Journal of Nutrition & Intermediary Metabolism 15 (2019) 35–41

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: www.elsevier.com/locate/jnim

The consumption of 12 Eggs per week for 1 year does not alter fasting serum markers of cardiovascular disease in older adults with early macular degeneration

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HIGHLIGHTS

- Serum total cholesterol, lipoprotein cholesterol and triglycerides levels did not change at any time during the consumption of 12 eggs per week for 1 year compared to baseline.
- Serum total cholesterol, lipoprotein cholesterol and triglycerides levels were not significantly different between the group that consumed 12 eggs per week for 1 year compared to the group that consumed no eggs for 1 year.
- Serum apolipoprotein (apo) A-1, apo B, lipoprotein $(Lp)_a$ and high sensitive C reactive protein (hsCRP) levels did not change at any time during the consumption of 12 eggs per week for 1 year compared to baseline.
- Serum glucose levels did increase significantly during the consumption of 12 eggs per week for 1 year compared to baseline but decreased back to baseline levels at 12 months.

ARTICLE INFO

Keywords:
Apolipoprotein A-1
Apolipoprotein B
C-reactive protein (CRP)
Cardiovascular disease (CVD)
HDL cholesterol
LDL cholesterol

ABSTRACT

Some studies suggest that eating more than one egg daily may increase risk of death from cardiovascular disease. The objective of this study was to determine the effects of consuming eggs on various serum markers associated with cardiovascular disease (CVD). Forty-five independently living adults diagnosed with early macular degeneration, but healthy otherwise were recruited into the study. Subjects were placed into the Intervention (n = 27) or Control group (n = 18) based on whether or not they would consume eggs. The Intervention group consumed 12 eggs per week while the Control group refrained from consuming any whole egg products for 1 year. Serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), glucose, apolipoprotein (apo) A-1 and apo B, lipoprotein $(Lp)_a$ and high-sensitive C reactive protein (hsCRP) were measured at baseline, 6, and 12 months. Serum low-density lipoprotein cholesterol (LDL-C) concentration was calculated via the Friedewald equation. Serum TC, TG, HDL-C, LDL-C, apo A-1, apo B, Lp_a and hsCRP concentrations did not change at any time in both the Intervention and Control groups compared to baseline nor were there any differences between the two treatment. Serum glucose concentrations did increase significantly in the Intervention group at 6 months compared to baseline (23%, $P < 0.05$) but decreased back to baseline concentrations at 12 months. This study suggests that the consumption of 12 eggs per week for 1 year does not significantly alter fasting serum lipids, lipoprotein cholesterol, or other biomarkers of CVD in older adults diagnosed with early macular degeneration.

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https://doi.org/10.1016/j.jnim.2018.11.004
Received 29 January 2018; Received in revised form 19 November 2018; Accepted 22 November 2018
Available online 28 November 2018
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Abbreviations

AMD	Age related macular degeneration
ANOVA	Analysis of Variance
Apo	Apolipoprotein
BMI	Body mass index
CVD	Cardiovascular disease
CHD	Coronary heart disease
HDL-C	High density lipoprotein cholesterol
hsCRP	High sensitive C reactive protein
IRB	Institutional Review Board:
Lp_a	Lipoprotein a
LDL-C	Low density lipoprotein cholesterol
DDR	Seven day dietary record
SEM	Standard error of the mean
TC	Total cholesterol
TG	Triglycerides

1. Introduction

One third of American adults suffers from cardiovascular disease (CVD) resulting partially from the adhesion of circulating cholesterol to the blood vessel wall, thereby forming atherosclerotic plaques that interfere with the normal vessel functionality [1]. As the plaque builds-up, it causes a narrowing of the arteries, thereby reducing the amount of oxygen delivered to tissues causing ischemia, which could result in stroke, sudden cardiac death, and myocardial infarction. There are different types of circulating cholesterol and not all of them pose threats to cardiac health. However, since low-density lipoprotein cholesterol (LDL-C) has been widely accepted as a major contributor to plaque buildup, elevated blood LDL-C is considered a major CVD risk factor.

