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Intake of up to 3 Eggs per Day Is Associated with Changes in HDL Function and Increased Plasma Antioxidants in Healthy, Young Adults[1–3]

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Abstract

Background: HDL function may be more important than HDL concentration in determining risk for cardiovascular disease. In addition, HDL is a carrier of carotenoids and antioxidant enzymes, which protect HDL and LDL particles against oxidation.

Objective: The goal of this study was to determine the impact of consuming 0–3 eggs/d on LDL and HDL particle size, HDL function, and plasma antioxidants in a young, healthy population.

Methods: Thirty-eight healthy men and women [age 18–30 y, body mass index (in kg/m^2) 18.5–29.9] participated in this 14-wk crossover intervention. Subjects underwent a 2-wk washout (0 eggs/d) followed by sequentially increasing intake of 1, 2, and 3 eggs/d for 4 wk each. After each period, fasting blood was collected for analysis of lipoprotein subfractions, plasma apolipoprotein (apo) concentration, lutein and zeaxanthin concentration, and activities of lecithin-cholesterol acyltransferase, cholesteryl ester transfer protein, and paraoxonase-1.

Results: Compared with intake of 0 eggs/d, consuming 1–3 eggs/d resulted in increased large-LDL (21–37%) and large-HDL (6–13%) particle concentrations, plasma apoAI (9–15%), and lecithin-cholesterol acyltransferase activity (5–15%) ($P < 0.05$ for all biomarkers). Intake of 2–3 eggs/d also promoted an 11% increase in apoAII ($P < 0.05$) and a 20–31% increase in plasma lutein and zeaxanthin ($P < 0.05$), whereas intake of 3 eggs/d resulted in a 9–16% increase in serum paraoxonase-1 activity compared with intake of 1–2 eggs/d ($P < 0.05$). Egg intake did not affect cholesteryl ester transfer protein activity.

Conclusions: Intake of 1 egg/d was sufficient to increase HDL function and large-LDL particle concentration; however, intake of 2–3 eggs/d supported greater improvements in HDL function as well as increased plasma carotenoids. Overall, intake of ≤3 eggs/d favored a less atherogenic LDL particle profile, improved HDL function, and increased plasma antioxidants in young, healthy adults. This trial was registered at clinicaltrials.gov as NCT02531958. *J Nutr* doi: 10.3945/jn.116.241877.

Keywords: eggs, healthy population, HDL function, lecithin cholesterol acyltransferase, plasma carotenoids, paraoxonase-1

Introduction

HDL is predominantly known for its role in reverse cholesterol transport (RCT)[4], the process by which excess cholesterol is removed from cells and transported back to the liver for excretion from the body (1). This ability to remove excess cholesterol is thought to explain why low HDL is related to cardiovascular disease (CVD) (2). Recently, evidence has emerged to suggest that composition and function of lipoproteins may be more important than concentration in determining CVD risk (3). For HDL, this includes its capacity to collect cholesterol as well as the antioxidant and anti-inflammatory roles of the lipoprotein particle.

For example, lipoprotein particle size is related to atherogenicity, with smaller LDL particles being more prone to oxidative modification and uptake into arterial walls (4). There are differing opinions regarding HDL particle size (5–10). However, the larger, more buoyant HDL appears to be most closely associated with decreased CVD risk (11). The main protein component of lipoproteins is composed of apos, which contribute to the regulation of lipoprotein formation, metabolism, and uptake (12). Some studies suggest that apo concentration may be a better predictor of CVD risk than plasma lipoproteins (13). Therefore, assessment of changes in apos may be indicative of the impacts of a dietary intervention on CVD risk.

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[3] Supplemental Figure 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[4] Abbreviations used: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; LCAT, lecithin-cholesterol acyltransferase; PON1, paraoxonase-1; RCT, reverse cholesterol transport.

