Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 261–267
Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Eggs distinctly modulate plasma carotenoid and lipoprotein subclasses in adult men following a carbohydrate-restricted diet☆

Gisella Mutungi[a], David Waters[a], Joseph Ratliff[a], Michael Puglisi[a], Richard M. Clark[a], Jeff S. Volek[a,b], Maria Luz Fernandez[a,b,*]

[a]Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA
[b]Department of Kinesiology, University of Connecticut, Storrs, CT 06269, USA

Received 2 June 2008; received in revised form 2 December 2008; accepted 15 December 2008

Abstract

We previously reported that carbohydrate restriction (CR) (10–15% en) during a weight loss intervention lowered plasma triglycerides (TG) by 45% in male subjects ($P<.001$). However, those subjects with a higher intake of cholesterol provided by eggs (640 mg additional cholesterol, EGG group) had higher concentrations of high-density lipoprotein (HDL) cholesterol ($P<.0001$) than the individuals consuming lower amounts (0 mg of additional cholesterol, SUB group). The objectives of the present study were to evaluate whether CR and egg intake (1) modulate circulating carotenoids and (2) affect the concentrations of plasma apolipoproteins (apo), lipoprotein size and subfraction distribution. CR decreased the number of large and medium very low-density lipoprotein cholesterol subclasses ($P<.001$), while small low-density lipoprotein (LDL) were reduced ($P<.001$). In agreement with these observations, a decrease in apo B ($P<.01$) was observed. In addition, CR resulted in a 133% increase in apo C-II and a 65% decrease in apo C-III ($P<.0001$). Although an increase of the larger LDL subclass was observed for all subjects, the EGG group had a greater increase ($P<.05$). The EGG group also presented a higher number of large HDL particles ($P<.01$) compared to the SUB group. Regarding carotenoids, CR resulted in no changes in dietary or plasma α- or β-carotene and β-cryptoxanthin, while there was a significant reduction in both dietary and plasma lycopene ($P<.001$). In contrast, dietary lutein and zeaxanthin were increased during the intervention ($P<.05$). However, only those subjects from the EGG group presented higher concentrations of these two carotenoids in plasma, which were correlated with the higher concentrations of large LDL observed in the EGG group. These results indicate that CR favorably alters VLDL metabolism and apolipoprotein concentrations, while the components of the egg yolk favor the formation of larger LDL and HDL leading to an increase in plasma lutein and zeaxanthin.

Keywords: Carbohydrate restriction; Eggs; Carotenoids; Lipoprotein subclasses; Apolipoproteins; Weight loss; Adult men

1. Introduction

Elevated levels of plasma low-density lipoprotein (LDL) cholesterol (LDL-C) constitute a well-recognized risk factor for coronary heart disease (CHD) [1]. Similarly, low levels of HDL cholesterol (HDL-C) and increased plasma triglycerides (TG) have been associated with CHD risk [2]. However, increasing evidence demonstrates that plasma apolipoproteins as well as lipoprotein subclasses can be better predictors of CHD than plasma TG or lipoprotein cholesterol [3]. For example, large VLDL particles that result from high TG production in the liver are highly susceptible to hydrolysis to form small dense LDL particles [4]. It is well known that normocholesterolemic individuals with higher concentrations of small, dense LDL particles are at increased risk for CHD [5] independent of plasma LDL-C concentrations. Small dense LDL particles tend to have longer residence periods in circulation due to their reduced binding affinity to the LDL receptor, making them more susceptible to oxidation [6]. Therefore, both small dense LDL and large VLDL particles are considered atherogenic. Similarly, when analyzing HDL particles, the protective effects of this lipoprotein are often amplified when there is a higher concentration of the larger HDL subclass [7].

In addition to lipoprotein subclasses, the levels of apolipoprotein (apo) B and the ratio of apo B/apo A-I have been reported to be more accurate predictors of CHD risk than lipoprotein cholesterol [8–10]. The apo B:apo A-I ratio is considered the best predictor for CHD risk because it reflects the cholesterol balance between atherogenic (apo B containing lipoproteins) and antiatherogenic lipoproteins [11]. Further, Onat et al. [12] have shown that apo B has a significant

Abbreviations: Apo, apolipoprotein; CHD, coronary heart disease; CHOL, dietary cholesterol; CR, carbohydrate restriction; CRD, carbohydrate restricted diets; EGG, egg group; HDL-C, HDL cholesterol; H-NMR, nuclear magnetic resonance; LDL-C, LDL cholesterol; LPL, lipoprotein lipase; LCAT, lecithin cholesterol acyltransferase; SUB, egg substitute group; TG, triglycerides; VLDL, very low-density lipoprotein.

☆ Supported by the Egg Nutrition Center/American Egg Board.

* Corresponding author. Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA. Tel.: +1 860 486 5547; fax: +1 860 486 3674.
E-mail address: maria-luz.fernandez@uconn.edu (M.L. Fernandez).

0955-2863/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2008.12.011

correlation with a greater number of parameters in the prediction of risks for CHD than does LDL-C. apo B is a marker for LDL and very low density lipoprotein (VLDL) particles; therefore, reductions in this apolipoprotein indicate reductions in the number of VLDL and LDL particles.

Carotenoids and cholesterol share common pathways in absorption and transport in the plasma compartment and the definitions of hypo- and hyper-responders exist for both [13,14]. Since carotenoids are transported in plasma by lipoproteins, changes in the concentrations of different lipoprotein subclasses are expected to affect carotenoid transport. In addition to containing cholesterol, eggs are good sources of lutein and zeaxanthin, two carotenoids that are selectively deposited in the macula and may be involved in protecting against age-related macular degeneration [15]. Thus, the appearance of these carotenoids in plasma may have implications for eye health.

