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Feasibility study of a docosahexaenoic acid optimized nutraceutical formulation on the macular levels of lutein in a healthy Mediterranean population.

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Short title: Optimized nutraceutical formulation with DHA on macular levels of lutein

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Abstract

Introduction: Macular pigment optical density plays a pivotal role in maintaining macular structure and functioning. Research shows that daily consumption of lutein reduces the risk of eye diseases such as age-related macular degeneration.

Objective: This study analyzes the influence of a supplementation containing lutein and antioxidant vitamins either with or without docosahexaenoic acid (DHA), with the main objective of identifying macular pigment optical density (MPOD) changes in both eyes at the end of the follow-up using the Visucam® retinograph. The secondary endpoint was to determine variation in the lutein and DHA levels in plasma and red blood cell membranes respectively.

Methods: One hundred healthy participants (200 eyes) aged 40-70 years (mean age 49.3 years, SEM=13.7) were randomized in a 1:1 ratio to receive daily one of the following supplements for 3 months: Lutein group (LT-G, n=49), Lutein /Docosahexaenoic acid group (LT/DHA-G, n= 51). The MPOD was measured at baseline and end of the follow-up by retinography (Visucam® retinograph). Lutein in plasma was determined by HPLC and DHA in red blood cell membranes was analyzed by gas chromatograph/mass spectrometer.

Results: From baseline, macular pigment optical density showed significantly higher values in the Lutein/DHA group than in the Lutein group at the end of the study ($p<0.0001$). Significantly higher Lutein in plasma ($p<0.0001$) and DHA ($p<0.0001$) levels in the red blood cell membrane were seen in the Lutein/DHA group than in the Lutein group at the 3-month follow-up.

Conclusion: Lutein supplementation improves macular pigment optical density in healthy subjects from a Mediterranean population being significantly increased in the presence of DHA. Therefore, our findings highlight the relevance of the adjunctive role of DHA for a better Lutein availability.

Key words: Macular pigment optical density, Lutein, Docosahexaenoic acid, Ocular health

Introduction

Several modifiable factors including nutrition have been studied in connection to the pathogenic mechanisms of eye diseases such as dry eyes, cataracts, glaucoma, and age-related macular degeneration (AMD) [1]. Vitamins, minerals, carotenoids, and long-chain polyunsaturated fatty acids (LC-PUFAs) have been studied in connection to ocular physiopathology [2], with some interesting results which will be discussed further.

The role of trace elements on eye diseases has been widely studied. It has been reported a high copper concentration in plasma samples from patients with diabetic retinopathy (DR), compared to control subjects [3]. In another study conducted on tear samples, Vinetskaia et al observed alterations in the levels of iron, magnesium and aluminum and, to a lesser extent, in the levels of copper and zinc in DR patients [4]. Instead, they found lower levels of zinc and higher of iron in tears of open-angle glaucoma patients. Decreased zinc levels in the retina have also been associated with the onset and progression of age-related eye diseases [5].

Carotenoids (also known as tetraterpenoids) are organic pigmented molecules that are produced by a wide variety of fruits (mango, papaya), vegetables (carrots, pumpkins), eggs (yolk), birds (pheasant, toucan, parrot), seafood (lobster, salmon, shrimp), and spices (saffron, paprika), which give these foods their yellow, orange, or red color. Leafy vegetables such as broccoli, kale, and spinach also contain high amounts of carotenoids. The major classes of carotenoids include the xanthophylls such as lutein (LT) and zeaxanthin (ZX) and carotenes (α-carotene, β-carotene, and lycopene). The human body does not synthesize any of these carotenoids [6]. Interestingly, White et al., have recently reported that availability of lutein in food (or pills) noticeably increases when consumed together with fat [7]. Specific xanthophylls are particularly located in the central retina, constituting the macular pigment. LT is the predominant xanthophyll in the retina, ZX in the macular periphery, and LT derived meso-ZX in the foveal area [8]. All of them play a pivotal role in maintaining the morphology and function of this structure [9]. Several studies have analyzed the role of macular pigment optical density (MPOD) in ocular health and disease, finding that low MPOD increases the risk of macular diseases such as AMD, while high MPOD protects the eyes against a variety of pathologies [10-12].

