Correlation between plasma levels of carotenoid and oxidized low density lipoproteins: A short human intervention study

Tiziana Bacchetti1, Domenico Tulli2, Simona Masciangelo1, Francesca Brugé3, Sonia Silvestri1, Patrick Orlando3, Luca Tiano3 and Gianna Ferretti*1

1Dipartimento di Scienze della Vita e dell’Ambiente, Università Politecnica delle Marche, 60100, Ancona, Italia
2Italsur s.r.l., Notaresco (TE), Italia
3Dipartimento di Scienze Cliniche Specialistiche e Odontostomatologiche, Università Politecnica delle Marche, 60100, Ancona, Italia

Abstract

The development of vegetable functional food products that provide benefits beyond their traditional nutritional value raised increasing interest. In fact several studies have shown that diet plays a role in the development of human diseases associated with oxidative damage (diabetes, cardiovascular disease and metabolic syndrome). Aim of the study was to evaluate the effects of daily intake of 300g of a frozen vegetable product containing red and green spinach, red and green chicory, red and green leaf chard (Italsur srl Notaresco, Italy) on plasma lipids and oxidized low density lipoproteins (ox-LDL). Furthermore, the bioavailability of lutein and β-carotene was investigated. The study included n=49 healthy volunteers (age ranged from 23 to 73 year), who consumed a portion of the vegetable product (300 g) every day for 2 weeks. The significant increase of plasma lutein and β-carotene after vegetable mix consumption for 2 weeks demonstrated that these phytonutrients are highly bioavailable. A significant reduction of plasma levels of total cholesterol (TC) and LDL-cholesterol (LDL-C) was observed after dietary intervention. The results demonstrated a significant decrease in plasma concentration of ox-LDL after treatment. Evaluation of ox-LDL represents a useful biochemical marker of lipid peroxidation; therefore the results demonstrated a decrease of lipid peroxidation of lipoproteins associated with the consumption of vegetable product. A significant negative correlation has been established between levels of plasma lutein and levels of ox-LDL before and after daily intake of the vegetable product. These results suggest that lutein may play a role in the protective effect against oxidation of LDL. In conclusion the increase of plasma carotenoids after dietary treatment for two weeks is associated with a protective effect against lipid peroxidation of lipoproteins.

Introduction

The frozen ready-to-eat vegetables market has been growing and deserves increasing attention due to modifications of lifestyle and changes of dietary habits. Accurate selection of vegetables can result in convenient, healthy and appealing products, which may contain interesting bioactive compounds. Among vegetable foods that could be defined as functional foods there are pigmented vegetables such as red spinach, green spinach, red chicory and red chard. Previous studies have demonstrated that they contain bioactive molecules and phytochemicals (polyphenols and carotenoids), vitamins (vitamin C, folate, and provitamin A), minerals (potassium, calcium, and magnesium) and fibers [1-5]. However their biological effects have been mostly investigated in vitro and in model animals [6-10].

Among phytonutrients, carotenoids have been widely investigated for their protective role against lipid peroxidation. In fact plasma carotenoid levels are associated with low density lipoproteins in particular [11]. The correlation between dietary intake of carotenoids and plasma carotenoid levels has been previously demonstrated [12-18]. Therefore the increase in plasma level of carotenoids after dietary intake of vegetable has been suggested as a useful nutritional marker of their bioavailability in human subjects. An increased plasma total antioxidant capacity has also been associated with a consumption of vegetable antioxidants [13,19,20]. The bioavailability of bioactive nutrients is modulated by different factors such as chemical composition of vegetable and processing [19,21,22].

In order to evaluate the nutritional properties of a ready to eat frozen product and the bioavailability of the main bioactive molecules, we investigated the plasma levels of carotenoids and antioxidant potential after intake of a experimental ready-to-eat product containing pigmented vegetables that are rich sources of polyphenols, carotenoids and other phytochemicals. Therefore we enrolled 48 volunteers whose diet was supplemented for two weeks with a daily portion of the vegetable product Moreover we studied the effect of the intake of the product on the markers of cardiovascular disease such as plasma lipid profile and levels of oxidized LDL.

