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ORIGINAL ARTICLE

Intake of up to 3 Eggs/Day Increases HDL Cholesterol and Plasma Choline While Plasma Trimethylamine-*N*-oxide is Unchanged in a Healthy Population

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Abstract Eggs are a source of cholesterol and choline and may impact plasma lipids and trimethylamine-*N*-oxide (TMAO) concentrations, which are biomarkers for cardiovascular disease (CVD) risk. Therefore, the effects of increasing egg intake (0, 1, 2, and 3 eggs/day) on these and other CVD risk biomarkers were evaluated in a young, healthy population. Thirty-eight subjects [19 men/19 women, 24.1 ± 2.2 years, body mass index (BMI) 24.3 ± 2.5 kg/m^2] participated in this 14-week crossover intervention. Participants underwent a 2-week washout with no egg consumption, followed by intake of 1, 2, and 3 eggs/day for 4 weeks each. Anthropometric data, blood pressure (BP), dietary records, and plasma biomarkers (lipids, glucose, choline, and TMAO) were measured during each intervention phase. BMI, waist circumference, systolic BP, plasma glucose, and plasma triacylglycerol did not change throughout the intervention. Diastolic BP decreased with egg intake ($P < 0.05$). Compared to 0 eggs/day, intake of 1 egg/day increased HDL cholesterol (HDL-c) ($P < 0.05$), and decreased LDL cholesterol (LDL-c) ($P < 0.05$) and the LDL-c/HDL-c ratio ($P < 0.01$). With intake of 2–3 eggs/day, these changes were maintained. Plasma choline increased dose-dependently with egg intake ($P < 0.0001$) while fasting plasma TMAO was unchanged. These results indicate that in a healthy population, consuming up to 3 eggs/day results in an overall beneficial effect on biomarkers associated with CVD risk, as documented by increased HDL-c, a reduced LDL-c/HDL-c ratio, and increased plasma choline in combination with no change in plasma LDL-c or TMAO concentrations.

Keywords Eggs · Cholesterol · HDL · LDL · TMAO · Choline

Abbreviations

ACE	Angiotensin-converting enzyme
BMI	Body mass index
BP	Blood pressure
CVD	Cardiovascular disease
FMO	Flavin monooxygenase
HDL-c	HDL cholesterol
LDL-c	LDL cholesterol
LC–MS/MS	Liquid chromatography with tandem mass spectrometry
TC	Total cholesterol
TAG	Triacylglycerol
TMA	Trimethylamine
TMAO	Trimethylamine-*N*-oxide
WC	Waist circumference

Introduction

For years, recommendations in the United States included a 300 mg/day limit on cholesterol intake [1]. In the newly-released Dietary Guidelines for Americans 2015–2020, these recommendations were removed for healthy populations due to the presence of only minimal evidence supporting a connection between cholesterol intake and cardiovascular disease (CVD) risk [2, 3]. However, confusion remains as to whether eggs, a rich source of cholesterol, should be included in a healthy dietary pattern. In addition

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to cholesterol, eggs contain high-quality protein, vitamins, minerals, choline, and carotenoids, and are relatively low in saturated fat (1.5 g/egg) [4]. The guidelines now state that cholesterol is "not a nutrient of concern" but that saturated fat intake should be limited [2]; thus, eggs fit these new recommendations.

A meta-analysis of 27 clinical studies found a minimal association between dietary and plasma cholesterol [5]. Most epidemiological data likewise show a minimal association between intake of ≥1 eggs/day and CVD risk/mortality among non-diabetic individuals [6–12]. Three meta-analyses corroborate this observation but suggest that the impact of egg intake in diabetics warrants further study [9, 12, 13]. Indeed, epidemiological data support a correlation between egg intake and risk of type-2 diabetes [14, 15]. Interestingly, clinical trials of egg intake by diabetic patients consistently report no increase in biomarkers associated with CVD risk, while some benefits have been observed [16, 17]. Results of intervention studies in non-diabetic populations also show that egg intake does not negatively impact biomarkers associated with CVD risk [18–21]. Despite these benefits and the new Dietary Guidelines, confusion remains.

