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Differences in response to egg-derived dietary cholesterol result in distinct lipoprotein profiles while plasma concentrations of carotenoids and choline are not affected in a young healthy population

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ABSTRACT

Background: It has been demonstrated that some individuals (approximately 1/3 of the population) can be classified as hyper-responders to dietary cholesterol.
Objective: The objective of this study was to evaluate whether the response to dietary cholesterol (provided by eggs) would mediate lipoprotein metabolism and the bioavailability of lutein, zeaxanthin, and choline, nutritional components present in eggs.
Methods: We recruited 36 healthy subjects who underwent a two-week washout period in which they consumed zero eggs. Participants were then consecutively fed 1, 2, and 3 eggs/day for 4 weeks each. In this analysis, we compared intake of 0 eggs/day to 3 eggs/day (540 additional mg of dietary cholesterol/day). Plasma lipids, lipoprotein particle subfractions, and plasma lutein, zeaxanthin, and choline were measured. We classified the normal plasma response to dietary cholesterol as an increase of 2.2 mg/dL for each 100 mg of dietary cholesterol/day. Therefore, those individuals with >12 mg/dL increase in plasma cholesterol after the intervention (n = 12) were considered hyper-responders while the remaining 24 subjects presented changes in plasma cholesterol ranging from −16 to 9 mg/dL and were considered normal responders.
Results: Compared to 0 eggs, hyper-responders had significant increases in plasma total, LDL and HDL cholesterol ($p < 0.001$) with no changes in the LDL/HDL ratio following intake of 3 eggs/day. They also had higher concentrations of large LDL ($p < 0.01$) with no changes in small LDL. Interestingly, both hyper- and normal responders had significant increases in large HDL particle concentration (12%) and plasma lutein (17%), zeaxanthin (30%), and choline (12%) ($p < 0.001$) compared to 0 eggs.
Conclusions: These results suggest that egg intake increases the bioavailability of carotenoids and choline present in eggs independently of individual responses to dietary cholesterol. Further, the observed increases in large HDL in all individuals could be associated with HDL being a major transporter of lutein and zeaxanthin in plasma.

1. Introduction

The concern on how dietary cholesterol affects plasma lipoproteins still exists in spite of the removal of the upper limit for dietary cholesterol in the 2015 Dietary Guidelines for Americans [1]. Part of the concern is due to the fact that certain individuals have a more robust response to dietary cholesterol, while the majority of the population does not experience fluctuations in plasma lipoproteins with a cholesterol challenge [2,3].

While the majority of individuals have a feedback mechanism to compensate for higher intake of cholesterol, by either decreasing absorption or reducing cholesterol synthesis [4], some individuals are more sensitive to cholesterol challenges. Individuals who experience an increase of >2.2 mg/dL plasma cholesterol for each 100 mg dietary cholesterol are classified as hyper-responders, while those with a lesser response are considered to have a normal response [5]. We have previously demonstrated such differences in response to dietary cholesterol in individuals challenged with additional 518 [3] or 640 mg of cholesterol for an extended time [2]. While the hyper-responders presented increases in both LDL and HDL cholesterol (about 1/3 of the population), the rest, considered to have the normal response [5], did not show increases in either lipoprotein following the same cholesterol challenge [2,3].

We have also shown that egg intake significantly increases plasma concentrations of the carotenoids lutein and zeaxanthin, and a protective

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role of these antioxidants has been demonstrated in individuals with metabolic syndrome [6,7]. Recent studies from our laboratory have also shown that eggs substantially increase plasma choline [8,9] and that those increases are not related to higher concentrations of trimethylamine oxide (TMAO), a postulated risk factor for heart disease [10]. To date, however, there has not been a study aiming at understanding whether the responses to dietary cholesterol via egg consumption are related to differential responses in plasma choline, lutein, and zeaxanthin in a young population.

For this study, we are analyzing data from our previous report where we challenged a young population with increasing concentrations of 0, 1, 2 and 3 eggs per day for 4 weeks each [7]. We hypothesized that the increases in plasma carotenoids and choline with egg consumption would be independent of the responses to dietary cholesterol.

