Original Article

Effects of Lutein Supplement on Serum Inflammatory Cytokines, ApoE and Lipid Profiles in Early Atherosclerosis Population

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Aim: The purpose of this study was to determine the effects of lutein supplement on serum cytokines, apoE and lipoprotein profiles in early atherosclerosis population.

Methods: Early atherosclerosis patients ($n=65$) were randomized to receive placebo (A + P, $n=31$) or 20 mg/d lutein (A + L, $n=34$) for 3 months.

Results: Serum lutein increased significantly compared to baseline after lutein supplements in A+L group ($p<0.001$). Lutein supplements resulted in a significant decrease in serum interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) at 3 month in A + L group ($p<0.05$). Intragroup comparison revealed a significant difference in the changes of serum MCP-1 between A + L and A + P groups ($p=0.021$). The serum low-density lipoprotein (LDL) and triglyceride (TG) significantly decreased in A + L group ($p<0.05$). The changes in serum lutein were negatively associated with those in serum LDL in A + L group ($r=-0.384$, $p=0.043$), while no such relationship was observed in A + P group ($r=0.087$, $p=0.685$).

Conclusion: An increase in serum lutein after supplementation can reduce inflammatory cytokines and regulate serum lipids, which may play important roles in early atherosclerosis.

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Key words; Lutein, Early atherosclerosis, Cytokines, ApoE, LDL

Introduction

Atherosclerosis is a pathologic status that underlies several important adverse vascular events, such as coronary disease, stroke, and peripheral arterial problems¹. It has become the leading cause of cardiovascular morbidity and mortality in western societies, accounting for a fifth of deaths worldwide². While the use of established medical therapies has reduced mortality from its complications, the adoption of a western lifestyle has led to a large increase in the prevalence of atherosclerosis throughout the world³; therefore, the prevention of atherosclerosis remains a major public health challenge for both China and for western countries.

Atherosclerosis is an inflammatory disease⁴. Inflammation is a key regulatory process that links multiple risk factors for atherosclerosis and its complications⁵. Oxidized low density lipoprotein (oxLDL) induces endothelial dysfunction and recruits monocytes and lymphocytes, initiating the development of atherosclerosis lesions. Thereafter, cytokines and chemokines secreted by activated macrophages perpetuate and amplify inflammatory responses⁶. In atherosclerosis patients, the extent of inflammatory infiltrates and their strategic location within protective fibers is associated with plaque rupture and/or thrombosis⁷. Furthermore, circulating inflammatory biomarkers, especially C-reactive protein, have been found to be associated with outcomes of cardiovascular events, even in the absence of dyslipidaemia⁸. These findings support the inflammatory nature of atherosclerosis; therefore, there is a hypothesis that antioxidants such as carotenoids could be used as an inexpensive means of the prevention and possibly treatment of atherosclerosis and its complications.

Lutein, one of the most abundant carotenoids in

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the human body⁹⁾, is believed to play a protective role against atherosclerosis. In an epidemiological study, subjects with the highest serum lutein ($0.42 \mu\text{mol/L}$) had 80% less arterial wall thickening than those with the lowest quintile of serum lutein ($0.15 \mu\text{mol/L}$), while no such relationship was observed with serum beta-carotene¹⁰⁾. Koh *et al.* also found that a high level of plasma lutein was associated with a decreased risk of acute myocardial infarction after adjustment for multiple risk factors for coronary heart disease in a Chinese population¹¹⁾. In animal experiments, a lutein-enriched diet could inhibit cholesterol accumulation and decrease oxidized LDL and inflammatory cytokines in the aorta of Guinea pigs¹²⁾. Other epidemiological studies, including the Atherosclerosis Risk in Communities (ARIC) study¹³⁾ and Health Professionals Follow-up Study¹⁴⁾, also found possible beneficial effects of a lutein-rich diet.

Aim

Although experimental and epidemiological studies suggest the possible benefits of lutein against atherosclerosis, randomized control trials are scarce. In addition, atherosclerotic plaque development and progression are associated with inflammatory changes from early stages. Atherosclerosis plaque could be considered to be the result of interactions among modified lipoproteins, inflammatory cytokines and elevated cholesterol. In early atherosclerosis, serum inflammatory markers are sensitive predictors of lesion development. In our study, we examined the effects of lutein supplementation on serum lipoproteins, apoE and cytokines in early atherosclerosis patients and verified the possible benefits.

