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Article in *Acta Scientifci Nutritional Health* · September 2021

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Anthropometric and Biochemical Effects of Consuming Omega-3 Fatty Acids Enriched Eggs

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Received: August 21, 2021

Published: September 18, 2021

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Abstract

Functional foods are products that control nontransmissible diseases. Egg is a low-cost food with high nutritional value and huge population intake. In the present study, the effect of Omega-3 fatty acids enriched eggs and non-enriched eggs on anthropometric measurements (weight, height, waist, hip, blood pressure, total fat percentage, visceral fat) and biochemical (lipid profile and glucose) were evaluated during 10 weeks, of 15 people with overweight or type I obesity. The participants were randomly assigned to receive a different treatment of enriched or non with Omega 3 eggs consumption with a dietary guideline or a specific eating plan. In the biochemical parameters, significant differences were observed for: total cholesterol ($p = 0.037$), HDL ($p = 0.001$) in treatment T4 and triglycerides ($p = 0.033$) in treatment T3. The adherence of each treatment was greater than 80%. In conclusion, enriched Omega-3 fatty acid eggs intake showed significant changes in biochemical indicators as total cholesterol, triglycerides and increase of HDL concentration.

Keywords: Omega-3; Nontransmissible Diseases; Lipid Profile; HDL; Triglycerides

Introduction

Non-transmissible diseases (NTDs) are the leading cause of death worldwide, contributing to the demise of 41 million people each year or 71% of global deaths [1]. Among NTDs, weight-related disorders (both obese and overweight conditions) the leading causes of mortality.

Concerningly, the overweight and obesity rate in the Americas is 24.6%, more than double the world average of 11.5% [2]. In Central America, one out of every five households includes at least one member who is overweight or obese. In Guatemala, Honduras and Costa Rica, 90% of individuals have a body fat percentage outside the normal range, with 70% of the population qualifying as overweight or obesity, 43% exhibiting high blood pressure, and at least 50% being physically inactive [3].

It is estimated that 60 to 70% of obese patients are dyslipidemic (high levels of plasma triglycerides, LDL and low levels of HDL) [4]. Atherogenic dyslipidemia (AD) is associated with inactive lifestyles, low fiber and high consumption of saturated and trans fatty acids. One way to counteract AD is with physical activity coupled with a diet low in carbohydrates and high in polyunsaturated fatty acids, such as Omega-3 fatty acids [5].

Omega-3 is an essential polyunsaturated fatty acid found in three dietary acids: alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA is found in vegetable oils such as flaxseed, soybeans, and canola while DHA and EPA are primarily found in fish such as tuna. Consumption of foods rich in ALA, EPA, or DHA increases circulating levels of Omega-3 in the body, thereby providing a litany of health benefits on car-

diovascular disease (atrial fibrillation, atherosclerosis, thrombosis, inflammation, and sudden cardiac death), diabetes, cancer, depression and various mental illnesses, age-related cognitive decline, periodontal disease, and rheumatoid arthritis [6].

Between 2015 to 2020, the dietary guidelines for Americans recommended a consumption of 230 grams of fish varieties per week, with the express goal of consuming 250 mg of EPA and DHA per day [7]. While consuming fish has numerous well-demonstrated nutritional benefits, per capita fish consumption among middle and lower-class families is low in Central American countries due to its high economic cost.

In contrast, eggs, particularly those derived from the common chicken (*Gallus gallus domesticus*) are a highly nutritious and affordable food source readily available throughout Central America. Eggs are rich in essential amino acids, which support the synthesis and maintenance of skeletal muscle mass [8]. Egg consumption in Central America has increased over the past years, as high production volumes and general favorability with consumers have led to its sale as a “commodity”. While commonly available egg variety are low in Omega-3 fatty acids, supplementing poultry feed with flax seed can significantly increase the amount of ALA in chicken eggs. Theoretically, these Omega-3 enriched eggs would accommodate the demand for this beneficial nutrient in addition to providing other benefits such as high protein value, all at an affordable cost to the consumer.

