British Journal of Medicine & Medical Research
3(4): 2203-2213, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Two Eggs per Day Increase Plasma Lutein and Zeaxanthin in a Pediatric Population Characterized by Low Intake of Fruits and Vegetables

Martha Nydia Ballesteros[2], Rosa M Cabrera[2], Socorro Saucedo[2] and Maria Luz Fernandez[1*]

[1]Department of Nutritional Sciences, University of Connecticut, Storrs, CT, USA.
[2]Centre for Food and Development, Hermosillo, Sonora, México.

Authors' contributions

This work was carried out in collaboration between all authors. Author MNB recruited the subjects, collected the data, analyzed plasma lipids and contributed to the writing of the paper. Authors RMC and SS contributed to recruitment and data collection; author MLF designed the experiment, analyzed the data and had a primary role in writing the manuscript. All authors read and approved the final manuscript.

Research Article

Received 2nd May 2013
Accepted 3rd July 2013
Published 23rd July 2013

ABSTRACT

Aims: Eggs are a good source of lutein and zeaxanthin, carotenoids known for their antioxidant properties. Mexican children have been shown to consume limited amounts of fruit and vegetables. The purpose of the current study is to determine whether the inclusion of eggs in the diet increases plasma carotenoids in this population.
Study Design: This study is a follow up on the effects of high egg intake on plasma lipids and atherogenic lipoproteins in children. Fifty four Mexican children (25 boys/29 girls) aged 8-12 y were randomly assigned to consume either 2 eggs/d (518 mg additional dietary cholesterol) (EGG period) or the equivalent amount of egg whites (SUB Period) in a cross-over design for 4 wk. After a 3 wk washout, children were crossed over to the alternate treatment.
Methodology: 3-day dietary records, plasma carotenoids and apolipoproteins were measured at the end of the EGG and SUB Periods**.**
Results: In agreement with the lack of effects of eggs in increasing atherogenic

**Corresponding author: Email: maria-luz.fernandez@uconn.edu;*

lipoprotein profiles, plasma apolipoprotein B concentrations did not change between periods indicating that increases in plasma cholesterol were not associated with higher number of LDL particles. Although the values for apo C-III were high compared to other pediatric populations, they were not affected by egg intake. Dietary records indicated low intake of carotenoids, especially during the SUB period. Plasma lutein and zeaxanthin were increased during the EGG period from 0.235 ± 0.071 to 0.280 ± 0.147 μmol/L ($P<0.001$) and 0.044 ± 0.019 to 0.051 ± 0.031 μmol/L ($P<0.001$), respectively.
Conclusions: These results suggest that the eggs are a good source of lutein and zeaxanthin in this population and that the increases in LDL size during the egg period may also be related to a better transport of these carotenoids in plasma.

Keywords: Eggs; Mexican children; lutein; zeaxanthin; LDL/HDL ratio.

1. INTRODUCTION

Dietary guidelines that target reductions in coronary heart disease (CHD) risk, recommend no more than 300 mg/day of dietary cholesterol, a recommendation that limits egg intake [1]. There are a number of epidemiological studies that do not support a relationship between egg intake and CHD incidence [2,3]. Furthermore, the Lipid Research Clinics Prevalence Follow-up Study [4], which examined both men and women (n=4546) reported no significant relationships between CHD deaths and dietary cholesterol intake. Several other studies [5,6,7] have also failed to find associations between CHD incidence and egg intake. More recently, lack of correlations between egg intake and risk for coronary heart disease or stroke, have been reported [8,9].

Epidemiological studies indicate an inverse relationship between intake of lutein and zeaxanthin, and age related macular degeneration [10]. Clinical interventions have demonstrated that egg intake increases macular pigment density [11]. These carotenoids may also play a role in decreasing the susceptibility of the LDL particle to oxidation [12].

In spite of the modifications of the American Heart Association from their previous policy of eating only two eggs per week, to permitting the consumption of one yolk per day [13], the majority of the population is still concerned in regards to egg intake. These recommendations create uncertainty in those individuals who might derive health benefits by including eggs in their diets.

In a National survey conducted to evaluate risk for heart disease in Mexico, the Northern States including Sonora and Sinaloa were reported to have the highest levels of plasma LDL cholesterol and triglycerides compared to the rest of the country [14]. Data from The National Census on Health and Nutrition reports that similar to developed countries, the number one cause of death in Mexico is CHD [15]. Previous studies have clearly demonstrated that not only adults [16,17] but also children from this area of Mexico [18] present the lipid profiles characteristic of the metabolic syndrome and insulin resistance [19], which greatly increase the risk for diabetes and heart disease later in life.

