Compared to an Oatmeal Breakfast, Two Eggs/Day Increased Plasma Carotenoids and Choline without Increasing Trimethyl Amine N-Oxide Concentrations

Amanda Missimer, Maria Luz Fernandez, Diana M. DiMarco, Gregory H. Norris, Christopher N. Blesso, Ana Gabriela Murillo, Marcela Vergara-Jimenez, Bruno S. Lemos, Isabel Medina-Vera, Olga V. Malysheva & Marie A. Caudill

To cite this article: Amanda Missimer, Maria Luz Fernandez, Diana M. DiMarco, Gregory H. Norris, Christopher N. Blesso, Ana Gabriela Murillo, Marcela Vergara-Jimenez, Bruno S. Lemos, Isabel Medina-Vera, Olga V. Malysheva & Marie A. Caudill (2018): Compared to an Oatmeal Breakfast, Two Eggs/Day Increased Plasma Carotenoids and Choline without Increasing Trimethyl Amine N-Oxide Concentrations, Journal of the American College of Nutrition, DOI: 10.1080/07315724.2017.1365026

To link to this article: https://doi.org/10.1080/07315724.2017.1365026

Published online: 09 Jan 2018.
Compared to an Oatmeal Breakfast, Two Eggs/Day Increased Plasma Carotenoids and Choline without Increasing Trimethyl Amine N-Oxide Concentrations

Amanda Missimer, Maria Luz Fernandez, Diana M. DiMarco, Gregory H. Norris, Christopher N. Blesso, Ana Gabriela Murillo, Marcela Vergara-Jimenez, Bruno S. Lemos, Isabel Medina-Vera, Olga V. Malysheva, and Marie A. Caudill

ABSTRACT
Background: Habitual consumption of eggs has been hypothesized to positively modify biomarkers of cardiovascular disease risk through proposed antioxidant properties.
Objectives: To examine this relationship, 50 young, healthy men and women were enrolled into a randomized crossover clinical intervention.
Methods: Participants consumed either 2 eggs per day or one packet of oatmeal a day for 4 weeks, followed by a 3-week wash-out and crossed over to the alternate breakfast. Fasting blood samples and peripheral blood mononuclear cells (PBMCs) were collected at the end of each intervention period.
Results: Increases in plasma large high-density lipoprotein (HDL) and large low-density lipoprotein (LDL) particle concentrations as measured by nuclear magnetic resonance were found following egg consumption (p < 0.001, p < 0.05), respectively, with increases in apolipoprotein concentration as well (p < 0.05). Though there was no difference in the intake of antioxidants lutein and zeaxanthin, a significant increase in plasma concentrations of these carotenoids was observed (p < 0.001) after egg consumption. There was no change in lecithin–cholesterol acyl transferase, cholesteryl ester transfer protein, or paraoxonase-1 arylesterase activities between breakfast interventions. Dietary and plasma choline were both higher following egg consumption compared to oatmeal consumption (p < 0.001); however, there was no change in plasma trimethylamine N-oxide (TMAO) concentrations. Two eggs per day had no impact on PBMC gene expression related to cholesterol metabolism, oxidation, or TMAO production.
Conclusions: These results suggest that compared to oatmeal, consumption of 2 eggs for breakfast provided increased plasma carotenoids and improved biomarkers of cardiovascular disease (CVD) risk while not affecting TMAO levels in this population.

Abbreviations: ABCAI, ATP-binding cassette transporter AI; ABCG1, ATP-binding cassette transporter G1; Apo, apolipoprotein; CD36, cluster of differentiation 36; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; EGGS, 2 large eggs; OATS, one packet of oatmeal; FM03, flaxin containing monoxygenase 3; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LDLR, LDL receptor; PBMC, peripheral blood mononuclear cells; PC, phosphatidylcholine; PL, phospholipids; PON-1, paraoxonase-1; RCT, reverse cholesterol transport; SR-B1, scavenger receptor class B type 1; TG, triglycerides; TMAO, trimethylamine N-oxide

