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ORIGINAL RESEARCH

Enhancing Endothelial Function with Nutrient-Enriched Table Hen Eggs: A Randomized Study in Patients Recovering from Acute Coronary Syndrome

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Purpose: This study investigated the effect of consumption of table eggs enriched with n-3 polyunsaturated fatty acids (n-3 PUFA), lutein, vitamin E and selenium on microvascular function, oxidative stress and inflammatory mediators in patients after acute coronary syndrome (ACS).

Patients and Methods: In a prospective, randomized, interventional, double-blind clinical trial, ACS patients were assigned to either the Nutri4 (N=15, mean age: 57.2 ± 9.2 years), or the Control group (N=13; mean age 56.8 ± 9.6 years). The Nutri4 group consumed three enriched hen eggs daily for three weeks, providing approximately 1.785 mg of vitamin E, 0.330 mg of lutein, 0.054 mg of selenium and 438 mg of n-3 PUFAs. Biochemical parameters, including serum lipids, liver enzymes, nutrient concentrations, serum antioxidant enzyme activity (catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD)), and markers of oxidative stress (thiobarbituric acid reactive substances (TBARS) and ferric reducing ability (FRAP)), were assessed before and after the dietary interventions. Additionally, arterial blood pressure, heart rate, body composition, fluid status, anthropometric measurements, and skin microvascular blood flow responses to various stimuli (postocclusive reactive hyperemia (PORH), acetylcholine- (Ach ID), and sodium nitroprusside- (SNP ID)) were measured using laser Doppler flowmetry (LDF) throughout the study.

Results: The intake of Nutri4 eggs led to a significant reduction in LDL cholesterol levels, while the levels of total cholesterol remained within the established reference values. Consuming Nutri4 eggs resulted in a 12.7% increase in serum vitamin E levels, an 8.6% increase in selenium levels, and demonstrated a favorable impact on microvascular reactivity, as evidenced by markedly improved PORH and ACh ID. Nutri4 eggs exerted a significant influence on the activity of GPx and SOD, with no observed changes in TBARS or FRAP values.

Conclusion: The consumption of Nutri4 eggs positively influenced microvascular function in individuals with ACS, without eliciting adverse effects on oxidative stress.

Keywords: acute coronary syndrome, microvascular function, n-3 PUFA, lutein, selenium, vitamin E

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Introduction

Coronary artery disease, characterized by the formation of atherosclerotic plaque in the epicardial coronary arteries, remains the leading cause of mortality worldwide despite significant therapeutic advancements.[1,2] Depending on the process affecting the coronary arteries and consequently the clinical presentation, patients can be categorized as having acute or chronic coronary disease.[3,4] ACS refers to a group of conditions involving sudden disruption of blood flow in the cardiac muscle due to coronary artery obstruction. It encompasses conditions such as unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI).[3] Contributing factors to the pathogenesis of ACS commonly include dyslipidemia, arterial hypertension, and diabetes mellitus.[5] All these conditions are underlined by endothelial dysfunction.

Endothelial dysfunction occurs when there is endothelial damage and an imbalance between vasodilators and vasoconstrictors.[6] High levels of low-density lipoprotein cholesterol (LDL), often associated with hypercholesterolemia, elevate oxidative stress and inflammation. This process ultimately diminishes the availability of nitric oxide (NO), an essential endothelial vasodilator.[7] NO is released by endothelial cells in response to different stimuli such as acetylcholine, bradykinin or shear stress, causing vascular smooth muscle relaxation and vasodilation.[7,8] Additionally, to maintain vascular tone, the endothelium releases vasoconstrictive factors such as endothelin-1, thromboxane, and prostaglandin F2α. Elevated oxidative stress and the generation of pro-inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF α) play a role in the initiation of endothelial dysfunction. The imbalance between the production of reactive oxygen species (ROS) and the defensive mechanisms provided by enzymatic and non-enzymatic antioxidants amplifies the severity of endothelial dysfunction.[9] Additionally, oxidative stress has gained acknowledgment as a novel risk factor implicated in the onset of ACS, influencing prognosis, quality of life, and the survival of patients.[10] Apart from its correlation with atherosclerosis, oxidative stress has the potential to trigger oxidative alterations or harm via lipid peroxidation at the deoxyribonucleic acid (DNA) and protein levels. This process can have detrimental consequences on the structure and functionality of the cardiovascular system.[11]

Given that ACS is a critical and urgent condition, being the primary contributor to mortality, emphasizing its prevention holds significant importance. Numerous extensive randomized clinical trials have illustrated that incorporating n-3 PUFA into the diet enhances the outlook for individuals who have recently experienced a myocardial infarction.[12,13] The potential benefits of consuming n-3 PUFA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are robust and extend to lowering blood triglyceride levels, arterial blood pressure (BP), and the likelihood of sudden cardiac death.[14] A recent investigation conducted by Stupin et al unveiled significant enhancements in microvascular endothelium-dependent vasodilatation among individuals consuming n-3 PUFA enriched eggs. This improvement was accompanied by a simultaneous reduction in pro-inflammatory serum markers such as interferon gamma (INF-γ) and an elevation in anti-inflammatory cytokines like interleukin 10 (IL-10). These observations offer preliminary insights into the potential mechanisms that may underlie the improvement of microvascular function through dietary modifications.[15] Eicosanoids, serving as tissue hormones engaged in inflammatory processes and immune system responses, arise from either n-3 or n-6 PUFA, each showcasing distinct structural characteristics and effects. Eicosanoids derived from n-3 PUFA display anti-aggregatory, anti-inflammatory, and vasodilatory properties, in contrast to their n-6 PUFA counterparts, which exhibit opposing effects. The specific type of eicosanoid generated is contingent upon the competitive interplay between n-3 and n-6 PUFA, as well as the involvement of enzymes in the eicosanoid synthesis process.[16–19] Moreover, n-3 polyunsaturated fatty acids (PUFA), along with n-6 PUFA like arachidonic acid, act as precursors for oxylipins and their subgroups of eicosanoids, which encompass prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT). These molecules play crucial roles as mediators and regulators of inflammation.[20,21] Consequently, it is plausible to hypothesize that an increased dietary intake of n-3 PUFA may modify the oxylipin profile, potentially yielding beneficial effects on vascular function and inflammation in individuals with coronary disease. Additionally, enhancing food with trace elements possessing antioxidant properties, such as selenium, lutein, and vitamin E, may prove advantageous in combination with n-3 PUFA, which have previously demonstrated cardioprotective effects.[22–24]

The main aim of this study was to evaluate the effects of consuming table hen eggs enriched with functional nutrients such as n-3 PUFA, vitamin E, selenium, and lutein on microvascular reactivity, lipid profile, and biomarkers of oxidative stress, including TBARS and FRAP, as well as antioxidant enzyme activity in patients with ACS following the prescribed dietary protocol.

Materials and Methods

Study Design and Participants

The research was structured as a forward-looking, randomized, interventional, and double-blind clinical study. The initial recruitment of participants occurred at the Department of Heart and Vascular Diseases, Osijek (Osijek University Hospital, Croatia). Subsequent analyses were carried out at the Laboratory for Clinical and Sport Physiology, Department of Physiology and Immunology, Faculty of Medicine Osijek, University of Osijek, Croatia, spanning from September 2021 to March 2022. A total of 28 adults (24 men and 4 women) aged 18 and above, with a history of ACS, were enrolled in the study. Inclusion criteria comprised individuals with ACS, encompassing STEMI, NSTEMI, and unstable angina pectoris. Exclusion criteria involved refusal to participate, hereditary metabolic, autoimmune, and other systemic diseases, known malignancies, uncontrolled hypertension, recent surgery (within 3 months), recent severe trauma (within 6 months), renal insufficiency (except in diabetic patients with Creatinine Clearance (CrCl) > 60mL/min), recent cerebrovascular insult (within 6 weeks), neurodegenerative disease and epilepsy, significant anemia (Hb < 110 [men], < 100 [women]), active bleeding, post-resuscitation status (within 3 months), therapy significantly affecting vascular or immune function (monoclonal antibodies, immunosuppressants, systemic corticosteroids), chronic respiratory insufficiency and chronic hypoxemia, sepsis and active infections with systemic inflammatory response (eg active tuberculosis), untreated thyroid disease, active alcohol and drug abuse, and liver failure. The study included individuals who were not undergoing statin therapy before the onset of ACS and hospital admission. High-dose statin therapy was initiated in both the Control and Nutri4 groups after enrollment in the nutritional protocol. Before being incorporated into the protocol, participants were instructed to abstain from using n-3 PUFA-rich supplements (eg, capsules) during the three-week dietary regimen.