The objectives of this study were to evaluate whether carbohydrate restriction and egg intake (1) modulate circulating carotenoids and (2) affect lipoprotein subclasses, apolipoproteins and lipoprotein size to gain a better insight into the type of particles generated by dietary interventions which result in significant reductions of plasma TG (carbohydrate restriction) and increases in HDL-C (egg intake) [16]. Our hypothesis was that eggs, as a source of dietary cholesterol (CHOL) and an effective delivery vehicle of carotenoids, would promote the formation of larger lipoproteins carrying higher concentrations of plasma carotenoids. In addition, we hypothesized that the beneficial changes in lipoprotein metabolism induced by CR [17] would not be altered by increased egg consumption.

2. Experimental procedure

2.1. Materials

Liquid whole eggs and cholesterol-/fat-free eggs were purchased from Vistar Corporation (Windsor, CT, USA). Both egg products are identical in color and consistency, the difference being the removal of the yolk in the cholesterol-/fat-free eggs. Kits for total cholesterol (TC) and triglycerides were from Roche Diagnostics (Indianapolis, IN, USA). Plates for cholesterol ester transfer protein analysis were from BioVision (Mountain View, CA, USA). Kits for apolipoproteins were from LINCO Research (St. Charles, MO, USA).

2.2. Study design

We recruited 31 men between the age of 40 and 70 years with a body mass index (BMI) of 26–37 kg/m^2 from the University and the surrounding community and randomly assigned the subjects to consume the equivalent of 3 liquid eggs per day (EGG) or the same amount of egg substitute (SUB). Subjects were excluded from the study if they were taking lipid-lowering drugs or have been enrolled in the previous month in a weight loss intervention. Subjects were asked to consume the daily portion of the EGG or SUB. No guidelines were given regarding preparation method but most subjects consumed scrambled eggs during breakfast. Compliance was assessed by the returning of the uneaten portion every week. Three individuals dropped out of the study due to compliance issues. Subjects were excluded if they had hypothyroidism, documented heart disease, Type I diabetes, gout or egg allergies. All subjects followed a carbohydrate-restricted diet (CRD) during 12 weeks. Similar to other studies in our laboratory [16], carbohydrates were restricted to 10–15% of total energy, 25–30% from protein and 55–60% from fat. This was a parallel randomized placebo-controlled single-blinded study. The substitute had the same color and consistency as the eggs. Subjects were matched by BMI and age, and the subjects who completed the study were 15 from the EGG and 13 from the SUB group. These were free-living subjects who were not provided with any other foods apart from either eggs or egg substitute to consume as part of their diet, and no restrictions were given towards energy intake. Subjects received individual and personalized dietary counseling from registered dietitians prior to the dietary intervention. Detailed dietary booklets, specific to each dietary treatment, were provided outlining dietary goals; lists of appropriate foods, recipes and sample meal plans and food record log sheets. No explicit instructions were provided regarding caloric intake for either diet to allow expression of any noncognitive aspects on food intake. Subjects received weekly follow-up counseling during which body mass was measured, compliance was assessed, and further dietetic education was provided. A 3-day weighed food records was obtained at baseline to assess habitual nutrient intake, and 5-day records were completed during Weeks 1, 6 and 12 of the intervention.

Subjects were given specific instructions on the type of foods that had to be avoided as a result of following a CRD, and they could not consume any additional eggs apart from what was provided to them weekly. Subjects in the EGG group were taking an additional 640 mg/d of cholesterol, while those in the SUB group did not have any CHOL contribution from this product. They could take unlimited amounts of meat and fish; moderate amounts of cheese, vegetables and salad dressings with low carbohydrate content and small amounts of seeds and nuts. There were no restrictions on the type of fats consumed. Subjects were asked to maintain their normal routine of physical activity during the course of this study, which was monitored by an exercise diary. All study protocols were approved by the University of Connecticut Institutional Review Board, and all subjects signed an informed consent before participating in the study.

2.3. Blood collection

After an overnight, fast blood was collected into EDTA and heparinized tubes from an antecubital vein, and the blood was immediately centrifuged at 2000×*g* for 20 min. Preservatives (1 ml/L sodium azide, 1 ml/L phenyl methyl sulphonyl fluoride (PMSF) and 5 ml/L aprotinin) were added to the plasma once separated from red blood cells. The plasma was then aliquoted, and analysis for plasma lipids was performed shortly, while the rest of the plasma samples were frozen at −80°C for further analysis.

2.4. Plasma lipids

Total cholesterol and TG were measured by enzymatic methods, and HDL cholesterol, by determining cholesterol concentration in the supernatant after precipitation of apo B-containing lipoproteins, as previously reported [16].

2.5. HDL, LDL, and VLDL particle size and number

Nuclear magnetic resonance (H-NMR) analysis was performed on 400-MHz NMR analyzer (Bruker BioSpin, Billerica, MA, USA) as previously described [17]. Briefly, lipoprotein subclasses of different sizes produce a distinct lipid methyl signal the amplitude of which is directly proportional to lipoprotein particle concentration. NMR simultaneously quantifies >30 lipoprotein subclasses that are empirically grouped into nine smaller subclasses based on particle diameters: large VLDL (>60 nm), medium VLDL (27–35 nm), small VLDL (23–27 nm), intermediate-density lipoprotein (IDL), large LDL (21.2–23 nm), medium LDL (19.8–21.2), small LDL (18–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm) and small HDL (7.3–8.2 nm). Weighted average lipoprotein particle sizes in diameters were calculated based on the diameter of each lipoprotein subclass multiplied by its respective relative concentration.

2.6. Apolipoproteins and lecithin cholesterol acyl transferase activity

Apolipoproteins were measured using LINCOplex: Multiplex Biomarker Immunoassay for Luminex Instrumentation/xMAP Technology. The technique uses fluorescently labeled microsphere beads with antibodies to each individual apolipoprotein [18]. The method by Ogawa & Fielding [19] was used for lecithin cholesterol acyl transferase (LCAT) determination.