Among the omega-3 (ω-3) LC-PUFAs, the dietary precursor linolenic acid (ALA, C18:3) is transformed with difficulties to both eicosapentaenoic acid (EPA; C20:5 ω3) and docosahexaenoic acid (DHA; C22:6 ω3), which are the major LC-PUFAs active types in humans. The retina is the human tissue that contains the highest lipid profiles enriched in DHA LC-PUFA, which plays a pivotal role in the regulation of retinal morphology and functioning [13]. A variety of reports have shown that ω-3 LC-PUFAs supplementation (from food, as well as from oral capsules), induces positive responses on global health [14] and on pathologic situations including degenerative and aging-related disorders [15]. Long chain omega-3 (LC- ω-3) fatty acids are incorporated into the cell membrane phospholipids. Harris reported that the ω-3 index measures the percentage of the LC- ω-3 PUFAs (EPA+DHA) in relation to total red blood cell membranes (RBCMs) fatty acids [16], so the RBCMs have been proposed as a precise marker of dietary fatty acid intake [17]. Epidemiological and

experimental studies have demonstrated that the RBCM EPA and EPA+DHA (as markers of ω-3 dietary PUFA status) were closely related with neovascular AMD [18].

Previous studies reported that MPOD did not change significantly when LT and DHA were administered together [19]. In fact, the authors argued only that LT and DHA worked synergistically to improve the entire MPOD spatial profile [19].

Summing up the current scientific evidence and taking into account the importance of xanthophylls for vision, we designed this study to analyze the effects of an optimized nutraceutical formulation regime over 3 months, based on a high content DHA triglyceride. We have determined the DHA variation in the RBCMs and the plasma and macular levels of LT in an adult healthy Mediterranean population.

Materials and Methods

The present work adheres to the Ethical Principles for Medical Research Involving Human Subjects [Declaration of Helsinki (Edinburgh, 2000)], the Ethics Committee standards of the study centers and the Spanish Agency of Medicines and Medical Devices (AEMPS). Signed informed consent was obtained from all the participants. EudraCT number 2019-002356-16 for the sponsor's protocol code number 36/16.

Study Design

A prospective interventional multicenter double masked study was conducted between 2016-17/2017-18 in the Mediterranean area of Valencia (Spain). The main purpose was to evaluate the effect on MPOD of two commercial supplements having a similar formulation relating vitamins and minerals, close to the one used in the Age-Related Eye Disease Study (AREDS). The only difference between them was the presence of LT (formula 1) or LT+DHA (formula 2) given in a subject-masked fashion over 3 consecutive months, as measured by the Visucam® retinograph. The secondary endpoint was to look at the effects of both supplement regimes on the plasma levels of LT, and of DHA in the RBCM, via standard laboratory assays.

Patient Management

For this study, 125 healthy potential participants were initially selected in ophthalmologic appointments during an ordinary visit in the study centers. These initially potential participants were interviewed according to the inclusion/exclusion criteria (Table 1), extensively informed about the study characteristics, and provided written informed consent for participation in the study. Briefly, the following baseline interview data were recorded: socio-demographics, personal/familial background and lifestyle (nutrition). At the first visit, patients underwent a systematized ophthalmic examination in both eyes including best corrected visual acuity (BCVA), intraocular pressure (IOP) and ocular fundus examination and/or retinographies. Of the potential participants, 110 suitable participants aged 40-70 years were recruited and homogeneously randomized in a 1:1 ratio to receive the following treatments for 3 months: Lutein group (LT-G, n=55): Formula 1, dosing 6 mg

of LT per day; or lutein plus DHA group (LT/DHA-G, n=55): Formula 2, dosing 6 mg of LT and 700 mg of a concentrated DHA triglyceride daily (Table 2). This is a DHA triglyceride which has a high antioxidant activity patented to prevent cellular oxidative damage [20-22]. Both food supplements were commercial formulations gently provided by the pharmaceutical company in similar boxes to the investigators in a masked fashion (BrudyRetina, box A, and BrudyRetina box B; Brudylab, SL, Barcelona, Spain). Neither the investigators nor the participants knew the specific composition of the two formulas. The commercialized nutraceuticals were given to the participants without any charge. Blood samples were collected in fasting condition to analyze the plasma levels of LT and the RBCM DHA content by high performance liquid chromatography (HPLC) and gas chromatography (GC), respectively. At the end of the 3-month supplementation period, the participants were visited again to perform the same protocol, as reflected in the Figure 1.