In addition, to this high predisposition for CHD, Mexican children from this region consume limited amounts of fruits and vegetables [20], which can greatly increase the oxidation capacity in plasma. However, these children consume eggs as part of their regular diet. We previously reported that egg consumption does not increase the risk of heart disease in this population [18]. Our current hypothesis is that that egg consumption would favorably affect

the concentrations of plasma lutein and zeaxanthine, which may increase plasma antioxidant capacity in a pediatric population characterized by low intake of fruits and vegetables.

2. SUBJECTS AND METHODS

2.1 Experimental Protocol

The Experimental protocol was approved by the University of Connecticut Institutional Review Board and by the Review Board of CIAD A.C. The experimental design has been described in detail previously [18]. Briefly, the protocols were explained to the children and parents signed the consent form. Children from the School Mauricio Kelly (Hermosillo, Mexico) were provided free breakfast during 2 two month intervals. They were randomly allocated to consume 2 eggs (additional 518 mg of cholesterol) per day or the equivalent amount of egg whites (0 additional mg of dietary cholesterol). After a three week wash out period, children were allocated to the alternate diet. Based on the mean differences in LDL and HDL-C concentrations and standard deviations following egg intake, from our previous studies, we estimated that a total of 50 children would be needed to identify differences in plasma lipids and plasma carotenoids [21,22]. We recruited 60 children to allow for attrition and 54 subjects (25 boys and 29 girls) completed the study.

Children were asked not to change their regular diet during the whole intervention. Three-day weighed food records [23] were used to evaluate cholesterol and carotenoids intake. The individuals in charge of preparing meals at home were given food scales (Ohaus CS 2000) and a chart to record the child's daily intake [23]. Diet intake was analyzed using the ESHA Food processor program (ESHA, Food Processor, 7.20, ESHA Research Editor, 1998). Regional foods that were not included in the data-base were analyzed for individual components and added to the data-base (Ballesteros et al., unpublished data). Parents in agreement with children completed three 24-h dietary records during each treatment period.

2.2 Plasma Lipids, Apolipoproteins and Insulin

Fasting blood samples were collected on two separate days for each child to account for day to day variability and plasma lipids were measured at baseline, during the washout and at the end of each dietary period as previously reported [18]. Apolipoprotein (apo) B concentrations were determined using an immunoturbidimetric method and turbidity was measured in a microplate spectrophotometer at 340 nm [24]. Plasma apo CIII was measured from 150µl of sample using WAKO kits in a Hitachi Autonalyzer 740 as previously reported [25]. Plasma insulin was measured using a Linco RIA kit (Linco Research, Inc, St. Charles, MI) that utilizes the double-antibody/PEG technique [26].

2.3 Plasma Carotenoids

Plasma (200µl) was prepared for HPLC analysis by combining each sample with an internal standard of 50 µl ethyl-β-apo-8`-carotenoate (Fluka, Ronkonkoma, NY) and 200 µl ethanol containing butylated hydroxytoluene, similar to the method previously described [27]. Briefly, the sample was extracted three separate times using a hexane carrier containing butylated hydroxytoluene while centrifugation facilitated the phase separation. The solvent was removed with a stream of nitrogen and the resulting hexane layers were reconstituted with 100 µl of 2-propanol and placed into HPLC injection vials. A Waters HPLC system equipped with a Varian column (100x4.6 mm Microsorb-MN 100-3 C-18), and preceded by an Upchurch C-18 guard column (Upchurch Scientific, Oak Harbor, WA) was used to analyze

the carotenoid content of the plasma. The isocratic mobile phase contained 80% acetonitrile, 15% dioxane, 2.5% methanol, 2.5% 2-propanol, 0.01% triethylamine, and 0.01% ammonium acetate. The internal standard and carotenoid content of the plasma was detected at 450 nm. All solvents were HPLC grade and were filtered and degassed before use. Standard curves were compiled from HPLC purified lutein and zeaxanthin.