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Both cholesteryl ester (CE) transfer protein (CETP) and lecithin-cholesterol acyltransferase (LCAT) are involved in RCT. CETP is a plasma protein that facilitates lipid exchange between lipoproteins by aiding primarily in the equimolar transfer of CEs from HDL to VLDL, chylomicrons, or to a lesser extent, LDL in exchange for TGs (14). The transfer of CEs from HDL to these TG-rich lipoproteins is an important part of RCT, because CEs are transported back to the liver by TG-rich lipoproteins (14, 15). A downside to this exchange is that HDL TG enrichment reduces its ability to take up cholesterol and interact with the hepatic transporters responsible for its uptake (16). Hydrolysis of HDL TGs also leaves behind smaller HDL particles, which as discussed above, may or may not be advantageous. Therefore, CETP is considered to have both pro- and antiatherogenic roles. LCAT is a primarily HDL-associated enzyme that catalyzes the conversion of free cholesterol into CEs, allowing for incorporation and retention of cholesterol in the HDL particle core. This is a necessary step in the process of HDL maturation and stabilization; thus, it is hypothesized that LCAT activity promotes RCT (17).

Aside from its cholesterol-carrying capacity, HDL also has antioxidant capabilities. HDL is the main carrier of paraoxonase-1 (PON1), an enzyme that protects both HDL and LDL against oxidative modification (18, 19). Lastly, lipoproteins are transport vehicles for carotenoids, lipophilic compounds with strong antioxidant capabilities (20). Carotenoids are divided into 2 groups (21); the hydrocarbon carotenes predominately associate with LDL, whereas oxygen-containing xanthophylls are primarily transported by HDL (22). Of interest to the present research are lutein and zeaxanthin, xanthophylls that are present in egg yolk (23, 24).

Previous studies have found egg intake to be associated with increases in HDL and LDL particle size, LCAT activity, and plasma apoAII and carotenoid concentrations (23–28). However, most studies have been conducted in nonhealthy populations, and egg intake was often coupled with other dietary restrictions. Therefore, the goal of the present study was to determine how intake of an increasing number of eggs (0, 1, 2, and 3 eggs/d) affects lipoprotein composition and function in relation to CVD risk in a young, healthy population. We hypothesized that consuming 2–3 eggs/d would be sufficient to promote favorable changes in the parameters discussed above without negatively affecting the overall lipoprotein profile or HDL function.

Methods

Study design. We recruited 40 men and women [age 18–30 y, BMI (in kg/m^2) 18.5–29.9] with blood pressure ≤140/90 mm Hg and a healthy lipid panel (glucose ≤126 mg/dL, total cholesterol ≤240 mg/dL, TG ≤500 mg/dL) for participation in this 14-wk crossover dietary intervention. Informed consent was obtained from all participants before screening, and all portions of this protocol were approved by the University of Connecticut Institutional Review Board. Qualifying participants underwent a 2-wk washout period, during which 0 eggs/d were consumed, followed by sequentially increasing intake of 1, 2, and then 3 eggs/d for 4 wk each. Fasting plasma (30 mL) was collected at the end of each dietary period, and serum (10 mL) was collected after intake of 1, 2, and 3 eggs/d.

No restrictions were placed on how participants ate the eggs; they could be consumed in any fashion and at any time of day. Compliance to the dietary intervention was assessed weekly when participants arrived to pick up eggs. All eggs (large, grade A, white) were provided to participants and were purchased at a local supermarket (Big Y). Participants were asked not to consume any eggs outside of those provided to them. Aside from egg intake, participants were instructed to maintain their normal dietary habits throughout the study. This was assessed by completion of 3-d dietary records during each arm of the intervention. Dietary intake was assessed by using the Nutrition Data System for Research software (2013) developed by the Nutrition Coordinating Center, University of Minnesota.

This study was powered to detect a 10% difference in HDL (80% power with 2-sided significance level of $\alpha = 0.05$) with $n = 35$ subjects. We recruited 40 subjects to allow for attrition.

Lipoprotein particle size. Plasma VLDL, LDL, and HDL particles by subclass (small, medium, and large) as well as overall mean LDL, VLDL, and HDL particle sizes were determined by using proton NMR spectroscopy (26). NMR analysis was performed by LipoScience, Inc.

Apolipoproteins. ApoAI, apoAII, apoCII, apoCIII, and apoE were quantified simultaneously in plasma by using multiplex technology with a commercially available kit (EMD Millipore) and a Luminex MAGPIX instrument (Luminex Corporation). Intra-assay variation for this method was 4.7%.