2. Materials and methods

a. 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) 18.5–29.9 kg/m^2, blood pressure <140/90 mmHg, fasting triglycerides <500 mg/dL, fasting glucose <126 mg/dL, or fasting total cholesterol <240 mg/dL as previously reported [7,8]. Thirty-six participants finished the intervention, which consisted of eating 0 eggs for 2 weeks (washout period) followed by 1 egg/day for 4 weeks, 2 eggs/day for 4 weeks, and 3 eggs/day for 4 weeks. Comparisons for this study are done between 0 eggs and 3 eggs/day to better analyze the hyper-response to dietary cholesterol of some individuals. During each intervention period, participants completed 3-day dietary and exercise records, and fasting blood samples were collected as previously described [7]. Dietary intake was analyzed using Nutrition Data System for Research (NDSR) 2014 (Nutrition Coordinating Centre, University of Minnesota) to quantify carotenoid and choline intake. Participants were classified as hyper-responders to dietary cholesterol if they experienced an increase in plasma cholesterol >12 mg/dL in response to the additional 540 mg of dietary cholesterol provided by the 3 eggs. The dependent variables in this study are plasma cholesterol, carotenoids and choline.

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 NCT02531958.

2.1. Plasma lipids and lipoprotein particles

Plasma lipids were measured by use of Cobas C-111 analyzer and LDL cholesterol (LDL-C) was calculated by the Friedewald equation [11]. Lipoprotein particle number, size, and concentration were determined by Nuclear Magnetic Resonance (NMR) spectroscopy as previously reported [12].

2.2. Plasma lutein and zeaxanthin

Lutein and zeaxanthin were extracted from plasma as described previously [7]. Briefly, analysis was performed by using reverse-phase high performance liquid chromatography (HPLC) using a Shimadzu Prominence UFLC (Shimadzu Corporation, Kyoto, Japan). Carotenoid concentrations were determined by comparing area under the curve of chromatogram peaks to standard curves generated with purified lutein and zeaxanthin standards (Sigma-Aldrich, St. Louis, MO). A standard curve for *trans*-β-apo-8′-carotenal was also generated for determination of carotenoid recovery efficiency.

2.3. Plasma choline

Plasma concentrations of free choline were measured in duplicate on different days by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [13], with modifications based on instrumentation [14]. The inter-assay CV was <3.5% for each metabolite based on duplicate measures and <5% based on in-house controls.

2.3.1. Statistical analysis

All statistical analysis was carried out using SPSS for Windows Version 25 (IBM Corp). Data were analyzed by two-way ANOVA with one factor being diet (0 vs 3 eggs) and the other being response (hyper-response vs normal response). Data are presented as mean ± SD and P values < 0.05 were considered to be significant.

3. Results

3.1. Plasma lipids and LDL/HDL ratio

Plasma total cholesterol, LDL-C, and HDL cholesterol (HDL-C) were significantly increased by intake of 3 eggs/day as compared to 0 eggs only in those individuals classified as hyper-responders ($p < 0.001$). These parameters did not change in participants classified with the normal response (Table 1). Interestingly, for the subjects with a normal response, LDL-C was reduced after the consumption of 3 eggs resulting in a lower LDL/HDL ratio ($p < 0.05$), while the LDL/HDL ratio was maintained for hyper-responders (Table 1).

3.2. LDL and HDL subfraction concentration

Compared to 0 eggs, large LDL particle concentration was significantly increased after 3 eggs only among cholesterol hyper-responders ($p < 0.001$) (Table 2). However, response classification (hyper vs normal) and diet (0 versus 3 eggs) did not affect plasma concentrations of small LDL or small and medium HDL particles ($p > 0.05$). In contrast, large HDL particles were present in higher concentrations after the intake of 3 eggs for all subjects ($p < 0.01$) (Table 2).

3.3. Lutein and zeaxanthin

Plasma lutein and zeaxanthin were increased in all subjects, independent of response classification, as indicated in Fig. 1. Lutein concentrations increased by 17% compared to 0 eggs and zeaxanthin concentrations increased by 30% after the 3 eggs/day period. In contrast, dietary lutein and zeaxanthin did not differ between 0 and 3 eggs per day ($p > 0.05$) indicating that these carotenoids, while present in small amounts in eggs, are highly bioavailable.

3.4. Choline

Plasma choline was higher in all subjects after consuming 3 eggs/day, independent of cholesterol response classification ($p < 0.01$). Similarly, dietary choline was higher during the egg period ($p < 0.05$) (Fig. 2).

