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Чи корисне яйце для очей? Додавання лютеїну до функціональних харчових продуктів впливає на стан ділянки жовтої плями в молодих здорових осіб

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Лютеїн є каротиноїдом і одним з основних пігментів з антиоксидантними властивостями в ділянці жовтої плями ока. Метою цього дослідження було визначити вплив споживання лютеїну у вигляді функціональних харчових продуктів (курячих яєць, збагачених n-3 ПНЖК, селеном, вітаміном D і лютеїном) на морфологію жовтої плями в молодих здорових осіб, використовуючи оптичну когерентну томографію (ОКТ).

Учасники та методи. Рандомізоване, подвійне сліпе, плацебо-контрольоване пілотне дослідження проводилося на одинадцяти здорових особах обох статей (середній вік від 18 до 28 років), віднесених у групу Nutri4 і контрольну групу. Протягом тритижневого протоколу група Nutri4 споживала збагачені поживними речовинами курячі яйця, а контрольна група споживала звичайні. На початку та в кінці протоколу учасники проходили офтальмологічні обстеження заднього сегмента ока методом ОКТ. Щоб визначити біохімічні показники крові (концентрація поживних речовин у сироватці, ферменти печінки, ліпідний профіль сироватки крові, маркери запалення), було взято венозну кров.

Результати. Споживання яєць Nutri4 призвело до значного підвищення концентрації n-3 ПНЖР, лютеїну та вітаміну Е в сироватці крові. Після протоколу харчування в групі Nutri4 середня товщина макулярної ділянки (нижній зовнішній шар жовтої плями) збільшилася, тоді як у контрольній групі не було виявлено суттєвих змін у товщині сітківки. Після відповідного протоколу харчування в групі Nutri4 рівень сечовини під-

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вищився, а гамма-глутаміламінотрансфераза суттєво зменшилася, а в контрольній групі збільшився рівень аспартатамінотрансферази.

Висновки. Споживання збагачених поживними речовинами курячих яєць має позитивний вплив на товщину та об'єм сітківки.

Ключові слова: функціональні харчові продукти, лютеїн, жовта пляма, оптична когерентна томографія, яйця.

Can an eye benefit from an egg? Addition of lutein in functional food products affects the macula lutea of young healthy individuals

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Objectives: Lutein is a carotenoid and one of the primary pigments with antioxidative effects found in the macula lutea of an eye. The present study aimed to determine the effects of lutein consumption in the form of functional food (hen eggs enriched with n-3 PUFAs, selenium, vitamin D and lutein) on macula lutea morphology by using an optical coherence tomography (OCT) in young healthy subjects.

Participants and Methods: Randomized, double-blind, placebo-controlled pilot study included eleven healthy young subjects of both sexes (average age 18 to 28 years), assigned to a Nutri4 group and a control group. The Nutri4 group consumed nutritionally enriched hen eggs, while the control group consumed regular ones during the three-week protocol. At the beginning and end of the protocol, the subjects underwent ophthalmological examinations of the posterior eye segment by OCT. Venous blood was sampled to determine biochemical blood parameters (serum concentration of nutrients, liver enzymes, serum lipid profile, inflammatory markers).

Results: Consumption of Nutri4 eggs led to a significant increase in n-3 PUFAs, lutein and vitamin E concentrations

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in serum. The average macular thickness (lower outer layer of the macula) was increased in the Nutri4 group, while no significant change was found in the retinal thickness of the control group after the dietary protocol. Urea was raised, and gamma-glutamyl aminotransferase significantly decreased in the Nutri4 group, while aspartate aminotransferase was increased in the control group after the corresponding dietary protocol.

Conclusion: The consumption of enriched hen eggs has a beneficial effect on the thickness and volume of the retina.

Keywords: Functional food, lutein, macula lutea, optical coherence tomography, eggs.

Introduction

The eye is a highly vascularized organ, and the vascularization itself is essential for supplying the nutrients and oxygen necessary for the cells to function. Therefore, the eye's retina is susceptible to oscillations of oxygen and nutrients, and any imbalance causes damage and eventually leads to the development of eye diseases [1]. Lutein is an essential carotenoid belonging to the xanthophyll subclass [2]. Lutein is known for its preventive properties against eye-related diseases, such as age-related macular degeneration (AMD) [3], cataract [4], diabetic retinopathy [5] and uveitis [6]. Due to its high antioxidant potential and ability to filter blue light, lutein protects the eyes from radiation and prevents the photoaging process of the skin [7], absorbing 40% to 90% of incident blue light, depending on its concentration [5]. Lutein is abundant in leafy green vegetables and brightly colored fruits, and its highest concentration is in spinach, cabbage, kale and egg yolks [8]. The bioavailability of lutein from eggs is much higher than that of other food sources due to the egg's high lecithin content, a free non-esterified form of lutein and simple food matrix [9]. The intake of lutein from dietary supplements can vary between 28.7 and 43.1%. For these reasons, new approaches to formulating lutein supplements and drug delivery therapies, such as lipid nanoparticles and nanocrystals, have been developed [10].

Interestingly, a diet rich in n-3 PUFAs is thought to provide long-term benefits for several chronic eye conditions, including dry eye syndrome (DED) and age-related macular degeneration (AMD) [11]. This diet improves the cellular response of the retina to ischemic, oxidative, and inflammatory damage [12]. Docosahexaenoic acid (DHA) is involved in maintaining the structural and functional properties of the retina [13]. Epidemiological studies have shown a lower risk of developing early-stage AMD and a progression to vision-threatening late forms of AMD owing to n-3 PUFAs intake [14].

Some well-known antioxidants have been investigated in ocular diseases, too. For example, vitamin E is a free radical scavenger, ubiquitous in the human body [15]. Besides its many protective effects, vitamin E participates in the modulation of telomerase activity, thus preventing the development of retinopathy [16]. Epidemiological studies suggest a beneficial effect of vitamin E on AMD progression [17]. Glutathione peroxidase, selenoprotein P (SeP), and selenoprotein W (SeW) are antioxidant selenoproteins that reduce the formation of reactive oxygen species (ROS), such as hydrogen peroxide or lipid hydroperoxides [18]. SeP is a selenium-transport protein that is produced by the lacrimal gland. It is secreted in tears to supply selenium to the corneal epithelium. In DED, selenoprotein levels in tears are reduced, and it is thought that selenium deficiency leads to \the impaired antioxidant capacity of tears [19]. Recently, more interest has risen in natural food products, i.e., functional foods that contain components that benefit body functions beyond their nutritional values [20]. Poultry is especially suitable for functional food products due to the ability to use physiological and metabolic processes to deposit useful ingredients from food in meat and eggs [20]. Eggs can be enriched with functional ingredients by modifying the rations of food for laying hens, resulting in "designed" eggs that meet the definition of functional food [21].