CETP and LCAT. CETP activity in plasma was assessed by using a commercially available assay kit (BioVision, Inc.). Plasma was incubated with a self-quenching donor molecule and an acceptor molecule, and the decrease in fluorescence intensity over time was measured to calculate CETP activity. The intra-assay variation for this method was 5%. LCAT activity in plasma was also measured by using a commercially available fluorometric assay kit (Cell Biolabs, Inc.). The strength of the fluorescence signal after an incubation period was indicative of relative LCAT activity. The intra-assay variation for both methods was <1.5%. Fluorescence output for both assays was measured by using a BioTek Synergy 2-plate reader with Gen5 Software (BioTek Instruments, Inc.).

PON1. PON1 activity, rather than expression, is a better predictor of CVD risk (29). Serum PON1 arylesterase activity toward phenyl acetate was measured spectrophotometrically by using an in-house 96-well microplate assay that has been described previously (30) and a Biotek Epoch instrument with Gen5 Software (BioTek Instruments, Inc.). The intra-assay variation for this method was 4%. We did not assess PON1 activity after the 0-egg/d period because serum was not isolated at this time point.

Plasma carotenoids. Lutein and zeaxanthin were extracted from plasma as described previously (24). Briefly, carotenoids were extracted in a series of steps by using a 2:1 (vol:vol) chloroform:methanol mixture followed by hexane. Samples were dried under nitrogen gas and reconstituted in ethanol for analysis. A known concentration of internal standard (trans-β-apo-8′-carotenal; Sigma-Aldrich) was added to each sample before extraction to calculate carotenoid recovery. All work was completed in the dark to limit carotenoid oxidation.

Analysis of plasma lutein and zeaxanthin concentrations was conducted by using reverse-phase HPLC. Carotenoid concentrations were measured on a Shimadzu Prominence UFLC (Shimadzu Corporation) fitted with a C30 3-μm, 150 × 4.6–mm carotenoid column (YMC America) with a guard column. Lutein and zeaxanthin concentrations were determined by comparing the AUC of chromatogram peaks to standard curves generated with purified lutein and zeaxanthin standards (Sigma-Aldrich). A standard curve for trans-β-apo-8′-carotenal was also generated for determination of carotenoid recovery efficiency.

Statistical analysis. All variables were analyzed by repeated-measures ANOVA, with least significant difference post hoc analysis when appropriate. Two-factor ANOVA was used to test for differences between the sexes. Finally, Pearson correlations were conducted between positive outcomes. Statistical analysis was conducted with SPSS for Windows, version 22 (IBM Corp). The level of significance for all tests was set at $P < 0.05$. Data are reported as means ± SDs.

Results

Participant characteristics and dietary intake. Thirty-seven men and women completed the intervention (age 24.1 ± 2.2 y, BMI 24.3 ± 2.5). BMI, waist circumference, systolic blood pressure, fasting plasma glucose, and fasting plasma TG did not

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TABLE 1 Lipoprotein particle concentrations from fasting plasma of young, healthy individuals after intake of 0 eggs/d for 2 wk and daily intake of 1, 2, and 3 eggs for 4 wk each[1]