Introduction

There is a well-known inverse relationship between plasma high-density lipoprotein cholesterol (HDL-C) concentrations and cardiovascular disease (CVD) (1). The primary mechanism for this relationship is the role of HDL in reverse cholesterol transport (RCT) and CVD protection (2). RCT is a process where excess cholesterol is effluxed out of cells to HDL and then transported back to the liver for excretion (3). The HDL-mediated efflux from cholesterol-laden macrophages is particularly important in the prevention of atherosclerosis or the deposition of excess lipids into artery walls (4). Beyond RCT, HDL has been proposed to have various other functions, including anti-inflammatory, antioxidant, antithrombotic, and antimicrobial properties (5). Though interaction with various proteins, of which over 100 have been identified, HDL functionality can span beyond RCT to protect against CVD and other health-related complications (6).

Specific proteins involved in HDL metabolism and RCT such as lecithin–cholesterol acyl transferase (LCAT), cholesteryl ester transfer protein (CETP), apolipoprotein (apo) AI, and paraoxonase-1 (PON1) have been linked to HDL functionality (7). These proteins have been suggested to have a role in HDL remodeling, cholesterol efflux, and antioxidant activity (8). The ability for HDL to be remodeled in circulation has been an area of interest, because current literature is still uncertain regarding the most effective HDL species. At present, it is...
hypothesized that larger HDL particles will be able to transport more cholesterol for excretion than smaller HDL, as well as have a larger surface area to carry beneficial antioxidants and proteins (9). The role of HDL as a carrier of antioxidants is relevant to its ability to prevent and reverse CVD (10).

Increased circulating HDL-C has been suggested to be a marker of increased RCT and to have potential in reducing risk of atherosclerosis development (11). Studies have shown the ability of eggs to increase HDL-C concentration in circulation, which may improve RCT, by the proposed ability to carry more cholesterol to the liver for excretion (12–14). Egg-derived phospholipids (PLs) may play a role in the ability of HDL to accept excess cholesterol from cells during the early stages of RCT (15). Specifically, dietary PLs have been observed to be preferentially incorporated into HDL particles, which have a postulated role in beneficial remodeling of HDL (15,16). Additionally, whole egg consumption has been associated with the upregulation of genes associated with RCT, such as cholesterol efflux transporters, ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1), HDL receptor, and scavenger receptor class B type 1 (SR-BI), which promotes selective uptake of HDL cholesteryl ester by the liver and targets removal of cholesterol via excretion (17).

Nutritionally, eggs are a source of high-quality protein and also contain essential vitamins and minerals, antioxidant carotenoids lutein and zeaanthin, choline, cholesterol, and numerous PLs (18,19). Previous studies have associated habitual egg consumption with decreased energy intake, increased satiety, and weight loss in certain populations (20,21). High-quality proteins or complete proteins contain sufficient amounts of the 9 essential amino acids and are easily and highly digestible (19). Proteins can increase satiety or the feeling of fullness, as well as increase energy expenditure (22).

Though egg consumption has been met with controversy, numerous studies in our laboratory have demonstrated the lack of negative effects on CVD biomarkers with dietary cholesterol consumption and even some additional beneficial effects of daily whole egg intake (13,23,24). Studies in which participants have consumed whole eggs have shown improvements in the biomarkers associated with CVD risk and improvement in the markers of chronic inflammation, glucose intolerance, and insulin resistance in several distinct populations: children, individuals with metabolic syndrome, and individuals with type 2 diabetes (12,14,25). Further, due to recent evidence linking fasting trimethylamine N-oxide (TMAO) concentrations to CVD, eggs have become an area of interest again because they are a rich source of choline in the diet (26). Choline, along with other components such as carnitine and betaine, can be metabolized by gut microbiota to form trimethylamine (TMA), which is then oxidized by hepatic flavin-containing monoxygenases (FMOs) into TMAO (27). However, choline in eggs is present as phosphatidyl choline (PC), which is better absorbed in the small intestine and will be less affected by the microbiota in the large intestine; therefore, we hypothesize no change in TMAO levels with the consumption of 2 eggs for breakfast. Furthermore, to understand the effect of egg consumption on HDL functionality, we hypothesized that the consumption of 2 eggs per day, compared to an oatmeal breakfast, would promote favorable changes to the HDL profile and increase carotenoid content without negatively affecting TMAO concentrations or CVD risk biomarkers.

