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Review Article

# Carotenoids in Cardiovascular Disease Prevention

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**Abstract**

Cardiovascular Disease (CVD) remains a major cause of morbidity and mortality in developed society. Since oxidative stress and inflammation are key players in the etiology of CVD, it is conceivable that natural antioxidants such as carotenoids, existing in fruits and vegetables, may help in preventing the CVD onset.

More than 700 carotenoids have been identified, among them β-carotene, α-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin are the major dietary carotenoids with antioxidant properties likely linked to the ability in scavenging free radicals such as lipid peroxyl radicals, reactive oxygen species (ROS), and nitric oxide (NO).

Increased ROS generation has been associated with a functional inactivation of NO due to the reaction with superoxide anione ($O_2$-), leading to peroxynitrite (ONOO-) formation and subsequent reduction in vascular NO bioavailability that characterizes the early stage of atherosclerosis.

Carotenoids, at least in part, by directly removing $O_2$-, have been shown to restore NO endothelial bioavailability. Hence, they may be considered potential anti-oxidant modulators of endothelial response to pro-oxidant/inflammatory stimuli.

More recently, several *in vitro* and *in vivo* experiments have demonstrated that carotenoids are able to reduce inflammation and oxidative stress through the regulation of various cellular functions, thus supporting the epidemiological studies indicating a strong correlation between dietary carotenoid consumption and decreased risk of CVD. However, since human intervention studies are controversial, the *in vivo* mechanism/s underlying the carotenoid's cardiovascular protective activities is still little known.

This review aims to outline the current situation of relations between the main dietary carotenoids and prevention of CVD.

## ABBREVIATIONS

CVD: Cardiovascular Disease; NO: Nitric Oxide; ROS: Radical Oxygen Species; HUVECs: Human Umbilical Vein Endothelial Cells; CHD: Coronary Heart Disease; MI: Myocardial Infarction; SCD: Sudden Cardiac Death; TNF-α: Tumor Necrosis Factor-alpha; NF-kB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; ICAM-1: Intercellular Adhesion Molecule-1; VCAM-1: Vascular Cellular Adhesion Molecule-1; LPS: Lipopolysaccharide; SMC: Smooth Muscle Cells; PDGF: Platelet Derived Growth Factor; MAPK: Mitogen-Activated Protein Kinase; SOD: Superoxide Dismutase; CAT: Catalase; GSPHPx: Gluthathione Peroxidase; MCP-1: monocyte chemotactic protein-1; IL-8: Interleukin-8; IL-1: Interleukin-1; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; ox-LDL: oxidized-Low Density Lipoprotein; HMG-CoA: 3-Hydroxy-3Methylglutaryl-CoA; BMI: Body Mass Index.

## INTRODUCTION

Cardiovascular disease (CVD) remains a leading cause of mortality and disability around the world. There is a massive body of epidemiological studies suggesting that Mediterranean countries have a lower rate of CVD mortality when compared to other regions of Western Europe and the United States [1]. The reduced rate of CVD mortality can be explained, at least in part, by the dietary culture of the Mediterranean region that includes consumption of large amounts of fruit and vegetables.

Vegetables and fruit represent important sources of carbohydrates, dietary fiber, antioxidant vitamins, minerals, polyphenols, and various phytochemicals and, in particular, they are the main asset of carotenoids in the human diet [2,3].

Carotenoids are liposoluble C-40-based isoprenoid pigmented

(yellow, orange, and red) compounds that are synthesized by plants and microorganism but not by animals [4].

More than 700 carotenoids have been characterized; about 50 of them consumed in the human diet [5,6]. Approximately 10-15 carotenoids represent most of the dietary intake, and these are found in measurable concentrations in human blood and tissues [7,8]. Among them, β-carotene, α-carotene and β-cryptoxanthin are the major carotenoids having significant pro-vitamin A activity, while lutein, lycopene, and zeaxanthin are not converted into active retinoids by humans [9], their structure are illustrated in (Figure 1).

Various biological effects have been ascribed to carotenoids. One of these is the antioxidant and scavenging capacity related to their structure [10,11]. Indeed, the conjugated double bond structure is primarily responsible for the chemical reactivity of carotenoids with free radicals such as the peroxyl, hydroxyl, and superoxide radicals. Of note, carotenoids have proved able to prevent or decrease oxidative damage to DNA, lipid and proteins [5,12].

Conversely, a number of reports claim that carotenoids can act as pro-oxidant molecules and increasing the total radical yield in a system [13,14]. The key factor to determine the switch of carotenoids from antioxidant to pro-oxidant is the oxygen partial pressure ($pO_2$) and the carotenoid concentration [15,16]. At higher $pO_2$ a carotenoid radical can react with molecular oxygen to generate a carotenoid-peroxyl radical which can act as a pro-oxidant by promoting oxidation of unsaturated lipids.

Although the antioxidant properties of carotenoids have been proposed as the main mechanism behind their beneficial effects,

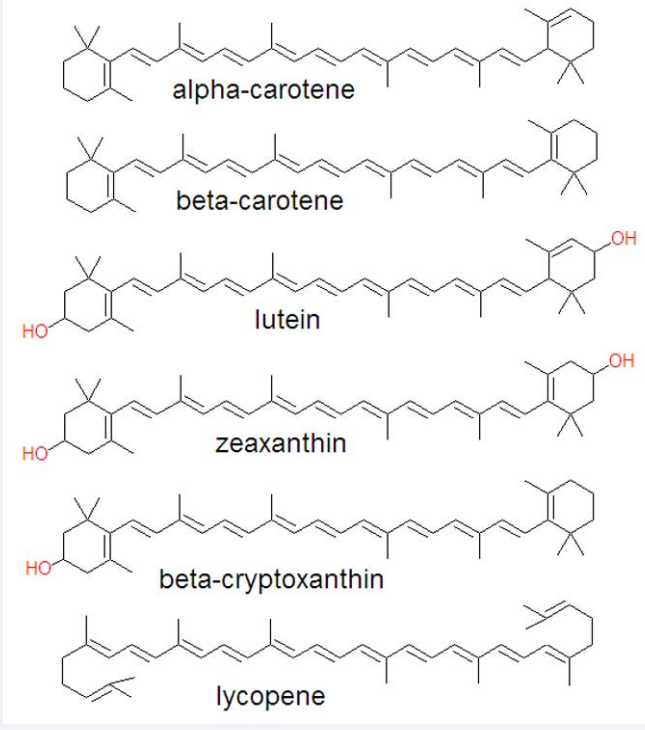


**Figure 1** Chemical structure of the main carotenoids in the human diet.

recent studies are also beginning to show that these compounds may mediate their effects via other mechanisms such as cell growth regulation, gap-junction communication, modulating gene expression and others [17].

Several epidemiological reports have shown a correlation between elevated dietary carotenoid intake and circulating levels and prevention of CVD [18-21]. For example a relationship has been demonstrated between circulating carotenoid concentrations and several markers of inflammation, oxidative stress, and endothelial dysfunction [22], which are known to be associated with CVD [23-25].

At present, it is well recognized that atherosclerosis is an inflammatory disease [26], and some evidence suggest that beneficial effects of carotenoids may result from modulation of the inflammatory responses [20-24].

Furthermore, carotenoids and vitamins could have an antioxidant-mediated tempering influence on endothelial function and inflammation, thereby reducing the risk of atherosclerosis [27].

In this regard, we recently demonstrated *in vitro* that β-carotene and lycopene reduce the inflammatory response in tumor necrosis factor-α (TNF-α)-treated human umbilical vein endothelial cells (HUVECs). This effect was mainly due to the redox balance protection and to the maintenance of NO bioavailability [28]. In line with our data other *in vitro*, *in vivo* and human studies support the idea that carotenoids may exert their cardiovascular protective action by increasing NO bioavailability [29-31], as shown in (Figure 2).

However, most findings published to date indicate that such benefits stem from carotenoids' anti-inflammatory action, though the mechanism/s underlying this vascular activity is/are still largely unknown.

Moreover, since carotenoids are a complex group of chemicals, and studies of the health effects of carotenoids are very heterogeneous, it is difficult to perform a meta-analysis or even a detailed systematic review of the health effects of carotenoids.

To date, plasma concentrations of carotenoids are considered useful biomarkers of total dietary intake of vegetables and fruit [32] and epidemiological studies have provided convincing evidence in support of the protective role of carotenoids in CVD [33]. However, these observations need to be better validated on the one hand by carrying out *in vitro* studies on the molecular mechanisms and, on the other hand by conducting well-controlled human intervention studies in the future.

Although several studies have already showed the relationship between carotenoids and CVD, none of them has covered *in vitro*, animal, epidemiological and human studies (Table 1). Hence, we believe that this review may add a piece of knowledge about the relations between the carotenoids and prevention of CVD.