	0 eggs/d	1 egg/d	2 eggs/d	3 eggs/d	P
VLDL and CMs					
Total	53.1 ± 14.7	48.4 ± 15.5	51.8 ± 18.7	51.9 ± 17.4	NS
Large (60–100 nm), nmol/L	3.2 ± 2.4	3.2 ± 2.5	3.3 ± 3.0	3.1 ± 2.4	NS
Medium (40–60 nm), nmol/L	16.2 ± 10.8	14.8 ± 10.9	15.0 ± 11.2	15.2 ± 11.5	NS
Small (30–40 nm), nmol/L	33.8 ± 13.9	30.4 ± 11.9	33.5 ± 16.1	33.6 ± 13.8	NS
Mean particle size, nm	47.2 ± 5.4	48.1 ± 6.1	46.6 ± 5.8	46.8 ± 7.0	NS
LDL					
Total, nmol/L	923 ± 243[a]	986 ± 184[b]	995 ± 224[b,c]	1050 ± 297[c]	0.003
Large (23–30 nm), nmol/L	299 ± 165[a]	363 ± 162[b]	396 ± 200[b]	410 ± 211[b]	0.004
Small (18–23 nm), nmol/L	452 ± 181	429 ± 204	410 ± 193	396 ± 202	NS
Mean particle size, nm	20.7 ± 0.5	20.8 ± 0.5	20.9 ± 0.5	20.9 ± 0.5	NS
HDL					
Total, μmol/L	36.7 ± 5.5	37.5 ± 4.5	38.3 ± 5.1	37.7 ± 4.8	NS
Large (10–13 nm), μmol/L	9.0 ± 3.0[a]	9.6 ± 3.2[b]	9.9 ± 3.7[b,c]	10.2 ± 3.6[c]	0.03
Medium (8.2–10 nm), μmol/L	14.1 ± 4.9	14.7 ± 6.6	14.6 ± 5.9	14.7 ± 5.8	NS
Small (7.3–8.2 nm), μmol/L	14.6 ± 4.5	13.7 ± 6.0	14.9 ± 6.9	14.2 ± 6.6	NS
Mean particle size, nm	9.6 ± 0.2	9.6 ± 0.4	9.6 ± 0.4	9.6 ± 0.4	NS

[1] Values are means ± SDs (n = 35). Means without a common superscript letter differ, $P < 0.05$ by repeated-measures ANOVA with least significant difference post hoc analysis. NS, $P \geq 0.05$. CM, chylomicron.

change, whereas diastolic blood pressure decreased with egg intake ($P < 0.05$) (data not shown). The lack of change in weight during the intervention suggests that participants adjusted to the additional calorie intake associated with egg consumption. No sex differences were noted in any of the measured parameters in response to egg intake.

Lipoprotein particle size. Most relevant to lipoproteins, cholesterol and total fat intake (percentage of kilocalories) increased in a dose-dependent manner with daily egg consumption ($P < 0.05$) (data not shown). No changes were observed in VLDL particle size or concentration of each VLDL subfraction. There was no difference in plasma LDL between 0 (84 ± 25 mg/dL), 2 (78 ± 21 mg/dL), and 3 eggs/d (83 ± 24 mg/dL), whereas intake of 1 egg/d resulted in lower LDL (74 ± 16 mg/dL) than the other intakes ($P < 0.05$). However, LDL total particle concentration increased dose dependently with egg intake ($P < 0.05$) (**Table 1**). This can be attributed to an increase in large-LDL particle concentration with 1–3 eggs/d compared with 0 eggs/d ($P < 0.05$), whereas the concentration of small- and medium-sized particles remained unchanged.

HDL was increased by intake of 1 (64 ± 14 mg/dL), 2 (65 ± 15 mg/dL), and 3 eggs/d (65 ± 13 mg/dL) compared with 0 eggs/d (61 ± 14 mg/dL) ($P < 0.05$). The concentration of large HDL particles likewise increased in a dose-dependent manner with egg intake ($P < 0.05$). However, the concentration of small- and medium-sized HDL particles, total HDL particle concentration, and HDL mean particle size did not change (Table 1).

Apolipoproteins. In concordance with the increase in large HDL, we observed an increase in concentrations of apoAI with intake of 1–3 eggs/d ($P < 0.05$) and apoAII with intake of 2–3 eggs/d ($P < 0.05$) compared with smaller quantities of eggs (**Table 2**). Plasma concentrations of apoCII, apoCIII, and apoE did not change. We also observed a positive correlation between apoAI and large-HDL particle concentration ($r = 0.39$; $P = 0.0001$) (**Figure 1**).

CETP and LCAT activities. No change was observed in CETP activity during any arm of the dietary intervention (**Figure 2**A). LCAT activity increased in a dose-dependent manner with egg intake ($P < 0.05$) (Figure 2B).