Participants and Methods

Subjects

The subjects of this study were from a follow-up visit of men and women in the The Beijing Atherosclerosis Study, a randomized, double-blind, placebo-controlled interventional trial that was designed to investigate the effects of lutein on in the prevention of atherosclerosis¹⁵⁾. The study participants consisted of individuals with early atherosclerosis diagnosed by their carotid intima media thickness (IMT)¹⁶⁾ (as $\text{IMT} > 750 \mu\text{m}$ under 59 years old or $\text{carotid IMT} > 850 \mu\text{m}$ over 60 years old). Subjects of the present study were 65 patients after diagnosis. The patients were randomly assigned to receive 20 mg/d lutein (A+L, $n=34$) or a placebo control (A+P, $n=31$) for three months. The study was approved by the ethics com-

mission of Peking University Health Science Department and all participants gave written informed consent.

Lutein Sources and Study Designs

Study substances were suspended in wild camellia oil and given in acid-soluble gelatin capsules. A placebo containing wild camellia oil (100%) was supplied in identical-looking capsules. All the subjects were instructed to consume one capsule per day immediately after lunch and to store the study substance at room temperature. All participants were encouraged to maintain their usual lifestyle and dietary habits. Compliance was assessed at the end of the study using pill counting, food records, and measurement of plasma lutein levels.

Anthropometric Parameters, Blood Pressure, and Blood Collection

Body weight and height were measured in the morning without shoes to calculate the body mass index (BMI) (kg/m^2). Blood pressure (BP) was measured in the left arm of seated patients after a 20-min rest. Study subjects were interviewed for smoking behavior and drug administration during their visits. After a 12-h fasting period, venous blood specimens were collected in EDTA-treated and plain tubes, centrifuged to produce plasma or serum, and stored at -70°C until analysis.

Serum Lipid Profile, Fasting Glucose, ApoE and Cytokines Analysis

Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and glucose were measured within 2 days of collection by an auto-analyzer at Beijing Lawke Health Laboratory.

Apolipoprotein E (ApoE) and inflammatory cytokines, including monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), endothelin-1 (ET-1), interferon- γ (IFN- γ), and vascular cell adhesion molecule-1 (VCAM-1), were analyzed by a specific, commercially available ELISA kit (R&D Systems, Minneapolis, MN) with an ELISA reader at 450 nm, in accordance with the manufacturer's instructions. Sensitivity of the assay was 1.0 pg/mL for all the cytokines and the intra- and inter-assay coefficients of variation (CV) were 4.8% and 5.6%, respectively.

Plasma levels of lutein, zeaxanthin, β -carotene and lycopene were analyzed by high pressure liquid chromatography (HPLC) derived from the method described by Yeum *et al.*¹⁷⁾. In brief, 500 μL serum, 500 μL internal standard (8-apo- β -carotenal) and 500