Adding to this confusion is the controversy regarding trimethylamine-*N*-oxide (TMAO), [22]. TMAO is a metabolite of choline; choline is converted by gut microbes into trimethylamine (TMA) which is absorbed and oxidized into TMAO [22]. Plasma TMAO is associated with an increase in the occurrence of cardiovascular events and elevated risk of CVD [23–26]. Because eggs are a rich source of choline, it is hypothesized that regular intake may promote elevation of plasma TMAO [22]. Three studies have examined the postprandial response to egg intake; each observed a spike in plasma TMAO, though this increase appears to be transient [25, 27, 28].

Therefore, we sought to examine whether egg intake has an impact on fasting plasma TMAO concentrations. It is also unclear whether there is a dose-dependent relationship between number of eggs consumed and change in plasma TMAO [29]. The present 14-week crossover dietary intervention was designed to examine the impact of consuming 0, 1, 2, and 3 eggs/day on plasma lipids, choline, TMAO, and other biomarkers of CVD risk to determine an optimal egg intake in a population in which CVD risk is not a concern. Thus, we chose young, healthy individuals. We hypothesized that intake of up to 3 eggs/day would not increase these biomarkers of CVD risk in this population. The results discussed below support this hypothesis and suggest that despite the increase in cholesterol and choline intake, plasma lipids and TMAO concentrations are not negatively altered.

Materials and Methods

Experimental Design

We recruited 40 healthy men and women age 18–30 years and with a body mass index (BMI) 18.5–29.9 kg/m^2 for participation in this crossover dietary intervention. Requirements for inclusion were a normal blood pressure (BP), healthy lipid profile, and willingness to consume eggs daily for 12 weeks. Current or past diagnoses of heart disease, stroke, diabetes, cancer, liver disease, renal disease, or any severe infectious disease were cause for exclusion. Additional exclusion criteria included smoking, use of glucose or lipid-lowering medications or supplements, individuals with BP ≥ 140/90 mm Hg (average of three measurements), total cholesterol (TC) ≥240 mg/dL, plasma triacylglycerol (TAG) ≥500 mg/dL, plasma glucose ≥126 mg/dL, or allergy to eggs.

This study was powered to detect a 10% difference in HDL cholesterol (HDL-c) (80% power with two-sided significance level of $\alpha = 0.05$) between intake of 0 and 3 eggs/day [30]. An $n = 35$ was sufficient to detect differences in HDL-c with increasing egg intake. We recruited 40 subjects to allow for attrition. All study protocols were approved by the University of Connecticut Institutional Review Board (protocol #H15-227) and informed consent was obtained from all participants prior to screening. This trial is registered at clinicaltrials.gov, trial #NCT02531958.

Upon qualification for participation, individuals began a 2-week washout period during which they were asked to abstain from intake of eggs or any foods in which eggs are a main component. A list of foods to avoid was provided to the participants. Following the washout period, subjects were fed 1 egg/day for 4 weeks, 2 eggs/day for 4 weeks, and then 3 eggs/day for 4 weeks. All variables described below were assessed after the 2-week washout and following consumption of 1, 2 and 3 eggs/day. All eggs (grade A, white, large size) were purchased from the same local store (Big Y, Tolland, CT, USA). Participants could prepare the eggs any way they wanted and could consume them at any time of day.

Dietary and Exercise Records

Participants were advised to maintain their normal dietary and exercise habits for the duration of the study; this was monitored by completion of 3-day dietary and exercise records during each arm of the intervention. Following enrollment in the study, individuals were given instructions on how to properly record dietary intake and exercise habits. Completed dietary records were analyzed using

Nutrition Data Systems for Research software (2013), developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA.

Body Weight, BMI, and Waist Circumference

Height was measured using a portable stadiometer to the nearest 0.5 cm. Weight was measured to the nearest 0.1 kg using an electronic scale; height and weight were used to calculate BMI. Waist circumference (WC) was measured to the nearest 0.5 cm directly against the skin at the top of the iliac crest using a flexible measuring tape. Three measurements were taken and averaged.

Blood Pressure

BP was measured after participants were seated quietly for 5 min using an Omron HEM 7320-Z portable automatic blood pressure cuff. Three measurements were taken ~1 min apart and averaged.

Plasma Lipids and Glucose

At the end of each arm of the intervention, plasma (70 mL) was collected from each participant into EDTA-coated vacutainer tubes following a 12-h fast. Plasma glucose, TAG, TC, and HDL-c were measured using a Cobas C111 automated clinical analyzer (Roche Diagnostics, Indianapolis, IN, USA). LDL cholesterol (LDL-c) was calculated using the Friedewald Equation [31] and was used to calculate the LDL-c/HDL-c ratio.