The study population consisted of 28 adults diagnosed with ACS, including ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI), and unstable angina (UA). Among them, 25 patients were diagnosed with STEMI, two with NSTEMI, and one with UA. The mean age of the participants was 58 years, with a range of 45 to 72 years. The majority of the participants were male (85.7%). Regarding revascularization procedures, all 28 patients had undergone percutaneous coronary intervention (PCI). Baseline medications varied among the participants. The most commonly prescribed medications included antihypertensives, antiplatelets, anticoagulants, statins, and antidiabetic medications. Other medications such as analgesics, sedatives, and medications for comorbidities were also reported among the study participants. Primary endpoint was microvascular reactivity, while secondary endpoint were markers of oxidative stress (TBARS and FRAP), evaluation of antioxidant enzyme activities (CAT, GPx, and SOD), determination of anthropometric and hemodynamic parameters; examination of blood profile, alterations in serum biochemical parameters, lipid profile, and liver enzyme parameters; assessment of free fatty acids, lutein concentration, vitamin E, and selenium in serum sample.

This research constitutes a component of a registered clinical trial examining the impacts of n-3 PUFA, vitamin E, selenium, and lutein enriched eggs on microvascular reactivity (ClinicalTrials.gov ID: NCT04564690).

Study Protocol

Subsequent to their hospitalization, individuals willingly enrolled in the research protocol by providing written informed consent. The study received approval from the Ethics Committee of the Faculty of Medicine Osijek (Code: 2158–61-46-22-38) and the Ethics Committee of the Osijek University Hospital (Code: R2-8262/2020), thus ensuring compliance with the Declaration of Helsinki. Upon enrolment, participants were randomly assigned to two groups through a simple coin toss (letter-1 for the Control group, heads-2 for the experimental group). The Control group consumed regular table eggs, whereas the experimental group (Nutri4 group) consumed table eggs enriched with n-3 polyunsaturated fatty acids

(PUFA), vitamin E, lutein, and selenium. The dietary research protocol spanned three weeks, during which participants attended two study visits. The initial visit marked their entry into the protocol, involving the distribution of food diary forms for monitoring food consumption and the collection of initial measurements. The second visit occurred after three weeks of consuming either functionally enriched or regular table eggs, during which measurements were repeated, and participants submitted their diet diaries. Throughout the dietary protocol, each participant consumed three hard-boiled table eggs (commercial size L) every morning. A total of 63 table hen eggs, collaboratively produced with partners from the Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Croatia, were consumed by each subject during the duration of the protocol. The principal investigator maintained regular contact with the subjects to ensure compliance to the egg consumption instructions and accurate completion of the food diary forms. All subjects were instructed to adhere to the study dietary protocol without making any significant changes to their usual diet, except for the consumption of eggs. They were also advised against consuming other foods rich in n-3 PUFAs or supplementing with n-3 PUFAs during the study. To promote strict adherence, subjects were frequently contacted by telephone and instructed to maintain a detailed diet diary, which served as a tool to monitor their dietary habits. The diet diaries used in the study were based on the work of Kolobarić et al.[25] Both the subjects and the researchers involved in this study remained completely blinded. Data from all subjects were coded and assigned a number by a researcher who had no direct contact with the participants.

Collaborative efforts with partners from the Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Croatia, resulted in the production of table hen eggs. Table 1 illustrates the nutrient content and fatty acid profile of the edible portion of both regular and Nutri4 table hen eggs. Significantly, lower concentrations of vitamin E, lutein, and selenium were found in regular table hen eggs compared to Nutri4 eggs.

Table 1 Nutrients Content and Fatty Acids Profile of Edible Part of Regular and Nutri 4 Table Hen Eggs

Parameters	Regular Eggs	Nutri 4 Eggs
	mg/60 g Egg (Edible Part)**	
	Regular Eggs	Nutri4 Eggs
Lutein (mg)	0.110	0.616*
Vitamin E (mg)	0.595	1.098*
Selenium (mg)	0.0183	0.0231*
Fatty acids		
∑SFA	1566 ± 346	1442 ± 185
∑MUFA	1976 ± 189	2419 ± 139
∑n-3 PUFA	146 ± 20	342 ± 25*
ALA	71 ± 11	189 ± 16*
EPA	n.d.	19 ± 2*
DHA	75 ± 11	135 ± 11*
∑n-6 PUFA	1263 ± 148	747 ± 46*
LA	1165 ± 140	702 ± 43
AA	89 ± 9	44 ± 4*
∑n-6 / ∑n-3 PUFA	8.71	2.18

Notes: **L size table egg with an average weight of 68 g has about 60 g of edible portion. Data are presented as mean ± standard deviation. Statistical analysis: *Statistically significant differences within-group change ($p<0.05$). Nutri 4 Eggs, functionally enriched hen eggs with n-3 PUFA, vitamin E, selenium, and lutein.

Abbreviations: ∑SFA, saturated fatty acids (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C23:0); ∑MUFA, monounsaturated fatty acids (C14:1, C16:1, C18:1n9t, C18:1n9c, C20:1n9, C22:1n9); ∑n-6 PUFA, polyunsaturated fatty acids (C18:2n6c, C18:3n6, C20:3n6, C20:4n6, C22:2n6); LA, linoleic acid (C18:2n6c); AA-arachidonic acid (C20:4n6); ∑n-3 PUFA, polyunsaturated fatty acids (C18:3n3, C20:3n3, C20:5n3, C22:6n3); ALA, alpha linolenic acid (C18:3n3); EPA, eicosapentaenoic acid (C20:5n3); DHA, docosahexaenoic acid (C22:6n3); n.d., not detected.

The findings reveal noteworthy distinctions in various parameters between the two table egg types. Regarding nutrient content, Nutri4 eggs demonstrated significantly elevated concentrations of vitamin E, lutein, and selenium in comparison to regular eggs (Table 1). This underscores the enhanced nutritional profile of Nutri4 eggs relative to regular eggs. The increased levels of vitamin E, lutein, and selenium in Nutri4 eggs suggest their potential as a functional food source rich in these beneficial nutrients. Moreover, the modified fatty acid profile of Nutri4 eggs, featuring reduced n-6 polyunsaturated fatty acids (PUFA) and increased n-3 PUFA, particularly alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), aligns with emerging research emphasizing the importance of a balanced intake of n-3 and n-6 fatty acids for human health.