CVD prevention guidelines recommend reducing the consumption of foods containing saturated and *trans* fats, nutrients that may increase serum LDL-C [2]. Over the years, foods such as eggs have come under fire for their perceived role in contributing to CVD. The recommended daily limit for dietary cholesterol until recently was 300 mg for healthy individuals and 200 mg for patients with CVD, a single egg yolk contains 186–200 mg of cholesterol [3]. Although this limit was removed in the 2015 edition of the Dietary Guidelines [4], this change should not suggest that dietary cholesterol is no longer important to consider when examining healthy dietary patterns for all individuals. The high cholesterol content of egg yolks may appear to support findings based on the correlation of dietary egg consumption with increased total carotid plaque area [5–7], while others have not observed this [8]. While these studies observed an association between dietary egg consumption and total plaque area, there was insufficient data collected on other possible confounders including; saturated fat intake, exercise activities, and waist circumference (although BMI was included) of study participants [9]. A recent review examining the overall cardio-metabolic health effects of egg consumption suggests that eggs maybe consumed safely as part of a healthy diet in both the general population and those people at a higher risk of CVD [10].

Prior studies by our laboratory have investigated the effects of egg-rich diets on serum lipids and lipoprotein cholesterol concentrations but in shorter durations. Goodrow et al. [11] examined the consumption of one egg per week for 5 weeks, and showed that serum concentrations of total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were not affected by the consumption of eggs. In another study, Handelman et al. [12] investigated a diet consisting of 1.3 eggs per week for the same length of time but showed that serum LDL-C concentrations increased approximately 10%. Differences in these studies involved different sample sizes, mean age of the subjects, and gender composition; potentially contributing to the variation in their findings. In another study, Greene et al. [13] demonstrated that consuming three eggs per day produced a statistically significant increase in both serum LDL-C and HDL-C concentrations in individuals between 29 and 60 years of age. In comparison, another study from our laboratory [14] examined the consumption of 2 and 4 egg yolks per day for 5 weeks each on serum lipids and lipoprotein concentrations and showed that serum HDL-C increased significantly by 5% with both 2 and 4 egg yolks while serum LDL-C increased significantly by 8% with the 4 egg yolk diet compared to slight decrease with the 2 egg yolk diet.

The purpose of the current study was to expand our knowledge of previous studies by examining the longer term effects of consuming 12 eggs per week for 1 year on serum lipids, lipoprotein cholesterol, and other select CVD biomarkers in older patients with early macular degeneration as part of a study that was examining the effect of egg consumption as form of dietary treatment and/or slowing down the progression of the disease.

2. Methods

2.1. Subjects and study design

One hundred and twenty-two subjects were recruited from an existing patient population at Nashua Eye Associates who were previously diagnosed with early or intermediate stage DRY aged-related macular degeneration (AMD) but were healthy otherwise. Subjects who fulfilled the inclusion criteria (n = 45), needed to receive permission from their primary care physician, and sign the informed consent. Inclusion criteria for the protocol included; newly diagnosed participants with early Dry AMD within the last 2 months, ≥ 50 years of age, and were willing to have blood drawn for measurement of blood analytes. Exclusion criteria included; anyone who was unwilling to stop taking oral supplements containing lutein and/or zeaxanthin, was allergic to eggs, and was < 50 years of age. Once identified, laboratory personnel met with participants to provide them with a brief overview of the study, how to fill out a 7 Day Dietary Record (DDR), fasting (> 12 h) procedures for blood draws, and what was expected of them for the successful completion of the study. Included in this meeting were issues of compliance; especially with the consumption of lutein-free oral supplements, the consumption of 12 eggs per week by the Intervention group, and no egg consumption by the Control group. Participants placed in the Intervention group were instructed to consume no more than 2 eggs per day, 12 eggs per week and not to go > 2 days without eating eggs. Research personnel were also available to answer questions from participants on how to incorporate 2 eggs into the typical diet.