4. Discussion

Here, we present data showing that intake of 3 eggs/day results in increases of both LDL-C and HDL-C with no changes in the LDL/HDL ratio as compared to 0 eggs/day [2,3] in individuals who are hyper-responsive to dietary cholesterol. Approximately 1/3 of our study participants fell into this category. An interesting observation in these hyper-responders is that consumption of 3 eggs/day led to the formation of higher concentrations of larger LDL particles, indicating that the increases in cholesterol observed in these individuals tended to distribute into less atherogenic particles [15]. The other 2/3 of the participants experienced no significant change in HDL-C but a significant decrease in plasma LDL-C, resulting in a lower LDL/HDL ratio after the 3 eggs/day period.

The novelty of this study is that we have shown that all individuals, regardless of their response to dietary cholesterol, had increased plasma concentrations of lutein, zeaxanthin, and choline following egg intake.

Table 1
Plasma lipoprotein concentrations in participants classified as hyper-responders or normal responders to dietary cholesterol provided by eggs.

Parameter	Hyper-responders (n = 12)[a]		Normal Response (n = 24)		Diet Effect	Response Effect
	0 Eggs	3 Eggs	0 Eggs	3 Eggs	P value	P Value
Total cholesterol (mg/dL)[b]	152 ± 32[a]	179 ± 25[b]	164 ± 32[a]	155 ± 25[a]	0.001	<0.001
LDL cholesterol (mg/dL)	75 ± 25[a]	92 ± 35[b]	88 ± 23[b]	78 ± 16[a]	NS[c]	<0.001
HDL cholesterol (mg/dL)	61 ± 9[a]	70 ± 11[b]	61 ± 12[a]	62 ± 13[a]	0.001	<0.001
Triglycerides (mg/dL)	80 ± 31[a]	85 ± 37[a]	80 ± 22[a]	72 ± 22[a]	NS	NS
LDL/HDL	1.3 ± 0.8[a]	1.4 ± 0.8[a]	1.5 ± 0.5[b]	1.3 ± 0.4[a]	NS	<0.01

[a]Values are expressed as mean ± SD for the number of subjects indicated in parentheses.
[b] Values in the same row with different superscripts are significantly different ($p < 0.05$ for interaction effect).
[c] NS = non-significant.

Table 2
Plasma lipoprotein particle subfractions in participants classified as hyper-responders or normal responders to dietary cholesterol provided by eggs.

Parameter	Hyper-responders (n = 12)[a]		Normal Response (n = 24)		Diet Effect	Response Effect
	0 Eggs	3 Eggs	0 Eggs	3 Eggs	P Value	P Value
Large LDL (mmol/L)[b]	234 ± 130[a]	440 ± 231[b]	331 ± 174[a]	393 ± 202[a]	0.001	<0.001
Small LDL (mmol/L)	75 ± 25[a]	92 ± 35[a]	88 ± 23[a]	78 ± 16[a]	NS[c]	NS
Large HDL (μmol/L)	9.3 ± 2.5[a]	11.2 ± 3.7[b]	8.8 ± 0.7[a]	9.7 ± 0.7[b]	<0.01	<0.001
Medium HDL (mmol/L)	80 ± 31[a]	85 ± 37[a]	80 ± 22[a]	72 ± 22[a]	NS	NS
Small HDL (mmol/L)	1.30 ± 0.75[a]	1.38 ± 0.79[a]	1.50 ± 0.50[a]	1.3 ± 0.36[a]	NS	NS

[a]Values are expressed as mean ± SD for the number of subjects indicated in parentheses.
b Values in the same row with different superscript are significantly different ($p < 0.05$ for interaction effect).
[c] NS = non-significant.

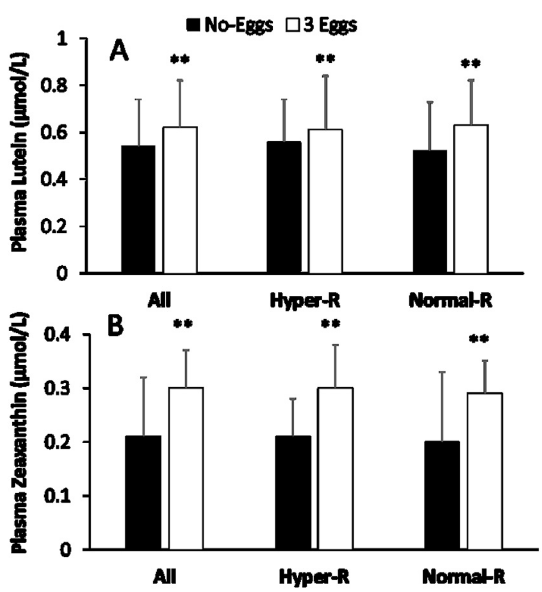

Fig. 1. Plasma lutein (Panel A) and plasma zeaxanthin (Panel B) for all individuals (n = 36) and for those classified as hyper-responders (Hyper-R) (n = 12) or with normal response (Normal-R) (n = 24). ** indicates significantly different at $p < 0.01$.