PON1. PON1 activity was significantly increased with intake of 3 eggs/d compared with 1–2 eggs/d ($P < 0.05$) (**Figure 3**A). We observed a positive correlation between PON1 activity and apoAII concentration ($r = 0.25$; $P < 0.05$) (**Supplemental Figure 1**A). PON1 activity was also positively correlated with LCAT activity ($r = 0.32$; $P < 0.05$) (Supplemental Figure 1B).

Plasma carotenoids. We evaluated the intake of the antioxidant carotenoids lutein and zeaxanthin, which are present in small amounts in egg yolk. Despite the fact that individuals were consuming up to 3 eggs/d, we observed no change in lutein and zeaxanthin intake (**Figure 4**A). However, plasma lutein and zeaxanthin concentrations were increased with intake of 2–3 eggs/d compared with 0–1 eggs/d ($P < 0.05$) (Figure 4B).

Discussion

In this study we observed that increasing egg intake resulted in significant changes in HDL metabolism that can be related to enhanced RCT as well as higher concentrations of plasma

TABLE 2 Plasma apo concentrations in young, healthy individuals after intake of 0 eggs/d for 2 wk and intake of 1, 2, and 3 eggs/d for 4 wk each[1]

	0 eggs/d	1 egg/d	2 eggs/d	3 eggs/d	P
ApoAI, mg/L	320 ± 65[a]	350 ± 84[b]	384 ± 106[b]	370 ± 84[b]	0.003
ApoAII, mg/L	152 ± 37[a]	162 ± 34[a]	170 ± 35[b]	169 ± 36[b]	0.013
ApoCII, μg/L	48 ± 30	52 ± 27	55 ± 28	53 ± 29	NS
ApoCIII, mg/L	100 ± 45	109 ± 40	113 ± 44	110 ± 37	NS
ApoE, mg/L	29 ± 10	32 ± 9	34 ± 10	34 ± 9	NS

[1] Values are means ± SDs (n = 36). Means without a common superscript letter differ, $P < 0.05$ by repeated-measures ANOVA with least significant difference post hoc analysis. NS, $P \geq 0.05$. Apo, apolipoprotein.

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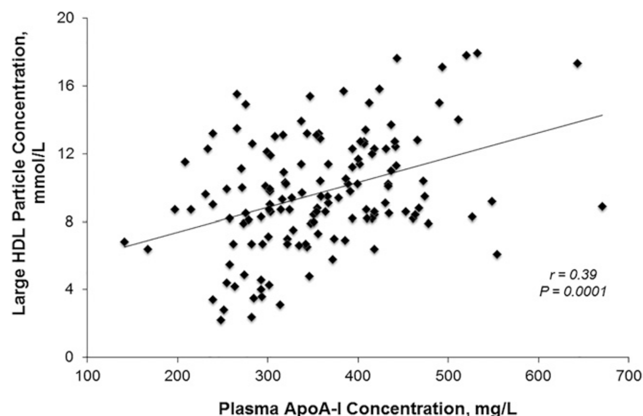

FIGURE 1 Pearson correlation between plasma apoAI and large-HDL particle concentrations in 36 young, healthy men and women after a 2-wk intake of 0 eggs/d and consumption of 1, 2, and 3 eggs/d for 4 wk each. apo, apolipoprotein.

antioxidants. Thus, we demonstrated that egg intake results in favorable HDL-related outcomes in a young, healthy population.

Increasing egg intake and LDL and HDL metabolism. Lipoprotein particle size is related to particle atherogenicity. For example, small LDL particles are more susceptible to oxidation, making them more likely to contribute to arterial lesions (4). Because large LDL particles are less susceptible to such modification, they are considered the least atherogenic LDL subfraction. Indeed, large LDL was not associated with increased CVD risk in a large 11-y follow-up study (5). Therefore, a lipoprotein profile with relatively more large and fewer small LDL particles is considered favorable. In the present study, we observed an increase in large LDL particles, which suggests a less atherogenic LDL profile.