Materials and methods

Subjects

The study was conducted during September–November 2012. The inclusion criteria for subjects were: not taking vitamins, minerals,

Correspondence to: Gianna Ferretti, Dipartimento di Scienze Cliniche Specialistiche e Odontostomatologiche, Università Politecnica delle Marche, 60100, Via Brecce Bianche, 60131 Ancona, Italia; E-mail: g.ferretti@univpm.it

Key words: pigmented vegetable, functional foods, oxidized-LDL, plasma lipids, carotenoids, antioxidant

Received: February 20, 2016; Accepted: March 22, 2016; Published: March 25, 2016
Correlation between plasma levels of carotenoid and oxidized low density lipoproteins: A short human intervention study

Bacchetti T (2016) Correlation between plasma levels of carotenoid and oxidized low density lipoproteins: A short human intervention study

Volume 3(2): 283-288

Integr Food Nutr Metab, 2016         doi: 10.15761/IFNM.1000144

The exclusion criteria were: diagnosed diseases such as allergies, cancer, diabetes, obesity, hypertension, mental diseases, gastrointestinal or renal diseases, as well as intake of drugs related to these pathologies, alcohol consumption >30 g/day, vegetarian diet. None of the female subjects was pregnant or lactating. Volunteers were recruited in the Polytechnic University of Marche (UNIVPM), Italy.

Forty-eight subjects followed the diet, 22 of them (45.8%) were males. The median age was 40.5 years (1st–3rd quartile: 36 – 47 years) and median BMI 23.6 kg/m² (1st–3rd quartile: 21.8 – 26.4 kg/m²). The mean plasma lipid measurements for the subjects are summarized in Table 1.

**Intervention study**

The intervention phase consisted of a 2-week period which included daily consumption of a portion (300 g) of the vegetable frozen product supplied by Italsur srl (Notaresco, Teramo, Italy). The vegetable frozen product contains the same proportions of red spinach (*Spinacia oleracea*), green spinach (*Spinacia oleracea*), red chicory and green chicory and a lower percentage of green chard (*Beta vulgaris L. var. cicla*) and red chard (*Beta vulgaris L. var. cicla*). Macronutrients contained in a portion of the final product were evaluated [23]. Water-soluble vitamins were quantified by high-performance liquid chromatography/electrospray ionization-mass spectrometry [24]. Carotenoids were analyzed using high performance liquid chromatography (HPLC) [25]. Total polyphenols were evaluated following Xu et al. [26]. Total antioxidant potential was evaluated by ORAC assay [4]. Table 2 shows the percentage of the energy, macro and micronutrients found in the vegetable product.

The product was consumed after cooking for 15 minutes with 2 tablespoons extra virgin olive oil. Intake of the vegetable product was included in the normal daily diet and no specific time of consumption or accompanying meal was established. Subjects were recommended to maintain their habitual dietary intake (especially as regard to their consumption of a provided list of foods with high carotenoids, polyphenol and vitamin C contents) and their usual physical activity or other lifestyle habits. Moreover, they were requested to record any sign of illnesses, medications, and any deviations from their experimental diets. No subject was reported to have side effects and thus, no one
dropped out during the experimental period. At the beginning of the intervention (baseline, T0) and at the end (after 2 weeks, T15), fasting blood samples were collected. The study was performed in accordance with the Helsinki Declaration of Human Studies and approved by the Ethical Committee of the "Azienda Ospedaliero-Universitaria Ospedali Riuniti" Ancona (Italy) (Protocol number 211525). All participants signed an informed consent document.

**Analytical determinations**

At the beginning of the study and after two weeks of dietary treatment, fasting blood samples (10 mL) were collected from each subject by venipuncture from the antecubital vein: 5 mL were placed in heparin tubes for haematological measurements, while 5 mL were placed in tubes without any anticoagulant and centrifuged at 1500 g for 10 min at 4°C for serum separation. Plasma and serum aliquots were prepared and stored at −80°C until analysis. Analyses did not commence until the full intervention study was completed and all samples from each subject were analysed within one batch to reduce interbatch variation.