However, a lack of clinical trials at the regional level mean few affordable dietary alternatives exist to address the problems of obesity. Therefore, the following study aims to evaluate the biochemical and anthropometric changes following a diet supplemented with Omega-3 enriched eggs in people with overweight and obesity. Our findings suggest such a diet can improve their Body Mass Index, Blood Pressure, waist-hip index and their lipid profile (increase HDL, reduce cholesterol, triglycerides and LDL).

Materials and Methods

Location and production of Omega-3 enriched eggs

The study was carried out at Zamorano Pan American Agricultural School, University of Agriculture, located in the Francisco Morazan Department, Honduras. Omega-3 enriched eggs were produced under the project “Effects of Zamofeed on the profile of fatty

acids, cholesterol and pigmentation of the egg yolk”. Adding flaxseed by 3% to the hens’ diet increased the amount of α -linolenic acid (ALA) resulting a product (egg) with an average mass of 60 grams, 325 mg of Omega-3 and a reduction of 27.8 mg cholesterol relative to control eggs.

Fatty acid profile of omega-3 enriched eggs

To evaluate the profile of the eggs, the yolks were extracted and fatty acid (AOCS Ce 2-66 and AOCS Ce1j-07) and cholesterol (AOAC 994.10) content were determined via gas chromatography.

Study description

The clinical trial was previously approved by the Ethics Committee of Honduras belonging to the Autonomous University of Honduras. The research corresponds to an observational Phase I case-control clinical study. The sampling was non-probabilistic with desire to participate and the signing of an informed consent.

Study population

For the study, 16 workers of both sexes with ages between 28 and 47 years were selected via non-probabilistic random sampling. The selection criteria were: desire to participate voluntarily in the study, body mass index between 25-29.9kg (overweight) or 30-34.9kg (Type I obesity), regular consumption of egg and not being allergic or intolerant to eggs. The excluded population were people with mental difficulties, body mass index ≤ 24.9 or ≥ 35 , people intolerant or allergic to eggs.

Organization of the study population

Four participants were distributed in each treatment. The participants were randomly assigned to their treatment through the “Random” function of the Excel program. At the same time, each treatment was randomly assigned either a dietary guideline or a detailed, specific eating plan. The four treatments were:

T1. Five eggs without Omega-3 (Control) per person, per week, in conjunction with a specific eating plan.

T2. Three eggs without Omega-3 (Control) per person, per week, in conjunction with a dietary guideline.

T3. Five Omega-3 eggs (325 mg) per person, per week, in conjunction with a dietary guideline.

T4. Three Omega-3 eggs (325 mg) per person, per week, in conjunction with a specific eating plan.

Evaluation of anthropometric measurements and blood pressure

Weight (kg) and height (m) were measured to determine the body mass index (IMC) (kg/m^2). OMRON HBF-514C bioelectric impedance equipment was used to determine the weight. HBF-514C. A portable stadiometer 212 was used to determine height. To determine the waist-hip index ($\text{WHR} = \text{waist cm} / \text{hip cm}$), waist and hip circumference measurements were collected using an inextensible SECA 201 tape measure. The values obtained, allowed the participants to be categorized, according to the ranges stipulated by the National Heart Lung Blood Institute [8].

To determine the body composition (percentage of fat and visceral fat), the OMRON bioelectric impedance equipment model HBF-514C was used, which consists of six low-voltage electrical conduction sensors. The values obtained were classified according to the "Organization World Health" [9] for body fat and Omron Healthcare [10] for visceral fat. Blood pressure was measured with an OMRON M3 digital blood pressure monitor, precision class 1. The results provided by the cuff were categorized according to the "American Heart Association" [11].

Biochemical analysis (plasma lipid profile, glucose)

Total cholesterol, triglycerides, HDL and LDL were included in the lipid profile. Blood samples (35 μL) were taken from the participants via capillary and the DS Lipidocare portable was used to obtain the values of the mentioned lipid indicators. These values allowed the classification of the participants according to the ranges stipulated by the "National Institute of the Heart, Lung and Blood" [12]. The initial samples were collected as a reference value for comparison at the end of the study, after 10 weeks of the study. People fasted and did not smoke prior to data collection.