Determination of Anthropometric and Hemodynamic Parameters

Anthropometric measurements were undertaken on the participants during the initial and subsequent study visits, adhering to the anthropometry examination protocol and procedures outlined in the Anthropometry Procedures Manual.[26] To evaluate body fluid composition, the Maltron Bioscan 920-II, a four-channel portable impedance meter manufactured by Maltron International Ltd. (Rayleigh, Essex, UK), was employed. Additionally, the subjects' blood pressure (BP) and heart rate (HR) were measured three times at each study visit using the OMRON M3, an automatic oscillometric monitor from OMRON Healthcare Inc. (Osaka, Japan). This methodology was consistent with the description provided by Breškić Ćurić et al, and the mean value derived from the three measurements was recorded and presented for subsequent analysis.[27]

Analysis of Biochemical Parameters, Free Fatty Acids, Lipid Profile, Lutein Concentration, Vitamin E, and Selenium in the Serum Samples

Biochemical analyses encompassed the evaluation of various markers utilizing venous blood samples obtained from the participants during both study visits. The parameters assessed included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), high-sensitivity C-reactive protein (hsCRP), fasting blood glucose concentration, urea, creatinine, urate, sodium (Na), potassium (K), calcium (Ca), total iron (Fe), ferritin, and transferrin. These analyses were conducted at the Department of Clinical Laboratory Diagnostics, Osijek University Hospital, Osijek, Croatia, utilizing standardized laboratory methods. The blood count was determined using a Sysmex XN 1000 hematological counter (Sysmex, 2017)[28] while the biochemical parameters were measured using the Olympus AU680 device from Beckman Coulter (Čvorišćec et al, 2009).[29]

The serum samples of the participants were utilized to determine the free fatty acid profile, with venous blood samples collected during both study visits and stored at −80 °C until analysis. The concentration of free fatty acids in the serum of individuals with ACS was ascertained through gas chromatography-tandem mass spectrometry (GC-MS/MS) at the Bioanalytical Laboratory of BIOcentar Zagreb. The methodologies followed for these analyses were in accordance with the protocols outlined by Stupin et al[15] and Wang et al.[30] The instrumental setup employed for the analyses consisted of a mass spectrometer (Thermo Fisher GC Trace 1300 coupled with TSQ 9000 Triple Quadrupole), and a standard mixture of fatty acid methyl esters (FAME MIX) dissolved in methylene chloride (Supelco Inc., Bellefonte, PA, USA) at a concentration of 30 mg/mL.

Lutein concentrations in serum samples were assessed following the procedures outlined in the protocol by Tzeng et al[31] and Leeson et al.[32] The preparation of serum samples involved the addition of deionized water and 0.01% ascorbic acid dissolved in absolute ethanol. Following mixing, hexane was introduced, and the resulting mixture underwent centrifugation. The ensuing supernatant was subjected to evaporation, and the concentration of lutein was determined using high-performance liquid chromatography (HPLC). The analysis employed a high-resolution end-capped HPLC column (Hypersil; particle size 5 µm; 4.6×250 mm) and a C18 analytical column (Shim-pack GIST; particle size 5 µm; 250×4.6 mm). The HPLC LC-30 NEXERA (Shimadzu, Japan, 2018) instrument was utilized for the analysis, and all samples underwent triplicate analyses.

The spectrophotometric measurement of vitamin E concentration utilized a modified version of the method developed by Jargar et al.[33] The process involved creating a mixture consisting of the subject's serum, absolute ethanol (for serum protein denaturation), and xylene (to facilitate separation of the supernatant from proteins). Subsequently, this mixture underwent centrifugation at 3000 g for 10 minutes. The resultant supernatant was combined with 2.2-bipyridyl and FeCl3, resulting in a pink coloration of the mixture. Following a 2-minute incubation period, the absorbance of the mixture was measured at 492 nm using a spectrophotometer (PR 3100 TSC Microplate Reader, BioRad Laboratories, Hercules, California). The absorbance readings corresponded to the concentration of vitamin E. The methodology applied in this process drew inspiration from the work of Šušnjara et al.[34]

For the determination of selenium concentration in serum, the adopted methodology involved pre-digesting serum samples in a CEM Mars 6 (CEM, Matthews, NC, USA) using a 5:1 mixture of ultra-pure HNO3 and H2O2 at 180°C for 1 hour. Subsequent to digestion, the selenium concentration in the digested sample solutions was assessed using inductively coupled plasma mass spectrometry (ICP-MS) through three replicates. The ICP-MS instrument employed for the analysis was an Agilent 7500a (Agilent Technologies Inc., California, USA).[35]

Determination of TBARS and FRAP

Spectrophotometry was utilized for the measurement of TBARS to quantify the products resulting from lipid peroxidation, serving as an indicator of oxidative stress levels. To eliminate potential interference from other substances, including proteins, trichloroacetic acid was introduced to precipitate the proteins. It is crucial to acknowledge that the TBARS method lacks specificity due to the binding of thiobarbituric acid with various substances.

The assessment of the FRAP of plasma also involved spectrophotometry to gauge the antioxidant capacity of the blood samples. In this approach, Fe3+-TPTZ (2,4,6-tris(2-pyridyl)-striazine) undergoes reduction to Fe2+-TPTZ in the presence of antioxidants, leading to a blue discoloration. The TBARS and FRAP measurements adhered to a well-established protocol previously outlined in our laboratory. This protocol ensured the precise and reliable determination of TBARS and FRAP values in the samples.[36]

Spectrophotometric Antioxidant Enzyme Activities Assay

The analysis of antioxidant enzyme activities in the subjects' serum was carried out using a Lambda 25 UV/Vis spectrophotometer with UV WinLab 6.0 software support (PerkinElmer, Waltham, MA, USA). The methods employed for these assessments were outlined in the studies conducted by Ćosić et al[36] and Mihaljević et al.[37] Enzyme activities were expressed as U (mg protein)$^{-1}$. The determination of catalase (CAT) activity utilized the spectrophotometric method developed by Aebi (1984).[38] Measurement of glutathione peroxidase (GPx) activity was conducted at 340 nm using the Wendel method (1981).[39] Additionally, the assessment of superoxide dismutase (SOD) activity followed the procedure described by Flohe and Otting (1984).[40]

Evaluation of Microvascular Reactivity in Response to Vascular Occlusion and Acetylcholine and Sodium Nitroprusside Administration

Microvascular reactivity assessments were conducted at each study visit, employing laser Doppler flowmetry (MoorVMS-LDF, Axminster, UK). The evaluation included measuring microvascular reactivity in response to post-occlusive reactive hyperemia (PORH). Additionally, skin microvascular flow was measured in response to the application of acetylcholine (ACh), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP), an endothelium-independent vasodilator. The evaluation procedure required the subject to recline in a temperature-controlled room (23.5±0.5°C) for 30 minutes. A laser Doppler probe, approximately 13–15 cm in length, was affixed to the subject's left volar forearm from the wrist. Following a 5-minute baseline blood flow measurement, a pneumatic cuff was applied around the upper arm to induce occlusion of the brachial artery with a pressure 30–50 mmHg above the subject's systolic pressure. The occlusion lasted for 1 minute, after which the cuff was released to induce PORH, and measurements continued for an additional 1–2 minutes. Subsequent to the PORH test, ACh and SNP were administered using a laser Doppler probe and iontophoresis. Microcirculatory blood flow was expressed in arbitrary perfusion units during the test.

The value of microcirculatory blood flow was determined by calculating the area under the curve (AUC) during baseline flow and during the administration of ACh or SNP using an appropriate program.[41,42]

Statistical Analysis

Numerical variables were succinctly presented utilizing either mean and standard deviation or median and interquartile range, contingent on their distribution. The normality of distributions was gauged using the Shapiro–Wilk test. Disparities within the Control and Nutri4 groups before and after the dietary protocol were scrutinized via paired t-tests for normally distributed data and Wilcoxon signed-rank tests for non-normally distributed data. Comparative analysis of absolute changes between the groups was conducted using independent t-tests for normally distributed data and Mann–Whitney *U*-tests for non-normally distributed data. A significance level of $\alpha = 0.05$ and a statistical power of 80% were applied throughout. The statistical analyses were carried out using XLSTAT version 2019.2.2 (Addinsoft, New York, NY, USA).

Results

Age, Anthropometric Characteristics, Body Composition and Body Fluids of Patients with Acute Coronary Syndrome

The age, anthropometric characteristics, body composition, and body fluids of patients with ACS were evaluated. The descriptive statistics for anthropometric parameters, body composition, and body fluids are presented in Table 2. The subjects in both study groups exhibited comparable anthropometric measurements and body composition, with no significant differences observed in body mass index (BMI), waist-hip ratio (WHR), percentage of fat tissue (FFM%), percentage of body fat (FAT%), share of total body fluid (TBW%), proportion of extracellular fluid (ECW%), proportion of intracellular fluid (ICW%) (Table 2).