**Plasma lipids and glycaemia**

Serum glucose, triacylglycerols (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) were analyzed by commercial kits (Chemadiagnostica, Jesi, Italy). Low density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula.

| Table 1. Plasma lipid profile, levels of ox-LDL and total antioxidant capacity subjects in plasma of LDL in plasma of subjects at baseline and after 2week period which include a daily portion of the ready-to-eat product containing pigmented vegetables. (Data are shown as mean ± standard deviation). A p < 0.05 was considered statistically significant. |
|---|---|
| **Baseline** (n=49) | **After 2-weeks** (n=49) | **P** value |
| TC (mmol/L) | 4.87 ± 0.56 | 4.55 ± 0.52 | p=0.001 |
| LDL-C (mmol/L) | 2.80 ± 0.56 | 2.53 ± 0.52 | p=0.001 |
| HDL-C (mmol/L) | 1.65 ± 0.28 | 1.65 ± 0.30 | p=0.5 |
| TG (mmol/L) | 0.91 ± 0.41 | 0.87 ± 0.37 | p=0.06 |
| Fasting glucose (mmol/L) | 4.88 ± 0.43 | 4.83 ± 0.31 | p=0.07 |
| Total antioxidant capacity (mmol TE/L) | 14634 ± 3492 | 16218 ± 3834 | p=0.001 |
| ox-LDL (U/L) | 41.7 ± 16.1 | 32.6 ± 12.2 | p=0.001 |
| ox-LDL:LDL-C (Ummol) | 15.2 ± 5.6 | 13.2 ± 5.2 | p=0.001 |

| Table 2. Macronutrients, vitamins and phytochemicals in a portion (300 g) of vegetable product containing red and green spinach, red and green chicory, red and green leaf chard (Italsur srl Notaresco, Italy). (Data are reported as mean ± standard deviation of data from analyses carried out in samples from different batch and different years (2011 and 2013). |
|---|---|
| **Composition of a portion of vegetable product (300 g)** | **Energy (kcal)** | 18 ± 2 |
| **Protein (g)** | 7.8 ± 1.1 |
| **Carbohydrate(g)** | 11.0 ± 2.2 |
| **Fiber(g)** | 8.5 ± 0.18 |
| **Fat(g)** | 0.63 ± 0.01 |
| **Saturated fatty acids(g)** | 0.15 ± 0.01 |
| **Monounsaturated fatty acids (g)** | 0.06 ± 0.01 |
| **Vitamin C (mg)** | 85 ± 3.7 |
| **Folate (mg)** | 0.33 ± 2.7 |
| **Alpha-tocopherol (mg)** | 3.9 ± 0.30 |
| **Nicin (mg)** | 1.2 ± 0.05 |
| **Retinol (mg)** | 3.2 ± 0.20 |
| **Riboflavin (mg)** | 0.27 ± 0.03 |
| **Thiamine (mg)** | 0.18 ± 0.01 |
| **Total Polyphenols** | 530 ± 82 |
| **Caffeic acid (mg)** | 80.1 ± 3.7 |
| **Chlorogenic acid (mg)** | 18.2 ± 1.5 |
| **Cumaric acid (mg)** | 3.4 ± 0.34 |
| **Ferulic acid (mg)** | 2.3 ± 0.11 |
| **Betacyanin (mg)** | 26.7 ± 1.5 |
| **Luteolin (mg)** | 4.7 ± 0.9 |
| **Kaempferol (mg)** | 1.23 ± 0.17 |
| **Quercetin (mg)** | 1.91 ± 0.11 |
| **Isohamnetin (mg)** | 1.32 ± 0.02 |
| **β-carotene (mg)** | 11.1 ± 0.8 |
| **Zeaxanthin (mg)** | 0.7 ± 0.1 |
| **Lutein (mg)** | 29.6 ± 0.30 |
| **Total ORAC (mmol/TEI)** | 5.8 ± 0.1 |
Plasma carotenoids

Carotenoids (β-carotene and lutein) were quantified in plasma of subjects by high-performance liquid chromatography (HPLC) system, using a single dilution step after extraction with propanol (1:5, v/v) and vigorous vortexing of 250 μL of extraction mixture. This mix was centrifuged for 2 min at 20,000 g at 4°C. Forty microliters of super natant were injected into the HPLC with electrochemical detector (ECD) by Shiseido (Tokyo, Japan), using a pre-separation concentrating column 50 × 2.0 mm ID 5 μm, separation C18 column 150 × 2.0 mm ID 3 μm, and a post-separation reducing column CQR 20 × 2.0 mm, all from Shiseido. For each carotenoid quantified, two mobile phases were used. Mobile phase 1 for loading and concentrating the sample (50 mM sodium perchlorate in methanol/water 95:5, v/v) was the same for both mobile phases, while mobile phase 2 was 50 mM sodium perchlorate in methanol/isopropanol (80:20, v/v) for lutein and 50 mM sodium perchlorate in methanol/isopropanol (98:2, v/v) for β-carotene. Moreover, flow rate was 200 μL/min for phase 1 in both analyses. Flow rates for phase 2 were 300 and 80 μL/min for β-carotene and lutein, respectively. Total chromatographic run times and retention times were 24 min/12.3 min for β carotene and 21 min/9.8 min for lutein [12].