2.7. Carotenoid analysis

To determine carotenoid content of egg and egg substitute, a 100-μl aliquot was placed in a screw-top test tube with 1 ml pyrogallol in reagent alcohol and 5 ml 15% potassium hidroxyde (KOH) in methanol, as previously described [13]. An internal standard (ethyl-β-apo-8′-carotenoate) was added to the sample and reconstitute with 2-propanol in preparation for high-performance liquid chromatography (HPLC) analysis.

Plasma samples were prepared for HPLC analysis as previously described [13]. Briefly, 200 μl of serum was mixed with an equal volume of absolute ethanol containing butylated hydroxytoluene and ethyl-β-apo-8′-carotenoate (internal standard). The sample was then extracted three times with hexane-containing butylated hydroxytoluene. Samples were centrifuged to facilitate phase separation. The hexane layers were combined, and the solvent removed with a stream of nitrogen. The residuals were reconstituted with 100-μl 2-propanol and placed in HPLC injection vials.

Carotenoids were analyzed using a Waters HPLC system. A Varian HPLC column (100×4.6-mm microsorb-MN 100-3 C-18) preceded by an Upchurch C-18 guard column (Upchurch Scientific, Oak Harbor, WA, USA) with an isocratic mobile phase consisting of 80% acetonitrile: 15% dioxane: 2.5% methanol: 2.5% 2-propanol: 0.01% triethylamine: 0.01% ammoniun acetate. Detection of internal standard and carotenoids was at 450 nm. All solvents used were HPLC grade and were filtered and degassed before use. Standard curves were compiled from HPLC purified lutein, zeaxanthin and commercially purchased α-carotene, β-carotene, lycopene and β-cryptoxanthin.

2.8. Statistical analyses

A two-way repeated-measures analysis of variance (ANOVA) was used to determine diet effects and time effects on plasma lipids, apolipoproteins, lipoprotein subclasses and plasma and dietary carotenoids. Individual responses to the intervention over time was the repeated measure and EGG vs. SUB, the between-subject factors. P value of <.05 was considered significant. SPSS version 13.0 for windows was used to perform the statistical analyses, and the data values are reported as mean±S.D.

3. Results

3.1. Diet

Subjects complied with the diet as assessed by urinary ketones and dietary records, as previously reported [16]. Both groups spontaneously reduced energy intake by 22% (P<.05), although they were not instructed to reduce the amount of calories. Also, the groups had similar contribution to total energy from fat and carbohydrate both at baseline and after 12 weeks. The energy contribution from carbohydrate was 42.4±8.3% for the EGG and 41.5±9.5% for the SUB group at baseline, and 14.9±9.3% for the EGG and 19.9±12.1% for the SUB group at the end of the intervention. Similarly dietary fat went from a mean of 39.5% to 55% at Week 12, and protein, from 17.9% at baseline to 25.7% at the end of the intervention [17]. There were no differences in the percentage of saturated fat, monounsaturated fat or polyunsaturated fat between groups at baseline or post intervention. In contrast, CHOL was 319±150 mg/day for the EGG group at baseline and 827±192 mg at the end of the intervention, while the SUB group was consuming 354±170 mg/day cholesterol at baseline and 277±100 mg/d at the end of the intervention (P<.0001 between groups).

3.2. Weight

There were very significant decreases in weight for all subjects as previously reported [16]. Subjects from the EGG group had a reduction of 6.7 kg after 12 weeks (from 98.9±15.3 to 92.2±12.7 kg), while the SUB group had a weight reduction of 5.9 kg (from 97.6±19.9 to 91.7±15.7 kg).

3.3. Plasma lipids

Plasma lipids for this intervention have been reported previously [16]. In this population, plasma total cholesterol did not change significantly from baseline to post-intervention. Subjects from the EGG group had 198.3±42.1 mg/dL at baseline and 202.2±41.8 mg/dL post-intervention while those from the SUB group had 188.3±33.7 mg/dL at baseline and 187.3±39.5 post-intervention. Plasma TG were reduced for all subjects by 45% (from a mean of 120±59.4 to 73.4±26.9 mg/dL post intervention). HDL-C increased in the EGG group only from 47.6±15.1 to 57.1±15.1 mg/dL and remained unchanged for the SUB group [16].

3.4. Mean apolipoprotein size

As shown in Fig. 1A, there were no significant changes observed in the mean diameter of VLDL between baseline and post-intervention for either group. In contrast, there was a significant increase in LDL diameter for all subjects after 12 wk of the intervention (Fig. 1B). HDL diameter only increased in the EGG group (Fig. 1C) (P<.05).

3.5. Number of VLDL particles

The number of all VLDL particles sizes was reduced following the intervention for all subjects independent of the group (Table 1). Total VLDL was reduced from 87.1±34.9 at baseline to 56.2±26.0 nmol/L at Week 12 on the EGG group, while the SUB group was reduced from 79.0±25.3 to 59.8±27.5 nmol/L (P<.0001). The reduction in total VLDL particles was associated with the large and medium VLDL for all subjects following the CRD (Table 1). The reduction in the small VLDL particles was borderline significant (P=.065).

3.6. Number of IDL and LDL particles

Diet effects on IDL, large, medium and small LDL particles are shown in Table 2. These changes are from baseline to the end of the intervention (Week 12). There were no changes observed on the total IDL or LDL particle numbers in either group. Although all subjects had an increase in the number of large LDL post intervention, subjects from the EGG group had a greater increase in large LDL compared to the SUB group (P<.01). The large LDL number of particles increased from 381.3±181 to 541.7±251.8 nmol/L (P<.0001) in the EGG group, while in the SUB group, there was a more modest increase from 340.6±129.3 to 380.8±143.4 nmol/L. The smaller LDL particles were decreased for all subjects following the CRD independent of the group (Table 2).