Our studies so far have shown that consumption of n-3 PUFAs enriched eggs could have had

a beneficial effect on microvascular function by increasing the microvascular reactivity of healthy young subjects and athletes [22, 23] and that n-3 PUFAs functionally enriched eggs have anti-inflammatory potential [24, 25]. Furthermore, dietary intake of enriched eggs with four nutrients (n-3 PUFA, vitamin E, lutein and selenium) has a protective effect even in non-inflammatory conditions in healthy young subjects [26].Taken together, the present study's hypothesis is that increased consumption of a functional food diet containing lutein, along with n-3 PUFAs, selenium and vitamin E, may have a beneficial effect on the eye, particularly the retina. This study aimed to determine the impact of functionally enriched hen egg consumption on the posterior segment of the eye in young, healthy individuals.

Materials and Methods

Population and Study Protocol

This was a randomized, double-blinded, prospective, placebo-controlled interventional study (Figure 1), a pilot study as a part of the study where the influence of enriched hen eggs on microvascular function and anti-inflammatory effect in healthy young subjects have been investigated [26]. This study involved eleven healthy young subjects of both sexes, students of the Josip Juraj Strossmayer University in Osijek, aged 18 to 28. Subjects did not have a positive history of cardiovascular disease, were non-smokers, and did not take anti-inflammatory drugs and contraceptives. Exclusion criteria for subjects were previously known presence of hypertension, coronary heart disease, diabetes, hyperlipidemia, kidney damage, cerebrovascular diseases and peripheral vascular diseases. The participants were volunteers who reviewed the research protocol and signed informed consent. The research protocol and procedure met the standards of the latest edition of the Declaration of Helsinki, and the research was approved by the Ethics Committee of the Faculty of Medicine in Osijek (Class: 602-04 / 20-08 / 07, Number: 2158-61-07-20-185).

The study was performed in the frame of the Scientific Center of Excellence for Personalized Health Care University in Osijek, at the Faculty of Medicine Osijek, Laboratory for Molecular and Clinical Immunology, Laboratory for Clinical Physiology and Physiology of Sport and in the Clinic for Ophthalmology of the Clinical Hospital Center Osijek. Measurements were done twice, before and after three weeks of dietary protocols. Subjects were divided into two study groups: a control group consuming regular hen eggs (CTRL, n=6) and a group consuming enriched eggs (Nutri4, n=5). The composition of eggs is presented in Table 1. Participants consumed three eggs per day for 21 days. Nutri4 group consumed approximately 3.29 mg/per day of vitamin E, 1.85 mg per day of lutein, 0.06 mg/per day of selenium and 1026 mg/per day of n-3 PUFAs in 3 eggs per day for three weeks while the control group consumed regular hen eggs produced on the same farm (approximately 1.79 mg/per day of vitamin E, 0.33 mg/per day of lutein, 0.05 mg/per day of selenium and 438 mg/per day of n-3 PUFAs) in 3 eggs per day for three weeks, a product of the poultry farm (Marijančanka d.o.o., Marijanci). All subjects have kept dietary diaries to keep records of daily food intake.

The eggs were of the same L commercial size to reduce the possibility of noticing the difference between regular and Nutri4-enriched hen eggs. Neither subjects nor researchers had access to information about which experimental group a particular subject belonged to until the end of the protocol. On the first and last day of the three-week protocol, venous blood was sampled to determine the blood's biochemical characteristics, and a detailed ophthalmological examination and OCT examination were performed.

Measurement of Anthropometric Characteristics, Biochemical and Hematological Parameters

At each visit, the subjects were measured for body weight and height to determine the body mass index (BMI) and the waist to hips ratio (WHR). BMI was calculated from the ratio of body weight in kilograms and body height in meters squared, and WHR from the ratio of waist circumference to hips in centimeters.

After 30 minutes of rest in a quiet room, fasting venous blood was sampled during each visit. From the preparations of venous blood – serum, plasma and whole blood, biochemi-