Materials and methods

Experimental design

The participant population for this study was young, healthy men and women between the ages of 18 and 30 years with a body mass index (BMI) ≥ 18.5 and ≤ 30 kg/m², blood pressure < 140/90 mmHg, fasting triglycerides < 500 mg/dL, fasting glucose < 126 mg/dL, or fasting total cholesterol < 240 mg/dL. Based on the standard deviation from our previous studies and using a Z value of 1.96 (95% confidence interval), we needed to recruit 40 patients to detect a 20% difference in HDL-C between groups (12,14). Fifty participants (25 men and 25 women) were recruited, to allow for attrition. The study was a crossover design in which participants were randomly allocated to consume either 2 large grade A eggs (EGGS) per day or 1 packet of oatmeal (OATS) per day (384 g/d) for breakfast for 4 weeks each. The macronutrient composition of eggs and oatmeal were as follows: 2 large eggs: 12 g protein, 0 g carbohydrates, 10 g fat; 1 packet of oatmeal: 4 g protein, 33 g carbohydrates, 2 g fat. There was a 3-week washout in between the intervention periods during which participants did not consume eggs or oatmeal. Subjects were allowed to consume eggs and oatmeal as desired, and daily compliance was monitored by self-report. Following each intervention period, participants completed 3-day dietary and exercise records and fasting blood samples were collected as previously described (28). Dietary intake was analyzed using Nutrition Data System for Research 2014 (Nutrition Coordinating Centre, University of Minnesota, St. Paul, MN) to quantify carotenoid and choline intake. This study was approved by the Institution Review Board at the University of Connecticut (protocol #H14-032). This trial is registered at ClinicalTrials.gov as NCT02181244. Baseline characteristics of subjects as well as dietary intake have been previously reported (28).

Lipoprotein particle size

Fasting plasma was analyzed following egg or oatmeal consumption to determine total lipoprotein particle number, size, and concentration using nuclear magnetic resonance. This technique provides information on individual lipoprotein particle subfractions by counting the number of lipoprotein particles available in a sample. Nuclear magnetic resonance simultaneously quantifies >30 lipoprotein fractions that are grouped into 10 subclasses based on diameter: large very-low-density lipoprotein (VLDL) (>60 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), intermediate density lipoprotein (IDL) (23–27 nm), large LDL (21.2–23 nm), medium LDL (19.8–21.2 nm), small LDL (18.8–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm). Analysis was performed by LipoScience, Inc. (Raleigh, NC).

Plasma apolipoproteins

Plasma apolipoproteins (A-I, A-II, B-100, C-II, C-III, E) were quantified using a commercially available human apolipoprotein multiplex assay kit (EMD Millipore, Bedford, MA) and analyzed by a Luminex MAGPIX analyzer (Luminex Corporation, Austin, TX). This procedure quantifies apolipoproteins in plasma using antibody-immobilized fluorescent dye-labeled
microspheres. The intra-assay variability is less than 5% for plasma apolipoproteins (12).

**LCAT, CETP, PON1, and inflammatory markers**

LCAT and CETP activities were measured using commercially available specific activity kits. LCAT activity was measured following incubation with fluorescently labeled substrate, with the strength of the fluorescence signal over time indicating relative activity (Cell Biolabs, Inc. San Diego, CA). CETP activity was determined from fasting plasma by measuring the transfer of a fluorescent neutral lipid from a donor to acceptor molecule (BioVision, Inc, Milpitas, CA). The mediated fluorescence was measured by microplate reader (BioTek Instruments, Winooski, VT). The intra-assay variability for both assays was <5%.

PON1 aroylseresterase activity was measured using spectrophotometric methods as previously described (29). This is a measure of PON1 activity toward phenyl acetate in fasting serum, with an intra-assay variability of 4%. Plasma tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were measured using commercially available kits (Abcam Inc., Cambridge, MA), and plasma C reactive protein (CRP) was measured using an automated clinical chemistry analyzer (Cobas c111, Roche Diagnostics, Holiston, MA).

