Lipoprotein Changes Following Consumption of Lutein-enriched Eggs are Associated with Enhanced Lutein Bioavailability

Aize Kijlstra[1], Sanne M. van der Made[2], Jogchum Plat[2], Tos T.J.M. Berendschot[1,*]

[1]University Eye Clinic Maastricht, Maastricht University Medical Centre, Maastricht, The Netherlands
[2]Department of Human Biology and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands
*Corresponding author: t.berendschot@maastrichtuniversity.nl

Abstract Lutein is concentrated in the retina and since it cannot be synthesized by the human body, its uptake depends on nutritional intake. Lutein-enriched eggs are a good lutein source, but whether changes in lipoprotein status following lutein-enriched egg consumption may affect an individual's lutein response is not yet clear. Data from three intervention trials with lutein-enriched eggs or products made from the enriched egg yolks were combined (n=294) and analyzed to investigate the dynamics of the lutein response in relation to lipoprotein levels. Cross sectional correlation was tested at baseline between lutein and lipoprotein profiles in all participants. Subsequently two groups were selected from the combined database whereby individuals receiving lutein-enriched egg yolks (n=137) were compared with controls not receiving eggs (n=117). Significant correlations between blood lutein concentrations and total cholesterol (r=0.309; p<0.001), HDL-C (r=0.246; p<0.001), LDL-C (r=0.241; p<0.001), ApoA1 (r=0.301; p<0.001), and ApoB100 (r=0.199; p<0.005) concentrations, but not with serum triglycerides were found at baseline. Following a three to twelve month intervention, blood lutein concentrations increased from 238 to 463 ng/ml (p<0.001) in the lutein group, whereas levels in controls remained unchanged. The lutein increase in the lutein-enriched egg group correlated significantly with changes in total cholesterol, HDL-C, LDL-C, ApoA1 and ApoB100 concentrations. To conclude, individuals showing the largest lipoprotein increase following egg consumption were also those with the strongest increase in blood lutein concentration. This indicates that therapies directed at altering lipoprotein levels may indirectly affect lutein bioavailability.

Keywords: *LDL-cholesterol, HDL-cholesterol, apolipoprotein A-I, apolipoprotein B100, carotenoid, xanthophyll, dietary intervention*

Cite This Article: Aize Kijlstra, Sanne M. van der Made, Jogchum Plat, and Tos T.J.M. Berendschot, "Lipoprotein Changes Following Consumption of Lutein-enriched Eggs are Associated with Enhanced Lutein Bioavailability." *Journal of Food and Nutrition Research*, vol. 5, no. 6 (2017): 362-369. doi: 10.12691/jfnr-5-6-2.

1. Introduction

Lutein, together with zeaxanthin and meso-zeaxanthin belongs to the three carotenoids that constitute macular pigment in primate retina's [1]. The macular localization of lutein serves important functions including light absorption, oxygen radical scavenging and regulation of inflammation [2]. Evidence for the importance of macular pigment comes from the observation that eliminating lutein from the diet of monkeys leads to degenerative changes in their retinas [3] and that patients with acquired macular pigment loss (Macular Telangiectasia, Sjögren-Larsson syndrome) suffer from serious visual handicap [4,5] and show signs of macular degeneration at an early age [6].

Both epidemiological and intervention studies clearly support a beneficial role for lutein in preventing the development of age related macular degeneration [1]. Lutein cannot be synthesized by humans and its presence in the human body thus depends on the consumption of dietary sources like certain fruits, vegetables or animal products such as eggs [7,8]. To prevent progression of age related macular degeneration (AMD), many ophthalmologists are currently recommending patients to take commercially available over-the-counter supplements containing lutein, as based on the evidence from the Age-Related Eye Disease Study 2 (AREDS2) [9,10,11]. Alternatively, individuals may be advised to increase their consumption of carotenoid rich foods including dark green, leafy vegetables [12] or to take functional foods such as lutein-enriched eggs or products prepared from these egg yolks [13,14,15]. In the past decade we have conducted studies to investigate the effect of lutein-enriched egg yolks on various lutein response parameters including blood lutein concentrations, macular pigment response and visual function parameters [13,16]. In view of the fact that consumption of egg yolks increases dietary cholesterol intake, we concomitantly assessed the cholesterol status of the participants of our studies [14,17,18]. These studies showed that the daily consumption of lutein-enriched egg yolks can lead to a doubling of blood lutein concentrations,

as well as a significant improvement in macular pigment optical density (MPOD) and visual acuity, without markedly elevating circulating lipids and lipoproteins [14,16,17,18].

Lutein is transported through the circulation incorporated into lipoproteins [19,20,21]. Controversial results have been reported, whereby blood lutein concentrations were either found to correlate with high-density lipoprotein cholesterol (HDL-C) alone [22,23] or with both HDL-C and low-density lipoprotein cholesterol (LDL-C) [20,24,25]. The reasons for this controversy are not yet clear and merit further investigation. Whether changes in lipoprotein status following lutein-enriched egg consumption may affect the individual lutein response is also not yet clear. Therefore, the present work was carried out to investigate whether changes in lipoprotein status following lutein-enriched egg consumption may affect the individual lutein response. Our study confirms the correlation of blood lutein concentrations with both HDL-C and LDL-C concentration, whereas a novel finding was the observation that individuals with the largest HDL-C and LDL-C increase following egg consumption were also those with the strongest increase in their blood lutein concentration.

2. Methods

2.1. Subjects

Three studies performed in the Maastricht University Medical Centre, Maastricht, The Netherlands aimed at assessing the use of specialty eggs on blood lutein status, were reanalyzed to study the role of lipoprotein profiles on lutein bioavailability [13,14,16,18]. All the studies had in common that the blood lutein concentrations as well as the lipoprotein profiles were performed by the same labs using the same methods. The specialty eggs were produced by the same provider (Newtricious R&D, Oirlo, The Netherlands) and were obtained by feeding laying hens with a special feed enriched with natural lutein and zeaxanthin. Dairy drinks used as vehicles for the specialty egg yolks were also produced by Newtricious R&D.