## *In vitro* studies

There are several advantages in employing cellular models to investigate the effect of carotenoids on inflammatory- and oxidative-pathways. These include the ability to investigate, under well-defined conditions, specified carotenoid concentrations and

specific types of cells and therefore allowing for a large number of investigations that are suitable for hypothesis building and studying mechanistic effects. However, the limitations of these cellular studies include the difficulty to conduct long-term studies due to limited cell life; the missing interactions with other cells present *in vivo*.

Many *in vitro* studies, especially those that include cellular models, have aided in establishing a link between carotenoids, oxidative stress, and inflammation [34].

Moreover, several studies highlight the beneficial effect of carotenoids in maintaining endothelial NO bioavailability, suggesting their ability to preserve endothelial function and more in general vascular health (Figure 2).

In this regard, as mentioned above, we recently demonstrated that treatment with β-carotene or lycopene significantly decrease reactive oxygen species (ROS) and nitrotyrosine (an index of ONOO-) levels, thus improving NO bioavailability. In parallel it down-regulates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)-dependent adhesion molecule expression and monocyte–HUVEC interaction, [28] (Figure 2).

In addition, some *in vitro* studies have suggested that carotenoids significantly inhibit TNF-α-induced intercellular and vascular adhesion molecules (ICAM-1 and VCAM-1) expression in both vein and arterial endothelial cells [35] and have barrier integrity activity, as well as inhibitory activity on cell adhesion and migration to endothelium by blocking the activation of NF-κB, CD14 and TLR4 expression and production of TNF-α [36].

However, one should also consider that in TNF-α-stimulated endothelium NO rapidly reacts with superoxide anion ($O_2$-) to form a stable potent oxidant ONOO- resulting in decreased vascular relaxation, and contributing to the up-regulation of NF-kB-dependent cellular response. Thus, the general effect of anti-oxidant molecules on the biological function of NO is likely to be due, at least in part, to direct removal of $O_2$- [37,38]. More recently, sung et al. [39] demonstrate a novel beneficial effect of

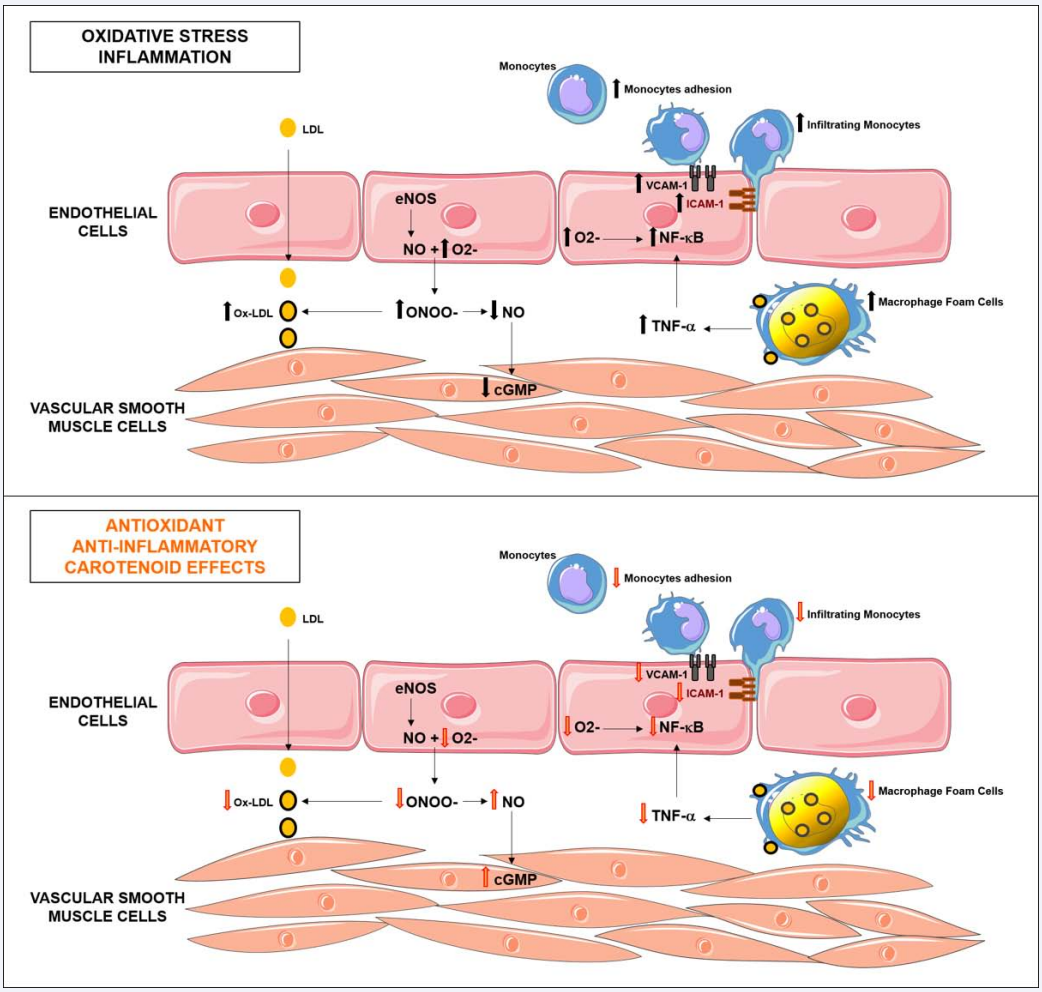


**Figure 2** The protective antioxidant and anti-inflammatory carotenoid effects on vascular cells. Under oxidative condition NO may react with O2- to form ONOO-, this lead to the decrease of NO bioavailability leading to endothelial dysfunction, enhanced LDL peroxidation and chronic vascular inflammation. This is associated to lipid accumulation in the arterial wall, NF-κB activation that in turn triggers the up-regulation of VCAM-1 and ICAM-1. All these events generate a negative loop, which might be interrupted by antioxidant and anti-inflammatory effect of the carotenoids. eNOS, endothelial nitric oxide synthase; NO, nitric oxide; O2-, superoxide anion; ONOO-, peroxynitrite; cGMP, cyclic guanosine monophosphate; LDL, low density lipoprotein; ox-LDL, oxidized low density lipoprotein; TNF-α, tumor necrosis factor alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

**Table 1:** Major cited studies on the positive and negative effects of carotenoids on Cardiovascular Diseases.

| POSITIVE CITED STUDIES | | | | |
|---|---|---|---|---|
| **Study type** | **Cell type, animal type or specimen** | **Carotenoids investigated** | **Main effects** | **Reference** |
| *In vitro* | HUVECs | β-carotene and lycopene | Reduced inflammation and oxidation and increased NO bioavailability | Di Tomo et al. [28] |
| *In vitro* | HUVECs | lycopene | Inhibited VCAM-1 and ICAM-1 | Hung et al. [35] |
| *In vitro* | HUVECs | lycopene | Inhibited leucocyte adhesion and migration | Bae et al. [36] |
| *In vitro* | HUVECs | lycopene | Inhibited cyclic strain-induced endothelin-1 and suppressed ROS | Sung et al. [39] |
| *In vitro* | HUVECs | lycopene | Reduced oxidative injury and apoptosis | Tang et al. [40] |
| *In vitro* | Neutrophils | β-carotene and annatto | Modulated the production of ROS and NO | Rossoni-Junior et al. [41] |
| *In vitro* | Macrophages | β-carotene | Reduced inflammation and scavenged intracellular ROS | Bai et al. [42] |
| *In vitro* | Macrophages | astaxanthin | Inhibited macrophages activation | Kishimoto et al. [44] |
| *In vitro* | Macrophages | lycopene | Regulated cholesterol synthesis and efflux | Palozza et al. [47] |
| *In vitro* | Macrophages | lycopene | Prevented oxidative stress, cell cycle arrest and apoptosis | Palozza et al. [48] |
| *In vitro* | Rat Smooth Muscle Cells | lycopene | Inhibited PDGF-BB-induced signaling pathway | Lo et al. [49] |
| *In vitro* | Platelet | lycopene | Inhibited platelet activation | Hsiao et al. [51] |
| Animal | Rats | b-carotene and annatto | Decreased oxidation and inflammation | Rossoni-Junior et al. [41] |
| Animal | Rats | lycopene | Suppressed oxidative stress and reduced myocardial injury | Bansal et al. [54] |
| Animal | Rats | lycopene | Reduced oxidative stress | Breinholt et al. [55] |
| Animal | Rats | lycopene | Reduced inflammation | Liu et al. [56] |
| Animal | Rats | lycopene | Reduced oxidative stress | Zhu et al. [57] |
| Animal | Rabbits | lycopene | Increased HDL and reduced plaque area in the aorta | Verghese et al. [58] |
| Animal | Rabbits | lycopene | Reduced LDL, total cholesterol, triaglycerol and reduced plaque area in the aorta | Hu et al-. [59] |
| Animal | Rats | lycopene and tomato | Reduced oxidative stress | Gitenay at al. [62] |
| Animal | Hamsters | tomato paste rich in lycopene | Lowered lipids and reduced oxidative stress | Hsu et al. [63] |
| Animal | Mice | astaxanthin | Ameliorated endothelial function | Khan et al. [64] |
| Animal | Rats | astaxanthin | Ameliorated endothelial function | Zhao et al. [65] |
| Animal | Mice | lycopene and dietary mixture | Reduced atherosclerotic lesion | Verschuren et al. [67] |
| Epidemiological | _ | vegetables rich carotenoids | Reduced CHD risk | Liu et al. [70] |
| Epidemiological | _ | α-carotene and β-carotene | Lowered risk of atherosclerosis | D'Orico et al. [71] |
| Epidemiological | _ | lycopene | Reduced oxidative stress | Buijsse et al. [72] |
| Epidemiological | _ | β-carotene | Lowered incidence of CVD | Gey et al. [73] |
| Epidemiological | _ | β-carotene | Reduced MI risk | Kardinaal et al. [74] |
| Epidemiological | _ | β-carotene | Reduced MI risk | Klipstein-Grobusch et al. [76] |
| Epidemiological | _ | carotene and vitamin E | Reduced CHD risk | Rimm et al. [77] |
| Epidemiological | _ | β-carotene | Low carotenoid concentrations increased CVD mortality risk | Karppi et al. [78] |
| Epidemiological | _ | lycopene, lutein and β-carotene | Reduced oxidation of LDL | Karppi et al. [79] |
| Epidemiological | _ | β-carotene | Reduced SCD risk | Karppi et al. [80] |
| Epidemiological | _ | lutein, zeaxanthin and lycopene | Lowered risk of atherosclerosis | Xu et al. [81] |