There is a discrepancy concerning the relation between size and atherogenicity of HDL particles. One hypothesis is that larger HDL particles are indicative of increased RCT. This is supported by findings that large-HDL particle concentration is inversely associated with CVD (5, 6, 31), whereas small-HDL particle concentration is positively associated with CVD risk (6, 31). The opposing hypothesis is that smaller particles are preferable because they have the capacity to accept more cholesterol, whereas large particles are full and do not have this capacity (32). Some evidence suggests that smaller HDL particles carry more antioxidants (9, 10). Despite these apparent benefits, small-HDL particle concentration was not associated with decreased CVD in a study of >27,000 women (5). Thus, evidence suggests that large-HDL particle concentration indicates a healthier HDL phenotype, although it is unclear whether this is reflective of enhanced RCT.

In the present study we observed an increase in the concentration of large HDL particles as well as an increase in concentration of lutein and zeaxanthin and activity of PON1, which are HDL-associated antioxidants. It is notable that PON1 is typically found on small HDL (9), yet PON1 activity increased despite no observed change in concentration of small HDL particles. Thus, it appears this shift toward a population of larger HDL particles did not negatively affect HDL antioxidant properties. Although results have been mixed (10), the overall evidence seems to point toward large HDL as being equally or more anti-atherogenic than the smaller subfractions.

The main protein associated with HDL, apoAI, is responsible for facilitating the interaction of HDL with cellular cholesterol efflux transporters, which is the initial step in RCT (33). It is this mechanism that likely explains the correlation we observed between apoAI and large-HDL particle concentration. In the presence of more apoAI, more cholesterol can be taken up from cells, possibly explaining the increase in large HDL particles. Because CVD risk is more closely associated with the concentration of large HDL particles, this change may be considered anti-atherogenic (11). ApoAI also has exposed cysteine residues that confer antioxidant capacity to the HDL particle (34, 35). Therefore, an increase in apoAI concentrations may be indicative not only of increased potential for RCT but also increased antioxidant capacity of HDL.

The second most-abundant HDL-associated protein, apoAII, is important for stabilization of the HDL particle (36). Similar to

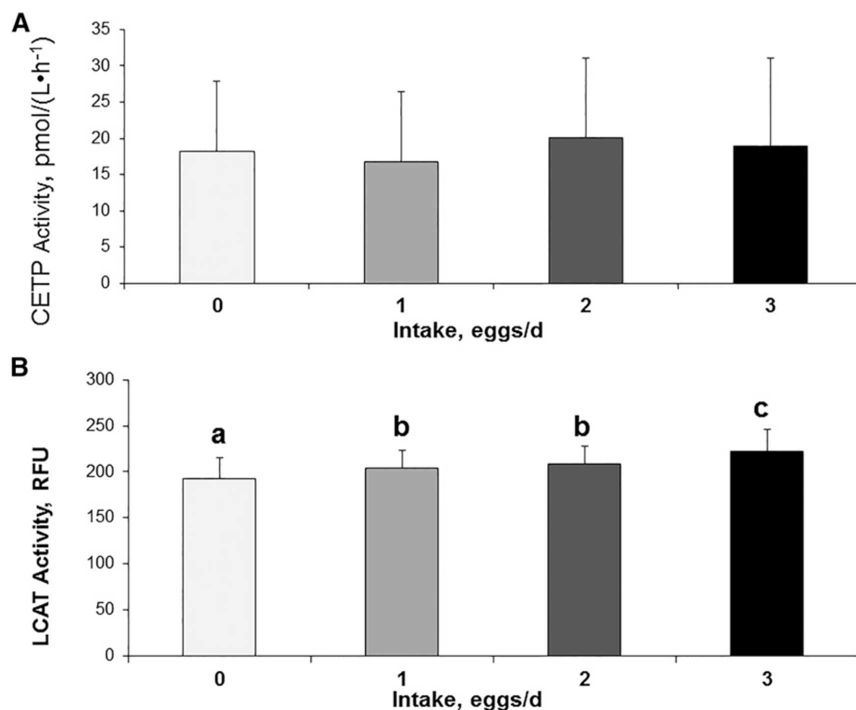

FIGURE 2 Plasma activity of CETP (A) and LCAT (B) in young, healthy men and women after a 2-wk intake of 0 eggs/d and consumption of 1, 2, and 3 eggs/d for 4 wk each. Values are means ± SDs for 36 individuals. Means without a common letter differ, $P < 0.05$ by repeated-measures ANOVA with least significant difference post hoc analysis. CETP, cholesteryl ester transfer protein; LCAT, lecithin cholesterol transfer protein; RFU, relative fluorescence unit.