The first study (Short Egg Study-I) was performed from September 2007 to February 2008 and included healthy individuals, who were 18 years and older [13,17]. Exclusion criteria included smoking, diabetes, having heart disease, lipid metabolic diseases, ocular media opacities or other ocular diseases and egg allergy. Individuals taking supplements containing lutein and/or zeaxanthin in the past six months, and those with a body mass index > 30 kg/m2 were also excluded. Subjects with a high MPOD score above 0.55 were also excluded. This was done to be able to detect a possible MPOD increase following lutein supplementation. Mean MPOD in the Dutch population is 0.33 (SD: 0.187) [26]. A total of 97 subjects (male: n=42; female: n=55) with a mean age of 47 years were included for the current analysis. Participants in this first study were randomly divided into five intervention groups. The study compared the effect of the daily consumption of specialty eggs, either enriched for zeaxanthin (n=19) or lutein (n=19) (two groups), with a group receiving a buttermilk drink containing lutein-enriched egg yolks (n=20; 1 mg lutein per egg yolk), a group consuming normal eggs (n=19) and an untreated control group (n=20) [13,17]. Subjects participating in this "Short Egg Study-I" consumed the eggs or egg containing buttermilk drink on a daily basis for a total time period of twelve weeks. Lutein and lipoprotein/lipid concentration in the blood was measured at baseline and at the end of the study.

The second study (Long Egg Study) was performed between October 2009 and December 2011 and was a one-year, randomized, double blind, placebo-controlled intervention trial in elderly subjects with ocular drusen and/or retinal pigment abnormalities who had not yet been diagnosed with AMD [16]. Subjects were included if they were 50 years or older and if they had drusen and/or retinal pigment epithelium alterations in at least one eye as evidenced by fundus photographs. Visual acuity had to be >0.5, and participants should not exhibit ocular media opacities. Subjects were not allowed to use nutritional supplements containing lutein or zeaxanthin. Further exclusion criteria included diabetes, cardiovascular disease, lipid metabolic disease demanding lipid-lowering treatment and egg allergy. A total of 89 subjects (male: n=29; female: n=60) with a mean age of 62 years were included for the current analysis. Subjects analyzed at baseline in the experimental (lutein) group (n=47) took a lutein-enriched egg-yolk containing dairy drink (1.5 yolks; 1.4 mg lutein) whereas controls (n=42) received a similar color matched buttermilk drink without egg yolks. Subjects participating in this "Long Egg Study" consumed the control or egg yolk containing buttermilk drink (80 ml) on a daily basis for a total time period of twelve months. Blood lutein and lipoprotein/lipid concentration were measured at baseline and at the end of the study.

The third study (Short Egg Study II) was similar to the first study but was performed in an older age group of slightly hypercholesteremic individuals and was focused on the type of dairy drink (buttermilk or skimmed milk) as a vehicle [18]. Subjects were included if they were between 18 and 70 years old, had a fasting serum total cholesterol concentration between 5.3 – 8.6 mmol/l, a fasting plasma glucose < 7.0 mmol/l, a BMI between 23 – 34 kg/m2, were non-smoking and were willing to stop their usual egg consumption for the duration of the study. Exclusion criteria as detailed in the original study [18] included unstable body weight (weight gain or loss of more than 3 kg in the past 3 months), egg allergy or intolerance, use of medication or diet affecting serum lipid and/or glucose metabolism, cardiovascular disease, unwillingness to stop consumption of vitamin supplements, fish oil capsules or products rich in plant stanol or sterol esters 3 weeks before the start of the study, alcohol abuse defined as consumption of > 21 alcohol consumptions a week for men and > 14 for women, drug abuse, pregnancy/breastfeeding or having donated blood within one month prior to the start of the study or planning a donation during the study. A total of 108 subjects (male: n=55; female: n=53) with a mean age of 58 years were included for the current analysis. It included four groups taking a daily portion of 80 ml skimmed milk with (n=27) or without (n=27) lutein-enriched egg yolks (1.5 yolks; 1.4 mg lutein) or 80 ml buttermilk with (n=26) or without (n=28) lutein-enriched egg yolks (1.5 yolks; 1.4 mg lutein) during a 12-week period.

Participants for the three studies mentioned above were recruited through advertisements in local newspapers, university-, and hospital buildings. Flow diagrams showing the enrollment, randomization and distribution of participants are described in our earlier reports [13,14,16,17,18]. Each study was conducted according to the declaration of Helsinki and was approved by the Medical Ethical Committee of Maastricht University Medical Centre. Informed consent was obtained from all participants and the studies were registered at ClinicalTrials.gov (Short Egg Study I: NCT00527553; Short Egg Study II: NCT01566305; Long Egg Study: NCT00902408).

2.2. Blood Sampling and Chemical Analysis

Fasting blood samples were taken after an overnight fast by venipuncture using 10 mL serum and EDTA coated tubes (Becton, Dickinson and Company, Franklin Lakes, NY, USA).

Lutein concentrations in blood were analyzed using high performance liquid chromatography (HPLC) as described elsewhere [18]. The concentration of serum total cholesterol (CHOD-PAP method; Roche, Basel, Switzerland), HDL cholesterol (Phosphotungstate precipitation method; Roche, Basel, Switzerland), triglycerides (GPO-Trinder; Sigma Diagnostics, St. Louis, USA) and apolipoproteins A-I and B100 (UNI-KIT ApoAI and ApoB100; Roche, Basel, Switzerland) were determined according to the manufacturer's instructions. Serum LDL cholesterol concentration was calculated using the Friedewald equation.The title should be formatted in an hourglass style; the first line longer than the second, the second line shorter than the third. Use numerical superscript callouts as shown in this template to link authors with their affiliations. Corresponding author should be denoted with an asterisk as shown. Email address is compulsory for the corresponding author.

2.3. Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corporation, Armonk, NY, USA). Pearson's correlation analyses were performed to investigate the relation between various parameters. Differences between the start and endpoint of various parameters were determined by the independent and paired samples T test. Multivariate linear regression analysis was performed to compare the relative contribution of various parameters on the lutein changes following lutein-enriched egg consumption.

3. Results

We reanalyzed data from three earlier studies performed by our group concerning the supplementation of volunteers with lutein-enriched egg yolks. The association between lutein dynamics following supplementation with lipoprotein status was however not yet analyzed in these studies and was therefore the subject of the study presented here. To obtain a larger sample size, we combined data from these three studies.