The mean age of the patients in both the Control and Nutri4 groups (56.8 ± 9.6 years and 57.2 ± 9.2 years, respectively) aligns with the typical age range of individuals affected by ACS. The mean height for both groups (Control: 173.3 ± 7.9 cm; Nutri4: 173.7 ± 6.0 cm) falls within the expected range for the general population. Additionally, the mean BMI values (Control: 30.0 ± 5.7 kg/m2; Nutri4: 28.3 ± 3.6 kg/m2) indicate that the patients in both groups had a similar distribution of body weight relative to height and were adipose.

Table 2 Comparison of Anthropometric and Body Composition Parameters Between the Control and Nutri 4 Groups

Parameters	Control	Nutri 4	*p*
Age (years)	56.8 ± 9.6	57.2 ± 9.2	0.924
Height (cm)	173.3 ± 7.9	173.7 ± 6.0	0.896
BMI (kg/m^2)	30.0 ± 5.7	28.3 ± 3.6	0.377
WHR	0.97 ± 0.07	0.98 ± 0.06	0.947
FFM%	67.7 ± 10.6	68.3 ± 9.0	0.887
FAT%	32.3 ± 10.6	31.7 ± 9.0	0.887
TBW%	50.3 ± 7.9	50.1 ± 7.5	0.936
ECW%	43.7 ± 2.7	43.4 ± 1.8	0.740
ICW%	56.3 ± 2.7	56.6 ± 1.8	0.740

Notes: The values are presented as mean ± standard deviation (SD). No statistically significant difference ($p<0.05$) was observed according to the Mann–Whitney *U*-test. Nutri 4, chicken eggs enriched with n 3 PUFA selenium, vitamin E and lutein.

Abbreviations: BMI, body mass index; WHR, waist-hip ratio; FFM%, percentage of fat tissue; TBW%, share of total body fluid; ECW%, proportion of extracellular fluid; ICW%, proportion of intracellular fluid.

Blood Profile, Changes in Serum Biochemical Parameters, Lipid Profile, and Liver Enzyme Parameters in Patients with Acute Coronary Syndrome

The Table 3 showcases the changes observed before and after the dietary protocols, along with the absolute change observed, including blood count parameters, biochemical parameters, lipid profile, and liver enzymes in the serum samples in ACS patients consuming regular or enriched table eggs. The statistical significance of the within-group differences (p^a) and between-group differences (p^b) is indicated. For blood count parameters (leukocytes, erythrocytes, hemoglobin, hematocrit, and platelets), no significant differences were observed between groups.

Regarding the biochemical parameters, results demonstrated that the concentration of Na increased in both studied groups, with a statistically significant rise observed in the Nutri4 group. Similarly, the concentration of Fe increased in both groups; however, a statistically significant elevation was found only in the Nutri4 group following the dietary protocol. Transferrin levels demonstrated a significant increase in the Control group, but not in Nutri4 group.

A noteworthy decrease in ferritin levels was identified as statistically significant within the Control group post-implementation of the dietary protocol. Significantly, the Nutri4 group exhibited a substantial reduction in fibrinogen concentration following the dietary intervention. The noteworthy reduction in fibrinogen levels observed in the Nutri4 group implies that the consumption of Nutri4 eggs might exert a favorable influence on blood clotting factors, potentially diminishing the risk of unfavorable cardiovascular events. Additionally, both investigated groups exhibited a significant decrease in hsCRP values upon the conclusion of the dietary protocol.

The lipid profile parameters were also assessed (Table 3). HDL-cholesterol was significantly increased in the Nutri4 group, while LDL-cholesterol was significantly decreased in the Nutri4 group compared to the Control group. Lastly, the liver enzyme levels, such as AST, ALT, and GGT, were analysed. AST was significantly decreased in the Nutri4 group, while ALT and GGT levels did not significantly change.

Biomarkers of Oxidative Stress and Antioxidative Defence in Patients with ACS: Lutein, Vitamin E, Selenium, n-6/n-3 PUFAs Ratio, Antioxidant Enzyme Activities, and Oxidative Stress Biomarkers

The impact of consuming regular and enriched eggs on serum concentrations of lutein, vitamin E, selenium, n-6/n-3 PUFAs ratio, oxidative stress marker (TBARS), antioxidant capacity (FRAP), and serum activity of antioxidant enzymes (CAT, GPx, SOD) is detailed in Table 4.

The outcomes revealed that both the Control and Nutri4 groups witnessed an elevation in lutein concentration post dietary protocol, although the difference lacked statistical significance. Upon comparing the absolute changes in lutein concentration between the two groups, it was noted that the Control group manifested a larger increase compared to the Nutri4 group. Consequently, the consumption of eggs did not yield a significant impact on lutein concentration in the serum of ACS patients. Concentrations of vitamin E displayed a significant increase post dietary protocols, particularly in the Nutri4 group compared to baseline, unlike the Control group. A notable discrepancy was observed in absolute changes in vitamin E concentration between the two groups pre- and post-dietary protocols. The Control group exhibited a reduction in vitamin E concentration, while the Nutri4 group demonstrated a substantial elevation in vitamin E concentration following the dietary protocol. Serum concentrations of selenium significantly increased following the dietary protocols in both the Control and Nutri4 groups (Table 4). However, the absolute changes in selenium concentration before and after the dietary protocol between the two groups were comparable. The serum selenium concentration demonstrated a significant increase in both groups following the dietary protocols, with higher levels observed in the Nutri4 group. A comprehensive analysis of free fatty acids, examining 37 free fatty acids in the serum sample, was conducted. Only the concentrations of detectable fatty acids (above the quantification limit) are presented in Table S1. Overall, the consumption of enriched eggs resulted in a reduction of the n-6/n-3 ratio (Table 4). The serum fatty acid profile in ACS patients after the dietary protocol with regular eggs, compared to baseline measurement in the Control group, did not exhibit significant changes, and the n-6/n-3 ratio decreased, albeit not significantly.

CAT activity experienced a decrease in both the Control and Nutri4 groups following the dietary protocol. In the Control group, GPx activity significantly decreased post-dietary protocol, whereas it remained unchanged in the Nutri4

Table 3 Effects of Dietary Protocol on Blood Count, Biochemical, Lipid Profile, and Liver Enzyme Parameters: a Comparison Between Control and Nutri 4 Groups Using Serum Samples

