 3-39: The effect of DHA on the VF-14 Questionnaire score for Patient #8.

The effect of DHA on the visual acuity of Patient #8 is shown on Figure 3-40. The right eye had a vision increase of three lines on the EDTRS scale in the first treatment period with placebo. The vision then decrease to return to baseline and ultimately gained one line of vision at the end of the trial while on placebo. For the left eye, the visual acuity improved by one line in the first treatment period with placebo. The improved vision was maintained until the end of the trial. The change in vision did not correspond to the changes in the plasma DHA levels.

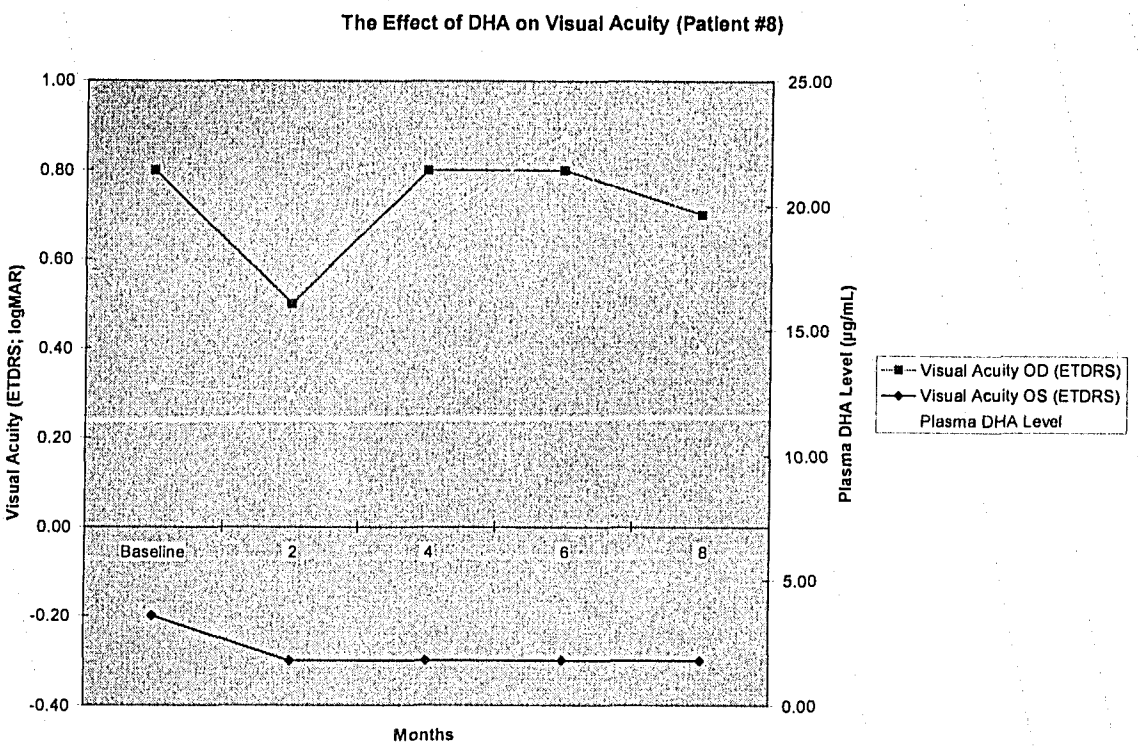

Figure 3-40: The effect of DHA on the visual acuity (EDTRS) of Patient #8.

Overall Analysis

Taken together, the individual analysis allowed a complete examination into the effect of DHA on the outcome measures. Each patient had undergone two crossovers in the clinical trial. In this section, each crossover and the carryover effects of DHA will be examined.

First Crossover

The first crossover was analyzed by the Wilcoxon Signed Ranks Test, which is a non-parametric test equivalent to the paired T-test. The group taking the DHA supplements is compared to the group taking the placebo treatment during the first

crossover. The DHA treatment data in the first treatment period for patients in Group 1 is combined with DHA treatment data in the second treatment period for patients in Group 2. Similarly, the placebo treatment data in the second treatment period for patients in Group 1 are combined with the placebo treatment data in the first treatment period for patients in Group 2. A representative table of the data is seen in Table 3-10 outlining the mfERG results of the right eye.

Table 3-10: The central mfERG amplitudes of the right eye in patients after the first treatment period.

Group*	mfERG amplitude DHA (A)	mfERG amplitude placebo (B)	Difference in Amplitude (B-A)	Rank of the Absolute Difference
1	13.18	14.23	1.05	2
1	13.00	10.15	-2.85	7
1	11.33	9.00	-2.33	6
1	9.40	12.90	3.50	8
2	7.70	8.83	1.13	3
2	12.08	10.03	-2.05	5
2	12.13	12.45	0.32	1
2	10.05	11.20	1.15	4

* Group 1: Patients on DHA in the first treatment period; 2: Patients on placebo in the first treatment period.

The patients are divided into two groups depending on the type of treatment taken in the first treatment period. Hence, half the patients took DHA first and half took placebo first before switching over to the other treatment. The data, as shown in the Table 3-10, shows the compilation of the mfERG amplitudes the DHA and placebo after the treatment periods. The difference in amplitudes between the two treatment periods (DHA vs. placebo) are ranked based on their absolute values. The sum of the ranks for the positive differences and negative differences are tallied, and compared using the Wilcoxon Signed Ranks Test (Table 3-11). This test is an equivalent of the

paired t-test. The paired t-test assumes that the (population) distribution of the differences has a normal distribution. This assumption cannot be made as our trial consists of only eight patients.

Table 3-11: The comparison of central mfERG amplitudes of the right eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

ERG_R1_A: central mfERG amplitude with DHA, first crossover
ERG_R1_B: central mfERG amplitude with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R1_B - ERG_R1_A	Negative Ranks	3[a]	6.00	18.00
	Positive Ranks	5[b]	3.60	18.00
	Ties	0[c]		
	Total	8		

a. ERG_R1_B < ERG_R1_A

b. ERG_R1_B > ERG_R1_A

c. ERG_R1_B = ERG_R1_A

Test Statistics[b]

	ERG_R1_B - ERG_R1_A
Z	.000[a]
Asymp. Sig. (2-tailed)	1.000

a. The sum of negative ranks equals the sum of positive ranks.

b. Wilcoxon Signed Ranks Test

The sum of ranks for the positive differences (higher amplitude with placebo) and negative differences (higher amplitude with DHA) are the same for the average central rings mfERG amplitudes of the right eye. The lack of difference between the two sum of ranks equates to an Z score of zero and a statistical significance of one (not statistically significant) (Table 3-11).

For the average peripheral rings mfERG amplitude of the right eye, the sum of the negative ranks (higher amplitude with DHA) is higher than the sum of the positive ranks. The difference between the two sums had a Z score of -1.12 and is not statistically significant (Table 3-12).

Table 3-12: The comparison of peripheral mfERG amplitudes of the right eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

ERG_R2_A: peripheral mfERG amplitude with DHA, first crossover
ERG_R2_B: peripheral mfERG amplitude with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R2_B - ERG_R2_A	Negative Ranks	6[a]	4.33	26.00
	Positive Ranks	2[b]	5.00	10.00
	Ties	0[c]		
	Total	8		

a. ERG_R2_B < ERG_R2_A

b. ERG_R2_B > ERG_R2_A

c. ERG_R2_B = ERG_R2_A

Test Statistics[b]

	ERG_R2_B - ERG_R2_A
Z	-1.120[a]
Asymp. Sig. (2-tailed)	.263

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

The comparison of the average central rings mfERG amplitudes of the left eye are shown in Table 3-13. The sum of the positive ranks is higher than the sum of the negative ranks indicating that the amplitudes were higher while the patients were on

placebo treatment. The difference between the ranks is not significant with a Z score of -0.84 and p value of 0.401.

Table 3-13: The comparison of central mfERG amplitudes of the left eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

ERG_R1_A: central mfERG amplitude with DHA, first crossover
ERG_R1_B: central mfERG amplitude with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R1_B - ERG_R1_A	Negative Ranks	3[a]	4.00	12.00
	Positive Ranks	5[b]	4.80	24.00
	Ties	0[c]		
	Total	8		

a. ERG_R1_B < ERG_R1_A

b. ERG_R1_B > ERG_R1_A

c. ERG_R1_B = ERG_R1_A

Test Statistics[b]

	ERG_R1_B - ERG_R1_A
Z	-.840[a]
Asymp. Sig. (2-tailed)	.401

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

For the peripheral rings mfERG amplitudes of the left eye, the comparison shows the sum of ranks is higher for the positive ranks than the negative ranks, similar to the central mfERG amplitudes (Table 3-14). The resulting Z score is -0.42 and the p value is 0.674, which is not statistically significant.

Table 3-14: The comparison of peripheral mfERG amplitudes of the left eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

ERG_R2_A: peripheral mfERG amplitude with DHA, first crossover
ERG_R2_B: peripheral mfERG amplitude with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R2_B - ERG_R2_A	Negative Ranks	3[a]	5.00	15.00
	Positive Ranks	5[b]	4.20	21.00
	Ties	0[c]		
	Total	8		

a. ERG_R2_B < ERG_R2_A

b. ERG_R2_B > ERG_R2_A

c. ERG_R2_B = ERG_R2_A

Test Statistics[b]

	ERG_R2_B - ERG_R2_A
Z	-.420[a]
Asymp. Sig. (2-tailed)	.674

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

The data comparison of the EOG Arden ratios of the right eye is shown in Table 3-15. For the first crossover, the sum of ranks for the positive ranks is higher than that for the negative ranks. This result means that the Arden ratio under placebo treatment was higher compared to that of DHA treatment. The Wilcoxon Signed Ranks Test gave a Z score of -1.829 with a p value of 0.067. The p value is considered statistically insignificant, but is considered to be "probably" significant.

Table 3-15: The comparison of EOG Arden ratio of the right eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

EOG_OD_A: EOG Arden ratio with DHA, first crossover
EOG_OD_B: EOG Arden ratio with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
EOG_OD_B - EOG_OD_A	Negative Ranks	2[a]	2.50	5.00
	Positive Ranks	6[b]	5.17	31.00
	Ties	0[c]		
	Total	8		

a. EOG_OD_B < EOG_OD_A

b. EOG_OD_B > EOG_OD_A

c. EOG_OD_B = EOG_OD_A

Test Statistics[b]

	EOG_OD_B - EOG_OD_A
Z	-1.829[a]
Asymp. Sig. (2-tailed)	.067

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

For the left eye, the EOG Arden ratio behaved similar to that of the right eye. The ratio is higher during the placebo treatment than the DHA treatment. The sum of ranks for the positive ranks was higher than the negative ranks (Table 3-16). Statistically, the comparison shows a Z score of -1.262 and a p value of 0.207. This result is statistically insignificant.

Table 3-16: The comparison of EOG Arden ratio of the right eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

EOG_OS_A: EOG Arden ratio with DHA, first crossover
EOG_OS_B: EOG Arden ratio with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
EOG_OS_B - EOG_OS_A	Negative Ranks	3[a]	3.00	9.00
	Positive Ranks	5[b]	5.40	27.00
	Ties	0[c]		
	Total	8		

a. EOG_OS_B < EOG_OS_A

b. EOG_OS_B > EOG_OS_A

c. EOG_OS_B = EOG_OS_A

Test Statistics[b]

	EOG_OS_B - EOG_OS_A
Z	-1.262[a]
Asymp. Sig. (2-tailed)	.207

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

The patients' subjective vision, as measured by the VF-14 questionnaire, was more improved during the placebo treatment than the DHA treatment (Table 3-17). The sum of ranks for the positive ranks is higher than that of the negative ranks. The resulting Z score is -0.931 with a p value of 0.352. The difference between the sum of ranks for the questionnaire score is statistically insignificant.