**Plasma carotenoids and TMAO**

The carotenoids lutein and zeaxanthin were extracted from plasma using a mixture of 2:1 chloroform : methanol, 0.85% NaCl, and unfiltered hexane. Analysis of samples was done using high-performance liquid chromatography on a Shimadzu Prominence UFLC (Shimadzu Corporation, Kyoto, Japan) with a C30 3 mm × 4.6 mm carotenoid column (YMC America, Allentown, PA) and guard column. An internal standard (trans-β-apo-8’-carotenal, Sigma-Aldrich, St. Louis, MO) was added to each sample prior to extraction to determine carotenoid recovery efficiency. External standards of purified lutein and zeaxanthin (Sigma-Aldrich) were used to calculate a standard curve for measurement of plasma carotenoid concentrations. Plasma TMAO was measured by liquid chromatography coupled with tandem mass spectrometry as previously described (30).

**Gene expression**

Peripheral blood mononuclear cell (PBMC) isolation was carried out following each breakfast intervention period. Fasting blood (40 mL) was collected from subjects into EDTA vacutainer tubes and kept on ice. Blood was diluted with 10 mL sterile 10× phosphate-buffered saline and mixed gently. Blood was layered over Histopaque solution and centrifuged at 400 × g for 35 minutes using a Beckman Coulter (Simsbury, CT) centrifuge with a swing-bucket rotor to separate the PBMC buffy coat. Buffy coats were collected and diluted with phosphate-buffered saline, washed twice, and resuspended in RPMI medium. For storage, samples were diluted 1:1 with cryopreservation media (RPMI containing 20% fetal bovine serum and 10% dimethyl sulfoxide) and frozen at a controlled rate in Cool Cell containers (BioCision, LLC, Hill Valley, CA) at −80°C for at least 24 hours. PBMC samples were then transferred to liquid nitrogen for long-term storage.

PBMC expression of genes related to cholesterol metabolism, transport, oxidation, and TMAO production were determined using quantitative real-time RT-PCR (qRT-PCR). RNA was extracted from freshly isolated PBMCs using TRIzol reagent (Invitrogen, Inc., Waltham, MA) according to the manufacturer’s instructions and using methods previously described (16). Synthesis of complementary DNA (cDNA) was performed as previously described using a Bio-Rad C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA) (29). Gene expression was measured using an iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA) procedure with qRT-PCR analysis. Primer sequences were designed according to the GenBank database (Table 1). Expression levels of each target gene were calculated from threshold cycle (Ct) values, converted to relative expression using the comparative 2-ΔΔCt method, and normalized to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression.

**Statistical analysis**

All statistical analyses were performed using SPSS Version 22. All data are reported ± standard deviation (SD) unless noted otherwise, and significance was set at p < 0.05. Paired t tests were used to assess differences between dietary treatments.

**Results**

**Diet and exercise**

There were no changes in level of activity between the 2 breakfast periods as documented by the exercise records. There were some changes between diets as previously reported (28). Participants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMGCR</td>
<td>5’-CCAGTTGGTGCTCTTCCA-3’</td>
<td>5’-TCGAGCGAAGCTTCACTCT-3’</td>
</tr>
<tr>
<td>LDLR</td>
<td>5’-ACTGCGTGGACTCCAAATTCTAC-3’</td>
<td>5’-GTTGCCCCCGTTCA-3’</td>
</tr>
<tr>
<td>CD36</td>
<td>5’-GGCTTGGACGGAAGCTTG-3’</td>
<td>5’-AGGTCTCAACTGGCTATGAA-3’</td>
</tr>
<tr>
<td>SRA</td>
<td>5’-AAAGCCTGATCCACTACGCGCCT-3’</td>
<td>5’-CCGGCGTTGTGACAGTGA-3’</td>
</tr>
<tr>
<td>FMO3</td>
<td>5’-TGTTGGCATCAATGGATTTGG-3’</td>
<td>5’-CTGGTTTATTATAGCCTGCCG-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-TGTGGGCACTAATGGATTTGG-3’</td>
<td>5’-ACACATATTTGCCGTCG-3’</td>
</tr>
</tbody>
</table>