	Control		p^a	Nutri 4		p^a	Absolute Change After Dietary Protocol		p^b
	Before	After		Before	After		Control	Nutri 4	
Leukocytes (x10^9/L)	9.4 ± 2.7	8.5 ± 1.8	0.050	8.2 [7.6–10.7]	8.5 ± 2.3	0.459	−0.9 ± 1.4	−0.5 ± 1.8	0.534
Erythrocytes (x10^{12}/L)	4.6 ± 0.5	4.8 ± 0.4	0.140	4.9 ± 0.3	4.9 ± 0.3	0.370	0.2 ± 0.3	−0.1 ± 0.2	0.070
Hemoglobin (g/L)	139.7 ± 13.3	142.5 ± 8.7	0.263	145.5 ± 8.2	141.9 ± 11.1	0.069	2.8 ± 8.4	−3.6 ± 6.8	0.041
Hematocrit (%)	0.4 ± 0.0	0.4 ± 0.0	0.121	0.4 ± 0.0	0.4 ± 0.0	0.312	0.0 ± 0.0	0.0 ± 0.0	0.055
Platelets (x10^9/L)	255.0 ± 57.0	232.2 ± 53.7	0.125	253.1 ± 56.9	246.0 ± 57.6	0.720	−22.8 ± 47.9	−26.0 [−52.5–13.0]	0.955
Urea (mmol/L)	5.7 ± 2.7	6.1 ± 1.8	0.499	6.0 ± 1.6	5.4 ± 1.3	0.139	0.4 ± 2.3	−0.5 ± 1.3	0.168
Creatinine (µmol/l)	84.6 ± 18.1	85.1 ± 16.5	0.851	77.7 ± 14.1	80.0 ± 15.7	0.302	0.5 ± 8.7	2.3 ± 8.4	0.568
Sodium (mmol/l)	138.7 ± 4.7	141.0 ± 2.9	0.060	139.2 ± 2.6	141.1 ± 1.8	0.017	2.3 ± 4.0	1.9 ± 2.8	0.773
Potassium (mmol/l)	4.1 ± 0.3	4.3 ± 0.3	0.120	4.0 ± 0.3	4.1 ± 0.2	0.761	0.1 ± 0.3	0.0 ± 0.4	0.432
Glucose (mmol/L)	6.6 ± 2.1	5.7 ± 0.8	0.094	6.0 ± 1.0	5.6 ± 1.0	0.192	−0.9 ± 1.8	−0.3 ± 1.0	0.307
hsCRP (mg/L)	14.6 ± 13.0	4.0 ± 4.6	0.017	18.3 ± 18.5	5.6 ± 5.9	0.023	−10.6 ± 13.8	−12.7 ± 19.2	0.752
Calcium (mmol/L)	2.5 ± 0.5	2.4 ± 0.1	0.713	2.3 ± 0.1	2.4 ± 0.1	0.166	−0.1 ± 0.5	0.0 ± 0.1	0.510
Iron (mmol/L)	10.3 ± 4.4	12.6 ± 3.0	0.183	11.0 ± 5.7	14.8 ± 5.8	0.035	2.3 ± 5.9	3.8 ± 6.3	0.530
Transferrin (g/L)	2.2 ± 0.4	2.4 ± 0.4	0.015	2.4 ± 0.4	2.4 ± 0.4	0.691	0.1 ± 0.2	0.0 ± 0.2	0.049
Ferritin (µg/l)	236.1 ± 106.2	200.6 ± 118.2	0.039	238.5 ± 269.7	204.4 ± 195.7	0.265	−35.5 ± 55.4	−34.1 ± 113.8	0.967
Fibrinogen (g/L)	4.0 ± 0.7	3.6 [3.3–3.6]	0.129	4.2 ± 1.0	3.5 [3.4–3.8]	0.049	−0.3 ± 0.5	−0.1 [−0.9–0.1]	0.900
Cholesterol (mmol/L)	4.38 ± 0.9	4.09 ± 1.2	0.413	4.37 ± 1.7	3.67 ± 1.0	0.088	−0.30 ± 1.3	−0.70 ± 1.5	0.449
Triglycerides (mmol/L)	1.49 ± 0.4	1.51 ± 0.8	0.898	1.29 ± 0.3	1.45 ± 0.8	0.302	0.02 ±0.6	0.15 ±0.6	0.557
HDL cholesterol (mmol/L)	1.03 ± 0.3	1.14 ± 0.3	0.0004	1.01 ± 0.2	1.03 ± 0.2	0.646	0.11 ±0.1	0.02 ±0.2	0.065
LDL cholesterol (mmol/L)	2.89 ± 0.8	2.51 ± 1.1	0.259	2.89 ±1.4	2.14 ± 0.7	0.0001	−0.38 ±1.2	−0.75 ±1.2	0.414
AST (U/L)	40.0 [22.0–70.0]	29.3 ± 9.4	0.027	29.0 [23.0–91.0]	27.0 [22.5–32.0]	0.108	−28.2 ± 40.8	−1.0 [−53.0–2.0]	0.518
ALT (U/L)	50.9 ± 27.8	40.8 ± 24.1	0.338	35.0 [24.0–50.5]	31.0 [27.5–44.0]	0.470	−10.1 ± 29.3	−4.2 ± 15.4	0.504
GGT (U/L)	33.0 [23.0–55.0]	32.0 [21.0–44.0]	0.037	32.0 [24.0–43.0]	32.0 [25.0–43.0]	0.850	−3.0 [−0.8–0.00]	0.0 [−5.5–5.0]	0.212

Notes: The results are expressed as mean ± standard deviation for normally distributed data, or as median and [interquartile range] for data that do not follow a normal distribution. *p*-values highlighted in bold indicate statistically significant differences ± $p<0.05$), where p^a represents within-group differences ± paired *t*-test or Wilcoxon signed-rank test), and p^b represents differences between groups ± independent *t*-test or Mann–Whitney *U*-test). Nutri 4, hen eggs enriched with n-3 PUFAs, selenium, vitamin E, and lutein.

Abbreviation: hsCRP, high-sensitivity C-reactive protein.

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Table 4 Impact of Consuming Regular (Control Group) and Enriched Hen Eggs (Nutri4 Group) on Serum Levels of Lutein, Vitamin E, Selenium, n-6/n-3 PUFAs Ratio, Oxidative Stress Marker (TBARS), Antioxidant Capacity (FRAP), and Serum Activity of Antioxidant Enzyme (CAT, GPx, SOD) in Patients with Acute Coronary Syndrome (ACS)

	Control			Nutri4			Absolute Change After Dietary Protocol		
	Before	After	p^a	Before	After	p^b	Control	Nuti 4	p^b
Lutein (µmol/L)	0.072 ± 0.03	0.106 ± 0.07	0.116	0.130 ± 0.05	0.138 ± 0.06	0.798	0.035 ± 0.05	0.008 ± 0.001	0.477
Vitamin E (µg/mL)	9.7 ± 4.3	9.5 ± 3.8	0.527	10.2 ± 4.3	11.5 ± 4.0	0.0001	−0.2 ± 1.1	1.3 ± 0.8	0.001
Selenium (µg/L)	66.6 ± 14.0	75.3 ± 14.5	0.025	71.1 ± 7.2	77.2 ± 10.5	0.035	8.8 ± 9.6	6.1 ± 7.7	0.503
n-6 / n-3 PUFAs	17.1 ±6.61	15.7 ±6.61	0.692	17.8 ±7.638	11.5 ±5.93	0.032	−1.4 ±6.5	−6.3 ±3.9	0.230
TBARS (µM MDA)	0.36 ± 0.19	0.36 ± 0.16	0.968	0.20 ± 0.16	0.21 ± 0.14	0.752	0.001 ± 0.13	0.01 ± 0.09	0.891
FRAP (mM/L Trolox)	0.33 ± 0.11	0.35 ± 0.10	0.210	0.28 ± 0.05	0.30 ± 0.05	0.359	0.02 ± 0.06	0.01 ± 0.05	0.644
CAT (U/mg protein)	4,9 ± 1.5	4,5 ± 2.5	0.661	8,6 ±4.4	6,5 ±1.5	0.185	−0,3 ±2.4	−2,1 ± 4.6	0.296
GPx (U/mg protein)	0.0126 ± 0.0016	0.0111 ±0.0012	0.002	0.0109 ±0.0011	0.0112 ±0.0013	0.313	−0.0016 ±0.0013	0.0003 ±0.0008	0.0002
SOD (U/mg protein)	6,3 ± 1.1	5,6 ± 1.1	0.093	6,1 ± 0.8	6,5 ± 0.6	0.031	−0,7 ± 1.2	0,4 ± 0.6	0.012

Notes: Data are presented as mean ± standard deviation. *p*-values indicate statistically significant differences ($p<0.05$), with p^a representing within-group differences (paired *t*-test), and p^b representing between-group differences (independent samples *t*-test).: n-6/n-3 PUFAs ratio.
Abbreviations: PUFA, polyunsaturated fatty acids; TBARS, thiobarbituric acid reactive substances; FRAP, iron-reducing plasma capacity; CAT, catalase antioxidant enzyme activity; GPx, glutathione peroxidase; SOD, superoxide dismutase.

group. A notable disparity in GPx activity changes emerged when comparing the Control and Nutri4 groups. The Control group exhibited a decrease in GPx activity, while the Nutri4 group demonstrated an increase. SOD activity remained unaltered in the Control group due to the dietary protocol, whereas in the Nutri4 group, SOD activity significantly increased post-dietary protocol. A significant difference in SOD activity changes between the Control and Nutri4 groups was evident, with an increase in the Control group and a decrease in the Nutri4 group.