The study was divided into two phases. Phase 1 involved the execution of a 6-week wash-out run-in period. During this time, all participants were instructed not to eat any pure egg products. At the conclusion of the six-week trial, all participants were subjected to the collection of minimum 12-h fasting blood samples (baseline). During the fifth week of Phase I, dietary daily-recorded intake (DDRs) was collected from all participants that reflected their nutritional intake over a 7-day period. This served both as the basis for dietary analysis, as well as a measure of participant compliance. Phase 2 of the study consisted of a 12-month period in which all participants were assigned to either the Intervention group (12 eggs per week for 12 months) or the Control group (no eggs for 12 months). Assignment into either group was not completely random unfortunately since some subjects were not willing to consume eggs or currently avoided them and thus were placed into the Control group. Grocery store vouchers were supplied to each participant in order for them to purchase their own dozen eggs on a weekly basis. Greater than 12-h fasting blood samples were collected from all participants at both six and 12 months. Seven day DDRs were collected during the third week of each month for 12 months from all participants. Research personnel also made phone calls on a weekly basis to all subjects as a measure of compliance as well. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and the use of human subjects for this study was approved by the University of Massachusetts, Lowell, Institutional Review Board (IRB). Written informed consent was obtained from all subjects. All subjects were given a cash incentive and a certificate of participation at the end of the study.

2.2. Dietary analysis

In addition to the procedures mentioned above, additional control measures were implemented. This included the confirmation of no egg consumption for the Control group, as well as confirmation of the consumption of 12 eggs per week for the Intervention group. Phone calls to participants were made twice a week to remind them of their respective consumption requirements, and to fast 12 h prior to blood sample collection. Nutrient analysis was performed using EvaluEat, version 1.2 dietary analysis tool (Pearson Education, Benjamin Cummings, 2004). Dietary intakes of the macronutrients, including; fat, saturated fat, carbohydrate, and fiber were assessed.

2.3. Blood collection

Blood samples were collected from each participant at baseline, 6 months and 12 months. Samples were separated by centrifugation at 1500 × *g* for 12 min at 4 °C, serum was collected and divided into aliquots, and stored at −80 °C. Serum lipid, lipoprotein cholesterol, and glucose concentrations were analyzed at baseline, 6 and 12 months in duplicate and the mean for the two runs was used in statistical analyses. Serum apolipoprotein (apo) A-1, apo B, lipoprotein $(Lp)_a$, and high sensitive c-reactive protein (hsCRP) levels were analyzed at baseline and 12 months also in duplicate.

2.4. Biochemical analyses

Serum TC, HDL-C, TG, hsCRP, Apo A-1, Apo B, Lp_a, and glucose concentrations were measured using a Cobas Mira Plus Clinical Chemistry Autoanalyzer (Roche, Branchburg, NJ). Serum TC [15], TG [16] and glucose concentrations were obtained by enzymatic methods. Cholesterol, triglyceride, and glucose reagents were supplied by Sekisui Diagnostics (Framingham, MA, USA) and followed the procedure outline by the company. Serum HDL-C concentration was measured using the Genzyme HDL Ultra Cholesterol Reagent (Sekisui Diagnostics, Framingham, MA, USA). Serum LDL-C concentration was calculated via the Friedewald equation [17]. Serum apo A-1, apo B, Lp_a and hsCRP concentrations were measured by immunoturbidimetric assay (Sekisui Diagnostics, Framingham, MA).

2.5. Statistical analyses

Sigma Stat version 3.1 (Jandel scientific, San Jose, CA, USA) and SPSS software (version 20, Armonk, New York, USA) were used for statistical analysis. Serum lipids and glucose as well as the 7 DDR data were analyzed utilizing a two-way repeated-measures analysis of variance (ANOVA) with time point of blood draw (0, 6, and 12 months) as within-subject factors and treatment (Intervention vs Control) as between-subject factors. Paired *t*-Test was used to determine the significance of changes in serum apo A-1, apo B, Lp_a, and hsCRP levels within subjects between baseline and 12 months. A simple *t*-test was executed for the determination of significant changes in serum apo A-1, apo B, Lp_a, hsCRP levels between treatment groups at baseline and 12 months. All values are expressed as mean ± SEM and statistical significance was set at $P \leq 0.05$.