Blood sugar levels were determined using the ACCU-CHEK Rapid Test Kit. The sample was collected by capillary action using a test strip provided by the equipment manufacturer. To determine the category in which the participants were found, the ranges stipulated by the "National Institute of Diabetes and Digestive Kidney Diseases" [13].

Adherence to treatment

Participants' adherence to the assigned treatment was measured through the Morisky-Green test, which consists of a series of four questions with a dichotomous answer (yes/no). This questionnaire was applied in the middle of the study and at the end of the study.

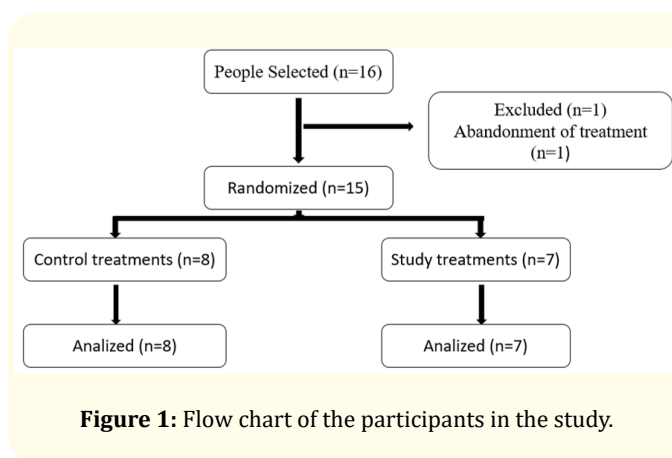
Statistical analysis

The "Statistical Analysis System" (SAS®) version 9.4 program was used. The data were obtained through two measurements carried out in the same experimental unit, using a T-student test for paired samples and to evaluate the significance between treatments. A Duncan test was performed with a significance level of $P \leq 0.05$.

Results

Characterization of participants

16 participants started the study. During the process one of them left the treatment, resulting in 15 total participants ($n = 8$ men and $n = 7$ women). Every participant was randomized and put in each of the four treatments. Then, people under control and treatments groups (Omega-3 enriched eggs), were observed in order to determinate the influence of eggs in the serum lipid profile.



The anthropometric indicators evaluated were: age, height, weight, Body Mass Index (BMI), Waist-to-Hip-Ratio (WHR), systolic and diastolic blood pressure, glucose, total mass body fat, visceral fat, total cholesterol, triglycerides, HDL and LDL.

Anthropometric and blood pressure measures

At the beginning of the study participants were overweight or had obesity type I (25 - 29.99 and 30 - 34.9 kg/m²), with WHR between 0.92 and 1.00 ranges correlating to medium and high cardio-metabolic risk, respectively. Treatment groups T1 and T3 displayed normal ranges of diastolic blood pressure (<80 mm/Hg) however, treatments T2 and T4 had prehypertension ranges (80-89 mm/Hg). Values of total mass body fat and visceral fat were outside the normal ranges for all groups, with an average total mass body fat (>33%) in women and (>20%) in men and visceral fat percentage between (10-14%) in the general population. The only exception was treatment group T1, which presented normal ranges of visceral fat ($\leq 9\%$).

End of study metrics were as follows. Participants who ate eggs enriched with Omega-3 (T3 and T4) exhibited reduced weight, BMI and WHR. Participants in group T2 reduced their systolic pressure to a normal range (<120 mm/Hg) and participants in treatment T1 increased their levels of visceral fat to a high range (10-14%), though this is comparable to the starting visceral fat for all other treatment groups. All these changes displayed statistical significance. Table 1 shows initial and final values of anthropometric measures and blood pressure for the four treatments.