2.4 Data Analysis

Repeated measures ANOVA was utilized to determine dietary treatment effects on plasma lipids, apolipoproteins B and C-III, insulin and both dietary and plasma carotenoids between the EGG and SUB periods. The repeated measure was time and the between subjects factor was gender. $P<0.05$ was considered statistically significant. SSPS version 11.5 was used for statistical analysis

3. RESULTS AND DISCUSSION

3.1 Diet

The main differences in diet have been previously reported [18]. All children irrespective of gender consumed more total fat, saturated and monounsaturated fat and vitamin E during the EGG period while they consumed more carbohydrate and protein during the SUB period ($P<0.05$) [18]. The amount of dietary cholesterol was higher ($P<0.001$) during the EGG period with boys consuming 677 ± 121 mg versus 171 ± 127 and girls consuming 614 ±158 mg versus 125 ± 66 during the EGG and SUB periods, respectively. The amount of dietary cholesterol during the egg period corresponds to 2X the recommendations in US. However, it is important to note that many countries including European Union, Korea, India, Australia and New Zealand among others, do not have an upper limit for dietary cholesterol [28].

3.2 Plasma Lipid, Apolipoproteins and Insulin

Both LDL and HDL cholesterol were higher during the EGG period for both genders ($P<0.05$) (Table 1); however the LDL/HDL was maintained during both dietary periods. Plasma apolipoproteins B and C-III as well as plasma insulin were not affected either by gender of by dietary intervention.

3.3 Dietary and Plasma Carotenoids

Result from carotenoid intake in mg/d is presented in Table 2. As predicted by the diet, the concentrations of lutein + zeaxanthin were higher during the EGG period ($P<0.01$). In addition dietary lycopene was also higher during the EGG period. The concentrations of the other carotenoids: α and β carotene, and cryptoxanthine were not different between periods or between gender.

The concentrations of plasma carotenoids were in agreement with dietary intake (Table 3). Plasma lutein and zeaxanthin were higher during the egg period both in boys and girls ($P < 0.05$). While there were no significant differences in the other plasma carotenoids although dietary lycopene was higher during the EGG period.

Table 1. Plasma LDL cholesterol (LDL-C), HDL-cholesterol (HDL-C), LDL/HDL ratio, apolipoprotein (apo) B apo C and apo E of boys and girls during the EGG or egg substitute (SUB) dietary periods[1]

	Boys		Girls		Diet effect	Normal Range
	EGG	SUB	EGG	SUB	(P value)	
LDL-C (mg/dL)	73.7 ± 17.2	66.2 ± 14.1	73.7 ± 14.1	68.5 ± 17.7	< 0.01	70-100 mg/dL
HDL-C (mg/dL)	52.1 ± 8.9	48.3 ± 8.2	47.5 ± 9.7	46.1 ± 8.9	<0.025	40-70 mg/dL
LDL/HDL	1.45 ± 0.43	1.39 ± 0.52	1.63 ± 0.51	1.55 ± 0.49	NS	< 3.5
Apo B (mg/L)	596 ± 99	620 ± 136	586 ± 107	616 ± 95	NS	630-1114 mg/L
Apo C-III (mg/L)	105 ± 28	120 ± 33	116 ± 58	111 ± 33	NS	5.5- 95 mg/L
Insulin (µU/ml)	12.4 ± 6.0	13.1 ± 7.4	11.9 ± 5.9	13.0 ± 5.7	NS	2.0-20 µU/ml

[1]*Values are expressed as mean ± S.D for N= 25 boys and N= 29 girls. There were no gender or interactive effects for any of plasma lipids, apo B, Apo B or Apo C*

Table 2. Dietary Carotenoids in Boys and Girls during the EGG or Egg Substitute (SUB) periods[1]

(mg/d)	Boys		Girls		Diet Effect
	EGG	SUB	EGG	SUB	(P value)
Lutein+ Zeaxanthin	538 ± 330	271 ± 294	632 ± 432	264 ± 343	< 0.01
β-Carotene	962 ± 1130	716 ± 675	616 ± 633	803 ± 1019	NS
α-Carotene	219 ± 456	84 ± 128	91 ± 164	86 ± 178	NS
Lycopene	3282 ± 3158	1680 ± 1872	1740 ± 2318	1407±1794	P < 0.05
Cryptoxanthine	46 ± 55	43 ± 90	37 ± 92	59±153	NS

[1] *Values are expressed as mean ± S.D for N= 25 boys and N= 29 girls. There were no gender or interactive effects for dietary carotenoids*