Measurements of Blood Flow and Vascular Reactivity in the Skin Microcirculation of Patients with ACS

The impact of consuming regular and enriched eggs on changes in PORH, ACh ID, and SNP ID values in patients with ACS is depicted in Figures 1–3. PORH values remained unaltered within the Control group but exhibited a significant increase in the Nutri4 group (Figure 1A). A notable difference in absolute changes of PORH was noted between the two groups (Figure 1C), indicating a decrease in the Control group and an increase in the Nutri4 group post-dietary protocol (Figure 1B). Following the dietary protocol, ACh ID in the Control group decreased without statistical significance, while in the Nutri4 group, it significantly increased (Figure 2A). A significant difference in absolute changes of ACh ID was evident between the two groups (Figure 2C), showcasing a decrease in the Control group and an increase in the Nutri4 group post-dietary protocol (Figure 2B). In the Control group, SNP ID values remained unaltered, whereas in the Nutri4 group, SNP significantly increased (Figure 3A and B). No significant difference in absolute changes of SNP ID was observed between the two groups after the dietary protocol (Figure 3C).

Discussion

In this study, we explored the favorable effects of a diet enriched with n-3 PUFA, lutein, vitamin E, and selenium on microvascular function in ACS patients. The key outcomes are summarized as follows: a) Consumption of enriched eggs led to a significant reduction in the n-6/n-3 PUFA ratio, accompanied by a noteworthy decrease in LDL cholesterol levels; b) Consumption of enriched eggs resulted in a significant decrease in hsCRP and fibrinogen levels in ACS patients, indicating a reduction in inflammation and coagulation; c) Consumption of enriched eggs led to a significant increase in SOD activity. d) Additionally, the consumption of enriched eggs had a significant impact on the microvascular function of ACS patients, evidenced by increased microvascular blood flow in response to PORH and ACh ID. Collectively, these results demonstrate improvements in microvascular reactivity parameters and a decrease in pro-inflammatory and pro-coagulatory conditions in the vasculature of ACS patients, supporting the hypothesis that an enriched diet is beneficial for this population.

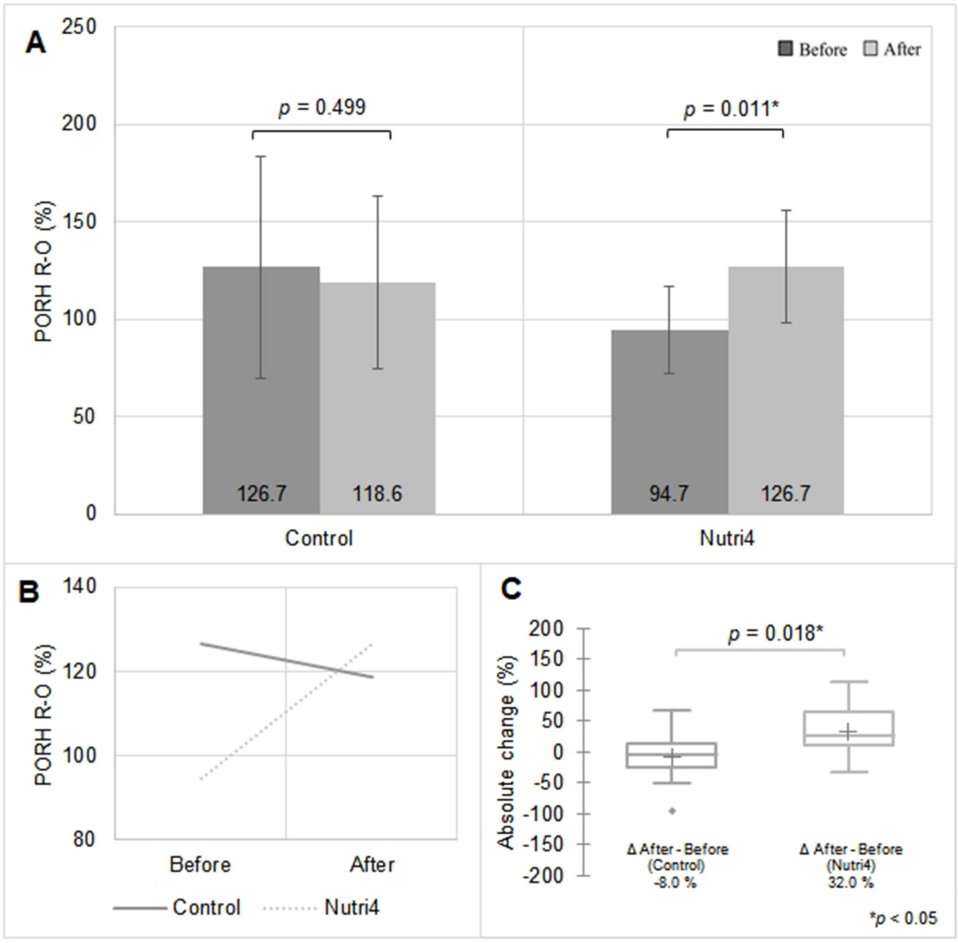

Figure 1 Effect of Dietary Intake of Regular and Nutrient-Enriched Table Eggs on PORH Parameters in Patients with ACS: (**A**) Comparison within Each Investigated Group, (**B**) Trend Display of PORH Changes in Each Investigated Group during Dietary Protocol, (**C**) Absolute Changes Between Investigated Groups during Dietary Protocol.
Notes: Data are expressed as the mean value ± standard deviation. Dietary protocol assessed via paired t-tests (Nutri 4) and Wilcoxon signed-rank tests (Control) pre/post egg consumption. Group differences in absolute changes analysed using independent t-tests. *p**indicates significance ($p<0.05$). Nutri 4, chicken eggs enriched with n-3 PUFA selenium, vitamin E and lutein.
Abbreviations: PORH, Postocclusive reactive hyperemia; ACS, acute coronary syndrome.

The results of this study suggest that the consumption of hen eggs enriched with n-3 PUFA, vitamin E, selenium, and lutein may positively affect serum biochemical parameters in ACS patients. N-3 PUFA, a healthy fat type, has demonstrated anti-inflammatory properties and a reduced risk of heart disease.[27] Transferrin levels demonstrated a significant increase in the Control group, but not in the Nutri4 group. Transferrin, a protein involved in iron transport, has been investigated in the context of cardiovascular health. Although the specific role of transferrin in ACS is still under investigation, previous studies have reported associations between transferrin levels and cardiovascular risk factors.[43] Additionally, a noteworthy decrease in ferritin levels was identified as statistically significant within the Control group post-implementation of the dietary protocol. Significantly, the Nutri4 group exhibited a substantial reduction in fibrinogen concentration following the dietary intervention. Fibrinogen, recognized as a pivotal factor in blood clotting, has been associated with elevated cardiovascular risk. The proposition of reducing fibrinogen levels has been put forth as a strategy to mitigate the risk of cardiovascular events.[44]

Furthermore, the body composition parameters, including FFM% and FAT%, were comparable to previous findings in patients with ACS (Breškić Ćurić et al, 2021).[27] The mean values for FFM% indicate a typical composition of lean tissue and adipose tissue in these individuals. Evaluation of body fluid parameters, such as total body water (TBW%), extracellular water (ECW%), and intracellular water (ICW%), also aligns with the existing literature on ACS. The mean values for TBW%, and ICW% indicate a similar distribution of body fluid compartments in these patients.