| | | | | |
|---|---|---|---|---|
| Epidemiological | – | lutein, zeaxanthin, β-cryptoxanthin and α-carotene | Protected the early stage of atherosclerosis | Dwyer et al. [82] |
| Epidemiological | – | carotenoids in Mediterranean diet | Reduced endothelial damage and improved the regenerative capacity of endotheliun | Marin et al. [83] |
| Epidemiological | – | carotenoids in fruit and vegetables | Promoted cardiovascular health | Holt et al. [84] |
| Epidemiological | – | carotenoids in Mediterranean diet | Reduced oxidative stress and modulated inflammation | Azzini et al. [85] |
| Epidemiological | – | lycopene | Reduced MI risk | Ko]hlmeier et al. [18] |
| Epidemiological | – | lutein, zeaxanthin, β-cryptoxanthin | Serum levels inversely associated with all-cause of mortality | De Waart et al. [89] |
| Epidemiological | – | lycopene | Serum levels inversely associated with CVD | Petyaev et al. [90] |
| Human Intervention | – | Carotenoids and other dietary constituents | Reduced all-cause of mortality and reduced incidence of MI, stroke, and hypertension | Yang et al. [91] |
| Human Intervention | – | carotenoids in vegetables | Reduced CHD risk | Liu et al. [95] |
| Human Intervention | – | lycopene | Alleviated oxidative stress | Chen et al. [97] |
| Human Intervention | – | lycopene and tomato product | Reduced plasma LDL cholesterol | Palozza et al. [98] |
| Human Intervention | – | lutein and lycopene | Decreased intima-media thickness | Zou et al. [99] |
| Human Intervention | – | Tomato products | Lowered postprandial oxidative stress | Burton-Freeman et al. [100] |
| Human Intervention | – | lycopene | Improved endothelial function in CVD patients | Gajendragadkar et al. [21] |
| Human Intervention | – | lycopene | Reduced oxidative stress and improved endothelial function | Kim et al. [102] |
| Human Intervention | – | lycopene | Ameliorated serum cholesterol levels and blood pressure | Ried et al. [106] |
| Human Intervention | – | astaxanthin | Reduced oxidation and inflammation | Guerin et al. [107] |
| Human Intervention | – | astaxanthin | Improved oxidative stress | Choi et al. [109] |
| Human Intervention | – | astaxanthin | Prevented oxidative damage | Kim et al. [110] |
| Human Intervention | – | astaxanthin | Decreased oxidative stress and inflammation and enhanced immune response | Park et al. [111] |
| Human Intervention | – | astaxanthin | Increased serum HDL and adiponectin | Yoshida et al. [113] |
| **FAILED AND NEGATIVE CITED STUDIES** | | | | |
| **Study type** | **Cell type, animal type or specimen** | **Carotenoids investigated** | **Main effects** | **Reference** |
| Animal | Rabbits | lycopene-rich tomatoes | Did not reduce atherosclerosis | Frederiksen et al. [60] |
| Animal | Rabbits | lycopene | Did not improved aorta lesions | Lorenz et al. [61] |
| Animal | Rabbits | astaxanthin | Did not prevent atherogenesis | Jacobsson et al. [66] |
| Epidemiological | _ | β-carotene | Did not reduce LDL oxidation | Reaven et al. [86] |
| Epidemiological | _ | β-carotene | Increased all-cause of mortality | Bjelakovic et al. [87] |
| Epidemiological | _ | α-carotene and lycopene | Increased mortality risk | Shardell et al. [88] |
| Human Intervention | – | β-carotene | Did not produce any significant reductions of vascular disease | MRC/BHF Heart Protection Study [92] |

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|---|---|---|---|---|
| Human Intervention | – | β-carotene | Increased CHD risk | Rapola et al. [93] |
| Human Intervention | – | β-carotene | No cardiovascular benefit | Cook et al. [94] |
| Human Intervention | – | β-carotene | No impact on all-cause of mortality | Hennekens et al. [96] |
| Human Intervention | – | lycopene | No evidence on CVD prevention | Riccioni et al. [101] |
| Human Intervention | – | lycopene from cooked tomatoes | No improvement in lipid peroxidation rate and lipid profile | Bose et al. [103] |
| Human Intervention | – | tomato-based drink | No effect on inflammation and oxidation | Riso et al. [104] |
| Human Intervention | – | tomato products | No improvement in endothelial function | Stangl et al. [105] |

**Abbreviations:** CVD: Cardiovascular Disease; NO: Nitric Oxide; ROS: Radical Oxygen Species; HUVECs: Human Umbilical Vein Endothelial Cells; CHD: Coronary Heart Disease; MI: Myocardial Infarction; SCD: Sudden Cardiac Death; ICAM-1: Intercellular Adhesion Molecule-1; VCAM-1: Vascular Cellular Adhesion Molecule-1; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

lycopene through regulation of endothelin-1 (ET-1) expression, a powerful vasopressor synthesized by endothelial cells that plays a crucial role in the pathophysiology of CVD. More in detail, they showed that lycopene inhibits cyclin strain-induced ET-1 expression through the suppression of ROS generation and induction of heme oxygenase-1 in HUVECs.

Within this scenario, carotenoids may be considered potential anti-oxidant modulators of endothelial response to pro-oxidant/inflammatory stimuli.

The effects of lycopene on oxidative injury and apoptosis in endothelial cells following exposure to $H_2O_2$ were investigated by Tang *et al.* [40] using human vascular endothelial cells. Pre-treatment with lycopene dose-dependently decreased malondialdehyde (MDA) contents in $H_2O_2$-treated cells. Lycopene also significantly reduced the number of cells undergoing apoptosis in response to $H_2O_2$ inhibiting the up regulation of p53 messenger ribonucleic acid (mRNA) and caspase3 mRNA. These results suggest that protecting endothelial cells from oxidative injury may be one of the mechanisms underlying the cardiovascular-related beneficial effects of lycopene.

In agreement with the hypothesis that carotenoids might exert their protective role through oxidative stress reduction, Rossoni-Junior *et al.* [41] showed that neutrophils from diabetic animals produce significantly more ROS and NO than their respective controls and that supplementation with β-carotene and annatto (which has been identified as a carotenoid having antioxidative effects) is able to modulate the production of these species.

In previous study, Bai *et al.* [42], already supported the idea that carotenoids may protect against oxidative stress. In fact, they suggest that β-carotene, in RAW264.7 cells stimulated with lipopolysaccharide (LPS), possesses anti-inflammatory activity by functioning as a potential inhibitor for redox-based NF-kappa B activation, probably by directly scavenging the intracellular ROS.