Table 3. Plasma Carotenoids in Boys and Girls during the EGG or Egg Substitute (SUB) periods[1]

µmol/L	Boys		Girls		Diet Effect
	EGG	SUB	EGG	SUB	(P value)
Lutein	0.273 ± 0.105	0.237 ± 0.068	0.287 ± 0.176	0.237 ± 0.075	< 0.05
Zeaxanthin	0.045 ± 0.030	0.044 ± 0.020	0.053 ± 0.034	0.044 ± 0.020	<0.05
β-Carotene	0.135 ± 0.092	0.135 ± 0.064	0.147 ± 0.089	0.163 ± 0.108	NS
α-Carotene	0.022 ± 0.047	0.016 ± 0.029	0.016 ± 0.022	0.017 ± 0.022	NS
Lycopene	0.190 ± 0.090	0.222 ± 0.100	0.161± 0.093	0.193 ± 0.100	NS
Cryptoxanthine	0.082 ± 0.039	0.108 ± 0.087	0.104 ± 0.056	0.119 ± 0.068	NS

[1] *Values are expressed as mean ± S.D for N= 25 boys and N= 29 girls. There were no gender or interactive effects for plasma carotenoids.*

In this study, we demonstrated that consumption of 2 eggs per day for 30 days by Mexican children, results in significant increases in plasma carotenoids, that may be possibly associated with the larger LDL size [18] and with the higher concentrations of large HDL [29] that has been observed following egg consumption. Interestingly, plasma apo B concentrations and the LDL/HDL ratio, two key markers of heart disease risk [30,31] did not change. These results also indicate that egg consumption provided higher concentrations of those plasma carotenoids that have been shown to decrease LDL oxidation [32] and protect against CHD [33,34].

We have previously shown that some of the children experienced increases in total cholesterol following the consumption of 2 eggs per day. However, the increases were both in LDL and HDL cholesterol with the consequent maintenance of the LDL/HDL ratio. It is also notable that LDL size was increased during the EGG period for all children irrespective of response classification [18].

It is well documented that the small dense LDL particles increase 3-fold the risk for CHD [35] due to their ability to become more easily oxidized, thus increasing their capability to penetrate the arterial wall and to begin the atherogenic process. In addition to our report in children [18], egg consumption has also been shown to increase LDL size in young adults [22], older individuals [36] and overweight subjects [37]. Large LDL do not become readily oxidized due their larger surface, which allows higher transport of antioxidants including Vitamin E [38] and carotenoids [37]. LDL size $\leq$ 26.8 nm is considered smaller LDL or pattern B while LDL > 26.9 nm is considered larger LDL or pattern A as measured by the Lipoprint system [39]. In our study, there were 36 children with pattern B after the SUB period and this number shifted to 31 during the EGG period indicating that eggs not only contributed to the increases in LDL size but also that there was a shift to the less atherogenic LDL profile.

This increase in LDL size is specifically beneficial to this population that is characterized by a large proportion of phenotype B, or increased concentration of smaller LDL particles [18]. Both adults and children in this region of Mexico are characterized by having an increased risk for CHD compared to the rest of the country [15]. Thus, dietary interventions aimed at reducing small LDL can be considered atheroprotective. These larger more buoyant LDL particles generated by egg intake [18,22,35,38] conveniently provide a larger surface for increased plasma transport of both lutein and zeaxanthin, the carotenoids present in eggs.

In addition, children did not have an increase in apoliprotein B during the EGG period. Apolipoprotein B has been reported to be a risk factor for heart disease independent of plasma cholesterol concentrations in clinical studies [27]. Consumption of eggs did not result in the formation of more apo B containing lipoproteins, which is in agreement with the generation of large LDL particles as previously observed [18]. Although Apo C-III was not affected by diet, values for this apolipoprotein were found to be high in these children when compared to those in adults [40]. Apo CIII is known to inhibit triglycericde hydrolysis by lipoprotein lipase [41,42], decreasing the removal of triglycerides from VLDL or chylomicrons. In humans, plasma apo CIII concentrations correlate with triglyceride levels [43] and apo C-III is higher in hypertriglyceridemic individuals [44]. Also plasma insulin was not affected by diet; however, the reported values are considered to be high for young children [45]. These elevated levels of both apo C-III and insulin indicate that these children might be at higher risk for heart disease and diabetes in agreement with the data from The Secretary of Health in Mexico [15].