Table 3-17: The comparison of VF-14 questionnaire in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

VF14_A: VF-14 questionnaire with DHA, first crossover
VF14_B: VF-14 questionnaire with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
VF14_B - VF14_A	Negative Ranks	2[a]	4.25	8.50
	Positive Ranks	5[b]	3.90	19.50
	Ties	1[c]		
	Total	8		

a. VF14_B < VF14_A

b. VF14_B > VF14_A

c. VF14_B = VF14_A

Test Statistics[b]

	VF14_B - VF14_A
Z	-.931[a]
Asymp. Sig. (2-tailed)	.352

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

For the visual acuity of the right eye, the comparison of DHA and placebo treatment is shown in Table 3-18. A lower score on the ETDRS system of visual assessment signifies a better visual acuity. Hence, when interpreting the sum of ranks, the negative ranks represent a worsening of vision and positive ranks represent an improvement of vision in the first crossover. The sum of ranks of positive ranks was greater than the negative ranks with a Z score of -1.134 and a p value of 0.257, which is not statistically significant.

Table 3-18: The comparison of the visual acuity of the right eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

VA_OD_A: visual acuity, right eye, with DHA, first crossover
VA_OD_B: visual acuity, right eye, with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
VA_OD_B - VA_OD_A	Negative Ranks	1[a]	2.00	2.00
	Positive Ranks	3[b]	2.67	8.00
	Ties	4[c]		
	Total	8		

a. VA_OD_B < VA_OD_A

b. VA_OD_B > VA_OD_A

c. VA_OD_B = VA_OD_A

Test Statistics[b]

	VA_OD_B - VA_OD_A
Z	-1.134[a]
Asymp. Sig. (2-tailed)	.257

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

The comparison of visual acuity of the left eye during the first crossover can be seen in Table 3-19. The sum of ranks for the negative ranks (worsening of vision) is greater than the sum for the positive ranks (improvement of vision). The Z score from the Wilcoxon Sign Ranked Test was -1.414 with a p value of 0.157. The difference between the two sum of ranks was not statistically significant.

Table 3-19: The comparison of the visual acuity of the left eye in patients taking DHA or placebo during the first treatment period using the Wilcoxon Signed Ranks Test.

VA_OD_A: visual acuity, left eye, with DHA, first crossover
VA_OD_B: visual acuity, left eye, with placebo, first crossover

Ranks

		N	Mean Rank	Sum of Ranks
VA_OS_B - VA_OS_A	Negative Ranks	2[a]	1.50	3.00
	Positive Ranks	0[b]	.00	.00
	Ties	6[c]		
	Total	8		

a. VA_OS_B < VA_OS_A

b. VA_OS_B > VA_OS_A

c. VA_OS_B = VA_OS_A

Test Statistics[b]

	VA_OS_B - VA_OS_A
Z	-1.414[a]
Asymp. Sig. (2-tailed)	.157

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

Second Crossover

In the second crossover, the patients in the Group 1 took placebo in the second treatment period and DHA in the third treatment period, while patients in Group 2 took the opposite. For the analysis, the placebo treatment data in the second treatment period for patients in Group 1 is combined with placebo treatment data in the third treatment period for patients in Group 2. Likewise, the DHA treatment data in the third treatment period for patients in Group 1 is combined with DHA treatment data in the second treatment period for patients in Group 2. The data is analyzed in the same manner as the first crossover by utilizing the Wilcoxon Sign Ranked Test.

The comparison of the central rings mfERG amplitudes of the right eye is shown in Table 3-20. The positive ranks represent amplitudes which were higher after the DHA treatment period than the placebo treatment period, whereas the negative ranks represent amplitudes higher after the placebo treatment period compared to the DHA treatment. The sum of ranks shows a higher sum for the positive ranks than the negative ranks, which shows the DHA amplitudes were higher than that of the placebo. The Wilcoxon Sign Ranked Test calculates the Z score to be -1.54 with a p value of 0.123, which is not statistically significant.

Table 3-20: The comparison of the central rings mfERG amplitudes of the right eye during the second crossover.

ERG_R1_B: central mfERG amplitudes, placebo treatment, second crossover
ERG_R1_C: central mfERG amplitudes, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R1_C - ERG_R1_B	Negative Ranks	2[a]	3.50	7.00
	Positive Ranks	6[b]	4.83	29.00
	Ties	0[c]		
	Total	8		

a. ERG_R1_C < ERG_R1_B
b. ERG_R1_C > ERG_R1_B
c. ERG_R1_C = ERG_R1_B

Test Statistics[b]

	ERG_R1_C - ERG_R1_B
Z	-1.540[a]
Asymp. Sig. (2-tailed)	.123

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

The peripheral rings mfERG amplitudes behaved similar to the central rings. The sum of ranks for the positive ranks is greater than the negative ranks signifying

that higher amplitudes prevail during DHA treatment (Table 3-21). The analysis reveals the Z score to be -0.42 with a p value of 0.674, which is statistically insignificant.

Table 3-21: The comparison of the peripheral rings mfERG amplitudes of the right eye during the second crossover.

ERG_R2_B: peripheral mfERG amplitudes, placebo treatment, second crossover
ERG_R2_C: peripheral mfERG amplitudes, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R2_C - ERG_R2_B	Negative Ranks	3[a]	5.00	15.00
	Positive Ranks	5[b]	4.20	21.00
	Ties	0[c]		
	Total	8		

a. ERG_R2_C < ERG_R2_B
b. ERG_R2_C > ERG_R2_B
c. ERG_R2_C = ERG_R2_B

Test Statistics[b]

	ERG_R2_C - ERG_R2_B
Z	-.420[a]
Asymp. Sig. (2-tailed)	.674

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

For the left eye, the comparison of the central rings mfERG amplitudes after either DHA or placebo treatment is shown in Table 3-22. The sum of ranks for the negative ranks (higher amplitudes after placebo treatment) is higher than the positive ranks (higher amplitudes during DHA treatment). The Z score is -0.98 with a p value of 0.327, which is not statistically significant.

Table 3-22: The comparison of the central rings mfERG amplitudes of the left eye during the second crossover.

ERG_R1_B: central mfERG amplitudes, placebo treatment, second crossover
ERG_R1_C: central mfERG amplitudes, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R1_C - ERG_R1_B	Negative Ranks	5[a]	5.00	25.00
	Positive Ranks	3[b]	3.67	11.00
	Ties	0[c]		
	Total	8		

a. ERG_R1_C < ERG_R1_B

b. ERG_R1_C > ERG_R1_B

c. ERG_R1_C = ERG_R1_B

Test Statistics[b]

	ERG_R1_C - ERG_R1_B
Z	-.980[a]
Asymp. Sig. (2-tailed)	.327

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

The peripheral rings mfERG amplitudes of the left eye behaved opposite to that of the central rings. The sum of ranks for the positive ranks is higher than the negative ranks demonstrating that DHA relate to higher mfERG amplitudes than placebo (Table 3-23). The analysis gives a Z score of -0.84 with a p value of 0.401, which is not statistically significant.

Table 3-23: The comparison of the peripheral rings mfERG amplitudes of the left eye during the second crossover.

ERG_R2_B: peripheral mfERG amplitudes, placebo treatment, second crossover
ERG_R2_C: peripheral mfERG amplitudes, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R2_C - ERG_R2_B	Negative Ranks	3[a]	4.00	12.00
	Positive Ranks	5[b]	4.80	24.00
	Ties	0[c]		
	Total	8		

a. ERG_R2_C < ERG_R2_B

b. ERG_R2_C > ERG_R2_B

c. ERG_R2_C = ERG_R2_B

Test Statistics[b]

	ERG_R2_C - ERG_R2_B
Z	-.840[a]
Asymp. Sig. (2-tailed)	.401

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

The data comparison of the EOG Arden ratios of the right eye is shown in Table 3-24. The sum of ranks for the negative ranks is higher than that for the positive ranks. This result means that the Arden ratio under placebo treatment was higher compared to that of DHA treatment. The Wilcoxon Signed Ranks Test gives a Z score of -0.56 with a p value of 0.575. The p value is considered statistically insignificant.

Table 3-24: The comparison of the EOG Arden ratio of the right eye during the second crossover.

EOG_OD_B: EOG Arden ratio of the right eye, placebo treatment, second crossover
EOG_OD_C: EOG Arden ratio of the right eye, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
EOG_OD_C - EOG_OD_B	Negative Ranks	5[a]	4.40	22.00
	Positive Ranks	3[b]	4.67	14.00
	Ties	0[c]		
	Total	8		

a. EOG_OD_C < EOG_OD_B

b. EOG_OD_C > EOG_OD_B

c. EOG_OD_C = EOG_OD_B

Test Statistics[b]

	EOG_OD_C - EOG_OD_B
Z	-.560[a]
Asymp. Sig. (2-tailed)	.575

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

For the left eye, the EOG Arden ratio behaved similar to that of the right eye. The ratio is higher after the placebo treatment than the DHA treatment. The sum of ranks for the positive ranks is higher than the negative ranks (Table 3-25). Statistically, the comparison shows a Z score of -0.421 and a p value of 0.674. This result is statistically insignificant.

Table 3-25: The comparison of the EOG Arden ratio of the left eye during the second crossover.

EOG_OS_B: EOG Arden ratio of the left eye, placebo treatment, second crossover
EOG_OS_C: EOG Arden ratio of the left eye, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
EOG_OS_C - EOG_OS_B	Negative Ranks	3[a]	7.00	21.00
	Positive Ranks	5[b]	3.00	15.00
	Ties	0[c]		
	Total	8		

a. EOG_OS_C < EOG_OS_B

b. EOG_OS_C > EOG_OS_B

c. EOG_OS_C = EOG_OS_B

Test Statistics[b]

	EOG_OS_C - EOG_OS_B
Z	-.421[a]
Asymp. Sig. (2-tailed)	.674

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

The patients' subjective vision, as measured by the VF-14 questionnaire, was more improved after the DHA treatment than the placebo treatment (Table 3-26). The sum of ranks for the positive ranks is higher than that of the negative ranks. The resulting Z score is -1.521 with a p value of 0.128. The difference between the sum of ranks for the questionnaire score is statistically insignificant.

Table 3-26: The comparison of the VF-14 questionnaire during the second crossover.

VF14_B: VF-14 questionnaire, placebo treatment, second crossover
VF14_C: VF-14 questionnaire, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
VF14_C - VF14_B	Negative Ranks	2[a]	2.50	5.00
	Positive Ranks	5[b]	4.60	23.00
	Ties	1[c]		
	Total	8		

a. VF14_C < VF14_B
b. VF14_C > VF14_B
c. VF14_C = VF14_B

Test Statistics[b]

	VF14_C - VF14_B
Z	-1.521[a]
Asymp. Sig. (2-tailed)	.128

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

For the visual acuity of the right eye, the comparison of DHA and placebo treatment is shown in Table 3-27. The sum of ranks of positive ranks is equal to the negative ranks giving a Z score of zero and a p value of one, which is not statistically significant. No difference was found in the visual acuity in either DHA or placebo treatment periods.