Table 1. Baseline characteristics of the combined studies

	N	Minimum	Maximum	Mean	Std. deviation
Age (years)	294	18.4	89.0	55.5	12.9
Lutein (ng/ml)	294	45.5	1080.5	230.5	132.2
Cholesterol (mmol/l)	294	3.16	9.93	6.07	1.12
HDL (mmol/l)	294	0.89	4.38	1.70	0.44
LDL (mmol/l)	294	1.12	7.39	3.82	1.00
ApoA1 (g/l)	294	0.96	2.41	1.45	0.24
ApoB100 (g/l)	294	0.36	1.74	1.02	0.23
Triglycerides (mmol/l)	292	0.25	3.68	1.21	0.64

In total, the three combined studies included 294 individuals with a mean age of 56 years. More females (n=168) were included than males (n=126). Data collected from all participants included blood lutein concentrations, various lipids and (apo)lipoproteins, and age of participants (Table 1).

3.1. Baseline Correlations

Pearson correlation analysis showed that baseline blood lutein concentrations correlated significantly with all lipoprotein and lipid parameters tested, i.e. serum total cholesterol (r=0.309; p<0.001), HDL-C (r=0.246; p<0.001), LDL-C (r=0.241; p<0.001), ApoA1 (r=0.301; p<0.001), and ApoB100 (r=0.199; p<0.005) concentrations, except serum triglyceride concentrations (Table 2; Figure 1-Figure 2). ApoA1 as the main surface protein of HDL-C and ApoB100 as the main surface protein of LDL-C showed significant correlations with each other (HDL-C/ApoA1: r = 0.83; P < 0.001 and LDL-C/ApoB100: r = 0.86; P < 0.001). Linear regression analysis showed that 6% of baseline lutein levels could be predicted by either HDL-C or LDL-C. Baseline lutein levels were best predicted (13.5%) by a model including HDL-C and LDL-C with adjustment for age and gender (p<0.001).
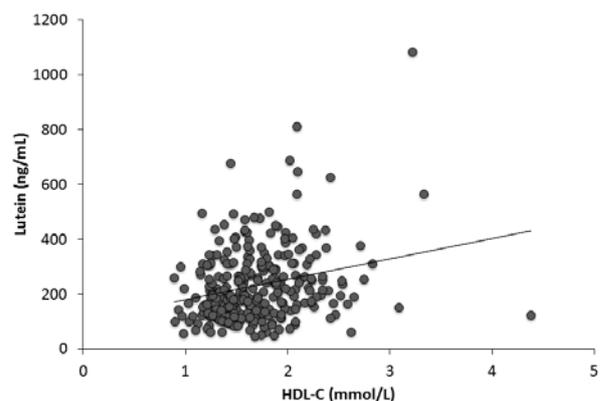

Figure 1. Correlation between baseline blood lutein levels and HDL-C (r2=0.061) in the combined study group (n=294)

3.2. Effects of Lutein Supplementation in Egg Yolks

The effect of lutein supplementation via the consumption of lutein-enriched specialty eggs showed a rapid rise of blood lutein concentration in all groups (Figure 3). The "Short Egg Study I" (1 mg daily lutein intake; 1.0 yolk/day) showed that lutein concentrations already started leveling off at six weeks following

intervention. Individuals in this study who received eggs (zeaxanthin enriched egg or normal egg) with a low lutein content (0.2 mg), only showed a moderate increase in their blood lutein levels at the end of the study period (12 weeks). The highest increase was seen in the "Short Egg Study II" group, whereby blood lutein concentrations increased from 273 ng/ml at baseline to 574 ng/ml at three months following the daily consumption of 1.4 mg lutein in a dairy drink containing lutein-enriched egg yolks (1.5 yolks/day). No difference was observed whether the eggs were dispersed in skimmed milk or buttermilk as already described in the original publication on this study [18]. Individuals in the Long Egg Study who received a daily egg yolk containing buttermilk beverage (1.4 mg lutein; 1.5 egg yolks/day) showed an increase in blood lutein concentrations from 206 ng/ml at baseline to 399 ng/ml at the end of the study (12 months), whereas blood lutein concentrations in the control group did not show any significant changes.

The analysis of the association between lipoprotein profiles and blood lutein changes following consumption of the lutein-enriched egg yolks was done by dividing the combined study participants into two groups. The lutein treated group contained individuals receiving lutein-enriched egg yolks (n=137), whereby we excluded the subgroups of the Short Egg Study I, that received a zeaxanthin enriched egg yolk (n=19) or a normal egg yolk (n=19) since these eggs only contained a low lutein (0.2 mg) content. The control placebo group (n=117) included all participants that received a dairy beverage without egg yolks and those from the control group from the Short Egg Study I that were left untreated.