Table 1
Demographic characteristic of the study population[a].

Height (in)	Baseline	6 Month	12 Month
Intervention (n = 27)	66.0 ± 0.83	66.2 ± 0.95	65.1 ± 0.80
Control (n = 18)	64.9 ± 0.87	64.7 ± 0.96	64.3 ± 0.92
Weight (lbs)			
Intervention (n = 27)	181.3 ± 10.3	186.4 ± 11.8	181.7 ± 11.1
Control (n = 18)	178.6 ± 13.9	178.5 ± 15.0	183.0 ± 19.2
BMI[b]			
Intervention (n = 27)	29.0 ± 1.27	30.8 ± 2.28	30.0 ± 1.66
Control (n = 18)	29.7 ± 2.07	30.1 ± 2.22	30.9 ± 2.86
Age			
Intervention (n = 27)	73.7 ± 1.55		
Control (n = 18)	77.3 ± 2.09		

[a] Values are Mean ± SEM. No significant differences exist between treatments and time.
[b] BMI = weight (lbs)/height $(in)^2$ x 704.

3. Results

3.1. Subject characteristics and diet

Of the 122 subjects interviewed for the study; 61 declined, 5 were declined by their primary care physician, and 11 dropped out or moved away during the 1 year, leaving 45 participants to finish the study, 14 males and 31 females (average age 75 years old) (Table 1). None of the subjects were removed from the study by their primary care physicians due to negative changes on serum lipids during the 12 months. There were no changes in height, weight or body mass index (BMI) in subjects during the course of the 12-month intervention (Table 1).

As expected, dietary cholesterol consumption was significantly greater in the Intervention group compared to the Control group at both the six- (288%; $P < 0.05$) and twelve- (345%; $P < 0.05$) month time point, as well as within the Intervention group when comparing baseline to six- (181%; $P < 0.05$) and twelve- (166%; $P < 0.05$) months (Table 2). Dietary protein, total fat, and saturated fat intake was significantly higher in the Intervention group compared to the Control group at six- (31%, 44%, and 43% respectively; $P < 0.05$) and twelve- (27%, 57%, and 60% respectively; $P < 0.05$) months (Table 2). In addition, the Intervention group consumed significantly less dietary carbohydrates at six- (−29%; $P < 0.05$) and twelve- (−23%; $P < 0.05$) months compared to baseline (Table 2).

3.2. Analysis of serum lipids and lipoprotein cholesterol and glucose measurements

The Intervention group had serum TC, LDL-C, TG, and HDL-C concentrations and TC/HDL-C and LDL-C/HDL-C ratios that were similar and were not statistically significant compared to the Control group at no time point during the 12-month study period (Table 3). Similarly, the Intervention group had no changes in serum TC, LDL-C, TG, and HDL-C concentrations and TC/HDL-C and LDL-C/HDL-C ratios compared to baseline after 12 months. Serum TC, LDL-C, HDL-C, and TG concentrations and TC/HDL-C and LDL-C/HDL-C ratios also were not different after 12 months in the Control group.

Serum glucose concentrations were not significantly different between the Intervention group and the Control group at any time point during the study (Table 3). The percent change between baseline and either the 6- or 12-month time points for the Control group were not statistically significant. However, the percent change between the 6- and 12-month time points for the Control group was decreased and approached significance ($P < 0.1$). On the other hand, the percent change from baseline to either the 6- or 12-month time point, for the Intervention group was increased and statistically significant for the 6-month time point (23%, $P < 0.05$). The percentage of change between 6 and 12 months for the intervention group was decreased and not

Table 2
Macronutrient intake as measured by the seven-day diet records (7DDR).