Table 3-27: The comparison of the visual acuity of the right eye during the second crossover.

VA_OD_B: visual acuity of the right eye, placebo treatment, second crossover
VA_OD_C: visual acuity of the right eye, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
VA_OD_C - VA_OD_B	Negative Ranks	2[a]	2.50	5.00
	Positive Ranks	2[b]	2.50	5.00
	Ties	4[c]		
	Total	8		

a. VA_OD_C < VA_OD_B

b. VA_OD_C > VA_OD_B

c. VA_OD_C = VA_OD_B

Test Statistics[b]

	VA_OD_C - VA_OD_B
Z	.000[a]
Asymp. Sig. (2-tailed)	1.000

a. The sum of negative ranks equals the sum of positive ranks.

b. Wilcoxon Signed Ranks Test

The comparison of visual acuity of the left eye after the first crossover can be seen in Table 3-28. The sum of ranks for the negative ranks (improvement of vision) is greater than the sum for the positive ranks (worsening of vision). The Z score from the Wilcoxon Sign Ranked Test is -0.577 with a p value of 0.564. The difference between the two sum of ranks was not statistically significant.

Table 3-28: The comparison of the visual acuity of the left eye during the second crossover.

VA_OS_B: visual acuity of the left eye, placebo treatment, second crossover
VA_OS_C: visual acuity of the left eye, DHA treatment, second crossover

Ranks

		N	Mean Rank	Sum of Ranks
VA_OS_C - VA_OS_B	Negative Ranks	2[a]	2.00	4.00
	Positive Ranks	1[b]	2.00	2.00
	Ties	5[c]		
	Total	8		

a. VA_OS_C < VA_OS_B
b. VA_OS_C > VA_OS_B
c. VA_OS_C = VA_OS_B

Test Statistics[b]

	VA_OS_C - VA_OS_B
Z	-.577[a]
Asymp. Sig. (2-tailed)	.564

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

Combined Crossover Analysis

The combined crossover analysis involves the amalgamation of data from the first and second crossovers. The DHA and placebo treatment data from the first and second crossovers are grouped together and analyzed collectively. As a result, sixteen sets of data (eight sets from each crossover) are available for the analysis simulating a trial consisting of sixteen patients compared to eight.

The comparison of the central rings mfERG amplitudes of the right eye is shown in Table 3-29. The sum of ranks for the negative ranks (higher amplitudes after DHA intake) was higher than the positive ranks (higher amplitudes after placebo

intake). The Z score is -0.724 and the p value is 0.469, which is statistically insignificant.

Table 3-29: The comparison of the central rings mfERG amplitudes of the right eye during the first and second crossovers.

ERG_R1_A: central mfERG amplitudes, DHA treatment, first and second crossover
ERG_R1_B: central mfERG amplitudes, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R1_B - ERG_R1_A	Negative Ranks	9[a]	9.11	82.00
	Positive Ranks	7[b]	7.71	54.00
	Ties	0[c]		
	Total	16		

a. ERG_R1_B < ERG_R1_A
b. ERG_R1_B > ERG_R1_A
c. ERG_R1_B = ERG_R1_A

Test Statistics[b]

	ERG_R1_B - ERG_R1_A
Z	-.724[a]
Asymp. Sig. (2-tailed)	.469

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

For the left eye, the peripheral rings mfERG amplitudes was higher after DHA consumption compared to placebo (Table 3-30). The sum of ranks shows the negative ranks are higher than the positive ranks. The analysis gives a Z score of -1.086 with a p value of 0.277. The higher amplitudes after the DHA treatment period are not statistically significant.

Table 3-30: The comparison of the peripheral rings mfERG amplitudes of the right eye during the first and second crossovers.

ERG_R2_A: peripheral mfERG amplitudes, DHA treatment, first and second crossover
ERG_R2_B: peripheral mfERG amplitudes, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R2_B - ERG_R2_A	Negative Ranks	11[a]	8.09	89.00
	Positive Ranks	5[b]	9.40	47.00
	Ties	0[c]		
	Total	16		

a. ERG_R2_B < ERG_R2_A

b. ERG_R2_B > ERG_R2_A

c. ERG_R2_B = ERG_R2_A

Test Statistics[b]

	ERG_R2_B - ERG_R2_A
Z	-1.086[a]
Asymp. Sig. (2-tailed)	.277

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

The comparison of the average central rings mfERG amplitudes of the left is shown in Table 3-31. The sum of the positive ranks was higher than the sum of the negative ranks indicating that the amplitudes were higher after the patients were on placebo treatment. The difference between the ranks is not significant with a Z score of -1.319 and p value of 0.187.

Table 3-31: The comparison of the average central rings mfERG amplitudes of the left eye during the first and second crossovers.

ERG_R1_A: central mfERG amplitudes, DHA treatment, first and second crossover
ERG_R1_B: central mfERG amplitudes, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R1_B - ERG_R1_A	Negative Ranks	6[a]	7.08	42.50
	Positive Ranks	10[b]	9.35	93.50
	Ties	0[c]		
	Total	16		

a. ERG_R1_B < ERG_R1_A

b. ERG_R1_B > ERG_R1_A

c. ERG_R1_B = ERG_R1_A

Test Statistics[b]

	ERG_R1_B - ERG_R1_A
Z	-1.319[a]
Asymp. Sig. (2-tailed)	.187

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

The peripheral rings mfERG amplitudes of the left eye behaved opposite to that of the central rings. The sum of ranks for the negative ranks is higher than the positive ranks demonstrating that DHA relate to higher mfERG amplitudes than placebo (Table 3-32). The analysis gives a Z score of -0.259 with a p value of 0.796, which is not statistically significant.

Table 3-32: The comparison of the average peripheral rings mfERG amplitudes of the left eye during the first and second crossovers.

ERG_R2_A: peripheral mfERG amplitudes, DHA treatment, first and second crossover
ERG_R2_B: peripheral mfERG amplitudes, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
ERG_R2_B - ERG_R2_A	Negative Ranks	8[a]	9.13	73.00
	Positive Ranks	8[b]	7.88	63.00
	Ties	0[c]		
	Total	16		

a. ERG_R2_B < ERG_R2_A
b. ERG_R2_B > ERG_R2_A
c. ERG_R2_B = ERG_R2_A

Test Statistics[b]

	ERG_R2_B - ERG_R2_A
Z	-.259[a]
Asymp. Sig. (2-tailed)	.796

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

The data comparison of the EOG Arden ratios of the right eye is shown in Table 3-33. The sum of ranks for the positive ranks is higher than that for the negative ranks. This result means that the Arden ratio under placebo treatment is higher compared to that of DHA treatment. The Wilcoxon Signed Ranks Test gives a Z score of -1.501 with a p value of 0.133. The p value is considered statistically insignificant.

Table 3-33: The comparison of the EOG Arden ratio of the right eye during the first and second crossovers.

EOG_OD_A: EOG Arden ratio, DHA treatment, first and second crossover
EOG_OD_B: EOG Arden ratio, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
EOG_OD_B - EOG_OD_A	Negative Ranks	5[a]	7.80	39.00
	Positive Ranks	11[b]	8.82	97.00
	Ties	0[c]		
	Total	16		

a. EOG_OD_B < EOG_OD_A

b. EOG_OD_B > EOG_OD_A

c. EOG_OD_B = EOG_OD_A

Test Statistics[b]

	EOG_OD_B - EOG_OD_A
Z	-1.501[a]
Asymp. Sig. (2-tailed)	.133

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

For the left eye, the EOG Arden ratio behaved similar to that of the right eye. The Arden ratio is higher after the placebo treatment than the DHA treatment. The sum of ranks for the positive ranks is higher than the negative ranks (Table 3-34). Statistically, the comparison shows a Z score of -1.19 and a p value of 0.234. This result is statistically insignificant.

Table 3-34: The comparison of the EOG Arden ratio of the left eye during the first and second crossovers.

EOG_OS_A: EOG Arden ratio, DHA treatment, first and second crossover
EOG_OS_B: EOG Arden ratio, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
EOG_OS_B - EOG_OS_A	Negative Ranks	8[a]	5.63	45.00
	Positive Ranks	8[b]	11.38	91.00
	Ties	0[c]		
	Total	16		

a. EOG_OS_B < EOG_OS_A

b. EOG_OS_B > EOG_OS_A

c. EOG_OS_B = EOG_OS_A

Test Statistics[b]

	EOG_OS_B - EOG_OS_A
Z	-1.190[a]
Asymp. Sig. (2-tailed)	.234

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

The patients' subjective vision, as measured by the VF-14 questionnaire, was more improved after the DHA treatment than the placebo treatment after the two crossovers (Table 3-35). The sum of ranks for the negative ranks is higher than that of the positive ranks. The resulting Z score is -0.440 with a p value of 0.66. The difference between the sum of ranks for the questionnaire score is statistically insignificant.

Table 3-35: The comparison of the VF-14 questionnaire during the first and second crossover.

VF14_A: VF-14 questionnaire, DHA treatment, first and second crossover
VF14_B: VF-14 questionnaire, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
VF14_B - VF14_A	Negative Ranks	7[a]	8.50	59.50
	Positive Ranks	7[b]	6.50	45.50
	Ties	2[c]		
	Total	16		

a. VF14_B < VF14_A
b. VF14_B > VF14_A
c. VF14_B = VF14_A

Test Statistics[b]

	VF14_B - VF14_A
Z	-.440[a]
Asymp. Sig. (2-tailed)	.660

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

The comparison of DHA and placebo treatment for the visual acuity of the right eye is shown in Table 3-36. As mentioned previously, a lower score on the ETDRS system of visual assessment signifies a better visual acuity. Hence, when interpreting the sum of ranks, the negative ranks represent a worsening of vision and positive ranks represent an improvement of vision in the first crossover. The sum of ranks of positive ranks is greater than the negative ranks in the combined crossover analysis with a Z score of -0.905 and a p value of 0.366, which is not statistically significant.

Table 3-36: The comparison of the visual acuity of the right eye during the first and second crossover.

VA_OD_A: visual acuity of the right eye, DHA treatment, first and second crossover
VA_OD_B: visual acuity of the right eye, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
VA_OD_B - VA_OD_A	Negative Ranks	3[a]	4.00	12.00
	Positive Ranks	5[b]	4.80	24.00
	Ties	8[c]		
	Total	16		

a. VA_OD_B < VA_OD_A
b. VA_OD_B > VA_OD_A
c. VA_OD_B = VA_OD_A

Test Statistics[b]

	VA_OD_B - VA_OD_A
Z	-.905[a]
Asymp. Sig. (2-tailed)	.366

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

The comparison of visual acuity of the left eye during the first crossover is shown in Table 3-37. The sum of ranks for the negative ranks (worsening of vision) is greater than the sum for the positive ranks (improvement of vision). The Z score from the Wilcoxon Sign Ranked Test is -0.447 with a p value of 0.655. The difference between the two sum of ranks is not statistically significant.

Table 3-37: The comparison of the visual acuity of the left eye during the first and second crossover.