Compared to US, children in this area of Mexico consume substantially less carotenoids in their diet [20]. For example US children have been reported to have plasma concentrations of 0.34 μmol/L [46], which is higher than the concentration of 0.28 μmol/L that was found in children after consuming the eggs in the current study. The low concentrations of lycopene, cryptoxanthin, β-carotene and lutein and zeaxanthin confirm dietary patterns of limited consumption of fruits and vegetables in this population [46]. Among Mexican children, the main dietary sources of lutein and zeaxanthin were orange juice, grape juice, quelites, corn tortillas, peaches and spinach in addition to the eggs consumed during the EGG period.

Studies suggest the potential contribution of the carotenoids lutein and zeaxanthin in the prevention of heart disease and stroke [10]. Specifically, lutein has been shown to exert cardio-protective effects in clinical studies [47]. Higher intake and higher plasma concentration of lutein are also associated with reduced risk of CHD [48]. Lutein has also been shown to be protective against oxidation and inflammation in animal [49] and cell studies [50]. In a study conducted in guinea pigs fed a hypercholesterolemic diet (0.25% cholesterol) in combination with lutein, there was a protective effect of this carotenoid against atherosclerosis at multiple points [32]. Compared to control guinea pigs, lutein-treated animals had fewer medium-small LDL particles, lower concentrations of oxidized LDL in plasma, lower amounts of both cholesterol and oxidized LDL in aorta and lower concentrations of inflammatory cytokines including IL-6 suggesting a protective anti-oxidant effect with this carotenoid in the presence of elevated cholesterol [32].

4. CONCLUSION

We conclude that whole egg consumption is a good alternative for increasing the circulation of plasma lutein and zeaxanthin two plasma carotenoids that have been shown to protect against inflammation [33], oxidation [10] and atherosclerosis development [32]. Further, eggs not only do not increase the risk of heart disease but they may also exert a protective effect due to increased concentrations of these carotenoids. As previously reported in children [18] and many other studies in adults [21,22,36,37] when plasma cholesterol is increased due to egg intake, there are increases in both LDL and HDL cholesterol, which results in maintenance of the LDL/HDL ratio a key marker of CHD risk [31]. Further eggs decrease the atherogenicity of LDL promoting the formation of larger LDL, and large HDL [36], which in turn can more easily transport carotenoids in plasma [37]. Thus increased consumption of eggs may help protect these children characterized by having elevated biomarkers for heart disease and by limited consumption of fruits and vegetables.

CONSENT

All authors declare that ‘written informed consent was obtained from the parents of the children.

ETHICAL APPROVAL

All authors hereby declare that all human studies have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

We thank David Waters for assisting in HPLC analysis of carotenoids. This study was supported by the Egg Nutrition Center.

COMPETING INTERESTS

This study was supported by an Egg Nutrition Center award given to MLF.

REFERENCES

1. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA. et al. Diet and lifestyle recommendations Revision 2006. A scientific statement from the American Heart Association Scientific Committee. Circulation. 2006;114(1):82-96.
2. Hu FB, Stampfer MJ, Rimm EB, Manson JE, Ascheiro A, Colditz GA. et al. A prospective study of egg consumption and risk of cardiovascular disease in men and women. JAMA. 1999;281(15):1387-94.
3. Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol. Am J Clin Nutr. 1997;65(6):1747-64.
4. Esrey KL, Joseph L, Grover SA. Relationship between dietary intake and coronary heart disease mortality: lipid research clinics prevalence follow-up study. J. Clin. Epidemiol. 1996;49(2):211-16.
5. McNamara DJ. Dietary cholesterol and atherosclerosis. Biochim Biophys Acta. 2000;1529(1-3):310-20.
6. Kritchevsky SB. A review of scientific research and recommendations regarding eggs. J Am Coll Nutr. 2004;(6 Suppl)23:596S-600S.
7. Krumholz HM, Seeman TE, Merrill SS, Mendez de Leon CF, Vaccarino V, Silverman DI et al. Lack of association between cholesterol and coronary heart disease mortality and morbidity and all-cause mortality in persons older than 70 years. JAMA 1994;272(27):1335-40.
8. Nakamura Y, Iso H, Kito Y, Ueshima H, Okada K, Konishi M et al. S. Egg consumption, serum total cholesterol concentrations and coronary heart disease incidence: Japan Public Health Center-based prospective study. Br J Nutr. 2006;96(5):921-8.
9. Qureshi AI, Suri FK, Ahmed S. Nasar A, Divani AA, Kimani JF. Regular egg consumption does not increase the risk of stroke and cardiovascular diseases. Med Sci. Monit. 2007:13(1):CR1-8.
10. Ribaya-Mercado JD, Blumberg JB. Lutein and zeaxanthin and their potential roles in disease prevention. J. Am Coll. Nutr. 2004:23(6 Suppl):567S-587S.
11. Vishwanathan R, Goodrow-Kotyla EF, Wooten BR, Wilson TA, Nicolosi RJ. Consumption of 2 and 4 egg yolks/d for 5 wk increases macular pigment concentrations in older adults with low macular pigment taking cholesterol-lowering statins. Am J Clin Nutr. 2009;90(5):1272-9.
12. Barona J, Jones JL, Kopek R, Comperatore M, Andersen C, Schwartz S, et al. A Mediterranean-style low-glycemic-load diet increases plasma carotenoids and decreases LDL oxidation in women with metabolic syndrome. J. Nutr Biochem. 2012;23(6):609-15.
13. American Heart Association; 2004. www.americanheart.org.