HMG-CoA = 3-hydroxy-3-methyl-glutaryl-coenzyme A, LDL = low-density lipoprotein, HMGCR = HMG-CoA reductase, LDLR = low-density lipoprotein receptor, CD36 = cluster of differentiation 36, SRA = scavenger receptor A, FMO3 = flavin monoxygenase 3, GAPDH = Glyceraldehyde 3-phosphate dehydrogenase.
consumed more fat ($p < 0.01$), more protein ($p < 0.01$), and less carbohydrate ($p < 0.001$) during the egg period (28).

**Lipoprotein particle size**

Following the consumption of 2 eggs per day, TC, HDL-C, and LDL-C were higher in plasma ($p < 0.05$) than after oatmeal consumption, as previously reported (28). In agreement with changes in plasma cholesterol concentrations, LDL and HDL particle concentrations were also modified (Figure 1). Total HDL particles were not different between treatment groups ($p = 0.12$); however, large HDL particle concentrations were higher during the egg breakfast compared to the oatmeal breakfast ($p < 0.0001$). No change was seen in medium HDL or small HDL concentrations between treatments (Figure 1A). Total LDL as well as large LDL ($p < 0.01$) particle concentrations were higher during the egg period, whereas the concentrations of small LDL were not different between dietary periods (Figure 1B). No differences in large, medium, or small VLDL were observed between treatments (data not shown). Surprisingly, no difference in PON1 activity was found, because this has been shown to increase with HDL-C and particle concentration (Table 2) (31).

Following egg consumption, plasma TNF-α was lower than following oatmeal, whereas there was no difference between intervention breakfasts in plasma inflammatory markers CRP and IL-6. The dietary intake records analysis revealed no significant difference in consumption between intervention breakfasts, even when 2 eggs were included in the habitual diet (Figure 2A). In contrast to dietary intake of carotenoids, plasma concentrations of lutein and zeaxanthin were significantly higher ($p < 0.001$) following egg versus oatmeal consumption (Figure 2B).

**Choline and TMAO concentrations**

According to dietary intake analysis, a significant increase in plasma choline was found following the consumption of 2 eggs per day; this finding was reflected in plasma concentrations

![Figure 1](image1.png)  
**Figure 1.** (A) HDL and (B) LDL lipoprotein particle concentration following consumption of 2 eggs or one packet of oatmeal for breakfast. Data are presented as mean ± SD; $n = 48$. *$p < 0.05$, ***$p < 0.001$.

**Table 2.** Plasma Apolipoproteins, Lecithin Cholesterol Acyltransferase, Cholesteryl Ester Transfer Protein, Paraoxonase-1, and Inflammatory Markers C-Reactive Protein, Tumor Necrosis Factor-α, and Interleukin-6.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EGGS</th>
<th>OATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I (mg/L)</td>
<td>144 ± 46</td>
<td>136 ± 44</td>
</tr>
<tr>
<td>Apo A-II (mg/L)</td>
<td>59 ± 12</td>
<td>58 ± 12</td>
</tr>
<tr>
<td>Apo B (mg/L)</td>
<td>18 ± 7</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>Apo C-II (mg/L)</td>
<td>24 ± 9</td>
<td>23 ± 9</td>
</tr>
<tr>
<td>Apo C-III (mg/L)</td>
<td>47 ± 15</td>
<td>44 ± 14</td>
</tr>
<tr>
<td>LCAT (RFU)</td>
<td>200.6 ± 26.6</td>
<td>205.6 ± 28.8</td>
</tr>
<tr>
<td>CETP (pmol/L·h⁻¹)</td>
<td>6.4 ± 12.2</td>
<td>6.4 ± 11.0</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.14 ± 0.22</td>
<td>0.16 ± 0.42</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>16.9 ± 4.4</td>
<td>17.9 ± 5.5</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>8.0 ± 3.3</td>
<td>7.6 ± 2.7</td>
</tr>
</tbody>
</table>

APO = apolipoprotein, PON1 = paraoxonase-1, LCAT = lecithin cholesterol acyl-transferase, CETP = cholesteryl ester transfer protein, CRP = C-reactive protein, TNF-α = tumor necrosis factor-α, IL = interleukin.