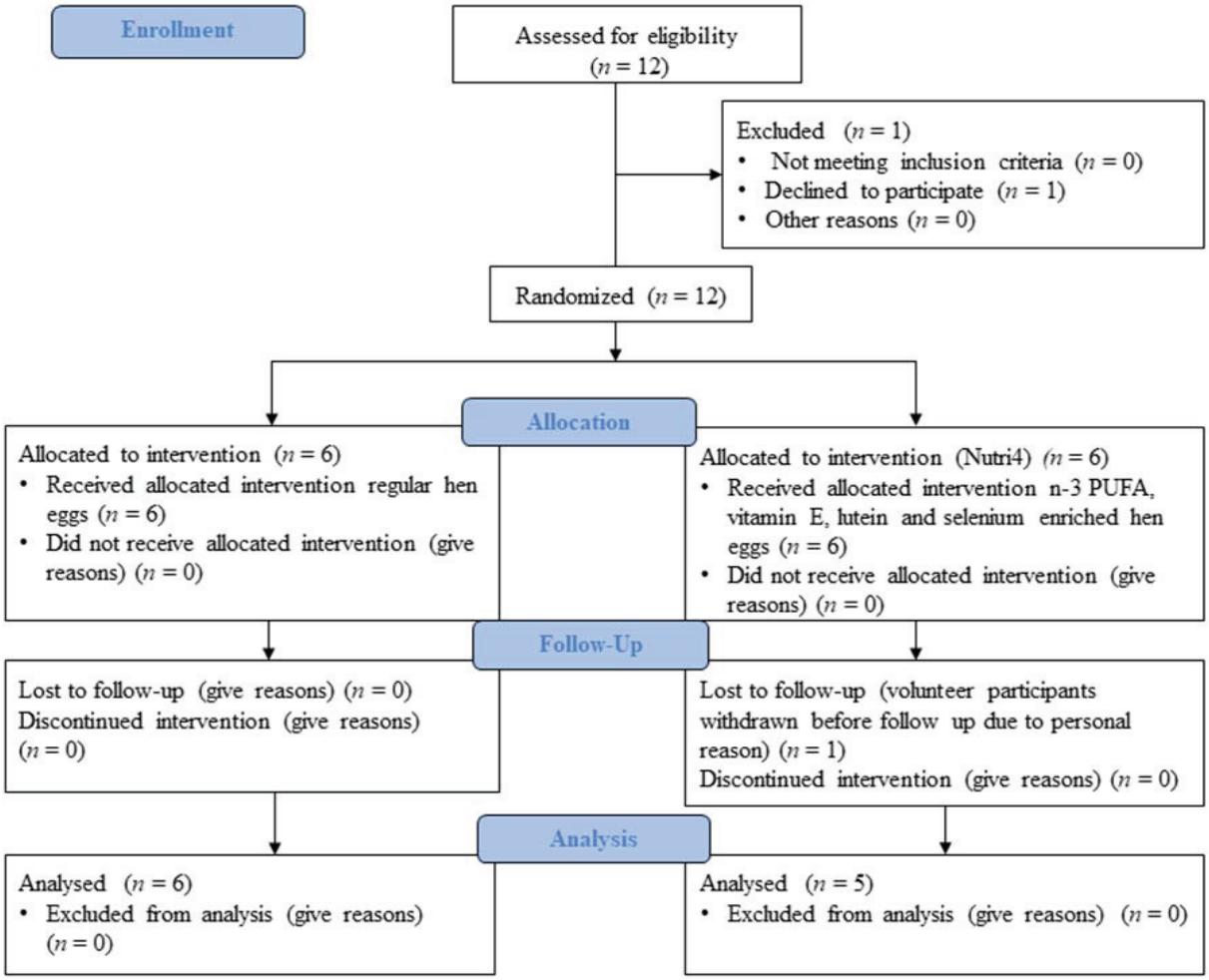


Figure 1. Consort diagram of enrollment of subjects

cal and hematological biomarkers were determined in the laboratory of the Department of Clinical Laboratory Diagnostics, Clinical Hospital Center Osijek. The following biochemical characteristics were assessed: triglyceride, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), C-reactive protein (CRP), blood glucose (GUK), urea, creatinine, urate, sodium, potassium, calcium, total iron and transferrin.

Measurements of Serum Fatty Acids Profile

The serum fatty acid profile was determined by gas-chromatography–tandem mass spectrometry (GC–MS/MS), a system by Thermo Fisher GC Trace 1300 coupled with a TSQ 9000 Triple Quadrupole. For the preparation of standard solutions, a solution of fatty acid methyl esters (FAME MIX) was purchased as 30 mg/ml of the total concentration of fatty acids in methylene chloride from Supelco (Supelco Inc., Bellefonte, PA, USA). Prior to analysis, samples were stored at – 80 °C and prepared for analysis according to Wang et al. [27]. Shortly, to 20 µl of serum sample in an Eppendorf tube, an additional 200 µl of saline, 1 mL of methanol and 2 mL of chloroform were added and mixed for 10 minutes on a rotary stirrer. Then, 500 µl of 1M NaCl was added and stirred for another 2 minutes. After stirring, the sample was centrifuged at room temperature for 10 minutes at 3000 rpm. After centrifugation, the layers were separated, and 1 mL of the lower chloroform layer was taken into a glass tube and evaporated to dryness under a stream of nitrogen. Fatty acid methylation was started by adding 1 mL of 14% methanolic BF 3 to the vaporized sample. The sample was then placed on a 75 °C thermoblock for 45 minutes. After

cooling, 1 ml of highly purified water and 1 ml of hexane were added and stirred for 2 minutes. After separating the layers, 600 µl of the upper hexane layer was evaporated to dryness. The sample was recovered with 600 µl of hexane and analyzed. Serum fatty acids profile analysis was performed at the BIOCentre's Bioanalytical Laboratory, BIOCentre – incubation center for biosciences, Zagreb, Croatia [23].

Measurements of Serum Vitamin E Concentration

Vitamin E concentrations in serum samples were determined according to the existing protocol [28]. First, absolute ethanol was used to denature serum proteins, and Xylene was used to separate the supernatant from proteins. The mixture was centrifuged at 3000g for 10 minutes, and the supernatant was separated. 2,2-bipyridyl and FeCl3 were then added to the supernatant, resulting in pink coloration. After 2 minutes of incubation, the absorbance was measured using a spectrophotometer (PR 3100 TSC Microplate Reader, Bio-Rad Laboratories, Hercules, California) at 492 nm, and the obtained absorbance was proportional to the serum vitamin E concentration. A five-point calibration curve was also made.

Serum Selenium Concentration Measurements

All samples for the measurement of serum Se concentration were digested in ultra-pure HNO3 and H2O2 (5:1 ratio) at 180 ° C for 60 minutes in a close microwave system CEM Mars 6 (CEM, Matthews, NC, USA). Se concentrations in solutions of digested serum samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) (ICP-MS, Agilent 7500a, Agilent Technologies Inc., California, USA). Each serum sample on the ICP was analyzed by internal pooled plasma control, and reference material NIST 1567b (wheat flour, National Institute of Standards and Technology, USA) was used to control the analytical method. All samples were analyzed in triplicate. Measurements were performed at the Department for Agroecology and Environment Protection, Faculty of Agrobiotechnical Sciences Josip Juraj Strossmayer University of Osijek.

Measurements of Serum Lutein Concentration

Lutein concentrations in serum samples were determined according to the existing protocols. In 200 ul of serum, we added 1 ml of deionized water and 0.01% ascorbic acid dissolved in absolute ethanol and stirred the mixture. Then we added 2 mL of hexane, stirred and centrifuged at 2500 RPM for 20 minutes. After centrifugation, we separated the supernatant, evaporated the supernatant and determined the concentration of lutein using HPLC. HPLC analysis was performed at the Department of Chemistry, Josip Juraj Strossmayer University of Osijek [29, 30].