VA_OS_A: visual acuity of the left eye, DHA treatment, first and second crossover
VA_OS_B: visual acuity of the left eye, placebo treatment, first and second crossover

Ranks

		N	Mean Rank	Sum of Ranks
VA_OS_B - VA_OS_A	Negative Ranks	3[a]	3.00	9.00
	Positive Ranks	2[b]	3.00	6.00
	Ties	11[c]		
	Total	16		

a. VA_OS_B < VA_OS_A
b. VA_OS_B > VA_OS_A
c. VA_OS_B = VA_OS_A

Test Statistics[b]

	VA_OS_B - VA_OS_A
Z	-.447[a]
Asymp. Sig. (2-tailed)	.655

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

Correlation Analysis

The crossover analysis covered the changes in the primary outcomes between DHA and placebo consumption. A correlation analysis is required to explore whether the primary outcomes are related to plasma DHA levels. Given the small number of patients in this clinical trial, the assumption of a normal distribution cannot be made. Thus, the Pearson correlation cannot be used, and the nonparametric method using Spearman's rank correlation is appropriate in this scenario. The Spearman's rank correlation will be used to analysis all the primary outcomes data with the corresponding plasma DHA data.

The correlation of the central rings mfERG amplitudes of the right eye is shown on Figure 3-41, and the analysis is conveyed in Table 3-38. The correlation coefficient (Spearman's rho) is 0.073 with a p value of 0.653. The slight positive correlation, DHA has a positive influence on the amplitudes, is statistically insignificant.

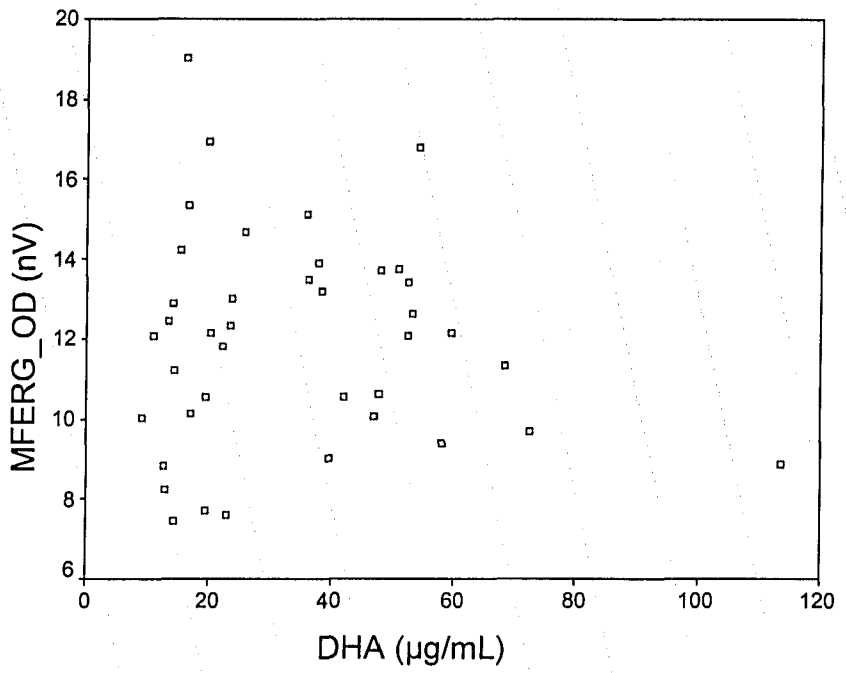

Figure 3-41: The effect of DHA on the average central rings mfERG amplitude of the right eye (MFERG_OD: central mfERG amplitude of the right eye).

Table 3-38: The correlation of DHA and the average central rings mfERG of the right eye using Spearman's rank correlation (MFERG_OD: central mfERG amplitude of the right eye).

Correlations

			MFERG_OD	DHA
Spearman's rho	MFERG_OD	Correlation Coefficient	1.000	.073
		Sig. (2-tailed)	.	.653
		N	40	40
	DHA	Correlation Coefficient	.073	1.000
		Sig. (2-tailed)	.653	.
		N	40	40

For the peripheral rings mfERG amplitudes, the analysis shows a negative correlation between DHA and the amplitudes (Figure 3-42). Higher plasma levels results in a lower mfERG amplitude. The correlation coefficient is -0.191 with a p value of 0.237, which is not statistically significant (Table 3-39).

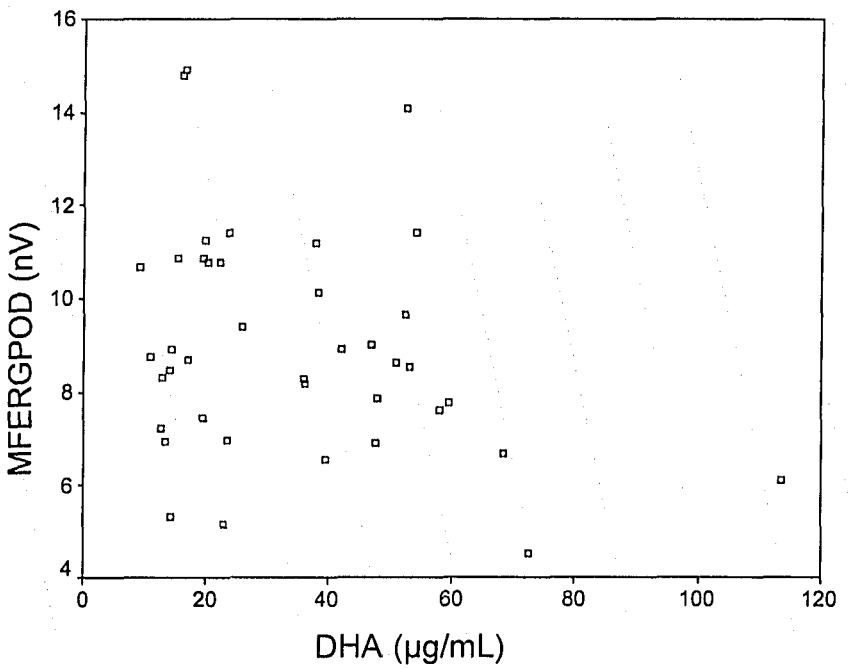

Figure 3-42: The effect of DHA on the average peripheral rings mfERG amplitude of the right eye (MFERGPOD: peripheral mfERG amplitude of the right eye).

Table 3-39: The correlation of DHA and the average peripheral rings mfERG of the right eye using Spearman's rank correlation (MFERGPOD: peripheral mfERG amplitude of the right eye).

Correlations

			MFERGPOD	DHA
Spearman's rho	MFERGPOD	Correlation Coefficient	1.000	-.191
		Sig. (2-tailed)	.	.237
		N	40	40
	DHA	Correlation Coefficient	-.191	1.000
		Sig. (2-tailed)	.237	.
		N	40	40

The correlation of DHA and the central rings mfERG amplitudes of the left eye is shown on Figure 3-43 and Table 3-40. The analysis shows a correlation of -0.372 and a p value of 0.018, which is statistically significant. Elevation of DHA results in lower central mfERG amplitudes of the left eye.

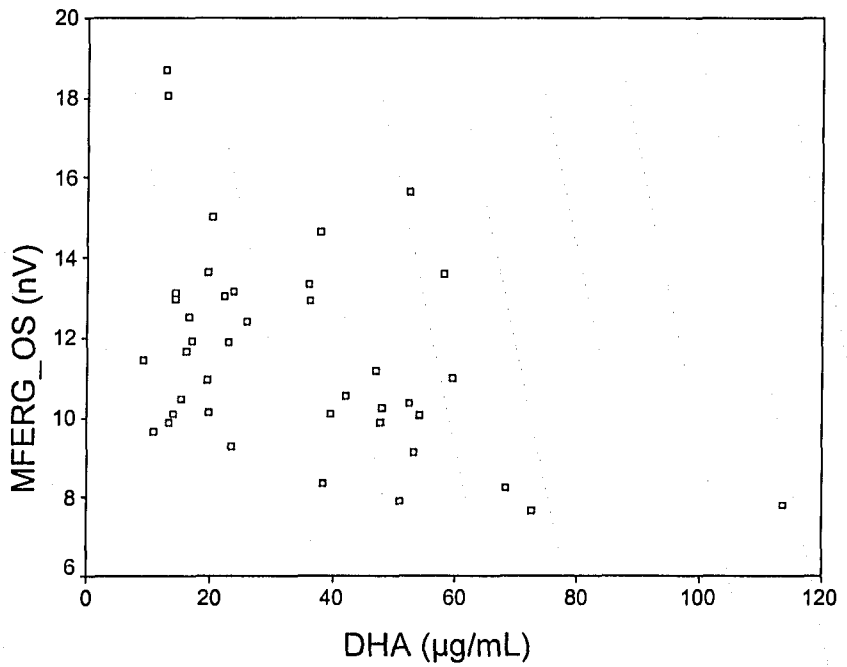

Figure 3-43: The effect of DHA on the average central rings mfERG amplitude of the left eye (MFERG_OS: central mfERG amplitude of the left eye).

Table 3-40: The correlation of DHA and the average central rings mfERG of the left eye using Spearman's rank correlation (MFERG_OS: central mfERG amplitude of the left eye).

Correlations

			MFERG_OS	DHA
Spearman's rho	MFERG_OS	Correlation Coefficient	1.000	-.372*
		Sig. (2-tailed)	.	.018
		N	40	40
	DHA	Correlation Coefficient	-.372*	1.000
		Sig. (2-tailed)	.018	.
		N	40	40

*. Correlation is significant at the 0.05 level (2-tailed).

For the average peripheral rings mfERG amplitudes of the left eye, the correlation coefficient was -0.234 with a p value of 0.147 (Figure 3-44 and Table 3-41). The negative correlation between DHA and peripheral mfERG amplitudes was not statistically significant.

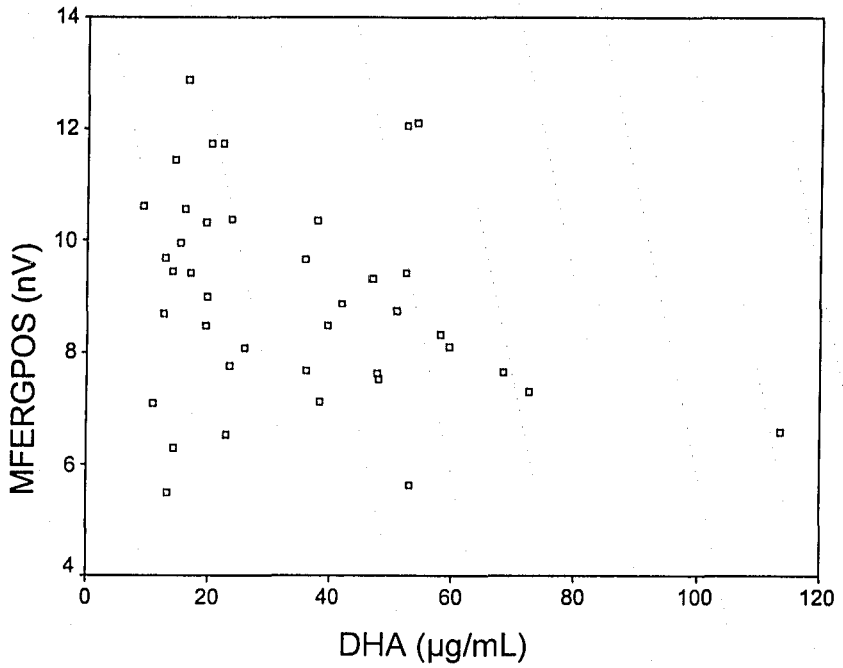

Figure 3-44: The effect of DHA on the average peripheral rings mfERG amplitude of the left eye (MFERGPOS: central mfERG amplitude of the left eye).