Mean age of both the experimental lutein egg yolk and the placebo groups was similar, whereas the lutein group included more females than the placebo group. Baseline concentrations of lutein, serum lipids and lipoproteins parameters (Table 3) were not significantly different between the placebo and lutein groups. Endpoint lutein concentration was significantly higher in the lutein intervention group as compared to the placebo group (p<0.001). Endpoint concentrations of the other parameters were not different between the two groups. Within group analysis for the placebo group showed that only the HDL-C concentration was slightly increased at the end of the study (p=0.034), although this significance was lost following Bonferroni correction for multiple comparisons.
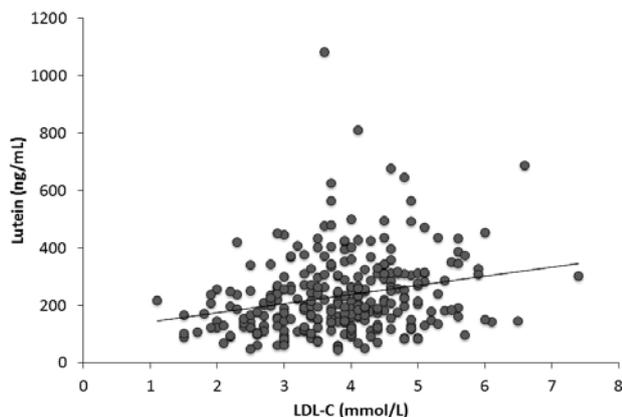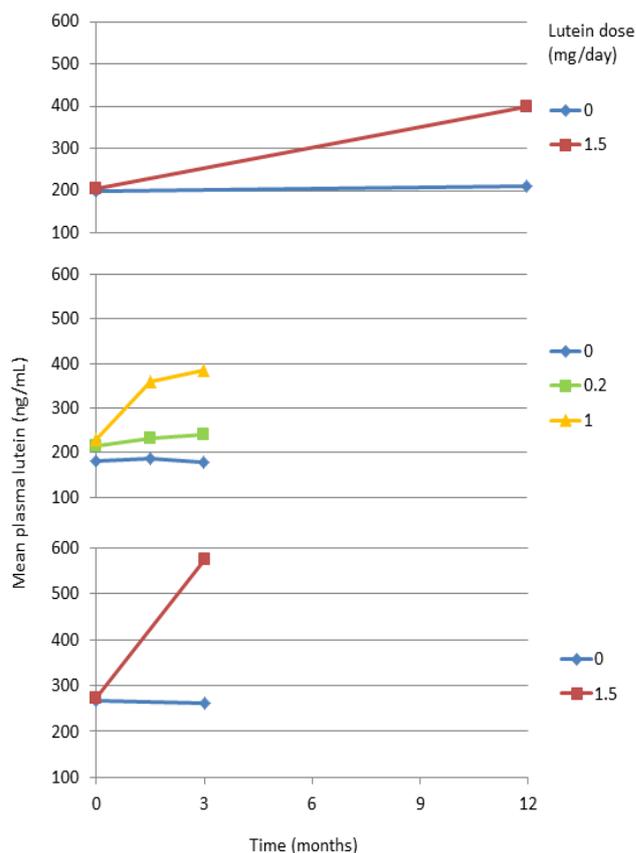

Figure 3. Effect of supplementation with lutein-enriched eggs on blood lutein concentration. LES: Long Egg Study; SES-I: Short Egg Study I; SES-II: Short Egg Study II. In SES-I, the data of the two groups receiving either a zeaxanthin enriched egg yolk or a normal egg yolk (lutein content 0.2 mg) were combined and are shown as a green line

Blood lutein concentrations in the combined lutein intervention group increased significantly from 238 ng/ml to 463 ng/ml (p<0.001; Table 3). There was also a significant 3% increase in serum total cholesterol concentrations and a significant 4% increase in serum LDL-C and ApoB100, whereas no significant changes were observed for serum HDL-C, ApoA1 or triglycerides (Table 3).

We subsequently investigated whether the observed lutein increase in the lutein intervention group was associated with the lipoprotein status at the endpoint of the studies. To compensate for the difference between intake of one or one and a half egg yolk in the different groups, we normalized the lutein increase for each participant by calculating the ratio of the blood lutein change in time versus daily lutein intake (Δ lutein/daily lutein intake; (ng/ml)/(mg)). The association between lipids and lipoproteins on the increase in lutein blood level following consumption of lutein-enriched eggs was analyzed using Pearson correlation analysis and showed a significant positive correlation between the normalized lutein change with LDL and total cholesterol but not with HDL-C at endpoint for the whole group (Table 4, Figure 4). Comparison of males with females showed that the associations observed for the whole group could almost completely be attributed to the female participants. ApoB100, as the main surface protein of LDL particles, also showed a concomitant correlation with the blood lutein increase following supplementation.

Figure 2. Correlation between baseline blood lutein concentration and LDL-C (r2=0.058) in the combined study group (n=294)

Table 2. Correlations between baseline blood lutein, various lipoproteins and lipids in the combined studies

		Lutein	Total Cholesterol	HDL-C	Apo AI	LDL-C	Apo B100	Triglycerides
	Pearson Correlation	1	0.309**	0.246**	0.301**	0.241**	0.199**	-0.015
Lutein	Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.001	0.797
	N	294	294	294	294	294	294	292
	Pearson Correlation		1	0.182**	0.247**	0.927**	0.846**	0.384**
Total Cholesterol	Sig. (2-tailed)			0.002	0.000	0.000	0.000	0.000
	N		294	294	294	294	294	292
	Pearson Correlation			1	0.833**	-0.123*	-0.146*	-0.390**
HDL-C	Sig. (2-tailed)				0.000	0.036	0.012	0.000
	N			294	294	294	294	292
	Pearson Correlation				1	-0.021	-0.006	-0.237**
ApoA1	Sig. (2-tailed)					0.717	0.917	0.000
	N				294	294	294	292
	Pearson Correlation					1	0.858**	0.312**
LDL-C	Sig. (2-tailed)						.000	0.000
	N					294	294	292
	Pearson Correlation						1	0.523**
ApoB100	Sig. (2-tailed)							0.000
	N						294	292
	Pearson Correlation							1
Triglycerides	Sig. (2-tailed)							
	N							292

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 3. Concentrations of blood lutein, lipids and lipoproteins at the start and end of the study for the combined control and lutein-enriched egg groups

	Combined control groups						P value	Lutein-enriched egg groups						P value
	Baseline			End of Study				Baseline			End of Study			
Lutein (ng/ml)	226.2	±	110.6	229.0	±	135.6	0.754	238.2	±	153.9	462.9	±	243.5	<0.001
Cholesterol (mmol/l)	6.13	±	1.10	6.20	±	1.11	0.196	6.15	±	1.15	6.34	±	1.19	0.003
HDL (mmol/l)	1.70	±	0.37	1.73	±	0.41	0.034	1.69	±	0.48	1.71	±	0.44	0.376
LDL (mmol/l)	3.88	±	0.96	3.91	±	0.99	0.538	3.88	±	1.05	4.05	±	1.07	0.003
ApoA1 (g/l)	1.43	±	0.22	1.46	±	0.23	0.066	1.43	±	0.24	1.45	±	0.24	0.263
ApoB100 (g/l)	1.05	±	0.23	1.05	±	0.24	0.489	1.03	±	0.24	1.06	±	0.23	0.004
Triglycerides (mmol/l)	1.22	±	0.56	1.24	±	0.63	0.727	1.27	±	0.70	1.27	±	0.76	0.809
Males/Females	52	/	65					55	/	82				
Mean Age (years)		57.2							55.4					

P values (two tailed) were calculated using the Paired Samples T Test.