Table 1. Demography and background characteristics

Characters	A + 20 mgL (n = 34)	A + Placebo (n = 31)	p value
Age, mean $\pm$ SD, y	57.8 $\pm$ 3.1	56.2 $\pm$ 4.6	0.152
Male, n (%)	16 (47.1)	11 (35.5)	0.344
BMI (Kg/m ²)	25.6 $\pm$ 3.1	25.9 $\pm$ 2.6	0.594
Hypertension, n (%)	5 (14.7)	8 (25.8)	0.264
Current or former smoker	10 (29.4)	8 (25.8)	0.930
Diabetes, n (%)	5 (14.7)	4 (12.9)	1.00 [▲]
Lipid-lowering medication, n (%)	8 (23.5)	11 (35.5)	0.290
Dyslipidemia, n (%)	13 (38.2)	11 (35.5)	0.818
Risk factors			
Blood pressure			
SBP, mmHg	125.6 $\pm$ 14.2	131.8 $\pm$ 14.7	0.083
DBP, mmHg	80.9 $\pm$ 9.0	83.6 $\pm$ 8.4	0.209
Lipid profile (mmol/L)			
CHO	5.20 $\pm$ 1.08	5.11 $\pm$ 0.83	0.714
LDL-C	2.98 $\pm$ 0.87	3.07 $\pm$ 0.72	0.641
HDL-C	1.37 $\pm$ 0.28	1.38 $\pm$ 0.24	0.891
Triglycerides	1.94 $\pm$ 1.17	1.81 $\pm$ 0.73	0.564
Fasting blood sugar, mmol/L	5.55 $\pm$ 1.35	5.42 $\pm$ 1.19	0.678
Serum carotenoids, μ mol/L			
Lutein	0.17 $\pm$ 0.10	0.13 $\pm$ 0.09	0.122
Zeaxanthin	0.03 $\pm$ 0.02	0.04 $\pm$ 0.03	0.430
β -carotene	0.08 $\pm$ 0.003	0.08 $\pm$ 0.002	0.756
Lycopene	0.087 $\pm$ 0.032	0.100 $\pm$ 0.036	0.235
Serum cytokines (pg/mL)			
IL-6, mean $\pm$ SD	197.38 $\pm$ 311.75	86.19 $\pm$ 110.29	0.090
Median (IQR)	67.82 (45.85-123.15)	63.80 (45.41-73.83)	0.232 [#]
IFN- γ , mean $\pm$ SD	98.67 $\pm$ 97.61	110.38 $\pm$ 120.77	0.698
Median (IQR)	72.94 (52.66-96.96)	58.77 (45.41-73.83)	0.442 [#]
ET-1, mean $\pm$ SD	2.40 $\pm$ 2.96	2.29 $\pm$ 3.28	0.905
Median (IQR)	1.27 (0.96-2.07)	1.1 (0.85-1.60)	0.305 [#]
MCP-1, mean $\pm$ SD	442.05 $\pm$ 174.52	399.17 $\pm$ 119.85	0.295
Median (IQR)	431.57 (311.54-563.13)	387.81 (318.62-482.4)	0.278 [#]
VCAM-1, mean $\pm$ SD	686.62 $\pm$ 514.63	675.05 $\pm$ 600.42	0.942
Median (IQR)	410.84 (311.05-1280.28)	395.68 (294.67-1179.85)	0.838 [#]
Serum apoE (μ g/mL)			
Mean $\pm$ SD	180.71 $\pm$ 95.66	131.51 $\pm$ 105.76	0.077
Median (IQR)	177.74 (123.18-208.37)	134.96 (85.37-196.51)	0.143 [#]

BMI, body mass index; CHO, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IL-6, interleukin-6; IFN- γ , interferon; ET-1, endothelin-1; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1; apoE, apolipoprotein E. The *p* value was based on independent-samples *t* test, Mann-Whitney test and chi-square test for binary characteristics. [▲], Fisher's exact test; [#], Mann-Whitney *U* test.

μ L ethanol were pipetted into a 5 mL disposable test tube and vortexed for 1 min. The mixture was extracted with 700 μ L hexane three times and the upper layer was collected and evaporated under nitrogen at 4°C. The residue was dissolved in solvent A (methanol : acetonitrile, 40:60 by vol.) and serum

carotenoids were quantified by reversed phase HPLC as described¹⁵).

Assessment of Dietary Intake

Dietary intake was assessed with a 24-h recall method for three days. Dietary energy and nutrient