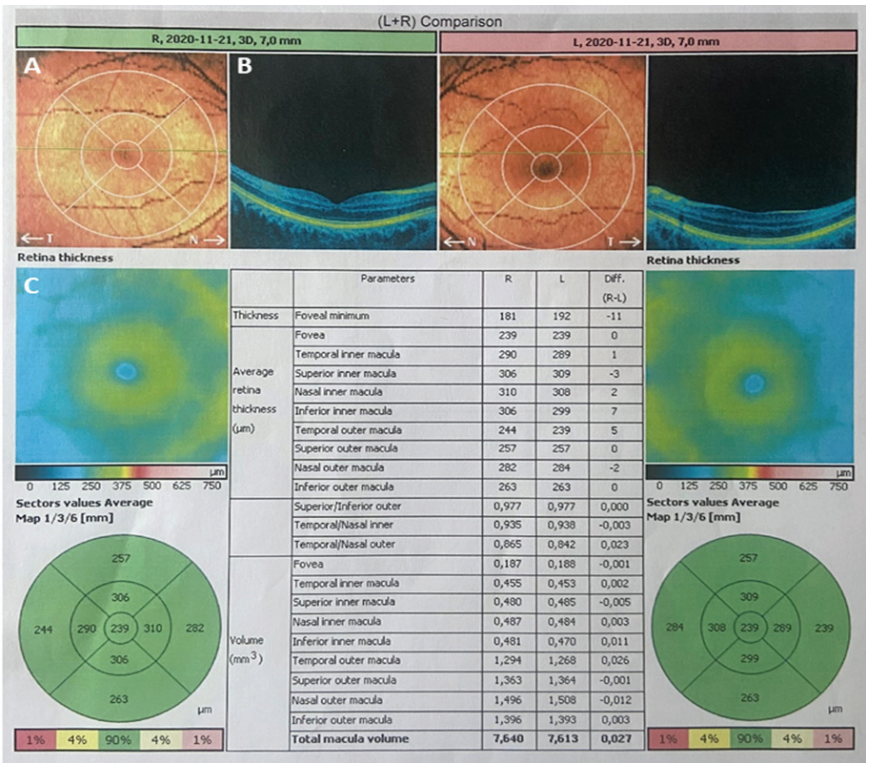
Ophthalmological Examination

An ophthalmological examination was performed before inclusion in the study and after dietary protocols. The socio-demographic data and personal, family, and ophthalmologic histories were taken from each patient, including questions about diseases and conditions considered to be risk factors (diabetes mellitus, hypertension, thyroid disease and other disbalance hormone and immune disease). General characteristics around the respondents included assessment of visual acuity (logMAR), emmetropia (%), myopia (%), hypermetropia (%), astigmatism (%), anterior segment pathology (%), posterior segment pathology (%) and intraocular pressure (mm Hg) in Table 2. Complete ophthalmological examination of both eyes included: measuring Best-Corrected Distance Visual Acuity (BCDVA) using ETDRS tables (expressed to two decimal places), biomicroscopy using Zeiss slit lamp (SL-120, Carl Zeiss, Germany), examination of the ocular background by indirect ophthalmoscopy on a biomicroscope (SL-120, Carl Zeiss, Germany), gonioscopy using Goldmann's indirect goniolet, intraocular pressure (expressed in mmHg, to one decimal place) using Goldmann's applanation method on a biomicroscope (SL-120, Carl Zeiss, Germany); OCT macula on the SOCT Copernicus device (Reichert / Optopol Technology, Inc., Depew, NY). All subjects were ordered for a follow-up examination three weeks after the first examination, i.e. from the beginning of egg consumption.

In this study, the SOCT Copernicus device (Reichert / Optopol Technology, Inc., Depew, NY) using the spectral region optical coherence tomography (OCT) method was used to obtain three-dimensional cross-sectional images of the human retina. The device produces a laser beam that focuses on the human retina. The light reflected from the internal structures of the retina is interferometrically analyzed using a device. The obtained data are computer processed to obtain images of the cross-section of the retina.

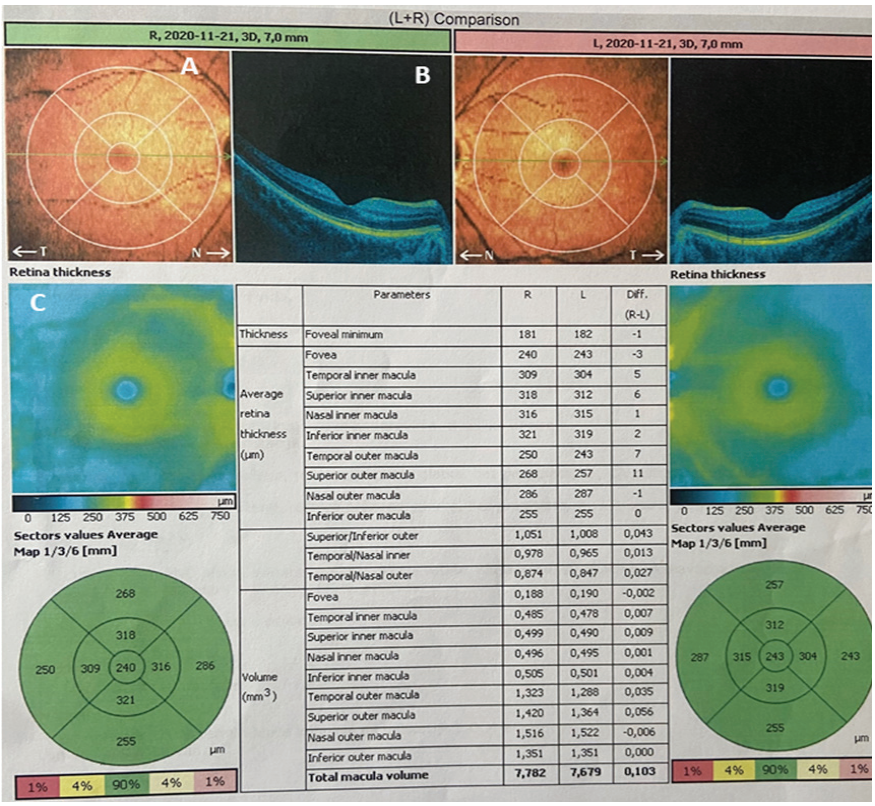
In the group of patients who ate enriched eggs, as well as in the control group, the OCT of the macula was recorded before the start of the study and after three weeks. Prior to the screening, each subject underwent mydriasis using a combination of 5% tropicamide and 10% phenylephrine. The same device settings and accompanying software were used for all respondents. An internal fixation light was used to center the scan field. OCT of the macular area includes a horizontal tomogram through the foveal area and maps and graphs of the macular area. There are a macular thickness map and a graph, a retinal nerve fiber layer map and a graph (RNFL), an RPE deformation map and a graph, an R / deformation map of IS / IOS layer, and many others.