Another carotenoid with antioxidant activity is astaxanthin, a xanthophyll carotenoid that have unique cell membrane actions. In cultured cells, astaxanthin protected the mitochondria against endogenous oxygen radicals, conserved their redox (antioxidant) capacity, and enhanced their energy production efficiency [43]. In addition, Kishimoto *et al.* [44] showed that astaxanthin could regulate the macrophageatherogenesis-related functions by suppressing the scavenger receptors up regulation, matrix metalloproteinase's activation, and pro inflammatory cytokines expression.

Since carotenoids may be considered efficient antioxidants, it has long been proposed that this property may be responsible for its beneficial effects.

However, reliable evidence indicate that carotenoids, and their metabolites, might modulate molecular pathways related with cell proliferation, acting at Akt, tyrosine kinases, mitogen activated protein kinase (MAP kinase) and growth factor signaling cascades [45].

Furthermore, recently other mechanisms such as modulation of lipid metabolism through control of cholesterol synthesis and oxysterol toxic activities have been evoked as relevant effects [46].

Consistent with this hypothesis, Palozza et al. [47] recently demonstrated that lycopene (0.5–2 mM) dose-dependently reduced the intracellular content of total cholesterol in THP-1 cells. They also showed that, in THP-1 cells, lycopene was able to reduce 7-ketocholesterol-induced ROS production, 8-hydroxydeoxyguanosine formation and to counteract 7-ketocholesterol-induced apoptosis by limiting caspase-3 activation [48]. Moreover, in the same cellular model lycopene prevented oxysterol-induced increase in pro-inflammatory cytokine secretion and expression. That effect was accompanied by inhibition of oxysterol-induced ROS production, mitogen-activated protein kinase (MAPK) phosphorylation and NF-κB activation. In addition, the carotenoid increased peroxisome proliferator-activated receptor γ levels in THP-1 macrophages. Taken all together, these data bring new information on the anti-atherogenic properties of lycopene, and on its action mechanisms in atherosclerosis prevention [45].

As above mentioned, carotenoids also modulate cell proliferation. In this regard, it has been demonstrated that lycopene binds platelet-derived growth factor (PDGF)-BB (homodimer form), which play an important role in the

progression of CVD, and inhibits PDGF-BB-induced intracellular signaling transduction pathway in rat vascular smooth muscle cells (SMC) [49].

Another key factor in atherogenesis is intravascular thrombosis [50]. Lycopene inhibited both aggregation in human platelets in a dose-dependent manner and the ATP-release reaction stimulated by agonists such as collagen or arachidonic acid. These results may suggest that tomato-based foods might be especially beneficial in the prevention of platelet aggregation and thrombosis [51].

Taking together, all the findings discussed above strongly indicate the protective effect of carotenoids in various cellular models.

Although most of the effects shown in *in vitro* studies cannot be directly transferred to the *in vivo* situation, these findings offer an opportunity to understand the mechanisms underlying the beneficial cardiovascular effects of carotenoids observed *in vivo*.

## Animal Studies

Compared to cellular studies, animal models allow for studying the effects under more complex, that is, realistic, physiological conditions. However, as a drawback, many animals metabolize carotenoids differently from humans [52].

In the last few years, several studies have been conducted in order to understand whether diet supplementation with carotenoids, administered alone or in combination with other molecules of nutritional interest, could exert the hypothesized protective cardiovascular effects in various animal models, which are mainly represented by mice, rats, hamsters and rabbits as will be described here.

By providing foods rich in carotenoids, several studies have aimed to reduce ROS and their negative impact on inflammation. For example, annatto extract or β-carotene added at 0.1% to the diet of rats for 7 days decreased ROS production in neutrophils and increased mRNA concentrations of superoxide dismutase (SOD), catalase (CAT), p22(phox), and p47(phox), which are components of the electron transfer elements of nicotinamide adenine dinucleotide phosphate oxidase [41].

Although, as just described, some authors have investigated the effect of foods containing different carotenoids, most of the studies relate to the effect of isolated carotenoids.

In this context, administration of lycopene in isolated form has received some attention because of its strong antioxidant effects in vitro [53]. Lycopene dissolved in olive oil reduced lipid peroxides levels and augmented glutathione levels as well as glutathione peroxidase (GSHPx) activity in male Wistar rats fed for 31 days [54]. In another experiment with female Wistar rats, lycopene was given for 2 week and the activity of glutathione reductase, GSHPx and SOD was significantly induced by various different doses of lycopene [55]. In a hyperhomocysteinemicrat model, lycopene reduced serum markers of inflammation such as VCAM-1, MCP-1, and IL-8, thus indicating anti-atherogenic effects [56].

Zhu J *et al.* [57] demonstrated that chronic lycopene treatment could attenuate endothelial dysfunction by reducing oxidative stress in streptozotocin-induced diabetic rats, indicating that chronic lycopene treatment might be useful in preventing the diabetic vascular complications associated with endothelial dysfunction.

Verghese *et al*. [58] recently demonstrated that dietary lycopene has a protective effect on cardiovascular disease in New Zealand male rabbits. Animals were fed on a normal diet, a high cholesterol diet and a high cholesterol diet containing various amounts of lycopene. The highest lycopene dose significantly reduced serum cholesterol, increased High Density Lipoproteins (HDL) cholesterol levels and reduced 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity as well as acyl-CoA-cholesterolacyl transferase activity. Of note, the highest dose of lycopene significantly reduced a plaque area in the aorta.

Accordingly with this finding, the comparison of lycopene and fluvastatin effects on atherosclerosis induced by a high-fat diet in adult male New Zealand white rabbits was lately published by Hu and colleagues [59]. The high-fat diet led to increased levels of total cholesterol, total triacylglycerol, Low Density Lipoprotein (LDL) cholesterol and IL-1. Lycopene was better than fluvastatin in reducing the changes in these parameters. Lycopene and fluvastatin also markedly reduced the formation of atherosclerotic plaques in the aorta compared to the situation in rabbits on a high-fat diet alone [59].

In contrast, Frederiksen *et al.* [60] did not show any effects from an intervention with extract of lycopene rich tomatoes when investigating male Watanabe heritable hyperlipidemic rabbits. The tomato extract had no effect on cholesterol and triacylglycerol levels in plasma, on cholesterol in lipoprotein fractions and on aortic atherosclerosis. Oxidation of plasma lipids was also unaffected by the intake of tomato extract. These results were recently confirmed by Lorenz *et al.* [61]. They showed that lycopene-treated New Zealand white rabbits, a part from reduced LDL cholesterol serum levels as well as reduced amounts of cholesteryl ester, did not improved the initial aorta lesions.

Some other animal studies investigate the potential role of tomatoes or tomato paste consumption and compared it to lycopene supplementation [62,63]. Interestingly,both studies, found that tomatoes had a higher potential than lycopene to affect oxidative stress-related parameters and plasmatic lipid profile, possibly due to the synergy of all the phytochemicals in tomatoes.

Recently several studies have suggested a cardiovascular protective role of astaxanthin Khan *et al.* [64] studied the effect of a proprietary astaxanthin prodrug (CDX-085) on thrombus formation, using a mouse model of arterial thrombosis. When compared to control mice, the CDX-085 fed group exhibited significant increases in basal arterial blood flow and significant delays in occlusive thrombus formation following the onset of vascular endothelial injury. Primary HUVECs and platelets isolated from Wistar-Kyoto rats treated with free astaxanthin demonstrated significantly increased levels of released NO and significantly decreased peroxynitrite levels. Thus, this study supports the potential of CDX-085 and its metabolite astaxanthin to treat or prevent thrombotic cardiovascular complications.

Interestingly, in spontaneously hypertensive rats,

astaxanthin-enriched diet reduces blood pressure and improves cardiovascular parameters by decreasing oxidative stress and improving NO bioavailability [30]. The ameliorative effect of astaxanthin was again recently demonstrated on endothelial dysfunction in streptozotocin-induced diabetes in rats where it inhibited the ox-LDL-LOX-1-eNOS pathway. This indicates that treatment with astaxanthin might be clinically useful for diabetic complications associated with endothelial dysfunction [65].

Conversely, Jacobsson *et al.* [66] evaluated the influence of alpha-tocopherol and astaxanthin on LD Loxidation lag time and atherosclerotic lesion formation in Watanabe heritable hyperlipidemic (WHHL) rabbits. They concluded that alpha-tocopherol but not astaxanthin prolonged the LDL oxidation lag time. The two antioxidative substances did not prevent atherogenesis in WHHL rabbits in this setting.

One notable recent study demonstrates that a dietary mix of fish oil, resveratrol, lycopene, catechin, α-tocopherol, and vitamin C, which was shown to be well tolerated in humans, improves lipid and inflammatory risk factors for CVD in a model of transgenic mice [67]. These findings support the concept of combination strategies with several bioactive nutrients and a systems-based, multi-target approach for complex multifactorial diseases, such as type 2 diabetes.