Variable	Anthropometric measures		
	Initial	Final	P
Weight (kg)			
T1	74.83 $\pm$ 3.6	75.20 $\pm$ 6.2	0.794
T2	79.43 $\pm$ 12.0	79.40 $\pm$ 11.9	0.965
T3	97.08 $\pm$ 10.0	95.33 $\pm$ 10.1	0.280
T4	99.50 $\pm$ 24.4	99.20 $\pm$ 25.7	0.777
Body Mass Index (kg/m ²)			
T1	28.50 $\pm$ 2.9	28.50 $\pm$ 3.8	1.000
T2	30.95 $\pm$ 4.6	31.00 $\pm$ 4.4	0.827
T3	33.43 $\pm$ 3.5	32.85 $\pm$ 3.2	0.352
T4	33.30 $\pm$ 7.5	33.20 $\pm$ 7.8	0.785
ICC			
T1	0.92 $\pm$ 0.0	0.93 $\pm$ 0.0	0.391
T2	0.95 $\pm$ 0.1	0.94 $\pm$ 0.0	0.666
T3	0.95 $\pm$ 0.1	0.92 $\pm$ 0.1	0.194
T4	1.00 $\pm$ 0.0	0.96 $\pm$ 0.0	0.225

Systolic blood pressure (mm/Hg)			
T1	111 $\pm$ 20.3	109 $\pm$ 19.1	0.576
T2	125 $\pm$ 15.9	116 $\pm$ 9.1	0.117
T3	111 $\pm$ 4.0	105 $\pm$ 9.9	0.438
T4	117 $\pm$ 10.0	119 $\pm$ 13.0	0.713
Diastolic blood pressure (mm/Hg)			
T1	76 $\pm$ 14.0	82 $\pm$ 10.2	0.512
T2	80 $\pm$ 19.6	72 $\pm$ 7.6	0.432
T3	75 $\pm$ 3.9	74 $\pm$ 6.7	0.876
T4	82 $\pm$ 6.0	92 $\pm$ 11.5	0.453
Body corporal total fat (%)			
T1	36.10 $\pm$ 9.2	33.93 $\pm$ 8.7	0.471
T2	38.30 $\pm$ 11.9	38.60 $\pm$ 10.9	0.617
T3	40.85 $\pm$ 11.6	41.08 $\pm$ 11.1	0.685
T4	37.83 $\pm$ 12.3	37.77 $\pm$ 13.3	0.930
Visceral fat (%)			
T1	9.00 $\pm$ 2.2	10.25 $\pm$ 1.0	0.391
T2	10.75 $\pm$ 3.3	11.00 $\pm$ 3.6	0.391
T3	11.25 $\pm$ 2.8	10.75 $\pm$ 2.9	0.181
T4	12.33 $\pm$ 3.5	12.00 $\pm$ 4.0	0.422

Table 1: Initial and final values of anthropometric measures and blood pressure for all treatment groups. $\pm$ DE represents media values, ($P < 0.05$) shows significant changes between initial and final measures.

Biochemical measures

At the beginning of the study, treatment T4 displayed normal ranges of blood glucose (<120 mg/dL) while the other treatment groups presented ranges of prediabetes (100-125 md/dL). Treatment group T4 exhibited high total cholesterol (200-239 mg/dL), while the other treatment groups were below the normal threshold (<200 mg/dL).

Groups T1-T3 displayed higher concentrations of triglycerides (200-499 mg/dL), while treatment T4 was in a normal range (<150 mg/dL). Concentrations of HDL amongst the four treatment groups were low (<40 mg/dL), as were the LDL values (<100 mg/dL), with only treatment T1 presenting an optimal concentration of LDL (100-129 mg/dL).

Blood glucose levels were unchanged following the conclusion of the study. Similarly, lipid profiles and total cholesterol values did not present significant changes, although nearly all treatments trended towards a decrease in these metrics, with the largest reductions occurring in treatments T3 and T4. Only treatment T1 demonstrated an increase in total cholesterol, changing their category from desirable to high (200-239 mg/dL).