Plasma total antioxidant capacity (PAT)

Plasma total antioxidant capacity (PAT) was measured using oxygen radical absorbance capacity (ORAC) adapted for semi-automated measurement on a 96-well microplate reader (Synergy HT; BioTek, Winooski, VT, USA) [27].

Markers of lipid peroxidation

In-vivo oxidized LDL were determined in plasma by a sandwich ELISA procedure using the murine monoclonal antibody mAB-4E6 as the capture antibody, and a peroxidase conjugated antibody against oxidized apolipoprotein B bound to the solid phase (ox-LDL, Mercodia AB, Uppsala, Sweden). Intra and inter-assay CVs were 2.82% and 7.29%, respectively. As LDL-C is considered a major determinant of AB, Uppsala, Sweden). Intra and inter-assay CVs were 2.82% and 7.29%, respectively. As LDL-C is considered a major determinant of

Statistical analysis

Mean value, standard deviation (DS), and mean standard error (SEM) were calculated. By using Student T test, we evaluated the significance of differences between mean values at study entry and following treatment. A p of 0.05 was considered statistically significant. Pearson correlation coefficients and their significance levels were calculated for linear regression analysis.

Results

Macronutrients and micronutrients contained in the frozen ready-to-eat product containing pigmented vegetables are summarized in Table 2. A portion of the product (300g) contains 8.5 ± 0.18 g and provides 34% of recommended daily intake of fiber (25 g/day). It contains high levels of vitamin C (85 ± 3.7 mg) and folate (0.33 ± 2.7 mg); a portion provides more than 100% of recommended dietary allowances (RDA) of both vitamins (Commission Directive 2008/100/EC). The levels of carotenoids were 11.1 ± 0.8 mg of β-carotene and 29.6 ± 0.30 mg of lutein (Table 2). As shown in Table 2, among polyphenols, phenolics acids and flavonols are the main components in the vegetable product. Anthocyanins and flavones have also been identified (Table 2). Using ORAC assay to evaluate total antioxidant capacity, the product ranks highest among vegetables [29]. In fact a portion of the frozen product provides 5.8 ± 0.1 mmol/TE.

Plasma lipids

Subjects at baseline were characterized by normal levels of the lipid profile (Table 1). A significant decrease occurred in TC, LDL-c after 15 days of diet are presented in Table 1. No significant difference was found in the absolute variation of HDL cholesterol levels and triacylglycerol levels (Table 1).

Carotenoids

Basal levels of lutein ranged from 0.16 μg/mL to 0.75 μg/mL and basal levels of β-carotene ranged from 0.07 μg/mL to 0.53 μg/mL, the median values are in agreement with previous studies by us and other authors (Figure 1) [13,30].

Figure 1 shows the variation in plasma concentrations of lutein and β-carotene after daily intake of the experimental product compared with baseline. A significant increase of plasma carotenoids (lutein and beta-carotene) was found after 15 days of supplemented diet (p<0.001).

Levels of oxidized LDL and total antioxidant properties

Plasma levels of ox-LDL were determined as biomarkers of lipid peroxidation during the study. The basal levels of ox-LDL were similar to those reported in previous studies by us and other studies on healthy subjects (Table 1) [13,28]. Significant decrease of the plasma levels ox-LDL and ox-LDL/LDL ratio was found after 15 days of supplemented diet (Table 1).

Relationship between plasma carotenoids and markers of lipid peroxidation

As shown in Figure 2 a significant negative correlation has been established between levels of plasma lutein and levels of ox-LDL (r=0.59, n=98, p<0.001) and ox-LDL/LDL-C (r=0.61, n=98, p<0.001) before and after daily intake of ‘Mix of chard, chicory and spinach’, no significant correlations were observed with plasma levels of β-carotene and levels of markers of lipid peroxidation.