## Epidemiological Studies

Several studies, over the past decades, have appeared in support of the carotenoids role in the prevention of CVD, mostly based on epidemiological studies showing a relationship between carotenoid plasma levels and CVD.

Recently, by analyzing sixty-two studies of plasma carotenoids in relation to health outcomes, a carotenoid health index has been proposed with risk categories as follows: very high risk: <1 μM, high risk: 1–1.5 μM, moderate risk: 1.5–2.5 μM, low risk: 2.5–4 μM, and very low risk: >4 μM [68]. Notably, over 95 percent of the USA population falls into the moderate or high risk category of the carotenoid health index [69].

The Physicians Health Study [70] found that coronary artery disease was less prevalent in men who ate vegetables rich in carotenoids. Moreover, recent studies, such as the coronary artery risk development in young adults (CARDIA) and the young adult longitudinal trends in antioxidants (YALTA) studies [22], have found that high plasma carotenoid concentrations are associated with reduced inflammation, oxidative stress, and endothelial dysfunction, three important characteristics of atherosclerosis, while the a lower incidence of atherosclerosis in individuals with higher plasma levels of β-carotene and α-carotene [71].

Recently, 139 Cretan (Greek) men aged 79 years and over were compared to men from Zutphen (The Netherlands). The Cretan men had approximately fourfold higher mean levels of lycopene as well as a lower level of oxidative stress and higher levels of antioxidants in plasma than men of the same age from Zutphen [72].

Early observational studies reported an association between a high dietary intake of β-carotene and reduced incidence of CVD [73,74].

In a case-control study, the risk of nonfatal acute myocardial infarction (MI) in women was inversely associated with daily intake of β-carotene-containing diet [75]. In the Rotterdam study [76], a population-based cohort study targeting the elderly, the daily intake of β-carotene through diet was inversely associated with the risk of MI.

Interestingly, in the American Health Professional's Study [77] conducted on 39,910 US males, the carotene intake was associated with a lower risk of Coronary heart disease (CHD) among current smokers but not nonsmokers.

Findings from a more recent study in 1031 Finnish men, found a more convincing correlation between serum β-carotene and reduction in risk of MI [78]. The same authors also suggested that dietary carotenoids might improve the content of *in vivo* oxidative modified LDL [79] and lately they showed that low serum β-carotene concentrations were associated to enhanced risk of sudden cardiac death (SCD) in middle-aged Finnish men [80].

Several other epidemiological studies showed an association between serum carotenoids and reduced risk of atherosclerosis [81,82]. In addition, recent findings showed that Mediterranean diet, in different age ranges, ameliorate endothelial function and multiple risk factors, including a better cardiovascular risk profile, reduced oxidative stress and modulation of inflammation [83-85].

However, evidence regarding the health benefits of carotenoids is controversial. In fact, in previous study [86] LDL oxidation was not reduced by β-carotene while in the same study vitamin E was found to be beneficial. Moreover, the Cochranre review [87] on antioxidant supplements and all-cause mortality found β-carotene and vitamin A to significantly increase all-cause mortality. Furthermore Studies of balanced carotenoid combinations are necessary for confirmation.

Likewise, recently, the effects of serum carotenoids and their interactions on mortality have been examined in a representative sample of US adults [88]. Interestingly, low α-carotene was the only carotenoid associated with CVD mortality. Very low serum total carotenoid, α-carotene, and lycopene concentrations may be risk factors for mortality, but carotenoids show interaction effects on mortality. Thus, studies of balanced carotenoid combinations are necessary for confirmation.

As regard the lycopene, several years ago the EURAMIC study [18] already suggested that this carotenoids may contribute to the protective effect of vegetable consumption on myocardial infarction risk. Subsequently, De Waart *et al.* [89] suggested that serum levels of individual carotenoids, particularly the oxygenated species, are inversely associated with all-cause mortality.

Recently, epidemiological data concerning lycopene and its potential cardiovascular health benefits have been extensively reviewed [4,90]. These promising epidemiological studies have prompted a number of small interventional studies, which have generally shown beneficial effects of lycopene on a range of outcome measures related to cardiovascular disease.

Based on these findings, it is clear that controversial

epidemiological data exist regarding the CVD-preventive effects of carotenoids.

## Human Intervention Studies

Human intervention studies, especially when conducted as a randomized, double-blind, and placebo controlled design, are still considered the "gold standard" in nutritional sciences for testing health effects of dietary compounds.

Recently, Yang *et al.* [91] reviewed the major publications relating to the potential effects on cardiovascular risk factors and outcomes of some common dietary constituents: carotenoids, flavonoid-rich cocoa, tea, red wine and grapes, coffee, omega-3 fatty acids, and garlic. Increased intake of some of these has been associated with reduced all-cause mortality or reduced incidence of myocardial infarction, stroke, and hypertension. However, although the evidence from observational studies, *in vitro* studies and animal studies showed linkages between carotenoids and prevention of cardiovascular disease and is supportive of beneficial effects for most of these foodstuffs taken as part of the diet, the potential benefits of using supplements derived from these natural products remain largely inconclusive.

The fact is that although many epidemiological studies have reported an association between β-carotene and the risk of CVD, several large randomized trials failed to reveal any reduction in CVD with β-carotene consumption. For instance, the MRC/BHF Heart Protection study [92], the α-tocopherol and β-carotene (ATBC) study [93] and the Women's Antioxidant Cardiovascular Study (WACS) [94] have all revealed the absence of beneficial effects due to the consumption of β-carotene.

Moreover, the prospective evaluation of the relation between vegetable intake and CHD risk in the Physicians' Health Study [95] concluded that the consumption of vegetables rich in carotenoids was associated with a reduced risk of CHD, but after 12 years of follow-up there was no impact from supplementation of β-carotene on CVD, cancer, or overall mortality among primarily non-smokers [96].

As described for β-carotene, less convincing results and a more complex landscape emerge when data from interventional studies on lycopene use in CVD patients are analyzed. There are multiple conflicting reports on how lycopene administration affects the progression of CVD and its outcomes [20,97]. However, there is a certain degree of reproducibility in scientific reports describing the reduction of cholesterol (LDL and total), upregulation of HDL [98], decrease in carotid artery intima-media thickness [99], and lowering of both plasma markers of oxidative damage [97] and postprandial oxidative stress [100] in patients treated with lycopene.

A recent review of the controlled clinical studies with lycopene in well-defined subject populations found no definite evidence for CVD prevention [101].

However, more recently a randomized controlled double-blind trial that analyzed the effect of oral lycopene supplementation on vascular function in patients with CVD showed an improvement in endothelial function in CVD patients but not in control healthy subjects [21].Beneficial effects of lycopene supplementation on oxidative stress and markers of endothelial function were also found in another randomized, placebo-controlled, double-blind study [102]. This study specially focused on middle-aged Korean men; hence, the results cannot be generalized to women. However, the data obtained demonstrated the anti-oxidative and anti-inflammatory effects of lycopene [102].

Unfortunately, no positive outcome emerges from studies on the effect of lycopene from cooked tomatoes or tomato-based drink on serum and plasma antioxidant enzymes, lipid peroxidation rate, lipid profile, markers of inflammation, immunomodulation, and oxidative stress [103-105].

A meta-analysis, employing human intervention trials between 1955 and September 2010, investigated the effect of lycopene on blood lipids and on blood pressure [106]. Ried and Fakler's meta-analyses [106] indicated that 25 mg daily of lycopene effectively reduced total cholesterol and LDL cholesterol in serum. Regarding the potential role of lycopene in the regulation of blood pressure, the clinical trials published to date are too low in number to offer any firm evidence of this. Although they do suggest lycopene has a lowering effect on systolic blood pressure, in particular in hypertensive subjects, further studies are necessary to prove these results.

Astaxanthin, a xanthophyll carotenoid, is a nutrient with a powerful antioxidative properties and with excellent safety and tolerability. This molecule demonstrated an important role in protection against oxidation and inflammation [107]. In fact, it showed to have the highest antioxidant activity toward peroxyl radicals among lutein, lycopene, α -carotene, β -carotene, α -tocopherol, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [108].

The real breakthrough with this nutrient, however, is that it produces clinically significant antioxidant benefits in human subjects, including groups especially vulnerable to oxidative stress, such as smokers, the obese, and the overweight.

In a Korean prospective, randomized, double-blind study [109], as taxanth in supplementation (3 weeks) improved oxidative stress biomarkers by suppressing lipid peroxidation and stimulating the activity of the antioxidant defense system in individuals with weight challenges. Another double-blind, randomized controlled trial was conducted by the same group [110]. The results suggest that astaxanthin supplementation might prevent oxidative damage in smokers by suppressing lipid peroxidation and stimulating the activity of the antioxidant system in smokers.