The representation used during the recording of the subjects was in the pseudo-color scale, where the highly reflective structures are shown in colors of larger wavelengths of the visible part of the spectrum (from green to red), and the structures that reflect the most are white. Low-reflecting structures are shown in colors of smaller visible spectrum wavelengths (from green to blue), and non-reflecting structures are black. For example, RNFL, IPL, and OPL are displayed as red, yellow, or light green, and INL and ONL – as blue or red. Any change that reflects light more strongly will result in shading of the changes behind it, while any lack of tissue that otherwise reflects light will result in a relatively enhanced reflection of tissues located distally (94). In addition to the classic B – views, graph processing and maps of macular thickness were made by compu-



	Parameters	R	L	Diff. (R-L)
Thickness	Foveal minimum	181	192	-11
Average retina thickness (µm)	Fovea	239	239	0
	Temporal inner macula	290	289	1
	Superior inner macula	306	309	-3
	Nasal inner macula	310	308	2
	Inferior inner macula	306	299	7
	Temporal outer macula	244	239	5
	Superior outer macula	257	257	0
	Nasal outer macula	282	284	-2
	Inferior outer macula	263	263	0
	Superior/Inferior outer	0,977	0,977	0,000
	Temporal/Nasal inner	0,935	0,938	-0,003
	Temporal/Nasal outer	0,865	0,842	0,023
Volume (mm^3)	Fovea	0,187	0,188	-0,001
	Temporal inner macula	0,455	0,453	0,002
	Superior inner macula	0,480	0,485	-0,005
	Nasal inner macula	0,487	0,484	0,003
	Inferior inner macula	0,481	0,470	0,011
	Temporal outer macula	1,294	1,268	0,026
	Superior outer macula	1,363	1,364	-0,001
	Nasal outer macula	1,496	1,508	-0,012
	Inferior outer macula	1,396	1,393	0,003
	Total macula volume	**7,640**	**7,613**	**0,027**

Figure 2. SOCT Copernicus device typical finding in Nutri4 group. A. Image of the fundus in which the position of the tomogram shown is marked with a green arrow. B. B-view of macular tomogram prose area of foveola. C. Macular thickness map



	Parameters	R	L	Diff. (R-L)
Thickness	Foveal minimum	181	182	-1
Average retina thickness (µm)	Fovea	240	243	-3
	Temporal inner macula	309	304	5
	Superior inner macula	318	312	6
	Nasal inner macula	316	315	1
	Inferior inner macula	321	319	2
	Temporal outer macula	250	243	7
	Superior outer macula	268	257	11
	Nasal outer macula	286	287	-1
	Inferior outer macula	255	255	0
	Superior/Inferior outer	1,051	1,008	0,043
	Temporal/Nasal inner	0,978	0,965	0,013
	Temporal/Nasal outer	0,874	0,847	0,027
Volume (mm^3)	Fovea	0,188	0,190	-0,002
	Temporal inner macula	0,485	0,478	0,007
	Superior inner macula	0,499	0,490	0,009
	Nasal inner macula	0,496	0,495	0,001
	Inferior inner macula	0,505	0,501	0,004
	Temporal outer macula	1,323	1,288	0,035
	Superior outer macula	1,420	1,364	0,056
	Nasal outer macula	1,516	1,522	-0,006
	Inferior outer macula	1,351	1,351	0,000
	Total macula volume	**7,782**	**7,679**	**0,103**

Figure 3. SOCT Copernicus device typical finding CTRL group. A. Image of the fundus in which the position of the tomogram shown is marked with a green arrow. B. B-view of macular tomogram prose area of foveola. C. Macular thickness map

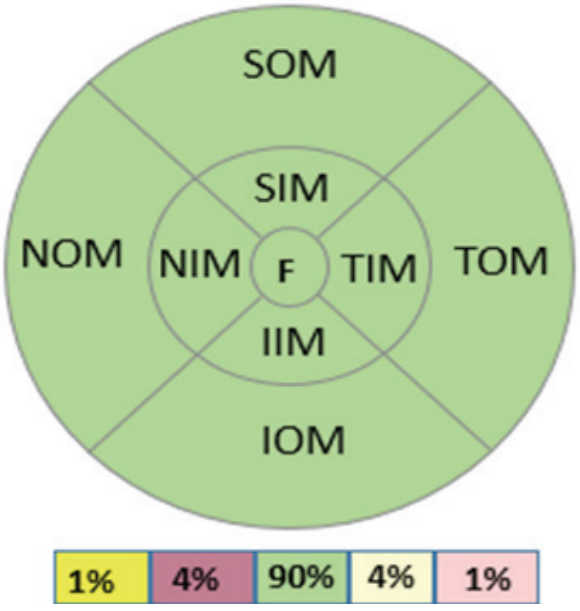


Figure 4. Graphical representation of the division of macular regions used in the formation of tabular data. F- fovea, TIM-temporal inner macula, SIM-superior inner macula, NIM-nasal inner macula, IIM-inferior inner macula, TOM-temporal outer macula, SOM-superior outer macula, NOM-nasal outer macula, IOM- inferior outer macula

ter processing, which provided a better insight into the morphology of the macula, and facilitated the interpretation of the findings. Representative findings of the Nutri4 group (Figure 2) and CTRL group (Figure 3) subject after dietary protocol made by the OCT method (Figure 4).