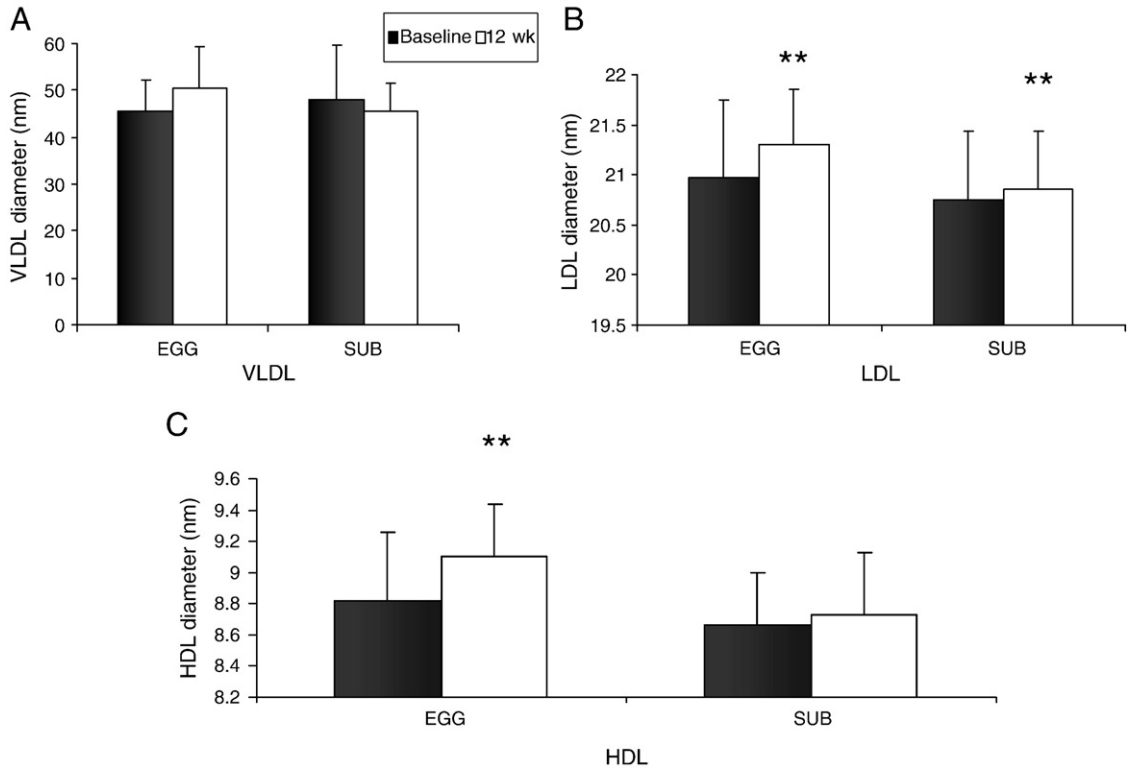

Fig. 1. Changes in VLDL (A), LDL (B) and HDL size (C) between baseline (white bars) and post intervention (Week 12) (black bars) for subjects in the EGG or SUB group. **Significantly different from baseline.

3.7. Number of HDL particles

Regarding HDL particles, there were no changes in the total or small HDL particles in either group (Table 3). Large HDL particles were increased in both groups. In the EGG group, HDL increased from 5.10±3.26 at baseline to 7.73±3.21 nmol/L, while in the SUB group, these particles were increased from 3.68±2.21 to 5.07±2.61, $P<.0001$. However, there was a greater increase in the EGG compared to the SUB group ($P<.01$). Medium HDL particles were reduced for all subjects, while no changes were observed in small HDL (Table 3). There was an increase in LCAT activity only on those subjects from the EGG group (Table 3). A total of 26 subjects had an increase in large HDL from the intervention — 15 from the EGG group and 11 from the SUB group (data not shown).

3.8. Apolipoproteins

Results for plasma apolipoproteins concentrations are shown on Table 4. All the effects observed for apolipoproteins are due to carbohydrate restriction since we found no differences between EGG or SUB groups. apo A-I concentrations did not change. This result is in agreement with the lack of effect in the number of HDL particles. In contrast apo-AII concentrations were reduced from baseline. Significant correlations were found between the reductions in apo-AII concentrations and the reduction in medium HDL ($r=0.553$, $P<.01$, data not shown). Plasma apo B concentrations were reduced for all subjects in agreement with the reductions in larger VLDL and smaller LDL. Apolipoproteins C-III and E were reduced ($P<.001$) after 12 wk, while apo C-II was increased by 133% ($P<.0001$) (Table 4).

3.9. Carotenoids

As indicated in Table 5, carotenoid consumption was for the most part not affected by carbohydrate restriction. Intake of α-carotene, β-carotene and β-cryptoxanthin were not different between baseline and 12 weeks. In contrast, dietary lycopene was

Table 1
Total number of VLDL, large, medium and small VLDL particles at baseline and following consumption of a CRD for 12 weeks in combination with either EGG or SUB

Variable (nmol/L)	EGG		SUB		P over time
	Baseline	12 Weeks	Baseline	12 Weeks	
Total VLDL	87±35	56±26	79±25	60±28	P<.0001
Large VLDL (35–60 nm)	3±4	1±1	4±6	1±3	P<.025
Medium VLDL (27–35 nm)	33±19	11±10	29±17	15±12	P<.0001
Small VLDL (23–27 nm)	51±18	44±21	46±15	43±20	P=.065

Values are means±S.D. EGG, n=15; SUB, n=13. Data were analyzed using repeated-measures ANOVA. *P* values are for time effects. There were no differences between diets in these parameters.