In the Park double-blind, randomized controlled trial [111], astaxanthin also significantly lowered C-reactive protein, a biomarker of systemic inflammation [112].

In addition, astaxanthinelevated HDL-cholesterol, lowered triglycerides in subjects with moderately elevated serum triglycerides compared to healthy subject in a double-blind randomized controlled trial [113].

Astaxanthin also significantly increased blood adiponectin levels a well-known hormone, which is produced by adipose tissue, cardiac and skeletal muscle, and vessel endothelia. Serum levels of adiponectin tend to be reduced in obese and/or diabetic

subjects, smokers, patients with coronary heart disease, and individuals with metabolic syndrome [114]. Although the results of this study suggest a normalization of adiponectin levels, 12 weeks of supplementation had no effect on BMI.

To date, the safety, bioavailability and effects of astaxanthin on oxidative stress and inflammation that have relevance to the pathophysiology of atherosclerotic CVD have been assessed only in a small number of clinical studies [115]. Hence, based on the cardiovascular studies described above, more cardiovascular clinical trials are recommended to better understand the role of carotenoids in the prevention of CVD.

## CONCLUSION

Increasing proofs indicates that a correctredox balance may be implicated in preserving health and longevity. Changing this equilibrium in favor of oxidation may cause pathological responses leading to functional disorders and disease. Despite the contradictions, promising epidemiological studies on nutrition, associating high levels of carotenoids with low levels of oxidative stress establishes that higher fruit and vegetable intakes tend to be associated with lower rates of heart and vascular diseases, including coronary heart disease and stroke.

However, to date, the biological role of carotenoids in the prevention and possibly in the treatment of cardiac and vascular diseases is only partially understood.

Thus, it is only through further studies that our knowledge of the crucial role played by carotenoids will improve and enable us to develop complementary approaches to the prevention, cure and management of cardiovascular diseases.

## REFERENCES

1. García-Fernández E, Rico-Cabanas L, Rosgaard N, Estruch R, Bach-Faig A. Mediterranean diet and cardiodiabesity: a review. Nutrients. 2014; 6: 3474-3500.
2. Johnson EJ. The role of carotenoids in human health. Nutr Clin Care. 2002; 5: 56-65.
3. Voutilainen S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. Am J Clin Nutr. 2006; 83: 1265-1271.
4. Böhm V. Lycopene and heart health. Mo Nutr Food Res. 2012; 56: 296-303.
5. Krinsky NI. Actions of carotenoids in biological systems. Annu Rev Nutr. 1993; 13: 561-587.
6. Crews H, Alink G, Andersen R, Braesco V, Holst B, Maiani G, Ovesen L . A critical assessment of some biomarker approaches linked with dietary intake. Br J Nutr. 2001; 86: 5-35.
7. G. Maiani, M. J. Caston, G. Catasta, E. Toti, I. G. Cambrodon, A. Bysted, et al. Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Mol Nutr Food Res. 2009; 53: 194-218.
8. G. Britton, S. Liaaen.Jensen and H. E. Pfander. Carotenoids-Handbook. Birkhauser Verlag, Basel. 2004.
9. Khoo HE, Prasad KN, Kong KW, Jiang Y, Ismail A . Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules. 2011; 16: 1710-1738.
10. Willcox BJ, Curb JD, Rodriguez BL. Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. Am J Cardiol. 2008; 101: 75D-86D.
11. E. Rodrigues, L. R. Mariutti and A. Z. Mercadante. Scavenging capacity of marine carotenoids against reactive oxygen and nitrogen species in a membrane-mimicking system. Mar Drugs. 2012; 10: 1784-1798.
12. Palozza P, Krinsky NI . Antioxidant effects of carotenoids in vivo and in vitro: an overview. Methods Enzymol. 1992; 213: 403-420.
13. Amengual J, Lobo GP, Golczak M, Li HN, Klimova T, Hoppel CL, Wyss A . A mitochondrial enzyme degrades carotenoids and protects against oxidative stress. FASEB J. 2011; 25: 948-959.
14. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr. 1995; 62: 1315-1321.
15. Burton GW, Ingold KU. Beta-Carotene: an unusual type of lipid antioxidant. Science. 1984; 224: 569-573.
16. Palozza P. Prooxidant actions of carotenoids in biologic systems. Nutr Rev. 1998; 56: 257-265.
17. Rao AV, Rao LG. Carotenoids and human health. Pharmacol Res. 2007; 55: 207-216.
18. Kohlmeier L, Kark JD, Gomez-Gracia E, Martin BC, Steck SE, Kardinaal AF, Ringstad J . Lycopene and myocardial infarction risk in the EURAMIC Study. Am J Epidemiol. 1997; 146: 618-626.
19. B. Buijsse, E. J. Feskens, D. Schlettwein-Gsell, M. Ferry, F. J. Kok, D. Kromhout and L. C. de Groot. Plasma carotene and alpha-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA). Am J Clin Nutr. 2005; 82: 879-886.
20. Ciccone MM, Cortese F, Gesualdo M, Carbonara S, Zito A, Ricci G, De Pascalis F. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediators Inflamm. 2013; 2013: 782137.
21. P. R. Gajendragadkar, A. Hubsch, K. M. Maki-Petaja, M. Serg, I. B. Wilkinson and J. Cheriyan. Effects of oral lycopene supplementation on vascular function in patients with cardiovascular disease and healthy volunteers: a randomised controlled trial. PLoS One. 2014; 9: 99070.
22. A. Hozawa, D. R. Jacobs, Jr., M. W. Steffes, M. D. Gross, L. M. Steffen and D. H. Lee. 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: 447-455.
23. S. P. Fortmann, E. Ford, M. H. Criqui, A. R. Folsom, T. B. Harris, Y. Hong, et al. CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: report from the population science discussion group. Circulation. 2004; 110: 554-559.
24. Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, Meyer J . Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation. 2001; 104: 1336-1342.
25. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med. 2002; 252: 283-294.
26. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nat Med. 2002; 8: 1211-1217.
27. van Herpen-Broekmans WM, Klöpping-Ketelaars IA, Bots ML, Kluft C, Princen H, Hendriks HF, Tijburg LB. Serum carotenoids and vitamins in relation to markers of endothelial function and inflammation. Eur J Epidemiol. 2004; 19: 915-921.

28. P. Di Tomo, R. Canali, D. Ciavardelli, S. Di Silvestre, A. De Marco, A. Giardinelli, et al. beta-Carotene and lycopene affect endothelial response to TNF-alpha reducing nitro-oxidative stress and interaction with monocytes. Mol Nutr Food Res. 2012; 56: 217-227.

29. Lee DK, Grantham RN, Mannion JD, Trachte AL. Carotenoids enhance phosphorylation of Akt and suppress tissue factor activity in human endothelial cells. J Nutr Biochem. 2006; 17: 780-786.

30. J. Monroy-Ruiz, M. A. Sevilla, R. Carron and M. J. Montero. Astaxanthin-enriched-diet reduces blood pressure and improves cardiovascular parameters in spontaneously hypertensive rats. Pharmacol Res. 2011; 63: 44-50.

31. T. W. George, E. Paterson, S. Waroonphan, M. H. Gordon and J. A. Lovegrove. Effects of chronic consumption of fruit and vegetable puree-based drinks on vasodilation, plasma oxidative stability and antioxidant status. J Hum Nutr Diet. 2012; 25: 477-487.

32. Carlsen MH, Karlsen A, Lillegaard IT, Gran JM, Drevon CA, Blomhoff R, Andersen LF . Relative validity of fruit and vegetable intake estimated from an FFQ, using carotenoid and flavonoid biomarkers and the method of triads. Br J Nutr. 2011; 105: 1530-1538.

33. Agarwal M, Parameswari RP, Vasanthi HR, Das DK. Dynamic action of carotenoids in cardioprotection and maintenance of cardiac health. Molecules. 2012; 17: 4755-4769.

34. Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress--implications of cellular signaling pathways and relation to chronic disease prevention. Nutr Res. 2014; 34: 907-929.

35. Hung CF, Huang TF, Chen BH, Shieh JM, Wu PH, Wu WB. Lycopene inhibits TNF-alpha-induced endothelial ICAM-1 expression and monocyte-endothelial adhesion. Eur J Pharmacol. 2008; 586: 275-282.

36. Bae JW, Bae JS. Barrier protective effects of lycopene in human endothelial cells. Inflamm Res. 2011; 60: 751-758.

37. Goszcz K, Deakin SJ, Duthie GG, Stewart D, Leslie SJ, Megson IL2, . Antioxidants in Cardiovascular Therapy: Panacea or False Hope? Front Cardiovasc Med. 2015; 2: 29.

38. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000; 87: 840-844.