Statistical Methods

The methods of descriptive statistics were applied. The sample size for a test strength of 0.85, a p-value of less than 0.05 and a minimum expected difference of 0.30 is five samples (subjects) per group. The results are presented as arithmetic mean and standard deviation. Data which do not follow a normal distribution are presented as Median (Min-Max). The normality of the distribution of variables was examined by the Shapiro-Wilk distribution normality test. The differences in the normally distributed numerical variables between the two independent groups were tested by the Student's t-test and, in the case of deviations from the normal distribution, by the Mann-Whitney U-test.

Table 1

Composition of nutrient ingredients in regular eggs and Nutri 4 eggs

An L-grade egg with an average weight of 68 g has about 60 g of edible portion		
Parameters	**Regular eggs**	**Nutri4 eggs**
Vitamin E (mg)	0.595 ± 0.174	1.098* ± 0.339
Lutein (mg)	0.11 ± 0.011	0.616* ± 0.085
Selenium (mg)	0.0183 ± 0.002	0.02305* ± 0.0012
Fatty acids		
∑SFA	1566 ± 346	1442 ± 185
∑MUFA	1976 ± 189	2419 ± 139
∑n-6 PUFA	1263 ± 148	747 ± 46*
LA	1165 ± 140	702 ± 43
AA	89 ± 9	44 ± 4*
∑n-3 PUFA	146 ± 20	342 ± 25*
ALA	71 ± 11	189 ± 16*
EPA	n.d.	19 ± 2*
DHA	75 ± 11	135 ± 11*
∑n-6/∑n-3 PUFA	8.71	2.18*
Numbers are mean ± standard deviation (SD). *p < 0.05 paired t-test Regular eggs vs. Nutri4 enriched hen eggs		

Results

The Association between Enriched Hen Eggs Consumption and Anthropometric, Biochemical and Ophthalmological Findings

The anthropometric and biochemical measurements are presented in Tables 2 and 3. There was no significant difference in creatinine, urate, sodium, potassium, glucose, hsCRP, calcium, iron, transferrin, cholesterol, triglycerides, HDL, LDL, HDL-C cholesterol (%) and ALT before and after respective protocol or between the groups. In the Nutri4 group, urea increased significantly after the dietary protocol, with the results being within the reference interval. After the dietary protocol, GGT was significantly reduced in the Nutri4 group but within the reference interval. In the CTRL group, after consuming regular hen eggs, there was a significant decrease in AST but within the reference interval.

There was no significant difference in the ophthalmic examination, visual acuity (logMAR), emmetropia (%), myopia (%), hypermetropia (%), astigmatism (%), anterior segment pathology (%), posterior segment pathology (%), intraocular pressure (mm Hg) before and after respective protocol or between the groups (Table 2).

The serum concentration of n -3 PUFA: DHA (μmol/L) (before 59.6 ± 26.1 vs after 122.1 ± 60.7) and EPA (μmol/L) (before 12.7 ± 2.9 vs after 16.6 ± 4.6), lutein (μmol/L) (before 0.102 ± 0.018 vs after 0.259 ± 0.087) and vitamin E (μg/mL) (before 7.63 ± 3.82 vs after 10.46 ± 2.57) was significantly increased in the Nutri4 group after the dietary protocol, while in the CTRL group, there was no significant change after respective dietary protocol. Likewise, there were no significant differences in selenium concentration after the dietary protocol in the CTRL and Nutri4 groups (Table 3).

Results of OCT Parameters of Anatomical Macular Regions of the Right and Left Eyes

In the CTRL group, there were no significant differences in F – fovea, TIM – temporal inner macula, SIM – superior inner macula, NIM – nasal inner macula, IIM – inferior inner macula, TOM – temporal outer macula, SOM – superior outer macula, NOM – nasal outer macula, IOM – inferior outer macula during the study period (Table 4). In the Nutri4 group, there was a significant increase in the average IPM – inferior outer macula thickness of both eyes after the dietary protocol, while there was no change in other measured parameters after the dietary protocol (Table 5).

Table 2

Influence of consumption of control and Nutri4 enriched hen eggs on anthropometric and ophthalmological parameters

Anthropometric prameters	Control group		Nutri4 group	
	Before	After	Before	After
N (F/M)	6		5	
Year	22 ± 1		20 ± 1	
BMI (kg/m2)	25.2 ± 5.7	25.2 ± 5.6	24.3 ± 2.8	24.8 ± 2.8
WHR	0.83 ± 0.09	0.83 ± 0.09	0.77 ± 0.05	0.77 ± 0.04
SBP (mmHg)	108 (100 – 119)	105 (100 – 107)*	120 (93 – 132)	115 (111 – 133)
DBP (mmHg)	69 (63 – 80)	74 (64 – 78)	71 (62 – 78)	71 (62 – 80)
MAP (mmHg)	83 (78 – 89)	83 (77 – 87)	88 (76 – 95)	85 (82 – 92)
HR (beats per minute)	78 ± 10	82 ± 8	82 ± 7	80 ± 12
Ophthalmic examination				
Visual acuity (logMAR)	0.1 ± 1.5	0.1 ± 1.5	0.1 ± 1.5	0.1 ± 1.5
Emetropia (%)	0		0	
Myopia (%)	2 (33%)		2 (40%)	
Hypermetropia (%)	3 (50%)		1 (20%)	
Astigmatismus (%)	1 (16%)		2(40%)	
Anterior segment pathology (%)	0		0	
Posterior segment pathology (%)	0		0	
Intraocular pressure (mm Hg)	14 ± 2.2	14.5 ± 2	13.6 ± 1.3	13.4 ± 1.2

Data are presented as mean ± standard deviation (SD). Data which does not follow a normal distribution are presented as Median (Min-Max). BMI – body mass index; WHR – waist to hip ratio; SBP – systolic blood pressure; DBP – diastolic blood pressure; MAP – mean arterial pressure; HR – heart rate; * P <0.05 before or after within the group; † P <0.05 difference between groups

Table 3

Influence of consumption of regular and Nutri4 enriched hen eggs on serum lutein concentration and biochemical parameters in healthy subjects