Triglycerides showed a significant reduction in treatments T3 (268.50 to 190.35 mg/dL) changing its category from high, to high-limit and T4 (379.33 to 274.45 mg/dL), which maintained its category. In order to decrease cardiovascular risks HDL values must be more than 50 mg/dL. Patients in treatment groups T3 and T4 nearly achieved this protective value. Although treatment T1 was close to 50 mg/dL, treatment T4 stood out due to its greater increase relative to its initial value. After study completion, every treatment belonged to the normal HDL category (40-60 mg/dL).

LDL was modestly reduced in participants who ingested Omega-3 eggs relative to the control treatments. However, none of the treatments presented significant differences.

Biochemical measures			
Variable	Initial	Final	P
Glucose (mg/dL)			
T1	105 ± 6.4	107 ± 6.7	0.736
T2	105 ± 17.3	108 ± 14.2	0.775
T3	103 ± 4.1	111 ± 8.6	0.197
T4	98 ± 5.0	91 ± 12.9	0.524
Total cholesterol (mg/dL)			
T1	182.25 ± 47.3	202.14 ± 60.2	0.085
T2	176.00 ± 37.6	172.13 ± 28.0	0.691
T3	159.00 ± 34.7	146.20 ± 19.7	0.220
T4	205.67 ± 38.6	198.05 ± 40.5	0.037
Triglycerides (mg/dL)			
T1	177.25 ± 43.8	168.95 ± 39.2	0.482
T2	307.75 ± 78.6	250.99 ± 106.7	0.096
T3	268.50 ± 185.0	190.35 ± 162.9	0.033
T4	98.20 ± 5.7	93.86 ± 1.1	0.383
HDL (mg/dL)			
T1	38.00 ± 8.6	49.30 ± 2.5	0.034
T2	30.50 ± 7.5	48.88 ± 3.7	0.021
T3	37.50 ± 8.3	49.51 ± 2.8	0.037
T4	31.33 ± 3.2	49.30 ± 4.5	0.001
LDL (mg/dL)			

T1	108.60 ± 51.6	119.05 ± 63.3	0.192
T2	83.75 ± 20.9	73.05 ± 26.4	0.392
T3	67.80 ± 13.1	58.62 ± 14.4	0.368
T4	98.20 ± 5.7	93.86 ± 1.1	0.383

Table 2: Initial and final values of biochemical measures of the four treatments.

± DE represents media values, (P < 0.05) shows significant changes between initial and final measures.

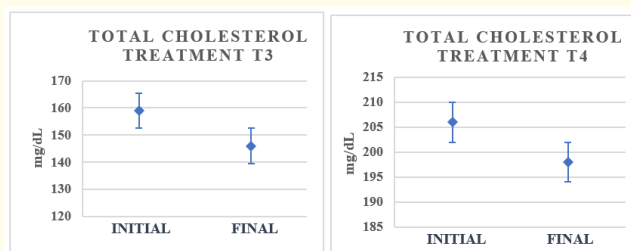


Figure 2: Initial and final values of total cholesterol for treatments T3 and T4.

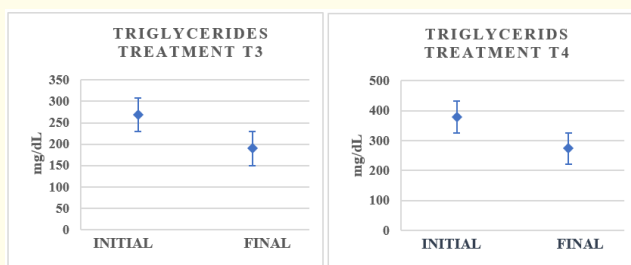


Figure 3: Initial and final values of triglycerides for treatments T3 and T4.