Table 4. Correlations between normalized lutein changes[1] and endpoint lipoprotein concentration following supplementation with lutein enriched eggs

	Females (N=82)		Males (N=55)		All (N=137)	
	Correlation	P value	Correlation	P value	Correlation	P value
Cholesterol	0.344**	0.002	0.203	0.137	0.293**	0.001
HDL-C	0.168	0.130	0.029	0.836	0.128	0.136
ApoA1	0.190	0.088	0.008	0.956	0.134	0.117
LDL-C	0.318**	0.004	0.227	0.096	0.280**	0.001
ApoB100	0.296**	0.007	0.266*	0.049	0.278**	0.001
Triglycerides	-0.047	0.681	-0.028	0.841	-0.041	0.643

[1]Lutein changes were calculated as Δ lutein/daily lutein intake; (ng/ml)/(mg))
*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

Table 5. Correlations between normalized lutein changes[1] (Δ lutein) and lipoprotein changes (Δ) between endpoint and baseline following supplementation with lutein enriched eggs.

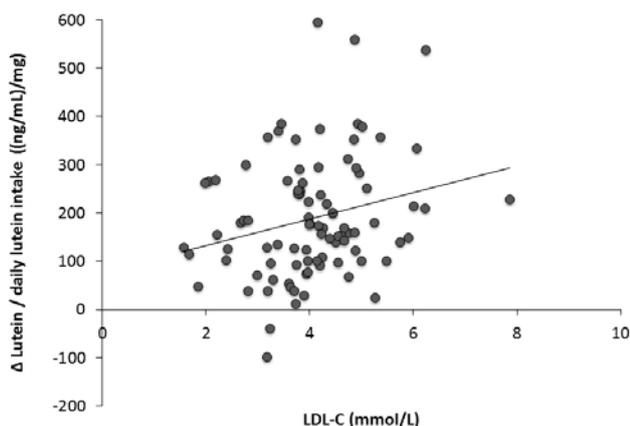|	Females (N=82)		Males (N=55)		All (N=137)	
	Correlation	P value	Correlation	P value	Correlation	P value
Δ Cholesterol	0.421**	0.000	0.315*	0.019	0.383**	0.000
Δ HDL-C	0.260*	0.018	0.063	0.649	0.207*	0.015
Δ ApoA1	0.333**	0.002	0.169	0.216	0.281**	0.001
Δ LDL-C	0.384**	0.000	0.313*	0.020	0.358**	0.000
Δ ApoB100	0.324**	0.003	0.375**	0.005	0.341**	0.000
Δ Triglycerides	-0.096	0.404	0.104	0.458	-0.015	0.869

Figure 4. Correlation between endpoint LDL-C (mmol/l) and normalized lutein change (Δ lutein/daily lutein intake ((ng/ml)/mg)) following lutein supplementation with lutein-enriched eggs (r^2=0.079; males and females combined)

3.3. Which Factors Predict Changes in Lutein Concentrations after Egg Yolk Intake?

We next questioned whether the lipoprotein profile at the beginning of the study might predict the lutein response following supplementation with lutein-enriched eggs. After performing a correlation analysis, none of the parameters tested at baseline were associated with the normalized lutein increase following consumption of lutein-enriched eggs (data not shown). Since LDL-C concentration at endpoint did correlate with the normalized lutein change following intervention, we investigated whether lipoprotein changes between the start and endpoint correlated with normalized lutein change. The lutein increase (Δ lutein) correlated significantly with all Δ lipoproteins tested during the intervention for the whole group (Table 5, Figure 5). The associations were strongest for females whereas in males significant associations were confined to Δ total cholesterol, ΔLDL-C and ΔApoB100. When comparing HDL-C with LDL-C changes, the strongest association (r= 0.358; p<0.001) was observed between normalized Δlutein and ΔLDL-C (Figure 5). Linear regression analysis showed that a model including LDL-C and HDL-C changes predicted 21.6% (p<0.001) of normalized lutein changes in females, but not in males. Predictions for both males and females showed that 17.2% (p<0.001) of normalized Δ lutein could be predicted by including both HDL-C and LDL-C in the model.

4. Discussion

This study shows that circulating blood lutein concentrations can double following consumption of lutein-enriched eggs or products prepared from these egg yolks and that this response is positively correlated with concomitant changes in serum lipoprotein levels. A cross sectional analysis confirmed earlier data from the Netherlands [24] and France [25] showing that blood lutein levels are associated with both HDL-C and LDL-C, but are in disagreement with earlier studies from Ireland [22] and the US [23,27] that showed that lutein levels were only associated with HDL-C, but not with LDL-C. The discrepancy between the Dutch and Irish data is probably not due to age differences between the studies, but may be due to the fact that the latter study [22] also included statin users, whereas this was not the case in our study. The discrepancy with the US studies may partially be explained by the fact that one of these studies [23] included young individuals (mean age: 23 years), whereas the mean age of participants in the our studies was 55.5 years. Mean LDL-C concentrations were lower in the US than in the current study. A higher LDL-C concentration in our study may allow a higher transport capacity for lutein and may thus explain why we did show a correlation between lutein and LDL-C. Another study from the US [27] investigated an older population (mean age 79 years) and the lack of an association between lutein and LDL-C levels might be due to a smaller sample size (n=33) as compared to our study (n=294). This latter study did show that LDL-C concentrations increased by 3.2 % following daily egg consumption, which is similar to the increase observed in our study. None of the studies mentioned above investigated the interaction between lipoprotein changes following egg consumption with the individual lutein response.