Parameters	Control group		Nutri4 group	
	Before	After	Before	After
1	2	3	4	5
Urea (mmol/L)	4.1 (3.6 – 7.9)	5.1 (4.2 – 7.7)	3.75 (3.6 – 4.9)	5.15 (4.2 – 5.9)*
Creatinine (μmol/l)	75 ± 21	73 ± 22	81 ± 22	78 ± 15
Urate (μmol/l)	314 ±60	312 ± 63	313 ± 56	315 ± 58
Sodium (mmol/l)	140 ± 1	138 ± 1	141 ± 1	139 ± 0.4
Potassium (mmol/L)	4.3 ± 0.4	4.2 ± 0.2	4.1 ± 0.1	4.1 ± 0.2
Glucose (mmol/L)	6.2 ± 1.5	5.2 ± 0.2	4.8 ± 1.1	4.6 ± 0.39
hsCRP (mg/L)	0.8 (0.23 – 4.29)	0.8 (0.13 – 3.61)	0.4 (0.2 – 1.7)	0.5 (0.23 – 1.31)
Calcium(mmol/L)	2.3 ± 0.07	2.4 ± 0.049	2.4 ± 0.07	2.4± 0.08
Iron (umol/l)	17.5 ± 7.1	12.8 ± 5.6	16.6 ± 5.3	20.6 ± 9.8
Transferrin (g/L)	2.9 ± 0.5	2.9 ± 0.9	2.6 ± 0.2	2.5 ± 0.3
Cholesterol (mmol/L)	4.3 ± 0.8	4.7 ± 0.8	4.5 ± 0.8	4.6 ± 0.8
Triglycerides (mmol/L)	1.04 ± 0.4	0.9 ± 0.3	1.1 ± 0.4	0.9 ± 0.2
HDL (mmol/L)	1.2 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.4
LDL (mmol/L)	2.6 ± 0.3	2.9 ± 0.8	2.6 ± 0.76	2.7 ± 0.15
HDL-C Kolesterol (%)	30.1 ± 6.9	29.3 ± 8.2	31.8 ±6.9	31 ± 8
AST (U/L)	22 (17 – 55)	18 (15 – 45)*	21 (14 – 39)	19 (15 – 45)
ALT (U/L)	25 (10 – 113)	23 (13 - 125)	21 (11 – 45)	18 (12 – 63)
GGT (U/L)	15 (9 – 51)	14 (8 – 56)	21 (14 – 37)	18 (13 – 33)*
Lutein (μmol/L)	0.131 ± 0.071	0.073 ± 0.115	0.102 ± 0.018	0.259 ± 0.087*†

Continuation of Table 3

1	2	3	4	5
Selenium (µg/L)	62.44 ± 9 .37	66.37 ± 10.09	64.88 ± 17.65	68.88 ± 3.082
Vitamin E (µg/mL)	9.87 ± 3.47	10.40 ± 3.78	7.63 ± 3.82	10.46 ± 2.57*
ALA (µmol/L)	14.2 ± 7.7	15 ± 5.1	20.4 ± 6.3	21.1 ± 8.0
DHA (µmol/L)	77.5 ± 18.9	78.9 ± 36.7	59.6 ± 26.1	122.1 ± 60.7 *
EPA (µmol/L)	20.1 ± 6.9	21.1 ± 4.6	12.7 ± 2.9	16.6 ± 4.6 *†
n/6 n/3	11.1	8.3	10.5	6.3*
Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Alpha-Linolenic acid (ALA); Data are presented as mean ± standard deviation (SD).; Data which does not follow a normal distribution are presented as Median (Min-Max). * P <0.05 before or after within the group; † P <0.05 difference between groups				

Table 4

Effect of consumption of regular hen eggs on the thickness of macular regions in control group

Parameter (µm)	**Control group**			
	Right eye		Left eye	
	Before	After	Before	After
SOM	269 ± 12	268 ± 12	265 ± 15	266 ±13
SIM	314 ± 16	314 ± 17	312 ± 16.5	312 ±15
TOM	288 ± 17	287 ± 16	248 ± 14	248 ± 10
TIM	315 ± 14	312 ± 17	300 ± 12	301 ± 11
IOM	260 ± 14	260±14.5	255 ± 10.8	255 ± 9
IIM	313 ± 1	313 ± 13.4	312 ± 13.9	312 ± 13
NOM	250 ± 9	252 ± 10.8	286 ± 15.2	288 ± 12
NIM	301 ± 11	301 ± 12	311 ± 16	312 ± 15
FOVEA	252 ± 31	232 ± 15	235 ± 18	233 ± 20
Results are expressed as mean ± standard deviation (SD), F- fovea, TIM-temporal inner macula, SIM – superior inner macula, NIM – nasal inner macula, IIM – inferior inner macula, TOM – temporal outer macula, SOM – superior outer macula, NOM – nasal outer macula, IOM – inferior outer macula				

Table 5

Effect of consumption of Nutri4 enriched hen eggs on the thickness of macular regions in Nutri4 group

Parameter (µm)	**Nutri4 group**			
	Right eye		Left eye	
	Before	After	Before	After
SOM	274 ± 15	276 ± 15	273 ± 17	275 ± 17
SIM	321 ± 22	320 ± 21	320± 20	321 ± 20
TOM	297 ±15	298 ± 18	252 ± 16	256 ± 16
TIM	321 ± 21	320 ± 21	301± 20	300 ± 20
IOM	269 ± 16	262 ± 14	269 ± 13	271 ± 12*†
IIM	316 ± 20	316 ± 21	313 ± 20	312 ± 21
NOM	258 ± 19	257 ± 17	300 ± 14	299 ± 15
NIM	303 ± 21	302 ± 22	319 ± 20	319 ± 21
FOVEA	251 ± 20	245 ± 21	246 ± 21	245 ± 21
Results are expressed as mean ± standard deviation (SD) * P <0.05 before or after within the group; † P <0.05 difference between groups), F – fovea, TIM – temporal inner macula, SIM – superior inner macula, NIM – nasal inner macua, IIM – inferior inner macula, TOM – temporal outer macula, SOM – superior outer macula, NOM – nasal outer macula, IOM – inferior outer macula				

Discussion

The aim of this study was to assess the impact of lutein, n-3 PUFA, selenium and vitamin E-enriched egg consumption on the visual function and retinal morphology of healthy human subjects. Consumption of enriched hen eggs led to increased concentrations of lutein, n-3 PUFAs and vitamin E in the serum of participants. Importantly, we observed an increase in CRT in one of the macular areas of the eye in subjects who were on a lutein-enriched diet following dietary protocol.