Ophthalmic Examination

Eye examination protocol in each eye included: BCVA, IOP, and ocular fundus inspection/photographs (Visucam® 200 pigment module; Carl Zeiss Meditec Iberia, Madrid, Spain). Each patient was seated and conveniently positioned for ocular examination under mesopic illumination. Three retinographies were taken consecutively from each eye at both study points according to the manufacturer's instructions. Retinographies were evaluated to obtain optimal quality for MPOD determinations according to previous reports [23,24]. Augmentation of the relative densitometric units (rdu) from baseline of the retinographs and their corresponding data from each eye were considered improvement and the results were recorded as percentages.

Sampling protocols

Blood sampling was scheduled at baseline and 3-month follow-up for biochemical assays. Antecubital vein blood was collected under fasting conditions at 8:00 a.m. from each participant. Tubes were centrifuged at 3000 rpm for 10 min and the plasma/erythrocytes were separated. Aliquots of the upper (plasmatic layer) and lower (erythrocytic layer) fractions from each participant were placed in separate Eppendorfs, labeled and stored at -80°C until processing. All experiments were performed in duplicate and the protocols are detailed below.

Laboratory Assays

Lutein analysis in plasma was obtained by using the modified method of Colmán-Martínez [25] extraction and determination of LT from the plasma samples was performed in twilight to avoid oxidation of the compounds. Plasma aliquots were thawed and protein was precipitated, which was followed by extraction with hexane. The extraction process was repeated twice more. The supernatants were dissolved in methyl tert-butyl ether (MTBE). Chromatographic analysis was carried out in a Waters 2795 Alliance HT HPLC system coupled to a Waters 2996 photodiode array detector (Waters Chromatography Corp., Milford, MA, USA). Chromatographic separation was performed on a reversed-phase column YMC (YMC America Inc., Allentown, PA, USA) at room temperature. The mobile phase consisted of a linear gradient of methanol, MTBE, and water. The gradient consisted of 6% MTBE to 56% linear gradient in 20 min, and then changed to 90% MTBE and was kept for 2 min and returned to initial conditions. The water was kept constant at 4%. The

flow rate throughout the run was 0.6 mL/min. The diode array detector (DAD) monitored the peaks at a range of 220 to 600 nm. The integration was performed with Waters® Empower software. LT was identified according to retention time and absorption spectra at 450 nm. The concentration in the samples was calculated using the LT standard curve. Fatty acid composition in RBCM were determined as methyl esters after methylation reaction, using the method of Lepage and Roy [26]. GC analysis was performed on a Shimadzu GCMS-QP2010 Plus gas chromatograph/mass spectrometer (Shimadzu, Kyoto, Japan), with a Shimadzu AOC-20i auto injector and a Shimadzu AOC-20s autosampler. The column used was a Suprawax-280 (Teknokroma, Barcelona, Spain). Operation conditions were as follows: the injector was used in splitless mode and the temperature was kept at 250°C. The temperature program was as follows: initial, 150°C with a 0.25 minutes hold, ramp at 35°C/min to 200°C, 8°C/min a 225°C with a 3.2 min hold, and then 80°C/min to 245°C with a 2.75 min hold. Helium was used as the carrier gas. The interface and ion source temperatures were 255°C and 200°C, respectively. The MS ionization mode was electron ionization. Fatty acid methyl esters (FAME) were identified through mass spectrometry and through comparison of the elution pattern and relative retention times of FAME with the reference FAME mixture (GLC-744 Nu-Chek Prep. Inc., Elysian MN, USA). The results were expressed in relative amounts (percentage molar of total fatty acids).

Statistical Analysis

The sample size was determined using the freeware GRANMO sample size calculation program v.7.12 (https://www.imim.cat/ofertadeserveis/software-public/granmo, Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain). Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 64 subjects are necessary in first group and 64 in the second to find as statistically significant a proportion difference, expected to be of 0.5 in group 1 and 0.75 in group 2. It has been anticipated a dropout rate of 10%. The ARCSINUS approximation was used to carry out these calculations. Microsoft Excel and IBM Statistical Package for the Social Sciences (SPSS) V.24.0 programs (SPSS Inc., Chicago, IL, USA) were used. For continuous variables, nonparametric (Mann–Whitney U) and parametric (t test) statistics were utilized, and the results were expressed as mean ± standard error of the mean (SEM). Categorical variables were expressed as percentages. A p value less than 0.05 was considered statistically significant, which was adjusted by Bonferroni correction when pairwise comparison in multiple groups was conducted. Correlation analysis was used to evaluate the association between the MPOD levels at baseline and the changes throughout the interventional study.