Our findings are in agreement with an earlier study that showed that the lipoprotein response following egg consumption associates with the blood carotenoid response [28]. Subjects for this study were selected on the basis of their plasma cholesterol response to egg consumption and included 10 men and 10 women classified as hyperresponders and 10 men and 10 women classified as hyporesponders. Following a daily consumption of one egg containing 600 µg lutein and zeaxanthin combined, the lutein concentration in blood increased by 182 and 147 ng/ml in the female and male hyperresponders, respectively, while the female and male hyporesponders had an increase of 91 and 79 ng/ml, respectively [28]. The lutein response in cholesterol hyperresponders was thus twice as high as in the hyporesponders. Similar to our study, the association between lipoprotein and lutein changes following egg consumption were strongest in females. The study was different from ours in that we investigated correlations between lipoprotein changes and lutein response whereas the responder study investigated the lutein response in preselected cholesterol responders following egg consumption.
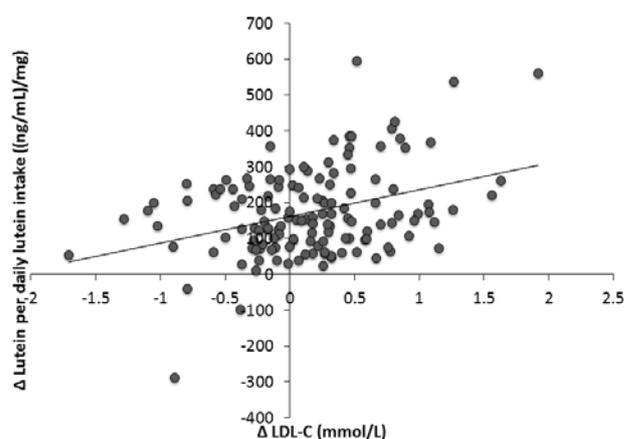

Figure 5. Correlation between lutein change (Δ lutein/daily lutein intake; (ng/ml)/mg) with change in LDL-C concentration (Δ LDL-C mmol/l) between endpoint and baseline following supplementation with lutein-enriched eggs (r^2=0.138; males and females combined)

Eggs are a good source of lutein whereby lutein responses following egg consumption are about two to three times higher as compared to lutein responses from a supplement or from spinach, which can be explained by the fact that eggs have a simple food matrix and due to the fat containing environment [29,30]. Following consumption of eggs, lutein is incorporated into mixed micelles in the lumen of the gastrointestinal tract, of which lutein is subsequently taken up by enterocytes [31]. This uptake from the mixed micelles is most likely a transporter-mediated process in which SRB1 situated in the apical enterocyte membrane is postulated as a most likely candidate [32]. In the enterocyte, lutein can possibly be cleaved by two enzymes: β,β-carotene-15,15'-monooxygenase (BCMO1) [33] and β,β-carotene-9',10'-oxygenase (BCO2) [34,35]. The remaining lutein is either bound within the enterocyte to lipid poor ApoA1 particles and secreted via a so called direct intestinal pathway or is incorporated into chylomicrons (chylomicron pathway) that are transferred from the enterocyte into the lymph and subsequently released into the bloodstream [36]. In the bloodstream, triglycerides in the chylomicrons are degraded by lipoprotein lipase and the remnants are rapidly taken up by the liver [37]. The question is whether chylomicrons themselves also deliver lutein to target tissues t, since genes involved in the processing of chylomicrons have been shown to be associated with lutein bioavailability [38]. Inside the hepatocytes the available lutein is incorporated into HDL-C or very low-density lipoprotein (VLDL) particles, which are then released into the bloodstream. In the bloodstream, the VLDL particles are converted to LDL particles. Following lutein supplementation, the lutein associated chylomicron levels peak between 4 and 8 hours [38], whereas lutein associated HDL-C and LDL-C peaks between 16 and 48 hours [31]. As a polar carotenoid, lutein is considered to reside on the surface of lipoproteins and may therefore easily undergo transfer between LDL and HDL particles [31,39].

HDL-C plays an important role in the transport of lutein to the retina as shown by experiments in the WHAM chick, which has a genetic defect in HDL-C assembly leading to a very low concentration of HDL-C in plasma as well as in the retina [40]. Data from this model as well as other findings indicate that selective retinal uptake of lutein is mediated by binding of the HDL-C lutein complex via the scavenger receptor class B member 1 (SRB1) receptor in the macula [41]. LDL levels are not altered in the WHAM chickens and the observation that other organs such as the heart or the eggs produced by these chickens, did not have a lower lutein level supports a role for LDL mediated lutein transport to these sites [40].

Taken together, the data support a role for both HDL-C and LDL-C in the transport of lutein in the blood. The exact mechanisms explaining the association between the lutein increase and HDL/LDL increase following egg consumption is however not yet clear. Our observations could merely reflect a higher transport capacity. Further experiments analyzing lutein distribution among various lipoprotein subtypes [20] following consumption of lutein-enriched eggs in cholesterol hyper and hyporesponders may shed more light on this issue. Lutein bioavailability has been shown to be genetically controlled [38,42], involving genes that are able to either control

lutein as well as chylomicron metabolism. Our study, as well as others concerned with the lutein response following egg consumption, has not taken the genetic background of the participants into account, but actually this should be considered in future studies.

In view of the close relationship between lipoprotein and lutein metabolism, future investigations should also be directed at possible adverse effects of cholesterol lowering agents on lutein bioavailability. Various drugs affecting cholesterol metabolism have been shown to be able to either increase or decrease blood lutein levels, which may depend on whether intestinal or other pathways have been affected [36,43]. Long-term statin use has been shown to be associated with a lower MPOD [23]. Ezitimibe, which is a selective intestinal cholesterol inhibitor, has been shown to inhibit lutein absorption in an in vitro model using Caco-2 cells [32]. Whether this drug affects lutein bioavailability in humans has not yet been reported. Many other drugs as well as functional foods affecting lipoprotein pathways are currently being employed on a large scale to prevent atherosclerosis. As yet, their effect on intestinal uptake of lutein and its subsequent transport to various organs, including the macular region of the eye, are however unclear and merit further investigation.

Acknowledgements

References

[1] Bernstein, P.S., Li, B., Vachali, P.P., Gorusupudi, A., Shyam, R., Henriksen, B.S. and Nolan, J.M., "Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease," *Prog. Retin. Eye Res*, 50, 34-66, Jan. 2016.

[2] Kijlstra, A., Tian, Y., Kelly, E.R. and Berendschot, T.T., "Lutein: More than just a filter for blue light," *Prog. Retin. Eye Res*, 31 (4), 303-315, Jul. 2012.

[3] Neuringer, M., Francis, P.J., Renner, L., Weiss, A. and Jeffrey, B.J., "*Atrophic macular degeneration in rhesus monkeys deficient in xanthophylls and n-3 fatty acids*," *Invest. Ophthalmol. Vis. Sci*, 51, 2785, 2010.