Values are presented as mean ± SD for $n = 48$.

* $p < 0.05$ compared to oatmeal.

(32) (Figures 3A and 3B). However, no difference was found in TMAO levels between the two breakfast interventions, despite the increase in dietary choline provided by eggs (Figure 3C).

**PBMC gene expression**

We further aimed to identify whether egg consumption would alter the expression of genes related to cholesterol metabolism—the LDL receptor and HMG-CoA reductase (HMGCR) and uptake of lipoprotein-derived cholesterol—and oxidation: cluster of differentiation 36 (CD36) and scavenger receptor A (SRA) and formation of TMAO (FMO3). No differences were seen in relative mRNA expression in either phase of breakfast consumption in this population (Figure 4).

**Discussion**

The health effects of habitual egg intake have been misunderstood by consumers, due to research that reports concern
around safety of consumption as well as those that, conversely, suggest potential for health benefits (33). Though dietary cholesterol has been deemed a nutrient of non-concern by the U.S. Department of Agriculture’s Dietary Guidelines Committee, the recently suggested link between plasma TMAO levels and CVD has put eggs back into the spotlight of controversy (26,34). To examine the safety and benefit of consuming 2 eggs per day, they were compared to a certified heart-healthy breakfast of oatmeal.

Lipoprotein particles have the ability to be diverse in composition and size, and dietary intake has been shown to have an impact on this phenomenon (9). Previous studies have shown the ability of egg consumption to have an impact on HDL and LDL particle size and number (12,23,31). In this study, we found a higher concentration of large HDL particles following the consumption of 2 eggs. The larger species of HDL are suggested to be the more anti-atherogenic variety, due to the proposed ability to carry more excess cholesterol from circulation back to the liver for excretion (7). It is hypothesized that the added dietary phospholipids with egg consumption may be driving the conversion to the large size HDL in circulation (23,35). Additionally, apoA-I, the apolipoprotein responsible for initial lipidation of HDL particles, was found to be higher following egg consumption. The greater abundance of apoA-I in combination with the larger HDL species suggests a shift to a more anti-atherogenic lipoprotein

Figure 2. (A) Dietary intake and (B) plasma concentrations of lutein and zeaxanthin following the consumption of 2 eggs or one packet of oatmeal for breakfast. Data are presented as mean ± SD, n = 48. *** p < 0.001. The straight arrow indicates no significant differences.

Figure 3. (A) Dietary choline, (B) plasma choline, and (C) plasma concentrations of TMAO following the consumption of 2 eggs or one packet of oatmeal for breakfast. Data are presented as mean ± SD, n = 48. *** p < 0.001. The straight arrow indicates no significant differences.
profile. ApoA-I has also been reported to have antioxidative properties and has been shown to reduce macrophage response to endotoxin stimulation (36). Previous studies utilizing the feeding of 1–3 eggs have also found an increase in large HDL particle size (31).

CETP is responsible for exchanging triglycerides (TG) for cholesterol esters between apoB-containing lipoproteins and HDL in an alternate pathway of RCT (37). According to our data, CETP was unchanged regardless of breakfast. Currently in the literature, there are mixed data on the connection of CETP to CVD risk. Though impairment of CETP activity has been suggested as a therapeutic target to reduce formation of cholesterol-rich LDL and TG-rich HDL, results have not been clear. Therefore, the finding of no difference in CETP activity between eggs and oatmeal could indicate that eggs are not affecting this parameter involved in RCT. The increase in large HDL particle concentration may also be a result of the higher consumption of PLs present in eggs. Previous studies have found egg PL to be preferentially incorporated into large HDL molecules, which may then enhance mobilization of cholesterol from cells (15,18).

Higher concentrations of large LDL particles were detected following the consumption of eggs. This modification is consistent with previous egg feeding studies (23,38,39). The larger species of LDL have been suggested to be less atherogenic, because studies have shown that small LDL is most likely to be oxidized in the endothelium and then taken up by macrophages, ultimately leading to the formation of foam cells and atherosclerotic plaque (4).