14. Posadas-Romero C, Tapia-Conyer R, Lerman-Garber I, Zamora-González J, Cardoso-Saldaña G, Salvatierra-Izaba B, et al. Cholesterol levels and prevalence of hypercholesterolemia in a Mexican adult population. Atherosclerosis. 1995;118(2):275-84.
15. Encuesta Nacional de Salud y Nutricion. Instituto Nacional de Salud Publica; 2006.
16. Romero AL, Romero JE, Galaviz S, Fernandez ML. Cookies enriched with psyllium and oat bran lower plasma LDL-cholesterol in normal and hypercholesterolemic men from Northern Mexico. J. Am. Coll. Nutr. 1998;17:601-608.
17. Vidal-Quintanar RL, Mendivil RL, Peña M, Fernandez ML. Lime-treated maize husks lower plasma LDL-cholesterol in normal and hypercholesterolemic adult men from northern Mexico. Brit. J. Nutr. 1999;81(4):281-88.
18. Ballesteros MN, Cabrera RM, Saucedo MS, Fernandez ML. Dietary cholesterol does not increase biomarkers for chronic disease in a pediatric population at risk from Northern Mexico. Am. J. Clin. Nutr. 2004;80(4):855-61.
19. Fernandez ML. The Metabolic Syndrome. Nutr Rev. 2007;64:S30-S34.
20. Ramírez-Silva I, Rivera JA, Ponce X, Hernández-Avila M. Fruit and vegetable intake in the Mexican population: results from the Mexican National Health and Nutrition Survey 2006. Salud Publica Mex. 2009;51(Suppl4):S574-85.
21. Herron KL, Vega-Lopez S, Ramjiganesh T, Conde-Knape K, Shachter N, Fernandez ML. Men classified as hypo- or hyper-responders to dietary cholesterol feeding exhibit differences in lipoprotein metabolism. J. Nutr. 2003;133(4):1036-42.
22. 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 (6):823-30.
23. Bingham SA. The dietary assessment of individuals: methods, accuracy, new techniques and recommendations. Dietary Survey Method. 1989;57:705-741.
24. Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A-I, A-II and B in serum. Clin. Chem. 1986;32(12):957-60.
25. Vega-Lopez S, Conde K, Vidal-Quintanar RL, Shachter NS, Fernandez ML. Sex and Hormonal Status Influence the Effects of Psyllium on Lipoprotein Remodeling and Composition. Metab. Clin Exp. 2002;51(4):500-7.
26. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: Advantages, limitations, and appropriate usage. Am J Physiol- Endocrinol Metabol. 2008;294(1):E15-E26.
27. 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(3):601-7.
28. Fernandez ML, Calle MC. Revisiting dietary cholesterol recommendations: does the evidence support a 300 mg/d limit? Current Atherosclerosis Reports. 2010;12(6):377-83.
29. Blesso CN, Andersen C, Barona J, Volek J, Fernandez ML. Whole egg consumption improves lipoprotein profiles and insulin sensitivity in individuals with metabolic syndrome. Metabolism. 2013;62(3):400-10.
30. 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 Jl. 2005;26(3):271-8.
31. Fernandez ML. The LDL to HDL cholesterol ratio, a more reliable clinical tool than LDL cholesterol to evaluate coronary heart disease risk. The Lipid Spin. 2009;4:4-6.
32. Kim JE, Leite JO, DeOgburn R, Smyth JA, Clark RM., Fernandez ML. A lutein-enriched diet prevents cholesterol accumulation and decreases oxidized LDL and inflammatory cytokines in the aorta of guinea pigs. J. Nutr. 2011;141(8):1458-63.