[4] Charbel Issa, P., van der Veen, R.L., Stijfs, A., Holz, F.G., Scholl, H.P., and Berendschot, T.T., "Quantification of reduced macular pigment optical density in the central retina in macular telangiectasia type 2," *Exp. Eye Res*, 89 (1), 25-31, Jun. 2009.

[5] van der Veen, R.L., Fuijkschot, J., Willemsen, M.A., Cruysberg, J.R., Berendschot, T.T. and Theelen, T., "Patients with sjogren-larsson syndrome lack macular pigment," *Ophthalmology,* 117 (5), 966-971, Jun. 2010..

[6] Theelen, T., Cruysberg, J.R., and Willemsen, M.A., "Macular fibrosis complicating macular pigment deficient maculopathy in sjogren-larsson syndrome," *Acta Ophthalmol,* 94 (7), e663-e664, Nov.2016.

[7] Calvo, M.M., "Lutein: A valuable ingredient of fruit and vegetables," *Crit. Rev. Food Sci. Nutr*, 45 (7-8), 671-696, 2005.

[8] Handelman, G.J., Nightingale, Z.D., Lichtenstein, A.H., Schaefer, E.J. and Blumberg, J.B., "Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk," *Am. J. Clin. Nutr*, 70 (2), 247-251, Aug. 1999.

[9] Aronow, M.E. and Chew, E.Y., "Age-related eye disease study 2: Perspectives, recommendations, and unanswered questions,". *Curr. Opin. Ophthalmol,* 25 (3), 186-190, May 2014.

[10] Broadhead, G.K., Grigg, J.R., Chang, A.A. and McCluskey, P., "Dietary modification and supplementation for the treatment of

age-related macular degeneration," *Nutr. Rev* 73 (7), 448-462, Jul. 2015.

[11] Age-Related Eye Disease Study 2 Research Group, "Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: Areds2 report no. 3," *JAMA ophthalmology* 132 (2), 142-149, Feb. 2014.

[12] Seddon, J.M., Ajani, U.A., Sperduto, R.D., Hiller, R., Blair, N., Burton, T.C., Farber, M.D., Gragoudas, E.S., Haller, J., Miller, D.T., et al., "Dietary carotenoids, vitamins a, c, and e, and advanced age-related macular degeneration. Eye disease case-control study group," *JAMA* 272 (18), 1413-1420, Nov. 1994.

[13] Kelly, E.R., Plat, J., Haenen, G.R., Kijlstra, A. and Berendschot, T.T., "The effect of modified eggs and an egg-yolk based beverage on serum lutein and zeaxanthin concentrations and macular pigment optical density: Results from a randomized trial," *PLoS One* 9 (3), e92659, 2014.

[14] van der Made, S.M., Kelly, E.R., Berendschot, T.T., Kijlstra, A., Lutjohann, D. and Plat, J., "Consuming a buttermilk drink containing lutein-enriched egg yolk daily for 1 year increased plasma lutein but did not affect serum lipid or lipoprotein concentrations in adults with early signs of age-related macular degeneration," *J. Nutr.* 144 (9), 1370-1377, Sep. 2014.

[15] Nolan, J.M., Meagher, K.A., Howard, A.N., Moran, R., Thurnham, D.I. and Beatty, S., "Lutein, zeaxanthin and meso-zeaxanthin content of eggs laid by hens supplemented with free and esterified xanthophylls," *Journal of nutritional science*, 5, e1 2016.

[16] van der Made, S.M., Kelly, E.R., Kijlstra, A., Plat, J. and Berendschot, T.T., "Increased macular pigment optical density and visual acuity following consumption of a buttermilk drink containing lutein-enriched egg yolks: A randomized, double-blind, placebo-controlled trial," *Journal of ophthalmology,* 9035745, 2016.

[17] Baumgartner, S., Kelly, E.R., van der Made, S., Berendschot, T.T., Husche, C., Lutjohann, D. and Plat, J., "The influence of consuming an egg or an egg-yolk buttermilk drink for 12 wk on serum lipids, inflammation, and liver function markers in human volunteers," *Nutrition,* 29 (10), 1237-1244, Oct. 2013.

[18] Severins, N., Mensink, R.P. and Plat, J., "Effects of lutein-enriched egg yolk in buttermilk or skimmed milk on serum lipids & lipoproteins of mildly hypercholesterolemic subjects," *Nutr. Metab. Cardiovasc. Dis*, 25 (2), 210-217, Feb. 2015.

[19] Romanchik, J.E., Morel, D.W. and Harrison, E.H., "Distributions of carotenoids and alpha-tocopherol among lipoproteins do not change when human plasma is incubated in vitro," *J. Nutr,* 125 (10), 2610-2617, Oct.1995.

[20] Goulinet, S.and Chapman, M.J., "Plasma ldl and hdl subspecies are heterogenous in particle content of tocopherols and oxygenated and hydrocarbon carotenoids. Relevance to oxidative resistance and atherogenesis. Arterioscler," *Thromb. Vasc. Biol,* 786-796, 17 (4), Apr. 1997..

[21] Cardinault, N., Abalain, J.H., Sairafi, B., Coudray, C., Grolier, P., Rambeau, M., Carre, J.L., Mazur, A. and Rock, E., "Lycopene but not lutein nor zeaxanthin decreases in serum and lipoproteins in age-related macular degeneration patients," *Clin. Chim. Acta*, 357 (1), 34-42, Jul. 2005.

[22] Loane, E., Nolan, J.M. and Beatty, S., "The respective relationships between lipoprotein profile, macular pigment optical density, and serum concentrations of lutein and zeaxanthin," *Invest. Ophthalmol. Vis. Sci,* 51 (11), 5897-5905, Nov. 2010.

[23] Renzi, L.M., Hammond, B.R., Jr., Dengler, M. and Roberts, R., "The relation between serum lipids and lutein and zeaxanthin in the serum and retina: Results from cross-sectional, case-control and case study designs," *Lipids Health Dis*, 11, 33, Feb. 2012.

[24] Broekmans, W.M., Berendschot, T.T., Klopping-Ketelaars, I.A., de Vries, A.J., Goldbohm, R.A., Tijburg, L.B., Kardinaal, A.F. and van Poppel, G., "Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin," *Am. J. Clin. Nutr*, 76 (3), 595-603, Sep. 2002.