Plasma Choline and TMAO

Plasma concentrations of free choline and TMAO were measured in duplicate on different days by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) [23, 32], with modifications based on instrumentation [33, 34]. Briefly, plasma samples were mixed with acetonitrile containing 0.1% formic acid and internal standards ($^{13}C_3$-TMAO; d13-choline). The supernatant was collected, mixed with acetonitrile containing 0.1% formic acid, and injected into the LC–MS/MS system. Settings for the LC–MS/MS analysis have been described previously [33]. The interassay CV was <3.5% for each metabolite based on duplicate measures and <5% based on in-house controls.

Statistical Analysis

Repeated-measures ANOVA was used to test for differences between treatments, with Fisher's LSD *post hoc* analysis where appropriate. All statistical analysis was conducted using SPSS version 22. Level of significance for all tests was set at $P < 0.05$. Data are reported as mean ± SD.

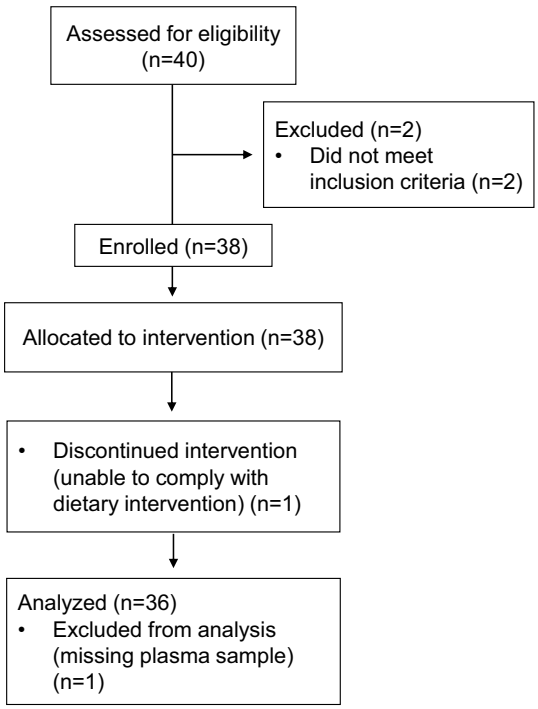

Fig. 1 Flow chart of a crossover dietary intervention in which $n = 37$ young, healthy individuals consumed 0 eggs/day for 2 weeks followed by ascending intake of 1, 2, and 3 eggs/day for 4 weeks each. Forty individuals were recruited for participation; two subjects were removed by the investigators because they did not fit the inclusion criteria, while 1 subject was removed from the study due to an inability to comply to the dietary intervention

Table 1 Participant characteristics at baseline of $n = 38$ young, healthy men and women enrolled in a 14-week crossover dietary intervention examining the impact of daily intake of varying quantities of eggs on biomarkers for cardiovascular disease risk

Age (years)	24.1 ± 2.2
Male gender (%)	50%
BMI (kg/m^2)	24.3 ± 2.5
Exercise (h/week)	2.2 ± 1.5
Waist circumference (cm)	85.8 ± 6.8
Systolic blood pressure (mmHg)	110 ± 12
Diastolic blood pressure (mmHg)	72 ± 9
Glucose (mg/dL)	95 ± 7
Triacylglycerol (mg/dL)	77 ± 23
Total cholesterol (mg/dL)	164 ± 29
HDL-c (mg/dL)	62 ± 15
LDL-c (mg/dL)	87 ± 21
LDL-c/HDL-c ratio	1.49 ± 0.50

Values are presented as means ± SD

HDL-c HDL cholesterol, *LDL-c* LDL cholesterol

Table 2 Macronutrient and micronutrient intake of $n = 37$ young, healthy men and women after a 2-week washout period during which no eggs were consumed and following daily intake of 1, 2, and 3 eggs for 4 weeks each