The activity of the enzyme LCAT, which is responsible for the esterification of cholesterol, was unchanged between breakfast treatments. In another study in which participants were fed a dose-dependent increase of eggs (1–3) for 4 weeks each, an increase in LCAT activity was found following the consumption of 3 eggs (31). This finding suggests that the consumption of dietary cholesterol from 2 eggs may not have an impact on increasing the function of the enzyme in a healthy population. In an egg feeding study in which 3 eggs were consumed in a population with metabolic syndrome, LCAT activity was increased. Authors connect LCAT activity to an increase in HDL particle size and number, because more dietary cholesterol is being esterified and RCT is enhanced (23). LCAT may also be more active in individuals classified as hyperresponders to dietary cholesterol. In a study in which individuals consumed 3 eggs per day, hyperresponders, regardless of gender, had higher activity of LCAT, in accordance with increased RCT (40).

PON1 is an HDL-associated enzyme with the proposed ability to protect against oxidative damage, inflammation, lipid peroxidation, and liver disease (41). In the present study, no differences in PON1 were found between the egg and oatmeal breakfasts. Low serum PON1 levels have been found in mice after consuming an atherogenic diet and correlated with a reduced capacity of HDL to prevent aortic plaque development (42). Thus, an unchanged PON1 is potentially due to only modest effects of eggs on inflammatory markers in this population. There were no differences between breakfasts in the inflammatory cytokine IL-6 and inflammatory marker CRP, whereas inflammatory cytokine TNF-α was lower following the consumption of 2 eggs per day. Because CRP is an acute phase response to a rising IL-6 concentration, results are consistent with reduced inflammation in this population (43). TNF-α, which is also part of the acute phase response, is an activator of both nuclear factor kappa light chain enhancer of activated β cells and mitogen-activated protein kinase pathways, which are major regulators of systemic inflammation (44). Because this population was young and healthy, an unchanged PON1 and reduced TNF-α may suggest that egg consumption helps maintain or reduce inflammatory levels.

Another factor possibly related to the observed decreases in inflammation relates to the antioxidant carotenoids lutein and zeaxanthin. According to dietary analysis, the intake of carotenoids, which are found in green leafy vegetables and in yellow foods such as egg yolk and corn, did not differ between breakfast treatments. However, plasma analysis of these carotenoids suggests higher concentrations of lutein and zeaxanthin in plasma when delivered by eggs compared to oatmeal, which confirms that lutein and zeaxanthin from eggs are highly bioavailable (23,39). HDL has been shown to carry carotenoids, which possess antioxidative properties (45). Both of these carotenoids are hypothesized to be part of the mechanism of how HDL has antioxidative effects on LDL, PL, and other tissues by preventing oxidation and protecting tissues from oxidative damage.

Figure 4. Expression of genes related to cholesterol metabolism, oxidation, and TMAO production. Data are presented as mean ± SD, n = 36.
stress. Studies have shown relationships between the carotenoids and upregulation of ABCA1 and promotion of cholesterol efflux (46). Lutein and zeaxanthin are distributed in most tissues but are found concentrated in the eye and have been shown to be important to the prevention of age-related macular degeneration (47). In addition, large HDL has been proposed to have the ability to carry a large percentage of lutein and zeaxanthin compared to other carotenoids, which may be the most relevant in preventing LDL oxidation and contributing to enhanced RCT (48). Previous studies have found increased lutein and zeaxanthin in plasma following the consumption of eggs (23,39,49).

A study by Tang et al. postulated a link between increased TMAO levels and risk of incidence for major cardiovascular events (26). TMAO is a metabolite that is generated in the liver by the enzyme FMO3 from TMA, a metabolite formed by gut microbiota from choline and various phospholipid species (50). Eggs are a naturally high source of choline, a bioactive lipid and essential nutrient (32); therefore, investigation into whether choline from eggs is related to plasma TMAO concentration is of interest. According to dietary analysis, when consuming 2 eggs per day, participants were receiving significantly more choline in their diet compared to the consumption of oatmeal. The lack of difference in TMAO levels between breakfasts suggests that egg-specific choline may not have a direct impact on plasma TMAO levels and thus have no relation to the impact on cardiovascular events. Because recent evidence has suggested that most Americans are consuming suboptimal amounts of choline, which is necessary for brain and nervous system function, 2 eggs per day are safe to consume without impact on TMAO levels (32).