39. L. C. Sung, H. H. Chao, C. H. Chen, J. C. Tsai, J. C. Liu, H. J. Hong, et al. Lycopene inhibits cyclic strain-induced endothelin-1 expression through the suppression of reactive oxygen species generation and induction of heme oxygenase-1 in human umbilical vein endothelial cells. Clin Exp Pharmacol Physiol. 2015; 42: 632-639.

40. Tang X, Yang X, Peng Y, Lin J. Protective effects of lycopene against H2O2-induced oxidative injury and apoptosis in human endothelial cells. Cardiovasc Drugs Ther. 2009; 23: 439-448.

41. J. V. Rossoni-Junior, G. R. Araujo, C. Padua Bda, M. M. Chaves, M. L. Pedrosa, M. E. Silva et al. Annato extract and beta-carotene modulate the production of reactive oxygen species/nitric oxide in neutrophils from diabetic rats. J Clin Biochem Nutr. 2012; 50: 177-183.

42. S. K. Bai, S. J. Lee, H. J. Na, K. S. Ha, J. A. Han, H. Lee, et al. beta-Carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-kappaB activation. Exp Mol Med. 2005; 37: 323-334.

43. Kidd P. Astaxanthin, cell membrane nutrient with diverse clinical benefits and anti-aging potential. Altern Med Rev. 2011; 16: 355-364.

44. Y. Kishimoto, M. Tani, H. Uto-Kondo, M. Iizuka, E. Saita, H. Sone, et al. Astaxanthin suppresses scavenger receptor expression and matrix metalloproteinase activity in macrophages. Eur J Nutr. 2010; 49: 119-126.

45. Palozza P, Parrone N, Simone RE, Catalano A. Lycopene in atherosclerosis prevention: an integrated scheme of the potential mechanisms of action from cell culture studies. Arch Biochem Biophys. 2010; 504: 26-33.

46. P. Palozza, R. Simone, A. Catalano, G. Monego, A. Barini, M. C. Mele, et al. Lycopene prevention of oxysterol-induced proinflammatory cytokine cascade in human macrophages: inhibition of NF-kappaB nuclear binding and increase in PPARgamma expression. J Nutr Biochem. 2011; 22: 259-268.

47. Palozza P, Simone R, Catalano A, Parrone N, Monego G, Ranelletti FO. Lycopene regulation of cholesterol synthesis and efflux in human macrophages. J Nutr Biochem. 2011; 22: 971-978.

48. P. Palozza, R. Simone, A. Catalano, A. Boninsegna, V. Bohm, K. Frohlich, et al. Lycopene prevents 7-ketocholesterol-induced oxidative stress, cell cycle arrest and apoptosis in human macrophages. J Nutr Biochem. 2010; 21: 34-46.

49. H. M. Lo, C. F. Hung, Y. L. Tseng, B. H. Chen, J. S. Jian and W. B. Wu. Lycopene binds PDGF-BB and inhibits PDGF-BB-induced intracellular signaling transduction pathway in rat smooth muscle cells. Biochem Pharmacol. 2007; 74: 54-63.

50. Kleinegris MC, Ten Cate-Hoek AJ, Ten Cate H. Coagulation and the vessel wall in thrombosis and atherosclerosis. Pol Arch Med Wewn. 2012; 122: 557-566.

51. Hsiao G, Wang Y, Tzu NH, Fong TH, Shen MY, Lin KH, Chou DS. Inhibitory effects of lycopene on in vitro platelet activation and in vivo prevention of thrombus formation. J Lab Clin Med. 2005; 146: 216-226.

52. Biehler E, Mayer F, Hoffmann L, Krause E, Bohn T . Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. J Food Sci. 2010; 75: 55-61.

53. Heber D, Lu QY. Overview of mechanisms of action of lycopene. Exp Biol Med (Maywood). 2002; 227: 920-923.

54. Bansal P, Gupta SK, Ojha SK, Nandave M, Mittal R, Kumari S, Arya DS . Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury. Mol Cell Biochem. 2006; 289: 1-9.

55. Breinholt V, Lauridsen ST, Daneshvar B, Jakobsen J. Dose-response effects of lycopene on selected drug-metabolizing and antioxidant enzymes in the rat. Cancer Lett. 2000; 154: 201-210.

56. Liu X, Qu D, He F, Lu Q, Wang J, Cai D, . Effect of lycopene on the vascular endothelial function and expression of inflammatory agents in hyperhomocysteinemic rats. Asia Pac J Clin Nutr. 2007; 16: 244-248.

57. J. Zhu, C. G. Wang and Y. G. Xu. Lycopene attenuates endothelial dysfunction in streptozotocin-induced diabetic rats by reducing oxidative stress. Pharm Biol. 2011; 49: 1144-1149.

58. M. Verghese, J. E. Richardson, J. Boateng, L. A. Shackelford, C. Howard, L.T. Walker et al. Dietary lycopene has a protective effect on cardiovascular disease in New Zealand male rabbits. J. Biol. Sci. 2008; 8: 268-277.

59. Hu MY, Li YL, Jiang CH, Liu ZQ, Qu SL, Huang YM, . Comparison of lycopene and fluvastatin effects on atherosclerosis induced by a high-fat diet in rabbits. Nutrition. 2008; 24: 1030-1038.

60. H. Frederiksen, S. E. Rasmussen, M. Schroder, A. Bysted, J. Jakobsen, H. Frandsen, et al. Dietary supplementation with an extract of lycopene-rich tomatoes does not reduce atherosclerosis in Watanabe Heritable Hyperlipidemic rabbits. Br J Nutr. 2007; 97: 6-10.

61. Lorenz M, Fechner M, Kalkowski J, Fröhlich K, Trautmann A, Böhm V, Liebisch G . Effects of lycopene on the initial state of atherosclerosis in New Zealand White (NZW) rabbits. PLoS One. 2012; 7: 30808.

62. Gitenay D, Lyan B, Rambeau M, Mazur A, Rock E. Comparison of lycopene and tomato effects on biomarkers of oxidative stress in vitamin E deficient rats. Eur J Nutr. 2007; 46: 468-475.

63. Hsu YM, Lai CH, Chang CY, Fan CT, Chen CT, Wu CH, . Characterizing the lipid-lowering effects and antioxidant mechanisms of tomato paste. Biosci Biotechnol Biochem. 2008; 72: 677-685.

64. S. K. Khan, T. Malinski, R. P. Mason, R. Kubant, R. F. Jacob, K. Fujioka, et al. Novel astaxanthin prodrug (CDX-085) attenuates thrombosis in a mouse model. Thromb Res. 2010; 126: 299-305.

65. Zhao ZW, Cai W, Lin YL, Lin QF, Jiang Q, Lin Z, Chen LL . Ameliorative effect of astaxanthin on endothelial dysfunction in streptozotocin-induced diabetes in male rats. Arzneimittelforschung. 2011; 61: 239-246.

66. Jacobsson LS, Yuan XM, Ziedén B, Olsson AG . Effects of alpha-tocopherol and astaxanthin on LDL oxidation and atherosclerosis in WHHL rabbits. Atherosclerosis. 2004; 173: 231-237.

67. L. Verschuren, P. Y. Wielinga, W. van Duyvenvoorde, S. Tijani, K. Toet, B. van Ommen, et al. A dietary mixture containing fish oil, resveratrol, lycopene, catechins, and vitamins E and C reduces atherosclerosis in transgenic mice. J Nutr. 2011; 141: 863-869.

68. Donaldson MS. A carotenoid health index based on plasma carotenoids and health outcomes. Nutrients. 2011; 3: 1003-1022.

69. Centers for Disease Control and Prevention (CDC). National Report on Biochemical Indicators of Diet and Nutrition—Vitamins A and E and Carotenoids; CDC: Atlanta, GA, USA. . 2008;

70. Liu S, Lee IM, Ajani U, Cole SR, Buring JE, Manson JE; Physicians' Health Study. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The Physicians' Health Study. Int J Epidemiol. 2001; 30: 130-135.

71. D'Odorico A, Martines D, Kiechl S, Egger G, Oberhollenzer F, Bonvicini P, Sturniolo GC . High plasma levels of alpha- and beta-carotene are associated with a lower risk of atherosclerosis: results from the Bruneck study. Atherosclerosis. 2000; 153: 231-239.

72. B. Buijsse, E. J. Feskens, J. Moschandreas, E. H. Jansen, D. R. Jacobs Jr, Kafatos A, et al. Oxidative stress, and iron and antioxidant status in elderly men: differences between the Mediterranean south (Crete) and northern Europe (Zutphen). Eur J Cardiovasc Prev Rehabil. 2007; 14: 495-500.