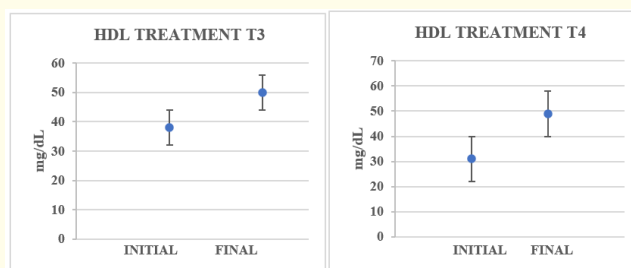


Figure 4: Initial and final values of HDL for treatments T3 and T4.

Adherence to treatment

Morisky-Green results are presented on figure 2 as percentages. Every treatment had a greater adherence than 80%, with treatment T3 demonstrating the highest percentage adherence.

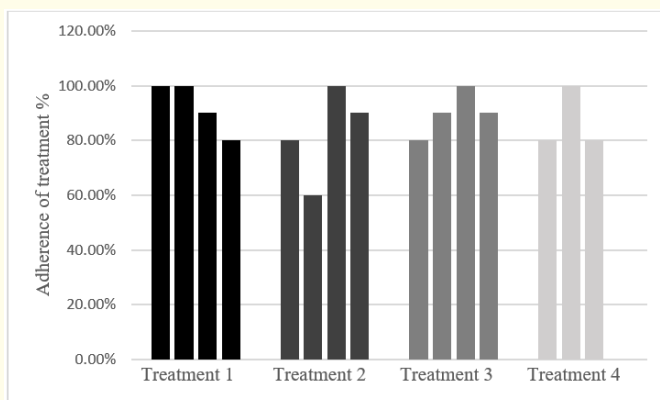


Figure 5: Treatment adherence per treatment.

Discussion

Our results suggest that the addition of flaxseed and phytosterols in poultry diets may provide health benefits to obese and overweight patients, likely due to the improved nutritional value of the eggs. The modification of poultry diet increase the polyunsaturated acids Omega-3 content in the egg yolk [14]. The polyunsaturated acids are macromolecules, which have a crucial role in the prevention and protection of people at risk for cardiovascular illnesses [15]. Lemahieu, *et al.* [16] determined that a flaxseed diet in poultry significantly increases linolenic, EPA and DHA in egg yolks.

This study showed that normal eggs and Omega-3 enriched eggs did not significant change the anthropometric measures of the participants. This statement agrees with a study of Ahmad, *et al.* [17], which concluded that a diet including Omega-3 enriched eggs did not significantly affect patient BMI.

Patients ingesting Omega-3 enriched eggs also did not display significant differences in blood pressure. However, treatment T3, which included enriched eggs with Omega-3 diet, demonstrated the greatest reduction in systolic pressure. Omega-3 decreases oxidative stress by inhibiting the formation of reactive oxidative species (ROS) and isoprostanes. This reduction increases the bio-

availability of nitric oxide, which in turn leads to vasodilatation and a reduction in blood pressure [18]. Excess ROS can alter cellular function, irreversibly damaging lipids, nucleic acids and cellular proteins. This oxidative damage can lead to numerous pathologies including hypertension, insulin resistance, neurodegenerative illnesses and so on [19]. Isoprostanes, including the especially potent F2, are strong vasoconstrictors [20].

Other potential benefits of consuming a diet rich in Omega-3 are derived in the fact that DHA can directly stimulate vascular smooth muscle through increased activity of K⁺ channels, which are dependent on Ca²⁺ ions. This activation leads to dilatation of blood vessels, which reduce blood pressure and improve vascular health [21]. Calcium and potassium channels are responsible for the contraction and relaxation, or vasodilation of blood vessels, respectively [22].

The lack of significant changes in blood glucose levels following dietary supplementation with Omega-3 enriched eggs is not surprising, given the fact that Omega-3 does not affect plasmatic glucose levels, nor HbA1c [23]. Despite this, treatment T4 reduced glucose levels, thereby maintaining a normal range throughout the study. Of note, while patients in group T4 suffered from type I obesity, which is frequently correlated with prediabetes [24], all blood glucose levels were in the normal range.