Table 3-41: The correlation of DHA and the average peripheral rings mfERG of the left eye using Spearman's rank correlation (MFERGPOS: peripheral mfERG amplitude of the left eye).

Correlations

			MFERGPOS	DHA
Spearman's rho	MFERGPOS	Correlation Coefficient	1.000	-.234
		Sig. (2-tailed)	.	.147
		N	40	40
	DHA	Correlation Coefficient	-.234	1.000
		Sig. (2-tailed)	.147	.
		N	40	40

The correlation of the EOG Arden ratio of the right eye is shown on Figure 3-45, and the analysis is shown in Table 3-42. The correlation coefficient (Spearman's rho) is 0.021 with a p value of 0.900. The slight positive correlation, DHA has a positive influence on the Arden ratio, is statistically insignificant.

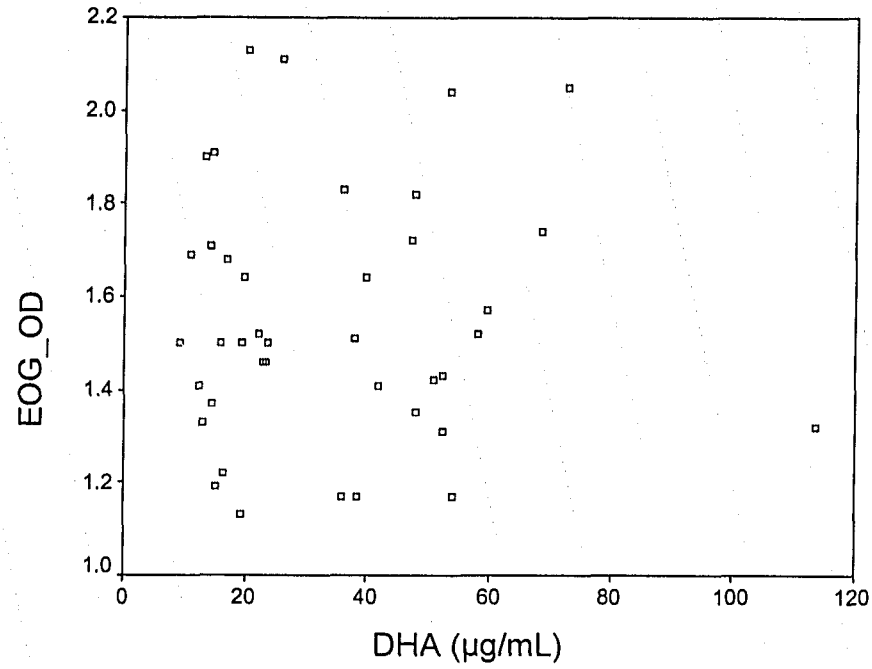

Figure 3-45: The effect of DHA on the EOG Arden ratio of the right eye (EOG_OD: EOG Arden ratio of the right eye).

Table 3-42: The correlation of DHA and the EOG Arden ratio of the right eye using Spearman's rank correlation (EOG_OD: EOG Arden ratio of the right eye).

Correlations

			EOG_OD	DHA
Spearman's rho	EOG_OD	Correlation Coefficient	1.000	.021
		Sig. (2-tailed)	.	.900
		N	40	40
	DHA	Correlation Coefficient	.021	1.000
		Sig. (2-tailed)	.900	.
		N	40	40

The correlation of DHA and the EOG of the left eye is shown on Figure 3-46 and Table 3-43. The analysis shows a correlation of 0.099 and a p value of 0.543, which is statistically insignificant.

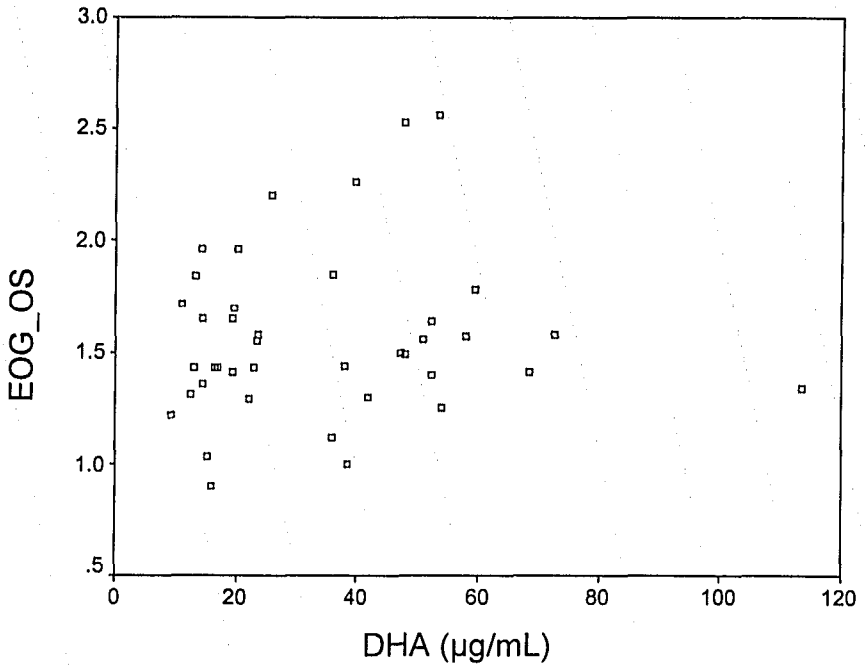

Figure 3-46: The effect of DHA on the EOG Arden ratio of the left eye (EOG_OS: EOG Arden ratio of the left eye).

Table 3-43: The correlation of DHA and the EOG Arden ratio of the left eye using Spearman's rank correlation (EOG_OS: EOG Arden ratio of the left eye).

Correlations

			EOG_OS	DHA
Spearman's rho	EOG_OS	Correlation Coefficient	1.000	.099
		Sig. (2-tailed)	.	.543
		N	40	40
	DHA	Correlation Coefficient	.099	1.000
		Sig. (2-tailed)	.543	.
		N	40	40

The patients' subjective vision, as measured by the VF-14 questionnaire, does not correlate with plasma DHA levels (Figure 3-47 and Table 3-44). The correlation coefficient is -0.111 suggesting a negative correlation between the VF-14 score and DHA plasma levels. The p value is 0.494, which is not statistically significant.

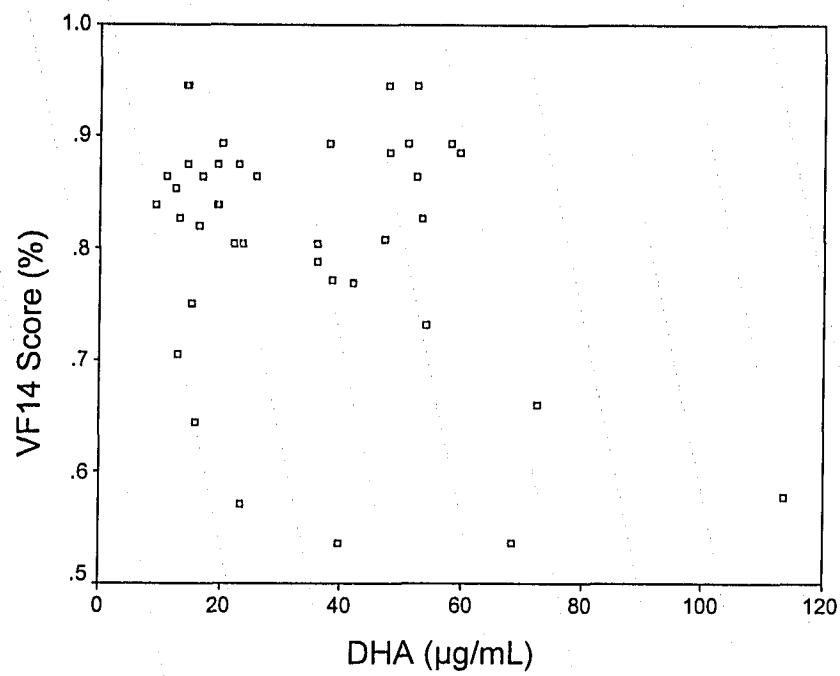

Figure 3-47: The effect of DHA on the VF-14 questionnaire score.

Table 3-44: The correlation of DHA VF-14 questionnaire score eye using Spearman's rank correlation.

Correlations

			VF14	DHA
Spearman's rho	VF14	Correlation Coefficient	1.000	-.111
		Sig. (2-tailed)	.	.494
		N	40	40
	DHA	Correlation Coefficient	-.111	1.000
		Sig. (2-tailed)	.494	.
		N	40	40

The correlation of the visual acuity of the right eye and plasma DHA levels is shown on Figure 3-48 and Table 3-45. The Spearman's rho correlation coefficient of 0.081 suggests a weakly positive correlation between DHA and visual acuity of the right eye, and the resultant p value of 0.621 is statistically insignificant.

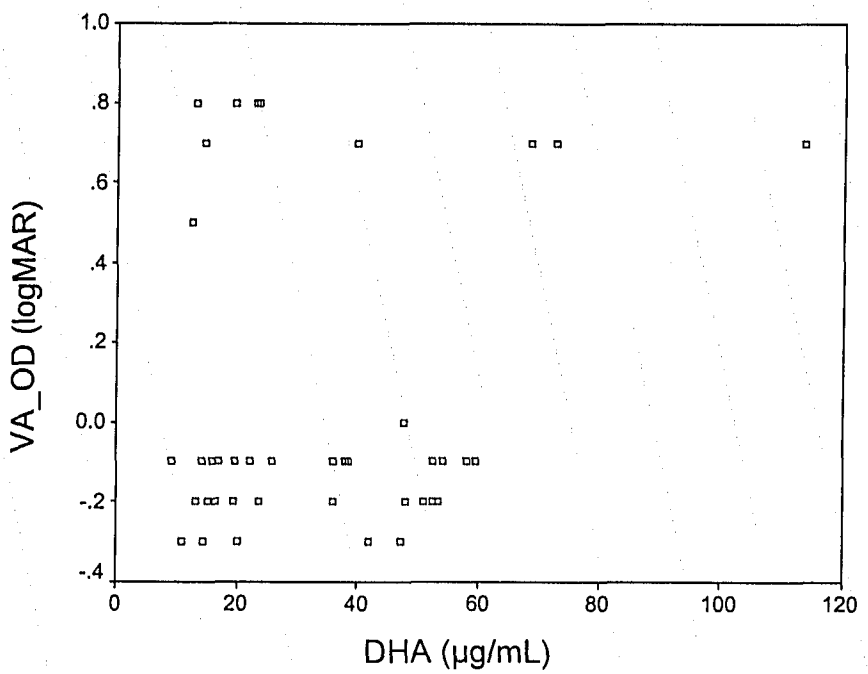

Figure 3-48: The effect of DHA on the visual acuity of the right eye (VA_OD: visual acuity of the right eye).

Table 3-45: The correlation of DHA and the visual acuity of the right eye using Spearman's rank correlation (VA_OD: visual acuity of the right eye).