Energy (Kcal)	Baseline	6 Months	12 Months
Intervention (n = 27)	1827.0 + 99.6	1507.9 + 79.5	1532.8 + 95.1
Control (n = 18)	1537.4 + 136.5	1248.0 + 99.2	1270.0 + 75.0
Protein (g/day)			
Intervention (n = 27)	75.0 + 4.03	70.9 + 3.53*	70.9 + 3.53*
Control (n = 18)	68.9 + 6.61	54.0 + 4.09*	54.5 + 3.77*
Carbohydrate (g/day)			
Intervention (n = 27)	217.7 + 18.8^{A}	154.0 + 8.98^{B}	166.9 + 13.1^{B}
Control (n = 18)	187.1 + 15.4	168.6 + 12.9	176.2 + 16.8
Fiber (g/day)			
Intervention (n = 27)	15.5 + 2.71	11.5 + 0.99	12.9 + 1.10
Control (n = 18)	15.4 + 1.91	14.1 + 1.39	15.0 + 1.77
Total Fat (g/day)			
Intervention (n = 27)	68.5 + 4.68	66.0 + 6.51*	65.6 + 4.63*
Control (n = 18)	56.8 + 6.23	45.8 + 4.42*	45.8 + 4.42*
Saturated Fat (g/day)			
Intervention (n = 27)	20.5 + 1.14	19.6 + 1.40*	20.0 + 1.49*
Control (n = 18)	18.6 + 2.56	13.7 + 1.57*	12.5 + 1.28*
Cholesterol (mg/day)			
Intervention (n = 27)	178.2 + 13.7^{A}	500.8 + 25.2*^{B}	473.5 + 25.4*^{B}
Control (n = 18)	165.8 + 19.0	128.8 + 18.1*	106.3 + 11.5*

*Significantly different at $p < 0.05$ between Intervention and Control.
Values in row not sharing a superscript are significantly different at $p < 0.05$ between time points.
Values are Mean ± SEM.

statistically significant.

3.3. Analysis of serum apo A-1, apo B, Lp_a, and hsCRP

In the Intervention group, serum apo A-1, apo B, apo B/A-1 ratio and serum Lp_a and hsCRP concentrations did not change after the 12 months of consuming eggs (Table 4). The same was true for the Control as well. Also, there were no differences between the Intervention and Control groups for serum apo A-1, apo B, apo B/A-1 ratio and serum Lp_a and hsCRP concentrations (Table 4).

3.4. Associations between lipoproteins and apolipoproteins

There was a significant positive association between percentage of change in serum LDL-C with serum apo B ($r^2 = 0.367$ and correlation of 0.606; $P < 0.001$). In addition, there was a significant positive association between percentage of change in serum HDL-C with apo A-1 ($r^2 = 0.337$ and correlation of 0.581; $P < 0.001$).

4. Discussion and conclusion

Over the years since the association between cholesterol and CVD was proposed, research has advanced our understanding of cholesterol metabolism. Scientists and clinicians now acknowledge the distinction between dietary and circulating cholesterol, and their non-linear relationship. This idea is supported by numerous reports over recent decades that investigated the effects of dietary egg consumption on human serum cholesterol. Houston et al. [18] studied the correlation between dietary egg consumption, blood cholesterol levels, and CVD incidence over 9 years. Based on data from 1941 participants belonging to the Health ABC Study cohort, a positive correlation among the three elements was found to exist only in older adults with non-insulin-dependent Type II diabetes [18]. Studies such as these advance previous findings [19] or reinforce that dietary egg consumption is likely to have negligible effects on CVD and stroke in healthy adults. Scrafford et al. [20] demonstrated that an egg-rich diet, defined as > 7 eggs per week, did not significantly raise the risk of coronary heart disease (CHD) mortality in both healthy and diabetic patients. Furthermore, Fernandez [21] suggests that the consumption of eggs might be negatively correlated with the progression of atherosclerosis. In a follow-up study, Blesso et al. [22] found that consuming 1 egg daily for 12 weeks improved the atherogenic lipoprotein profile and insulin resistance in 37 individuals between the ages of 30–70 with metabolic syndrome. Their study also reported no change in serum TC or LDL-C levels in subjects consuming egg yolks [22]. In fact, whole egg consumption was observed to raise serum HDL-C levels or the "good" cholesterol, while reducing serum TG levels, 8.6 mg/dL and −40.6 mg/dL, respectively, which was nearly twice as much as consuming yolk-free egg substitutes, 4.7 mg/dL and −26.2 mg/dL, respectively [22]. These results show that the increase in serum HDL-C concentrations was greater than the increase in serum TC or LDL-C concentrations.