Nutrient	0 eggs	1 egg	2 eggs	3 eggs
Energy (kcal/day)	2140 ± 647^{a}	1954 ± 641^{b}	2078 ± 650^{a}	2006 ± 626^{a}
Protein (%)	19.2 ± 5.6	19.2 ± 5.1	20.5 ± 5.5	20.5 ± 6.1
Fat (%)	33.1 ± 7.6^{a}	35.0 ± 8.3^{ab}	37.2 ± 8.6^{bc}	39.3 ± 7.6^{c}
Carbohydrate (%)	45.1 ± 10.7^{a}	44.2 ± 10.7^{a}	40.4 ± 10.2^{b}	38.5 ± 10.0^{b}
Total fiber (g/day)	26.1 ± 14.1^{a}	21.0 ± 10.5^{b}	22.1 ± 12.9^{b}	19.3 ± 9.6^{c}
Glycemic load	123.9 ± 45.8^{a}	114.4 ± 44.6^{a}	111.9 ± 46.4^{ab}	102.6 ± 42.8^{b}
Cholesterol (mg/day)	216 ± 115^{a}	390 ± 133^{b}	589 ± 142^{c}	743 ± 127^{d}
Selenium (mcg/day)	127.3 ± 45.3^{a}	133.1 ± 54.0^{ab}	148.0 ± 48.1^{bc}	154.0 ± 47.3^{c}
Choline (mg/day)	324.4 ± 120.2^{a}	420.9 ± 113.9^{b}	562.9 ± 133.3^{c}	696.4 ± 119.7^{d}
Vitamin D (µg/day)	6.1 ± 7.1^{ab}	5.6 ± 4.9^{a}	6.1 ± 3.1^{a}	8.3 ± 4.4^{b}
Sodium (mg/day)	3789 ± 1322	3662 ± 1620	3539 ± 1244	3506 ± 1332

Values are presented as means ± SD. Values with different superscripts differ at $P < 0.05$ as determined by repeated measures ANOVA with LSD *post hoc* analysis and adjustment for multiple comparisons

Results

Participant Characteristics

Thirty-eight participants qualified for participation; of these, thirty-seven participants completed the intervention. One participant was removed due to an inability to comply with the dietary intervention (Fig. 1). Participant characteristics at baseline are shown in Table 1.

Dietary Intake

Dietary and exercise patterns remained relatively consistent throughout the intervention. Dietary records also indicate that about 60% of the subjects typically consumed their eggs in the morning. Compared to 0 eggs/day, total kcal intake decreased with intake of 1 egg/day ($P < 0.05$) (Table 2). There was no difference in kcal intake between 0, 2, and 3 eggs/day, suggesting that the difference observed with 1 egg/day was random and not related to egg intake. Intake of protein (% energy) remained unchanged for the duration of the intervention. However, fat intake (% kcal) increased while intake of carbohydrates (% energy) decreased with intake of 2–3 eggs/day as compared to intake of 0–1 eggs/day ($P < 0.001$). Total fiber intake decreased with intake of 1 egg/day and remained lower for the duration of the intervention, with an additional decrease in fiber seen with intake of 3 eggs/day as compared to intake of 1–2 eggs/day ($P < 0.001$). Along with these changes, glycemic load decreased with increasing egg intake ($P < 0.05$).

Unsurprisingly, cholesterol intake increased dose-dependently with egg intake ($P < 0.0001$). Intake of selenium was increased with intake of 2 and 3 eggs/day ($P < 0.01$) and choline intake increased dose-dependently with egg consumption ($P < 0.0001$). Intake of 3 eggs/day also resulted in increases in vitamin D intake as compared to lower consumption of eggs ($P < 0.05$). Intake of sodium was unchanged for the duration of the intervention (Table 2).

Table 3 Anthropometrics and blood pressure (BP) of $n = 37$ young, healthy men and women after a 2-week washout period during which no eggs were consumed and following intake of 1, 2, and 3 eggs/day for 4 weeks each

	0 eggs	1 egg	2 eggs	3 eggs
BMI (kg/m^2)	24.2 ± 2.5	24.2 ± 2.5	24.2 ± 2.6	24.4 ± 2.7
Waist circumference (cm)	85.8 ± 6.8	85.8 ± 6.7	85.3 ± 6.5	86.0 ± 7.6
Systolic BP (mmHg)	113 ± 14	113 ± 13	112 ± 14	113 ± 11
Diastolic BP (mmHg)	76 ± 8^{a}	74 ± 8^{b}	74 ± 9^{b}	72 ± 9^{c}

Values are presented as means ± SD. Values with different superscripts differ at $P < 0.05$ as determined by repeated measures ANOVA with LSD *post hoc* analysis and adjustment for multiple comparisons

Anthropometrics, Blood Pressure, Plasma Lipids, and Glucose

BMI, WC, and systolic BP were unchanged for the duration of the intervention (Table 3). However, diastolic BP decreased with egg intake ($P < 0.05$).