Results

Demographics

The final number of participants was 100 (49 assigned to the LT-G and 51 assigned to the LT/DHA-G), and 200 eyes were analyzed. The rate of withdrawal was 10%, and withdrawals were due to deterioration of health, lack of motivation, confusing ocular fundus retinographs, and blood samples failing to be processed or showing confusing data.

The mean age of the LT-G was 44.6 ± 13.9 years vs 44.0 ± 13.5 years for the LT/DHA-G. The range of age of the participants is narrow enough to avoid an impact on the density of the crystalline lens, to prevent a confounding on the Visucam measurements. Distribution by gender was 51.1% men and 48.9% women in the LT-G and 40.8 % men and 59.2% women in the LT/DHA-G group.

Ophthalmic Examination

No changes in the BCVA and IOP values between groups were observed between the two study points. However, values of the MPOD were significantly higher at the 3-month follow-up than baseline in the eyes of both participant groups (Figure 2). Furthermore, there were noticeably higher values in the LT/DHA-G eyes (Figure 2B) than in the LT-G (Figure 2A). The eyes of the LT-G showed a 27.5 % increase in the right eye and a 32.2% in the left eye for MPOD, with a global increase of 29.0% in both eyes. The eyes of the LT/DHA-G showed an increase of 38.5% in the right eye and 40.6% in the left eye for MPOD, with a global increase of 39,6% in both eyes. However, 11 participants in the LT-G and 5 participants in the LT/DHA-G showed less than a 10% increase in MPOD concentration at the end of the study.

Laboratory Assays

The biochemical analyses demonstrated a statistically significant augmentation in plasma LT in the LT/DHA-G compared to the LT-G after 3 months of the supplement regime (Figure 3A). Moreover, significantly higher RBCM DHA levels were seen in the LT/DHA-G compared to the LT-G at the end of study (Figure 3B).

As shown in Figure 4A (LT-G) and 4B (LT/DHA-G), the MPOD showed a near-significant positive correlation with plasma LT in the group supplemented with formula 2 (LT/DHA-G, $r = 0.291$, $p = 0.085$), but not in the group supplemented with formula 1 (LT-G, $r = 0.014$, $p = 0.941$). Because MPOD correlated positively with LT plasma concentrations in the LT/DHA-G and this increased with the addition of DHA in the nutraceutical formulation, we compared the correlation coefficients of the MPOD and the RBCM DHA content of the total study population. There is a positive correlation of RBCM DHA content with MPOD was statistically significant ($r = 0.244$, $p = 0.0037$) (Figure 4C).

Discussion

We evaluated the effects of two oral supplements dosing the same daily amount of LT (in formula 1) and LT/DHA (in formula 2) on the MPOD in a healthy population. At 3-month follow-up, we found a significant increase in the MPOD levels in the participants randomly assigned to formula 2 (LT/DHA-G) compared to formula 1 (LT-G) ($p<0.0001$). Moreover, significantly higher plasma LT ($p<0.0001$) and DHA RBCM levels ($p< 0.0001$) was seen in the LT/DHA-G compared to the LT-G. Our data strongly indicate that nutraceuticals containing LT noticeably improve MPOD in healthy subjects, but with a significant increased dose-dependent response in the presence of DHA in triglyceride form (DHA-TG).

Studies similar to ours have also reported that the LT macular concentration is a modifiable nutritional factor in health and disease [12,27-31]. However, other authors did not found any

relationship between the nutritional intervention and changes in the LT macular levels, as in the work by Johnson et al. [19], which was carried out in a sample of 49 elder women without maculopathy. The MPOD did not change when LT and DHA were administered together. More recently, Korobelnik et al. [24] have suggested that patients with neovascular AMD did not show modified MPOD levels after 6 months of LT and ZX dietary supplementation, despite their plasma levels clearly showing continuous exposure to these xanthophylls. Olmedilla-Alonso et al. [32] also reported that neither the xanthophylls, the anthocyanins, nor a combination of these two (at the selected doses) were capable of increasing MPOD after 8 months of supplementation in postmenopausal women. Differences in the study design, the formulation as well as in the device used for analyzing the MPOD may be contributing to the difficulty in comparing between studies.