73. Gey KF, Puska P. Plasma vitamins E and A inversely correlated to mortality from ischemic heart disease in cross-cultural epidemiology. Ann N Y Acad Sci. 1989; 570: 268-282.

74. Kardinaal AF, Kok FJ, Ringstad J, Gomez-Aracena J, Mazaev VP, Kohlmeier L, Martin BC . Antioxidants in adipose tissue and risk of myocardial infarction: the EURAMIC Study. Lancet. 1993; 342: 1379-1384.

75. Tavani A, Negri E, D'Avanzo B, La Vecchia C. Beta-carotene intake and risk of nonfatal acute myocardial infarction in women. Eur J Epidemiol. 1997; 13: 631-637.

76. K. Klipstein-Grobusch, J. M. Geleijnse, J. H. den Breeijen, H. Boeing, A. Hofman, D. E. Grobbee, et al. Dietary antioxidants and risk of myocardial infarction in the elderly: the Rotterdam Study. Am J Clin Nutr. 1999; 69: 261-266.

77. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC, . Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med. 1993; 328: 1450-1456.

78. J. Karppi, J. A. Laukkanen, T. H. Makikallio, K. Ronkainen and S. Kurl. Low beta-carotene concentrations increase the risk of cardiovascular disease mortality among Finnish men with risk factors. Nutr Metab Cardiovasc Dis. 2012; 22: 921-928.

79. Karppi J, Nurmi T, Kurl S, Rissanen TH, Nyyssönen K. Lycopene, lutein and beta-carotene as determinants of LDL conjugated dienes in serum. Atherosclerosis. 2010; 209: 565-572.

80. Karppi J, Laukkanen JA, Mäkikallio TH, Ronkainen K, Kurl S. Serum Î²-carotene and the risk of sudden cardiac death in men: a population-based follow-up study. Atherosclerosis. 2013; 226: 172-177.

81. Xu XR, Zou ZY, Huang YM, Xiao X, Ma L, Lin XM, . Serum carotenoids in relation to risk factors for development of atherosclerosis. Clin Biochem. 2012; 45: 1357-1361.

82. J. H. Dwyer, M. J. Paul-Labrador, J. Fan, A. M. Shircore, C. N. Merz and K. M. Dwyer. Progression of carotid intima-media thickness and plasma antioxidants: the Los Angeles Atherosclerosis Study. Arterioscler Thromb Vasc Biol. 2004; 24: 313-319.

83. C. Marin, R. Ramirez, J. Delgado-Lista, E. M. Yubero-Serrano, P. Perez-Martinez, et al. Mediterranean diet reduces endothelial damage and improves the regenerative capacity of endothelium. Am J Clin Nutr. 2011; 93: 267-274.

84. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, Hong CP . Fruit and vegetable consumption and its relation to markers of inflammation and oxidative stress in adolescents. J Am Diet Assoc. 2009; 109: 414-421.

85. E. Azzini, A. Polito, A. Fumagalli, F. Intorre, E. Venneria, A. Durazzo, et al. Mediterranean Diet Effect: an Italian picture. Nutr J. 2011; 10: 125.

86. P. D. Reaven, A. Khouw, W. F. Beltz, S. Parthasarathy and J. L. Witztum. Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin E but not by beta-carotene. Arterioscler Thromb. 1993; 13: 590-600.

87. G. Bjelakovic, D. Nikolova, L. L. Gluud, R. G. Simonetti and C. Gluud. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev. 2012; 3: CD007176.

88. M. D. Shardell, D. E. Alley, G. E. Hicks, S. S. El-Kamary, R. R. Miller, R. D. Semba et al. Low-serum carotenoid concentrations and carotenoid interactions predict mortality in US adults: the Third National Health and Nutrition Examination Survey. Nutr Res. 2011; 31: 178-189.

89. De Waart FG, Schouten EG, Stalenhoef AF, Kok FJ. Serum carotenoids, alpha-tocopherol and mortality risk in a prospective study among Dutch elderly. Int J Epidemiol. 2001; 30: 136-143.

90. Petyaev IM. Lycopene Deficiency in Ageing and Cardiovascular Disease. Oxid Med Cell Longev. 2016; 2016: 3218605.

91. Yang Y, Chan SW, Hu M, Walden R, Tomlinson B . Effects of some common food constituents on cardiovascular disease. ISRN Cardiol. 2011; 2011: 397136.

92. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet. 2002; 360: 23-33.

93. J. M. Rapola, J. Virtamo, S. Ripatti, J. K. Huttunen, D. Albanes, P. R. Taylor et al. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet. 1997; 349: 1715-1720.

94. N. R. Cook, C. M. Albert, J. M. Gaziano, E. Zaharris, J. MacFadyen, E. Danielson, et al. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events

in women: results from the Women's Antioxidant Cardiovascular Study. Arch Intern Med. 2007; 167: 1610-1618.

95. Liu S, Lee IM, Ajani U, Cole SR, Buring JE, Manson JE; Physicians' Health Study. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The Physicians' Health Study. Int J Epidemiol. 2001; 30: 130-135.

96. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C . Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med. 1996; 334: 1145-1149.

97. J. Chen, Y. Song and L. Zhang. Effect of lycopene supplementation on oxidative stress: an exploratory systematic review and meta-analysis of randomized controlled trials. J Med Food. 2013; 16: 361-374.

98. Palozza P, Catalano A, Simone RE, Mele MC, Cittadini A . Effect of lycopene and tomato products on cholesterol metabolism. Ann Nutr Metab. 2012; 61: 126-134.

99. Z. Y. Zou, X. R. Xu, X. M. Lin, H. B. Zhang, X. Xiao, L. Ouyang, et al. Effects of lutein and lycopene on carotid intima-media thickness in Chinese subjects with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial. Br J Nutr. 2014; 111: 474-480.

100. B. Burton-Freeman, J. Talbot, E. Park, S. Krishnankutty and I. Edirisinghe. Protective activity of processed tomato products on postprandial oxidation and inflammation: a clinical trial in healthy weight men and women. Mol Nutr Food Res. 2012; 56: 622-631.

101. Riccioni G, Mancini B, Di Ilio E, Bucciarelli T, D'Orazio N. Protective effect of lycopene in cardiovascular disease. Eur Rev Med Pharmacol Sci. 2008; 12: 183-190.

102. Kim JY, Paik JK, Kim OY, Park HW, Lee JH, Jang Y, Lee JH . Effects of lycopene supplementation on oxidative stress and markers of endothelial function in healthy men. Atherosclerosis. 2011; 215: 189-195.

103. Bose KS, Agrawal BK. Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipid profile in coronary heart disease. Singapore Med J. 2007; 48: 415-420.

104. Riso P, Visioli F, Grande S, Guarnieri S, Gardana C, Simonetti P, Porrini M . Effect of a tomato-based drink on markers of inflammation, immunomodulation, and oxidative stress. J Agric Food Chem. 2006; 54: 2563-2566.

105. V. Stangl, C. Kuhn, S. Hentschel, N. Jochmann, C. Jacob, V. Bohm, et al. Lack of effects of tomato products on endothelial function in human subjects: results of a randomised, placebo-controlled cross-over study. Br J Nutr. 2011; 105: 263-267.

106. Ried K, Fakler P. Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. Maturitas. 2011; 68: 299-310.

107. Guerin M, Huntley ME, Olaizola M. Haematococcus astaxanthin: applications for human health and nutrition. Trends Biotechnol. 2003; 21: 210-216.

108. Naguib YM. Antioxidant activities of astaxanthin and related carotenoids. J Agric Food Chem. 2000; 48: 1150-1154.

109. Choi HD, Kim JH, Chang MJ, Kyu-Youn Y, Shin WG . Effects of astaxanthin on oxidative stress in overweight and obese adults. Phytother Res. 2011; 25: 1813-1818.

110. Kim JH, Chang MJ, Choi HD, Youn YK, Kim JT, Oh JM, Shin WG. Protective effects of Haematococcus astaxanthin on oxidative stress in healthy smokers. J Med Food. 2011; 14: 1469-1475.

111. Park JS, Chyun JH, Kim YK, Line LL, Chew BP. Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr Metab (Lond). 2010; 7: 18.

112. Pandolfi A. C-reactive protein: a potential new molecular link between inflammation, thrombosis and vascular cell proliferation? Cardiovasc Res. 2005; 68: 3-4.

113. 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.

114. Kajikawa Y, Ikeda M, Takemoto S, Tomoda J, Ohmaru N, Kusachi S. Association of circulating levels of leptin and adiponectin with metabolic syndrome and coronary heart disease in patients with various coronary risk factors. Int Heart J. 2011; 52: 17-22.

115. Fassett RG, Coombes JS. Astaxanthin: a potential therapeutic agent in cardiovascular disease. Mar Drugs. 2011; 9: 447-465.

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