Correlations

			VA_OD	DHA
Spearman's rho	VA_OD	Correlation Coefficient	1.000	.081
		Sig. (2-tailed)	.	.621
		N	40	40
	DHA	Correlation Coefficient	.081	1.000
		Sig. (2-tailed)	.621	.
		N	40	40

The visual acuity of the left eye correlates positively with plasma DHA levels (Figure 3-49 and Table 3-46). The Spearman's rho correlation coefficient is 0.401 suggesting that a higher level of plasma DHA corresponds to a higher visual acuity

score based on the ETDRS grading – a worsening of vision. The p value of 0.01 is statistically significant.

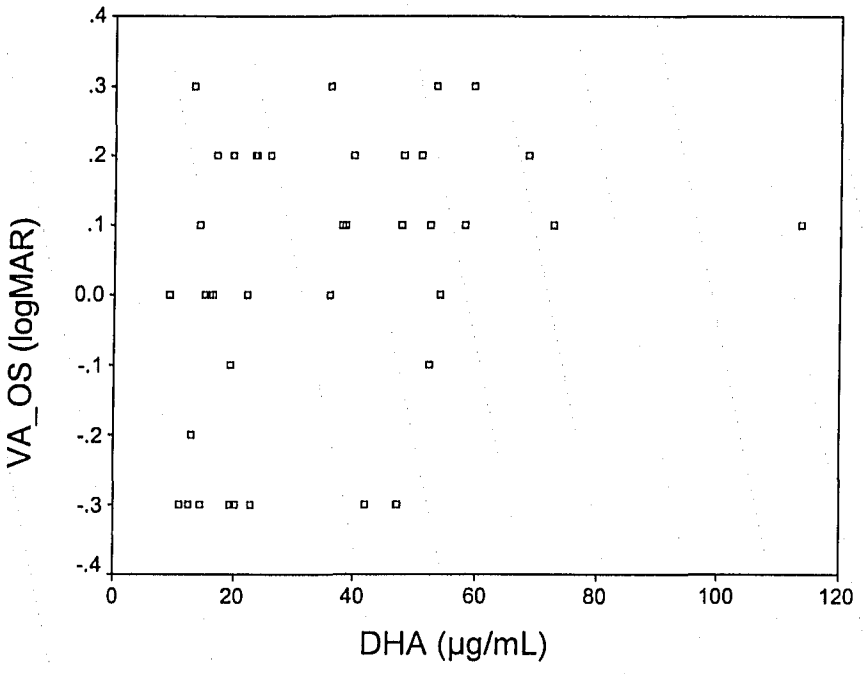

Figure 3-49: The effect of DHA on the visual acuity of the right eye (VA_OS: visual acuity of the left eye).

Table 3-46: The correlation of DHA and the visual acuity of the right eye using Spearman's rank correlation (VA_OS: visual acuity of the left eye).

Correlations

			VA_OS	DHA
Spearman's rho	VA_OS	Correlation Coefficient	1.000	.401*
		Sig. (2-tailed)	.	.010
		N	40	40
	DHA	Correlation Coefficient	.401*	1.000
		Sig. (2-tailed)	.010	.
		N	40	40

*. Correlation is significant at the 0.05 level (2-tailed).

Carryover Effects

The crossover trial relied on the basis that the DHA or placebo taken in one period will not be influential in the next period. In our trial, preclinical data from human and animal studies on DHA depletion and recovery was based upon findings from MacDonald and colleagues (2004) and Moriguchi's group (2001). A two month treatment period was established in the belief that this was adequate time to supplement and deplete DHA levels in the patients. A substantial carryover of the treatment leads to unreliable data and analysis. In our design of the clinical trial, the fourth treatment period, from month six to month eight, was utilized to examine the carryover effects of DHA, by having the patients continue on the same pill (DHA or placebo) as in the third treatment period. In Group 1, the patients were given DHA in the third and fourth treatment periods, and a carryover of DHA would give perhaps a continual rise or plateau of the plasma DHA level. In Group 2, the patients were given DHA in the second treatment period while receiving placebo in the rest (first, third, and fourth periods). This pattern gave an opportunity to assess carryover effects of DHA by comparing the first (placebo) and third (placebo after DHA) treatment periods. A carryover effect of DHA would be reflected in the third treatment period compared to the first treatment period. Also, the third and fourth treatment periods can be compared in this group of patients. A carryover effect of DHA would show a decline over the third period and into the fourth period. Taken together, the three possible time periods of assessing DHA carryover can be combined together for a single analysis (Table 3-47 and 3-48). As the number of patients in this trial is small, a

nonparametric test is used to analyze the data – the Wilcoxon Signed Rank Test (Table 3-47). The paired T-test is also used to assess the data which is based on a normal distribution, but it will allow an addition comparison and validation of the analysis (Table 3-48).

Table 3-47: The comparison of plasma DHA levels combined from three treatment periods used to assess carryover effects utilizing the Wilcoxon Signed Rank Test.

DHA_1: plasma DHA levels in periods before DHA treatment or the second period after DHA treatment.
DHA_2: plasma DHA levels in periods after DHA treatment

Ranks

		N	Mean Rank	Sum of Ranks
DHA_2 - DHA_1	Negative Ranks	2[a]	5.00	10.00
	Positive Ranks	10[b]	6.80	68.00
	Ties	0[c]		
	Total	12		

a. DHA_2 < DHA_1

b. DHA_2 > DHA_1

c. DHA_2 = DHA_1

Test Statistics[b]

	DHA_2 - DHA_1
Z	-2.275[a]
Asymp. Sig. (2-tailed)	.023

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

Table 3-48: The comparison of plasma DHA levels combined from three treatment periods used to assess carryover effects utilizing the paired t test.

DHA_1: plasma DHA levels in periods before DHA treatment or the second period after DHA treatment.
DHA_2: plasma DHA levels in periods after DHA treatment

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 DHA_1 - DHA_2	-14.5708	17.77905	5.13237	-25.8671	-3.2746	-2.839	11	.016

The analysis from Table 3-47 shows a sum of ranks for the positive ranks to be higher than the negative ranks with a Z score of -2.275 and a p value of 0.023. This result is statistically significant, and it suggests that periods after DHA treatment had higher plasma DHA levels than periods before DHA treatment periods or periods long after DHA treatment. In Table 3-48, the paired T-test gave a t score of -2.839 with a p value of 0.016, which is also statistically significant and supports the significant carryover effects of DHA.

Having examined the plasma DHA level carryover effects, the primary outcomes must be investigated for carryover effects as well. The first analysis will look at the carryover effects of the first crossover. Patients in Group 1 received DHA prior to placebo, and this was opposite to patients in Group 2. Therefore, one might expect the patients in Group 1 to gain a significant advantage in the primary outcomes during the placebo period (carryover from the DHA treatment initially) than patients in Group 2 who took placebo first. By taking the sum of a primary outcome in the first and second treatment periods, the two treatment groups may demonstrate a difference

in the sum, since Group 1 is influenced by DHA carryover effects. The analysis in Table 3-49 encompasses all the primary outcomes analysis for crossover in the first crossover using the Mann-Whitney Test, which is a nonparametric test equivalent to the two-sampled t test.

Table 3-49: The comparison of primary outcomes in Group 1 and 2 patients in the first crossover to examine for carryover effects using the Mann-Whitney Test.

Primary Outcomes	Group 1 Sum of Ranks	Group 2 Sum of Ranks	Mann-Whitney Z score	p value
Central mfERG OD	21	15	-0.866	0.386
Peripheral mfERG OD	18	18	0.000	1.000
Central mfERG OS	15	21	-0.866	0.386
Peripheral mfERG OS	16	20	-0.577	0.564
EOG OD	16	20	-0.577	0.564
EOG OS	19	17	-0.289	0.773
VF-14	14	22	-1.155	0.248
visual acuity OD	19.5	16.5	-0.447	0.655
visual acuity OS	22	14	-1.169	0.243

All the primary outcomes have p values greater than 0.05 and can be considered statistically insignificant. For each of the primary outcomes, the sum of the outcome in the first crossover between Group 1 and 2 did not show any difference.

For the second crossover, the data was treated in a similar manner where the second and third treatment periods are compared between the patients in Group 1 and 2. The second crossover should carry crossover effects for both treatment groups. Patients in Group 1 took placebo in the second treatment period, which follows the initial DHA treatment period, so one would expect this placebo period to contain carryover from the first period. Patients in Group 2 took DHA in the second treatment period followed by placebo in the third period. This placebo period would certainly

contain carryover effects. The two treatment groups then would both contain some carryover effects of DHA. Thus, a comparison of the two groups would likely yield a limited difference (Table 3-50).

Table 3-50: The comparison of primary outcomes in Group 1 and 2 patients in the second crossover to examine for carryover effects using the Mann-Whitney Test.

Primary Outcomes	Group 1 Sum of Ranks	Group 2 Sum of Ranks	Mann-Whitney Z score	P value
central mfERG OD	21	15	-0.866	0.386
Peripheral mfERG OD	17	19	-0.289	0.773
central mfERG OS	13	23	-1.443	0.149
Peripheral mfERG OS	19	17	-0.289	0.773
EOG OD	18.5	17.5	-0.145	0.885
EOG OS	17	19	-0.289	0.773
VF-14	17.5	18.5	-0.145	0.885
visual acuity OD	17.5	18.5	-0.154	0.878
visual acuity OS	22	14	-1.169	0.243

All of the primary outcomes show Mann-Whitney Z scores that have statistically insignificant p values (>0.05). The second crossover does not show any significant carryover effects according to this analysis.

A summary of the p values from the first, second, and combined crossovers is shown on Table 3-51. It also includes the correlation analysis and carryover effects analysis.

Table 3-51: Summary of the crossover, correlation, and carryover analysis.

Primary Outcome	1st Crossover p value	2nd Crossover p value	Combined Analysis p value	Correlation p value	1st Crossover Carryover	2nd Crossover Carryover
central mfERG OD	1.000	0.123	0.469	0.653	0.386	0.386
peripheral mfERG OD	0.263	0.674	0.277	0.237	1.000	0.773
central mfERG OS	0.401	0.327	0.187	0.018	0.386	0.149
peripheral mfERG OS	0.674	0.401	0.796	0.147	0.564	0.773
EOG OD	0.067	0.575	0.133	0.900	0.564	0.885
EOG OS	0.207	0.674	0.234	0.543	0.773	0.773
VF-14 questionnaire	0.352	0.128	0.660	0.494	0.248	0.885
visual acuity OD	0.257	1.000	0.366	0.621	0.655	0.878
visual acuity OS	0.157	0.564	0.655	0.010	0.243	0.243

Discussion

In this randomized, double-blinded, placebo-controlled, clinical trial, the effect of DHA on the macular function in eight patients with Best vitelliform macular dystrophy was examined. Individually, the patients displayed a variety of changes in visual and electrophysiological measurements. As a group, the analysis showed no statistical significance in the primary outcomes of the first, second, or combined crossovers. Correlation analysis between the plasma DHA level and the primary outcomes showed similar results compared to the crossover analysis with no statistical significance except for the central mfERG amplitudes and the visual acuity of the left eye (Table 3-51). Carryover analysis demonstrated significant carryover during the course of the entire clinical trial. The DHA supplementation resulted in an increase of plasma DHA level compared to the placebo treatment in these patients.