An important result of the present work is that DHA-TG reinforces the transport and uptake of LT into the macula, probably by inducing changes in the lipoprotein profile that, in turn, favors the availability of xanthophylls in the macula. We have confirmed the correlation between LT plasma levels and MPOD only in the LT/DHA-G, while no correlation was found in the LT-G group. Among the different determinants of MPOD considered, ω-3 LC-PUFAs dietary intake, especially with DHA, have been proposed as key factors [19,33]. These studies have shown that in total plasma phospholipids, analysis of the association between DHA level and MOPD were not significantly correlated. Contrary to plasma DHA level as a valid biomarker for LC-PUFAs dietary intake, by analyzing the population of both treatment groups (pre- and post-treatment), our study shows a significant positive relationship between RBCM DHA levels and MPOD. This positive correlation suggests an argument about the status of DHA within the retina and its role in relation to macular pigment concentration.

It has also been observed that some of our study participants (11% of the LT/DHA-G and 17% of the LT-G) displayed unnoticeable MPOD changes, despite having significantly higher plasmatic LT and RBC membrane DHA levels with respect to baseline (see Figures 2, 3, and 4). However, the proportion of non-response was lower among individuals assigned to formula 2 than formula 1, and appreciably lower than those reported by other authors in normal and pathologic eyes [34-36]. Interestingly, Obana et al. [37] have precisely described 3 types of response to administration regarding the increase in MPOD and serum LT levels in a sample of 36 healthy participants: retinal responders (with an increase of both MPOD and serum LT levels), retinal non-responders (neither MPOD nor serum LT concentration increases, but no MPOD changes), and retinal and serum non-responders.

The inter-individual variability in the absorption, circulation, and tissue responses of dietary carotenoids is widely accepted. Recent discoveries in several proteins involved in human carotenoid metabolism point to the fact that genetic variants in genes encoded for these proteins may affect their expression and subsequently the carotenoid availability and function [38]. Strong evidence indicates that gene variants related to carotenoid metabolism play a pivotal role in the uptake of LT and ZX into the macula. Feigl et al. [39] demonstrated in 20 healthy participants that MPOD levels can be related to high and low beta-carotene conversion BCMO1 genotypes and that the above effect may be different in pathologic retinas. Through a study of carotenoids in age-related eye

disease involving 1585 women, Meyers et al. [40] concluded that MPOD is a multi-factorial phenotype associated with variation in genes related to carotenoid transport, uptake, and metabolism that is independent of the well-known dietary and lifestyle influences on MPOD. In this scenario, we may hypothesize that the lower MPOD responses in some participants in the present study may be related to the decrease of oxidative stress and endoplasmic reticulum signaling pathways, involving polymorphisms that require extensive research, as reported by our group regarding the effects of single nucleotide polymorphisms in vitamin E on serum biomarkers for ocular diseases [41].

Nevertheless, our findings highlight the relevance of LT availability and the adjunctive role of DHA when assessing the relevance of nutraceuticals in macular health and disease. Supporting this, previous reports emphasize the potential role of ω-3 LC-PUFAs in late AMD prevention [42,43]. Recently, Arunkumar et al. [30] confirms the results from the AREDS2 supplement formulation [25] that oral formulations containing LT and ZX readily impact MPOD levels, lowering the rate of progression to advanced AMD and visual loss in the affected patients [44]. Furthermore, the Visucam® retinograph offered a suitable and relatively easy non-mydriatic tool for ocular fundus image and MPOD quantification for clinical studies.

Among the study limitations, the first is the relatively small sample size. Large cohorts have to be analyzed with a longer duration to better elucidate the role of LT and DHA in the healthy and pathologic macula. Second, our participants were selected with the intention of being well nourished, but tobacco habits were not taken into consideration. These two facts may partially interfere with the full generalizability of the results, especially when trying to extrapolate these data to AMD.