The current study produced no changes in the lipid profile, which are consistent with earlier studies conducted by us and others [11–14,23–25]. Goodrow et al. [11] found that consumption of 1 egg per day for 5 weeks produced slight yet statistically insignificant increases in serum TC and LDL-C concentrations, with no significant changes in LDL:HDL cholesterol or TC:HDL cholesterol ratios, key lipid biomarkers associated with CVD. Similar to previous studies [11,13,14], none of the present study's findings produced clinically significant lipid profile alterations. While the present study's cohort mean age was identical to that of Goodrow et al. [11] and Vishwanathan et al. [14], a mean of 79 years, while Greene et al. [13] ranged in age from 20 to 60, it should be noted that prior research has established an inverse association between age and serum LDL-C concentrations [26–28].

The addition of other biomarkers measured in the present study are believed to be relevant given the poor association between dietary cholesterol intake and serum lipid levels, and a growing understanding that serum lipid levels alone produce insufficient findings upon which to stage cardiovascular health status [26]. Moreover, additional biomarkers are relevant to the study of atherosclerosis due to growing recognition of the disease involving the dual effects of vascular lipid accumulation as well as the processes of inflammation [27]. In the present study, the apolipoproteins and Lp_a biomarkers were measured in order to learn more about whether increased egg consumption alters the metabolism of dietary cholesterols and influences vascular lipid accumulation. Serum hsCRP and glucose were also measured to assess the impact of egg consumption on major pro-inflammatory biomarkers that are also associated with CVD.

Apolipoproteins are components of lipoprotein particles that function by transporting cholesterol and triglycerides from their production sites to tissues and organs where they are utilized. Lp_a consist of an LDL-like particle and apo A attached to apo B of the LDL-like particle. Its physiological function in humans is still widely unknown but increased serum levels have been associated with CVD [29]. Apo A-1 is a major protein component of HDL and is recognized for its role in reverse transport of cholesterol back to the liver for catabolism [30–33]. Elevated serum HDL-C levels are clinically associated with a significant reduction in CHD morbidity and mortality [34–38]. The present study's findings showed that serum apo A-1 and HDL-C levels increased slightly from baseline to 6- and 12-months in the Intervention group and were positively correlated with one another, while serum apo A-1 and HDL-C levels in the Control group remained flat for the duration of the study. Apo B is a key protein in the transport of all cholesterol-carrying lipoproteins originating from the liver and intestinal tract. Serum apo B levels are widely considered the most biologically and analytically

Table 3
Serum lipid and lipoprotein cholesterol levels between baseline and 6 months and 12 months.