[25] Merle, B.M., Maubaret, C., Korobelnik, J.F., Delyfer, M.N., Rougier, M.B., Lambert, J.C., Amouyel, P., Malet, F., Le Goff, M., Dartigues, J.F., et al., "Association of hdl-related loci with age-related macular degeneration and plasma lutein and zeaxanthin: The alienor study," *PLoS One* 8, e79848, 2013.

[26] van der Veen, R.L., Berendschot, T.T., Hendrikse, F., Carden, D., Makridaki, M. and Murray, I.J., "A new desktop instrument for measuring macular pigment optical density based on a novel technique for setting flicker thresholds," *Ophthalmic Physiol. Opt*, 29 (2), 127-137, Mar. 2009.

[27] Goodrow, E.F., Wilson, T.A., Houde, S.C., Vishwanathan, R., Scollin, P.A., Handelman, G. and Nicolosi, R.J., "Consumption of one egg per day increases serum lutein and zeaxanthin concentrations in older adults without altering serum lipid and lipoprotein cholesterol concentrations," *J. Nutr,* 136 (10), 2519-2524, Oct. 2006.

[28] Clark, R.M., Herron, K.L., Waters, D. and Fernandez, M.L., "Hypo- and hyperresponse to egg cholesterol predicts plasma lutein and beta-carotene concentrations in men and women," *J. Nutr*, 136 (3), 601-607, Mar. 2006.

[29] Chung, H.Y., Rasmussen, H.M. and Johnson, E.J., "Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men," *J. Nutr*, 134 (8), 1887-1893, Aug. 2004.

[30] Tyssandier, V., Reboul, E., Dumas, J.F., Bouteloup-Demange, C., Armand, M., Marcand, J., Sallas, M. and Borel, P., "Processing of vegetable-borne carotenoids in the human stomach and duodenum," *Am. J. Physiol. Gastrointest. Liver Physiol*, 284 (6), G913-923, Jun. 2003.

[31] Yeum, K.J. and Russell, R.M., "Carotenoid bioavailability and bioconversion," *Annu. Rev. Nutr*, 22, 483-504, 2002.

[32] Sato, Y., Suzuki, R., Kobayashi, M., Itagaki, S., Hirano, T., Noda, T., Mizuno, S., Sugawara, M., Iseki, K., "Involvement of cholesterol membrane transporter niemann-pick c1-like 1 in the intestinal absorption of lutein," *J. Pharm. Pharm. Sci*, 15 (2), 256-264, 2012,.

[33] Hendrickson, S.J., Hazra, A., Chen, C., Eliassen, A.H., Kraft, P., Rosner, B.A. and Willett, W.C., "Beta-carotene 15,15'-monooxygenase 1 single nucleotide polymorphisms in relation to plasma carotenoid and retinol concentrations in women of european descent," *Am. J. Clin. Nutr,* 96 (6), 1379-1389. Dec. 2012.

[34] Amengual, J., Lobo, G.P., Golczak, M., Li, H.N., Klimova, T., Hoppel, C.L., Wyss, A., Palczewski, K. and von Lintig, J., "A mitochondrial enzyme degrades carotenoids and protects against oxidative stress," *FASEB J*, 25 (3), 948-959, Mar. 2011.

[35] Babino, D., Palczewski, G., Widjaja-Adhi, M.A., Kiser, P.D., Golczak, M. and von Lintig, J., "Characterization of the role of beta-carotene 9,10-dioxygenase in macular pigment metabolism," *J. Biol. Chem*, 290 (41), 24844-24857, Oct. 2015.

[36] Niesor, E.J., Chaput, E., Mary, J.L., Staempfli, A., Topp, A., Stauffer, A., Wang, H. and Durrwell, A., "Effect of compounds affecting abca1 expression and cetp activity on the hdl pathway involved in intestinal absorption of lutein and zeaxanthin," *Lipids* 49 (12), 1233-1243, Dec. 2014.

[37] Dallinga-Thie, G.M., Franssen, R., Mooij, H.L., Visser, M.E., Hassing, H.C., Peelman, F., Kastelein, J.J., Peterfy, M. and Nieuwdorp, M., "The metabolism of triglyceride-rich lipoproteins revisited: New players, new insight," *Atherosclerosis,* 211 (1), 1-8, Jul. 2010.

[38] Borel, P., Desmarchelier, C., Nowicki, M., Bott, R., Morange, S. and Lesavre, N., "Interindividual variability of lutein bioavailability in healthy men: Characterization, genetic variants involved, and relation with fasting plasma lutein concentration," *Am. J. Clin. Nutr,* 100 (1), 168-175, Jul. 2014.

[39] Tyssandier, V., Choubert, G., Grolier, P. and Borel, P., "Carotenoids, mostly the xanthophylls, exchange between plasma lipoproteins," *Int. J. Vitam. Nutr. Res*, 72 (5), 300-308, Oct. 2002.

[40] Connor, W.E., Duell, P.B., Kean, R. and Wang, Y., "The prime role of hdl to transport lutein into the retina: Evidence from hdl-deficient wham chicks having a mutant abca1 transporter," *Invest. Ophthalmol. Vis. Sci,* 48 (9), 4226-4231, Sep. 2007.

[41] Li, B., Vachali, P. and Bernstein, P.S., "Human ocular carotenoid-binding proteins," *Photochem Photobiol Sci* 9 (11), 1418-1425, Nov. 2010.

[42] Meyers, K.J., Mares, J.A., Igo, R.P., Jr., Truitt, B., Liu, Z., Millen, A.E., Klein, M., Johnson, E.J., Engelman, C.D., Karki, C.K., et al., "Genetic evidence for role of carotenoids in age-related macular degeneration in the carotenoids in age-related eye disease study (careds)," *Invest. Ophthalmol. Vis. Sci.* 55 (1), 587-599, Jan. 2014.

[43] Niesor, E.J., Gauthamadasa, K., Silva, R.A., Suchankova, G., Kallend, D., Gylling, H., Asztalos, B., Damonte, E., Rossomanno, S., Abt, M., et al., "Xanthophylls, phytosterols and pre-beta1-hdl are differentially affected by fenofibrate and niacin hdl-raising in a cross-over study," *Lipids,* 48 (12), 1185-1196, Dec. 2013.