Table 2
Total number of IDL, LDL, large, medium and small LDL particles at baseline and following consumption of a CRD for 12 weeks in combination with either EGG or SUB

Variable (nmol/L)	EGG		SUB		P over time
	Baseline	12 Weeks	Baseline	12 Weeks	
Total IDL	40.0±29.0	34.0±30.3	49.3±42.4	46.1±37.6	NS
Total LDL	1130±330	1152±211	1232±321	1185±276	NS
Large LDL (21.2–23 nm)	381±181[a]	542±252[b]	341±129[a]	381±143[a]	P<.0001
Medium Small LDL (19.8–21.2 nm)	146±73	116±52	176±66	150±54	P<.01
Very Small LDL (18–19.8 nm)	563±296	461±195	666±256	608±219	P<.05

Values are means±S.D. EGG, n=15; SUB, n=13. Data were analyzed using repeated-measures ANOVA. *P* values are for time effects. Different superscripts (a,b) in the same row indicate significant difference ($P<.05$). NS, nonsignificant ($P>.05$).

Table 3
Total number of HDL, large, medium and small HDL particles and LCAT activity at baseline and following consumption of a CRD for 12 weeks in combination with either EGG or SUB

Variable (μmol/L)	EGG		SUB		P over time
	Baseline	12 Weeks	Baseline	12 Weeks	
Total HDL	32.67±4.75	32.11±6.63	30.56±4.68	29.32±6.68	NS
Large HDL (8.8–13 nm)	5.10±3.26[a]	7.73±3.21[b]	3.68±2.21[c]	5.07±2.61[a]	P<.0001
Medium HDL (8.2–8.8 nm)	4.85±4.05	2.32±4.74	4.92±5.57	2.09±2.97	P<.01
Small HDL (7.3–8.2 nm)	22.70±3.51	22.07±4.32	21.96±6.21	22.11±4.34	NS
LCAT (μmol L^{-1} h^{-1})	19.9±13.5[a]	32.2±16.6[b]	25.6±7.7[a]	26.0±9.8[a]	P<.05

Values are means±S.D. EGG, n=15; SUB, n=13. Data were analyzed using repeated-measures ANOVA. P values are for time effects. Letters with different superscripts (a, b, c) in the same row indicate a diet effect (EGG vs. SUB) (P<.05). Tukey test was used as a post hoc test.

reduced from baseline for all subjects (P<.001). The dietary records were in agreement with the plasma concentrations of these carotenoids. Plasma concentrations of α-carotene, β-carotene and β-cryptoxanthine were not modified by the intervention (Table 6), while plasma levels of lycopene were reduced for all subjects (P<.05) (Table 6).

Dietary lutein and zeaxanthin were higher at Week 12 compared to baseline for all subjects, as shown in Fig. 2. However, subjects from the EGG group had higher concentrations of both lutein and zeaxanthin in plasma at Week 12, while the concentrations of these carotenoids did not change in subjects from the SUB group.

The individual changes in plasma lutein were different between groups. While 5 subjects from the SUB group had an increase in this carotenoid after 12 wk, 2 subjects had no change and 6 had a reduction compared to baseline. In contrast the 15 subjects for the EGG group increased plasma lutein. There was a significant correlation between changes in the number of large LDL and changes in plasma lutein (r=0.418, P<.01) (Fig. 3) suggesting that the higher number of these particles may be in part responsible for the higher concentrations of lutein. Similar results were found for zeaxanthin (data not shown).

4. Discussion

In this study, we have demonstrated that both carbohydrate restriction and whole egg intake significantly and distinctly alter the concentrations of plasma apolipoproteins and the distribution of lipoprotein subclasses. While CRD reduces plasma TG by decreasing both the total number of VLDL particles and the more atherogenic LDL and HDL subclasses, whole egg promotes the formation of larger LDL and HDL.

Table 4
Plasma apolipoproteins at baseline and following consumption of a CRD for 12 weeks in combination with either EGG or SUB

Variable (mg/L)	EGG		SUB		P over time
	Baseline	12 Weeks	Baseline	12 Weeks	
apo A-I	2150±640	2220±670	2000±410	1900±490	NS
apo A-II	450±180	380±120	470±130	350±100	P<.001
apo B	640±290	580±320	650±230	440±170	P<.025
apo C-II	107±34	233±96	101±42	196±61	P<.0001
apo C-III	240±80	84±30	250±100	69±18	P<.0001
apo E	100±25	85±30	91±30	62±17	P<.001

Values are means±S.D. EGG, n=15; SUB, n=13. Data were analyzed using repeated-measures ANOVA. P values are for time effects. There were no differences between diets in these parameters.

Table 5
Dietary carotenoids intake (mg/day) and plasma carotenoids (mmol/L) at baseline and after following consumption of a CRD for 12 weeks in combination with either EGG or SUB

	EGG		SUB		P over time
	Baseline	12 Weeks	Baseline	12 weeks	
Cryptoxanthin (mg/day)	0.26±0.59	0.25±0.76	0.53±1.41	0.44±0.69	NS
α-Carotene (mg/day)	0.79±1.2	0.55±0.49	0.46±0.74	0.38±0.53	NS
β-Carotene (mg/day)	3.90±.24	4.25±.49	4.97±3.78	3.77±2.99	NS
Lycopene (mg/day)	5.81±3.67	2.94±2.31	9.39±12.55	2.41±.87	P<.05
Lutein+zeaxanthin (mg/day)	2.05±1.18	4.31±4.15	3.21±.63	3.99±4.30	P<.05

Values are means±S.D. EGG, n=14; SUB, n=13. Data were analyzed using repeated-measures ANOVA. P values are for time effects. There were no differences between diets in these parameters.