TC (mmol/L)	Baseline	6 Months	12 Months
Intervention (n = 27)	4.55 ± 0.17 (4.01–4.82)	4.82 ± 0.19 (4.28–5.06)	4.74 ± 0.20 (4.11–4.93)
Control (n = 18)	4.68 ± 0.30 (3.84–5.19)	4.68 ± 0.30 (3.90–5.33)	4.59 ± 0.37 (3.79–5.47)
HDL (mmol/L)			
Intervention (n = 27)	1.41 ± 0.08 (1.17–1.55)	1.45 ± 0.07 (1.22–1.55)	1.50 ± 0.08 (1.27–1.65)
Control (n = 18)	1.43 ± 0.10 (1.18–1.67)	1.46 ± 0.10 (1.21–1.69)	1.44 ± 0.11 (1.21–1.69)
TG (mmol/L)			
Intervention (n = 27)	1.51 ± 0.12 (1.22–1.84)	1.69 ± 0.15 (1.38–2.15)	1.55 ± 0.12 (1.24–1.85)
Control (n = 18)	1.51 ± 0.11 (1.21–1.71)	1.44 ± 0.12 (1.23–1.75)	1.45 ± 0.09 (1.27–1.67)
LDL (mmol/L)			
Intervention (n = 27)	2.45 ± 0.14 (1.99–2.72)	2.59 ± 0.16 (2.14–2.80)	2.54 ± 0.17 (1.99–2.72)
Control (n = 18)	2.56 ± 0.24 (1.91–2.94)	2.64 ± 0.23 (2.04–3.11)	2.48 ± 0.31 (1.82–3.19)
TC/HDL-C			
Intervention (n = 27)	3.43 ± 0.23 (2.97–4.00)	3.46 ± 0.24 (2.90–3.61)	3.38 ± 0.22 (2.77–3.96)
Control (n = 18)	3.34 ± 0.26 (2.92–3.58)	3.27 ± 0.19 (3.03–3.3.99)	3.21 ± 0.16 (2.92–3.56)
LDL-C/HDL-C			
Intervention (n = 27)	1.88 ± 0.21 (1.47–2.32)	1.88 ± 0.15 (1.50–2.27)	1.84 ± 0.27 (1.35–2.23)
Control (n = 18)	1.80 ± 0.17 (1.47–19.7)	1.80 ± 0.12 (1.46–2.07)	1.71 ± 0.18 (1.43–2.03)
Glucose (mmol/L)			
Intervention (n = 27)	5.45 ± 0.32 (4.79–6.12)	*6.72 ± 0.68 (5.30–8.14)	6.39 ± 0.36 (5.63–7.14)
Control (n = 18)	6.16 ± 0.37 (5.36–6.95)	6.21 ± 0.38 (5.39–7.03)	5.73 ± 0.43 (4.81–6.65)

Values are Mean ± SEM. (95% CL).
* Significantly different from baseline at $p < 0.05$.

superior marker for all atherogenic particles and their relationship with CVD [30,39–42]. In the current study, serum apo B concentrations did not change in either group and was positively correlated with serum LDL-C levels. While both of these apolipoproteins physiological functions have yet to be fully understood, epidemiological and clinical studies suggest that they are valuable biomarkers for the early assessment and monitoring of coronary events and stroke in patients with CVD [43–46]. The findings of the current study help to provide additional evidence that the consumption of cholesterol, in this case in the form of eggs, maybe an important part of the mechanism by which humans can increase their serum apo A-I and HDL-C levels without altering their serum apo B and LDL-C levels.

Some studies have shown that an increase consumption of eggs and/or dietary cholesterol have led to problems with glucose homeostasis

Table 4
Serum biomarker concentrations between baseline and 12 months.

Apo A-1 (mg/L)	Baseline values	12 months	Mean Percentage Change
Intervention (n = 27)	167.24 ± 9.7 (147.26–187.21)	173.29 ± 8.76 (155.36–191.22)	7.63 (−2.29 to 17.55)
Control (n = 18)	168.36 ± 0.20 (146.82–189.9)	167.94 ± 11.8 (142.97–192.92)	−0.03 (−11.89 to 11.95)
Apo B (mg/L)			
Intervention (n = 27)	81.15 ± 4.56 (72.39–91.18)	81.86 ± 5.61 (70.29–93.41)	3.17 (−8.24 to 14.58)
Control (n = 18)	83.55 ± 6.93 (69.23–98.47)	83.53 ± 7.85 (66.98–100.08)	0.32 (−13.40 to 14.03)
hsCRP (mg/L)			
Intervention (n = 27)	3.42 ± 0.82 (1.74–5.10)	3.96 ± 0.92 (2.09–5.86)	9.78 (−4.50 to 24.13)
Control (n = 18)	2.70 ± 0.94 (0.73–4.67)	2.25 ± 0.51 (1.17–3.32)	−9.05 (−8.17 to 26.22)
Lp_a (mg/dL)			
Intervention (n = 27)	20.30 ± 3.9 (12.26–28.32)	17.19 ± 3.38 (10.24–24.13)	−9.31 (−25.17 to 6.54)
Control (n = 18)	20.30 ± 8.63 (7.89–32.17)	20.84 ± 8.31 (7.61–34.9)	9.08 (−9.98 to 28.13)
ApoB/ApoA-1			
Intervention (n = 27)	0.53 ± 0.048 (0.44–0.64)	0.51 ± 0.048 (0.41–0.61)	−0.025 (−0.115 to 0.164)
Control (n = 18)	0.50 ± 0.035 (0.44–0.58)	0.51 ± 0.037 (0.43–0.59)	0.026 (−0.138 to 0.190)