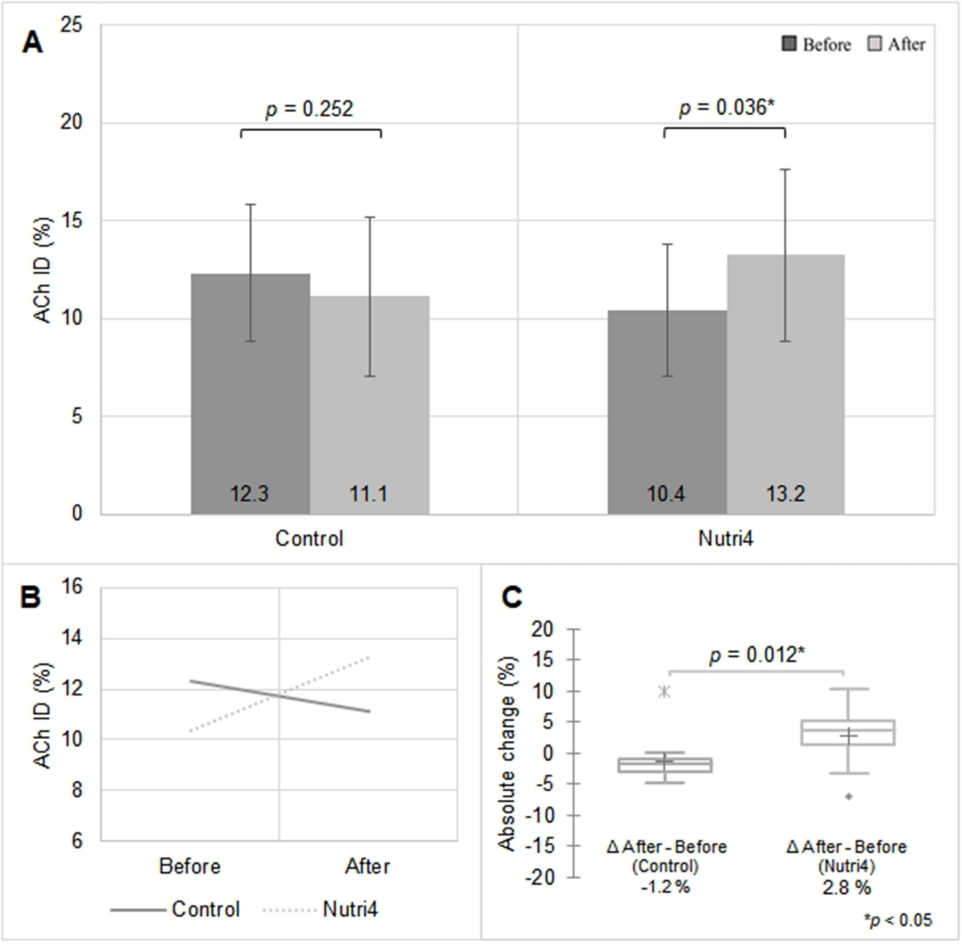

Figure 2 Effect of Consuming Regular and Functionally Enriched Hen Eggs on ACh ID Values in ACS Patients: (**A**) Comparison within Each Investigated Group, (**B**) Trend Display of ACh ID Changes in Each Investigated Group during Dietary Protocol, (**C**) Absolute Changes Between Investigated Groups during Dietary Protocol.
Notes: Data are expressed as the mean value ± standard deviation. Dietary protocol assessed via paired t-tests (Control and Nutri 4) pre/post egg consumption. Group differences in absolute changes analyzed using Mann-Whitney *U*-tests. *p** indicates significance ($p<0.05$). Nutri 4, chicken eggs enriched with n-3 PUFA selenium, vitamin E and lutein.
Abbreviations: Ach ID, Acetylcholine-induced coronary vasodilation; ACS, acute coronary syndrome.

Inflammation markers, including hsCRP, are widely recognized indicators of systemic inflammation and have been associated with cardiovascular disease. Previous studies have demonstrated that dietary interventions, encompassing the consumption of specific foods rich in anti-inflammatory compounds, can contribute to the reduction of hsCRP levels.[45] The significant decrease in hsCRP observed in the Nutri4 group suggests that Nutri4 egg consumption may possess anti-inflammatory properties, contributing to the improvement in inflammation markers, although this is not completely clear since there has also been a decrease in the Control group. Notably, vascular reactivity to stimuli, a crucial marker of endothelial function, can be influenced by dietary factors such as n-3 PUFA intake. This underscores the broader impact of dietary choices on cardiovascular health among ACS patients.[46,47]

Vitamin E, an antioxidant, may protect the heart and lower ACS risk.[48] Selenium, a trace mineral, could contribute to heart protection and improved blood flow.[22,49] Lutein, found in fruits and vegetables, may enhance eye health and reduce certain cancer risks.[50]

Supplementation of n-3 PUFA has the potential to reduce serum lipids, especially triglycerides, in individuals with hyperlipidemia.[51,52] It may also improve endothelial function and arterial stiffness in hypertensive patients with hypertriglyceridemia and high cardiovascular risk. In this study, the consumption of enriched eggs significantly reduced LDL-cholesterol levels in patients with ACS. Importantly, the enriched egg consumption did not adversely affect the lipid profile, demonstrating its safety in the cardiovascular disease population.[53,54]

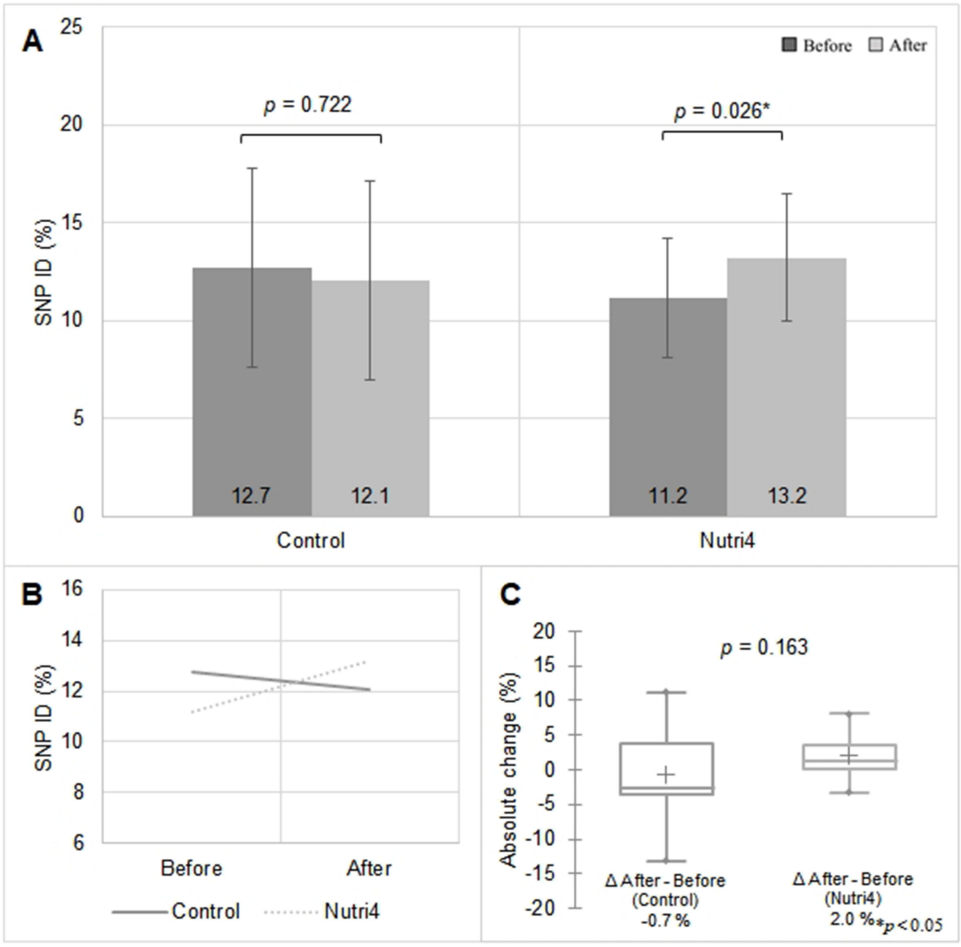

Figure 3 Influence of Consumption of Regular and Functionally Enriched Hen Eggs on SNP ID Values in ACS Patients: (**A**) Comparison within Each Investigated Group, (**B**) Trend Display of SNP ID Changes in Each Investigated Group during Dietary Protocol, (**C**) Absolute Changes Between Investigated Groups during Dietary Protocol.
Notes: Data are expressed as the mean value ± standard deviation. Dietary protocol assessed via paired t-tests (Control and Nutri 4) pre/post egg consumption. Group differences in absolute changes analyzed using independent t-tests. *p**Indicates significance ($p<0.05$). Nutri 4, chicken eggs enriched with n-3 PUFA selenium, vitamin E and lutein.
Abbreviations: SNP ID, Sodium nitroprusside-induced coronary vasodilation; ACS, acute coronary syndrome.