Patients with Best vitelliform macular dystrophy (BVMD) have an abnormal EOG in the presence of a normal ERG (Deutman, 1969). Given the localized foveal lesion present in patients, it seems logical that focal electrophysiological testing would be needed to reveal retinal dysfunction. Macular flicker ERGs in BVMD patients have reduced amplitudes indicating the involvement of the posterior pole retina in BVMD patients (Falsini et al., 1996). The mfERG allows mapping of retinal function through simultaneous testing of multiple retinal areas in a short period of time. Previous mfERG testing with BVMD patients showed decreased amplitudes in the central three rings, but normal amplitudes in the fourth and fifth rings (Scholl et al., 2002). Palmowski and colleagues (2003) also specifically demonstrated reduced amplitudes

in the waveforms of the central 6 degrees amongst BVMD patients. In our study, all the patients began the trial with mfERG amplitudes below the range of normal for the central rings (baseline data). The peripheral rings (3, 4, and 5) had amplitudes that were in the range of normal. Averaging of the central two rings and peripheral three rings allows a convenient and detailed analysis of data without compromising the principles established by Scholl and colleagues (2002). Scholl's group demonstrated a significant correlation between the visual acuity, the age of the patient, and the clinical stage of the disease in relation to the amplitudes of the mfERG. Poor visual acuity, old age, and advanced stage of disease inferred low amplitudes in the mfERG compared to a young patient with good vision and early stage disease. This type of analysis was not included in our study, but for our patients, the central two rings mfERG amplitude averaged between 8.25 to 19.03 nV and the peripheral three rings averaged between 6.98 to 14.80 nV. Amplitudes were relatively uniform between the different patients given their varied age and clinical stages.

The treatment with DHA brought in mixed mfERG results. Of the eight patients, 9 out of the 16 eyes had relatively the same or increased central rings mfERG amplitude while on DHA treatment compared to 7 eyes that had a decrease in amplitude. Although statistically insignificant, the central and peripheral rings amplitude for the right eye increased during periods of DHA treatment as demonstrated in the first, second, and combined crossover analysis. Correlation analysis was less conclusive with positive correlation for the central rings amplitude but negative for the peripheral rings. The left eye behaved completely opposite to the

right eye, with the central rings amplitude decreasing during placebo treatment periods. The exceptions are the peripheral rings amplitudes in the second and combined crossover analyses where amplitudes were higher during DHA treatment periods. This apparent inconsistency might be the result of the peripheral macula having less atrophy compared to the central macula and thus able to respond to DHA. Correlation analysis was negative for the left eye. Both the central and peripheral rings amplitude trended toward a decrease in correlation with higher DHA levels, with the peripheral rings being statistically significant. DHA has never been tested in patients with BVMD, but it has been test as a treatment for patients with retinitis pigmentosa (RP). Hoffman and colleagues (2004) designed a randomized, placebo-controlled clinical trial of DHA supplementation for X-linked RP patients. The four-year study showed significantly elevated RBC-DHA levels (red blood cell DHA level), correlation of cone ERG function to RBC-DHA, and less change in fundus appearance in the DHA supplemented group. The visual acuity, visual field, and more importantly, the rate of cone ERG functional loss was not significantly different between DHA and placebo (Hoffman et al., 2004). Another randomized, placebo-controlled clinical trial examined the benefits of DHA in patients with RP receiving vitamin A treatment (Berson et al., 2004a; Berson et al., 2004b). Specifically, this four-year study found no significant differences in the 30-Hz ERG amplitude, Humphrey field analyzer 30-2 sensitivity, and ETDRS visual acuity (Berson et al., 2004a). In subgroup analysis, the results showed that among patients not taking vitamin A prior to entry of the trial, those in the DHA group had a slower decline in

field sensitivity and ERG amplitude than those in the control group in the first two years (Berson et al., 2004b). After two years, the benefits disappear and both groups had no appreciable difference in the outcome measures.

Despite the lack of support of DHA supplementation in the adults, its benefits in the pediatric population are well documented. DHA supplementation in preterm infants has been shown to improve rod ERG thresholds (Uauy et al., 1990). Full-field ERGs performed on preterm infants fed with DHA based formula showed significantly improved rod thresholds, and analysis of the b-wave differences originated at the photoreceptor level (Birch et al., 1992a). These differences were only present at 37 weeks postpartum and disappear after 57 weeks. Correlation between ERG and DHA in term infants was shown by Hoffman and colleagues (2000). Animal studies complement these studies. Reduced amplitudes of the a- and b-waves of the ERG were found in rodents (Wheeler and Benolken, 1975; Weisinger et al., 1996), cats (Pawlosky et al., 1997), and baboons (Diau et al., 2003) fed with a diet lacking in DHA.

Observational studies in humans and animals provide evidence of the many effects of DHA supplementation; however, the mechanism underlying DHA function remains unclear. As mentioned, DHA status influences the ERG response and is related to the phototransduction pathway. Photoactivated rhodopsin binds to and activates the visual G-protein, which then activates a cyclic GMP (cGMP) specific phosphodiesterase (PDE) (Litman et al., 2001). Hydrolysis of cGMP by PDE causes the closing of cGMP-gated channels in the rod outer segment (ROS) plasma

membrane to change the transmembrane potential and initiate the neuronal response to light. Addition of DHA enhanced the formation of photoactivated rhodopsin and the G-protein (Litman et al., 2001; Niu et al., 2004). Another theory that has surfaced recently claims that a concentration gradient of DHA normally exists in the subretinal space between the rod outer segments (high concentration) and the RPE (low concentration), and the release of 11-cis retinal from interphotoreceptor retinoid-binding protein (IRBP) is facilitated when IRBP is exposed to sufficient DHA in the subretinal space (Chen et al., 1996; Chen et al., 1993). The lack of DHA impairs the release of 11-cis retinal which is essential for photoreceptor survival (Berson et al., 2004b).

In our clinical trial, the second primary outcome was the electro-oculogram (EOG). The EOG measures indirectly the standing potential of the eye by taking the light peak to dark trough ratio (Arden ratio) (Arden et al., 1962). Six out of eight eyes for both the left and right had an Arden ratio below 1.8, the cutoff for normal people. Further examination shows that only 5 out of the 8 eyes for both the left and right had an Arden ratio at or below 1.5, the range found by Cross and Bard (1974), Blodi and Stone (1990), and Pinckers and colleagues (1996). With DHA supplementation, 5 out of 8 right eyes displayed an increase in the EOG, while only 4 out of 8 left eyes had an increase in the Arden ratio. The first crossover showed a tendency towards placebo treatment for higher Arden ratios, with the right eye having a statistically significant trend. The second crossover and the combined crossover showed a tendency towards placebo as well, but statistically insignificant. The correlation analysis was positive,

although statistically insignificant, for both eyes meaning that increasing DHA levels correlate with higher Arden ratios.

DHA supplementation has never been tried in patients with BVMD. DHA has not been tested to demonstrate an increase in the EOG Arden ratio. The logic of our trial to utilize DHA to improve visual function in patients with BVMD resides in the underlying molecular mechanisms. BVMD is caused by a mutation in the VMD2 gene (chromosome 11q13), which encodes a protein, bestrophin, that functions as a chloride channel on the basolateral plasma membrane of the RPE (Forsman et al., 1992; Stone et al., 1992; Hou et al., 1996; Sun et al., 2002; Marmorstein et al., 2000). Bestrophin consists of either four (Sun et al., 2002) or six (Qu et al., 2004) transmembrane spanning helices, and a C-terminal cytoplasmic region with potential phosphorylation sites for protein kinase A, protein kinase C, and cGMP-dependent protein kinase. Marmorstein and colleagues (2002) has shown bestrophin to interact and dephosphorylated by a protein phosphatase 2A, and Sun and colleagues (2002) showed intracellular calcium plays a role in the channel's regulation. These interactions suggest that bestrophin participates in a variety of intracellular pathways.

The importance of chloride channels should not be overlooked. Disruption of RPE chloride transport can cause the accumulation of fluid in the subretinal space and subsequent retinal detachment (Bird, 1994; Weng et al., 2002). Members of the ClC chloride channel family have been proposed to be crucial for retinal function (Weng et al., 2002). Transgenic mice that were deficient in channels of this family were found to develop retinal degenerations and blindness within weeks after birth (Bosl et al.,

2001; Kornak et al., 2001). Weng and colleagues (2002) believe that oxidative stress plays a role in RPE aging and in retinal degenerations. They showed that peroxide (a product of oxygen metabolism and an oxidant) caused reversible inhibition of the RPE membrane chloride conductance. Peroxide exposure to RPE cells can cause mitochondrial DNA damage and apoptosis (Ballinger et al., 1999; Jin et al., 2001). For normal individuals with normal chloride conductance, oxidants may not cause cell and retinal damage as severe as individuals with impaired chloride conductance such as the case of BVMD patients.

One particular chloride channel, cystic fibrosis transmembrane conductance receptor (CFTR) is also affected by oxidants (Stutts et al., 1994). A defect in the CFTR gene causes cystic fibrosis. The defect in chloride transport produces pulmonary insufficiency and recurrent pulmonary infections leading to pulmonary failure and premature death (Wilmott and Fiedler, 1994). Most patients also have pancreatic insufficiency and ileal hypertrophy. Essential fatty acid abnormalities have been observed in patients with CF and may have a fundamental role in the symptoms and progression of the disease (Strandvik, 1992; Farrell et al., 1985; Freedman et al., 2004). Specifically, an imbalance exists between DHA and AA (arachidonic acid, an agonist of inflammation and mucus secretion) (Gilljam et al., 1986). Mice models with CF (cftr -/-) benefited from DHA supplementation where they corrected the lipid imbalance and reversed the observed pathology in the lungs, pancreas, and intestines. In a human trial, DHA supplementation in patients with CF resulted in improved respiratory function and recovery from infection (De Vizia et al., 2003).

The enticing effects of DHA on CF patients would suggest a role for DHA to play in other conditions that involve chloride channels, especially in patients with BVMD. Aside from chloride channels, DHA has demonstrated effects on potassium (Poling et al., 1995), sodium (Xiao et al., 1995), and calcium channels (Vreugdenhil et al., 1996). Human fetal retinal explants supplemented with DHA displayed an increase in gene expression with transcripts encoding proteins involved in lipid metabolism, retinal neurogenesis, and apoptosis protection (Rojas et al., 2003).

The VF-14 index was initially designed to measure the functional impairment caused by cataracts (Steinberg et al., 1994; Alonso et al., 1997). It was found to be a reliable and valid measure of functional impairment, and it provides information not given by visual acuity. The questionnaire's applicability was not limited to cataracts alone, but it was valid as a measure of functional impairment in patients with retinal disease (Linder et al., 1999; Mackenzie et al., 2002). In our trial, 3 patients had improved, 3 had the same, and 2 had worse VF-14 score during DHA supplementation. For the first crossover, placebo treatment seem to relate to higher VF-14 scores, while the second and combined crossover analysis pointed towards DHA having higher scores. Correlation analysis was also negative and conveyed a trend of lower VF-14 scores with higher plasma DHA levels. None of the trends were statistically significant. The apparent trend can be explained further in a later section regarding carryover.

The ETDRS logMAR chart was first developed by Bailey and Lovie (1976), enhanced by the National Academy of Science (1980), and further described by Ferris

and colleagues (1982). The use of a logarithm of the minimum angle of resolution (logMAR) chart for measuring visual acuity is considered the "gold standard" by which the outcomes of most clinical trials are judged (Hazel and Elliott, 2003). In our study, two patients had improved, four had the same, and two had a worsening of vision in the right eye. For the left eye, three patients had improved, one had the same, and four had worsening of vision during periods of DHA supplementation. The right eye had improved vision under the first and combined crossover analyses, and worsening of vision in the correlation analysis between DHA and ETDRS vision which were all statistically insignificant. On the other hand, the left eye had improved vision in the second crossover, but a worsening of vision in the first and combined crossover and a negative correlation with DHA which were all statistically insignificant.