Table 2. Three-Month End points and Risk Factors

	A + 20 mg L (<i>n</i> = 34)	A + Placebo (<i>n</i> = 31)	A + L vs A + Placebo [95% CI] (<i>p</i>)
Changes of serum lutein from baseline to 3 mo, $\mu\text{mol/L}$	0.40 ± 0.38 (0.19-0.60)*	0.005 ± 0.211 (-0.09-0.11)*	0.39 [0.17-0.61] (<0.001)
<i>P</i> , nonparametric analysis [#]	<0.001	0.915	<0.001
Cytokines (change from baseline)*			
Serum apoE ($\mu\text{g/mL}$)	-60.5 ± 100.5 <i>p</i> =0.005	-10.5 ± 77.0 <i>p</i> =0.529	-50.0 [-102.77-2.83] (0.063)
Serum IL-6 (pg/mL)	-148.6 ± 327.5 <i>p</i> =0.036	-35.6 ± 116.7 <i>p</i> =0.177	-113.0 [-265.05-39.11] (0.14)
Serum MCP-1 (pg/mL)	-108.8 ± 189.7 <i>p</i> =0.008	-11.97 ± 51.7 <i>p</i> =0.301	-96.8 [-182.94-10.71] (0.021)
Serum IFN- γ (pg/mL)	-3.0 ± 112.1 <i>p</i> =0.896	-44.5 ± 133.3 <i>p</i> =0.175	41.5 [-35.14-118.09] (0.28)
Serum VCAM-1 (pg/mL)	34.6 ± 679.3 <i>p</i> =0.827	-21.5 ± 856.0 <i>p</i> =0.912	56.1 [-559.2-447.0] (0.82)
Serum ET-1 (pg/mL)	-0.91 ± 2.96 <i>p</i> =0.132	-0.58 ± 2.28 <i>p</i> =0.270	-0.32 [-1.94-1.29] (0.69)
Risk factors (change from baseline)*			
LDL cholesterol, mmol/L	-0.28 ± 0.64 <i>p</i> =0.019	-0.16 ± 0.55 <i>p</i> =0.156	<i>p</i> =0.266
HDL cholesterol, mmol/L	0.052 ± 0.172 <i>p</i> =0.106	0.055 ± 0.231 <i>p</i> =0.233	<i>p</i> =0.954
Triglycerides, mmol/L	-0.712 ± 1.104 <i>p</i> =0.001	-0.225 ± 0.722 <i>p</i> =0.124	<i>p</i> =0.056
Blood glucose, mmol/L	-0.13 ± 0.45 <i>p</i> =0.128	-0.18 ± 0.75 <i>p</i> =0.053	<i>p</i> =0.656

CHO, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IL-6, interleukin-6; IFN- γ , interferon- γ ; ET-1, endothelin-1; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1; apoE, apolipoprotein E. Value are mean $\pm$ SD when appropriate. * (minimum, maximum). [#], Mann-Whitney *U* test. *P* values for change from baseline to 3 months for variables are based on paired *t*-test. The comparison between two groups is based on independent-samples *t* test.

content were calculated using a computer program based on the standard tables of China Food Composition¹⁸⁾.

Statistical Analysis

Continuous data are presented as the mean $\pm$ standard deviation (SD) and categorical variables are expressed as percentages. All continuous variables were tested for normal distribution using the Shapiro-Wilk test. Baseline characteristics were compared using the χ^2 test for categorical variables and independent-samples *t* test for continuous variables. Serum cytokines and apoE are also presented as the median $\pm$ interquartile range and tested by the Mann-Whitney *U* test. The three-month endpoints were assessed using the paired *t* test. Differences in the changes of variables from baseline to 3 months between the two groups

were tested by the independent-samples *t* test. Pearson's correlation coefficients were performed to examine the relationships between variables and are presented as *r* values. Two-sided *p* < 0.05 was considered significant. All analyses were conducted with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL).

Results

Baseline Clinical Characters and Treatment Delivery

All enrolled subjects (*n* = 65) completed the three-month study. Compliance was high and no serious adverse reactions were noted. There were no cross-overs during the 3-month follow-up period. At baseline, the study population age was 57.1 ± 4.4 ; 41.5% male; 13.8% had diabetes mellitus; 36.9% had dyslipidemia. Participants in the two groups were well

Fig. 1. Relationship between changes of serum lutein and LDL-cholesterol in different groups. r : correlation coefficient, Pearson's correlation analysis.

matched in terms of baseline demographics and performance variables. Baseline serum carotenoids, cytokines and apoE were also similar at baseline across the groups (Table 1).

Change of Serum Lutein Among Subjects

The lutein supplements resulted in a significant increase in serum lutein levels in the supplemented group compared to baseline ($p<0.001$) but not in the placebo group at 3 months (Table 2). Serum lutein increased by 0.40 ± 0.38 μmol/L in A+L and 0.005 ± 0.211 μmol/L in A+C groups, respectively. Compared with A+C group, A+L group showed a great improvement in serum lutein by 0.39 μmol/L (95% confidence interval, 0.17-0.61 μmol/L, $p<0.001$).

Inflammatory Cytokines and ApoE

After the 3-month follow up, the changes of inflammatory cytokines varied in two groups (Table 2). In A+P group, there were no significant changes in cytokines compared to baseline. In contrast, in A+L group, IL-6 and MCP-1 showed a significant decrease at 3 months ($p<0.05$). For MCP-1, A+L group demonstrated significant improvements compared with A+P group ($p=0.021$). Subjects assigned to A+L had great decreases in apoE than in A+P group, but these were not significant ($p>0.05$) (Table 2).