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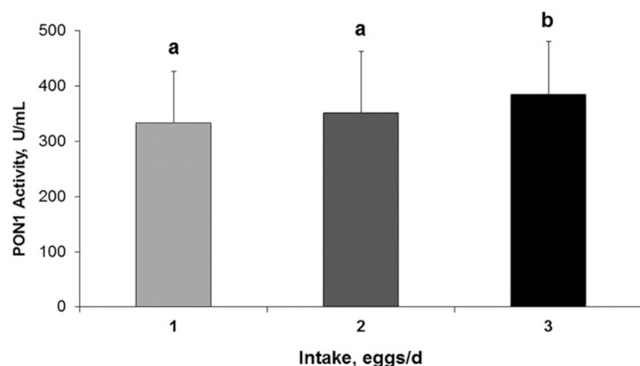

FIGURE 3 Serum PON1 activity in young, healthy men and women after intake of 1, 2, and 3 eggs/d for 4 wk each. Values are means ± SDs for 36 individuals. Means without a common letter differ, $P < 0.05$ by repeated-measures ANOVA with least significant difference post hoc analysis. PON1, paraoxonase-1.

apoAI, apoAII possesses antioxidant capabilities (8). Not all HDL particles contain apoAII, but those that do have improved antioxidant capacity because of the presence of the protein itself and also because it stabilizes PON1 (9). Moren et al. (9) found that PON1 on apoAII–containing HDL was more resistant to oxidation. In the present study we observed a positive correlation between apoAII concentration and PON1 activity, which may be partially explained by this stabilizing capability.

It has previously been shown that PON1 promotes RCT by enhancing cholesterol efflux to HDL (37). PON1 activity is positively correlated with LCAT activity as well, which likewise enhances RCT (38). This correlation was attributed to the ability of PON1 to prevent oxidation and inactivation of LCAT (38, 39). In the present study we likewise observed this correlation. This is suggestive of a healthier, more functional HDL profile. Moreover, egg intake increased LCAT activity. Because esterification of free cholesterol by LCAT is essential for RCT, increased LCAT activity indicates enhanced capacity for RCT (40).

The relation between CETP and CVD is complicated and not well understood. Because of its role in transferring TG to HDL, thus reducing the cholesterol-carrying capacity of the lipoprotein, CETP is considered proatherogenic (16). Inhibition of CETP has been proposed as a strategy for reduction of CVD risk by increasing HDL. However, clinical trials with CETP inhibitors have been unsuccessful for a variety of reasons (41–43). Although all 3 drugs led to increases in HDL, no substantial regression in CVD was observed (41, 43, 44). A possible reason is that the transport of CE back to the liver by LDL is an important component of RCT, a process which is reduced by CETP inhibition (14). Genetics has also been suggested to play a role in determining whether CETP acts in a more pro- or anti-atherogenic manner in a given individual (15). Despite the apparent dual roles of CETP in regulation of RCT and its relation with CVD risk, the intake of 1–3 eggs/d did not impact CETP activity in the present study. Thus, CVD risk was unchanged by egg intake according to this biomarker.

Increasing egg intake and plasma carotenoids. Compared with many colorful vegetables, eggs contain relatively little lutein and zeaxanthin (21). The important difference is that lutein and zeaxanthin from eggs are much more bioavailable (45). Because lutein and zeaxanthin are lipophilic compounds, their absorption requires micelle formation, which in turn requires ingestion of fat (21). One study found that carotenoid absorption from a green salad was low, but it was significantly increased when the salad was consumed with a full-fat dressing (46). Vegetables also contain fiber, which is known to disrupt micelle formation and interfere with the absorption of fat and other lipophilic compounds (21). For these reasons, vegetable-derived carotenoids have a very low bioavailability. Although lutein and zeaxanthin are present only in small amounts in eggs, 1 egg yolk also contains 5 g of fat. This amount is shown to be sufficient to facilitate the efficient absorption of carotenoids (46). Indeed, in the present study we observed an increase in plasma lutein and zeaxanthin concentrations after egg intake, even though carotenoid intake did not increase. It is important to note that intake of just 1 egg/d was not sufficient to promote this change. However, intake of 2–3 eggs/d did increase plasma lutein and zeaxanthin concentrations.