Enriched eggs exhibited significantly higher levels of n-3 PUFA (134.2%), vitamin E (84.5%), selenium (27.8%), and lutein (460%) compared to regular eggs. After a three-week dietary protocol, participants consuming enriched eggs experienced a substantial average increase of 12.7% in serum vitamin E concentration, which was not observed in the Control group. This finding aligns with a separate study involving young healthy participants, which also reported a significant increase in serum vitamin E concentration after consuming eggs enriched with n-3 PUFA, vitamin E, selenium, and lutein.[34]

Vitamin E supplementation or consumption of vitamin E-rich foods can elevate vitamin E levels in the serum.[55,56] A significant increase in serum selenium concentration was observed in the Nutri4 group, consistent with similar results reported in previous research.[34] The synergistic effect of vitamin E and selenium in eliminating lipid peroxides, formed as a result of excessive ROS interaction with PUFA, is well-known. Vitamin E acts directly as an antioxidant, while selenium serves as a cofactor for GPx enzymes.[57,58]

SOD, GPx, and CAT are enzymes that play a role in neutralizing reactive oxygen species (ROS) in the body. ROS are highly reactive molecules produced as byproducts of normal cellular metabolism, capable of damaging cells and tissues. In this study, GPx and SOD activity significantly increased in the Nutri4 group, with notable differences observed between participant groups. The higher activity of GPx and SOD in the Nutri4 group can be attributed to the increased

concentrations of vitamin E, selenium, and lutein in Nutri4 eggs, while n-3 PUFA likely do not directly impact the activity of antioxidative enzymes.[59,60]

Contrary to expectations, our study showed no significant differences in oxidative stress and antioxidative capacity within or between groups of ACS patients. These findings are consistent with previous research, indicating the need for further investigation into the impact of antioxidant concentration in enriched eggs, dosage, intervention duration, and characteristics of the studied population.[34]

Microvascular reactivity in patients with ACS refers to the ability of small blood vessels within the cardiac muscle to dilate or constrict in response to changes in blood flow or other stimuli. It is a crucial aspect of maintaining proper blood flow in the cardiac muscle and ensuring adequate supply of oxygen and nutrients. Several factors can affect microvascular reactivity in ACS, including inflammation, oxidative stress, and damage to the microvessels themselves. In some cases, microvascular dysfunction can contribute to the development of ACS by reducing blood flow in the cardiac muscle and potentially triggering the formation of a blood clot.[61] Importantly, lifestyle modifications such as smoking cessation, regular exercise, and maintaining a healthy diet can aid in improving microvascular function and reducing the risk of ACS. Given that microvascular function of the skin is impaired in most patients with ACS assessing PORH and local thermal hyperemia can provide valuable data on the state of microvascular function in ACS patients.[62] Present study demonstrates that the consumption of enriched eggs significantly improves vasodilation in response to PORH, ACh ID, and SNP ID in ACS patients.

The improvement in microvascular function observed in participants consuming enriched eggs can be explained by the increased concentration of n-3 PUFA in Nutri4 eggs, since there was significantly decreased ratio of n6/n3 in Nutri4 group. Other studies suggest that increased intake of n-3 PUFAs might enhance their integration into cellular membranes. N-3 PUFAs exhibit potential in improving endothelial function via various pathways: boosting nitric oxide (NO) production, regulating reactive oxygen species (ROS) by enhancing elimination, reducing inflammation, and promoting angiogenesis while mobilizing endothelial progenitor cells (EPCs). They activate both endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), and could also enhance other vasodilators.[63–70]

Stupin et al (2018, 2020)[15,42] obtained similar results in their studies, where PORH and ACh ID values significantly increased in young healthy individuals after consuming n-3 PUFA enriched eggs. Stirban et al[71] demonstrated that increased intake of EPA and DHA improves postprandial reactive hyperemia in hand microcirculation in individuals with type 2 diabetes. Vasileva et al[72] showed that daily consumption of 1.5 mg of n-3 PUFA for one month improves endothelial function and microcirculation (measured by LDF - laser Doppler flowmetry).

Considering the results obtained in this study, it can be concluded that consumption of enriched Nutri4 hen eggs significantly improves vasodilation in response to PORH, SNP ID, and ACh ID in patients with ACS. Taken together, these findings suggest that Nutri4 egg consumption may confer beneficial effects on certain biochemical parameters related to inflammation and blood clotting in patients with ACS.[27] Collectively, the consumption of enriched eggs demonstrated potential benefits in modulating antioxidant enzyme activities in patients with ACS. The increased activity of GPx and SOD in the Nutri4 group indicates a potential protective effect against oxidative stress. However, additional studies are imperative to unravel the underlying mechanisms and ascertain the clinical significance of these findings. The lack of a significant change in TBARS levels or FRAP suggests that egg consumption, both regular and enriched, may not significantly impact oxidative stress in ACS patients.

Study Limitations

While significant outcomes were observed within the three-week dietary intervention period, it is important to acknowledge the relatively short duration of our study. Future research endeavors should be directed towards investigating the long-term effects and sustainability of dietary interventions. Additionally, although our study explored the effects of the treatment on ACS patients, it is noteworthy that our sample may have limitations in generalizing the results to a wider population. Subsequent studies should include larger and more diverse samples to better understand the broader applicability of the findings. Additionally, variability in dietary adherence among participants may have influenced the final study outcomes. However, our patients had similar dietary patterns, based on the assessment of their dietary habits and daily food intake (via questionnaire or interview). Future investigations will pay additional attention to assessing and

ensuring consistent adherence to dietary interventions to enhance the reliability and relevance of the results. Finally, our study was conducted at a single center (UHC Osijek), which may limit the generalizability of the findings. Multi-center studies in the future will provide additional insights and a better understanding of the effects of a diet enriched with eggs on microvascular function in ACS patients.

Conclusion

The present study demonstrated that the intake of Nutri4 eggs was associated with enhanced microvascular reactivity in post-ACS patients, independent of changes in BP and HR. Moreover, while the consumption of enriched eggs resulted in a decrease in LDL-cholesterol levels, it is important to note that no significant differences were found between groups after the dietary intervention. This may be attributed to the sample size limitation, warranting further investigation. Nonetheless, these findings suggest that a well-designed dietary approach has the potential to positively impact cardiovascular system in individuals recovering from ACS. However, it is important to note that while the consumption of Nutri4 eggs did not significantly influence oxidative stress levels or antioxidant capacity in ACS patients, it did elevate the activity of GPx and SOD enzymes, indicating a potential role in mitigating oxidative stress. Furthermore, it is worth mentioning that the assertion regarding the safety of regular egg consumption (control group) should be interpreted cautiously. Although there were no significant adverse effects observed on the measured vascular parameters, there appears to be a slight worsening of microvascular reactivity, albeit with non-significant values, which warrants further investigation. Nonetheless, both regular and enriched eggs show promise for inclusion in the daily diet of ACS patients. By acknowledging these nuances, the study aims to provide a more comprehensive understanding of the implications of egg consumption on vascular system in ACS patients.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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