Serum Lipid Profile and Blood Glucose

Atherosclerosis risk factors demonstrated a greater decrease in LDL-cholesterol among individuals in the A+L group compared to baseline. Subjects in A+L group had a statistically significant improvement in serum triglyceride compared to baseline ($p=0.001$). The improvements of atherosclerosis risk factors between the two groups showed no significance (Table 2).

Relationship between Changes in Serum Lutein and LDL-Cholesterol

In all subjects, the change of lutein correlated negatively with changes in serum LDL-cholesterol ($r=-0.340$, $p=0.062$). In particular, for subjects in A+L group, the changes in serum lutein were significantly correlated with the change of LDL-cholesterol ($r=-0.384$, $p=0.043$; Fig. 1).

Discussion

In this randomized, double-blind, placebo-controlled study, lutein supplementation had beneficial effects on biomarkers of inflammatory cytokines, as evidenced by decreased IL-6 and MCP-1 levels in A+L group. In addition, the change in serum lutein in A+L group was significantly negatively correlated with the change in LDL-cholesterol. These results suggested that the antioxidant effects of lutein may play a

role in the prevention of atherosclerosis progression, which was closely related with inflammatory cytokines and LDL. The reductions in inflammatory cytokines after lutein supplementation were consistent with the previous findings that multiple inflammatory cytokines were inhibited in the aorta of guinea pigs following feeding with a lutein-enriched diet¹². More recently, Gao *et al.* also found that supplementation of xanthophylls (containing 40% lutein and 60% zeaxanthin) decreased proinflammatory and increased anti-inflammatory cytokines in hens and chicks¹⁹. In this study, lutein supplementation significantly increased serum lutein from baseline in the supplemented group compared to the placebo. This result confirms the compliance of the study subjects and the good response of participants to lutein supplementation.

Interleukin-6, a proinflammatory cytokine produced in various tissues, was demonstrated to be associated with both atherosclerotic plaque development and plaque destabilization effects in the atherosclerosis pathway²⁰. Recently, Boekholdt *et al.* pointed out that IL-6 and its receptor can activate an alternative trans-signaling pathway, mainly in endothelial cells and smooth muscle cells, and have a causative role in determining the risk of coronary heart disease²¹. As IL-6 has a key role in mediating inflammatory cascades and is concomitant with atherosclerosis complications²², the favorable impact of lutein supplementation on the IL-6 level seen in this study supported its function in the system inflammatory pathway and in atherosclerosis prevention. The improvement of IL-6 was greater in A + L group than in A + P group, although there was no significance. These results suggested that lutein might exert its biological function by inhibiting oxidative stress and regulating the inflammatory pathway²³, which were active in early atherosclerosis patients⁴.

Macrophages, differentiated from monocytes recruited from circulating blood, are believed to play key roles in atherosclerosis lesion initiation and early progression²⁴. Chemokine receptors on the surface of monocytes could interact with the chemokines secreted by activated endothelial cells and promote the directional migration of monocytes²⁵. MCP-1, a potent monocyte attractant, was demonstrated to be the key molecular link between oxidized lipoprotein and macrophage recruitment to the vessel wall in early atherosclerosis²⁶. In a mouse model of atherosclerosis, atherogenesis could be prevented or retarded by blocking chemokines or their receptors, which were related to monocyte entry²⁵. In our study, lutein supplementation significantly decreased MCP-1 in early athero-

sclerosis patients. From this result, lutein may play a beneficial role in preventing monocyte recruitment. As endothelial expression of MCP-1 is thought to be one of the biomarkers of endothelial dysfunction in early atherosclerotic lesions²⁶, this result suggested the possible beneficial effects of lutein on the endothelium. Overall, the findings from this study suggested that lutein may have possible benefits on endothelial function.