Plasma glucose and TAG did not change as a result of this intervention (Table 4). There were no significant differences in plasma TC between 0, 1, 2, and 3 eggs/day. Compared to 0 eggs/day, intake of 1 egg/day resulted in a 10.9% reduction in plasma LDL-c ($P < 0.05$). There was no difference in plasma LDL-c between 1 and 2 eggs/day or 2 and 3 eggs/day. Plasma LDL-c was higher following intake of 3 eggs/day as compared to 1 egg/day ($P < 0.05$),

Table 4 Plasma lipids and glucose of $n = 37$ young, healthy men and women after a 2-week washout period during which no eggs were consumed, followed by daily intake of 1, 2, and 3 eggs for 4 weeks each

	0 eggs	1 egg	2 eggs	3 eggs
Glucose (mg/dL)	95 ± 7	93 ± 8	94 ± 7	93 ± 7
Triacylglycerol (mg/dL)	79.5 ± 25.0	78.4 ± 27.9	80.6 ± 28.2	76.4 ± 28.0
Total cholesterol (mg/dL)	161 ± 30	154 ± 24	159 ± 28	163 ± 29
HDL-c (mg/dL)	61 ± 13[a]	64 ± 14[b]	65 ± 15[b]	65 ± 13[b]
LDL-c (mg/dL)	84 ± 25[a]	74 ± 16[b]	78 ± 21[ab]	83 ± 24[a]
LDL-c/HDL-c ratio	1.43 ± 0.59[a]	1.21 ± 0.38[b]	1.27 ± 0.47[bc]	1.32 ± 0.53[c]

Values are presented as means ± SD. Values with different superscripts differ at $P < 0.05$ as determined by repeated measures ANOVA with LSD *post hoc* analysis and adjustment for multiple comparisons

HDL-c HDL cholesterol, *LDL-c* LDL cholesterol

but not 0 eggs/day. HDL-c increased by 4.4% with intake of 1 egg/day and remained elevated for the duration of the intervention ($P < 0.05$) (Table 4). Intake of 1, 2, and 3 eggs/day all resulted in a significantly lower LDL-c/HDL-c ratio compared to intake of 0 eggs/day. The LDL-c/HDL-c ratio decreased by 15.4, 11.2, and 7.7% with daily intake of 1, 2 or 3 eggs, respectively, compared to 0 eggs (Table 4).

Plasma Choline and TMAO

Fasting plasma choline concentrations increased in a dose-dependent manner with increasing egg intake ($P < 0.0001$) (Fig. 2a). Despite this increase, fasting plasma TMAO remained unchanged for the duration of the intervention (Fig. 2b).

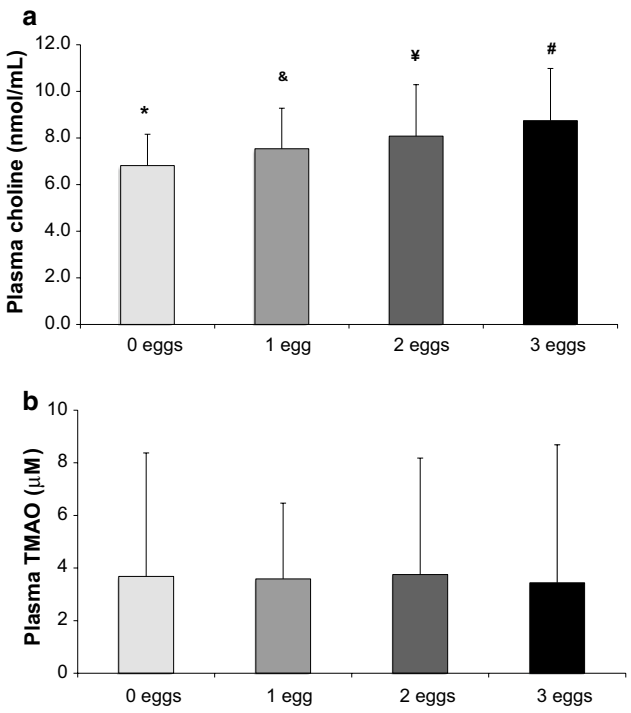

Fig. 2 Plasma choline (**a**) and trimethylamine-*N*-oxide (TMAO) (**b**) concentrations following a 2-week washout period (0 eggs/day), and after consuming 1, 2, and 3 eggs/day for 4 weeks each. Values are presented as means ± SD for $n = 36$ young, healthy men and women. *Bars* with different superscripts differ at $P < 0.05$ as determined by repeated measures ANOVA with LSD *post hoc* analysis and adjustment for multiple comparisons