The effect of DHA on visual acuity has been studied in adult and infant subjects. A case report of a patient with autosomal dominant Stargardt-like retinal dystrophy supplemented with DHA found improved mfERG amplitudes. This patient had a mutation in the ELOVL4 gene, which encodes for a fatty acid elongase and is presumably involved in the production of DHA (MacDonald et al., 2004). The recent clinical trial testing DHA on patients with retinitis pigmentosa (RP) (Hoffman et al., 2004) showed no clear benefit of DHA supplementation. In this four year prospective study, the difference between the visual acuity of the placebo and treatment group was 0.01 log units (logMAR) and statistically insignificant ($p=0.88$). The second study with RP patients examined the role of DHA in combination with vitamin A therapy.

ETDRS visual acuity was measured and the annual rate of decline over four years in terms of the number of letters lost per year was not significantly different between patients given DHA versus control (0.71 vs. 0.68, p=0.86) (Berson et al., 2004a). A specific subset of these patients was further examined. The authors discovered that patients who were previously not on vitamin A prior to entry into the trial had a slower decline in visual field sensitivity and electroretinogram amplitudes (Berson et al., 2004b). However, the annual rate of decline for the ETDRS visual acuity in terms of letters per year remained statistically insignificant between the DHA and the controlled groups (0.82 vs. 0.69, p=70). This also infers a disparity in the two treatment groups at the inception of the study.

Beside specific genetic diseases, DHA consumption has been examined with age-related macular degeneration (AMD). Cho and colleagues (2001) designed a prospective study to assess the relationship between intakes of total and specific types of fat and AMD. The prospective follow-up study include subjects from the Nurses' Health Study and the Health Professionals Follow-up Study totaling over forty-two thousand women and twenty thousand men at or over the age of 50 with no diagnosis of AMD. Fat intake was judged with a food-frequency questionnaire. Almost 600 patients were found to have AMD with visual loss of 20/30 or worse. Total fat intake was positively associated with risk of AMD (relative risk (RR) = 1.54, p=0.08). DHA had an inverse relation with AMD (RR=0.70, p=0.05), and consumption of four or more fish meals per week was associated with a 35% lower risk of AMD (RR=0.65, p=0.009) compared to three or less fish meals per month. In a small study of 21

patients (11 AMD and 10 healthy controls), fatty acid fractions in red blood cells were analyzed. Arachidonic acid (AA) and DHA were higher in the AMD group compared to the control group (Ouchi et al., 2002). The authors concluded that polyunsaturated fatty acids were vulnerable to free radicals and reactive oxygen species, and may be related to AMD induction. In a subsequent study by Seddon and colleagues (2003), a prospective cohort study of 261 participants with nonexudative AMD was followed over four years to monitor for progression to advanced AMD (geographic atrophy or neovascular disease). The study revealed that total fat and animal fat intake conferred a RR of 2.90 and 2.29, respectively. Essentially, all types of fats including vegetable and unsaturated fats led to higher risks of progression to advanced AMD. The only factor associated with a lower risk of AMD was in individuals with a higher fish intake (Cho et al., 2001; Seddon et al., 2003). The studies, however, did not provide satisfactory data on the use of DHA supplementation with different stages and types of AMD. Given our small cohort of patients, we were unable to correlate the stage of Best disease to clinical and electrophysiological improvement.

For the past two decades, researchers have studied the effects of DHA on infant visual and neurological development, and over 20 manuscripts have been published. The broad range of studies relied on numerous testing methods of visual function which included behavioral and electrophysiologic methods. Behavioral based tests stimuli used high-contrast square-wave gratings of two discrete luminances, where grating acuity are expressed in units of cycles per degree (Cy/degree) (SanGiovanni et al., 2000). The spatial frequency of cy/deg is a measure of visual

resolving power per degree in which higher values represent response to finer patterns. For easy comparison between experimental groups, conversion of measures to octaves is used, where one octave change represents a doubling or halving of the stimulus spatial frequency. Electrophysiologic tests employ square wave gratings or checkerboards to determine the visual evoked potential or VEP (SanGiovanni et al., 2000). The VEP measures the integrity of the visual system from the optic nerve to the occipital lobe via electroencephalographic activity recorded at the scalp (Odom et al., 2004).

SanGiovanni and colleagues (2000) examined 25 studies from 1992 to 1998, and conducted a meta-analysis to determine the efficacy of dietary DHA on the performance of visually-based tests in healthy, full-term infants. The analysis took into account the different behavioral tests used, the number of dietary groups, the sources of LCPUFAs (formula with DHA vs. formula without DHA vs. breast milk), the concentrations of DHA, and the study design (randomized vs. non-randomized). For randomized studies, acuity differences are significantly greater than zero at two months of age for behavioral-based measures (0.32 ± 0.09 octaves, $p=0.003$) (SanGiovanni et al., 2000). At this age, acuity differences were not significantly different from zero for electrophysiological-based outcomes. Non-randomized study designs showed statistically significant acuity differences for behavioral-based tasks at two months (0.49 ± 0.09 octaves, $p\leq0.001$) and four months (0.18 ± 0.08 octaves, $p=0.04$) of age. Acuity differences were also significant for electrophysiological-based tests at four months of age (0.37 ± 0.16 octaves, $p=0.02$). Combined study designs

revealed only statistically significant acuity differences for behavioral-based tests at two months of age (0.26 ± 0.10 octaves, $p \leq 0.0001$), and for electrophysiological-based tests, the results were significant at four months of age (0.26 ± 0.10 octaves, $p=0.009$) (SanGiovanni et al., 2000). The authors speculate as age increased, the magnitude of acuity difference approached zero, which may be explained by a convergent similarity in non-liquid diets with age.

A further study by SanGiovanni and colleagues (2000) of preterm infants echoed the same results. Analysis of randomized comparisons (formula with DHA vs. formula without DHA), based on four prospective trials, showed significant differences in visual resolution acuity at two (0.47 ± 0.14 octaves, $p \leq 0.001$) and four (0.28 ± 0.08 octaves, $p \leq 0.01$) months of age. Simmer (1999) concluded that DHA supplemented formula was better for early visual development compared to those on standard formula, but the effects were not significant after four months of age. Simmer and Patole (2005) provided an update to include an additional 11 studies that examined visual acuity over the first year, as measured by Teller acuity in six studies, by VEP in four studies, and by ERG in two studies, showed no significant differences in any visual assessment between supplemented and control infants.

DHA supplementation appears to have limited efficacy in both adult and infant. Given the results of our study, we agree with the sentiments of previous authors and researchers that the effects of DHA may be limited. However, an important factor that arose from our study, albeit unexpectedly, was the significantly large carry-over effects of the DHA. The patients' plasma DHA levels remain elevated post-DHA

supplementation. Analysis of the combined carryover in all the treatment periods showed the plasma DHA levels to be significantly higher in the periods following supplementation than before supplementation. When the analysis is broken down into the first and second crossovers, the carryover effects did not translate into significant change in the primary outcomes. The analysis may not reveal a correlation between the carryover of plasma DHA levels and primary outcomes given the small sample size. Even with a statistical insignificant result, the unknown effects of the carryover cannot be ignored as it has potential effects on the primary outcomes and correlation results in both individual and group data.

In our clinical trial, the patients were given a two month washout period after DHA supplementation. This period was thought to be a sufficient length of time for washout. In a pilot crossover trial determining the efficacy of fish oil in the treatment of angina pectoris in geriatric patients, a carryover effect was suspected in a group of patients that took fish oil prior to placebo (Aucamp et al., 1993). The authors found in the group of patients which took placebo prior to the fish oil had significant reduction in the number of anginal attacks and consumption of sublingual isosorbide dinitrate. The second group which took the fish oil first did not have this significant reduction even with a 16 week washout period. This result was attributed to a fish oil carryover effect (Aucamp et al., 1993). The study, however, did not state the composition of the fish oil capsules, and did not correlate results with plasma DHA levels. This information would have reinforced the speculated carryover phenomenon.

A second study, in which carryover effects were observed, examined the effect of fish oil in patients with lupus nephritis (Clark et al., 1993). The study was a crossover trial in which patients took alternately fish oil for one year followed by a ten week washout and one year of olive oil (placebo). The outcome measures were renal function, symptoms of disease activity, and serum lipid profiles. The study found no difference in the glomerular filtration rate, serum creatinine, or disease activity. Fish oil was shown to lower serum triglycerides and VLDL cholesterol. Carryover was shown when comparing platelet membrane phospholipid levels prior to fish oil and following washout. DHA levels after the washout period were elevated compared to baseline measurements before the start of the study. Although this data seems significant, the authors did warn that the placebo of olive oil turned out to have its own biological activity which in certain aspects was similar to the treatment effect. Clark and colleagues submit that the carryover effect may not be statistically significant given both placebo and fish oil had treatment and carryover effects. In our study, corn oil was used as the placebo. Corn oil has no omega-3 fatty acids, and it has been the placebo mainly used in the infant formula studies.

The results of our clinical trial showed minimal efficacy. Based on our sample size of eight patients, it was not sufficient to have results that are statistically significant. Given the variability of the results (relatively large standard deviation) and a small difference in the outcomes, a larger sample size is required to provide sufficient power. For a probability of 95% that the study will detect a treatment difference at a 0.05 significance level, a total of 106 patients will be needed to enter

the two treatment crossover study. This sample size holds true if the true difference (ie. outcome measures) between the treatments (DHA vs. placebo) is 0.5 times the within-patient standard deviation. The assumption of 0.5 times the within-patient standard deviation is made here because the standard deviation is large and the outcome measures are smaller in comparison. The value of 0.5 is strictly arbitrary. The main outcome of our study was that the carryover effect of DHA supplementation would affect all crossover-designed trials.

The uncertainty of the significant role of DHA remains given the lack of definitive clinical trials to prove the usefulness in the field of eye health and development. Our study raises more questions and provides new ideas for future directions. For our patients with Best vitelliform macular dystrophy, our clinical trial proved to be useful in evaluating the safety of the DHA as no patients suffered adverse effects with its consumption. The results were not suggestive of DHA efficacy in this small group of patients, but underlying trends in the data offer the hope that a larger clinical trial may show that DHA has significant benefits for patients with Best. The next clinical trial must address the issue of carryover, since it has now been shown, both in our trial and others, that DHA is retained by the body for a significant amount of time. A long-term prospective study is required to truly see the efficacy behind DHA as the benefits may need longer periods for fruition. The potential benefits of DHA on macular diseases in general are extremely important given the lack of any effective treatment to treat macular degeneration. An inexpensive oil such as DHA has the promise to bring significant health benefits with low economic impact. Also, an

examination of the physiology and molecular mechanism behind how DHA functions at the cellular level is crucial for better understanding of the clinical effects. Future experiments may include incorporating DHA into retinal pigment epithelial cells to investigate how DHA molecules affect chloride ion flow on wild type and mutant bestrophin using patch clamp techniques. X-ray crystallography can enhance our knowledge of how DHA may change the conformation of bestrophin in possibly allowing better ion flow. The story of DHA is just beginning.

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