33. Hozawa A, Jacobs DR Jr, Steffes MW, Gross MD, Steffen LM, Lee DH. Relationships of circulating carotenoid concentrations with several markers of inflammation, oxidative stress, and endothelial dysfunction: the Coronary Artery Risk Development in Young Adults CARDIA)/Young Adult Longitudinal Trends in Antioxidants (YALTA) study. Clin Chem. 2007;53(3):447-55.
34. Dwyer JH, Paul-Labrador MJ, Fan J, Shircore AM, Merz CN, Dwyer KM. Progression of carotid intima-media thickness and plasma antioxidants: the Los Angeles Atherosclerosis Study. Arterioscler Thromb Vasc Biol. 2004;24(2):313-9.
35. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res. 2002;43(9):1363-79.
36. 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.
37. Mutungi G, Waters D, Ratliff J, Puglisi MJ, Clark RM, Volek JS, et al. Eggs distinctly modulate plasma carotenoid and lipoprotein subclasses in adult men following a carbohydrate restricted diet. J Nutr Biochem. 2010;21(4):261-7.
38. Miwa K. Low density lipoprotein particles are small in patients with coronary vasospasm. Int J Cardiol. 2003;87(2-3):193-201.
39. Hoefner DM, Hodel SD, O'Brien JF, Branum EL, Sun D, Meissner I, et al. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. Clin Chem. 2001;47(2):266-274.
40. Herron KL, Vega-Lopez S, Ramjiganesh T, Conde-Knape K, Shachter N, Fernandez ML. Men classified as hypo- or hyper-responders to dietary cholesterol feeding exhibit differences in lipoprotein metabolism. J. Nutr. 2003;133(4):1036-42.
41. Brown WB, Baginsky ML. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. Biochem Biophys Res Comm 1972;46:375-382.
42. Shachter NS. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. Curr Opin Lipidol. 2001;12(3):297-304.
43. Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex V, Stampfer MJ, et al. VLDL, Apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. Circulation 2000;102(16):1886-92.
44. Frendenrich A, Giroux LM, Tremblay M, Krimbou L, Davingnon J, Cohn JS. Plasma lipoprotein distribution of apo C-III in normolipidemic and triglyceridemic subjects: comparison of the apo C-III to apo E ratio in different lipoprotein fractions. J Lipid Res. 1997; 38(7):1421-32
45. Lofgren IE, Herron KL, West KL, Zern TL, Brownhill R, Ilich-Ernst J, et al. A weight loss program favorably modifies anthropometrics and reverses the metabolic syndrome and insulin resistance in premenopausal women. J. Am Coll. Nutr. 2005;24(6):486-93
46. Burrows TL, Warren JM, Colyvas K, Garg ML, Collins CE. Burrows TL, et al. Validation of overweight children's fruit and vegetable intake using plasma carotenoids Obesity. 2009;17(1):162-8.
47. Dwyer JH, Paul-Labrador MJ, Fan J, Shircore AM, Merz CN, Dwyer KM. Progression of carotid intima-media thickness and plasma antioxidants: the Los Angeles Atherosclerosis Study. Arterioscler Thromb Vasc Biol. 2004;24(2):313-9.
48. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, et al. Heart Disease and Stroke Statistics—2006 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2006;113(6):e85-151.

49. Jin XH, Ohgami K, Shiratori K, Suzuki Y, Hirano T, Koyama Y, et al. Inhibitory effects of lutein on endotoxin-induced uveitis in Lewis rats. Invest Ophthalmol Vis Sci. 2006;47(6):2562-8.
50. Kim JH, Na HJ, Kim CK, Kim JY, Ha KS, Lee H, et al. The non-provitamin A carotenoid, lutein, inhibits NF-kappaB-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-kappaB-inducing kinase pathways: role of H(2)O(2) in NF-kappaB activation. Free Radic Biol Med. 2008;45(6):885-96.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=205&id=12&aid=1719