Discussion

In the last forty years, per capita egg intake in the United States has declined due to recommendations to limit cholesterol intake [35]. It was initially hypothesized that dietary cholesterol would increase plasma cholesterol and, therefore, CVD risk. Since these recommendations were released, however, research has revealed only a minimal correlation between egg intake and plasma cholesterol [5, 9]. Regular intake of eggs is also not linked to increases in biomarkers for CVD risk in non-diabetic adults [8, 9, 11–13].

In the present study, participants maintained their normal dietary habits except for the number of eggs consumed. With increasing daily egg intake, dose-dependent changes in nutrient intake were observed. One large egg contains ~180 mg cholesterol [36]. Dietary cholesterol increased 3.4-fold with intake of 3 eggs/day as compared to intake of 0 eggs/day. One egg also contains 5 g of fat [36], thus an increase in fat intake with egg consumption is unsurprising. Simultaneously, carbohydrate intake and dietary glycemic load decreased with increasing egg intake. One possible explanation is that many participants consumed the eggs in lieu of other carbohydrate- and fiber-rich breakfast foods such as cereals and bread products. This may explain the decrease in fiber intake with 2 and 3 eggs/day.

One large egg provides 6% DV of vitamin D, 35% DV of choline, and 17% DV of selenium [37, 38]. Intake of each nutrient increased significantly and dose-dependently with increasing egg intake. Vitamin D is a nutrient of concern in the United States [2]. Optimal plasma concentration of the vitamin is still being debated, but based on proposed concentrations, 30–70% of adults have low plasma

vitamin D [39, 40]. Inadequate plasma vitamin D has been associated with many conditions, including CVD, various types of cancer, osteoporosis, multiple sclerosis, and others [41, 42]. Therefore, the increase in vitamin D intake with consumption of 3 eggs/day is of importance. Participants of this study exceeded the RDA for selenium with intake of 0 eggs. Thus the increase in selenium intake with egg consumption is not of clinical significance. However, the recommendations for dietary choline were not being met with intake of 0 or 1 egg/day [43]. With intake of 2 and 3 eggs/day, participants were able to meet or exceed recommendations.

Along with changes to diet composition, egg intake was associated with favorable or no changes in biomarkers associated with CVD risk. Egg intake did not impact BMI, WC, systolic BP, fasting plasma glucose or TAG in a young, healthy population. However, egg intake was associated with decreased diastolic BP. Sodium intake was unchanged throughout the intervention, and therefore cannot explain this difference. However, it can be explained by the presence of bioactive peptides: endogenous or diet-derived peptides that can bind receptors, inhibit enzymes, or exert other physiological effects [44]. One impact of certain dietary protein-derived bioactive peptides is a reduction in BP [44–47].

Eggs are a source of bioactive peptides. In 2 recent studies, eggs were digested in simulated gastric conditions and ovalbumin-derived angiotensin converting enzyme (ACE)-inhibitory peptides were released [48, 49]. ACE is an important enzyme involved in the regulation of BP; inhibition of ACE reduces BP [48]. These ACE-inhibitory peptides may explain the reduction in diastolic BP observed in the present study. Because our participants had normal diastolic BP at baseline, this reduction is not clinically relevant. However, a future area of research may involve egg intake in individuals with elevated diastolic BP.

Past research shows that egg intake is associated with increases in plasma TC, a phenomenon attributable to increases in both LDL-c and HDL-c [50]. In the present study, however, LDL-c was actually lower with intake of 1 egg/day compared to intake of 0 eggs/day. Though LDL-c increased with intake of 2 and 3 eggs/day, values did not exceed baseline levels. Plasma TC likewise did not differ between intake of 0 and 3 eggs/day. Simultaneously, intake of 1 egg/day was sufficient to increase plasma HDL-c, an elevation that was maintained with intake of 2 and 3 eggs/day. Previous research indicates that a 1 mg/dL increase in HDL-c is associated with a 2–4% reduction in CVD risk [51]. In the present study, HDL-c increased by 3–4 mg/dL, which would translate to a 6–16% reduction in CVD risk as a result of consuming 1–3 eggs/day. It is also important to consider changes in HDL function as a result of egg intake; this data has been published elsewhere [52].