The reduction of serum LDL-cholesterol, triglyceride level and the correlation between changes in lutein and serum LDL-cholesterol levels in this study suggest that lutein may play a role in serum lipid profile regulation. LDL could be modified into the oxidized form of LDL (oxLDL) by reactive oxygen species (ROS), which play a central proatherogenic role in the arterial wall²⁷. Serum LDL cholesterol-related parameters could effectively predict the risk of cardiovascular diseases²⁸, and therapies aimed at lowering serum LDL-cholesterol had significant effects on the progression of atherosclerosis^{29, 30}. Although the mechanisms by which lutein exerts its biological functions in lipid regulation have not been well defined, evidence from an animal study suggested that xanthophyll carotenoids might lower serum LDL-cholesterol and triglyceride levels by increasing LDL uptake and fatty acid β -oxidation³¹. Yang *et al.* observed that the livers of mice fed astaxanthin, another xanthophyll carotenoid, had significantly higher greater mRNA levels of LDL receptor, 3-hydroxy-3methylglutaryl CoA reductase, and sterol regulatory element bind protein 2. They also found that mRNA abundance of several important enzymes in fatty acid β -oxidation, including carnitine palmitoyl transferase 1, acetyl-CoA carboxylase β and acyl-CoA oxidase, was significantly increased after astaxanthin supplementation. In humans, astaxanthin administration also showed a lipid-lowering effect³², consistent with the effect of lutein observed in this study. Because the structure of lutein is very similar to that of astaxanthin³³, it is probable that lutein exerts its function through the same mechanisms. Furthermore, serum apolipoprotein E, a determiner of triglyceride-rich lipoprotein metabolism³⁴, decreased after lutein supplementation in this study. In general, serum apoE could explain 20%-40% of variability in triglyceride levels in humans³⁴, and high levels of serum apoE appear to cause hypertriglyceridemia by stimulating triglyceride production and impairing VLDL lipolysis³⁵; therefore, the reduction of serum triglyceride in this study may be also due to, at least in part, decreased serum apoE after lutein supplementation. ApoE was synthesized from several cell lines, including hepatocytes,

macrophages, astrocytes and smooth muscle cells. Macrophages were found to be the cell type responsible for the production of apoE in various tissues in response to injury and repair³⁶⁾. For the early stage of atherosclerosis, recruitment of macrophages to injured vascular endothelial cells and their subsequent uptake of cholesterol were the major cellular events contributing to the development of atherosclerosis³⁷⁾. Macrophages would synthesize and secrete large quantities of apoE after taking up cholesterol³⁶⁾, which might be an important source of serum apoE in atherosclerosis patients. Evidence from *vitro* and *vivo* studies demonstrated that lutein could effectively suppress the inflammatory responses of macrophages³⁸⁾. Moreover, findings from our study and others suggested that lutein could inhibit the attraction of monocytes to the surface of injured vascular endothelial cells¹⁰⁾. This suggested that lutein might lower serum apoE by suppressing inflammatory responses and inhibiting the recruitment of macrophages. More studies are needed to confirm these results.

Conclusion

In this study, there were more female than male subjects. Because age and gender are important factors determining endothelial function³⁹⁾, the gender imbalance and the age distribution in this study should be noted in the generation of the results. Despite these limitations, 3 months of 20 mg/d lutein supplementation increased serum lutein and decreased serum inflammatory cytokines and serum TG and LDL-cholesterol. In addition, changes in serum lutein correlated negatively with those of serum LDL-cholesterol. These results add to the growing literature on the potential protective effects of lutein in atherosclerosis through anti-inflammatory effects and preservation of endothelial function.

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Conflict of Interest

None.