This study also indicates by both dietary records and plasma concentrations that CRDs do not limit intake of carotenoids with the exception of lycopene. The study also suggests that the food matrix (the lipids in the yolk) might influence the absorption and therefore the plasma appearance of certain carotenoids such as lutein and zeaxanthin.

4.1. CRD and VLDL metabolism

We have previously demonstrated that CR modifies lipoprotein metabolism by favorably reducing particles that are atherogenic and increasing those that are antiatherogenic [17]. In the present study, and as a result of CR, there was a reduction in the larger VLDL particles. This could be due to the increase of apo C-II concentration, which, in turn, facilitated the rapid clearance of triglyceride rich lipoproteins via the activation of lipoprotein lipase (LPL) [20] accounting for the observed decrease in the number of the large and medium VLDL particles. Adiels et al. [21] reported that overproduction of large VLDL particles in humans is driven by the high fat content in the liver, whereas elevated large VLDL particles is the major determinant of plasma TG [22]. Therefore, the observed decrease in large VLDL could also be due to the decline in hepatic fat content, reduced VLDL production and plasma TG [23]. Down-regulation of apo C-III in this study could also have contributed to the hydrolysis of the VLDL particle, since the presence of this apolipoprotein has been associated with LPL inhibition. Therefore its reduction could have resulted in the increased activation of LPL. The decrease in apo B concentrations can also explain the reduction in the large and medium VLDL particles.

4.2. CRD, CHOL and LDL metabolism

CRD resulted in an increase of larger LDL particles, whereas medium small and very small dense LDL particles were reduced. We assume that CHOL was responsible for further enhancing the

Table 6
Plasma carotenoids at baseline and after following consumption of a CRD for 12 weeks in combination with either EGG or SUB

Variable (mmol/L)	EGG		SUB		P over time
	Baseline	12 Weeks	Baseline	12 Weeks	
Cryptoxanthin	0.12±0.08	0.09±0.06	0.11±0.08	0.06±0.04	NS
α-Carotene	0.14±0.05	0.13±0.05	0.13±0.04	0.12±0.03	NS
β-Carotene	0.30±0.22	0.33±.25	0.25±.15	0.25±.12	NS
Lycopene	0.41±.25	0.32±.19	0.34±.20	0.20±.07	P<.05

Values are means±S.D. EGG, n=14; SUB, n=13. Data were analyzed using repeated-measures ANOVA. P values are for time effects. There were no differences between diets in these parameters.

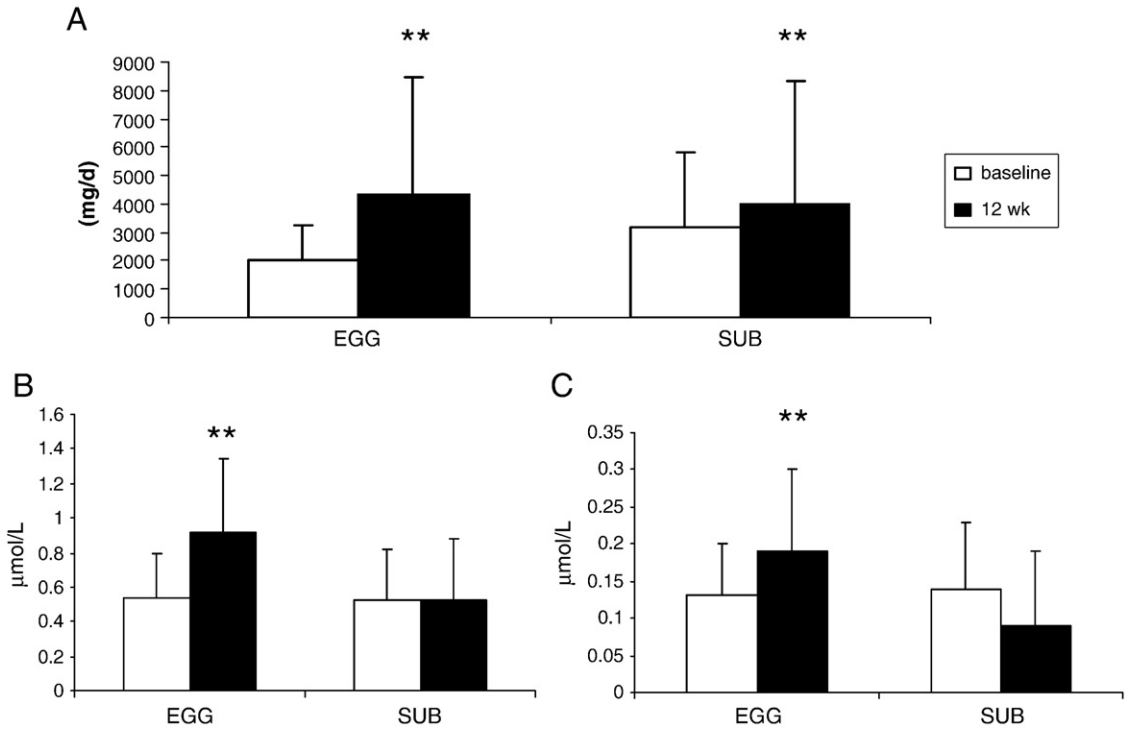

Fig. 2. Concentrations of dietary lutein+zeaxanthin at baseline and after 12 weeks for subjects in the EGG or SUB group (A). Changes in plasma lutein (B) and zeaxanthin (C) for subjects in the EGG and SUB groups. *Significantly different at $P<.01$.

increase in LDL size when both groups were compared since CHOL is the main difference between both diets. These observations are supported by similar studies investigating LDL metabolism in low carbohydrates interventions [24,25]. Also, in a weight loss study, Krauss et al. [26] found a reduction in small LDL-III and LDL-IV, whereas Feinman and Volek [27] reported improvement on LDL peak size in the absence of weight loss. CHOL has been shown to increase LDL size in other studies. A challenge of an additional 640 mg/d CHOL resulted in larger LDL particles in women [28] and in larger LDL in an older population [29]. However, results from this study suggest that CHOL further enhances the effects of CRD in increasing even more the size of LDL as it occurs when we compare the EGG versus the SUB group. Based on the clear relationship between small dense LDL and increased risk for coronary heart disease [27], the current findings are important.