As previously demonstrated, consumption of n-3 PUFAs enriched eggs has important beneficial effects by enhancing microvascular reactivity and supporting an anti-inflammatory environment [23]. In addition, recently, we have shown that consuming eggs enriched with these four nutrients has promoted inflammatory-resolving conditions in healthy young individuals [26]. Interestingly, in DED, the symptoms and signs of the disease are reduced by modulating the inflammation of the eye surface and by improving the lipid layer of the tear film [31, 32]. Moschos et al. showed that carotenoids have a protective effect on the macula in diabetic patients by showing a significant increase in central retinal thickness after two years of dietary supplementation [33]. Therefore, our finding in this study of a substantial increase in mean IPM-inferior outer macular thickness in subjects who consumed functionally enriched hen eggs is consistent with previously established evidence, and the absence of similar effects in the control group gives the notion of the protective effect of lutein and n-3 PUFAs supplementation validity. Kan et al. researched a sample of 360 subjects, who were divided into four groups: placebo control group, while the remaining three groups of subjects received chewable tablets of marigold extract, which contained lutein with lutein intake of 6 mg/day, 10 mg/day, and 14 mg lutein/day in tablet form once/day for 90 days, respectively. Subjects who consumed lutein reported reduction in eye fatigue, eye swelling and general eye discomfort compared to the placebo group, as well as increased tear secretion in the 10 mg and 14 mg lutein/day group at the end of 90-day trial. Macular pigment optical density (MPOD) was significantly increased in the groups that consumed lutein extract, while there was no significant increase in retinal thickness [34]. Thus, lutein consumption can affect several of macular and retinal parameters, depending on the duration of dietary or ingestion protocol. Taken all together, enhanced microvascular reactivity, as well as anti-inflammatory conditions that occur after consumption of enriched hen eggs [23–29] could have favorable effects on an eye function, including retinal blood flow and macula lutea morphology and function.

Egg yolk contains essential fatty acids, vitamins and minerals necessary for the normal functioning of the human organism. Enriching eggs with functional ingredients is easy due to the high-fat content of the egg yolk [20, 35]. By consuming meat and eggs enriched with functional elements, one can avoid consuming other food supplements and can affect the increase in the amount of functional ingredients in the blood and tissues, benefit the heart and reduce body weight, which is among the most critical health concerns related to the modern lifestyle [20, 36]. It is crucial to notice that despite the consumption of a relatively large amount of eggs in the three-week diet trial, the lipid profile of participants was not affected. Previously, we have demonstrated that consumption of n-3 PUFAs enriched hen eggs can decrease LDL cholesterol in patients with coronary artery disease [37].

Clinical epidemiological studies have reported the positive effects of dietary intake of n-3 PUFA on chronic inflammatory conditions [23, 38–40]. Metabolites of n-3 PUFA and n-6 PUFA play a key role in initiating and maintaining inflammation, breaking the inflammatory response, and blocking further cellular recruitment while promoting phagocytosis and tissue recovery [41]. For the same reasons, the intake ratio of n-3 and n-6 PUFA is important for the homeostasis of pro-inflammatory and anti-inflammatory metabolites [38, 42]. Eggs enriched in n-3 PUFA contain 5.3 times more ALA, 20 times more EPS, and 3.5 times more DHA than conventional eggs [43]. A recent study by Stupin et al. has shown that consumption of four-nutrients enriched hen eggs leads to a significant increase in levels of anti-inflammatory cytokine interleukin 10 (IL-10) and a decrease in levels of serum interferon gamma [23]. This is in line with other findings that increased serum concentrations

of n-3 PUFA from enriched hen's eggs can have anti-inflammatory properties, reducing the concentration of IL-6, MCP-1, TNF-α and hs-CRP, but also have a beneficial effect on microvascular reactivity in healthy young subjects [23, 44].

Eggs are rich in high-quality, easy-to-digest protein, with amino acid architecture similar to human protein [45]. In the Nutri4 group, urea levels were significantly higher due to the metabolic pathway of protein degradation, whose intake was increased due to the nutritional characteristics of the enriched diet, and GGT was considerably lower, which can be attributed to the total antioxidant capacity of the functional elements. In the CTRL group, a significant reduction in AST was found, which indicates a favorable effect of the nutritional components of hen eggs on overall liver function. Altogether, the results indicate an association between the intake of enriched and standard hen eggs and ophthalmological, anthropometric, and biochemical parameters in healthy subjects.

Further research in this field should focus on establishing an optimal dose of lutein in the form of functional food, considering the age and gender of subjects and pharmacodynamic/kinetic properties of lutein to exploit its potential in preventing and treating certain diseases. Larger sample sizes and longer follow-ups are necessary for these studies to ensure research quality.

In conclusions: Any significant increase in retinal thickness and volume is positive. In future research, it is necessary to pay attention to the external nasal macular region, where the retinal nerve fiber layer (RFNL) is localized, due to the possible connection between the action of functional elements on increasing macular pigment (MP), volume and thickness of nerve tissue.

Limitation of the present study: this was a pilot study; thus, a relatively small number of participants was included. However, despite this limitation, we were able to demonstrate a proof of concept that the intake of functional food could particularly affect certain organs or organ systems, such as the eye. Compared to some other studies that utilized supplements, the duration of the present protocol of enriched egg consumption was shorter. Nevertheless, some significant beneficial changes in retinal thickness were observed, which supported the hypothesis that dietary intake of nutrients can significantly impact health.

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Disclosures
The authors declared no conflict of interest.

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Institutional Review Board Statement: The study protocol and procedures conformed to the standards set by the latest revision of the Declaration of Helsinki and were approved by the Ethical Committee of the Science Center of Excellence, Josip Juraj Strossmayer University of Osijek (Cl: 602-04/14-08/06; No: 2158-610714-114) and Ethics Committee of the Medical Faculty Osijek (Cl: 602-04/20-08/07, Registration number: 2158-61-07-20147).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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