In order to examine the connection between HDL and cholesterol metabolism further, PBMC gene expression analysis was carried out in the areas of cholesterol metabolism, oxidation product uptake, and formation of TMAO. Because there was no difference in any of the gene expression data, it can be concluded that compared to an oatmeal breakfast, 2 eggs per day have no negative impact on the examined parameters. Despite the addition of ~360 mg/cholesterol per day from eggs, there was no impact on cholesterol metabolism, suggesting that the amount consumed is of no relevance to a properly functioning biosynthetic pathway. CD36 and SRA are principal receptors for the binding and uptake of oxidized LDL in macrophages (4). No difference in gene expression indicates a lack of increased uptake, which may be related to the species modification toward large HDL and LDL particles and away from the smaller species, which are more susceptible to oxidation. Lastly, expression of FMO3, the principle enzyme for conversion of TMA to TMAO, was unchanged, suggesting that the added choline consumed with the egg breakfast did not regulate the formation of TMAO from TMA via alterations in the transcription of FMO3.

There are several limitations to this study. First, the use of a young, healthy population that has little to no oxidative stress and inflammation at baseline means that these results do not translate to individuals who are in a pro-oxidant or pro-inflammatory state. In other studies in populations with metabolic syndrome and type 2 diabetes, a more profound impact of egg consumption on inflammation has been observed (14,51).

Additionally, we were limited by use of PBMC in a human population. This cell type, which is mainly composed of lymphocytes and monocytes, lacks necessary enzymes, such as those found only in the liver, for complete understanding of RCT. This young, healthy population had normal total cholesterol levels and additional intake did not impact relative gene expression for cholesterol efflux-related proteins ABAC1 and ABCG1. However, this study has many strengths, such as the collection of dietary and biological data to create a connection between nutrient intake and presence in plasma. This allowed us to determine the presence and impact of choline on both antioxidant carotenoid and TMAO concentrations, respectively.

Conclusions

The intake of 2 eggs per day for breakfast compared to one packet of oatmeal per day increased large HDL and LDL particle concentrations and apoA-I concentration in plasma while having no impact on other apolipoproteins or HDL-associated enzymes. Though dietary consumption of lutein and zeaxanthin did not differ between breakfasts, the presence of these carotenoids in plasma was higher with egg compared to oatmeal intake. Furthermore, higher concentrations in dietary choline and plasma choline following the egg breakfast did not impact TMAO concentrations. The consumption of 2 eggs per day may provide the necessary antioxidant capacity to lower inflammation and maintain TMAO levels, while potentially enhancing factors related to RCT.

Funding

This study was supported by funds provided by the Esperance Family Foundation and Egg Nutrition Center (ENC).

Author contributions

A.M. recruited and received consent of the subjects and was substantially involved in data collection and final draft of the article. M.L.F. designed the study, analyzed and interpreted all data, and provided final input for the article. D.D. participated in the determination of LCAT, CETP, PON-1, and carotenoids. She also provided input on the final draft. G.M. participated in gene expression studies and provided input in data analysis. C. N.B. provided input on all analyses and provided a substantial contribution to data interpretation and the final draft of the article. G.M. participated in data analysis and interpretation. M.V.-J. isolated the peripheral blood mononuclear cells for the study and provided input on data interpretation. B.L. and I.M. measured TMAO plasma concentrations and provided input on data interpretation. O.M. supervised the measurement of TMAO and helped in the generation of data. M.C. provided her lab for conducting TMAO measurements and contributed substantially to the final draft of the article.

ORCID

Maria Luz Fernandez http://orcid.org/0000-0002-1835-0792
Christopher N. Blesso http://orcid.org/0000-0002-4434-4839

References