It is important to note that these results apply to this subset of young, healthy individuals and should be extrapolated to other

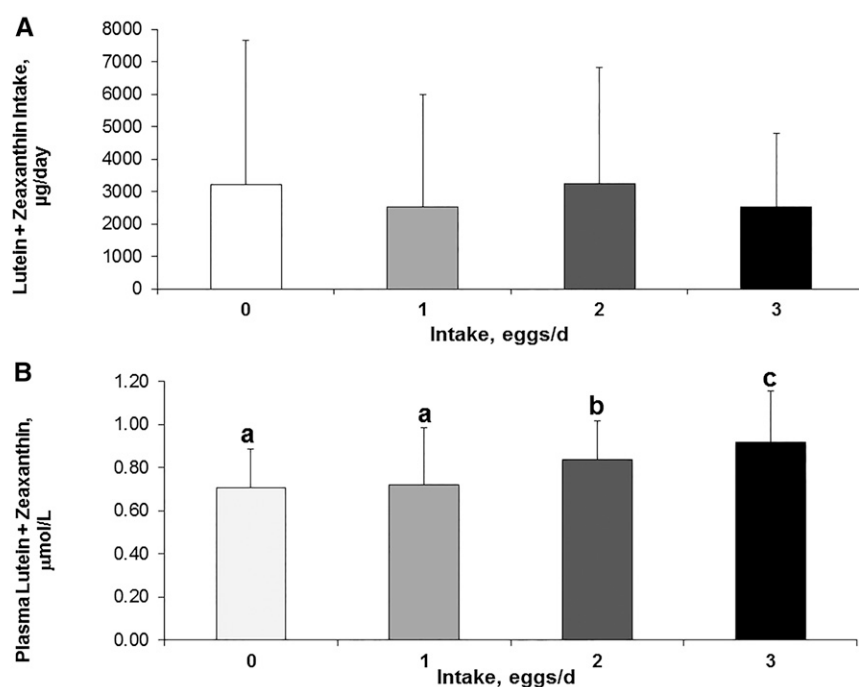

FIGURE 4 Dietary intakes (A) and plasma concentrations (B) of lutein and zeaxanthin in young, healthy men and women after a 2-wk intake of 0 eggs/d and consumption of 1, 2, and 3 eggs/d for 4 wk each. Values are means ± SDs for 36 individuals. Means without a common letter differ, $P < 0.05$ by repeated-measures ANOVA with least significant difference post hoc analysis.

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populations with care. In addition, we assessed biomarkers associated with CVD risk on a short-term basis; thus, no conclusions can be drawn regarding the long-term impacts of egg intake on more direct measures of CVD. A final limitation of these data is that we were unable to measure PON1 activity at baseline because of a lack of serum samples. Therefore, we were not able to determine the impact of eggs compared with no eggs on the activity of this enzyme. Regardless of this missing information, the results of this dietary intervention provide a comprehensive assessment of the impact of daily egg intake on HDL function and plasma antioxidant status. Finally, these data support the recent change to the dietary guidelines, which states that cholesterol is no longer a nutrient of concern. Thus, eggs can be included as part of a healthy dietary pattern without concern of elevating CVD risk in healthy individuals. Overall, 1 egg/d was sufficient to promote enhancements in HDL composition and function, and intake of 2–3 eggs/d supported greater enhancements in the function and composition of this lipoprotein.

Acknowledgments
DMD conducted all experiments and wrote the manuscript; GHN and CLM provided support with laboratory analysis and commented on data analysis and the manuscript; CNB provided input on data analysis; CNB and MLF provided input on the writing of the manuscript; MLF was responsible for the study design and statistical analysis and provided input on the completion of the manuscript. All authors read and approved the final manuscript.

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