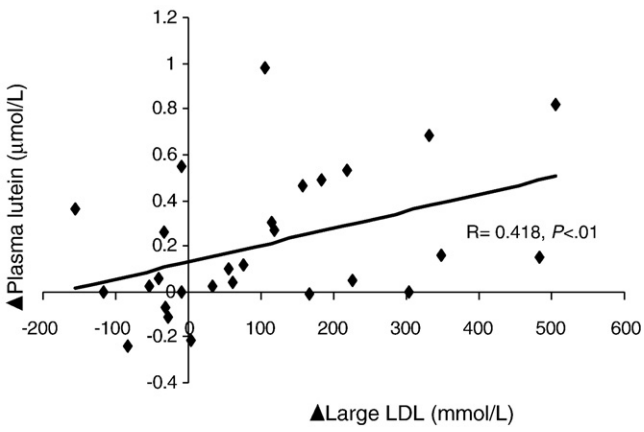

Fig. 3. Correlation between changes in lutein and changes in large LDL from baseline to 12 weeks.

4.3. CRD, CHOL and HDL metabolism

Studies following a low-carbohydrate diet with no restriction on calorie or dietary fat intake have demonstrated an increase in CHOL, which, in turn, contributes to the increase in plasma HDL-C [17]. These observations are supported by the results in this study, which suggest that in CR, CHOL is necessary for the increase in both HDL-C concentrations and the greater number of larger HDL particles. There was also an increase in LCAT activity associated with CHOL that can be explained by the increase observed in HDL particle size and in the number of large HDL. apo A-I did not change during the intervention in agreement with no changes observed in the total number of HDL particles. The observed reduction in apo A-II can be explained by the reduction of medium HDL particles observed in both groups. Tailleux et al. [30] reported that a reduction of apo A-II was associated with surviving myocardial infarction, suggesting that this apolipoprotein might be a risk marker of atherogenic particles. apo A-II has also been shown to inhibit cholesterol ester protein activity [30] and increase hepatic lipase [30,31], which are both potential beneficial effects of CHD.

4.4. CRD, CHOL and carotenoids

An interesting finding in this study was that CRD does not limit the intake of carotenoids with the exception of lycopene, as evaluated by dietary records and confirmed by plasma concentrations. During the intervention, subjects abstained from pasta and pizza, two major sources of lycopene for these individuals, explaining the low levels of plasma lycopene after 12 weeks. However, lycopene does not have to be limited on the CRD. Subjects could still have consumed tomatoes or tomato-based products if they had chosen to do it. In contrast, lutein and zeaxanthin intake was increased for all subjects at the end of the intervention due to the higher intake of green vegetables.

However, the higher concentrations of plasma lutein and zeaxanthin were only observed in those subjects from the EGG group. This

may be explained by the observation that carotenoids are better absorbed from eggs than from plant foods [32,33]. Based on our dietary records, the majority of dietary lutein and zeaxanthin in subjects from the EGG group was from the eggs since they were eating 3 eggs per day, while subjects from the SUB group consumed lutein and zeaxanthin mainly from plant sources in the form of salads and vegetables. The presence of fat in the egg yolk makes these carotenoids more readily available to be incorporated into the micelle and therefore optimizing their absorption in the intestine. The presence of dietary fiber, known to interfere with micelle formation, could be the cause on the lower bioavailability that was associated with low plasma levels of these carotenoids in the SUB group [32].

Lutein and zeaxanthin have important implications in health due to their potential dual role in protecting against CHD due to the lowering of inflammation and protection against LDL oxidation [33] and age-related macular degeneration [34]. In humans, the distribution of lutein and zeaxanthin between LDL and HDL particles is similar [35]. In this study, we observed an increase in large LDL and HDL for all subjects. However, these effects were more pronounced in subjects from the EGG group. The size of the lipoprotein particle may facilitate the uptake and incorporation of these carotenoids and, hence, increases their concentration in plasma. Thus, we speculate that there are two reasons of the higher plasma lutein and zeaxanthin in subjects from the EGG group. The first one is that a higher bioavailability of these carotenoids was facilitated by the egg matrix, and the second one is that CHOL increases the formation of larger LDL and HDL, which allows for a more efficient transport of lutein and zeaxanthin.

In summary, this study highlights the importance of measuring apolipoproteins and lipoprotein subclasses to fully understand the effects of diet on lipoprotein atherogenicity and heart disease risk. This study also provides additional information on the role of eggs in increasing lutein and zeaxanthin relative to the formation of the large and less atherogenic lipoprotein subclasses. Finally, this study is showing for the first time that carbohydrate restriction, with the exception of lycopene, does not affect dietary or plasma carotenoids.

Acknowledgments

This study was supported by a grant-in-aid from the Egg Nutrition Center to M.L.F.