References

- 1) Lusis AJ: Atherosclerosis. *Nature*, 2000; 407: 233-241
- 2) Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ: Global and Regional Burden of Disease and Risk Factors, 2001: Systematic Analysis of Population Health Data. *Lancet*, 2006; 367: 1747-1757
- 3) Rocha VZ, Libby P: Obesity, Inflammation, and Atherosclerosis. *Nat Rev Cardiol*, 2009; 6: 399-409
- 4) Ross R: Atherosclerosis: An Inflammatory Disease. *N Engl J Med*, 1999; 340: 115-126
- 5) Libby P, Ridker PM, Hansson GK: Inflammation in Atherosclerosis: From Pathophysiology to Practice. *J Am Coll Cardiol*, 2009; 54: 2129-2138
- 6) Tedgui A, Mallat Z: Cytokines in Atherosclerosis: Pathogenic and Regulatory Pathways. *Physiol Rev*, 2006; 86: 515-581
- 7) Mauriello A, Sangiorgi GM, Virmani R, Trimarchi S, Holmes DR Jr, Kolodgie FD, Piepgras DG, Piperno G, Liotti D, Narula J, Righini P, Ippoliti A, Spagnoli LG: A Pathobiologic Link between Risk Factors Profile and Morphological Markers of Carotid Instability. *Atherosclerosis*, 2010; 208: 572-580
- 8) Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ: Rosuvastatin to Prevent Vascular Events in Men and Women with Elevated C-Reactive Protein. *N Engl J Med*, 2008; 359: 2195-2207
- 9) Voutilainen S, Nurmi T, Mursu J, Rissanen TH: Carotenoids and Cardiovascular Health. *Am J Clin Nutr*, 2006; 83: 1265-1271
- 10) Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merz CN, Fogelman AM: Oxygenated Carotenoid Lutein and Progression of Early Atherosclerosis: The Los Angeles Atherosclerosis Study. *Circulation*, 2001; 103: 2922-2927
- 11) Koh WP, Yuan JM, Wang R, Lee YP, Lee BL, Yu MC, Ong CN: Plasma Carotenoids and Risk of Acute Myocardial Infarction in the Singapore Chinese Health Study. *Nutr Metab Cardiovasc Dis*, 2011; 21: 685-690
- 12) 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: 1458-1463
- 13) Iribarren C, Folsom AR, Jacobs DR Jr, Gross MD, Belcher JD, Eckfeldt JH: Association of Serum Vitamin Levels, LDL Susceptibility to Oxidation, and Autoantibodies against MDA-LDL with Carotid Atherosclerosis. A Case-Control Study. The Aric Study Investigators. *Atherosclerosis Risk in Communities. Arterioscler Thromb Vasc Biol*, 1997; 17: 1171-1177
- 14) Hak AE, Ma J, Powell CB, Campos H, Gaziano JM, Willett WC, Stampfer MJ: Prospective Study of Plasma Carotenoids and Tocopherols in Relation to Risk of Ischemic Stroke. *Stroke*, 2004; 35: 1584-1588
- 15) Zou Z, Xu X, Huang Y, Xiao X, Ma L, Sun T, Dong P, Wang X, Lin X: High Serum Level of Lutein May Be Protective against Early Atherosclerosis: The Beijing Athero-