Discussion

The phytochemical composition of pigmented vegetables has been identified (Table 2). Using ORAC assay to evaluate total antioxidant capacity, the product ranks highest among vegetables [29]. In fact a portion of the frozen product provides 5.8 ± 0.1 mmol/TE.
Correlation between levels of lutein and of oxidized LDL in plasma of subjects at baseline (\(\phi\)) and after 2 weeks period which included a daily portion of the ready-to-eat product containing pigmented vegetables (\(\phi\)).

Previously investigated [1-5]. However the biological effect on human subjects has not been previously studied.

A portion of the vegetable product contains about 30 mg lutein and 11 mg beta carotene. Serum carotenoid concentration increased after 2 weeks, beta-carotene concentration by 56% and lutein concentration by 94.5% compared with baseline. These data demonstrated that these phytonutrients in the product are bioavailable. In our previous study we have demonstrated that the daily intake for two weeks of a portion of the vegetable combination of black and red cabbage containing 1 mg lutein and 0.6 mg \(\beta\)-carotene, increased plasma \(\beta\)-carotene and lutein concentration by 80 and 204%, respectively compared with the baseline [13]. Other authors have demonstrated that three weeks of intake of 9.3 mg of \(\beta\)-carotene from spinach products increased serum concentration by 53% [31]. Martínez-Thomas et al. [32] have confirmed an increase of \(\beta\)-carotene by 114% after three weeks 3.9 mg of \(\beta\)-carotene contained in a fruit and vegetable soup. The interrelationship of different carotenoids present in food matrix affects carotenoid absorption and could explain the different percentage increase. In particular it is well known that lutein modulates \(\beta\)-carotene bioavailability [33].

Using the evaluation of the total antioxidant capacity, that represents a suitable biochemical parameter for assessing the overall antioxidant status, we demonstrated a significant increase. Our results demonstrated also that the daily consumption of the product for 2 weeks is associated with a significant reduction of total-cholesterol and LDL-C in absence of changes of HDL-C and TG levels.

A portion of the vegetable product contains 8.5 g of fibers and about a third (2.5 g) is represented by soluble fiber. Extensive research has shown that fibers play an important role in cholesterol metabolism by decreasing plasma TC and LDL-C, as demonstrated in a meta-analysis [34]. In general, most soluble fibers lower plasma total cholesterol more efficiently than water-insoluble fibers by decreasing LDL cholesterol without significantly affecting the HDL-C and TG levels [34]. Other phytochemicals contained in the product such as polyphenols could contribute to the LDL cholesterol lowering effect. This hypothesis is supported by previous studies, in-vivo, which has demonstrated that diets naturally rich in polyphenols improve plasma lipid profile [35-37]. Moreover, in vitro studies, have shown that individual polyphenols such anthocyanins, quercetin, kaempferol and luteolin are able to modulate cholesterol absorption [38,39] and lipid synthesis [40].

The decrease of oxidized LDL could be related to the modifications of carotenoid and/or other phytochemicals as suggested by previous human studies [12-17]. By the product analysis it was evidenced a high content of vitamin C. Previous studies have observed an inverse correlation between vitamin C concentrations and 8-epi-PGF2 concentrations in subjects at baseline and after 2 week of supplementation with the vegetable soup [41,42], moreover there is convincing evidence that vitamin C is a strong inhibitor of LDL oxidation [43], a recognized factor in the pathogenesis and progression of human atherosclerosis, by scavenging free radicals and other reactive species, and preventing their interaction to oxidize LDL [43]. All the aforementioned nutrients may have synergistically contributed to the protective effect associated with consumption of the vegetable product.

The statistical analysis of the effect of dietary intervention on biochemical parameters has demonstrated variability. A significant higher increase of lutein and \(\beta\)-carotene levels was observed in females. Moreover, subjects with values of TC, HDL-c, triacylglycerol, ox-LDL and ox-LDL/LDL ratio, over the median, had a higher decrease of the dependent variable. This variability in response must be associated with genetic or other characteristics of the individual [44].

Diet is considered the cornerstone for the prevention of age-related diseases, and a low-fat diet has been considered for decades as the most suitable alternative to achieve this goal. However, mounting evidence supports the efficacy of other alternatives, such as the Mediterranean diet and intake of foods rich in antioxidants and phytochemicals [45].