The LDL-c/HDL-c ratio is an independent predictor of CVD risk, with a value $\leq$2.5 indicating low risk [53]. In the present study, we observed a reduction or no change in LDL-c with an increase in HDL-c. Therefore, the LDL-c/HDL-c ratio was decreased by intake of 1, 2, or 3 eggs/day as compared to 0 eggs/day, equating to a reduction in CVD risk.

One egg contains 147 mg choline, primarily in the form of phosphatidylcholine (PC) [54]. Choline is metabolized by the intestinal microbiota into TMA, which is further oxidized into TMAO in the liver by the flavin monooxygenase (FMO) family of enzymes [22]. Fasting plasma TMAO has been associated with increased risk of cardiovascular outcomes [23–26]. For this reason, dietary components that elevate plasma TMAO are of concern. A large cross-sectional study found a positive association between intake of meat, fish, and cholesterol and plasma TMAO [26]. Three studies also showed that intake of $\geq$2 eggs resulted in a postprandial increase in plasma TMAO [24, 27, 28]. In each study, though, increases in plasma TMAO were observed only following ingestion of large amounts of choline at one time. The degree of response to choline ingestion also varied widely between individuals.

Despite the conversion of choline to TMAO by the gut microbiota, this nutrient is important for overall health. Choline is an essential component of cell membranes and is especially important in the nervous system [55]. In the present study, plasma choline increased with increasing egg intake while fasting plasma TMAO was unchanged. These data support the notion that the connection between choline intake and plasma TMAO is complex one.

It is important to consider that just because choline is consumed does not mean that it will be converted to TMAO. Consumption of choline in the form of PC may render the nutrient unavailable to the distal gut microbiota; it has been estimated that only 14% of choline from eggs is metabolized into TMAO [28]. The connection between choline and TMAO is also confounded by the large degree of inter-individual variability in composition of the gut microbiota [56]. Because only certain strains of bacteria are capable of converting choline to TMA [25], variation in the presence of these strains will impact the degree to which an individual responds to choline intake. There is also a high degree of interindividual variability in FMO activity [26]. These variations likely explain the wide range of response to choline or egg ingestion seen in previous studies. This also suggests that factors other than egg intake play a larger role in determining plasma fasting TMAO concentrations.

The fact that sustained intake of up to 3 eggs/day did not increase fasting plasma TMAO concentrations also suggests that the postprandial increases in TMAO observed in previous studies do not impact fasting plasma TMAO. Therefore, concerns regarding the impact of regular egg

intake on plasma TMAO in healthy individuals may be unfounded.

It is relevant to note that the relationship between plasma choline concentrations and biomarkers of CVD risk has also been controversial, with some studies suggesting the association is positive while others show it to be negative. Plasma choline has been positively correlated with plasma glucose, TAG, BMI, non-HDL-c, and certain pro-inflammatory cytokines in certain populations [57, 58]. Recently, however, it has been suggested that this association between choline and CVD risk is dependent on plasma TMAO concentrations rather than the concentration of choline itself [59]. Conversely, plasma choline has been shown to be negatively correlated with plasma homocysteine, a known biomarker associated with elevated CVD risk [60, 61]. Adequate choline intake has also been associated with reductions in certain biomarkers for CVD risk [57, 58]. Regardless, these findings are associative rather than mechanistic in nature and thus it cannot definitively be stated that choline intake or elevated plasma concentrations increase CVD risk. Moreover, though we observed an increase in plasma choline with egg intake, this value remained within the previously-established normal range [62, 63]. Thus, any increase in plasma choline caused by egg intake in this population should not be of clinical concern.

Although one limitation of this study is that the chosen population was young, healthy individuals, these results suggest that intake of up to 3 eggs/day does not increase clinical biomarkers associated with CVD risk in this population as estimated by traditional plasma lipid values as well as plasma TMAO concentrations. The data from this study therefore support the inclusion of eggs as part of a healthy dietary pattern in young, healthy adults.

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Compliance with Ethical Standards

Conflict of interest DMD, AM, and MLF have received funding from the Egg Nutrition Center. All other authors declare no conflicts of interest.

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