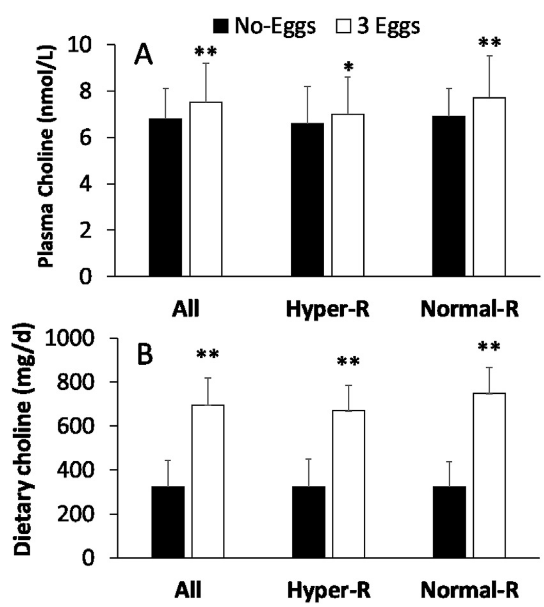

Fig. 2. Plasma (Panel A) and dietary choline (Panel B) for all individuals (n = 36) and for those classified as hyper-responders (Hyper-R) (n = 12) or with normal response (Normal-R) (n = 24). * indicates significantly different at $P < 0.05$ and ** indicates significantly different at $p < 0.01$.

Further, we observed increases in large HDL particle concentrations in all individuals, which has been associated with more effective reverse cholesterol transport [16,17].

An interesting observation in hyper-responders is that consumption of 3 eggs per day for 4 weeks led to the formation of higher concentrations of larger LDL particles indicating that the increases of cholesterol observed in these individuals tend to distribute into less atherogenic particles [18].

Although subjects with normal responses to dietary cholesterol did not present an increase in HDL cholesterol, they did have increases in the concentrations of large HDL and in LCAT activity suggesting a more efficient reverse cholesterol transport. It has been previously demonstrated that HDL particles are major carriers of the xanthophylls lutein and zeaxanthin [19,20]. Therefore, it appears than in all individuals the consumption of eggs leads to the formation of larger HDL, which in turn will be able to transport more lutein and zeaxanthin in plasma. Thus eggs appear to have two distinctive effects that result in increased concentration of these antioxidants in plasma: the fact that lutein and zeaxanthin are present in the lipid matrix in the egg, a situation that facilitates absorption [26] and that they form higher number of large HDL [25].

Eggs are a controversial food because they contain cholesterol, a nutrient that, in spite of the recent dietary recommendations, it is still regarded with suspicion by a number of agencies and certain individuals. On the other hand, they are excellent sources of choline and of highly bioavailable lutein and zeaxanthin [21]. Choline has a key role in cognitive function [22] and is a main structural component of membrane phospholipids [23]. However, most Americans do not meet the DRI for choline [24]. In addition, lutein and zeaxanthin have been shown to protect against atherosclerosis [25] and liver damage [26] in animal models and against inflammation [7] and age-related macular degeneration in humans [27]. Because eggs are a good source of these nutrients which are important for chronic disease prevention, and because our results suggest that egg intake results in anti-atherogenic changes to the plasma lipid profile, these findings increase support for the benefits of eggs in overall health.

5. Conclusions

From this study, we derive two important conclusions: (1) although hyper-responders have a more robust response to dietary cholesterol, they still maintain their LDL/HDL ratio, and the increased plasma cholesterol is distributed amongst the less atherogenic lipoprotein particles; and (2) egg intake provides similar benefits to all individuals in terms of increasing plasma lutein, zeaxanthin, and choline, independent of cholesterol response classification. These findings therefore support the notion that eggs can be a key component of a healthy diet for young, healthy adults, regardless of their physiological response to dietary cholesterol.

Declaration of competing interest

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