References

[1] Expert Panel on Detection EaToHBCiA. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). J Am Med Assoc 2001;285:2486–97.
[2] Yuan G, Al-Shali KZ, Hegele RA. Hypertriglyceridemia: its etiology, effects and treatment. CMAJ 2007;176:1113–20.
[3] Pischon T, Girman CJ, Sacks FM, Rifai N, Stampfer MJ, Rimm EB. Non-high-density lipoprotein cholesterol and apolipoprotein b in the prediction of coronary heart disease in men. Circulation 2005;112:3375–83.
[4] Zambon A, Bertocco S, Virtturi N, Polentarutti V, Vianello D, Crepaldi G. Relevance of hepatic lipase to the metabolism of triacylglycerol-rich lipoproteins. Biochem Soc Trans 2003;31:1070–4.
[5] Tornvall P, Karpe F, Carlson LA, Hamsten A. Relationships of low density lipoprotein subfractions to angiographically defined coronary artery disease in young survivors of myocardial infarction. Atherosclerosis 1991;90:67–80.
[6] Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992;93:189–99.
[7] Johansson J, Carlson LA, Landou C, Hamsten A. High density lipoproteins and coronary atherosclerosis. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients. Arterioscler Thromb 1991;11:174–82.
[8] Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. J Int Med 2006;259:437–46.
[9] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarctin in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937–52.
[10] Meisinger C, Loewel H, Mraz W, Koenig W. Prognostic value of apolipoprotein B and A-I in the prediction of myocardial infarction in middle-aged men and women: results from the MONICA/KORA Augsburg cohort study. Eur Heart J 2005;26:271–8.
[11] Lamarche B, Moorjani S, Lupien PJ, Cantin B, Bernard P, Dagenais G, et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec cardiovascular study. Circulation 1996;94:273–8.
[12] Onat A, Ozhan H, Can G, Hergenc G, Karabulut A, Albayrak S. Serum apolipoprotein B is superior to LDL-cholesterol level in predicting incident coronary disease among Turks. Anadolu Kardiyol Derg 2007;7:128–33.
[13] Clark RM, Herron KL, Waters D, Fernandez ML. Hypo- and hyper-response to egg cholesterol predicts lutein and beta-carotene plasma concentrations in men and women. J Nutr 2006;136:601–7.
[14] Furr HC, Clark RM. Intestinal absorption and tissue distribution of carotenoids. Nutr Biochem 1997;8:364–77.
[15] Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 2001;385:28–40.
[16] Mutungi G, Ratliff J, Puglisi M, Torres-Gonzalez M, Vaishnav U, Leite JO, et al. Dietary cholesterol from eggs increases HDL cholesterol in overweight men consuming a carbohydrate restricted diet. J Nutr 2008;138:272–6.
[17] Wood RJ, Volek JS, Liu Y, Shachter NS, Contois JH, Fernandez ML. Carbohydrate restriction alters lipoprotein metabolism by modifying VLDL, LDL and HDL subfraction distribution and size in overweight men. J Nutr 2006;136:384–9.
[18] Liu MY, Xydakis AM, Hoogeveen RC, Jones PH, Smith EO, Nelson KW, et al. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. Clin Chem 2005;51:1102–9.
[19] Ogawa Y, Fielding CJ. Assay of cholesteryl ester transfer activity and purification of a cholesteryl ester transfer protein. Methods Enzymol 1985;111:274–85.
[20] Hospattakar AV, Fairwell T, Ronan R, Brewer HB. Amino acid sequence of human plasma apolipoprotein C-II from normal and hyperlipoproteinemic subjects. J Biol Chem 1984;259:318–22.
[21] Adiels M, Taskinen MR, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, et al. Overproduction of large VLDL particles is driven by increased liver fat content in man. Diabetologia 2006;49:755–65.
[22] Hiukka A, Fruchart-Najib J, Leinonen E, Hilden H, Fruchart JC, Taskinen MR. Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subspecies in Type 2 diabetes. Diabetologia 2005;48:1207–15.
[23] Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard B. Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status: relation to plasma triglyceride and apolipoprotein B concentration. Insulin Atherosclerosis Study (IRAS) investigators. Arterioscler Thromb Vasc Biol 1997;17:64–71.
[24] Seshadri P, Iqbal N, Stern L, Williams M, Chicano KL, Daily DA, et al. A randomized study comparing the effects of a low-carbohydrate diet and a conventional diet on lipoprotein subfractions and C-reactive protein levels in patients with severe obesity. Am J Med 2004;117:398–405.
[25] Westman EC, Yancy Jr WS, Olsen MK, Dudley T, Guyton JR. Effect of a low-carbohydrate, ketogenic diet program compared to a low-fat diet on fasting lipoprotein subclasses. Int J Cardiol 2006;110:212–6.
[26] Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. Am J Clin Nutr 2006;83:1025–31.
[27] Feinman R, Volek J. Low carbohydrate diets improve atherogenic dyslipidemia even in the absence of weight loss. Nutr Met (Lond) 2006;3:24.
[28] Herron KL, Lofgren IE, Sharma M, Volek JS, Fernandez ML. A high intake of dietary cholesterol does not result in more atherogenic LDL particles in men and women independent of response classification. Metab Clin Exp 2004;53:823–30.
[29] Greene CM, Waters D, Clark RM, Contois JH, Fernandez ML. Plasma LDL and HDL characteristics and carotenoid content are positively influenced by egg consumption in an elderly population. Nutr Met 2006;3:6.
[30] Tailleux A, Duriez P, Fruchart JC, Clavey V. Apolipoprotein A-II, HDL metabolism and atherosclerosis. Atherosclerosis 2002;164:1–3.
[31] Jahn CE, Osborne JC, Schaefer EJ, Brewer HB. In vitro activation of the enzymic activity of hepatic lipase by apo A-II. FEBS Lett 1981;131:366–8.
[32] Castenmiller JJM, West CE, Linssen JPH, van het Hof KH, Voragen AGJ. The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. J Nutr 1999;129:349–55.
[33] Voutilainen S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. Am J Clin Nutr 2006;83:1265–71.
[34] Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, et al. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). Optometry 2004;75:216–30.
[35] Erdman JJ, Bierer T, Gugger E. Absorption and transport of carotenoids. In: Canfield L, Krinsky N, Olson J, editors. Carotenoids in Human Health. , New York: NY Acad. Sci.; 1993. p. 76–86.