Increase of adiposity and free fatty acids of obese patients leads to a homeostatic response which drives insulin resistance and hyperinsulinemia [25]. Insulin resistance is defined by ineffective insulin secretion, leading to nonresponsive insulin receptor cells. Insulin hypersecretion in the early stages of disease maintain relatively normal glycemic indices, leading to underreported cases of prediabetes [26]. These data suggest that while patients in treatment T4 may have had (pre-)diabetes, no metabolic anomalies could be detected using the tools and techniques available.

Following study, omega-3 did corporeal fat values in either men or women through completion of the study. Instead, each treatment group maintained high levels of corporeal fat, which fits with previous studies exploring this [27]. While we observed a reduction of visceral fat in treatments T3 and T4, these findings lack statistical significance. A previous study by Mello, *et al.* [28] confirms these findings: incorporation of Omega-3 in a high fat diet of obese mice after 10 weeks, leads to, observed beneficial effects in the reduction of visceral fat.

Some studies showed that Omega-3 fatty acids had the potential to reduce accumulated fat in the body, especially visceral fat [29]. Their proposed mechanism of action is that Omega-3 reduces oxidative damage and relieves changes in the antioxidant defense system and Krebs cycle, also Omega-3 to mitigate changes which could lead to a high fat diet [30].

The treatment groups receiving Omega-3 enriched eggs (T3 and T4), both displayed reductions in their cholesterol total values; however, only treatment T4 showed a statistically significant reduction. These findings align with the results from Shakoor, *et al.* [31], who demonstrated that the intake of two eggs enriched with Omega-3 for 5 weeks had the capability to reduce cholesterol by 12.5%. While the mechanism of action for the effect is still unclear, one study suggests that EPA confers a hypocholesterolemic effect by increasing bile flow and reverse transportation of cholesterol [32].

The National Lipid Association recommends a diet rich in Omega-3 fatty acids for patients with hypotriglyceridemic, as studies have shown this may decrease triglycerides levels. Our findings confirm the results of a previous study, where consuming Omega-3 enriched eggs significantly decreased triglycerides levels. Omega-3 fatty acids have low VLDL (very low-density lipoprotein) incorporation rates, which may increase the elimination rates of triglycerides in VLDL particles. Triglycerides are amphipathic molecules which assembly in blood lipoproteins (VLDL and chylomicrons) for transportation through the blood plasma to satisfying energetic needs.

Polyunsaturated fatty acids (PUFA) are high quality fats which benefit overall health. One study has shown that enriching eggs with PUFAs help to decrease LDL cholesterol. High density lipoproteins (HDL) synthesized in the gut and liver remove cholesterol of peripheral tissues, thereby conferring protection against atherogenesis. General consumption of eggs (Omega-3 enriched or not) is known to increase HDL levels.

In contrast, multiple studies have found that frequent consumption of eggs does not significantly alter levels of LDL cholesterol. However, our results from treatments T3 and T4 demonstrate a reduction in LDL, albeit without statistical significance. This reduction may be attributed to enriched Omega-3 consumption. A study by Valenzuela, *et al.* highlighted the beneficial effects on cardiovascular health conferred by polyunsaturated fatty acids Omega-3, which they argued was due to reduced LDL cholesterol.

To confirm the adherence by study participants, we performed clinical or therapeutic interviews, wherein an interviewer evaluates the effectivity and security of the prescribed treatments. To consider a treatment adherent, the medicine or treatment intake must be greater than 80%, while less than 80% is consider non-adherent. Every treatment group displayed greater than 80% adherence, allowing all treatment groups to be included as part of the study.

Conclusion

Enriched Omega-3 eggs intake showed significant changes in biochemical indicators. For total cholesterol profile ($p = 0.037$), triglycerides ($p = 0.033$) and increase of HDL concentration ($p = 0.001$).

Conflict of Interest

The authors express that there are no conflicts of interest when writing the manuscript.

Acknowledgement

Thank you to ITHC: the Institute for Technology in Health Care that was good to fund the biochemical analysis.

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Volume 4 Issue 10 October 2021

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