In summary**,** taking altogether, our data strongly demonstrate that supplementation with daily oral doses of LT (6 mg/d) and DHA (700 mg/d) for 3 months is effective in increasing circulating LT levels as well as MPOD in healthy eyes.

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Statement of Ethics

The present study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All subjects gave their written informed consent. The institute's ethic committee on human research approved the study protocol.

Disclosure

The authors report no conflicts of interest in this work.

Founding source

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Author Contributions

- Vicente Zanon-Moreno participated in work design, acquisition/analysis/ interpretation of data. Also participated in the final approval of the version to be published.
- Joan C. Domingo-Pedrol participated in the acquisition of data, analysis, and interpretation of data for the work.
- Silvia M. Sanz-Gonzalez participated in the acquisition and interpretation of data for the work. Also participated in the critical review of the draft version.
- Jorge Raga-Cervera participated in the acquisition of clinical data.
- Juan J. Salazar-Corral participated in the work design, in the critical review of the draft, and in the final approval of the version to be published.
- Maria D. Pinazo-Duran participated in the work design, acquisition of clinical data, in the critical review of the draft, as well as in the final approval of the version to be published

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Legends for Illustrations

Figure 1. Flow-chart showing classification and operative definition of the study participants. LT-G: lutein group (6 mg LT daily); LT/DHA-G: LT/Docosahexaenoic Acid group (6 mg LT and 700 mg DHA daily).

Figure 2. Basal and final MPOD of the right and left eyes of the LT-G group (A) and of the LT/DHA group (B). Data represents Mean ± SEM (n=47 in LT-G; n=49 in LT/DHA-G). MPOD: macular pigment optical density; RDU: relative densitometric units; RE: right eye; LE: left eye; LT-G: lutein group; LT/DHA-G: LT/Docosahexaenoic Acid group.

Figure 3. Basal and final plasmatic lutein concentrations (A) and RBCM DHA content (B) of both study groups. LT concentration was measured by high performance liquid chromatographic analysis and DHA content was determined by gas chromatography /mass spectrometry analysis. Data represents Mean ± SEM (n=38 in LT-G; n=36 in LT/DHA-G). LT-G: lutein group; LT/DHA-G: LT/Docosahexaenoic Acid group; RBCM: red blood cell membranes.

Figure 4. Scatterplots depicting the correlation between MPOD with the plasmatic lutein concentration ([LT]) in the LT-G (A, n=38), and in the LT-DHA/G (B, n=36). Correlation between MPOD with RBCM DHA levels including all the population studied was shown in (C, n=140). MPOD: macular pigment optical density; RDU: relative densitometric units; LT-G: lutein group; LT/DHA-G: LT/Docosahexaenoic Acid group; RBCM: red blood cell membranes.

Table 1. Inclusion and exclusion criteria for the study participants.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Individuals of both sexes Aged 40-70 years	Aged <40 or > 70 years
Healthy well-nourished persons	Dietary or alimentary disorders, or having relevant ocular or systemic diseases or treatments
Able to understanding the study goals and to provide signed informed consent	Unable to understand and to participate in the study

Table 2. Characteristics of both study formulas for the oral supplementation.

	Supplement containing LT FORMULA 1		Supplement containing LT/DHA FORMULA 2	
Omega-3 LC-PUFA	**Dose/day**	**%RI***	**Dose/day**	**%RI***
DHA (mg)	0		700	
EPA (mg)	0		85	
DPA (mg)	0		60	
VITAMINS				
C (mg)	80	100	53.4	66
E (mg α-TE)	12	100	8	66
B1 (mg)	1.1	100	0.74	66
B2 (mg)	1.4	100	0.94	66
B3 (mg)	16	100	10.6	66
B6 (mg)	1.4	100	0.94	66
B9 (µg)	200	100	133.4	66
B12 (µg)	2.5	100	1.66	66
MINERALS				
Zn (mg)	7.5	75	3.32	33
Cu (mg)	1	100	0.32	33
Se (µg)	55	100	18.32	33
Mn (mg)	2	100	0.66	33
OTHERS				
Lutein (mg)	6	-	6	-
Zeaxanthin (mg)	0,3	-	0,6	-
Glutathione (mg)	1	-	4	-

*%RI: Percentage of the Reference Intake; LT: Lutein; LT/DHA: Lutein plus DHA; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid