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Lycopene and Lutein and the Prevention of Atherosclerosis: is Supplementation Necessary?

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Abstract

Atherosclerosis and related cardiovascular diseases represent the leading cause of death worldwide. Oxidative stress, low grade inflammation and HDL dysfunction contribute to the development of this disease and therefore, dietary interventions that could counteract these factors are being studied. Special attention has been given to the potential protective role of carotenoids in atherosclerosis. These lipid soluble pigments synthesized by plants and abundant in fruits and vegetables have shown remarkable antioxidant capacity, suggesting that they could prevent LDL oxidation, one of the initial steps of atherosclerotic progression. Among all the carotenoids studied, lutein and lycopene have shown promising results, not only for their potential in protecting LDL from oxidation but also for modulating the expression of adhesion molecules and HDL functionality. Given their variable bioavailability and their multiple health claims, lutein and lycopene are usually found as supplements, however, the efficacy of these products in preventing or treating atherosclerosis remains unclear, since adequate serum concentrations could be achieved through a diet rich in fruits, vegetables and other carotenoids sources.

Keywords: *Carotenoids; Lutein; Llycopene; Atherosclerosis; Supplementation; Cardiovascular disease*

Abbreviations: Apo: Apolipoprotein; CCA: Common carotid artery; CRP: C reactive-protein; CVD: Cardiovascular disease; HDL: HDL-cholesterol, LDL-C: LDL-cholesterol, MDA: Malonaldehyde; TOAC: Total antioxidant capacity; IMT: Intima media thickness, NO: Nitric oxide; OxLDL: Oxidized LDL; PON1: Paraoxanse-1; SAA: serum amyloid A; TAOC: total antioxidant capacity

Atherosclerosis

Atherosclerosis is a chronic progressive disease of the arterial wall characterized by the proliferation of smooth muscle cells, the deposits of lipids and low grade inflammation [1-3]. The arterial regions more prone to atherosclerotic lesions are those with higher retention of cholesterol-rich, apolipoprotein (apo) B-containing lipoproteins [1,4,5]. These sequestered lipoproteins in the arterial wall are susceptible to be modified via oxidation, enzymatic and non-enzymatic cleavage or aggregation, which make these particles pro-inflammatory and pro-atherogenic [4]. For example, oxidized low-density lipoproteins (OxLDL) are ingested and accumulated into macrophages transforming them into cholesterol-rich foam cells which along with apoptotic cells, debris and cholesterol crystals form a necrotic core and luminal narrowing of arteries [4-6]. In addition, altered particles exhibit other atherogenic properties, like pro-inflammatory, immunogenic, apoptotic, and cytotoxic activities [6]. The lesion formation is usually a long and asymptomatic phase known as subclinical atherosclerosis, which is the precursor of clinical cardiovascular disease (CVD), including myocardial infarction and stroke [2,5]. Other clinical manifestations like coronary artery disease, cerebrovascular disease and peripheral arterial disease will occur in 2 of 3 men and 1 in 2 women after age 40 making atherosclerosis and its clinical complications are considered as the leading cause of death and loss of productive life years worldwide [2,7].

Dietary interventions for atherosclerosis

Vast evidence has shown that there is a relationship between nutrition and the progression of atherosclerosis. Studies have found that a high intake of carbohydrates, saturated and trans fats, as well as a low intake of antioxidant and fiber sources including fruits and vegetables, are positively related to atherosclerotic formation [8-11]. Due to the oxidative modification hypothesis, which states that oxidative stress plays an essential role the pathogenesis of atherosclerosis, dietary components with antioxidant capacity have shown a great potential in the prevention or treatment of this chronic disease as has been shown *in vitro* studies and in animal models [12,13]. Human trials have showed that subjects with higher plasma concentrations of antioxidant vitamins (vitamin C and vitamin E) have normal thickness for arterial walls and almost no plaque in carotid arteries, suggesting that antioxidants could be important protectors in the first phases of atherogenesis [14]. However, results from human dietary interventions using these antioxidant vitamins when the disease is already progressed have yielded controversial and disappointing results [12].

Other antioxidant compounds present in the diet that are not considered vitamins are carotenoids. This family of pigmented phytochemicals are synthesized by plants and some microorganisms where they provide protection against photo-damage and oxidation since these are very efficient scavenging singlet molecular oxygen and peroxyl radicals [11,15,16]. Although there is no recommended intake for these compounds, lower serum concentrations of carotenoids have been positively associated with increased risk of cardiovascular disease, age-related macular degeneration and certain types of cancer [17-20]. One problem with carotenoids is that many factors can affect their bio-accessibility (the amount available at the intestinal level for absorption) and thus, their potential bioactivity [21]. For this reason, in order to observe consistent physiological effects in humans, high doses only achieved by supplements have been employed.

The objective of this review is to summarize the available data, which correlates the intake of two carotenoids, lutein and lycopene- that have exceptionally high antioxidant activity compared to other carotenoids [22] and the progression of atherosclerosis in order to asses if supplementation with these compounds can be used as an effective dietary strategy to combat this chronic disease.

Lutein

Lutein is a carotenoid that belongs to the xanthophyll family (also known as oxycarotenoids). It has the peculiarity of having two hydroxyl groups in its structure, which determines its exclusive behavior in the body [23]. In foods, lutein is particularly abundant in green leafy vegetables and yellow foods such as corn and egg yolk [24]. The effect of lutein on eye health has been widely described, since this compound is selectively taken up by the retina where it provides protection against the oxidative blue light [25,26]. In addition, other potential benefits of lutein have been explored, based on the knowledge that lutein may also enhance immune function and protect against oxidative damage [25-27].

Although there is not recommended intake for lutein, intakes around 6 mg/day have been associated with positive effects in humans [28]; however intake from natural sources are around 1-2 mg/d [25,29,30] and the bio-availability of lutein depends on multiple factors, which means that serum concentrations are difficult to predict from dietary intake alone [26,30,31]. For this reason, in order to study the potential beneficial effects attributed to lutein, most researchers have used supplements with doses much higher than those achievable through regular diets. Epidemiological studies have assessed the potential role of lutein in the prevention of atherosclerosis by correlating serum lutein levels and atherosclerosis risk factors. For example, in the Los Angeles Atherosclerosis Study, a cohort of 269 adult women and 304 men with no history of cardiovascular disease were subject to ultrasound examination of carotid arteries, venipuncture and assessment of risk factors for atherosclerosis and cardiovascular disease at baseline and after an 18 month follow-up. The results show that plasma lutein concentration was positively correlated to HDL-cholesterol (HDL-C) concentrations and inversely correlated to the progression of intima-media thickness among non smokers and smokers, with a blocked progression in the highest plasma lutein quintile, suggesting a protective role of lutein [32]. The same authors conducted an animal supplementation model with apoE knockout mice, which develop severe atherosclerosis morphologically similar to the human situation. In this experiment, 10 mice were assigned to a chow diet (control) or a chow diet with added lutein (0.2% by weight) starting at 8 weeks of age. The results of this experiment show that lutein supplementation reduced the size of atherosclerotic lesions by 45% and decreased LDL oxidation in a dose-dependent manner [32].

Similar findings were also reported by Kim., *et al*. [27] in a study conducted with cholesterol challenged guinea pigs (0.25g cholesterol/100g body weight) randomly assigned to a hyper cholesterolemic diet alone or with supplementation of 3 mg per day of lutein for 12 weeks. While plasma LDL cholesterol (LDL-C) did not differ between groups, the lutein group showed lower concentrations of medium size LDL. Aortic cholesterol and malondialdehyde (MDA) concentrations were also lower in the supplemented group. Hematoxilin and eosin staining of the aortas showed focal intimal thickening in the control group, whereas less or no thickness was found in the lutein group. Furthermore, the concentration of OxLDL levels as well as aortic cytokines was significantly lower in lutein-supplemented guinea pigs. Finally, aortic cytokines were also lower in the lutein-supplemented guinea pigs compared to controls. These results suggest that lutein can exert antioxidant and anti-inflammatory properties that could protect the aortic tissue against cholesterol-induced atherosclerotic damage in guinea pigs [27].

With the hypothesis that these properties can also be observed in humans, Wang., *et al*. [33], conducted a randomized, double-blind, placebo-controlled trial of lutein supplementation with 117 healthy non smokers. Participants were randomly assigned to receive 10 or 20 mg/d of lutein or placebo for 12 weeks. Plasma carotenoid concentrations, total antioxidant capacity (TAOC), lipoprotein profile, and antioxidant enzymes activities were measured at baseline and after 6 and 12 weeks. In addition, biomarkers of oxidative damage to protein and lipids, and C- reactive protein (CRP) concentrations were also determined at baseline and after the 12 weeks of the trial. Results showed that plasma lutein concentrations and TAOC were significantly increased after 12 weeks of supplementation with both doses used. Also, the supplemented subjects showed a decrease in MDA plasma levels, being significant in the 20 mg group, suggesting that lutein decreases lipid peroxidation. CRP concentration (a marker for inflammation) also decreased in a dose-dependent manner after lutein supplementation, being significant between the 20 mg of lutein group and the placebo group. Serum CRP levels were inversely related to plasma lutein and TAOC for both treatment groups. These findings showed a possible beneficial effect of lutein supplementation and cardiovascular disease risk; however, the authors concluded that to make that assessment, longer and larger studies are needed [33].

A larger human study that evaluated the effect of plasma carotenoids and atherosclerosis was the Beijing atherosclerosis study. This case-control study had 125 subjects with early atherosclerosis and 107 controls aged 45 to 68 years. Using carotid ultrasonography, carotid intima media thickness (IMT) and arterial stiffness were measured. Serum carotenoids lutein, zeaxanthin and β-carotene were determined by high-pressure liquid chromatography (HPLC). Results showed that serum lutein concentrations were significantly lower in cases than in controls, a difference that was not significant in the case of zeaxanthin and β-carotene. Furthermore, these serum levels were inversely associated carotid IMT, suggesting that lutein may play a protective role in the progression of early atherosclerosis [34]. In addition to the antioxidant hypothesis, another mechanism that has been proposed for the role of lutein in cardiovascular protection, which involves inhibition of adhesion molecules to the cell surface of endothelial cells, a biomarker in the pathogenesis of atherosclerosis [25,35,36]. Martin,. *et al*. [22] showed that pre-incubation of human aortic endothelial cells (HAEC) with 0.9μ mol/l lutein before interleukin 1 beta (IL-1β (5 ng/ml) for 6 hours reduced vascular cell adhesion protein 1 (VCAM-1), E-selection and Intercellular Adhesion Molecule 1(ICAM-1) expression by 28%, 34%, and 14%, respectively [22].

Lycopene

Another carotenoid that has been associated with cardiovascular health is lycopene. This is an acyclic, not oxygenated and not provitamin A carotenoid present in red fruits and vegetables, being particularly abundant in tomatoes [37,38]. Several studies have found a negative correlation between high intake of tomatoes or tomato products and the risk of chronic diseases, most likely to be an effect of higher concentrations of lycopene in plasma [37,39,40]. Among carotenoids, lycopene is not only the most potent singlet oxygen quencher, but also appears to be the most effective in reducing both adherence of monocytes and expression of adhesion molecules to human aortic endothelial cells, suggesting an important role for lycopene in attenuating atherogenesis [22,41].

The intake of lycopene is higher when compared to lutein, probably because in addition to fresh fruits and vegetables, processed tomato products, such as juice, ketchup, paste, sauce and soup, are important dietary sources of lycopene, accounting for 50% of the daily intake of this carotenoid, which is approximately 3 mg/d [38]. Moreover, it has been reported that lycopene from these processed

food sources is more bioavailable in humans [42]. For these reasons, lycopene studies have been conducted using supplements and high doses. As the case with lutein, there is also epidemiological data that correlates plasma lycopene concentrations with atherosclerosis risk factors.

A study done with New Zealand rabbits showed that after following an atherosclerotic-high cholesterol challenge (0.5% cholesterol), animals that were supplemented with 5 mg/kg body weight of lycopene for a period of 4 weeks had significantly more lycopene plasma levels, which was correlated with a reduction of 50% in the levels of total cholesterol and LDL-C. The amount of esterified cholesterol accumulated in the aorta of the supplemented animals was also reduced. However, there was not a significant decrease in the extent of aortic surface lipid accumulation or the intima-media thickness in the lycopene treated rabbits, suggesting a protective -not a curative- role of lycopene in cholesterol-induced aortic lesions in rabbits [43]. In the Rotterdam study, the association between aortic atherosclerosis and the levels of the major serum carotenoids (α-carotene, β-carotene, α-cryptoxanthin, lutein, lycopene, and zeaxanthin) in a subsample of the elderly population was investigated (n = 108 cases and controls). Aortic atherosclerosis was assessed by presence of calcified plaques of the abdominal aorta. In an age- and sex-adjusted logistic regression model, serum lycopene (but no other carotenoids) was inversely associated with the risk of atherosclerosis [44]. Rissanen., *et al*. [18] studied the relationship between serum lycopene concentration and common carotid artery (CCA)-IMT in 1028 middle-aged men (aged 46–64 years old) in eastern Finland who were participants in the Kuopio Ischemic Heart Disease Risk Factor study and who were examined in 1991-1993. Findings were that male participants in the lowest quarter of serum lycopene concentration had a significantly higher mean CCA-IMT than did the other men in the study [18].

Carotenoids and HDL functionality

In addition to directly preventing the oxidation of LDL and the expression of adhesion molecules, another theory that may explain the potential benefits of lycopene against atherosclerosis progression is the improvement of HDL functionality. There is evidence that HDL has anti-atherogenic properties. Three main mechanisms have been proposed for these effects. The first one is that HDL and its role on reverse cholesterol transport, which is the transfer of cholesterol from extra-hepatic cells to the liver. This mechanism can take excess free cholesterol from cells like macrophages and transport it back to the liver for excretion, decreasing the formation of foam cells and the subsequent atherogenic lesion [45]. The second is that HDL may modulate endothelial function as adhesion of molecules to the cell surface of endothelial cells is a biomarker for early atherosclerosis [22].

Endothelial dysfunction is characterized by decreased bioavailability of nitric oxide (NO), a potent vasodilator, and increased affinity of the endothelial surface for leukocytes. The literature states that HDL can induce the activation of endothelial nitric oxide syntheses (eNOS), increasing NO release and thus, improving endothelial function [46]. Other studies have confirmed that HDL attenuates the expression of adhesion molecules such as VCAM-1, (ICAM)-1, and E-selection [45,47]. Lastly, HDL has shown ability to inhibit LDL oxidation given the content of antioxidants in the HDL particle, including carotenoids, the antioxidative properties of apo A-I; and the presence of antioxidant enzymes like paraoxonase 1 (PON1) [45]. However, during inflammatory states, the acute phase protein serum amyloid A (SAA), can displace apo AI from HDL, making it dysfunctional [6,48,49]. Dysfunctional HDL is considered pro-atherogenic because it lacks the ability of taking free cholesterol from macrophages, thus decreasing reverse cholesterol transport and promoting the accumulation of foam cells [50]. Moreover, dysfunctional HDL induces the release of pro-inflammatory cytokines that promote the recruitment of monocytes into vascular plaques. This HDL is also less effective in preventing LDL oxidation, due to decreased paraoxonase-1 (PON-1) activity [48].

The relationship between lycopene and HDL functionality was investigated by McEneny., et al. [48] in a study conducted with 54 moderately overweight individuals who were randomly allocated into one of three groups : control diet (< 10 mg lycopene/week), lycopene-rich diet (224-350 mg lycopene/week) and lycopene supplement (70 mg lycopene/week). Serum was taken at baseline and after the intervention. HDL characterization was assessed with ultracentrifugation, SAA was measured in HDL and in serum to assess systemic and HDL-associated inflammation and HDL functionality was determined by monitoring the activities of paraoxonase-1 (PON-1), cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyl transferase (LCAT). Results showed that the lycopene

intervention significantly increased HDL2 & 3, SAA was decreased in HDL3 following the lycopene-rich diet and in both serum and HDL3 after the lycopene supplementation. Baseline PON-1 activities were similar between the groups in serum and HDL2 & 3, However, following both lycopene interventions, the activity of PON-1 significantly increased in serum and HDL2 & 3. CETP activity decreased in serum after lycopene supplementation, and activity of LCAT increased in serum and HDL3 following both lycopene interventions. All these changes that lycopene exert over HDL in middle-aged subjects may explain why lycopene could be associated with the prevention of atherosclerosis and heart disease [48].

The HDL hypothesis can also be attributed to lutein. Blesso., *et al*. [51] showed that egg (a natural source of highly bioavailable lutein) consumption in patients with metabolic syndrome increased not only the concentration but the size of HDL particles, which also showed an increase of carotenoid content compared to the control group [51]. Unlike other carotenoids, lutein is mainly transported by HDL [52], and due to greater surface area, larger HDL particles are more suitable for xanthophyll transport [51]. This relationship between plasma lutein and larger HDL has been previously reported [53] and these larger HDL particles are also hypothesized to be more anti-atherogenic [51,54]. Lutein-rich HDL particles could be more effective in preventing the oxidation of LDL particles, given the fact that this polar carotenoid is located on the surface of lipoproteins [55]. However, further studies need to be done with the specific role of lutein and HDL functionality to support the hypothesis that this mechanism accounts for its anti-atherogenic properties.

Lutein and lycopene synergistic effects

Some authors suggest that there could be a synergistic effect when carotenoids are mixed together. An early study done with various carotenoids and their capacity to inhibit the formation of Thiobarbituric acid-reactive substances in multilamellar liposomes found that when 3 μM of lycopene and lutein are administered at the same time, their antioxidant capacity is increased when compared to each carotenoid alone [56]. Similar results were obtained in a human study in China, where 144 subjects aged 45-68 years were given either 20 mg lutein/d (n 48), 20 mg lutein/d + 20 mg lycopene/d (n 48) or placebo (n 48) for 12 months. At baseline and after the experimental period, the subjects' carotid artery intima-media thickness (CAIMT) was measured, as well as serum lutein and lycopene concentrations. Results showed that serum lutein concentrations increased significantly in the lutein group and in the combination group. This increase in serum carotenoid concentration was negatively correlated with CAIMT, thus suggesting that lutein and lycopene could protect against the progression of atherosclerosis in Chinese subjects and are being more effective when both were given together [57]. It is hypothesized that this happens because these carotenoids tend to arrange in different sites of membranes and lipoproteins, adding an enhanced protection against oxidative damage [58]. The mechanisms by which lutein and lycopene may protect against atherosclerosis are depicted in Figure 1.

Conclusion

Lutein and lycopene have been associated with three main effects that could be beneficial for atherosclerosis-risk patients: antioxidant capacity, inhibition of endothelial monocyte adhesion and adhesion molecules expression and HDL functionality enhancement. Most of the literature confirms that a higher intake of carotenoids correlates with higher concentrations of these compounds in plasma, and these plasma concentrations are inversely correlated to the incidence and progression of atherosclerosis in animals and humans. However, the evidence is not consistent in terms of the effects that high intakes of lutein and lycopene can exert over existing atherosclerosis lesions. It seems that while carotenoids may delay the progression of the disease, they cannot be considered as a cure or replace pharmacological therapy, physical activity and weight maintenance in high-risk patients. The inverse relationship between fruit and vegetables intake and the incidence of atherosclerosis cannot be attributed only to the presence of carotenoids in these food groups, since fruits and vegetables also have other bioactive components including fiber and polyphenols, which could have an additive effect in the protection against atherosclerosis.

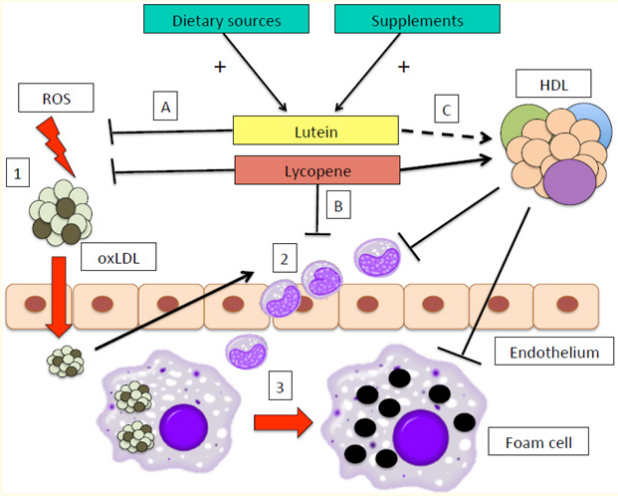

Figure 1: *Effects of lutein and lycopene in protecting against atherosclerosis. The numbers indicate the different steps that lead to atherosclerosis: 1) Reactive oxygen species (ROS) induce lipid per oxidation causing the formation of oxidized LDL (ox LDL); 2) ox LDL can trigger the expression of adhesion molecules by endothelial cells, which drive immune cell infiltration to the intima and 3) Infiltrated monocytes differentiate into macrophages, which uptake the ox LDL through scavenger receptors leading to the formation of foam cells, the first step in atherosclerosis progression. Lutein and Lycopene are hypothesized to contribute to the prevention of atherosclerosis via three pathways: A. Decreasing the oxidation of LDL particles by scavenging ROS, B: decreasing the expression of adhesion molecules and C: improving HDL functionality allowing for the efflux of free cholesterol from macrophages and enhancing reverse cholesterol transport.*

In order to recommend a proper dose for carotenoid supplements, it is important to consider that the bioavailability of these compounds is highly variable and multi factorial, which represent a difficulty in establishing a clear relationship between intake and physiological effects. Instead, it appears that establishing recommendations for serum carotenoid concentrations that have been associated with cardiovascular protection could be a more reliable approach. These concentrations of carotenoids can be achieved with diet alone; however, the use of supplements could be required in some cases. Despite the potential that lycopene and lutein may have in the protection against the development and progression of atherosclerosis, further clinical studies with diverse populations at risk are needed. A diet rich in fruits and vegetables (major sources of carotenoids), accompanied with a healthy lifestyle that includes physical activity, no smoking and weight control is still the best way to prevent atherosclerosis. The need for carotenoid supplements to prevent cardiovascular events remains controversial.

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