In conclusion the synergistic effects of phytochemicals (dietary fibers, polyphenols, carotenoids) present in the frozen ready to eat product could be responsible for the protective effects and benefits observed could be attributed to the complex mixture of phytochemicals present in whole foods. High levels of LDL-cholesterol and of oxidized LDL are biochemical markers for atherosclerosis [46,47]. Therefore we suggest that products rich in carotenoids and other phytochemicals could be inserted in dietary intervention aimed to prevent cardiovascular diseases and their complications.

Despite dietary health effects are influenced by genetic factors [44], an accurate selection of pigmented vegetable results in palatable, convenient and healthy product with physiological effects on plasma lipids and lipid peroxidation.

Authors’ contribution

All authors contributed to the intellectual development of this work and approved the final manuscript. TB and GF were responsible for the experimental design, coordination of research and preparation of the manuscript; TB and SM carried out evaluations of plasma biochemical parameters (plasma lipids, markers of lipid peroxidation) and participated in the preparation of the manuscript; D.T. was actively involved in the chemical and agronomic investigation of plant species used to make the frozen product; LT, FB, SS and PO carried out evaluation of plasma carotenoids and contributed to the preparation of the manuscript.

References

1. Chu YF, Sun J, Wu X, Liu RH (2002) Antioxidant and antiproliferative activities of common vegetables. J Agric Food Chem 50: 6910-6916. [Crossref]
2. Kugler F, Stintzing FC, Carle R (2004) Identification of betalains from petioles of differently colored Swiss chard (Beta vulgaris L. ssp. cicla [L.] Alef. Cv. Bright Lights) by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Agric Food Chem 52: 2975-2981.
3. Ninfali P, Bacchiocca M, Antonelli A, Biagiotti E, Di Gioacchino AM, et al. (2007)
Bacchetti T (2016) Correlation between plasma levels of carotenoid and oxidized low density lipoproteins: A short human intervention study

Characterization and biological activity of the main flavonoids from Swiss Chard (Beta vulgaris subsp. cyclocarpa). *Phytomedicine* 14: 216-221. [Crossref]

4. Ninfali P, Bacchiocca M (2003) Polyphenols and antioxidant capacity of vegetables under fresh and frozen conditions. *J Agric Food Chem* 51: 2222-2226. [Crossref]

5. Proteggente AR, Pannala A, Paganga G, Van Buren L, Wagner E, et al. (2002) The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radic Res* 36: 217-233. [Crossref]

6. Ko SH, Park JL, Kim SY, Lee SW, Chun SS, et al. (2014) Antioxidant Effects of Spinach (Spinacia oleracea L.) Supplementation in Hyperlipidemic Rats. *Preventive Food Nutr Med* 19: 19-26. [Crossref]

7. Lee JH, Son CW, Kim MY, Kim KH, et al. (2009) Red beet (Beta vulgaris L.) leaf supplementation improves antioxidant status in C57BL/6J mice fed high fat high cholesterol diet. *Nutr Res Pract* 3: 114-121. [Crossref]

8. Kim MY, Cheong SH, Kim MH, Son C, Yook HS, et al. (2009) Leafy vegetable mix supplementation improves lipid profiles and antioxidant status in C57BL/6J mice fed a high fat and high cholesterol diet. *J Med Food* 12: 877-884. [Crossref]

9. Lavelle V (2008) Antioxidant activity of minimally processed red chlorella (Cichorium intybus L.) and its derivatives: antioxidant and anti-inflammatory actions. *J Agric Food Chem* 56: 7194-7200. [Crossref]

10. Bhattacharjee AK, Jain M (2004) Spinacia oleracea L. protects against gamma radiation: a study on glutathione and lipid peroxidation in mouse liver. *Phytotherapy Research* 11: 607-615. [Crossref]

11. Wang W, Connor SL, Johnson EJ, Klein ML, Hughes S, et al. (2007) Effect of dietary lutein and zeaxanthin on plasma carotenoids and their transport in lipoproteins in age-related macular degeneration. *Am J Clin Nutr* 85: 762-769. [Crossref]

12. Barona J, Jones JJ, Kopec RE, Comperatore M, Andersen C, et al. (2012) A Mediterranean-style low-glycemic-load diet decreases LDL oxidation in women with metabolic syndrome. *J Nutr Biochem* 23: 609-615. [Crossref]

13. Bacchetti T, Vollini D, Manciagelo S, Gesuita R (2014) Effect of black and red cabbage on plasma carotenoid levels, lipid profile and oxidized low density lipoprotein. *J Funct Foods* 8: 128-137.