- sclerosis Study. *Atherosclerosis*, 2011; 219: 789-793
- 16) Dogan S, Kastelein JJ, Grobbee DE, Bots ML: Mean Common or Mean Maximum Carotid Intima-Media Thickness as Primary Outcome in Lipid-Modifying Intervention Studies. *J Atheroscler Thromb*, 2011; 18: 946-957
- 17) Yeum KJ, Booth SL, Sadowski JA, Liu C, Tang G, Krinsky NI, Russell RM: Human Plasma Carotenoid Response to the Ingestion of Controlled Diets High in Fruits and Vegetables. *Am J Clin Nutr*, 1996; 64: 594-602
- 18) Institute of Nutrition and Food Safety, Center of Disease Control: China Food Composition. Peking University Medical Press, Beijing, 2004
- 19) Gao YY, Xie QM, Jin L, Sun BL, Ji J, Chen F, Ma JY, Bi YZ: Supplementation of Xanthophylls Decreased Proinflammatory and Increased Anti-Inflammatory Cytokines in Hens and Chicks. *Br J Nutr*, 2012; Jan 25: 1-10. [Epub ahead of print]
- 20) Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B: How Much Is Too Much? Interleukin-6 and Its Signalling in Atherosclerosis. *Thromb Haemost*, 2009; 102: 215-222
- 21) Boekholdt SM, Stroes ES: The Interleukin-6 Pathway and Atherosclerosis. *Lancet*, 2012; 379: 1176-1178
- 22) Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, Wensley F, Higgins JP, Lennon L, Eiriksdottir G, Rumley A, Whincup PH, Lowe GD, Gudnason V: Long-Term Interleukin-6 Levels and Subsequent Risk of Coronary Heart Disease: Two New Prospective Studies and a Systematic Review. *PLoS Med*, 2008; 5: e78
- 23) Rydén M, Garvin P, Kristenson M, Leanderson P, Ernerudh J, Jonasson L: Provitamin a Carotenoids Are Independently Associated with Matrix Metalloproteinase-9 in Plasma Samples from a General Population. *J Intern Med*, 2012; Feb 28. doi: 10.1111/j.1365-2796.2012.2534x.x. [Epub ahead of print]
- 24) Moore KJ, Tabas I: Macrophages in the Pathogenesis of Atherosclerosis. *Cell*, 2011; 145: 341-355
- 25) Mestas J, Ley K: Monocyte-Endothelial Cell Interactions in the Development of Atherosclerosis. *Trends Cardiovasc Med*, 2008; 18: 228-232
- 26) Yadav A, Saini V, Arora S: MCP-1: Chemoattractant with a Role Beyond Immunity: A Review. *Clin Chim Acta*, 2010; 411: 1570-1579
- 27) Mitra S, Deshmukh A, Sachdeva R, Lu J, Mehta JL: Oxidized Low-Density Lipoprotein and Atherosclerosis Implications in Antioxidant Therapy. *Am J Med Sci*, 2011; 342: 135-142
- 28) Mizuno K, Nakaya N, Teramoto T, Yokoyama S, Ohashi Y, Ueki A, Takahashi S, Kubota Y, Nakamura H: Usefulness of LDL-C-Related Parameters to Predict Cardiovascular Risk and Effect of Pravastatin in Mild-to-Moderate Hypercholesterolemia. *J Atheroscler Thromb*, 2012; 19: 176-185
- 29) Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P: Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) Investigators: Statin Therapy, LDL Cholesterol, C-Reactive Protein, and Coronary Artery Disease. *N Engl J Med*, 2005; 352: 29-38
- 30) Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM, ASTEROID Investigators: Effect of Very High-Intensity Statin Therapy on Regression of Coronary Atherosclerosis: The Asteroid Trial. *JAMA*, 2006; 295: 1556-1565
- 31) Yang Y, Seo JM, Nguyen A, Pham TX, Park HJ, Park Y, Kim B, Bruno RS, Lee J: Astaxanthin-Rich Extract from the Green Alga *Haematococcus pluvialis* Lowers Plasma Lipid Concentrations and Enhances Antioxidant Defense in Apolipoprotein E Knockout Mice. *J Nutr*, 2011; 141: 1611-1617
- 32) Yoshida H, Yanai H, Ito K, Tomono Y, Koikeda T, Tsukahara H, Tada N: Administration of Natural Astaxanthin Increases Serum HDL-Cholesterol and Adiponectin in Subjects with Mild Hyperlipidemia. *Atherosclerosis*, 2010; 209: 520-523
- 33) Santocono M, Zurria M, Berrettini M, Fedeli D, Falcioni G: Influence of Astaxanthin, Zeaxanthin and Lutein on DNA Damage and Repair in Uva-Irradiated Cells. *J Photochem Photobiol B*, 2006; 85: 205-215
- 34) Huang Y: Mechanisms Linking Apolipoprotein E Isoforms with Cardiovascular and Neurological Diseases. *Curr Opin Lipidol* 2010; 21: 337-345
- 35) Huang Y, Liu XQ, Rall SC Jr, Taylor JM, von Eckardstein A, Assmann G, Mahley RW: Overexpression and Accumulation of Apolipoprotein E as a Cause of Hypertriglyceridemia. *J Biol Chem*, 1998; 273: 26388-26393
- 36) Mahley RW: Apolipoprotein E: Cholesterol Transport Protein with Expanding Role in Cell Biology. *Science*, 1988; 240: 622-630
- 37) Christopher K. Glass, Witztum JL: Atherosclerosis: The Road Ahead. *Cell*, 2001; 104: 503-516
- 38) Bian Q, Qin T, Ren Z, Wu D, Shang F: Lutein or Zeaxanthin Supplementation Suppresses Inflammatory Responses in Retinal Pigment Epithelial Cells and Macrophages. *Adv Exp Med Biol*, 2012; 723: 43
- 39) Skaug EA, Aspenes ST, Oldervoll L, Mørkedal B, Vatten L, Wisløff U, Ellingsen O: Age and Gender Differences of Endothelial Function in 4739 Healthy Adults: The Hunt3 Fitness Study. *Eur J Prev Cardiol*, 2012; [Epub ahead of print]