Values are Mean ± SEM. (95% CL).

and diabetes [19,47–49], which are associated with inflammation [50]. CRP is an inflammatory reference biomarker that has been shown to increase post-ingestion of dietary cholesterol [26]. In a cohort of 22,000 apparently healthy middle-aged men with no clinical evidence of diseases, patients with baseline serum hsCRP levels in the highest quartiles had a two-fold greater risk of stroke or peripheral vascular disease and a three-fold increase in risk of myocardial infarction [27]. The addition of measuring serum hsCRP in the current study along with the standard cardiovascular lipoprotein profiling was included because prior work found the serum lipoprotein levels had exhibited a poor association with serum hsCRP levels [51]. Tannock et al. [52] observed a poor correlation between changes in plasma level of CRP and corresponding levels of lipoproteins of varying densities in the setting of controlled increase in egg consumption. In the present study, serum hsCRP concentrations remained unchanged in the both the Control and Intervention groups, which is similar to previous findings [53,54]. Although serum glucose levels in the current study increased significantly initially (at the 6 month measurement) in the Intervention group, it decreased backed to towards the baseline value at the 12 month measurement. We don't know or understand why serum glucose levels increased and then dropped in the Intervention group. It is possible that the initial change in the subjects' diets may be partly responsible for this since the subjects in the intervention group consumed a lower carbohydrate diet and high protein and fat diet when on the eggs. More often than not, the subjects consumed their eggs in the morning as part of their breakfast which may have replaced higher carbohydrate choices like cereals, pancakes, and breads. However, it is possible that since the subjects were recording what they eat in food diaries, it is possible that they might have under- or over-estimated how much they ate or reported what they were supposed to eat which may have led to erroneous calculations on diet reporting. Also, serum glucose levels were never statistically different between treatment groups at any time point during the study. This finding is similar to recent studies that have also shown no increases in blood glucose levels or increase risk in developing type II diabetes [55–57].

The importance of this multidimensional approach is evident in the growing understanding that lipoprotein serum levels in isolation are poor indicators of cardiovascular health status. Nonetheless, there is a pressing need for continued research on this basis. While egg consumption has recently come under fire as contributing to worsening cardiovascular condition, these claims are based on the belief, not evidence how dietary cholesterol is related to changes in serum lipid profiles. The evolving view on cardiovascular health tracking, necessitates reconsideration of claims that are solely based on the dietary to serum lipid level relationship that the situation is far more complex than can be ascertained from the simple measurement of LDL-C, HDL-C, and similar traditional markers.

In conclusion, we have shown that the consumption of 12 eggs per week for 1 year does not alter serum lipids, lipoprotein cholesterol, glucose, apo A-1, apo B, Lp_a or CRP levels in free living individuals that have early AMD but are otherwise healthy.

CRediT authorship contribution statement

Hassan Aljohi: Data curation, Investigation, Formal analysis, Writing - original draft. **Mindy Dopler-Nelson:** Investigation, Methodology, Resources, Writing – review & editing. **Manuel Cifuentes:** Formal analysis, Data curation, Resources, Software, Validation, Writing – review & editing. **Thomas A. Wilson:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Acknowledgement

The authors have no conflict of interest to declare. The authors would like to thank Dr. Robert J. Nicolosi, Dr. Rohini Vishwanathan, Dr. John Dagianis and the Nashua Eye Associates, and Elizabeth Goodrow-Kotyla for their technical assistance and Maureen Faul for her administrative assistance. The authors would also like to thank the American Egg Board, Egg Nutrition Center, Washington, DC (T.A.W.) and the Massachusetts Lions Eye Research Fund Inc., New Bedford, MA (T.A.W.) for their funding support. The funding played no role in data collection, analyses, or interpretation.

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