14. Beck J, Ferracci L, Sun K, Fried LP (2008) Circulating oxidized low-density lipoproteins are associated with overweight, obesity, and low serum carotenoids in older community-dwelling women. *Nutrition* 24: 964-968. [Crossref]

15. Guaskins AJ, Rosner AJ, Mumford SL, Yeung E, Browne RW, et al. (2010) Adherence to a Mediterranean diet and plasma concentrations of lipid peroxidation in premenopausal women. *Am J Clin Nutr* 92: 1461-1467. [Crossref]

16. Haegaele AD, Gillette C, O'Neill C, Wolfe P (2000) Plasma xanthophyll carotenoids and reactions. *J Agric Food Chem* 56: 7194-7200. [Crossref]

17. Castenmiller JJ, Laurisen ST, Dragsted LO, van het Hof KH, Lissens JP, et al. (1999) beta-carotene does not change markers of enzymatic and nonenzymatic antioxidant activity in human blood. *J Nutr* 129: 2162-2169. [Crossref]

18. Martinez-Tomas R, Lurque E, Gonzalez-Silveira D, Sanchez-Campillo M (2011) Effect of the consumption of a fruit and vegetable soup with high in vitro carotenoid bioaccessibility on serum carotenoid concentrations and markers of oxidative stress in young men. *Eur J Nutr* 51: 221-229. [Crossref]

19. Kostic D, White WS, Olson JA (1995) Intestinal absorption, serum clearance, and interactions between lutein and beta-carotene when administered to human adults in separate or combined oral doses. *Am J Clin Nutr* 62: 604-610. [Crossref]

20. Brown L, Rosner B, Willett WW, Sacks FM (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr* 69: 30-42. [Crossref]

21. Amuzgi G, Bozzetto L, Costabile G, Giacco R, Mangione A, et al. (2014) Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial. *Am J Clin Nutr* 99: 463-471. [Crossref]

22. Qin Y, Xia M, Xu J, Hao YT (2009) Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am J Clin Nutr* 90: 485-492. [Crossref]

23. Zern TL, Wood RJ, Greene C, West KL (2005) Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *J Nutr* 135: 1911-1917. [Crossref]

24. Pal S, Ho N, Santos C, Dubois P, Mano J, et al. (2003) Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of ApoB100 from human HepG2 cells. *J Nutr* 133: 700-706. [Crossref]

25. Yoo SL, Xu Z, Zhang YY, Lu YH (2013) Black rice and anthocyanins induce inhibition of cholesterol absorption in vitro. *Food Funct* 4: 1602-1608. [Crossref]

26. Gebrardt R (2003) Variable influence of kaempferol and myricetin on in vitro hepatocellular cholesterol biosynthesis. *Planta Med* 76: 1071-1074. [Crossref]

27. Sanchez-Moreno C, Cano MP, de Ancos B, Plaza L (2004) Pulsed electric fields-processed orange juice consumption increases plasma vitamin C and decreases F2-isoprostanes, a marker of lipid peroxidation. *J Nutr Biochem* 15: 649-652. [Crossref]

28. Kaliora AC, Dedoussis GV, Schmidt H (2006) Dietary antioxidants in preventing hepatic lipid accumulation. *Nutr Metab Cardiovasc Dis* 16: 155-166. [Crossref]

29. Kaliora AC, Dedoussis GV, Schmidt H (2006) Dietary antioxidants in preventing hepatic lipid accumulation. *Nutr Metab Cardiovasc Dis* 16: 155-166. [Crossref]

30. Wang TT, Edwards AJ, Cledeninan BA (2013) Strong and weak plasma response to dietary carotenoids identified by cluster analysis and linked to beta-carotene 15,15′-monoxygenase 1 single nucleotide polymorphisms. *J Nutr Biochem* 24: 1538-1546. [Crossref]

31. Ros E, Martinez-Gonzalez MA, Estruch R, Salas-Salvadó J, Fito M, et al. (2014) Mediterranean diet and cardiovascular health: Teachings of the PREMIDED study. *Adv Nutr* 5: 3305-3315. [Crossref]
46. Hulthe J, Fagerberg B (2002) Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR study). *Arterioscler Thromb Vasc Biol* 22: 1162-1167.

47. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W (2005) Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. *Circulation* 112: 651-657.