Clinical evidence on the effects of antioxidant vitamins against bio-indicators associated with the development of cancer and atherosclerosis

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Abstract

Various oxygen species (such as hydroxyl-OH, peroxyl-ROO radicals), formed in human tissue cells by many endogenous and exogenous causes, cause extensive oxidative damage which according to a large body of scientific evidence links to initiation and development of aging, cardiovascular and other human diseases. This review paper first provides an overview of free radicals as initiators of oxidative stress with focus on LDL and DNA oxidative changes that are respectively associated with atherosclerosis and carcinogenesis. Based on common bio-indicators used to monitor LDL and DNA harmful oxidative processes, the paper provides a literature overview of the protective role that certain vitamins can exert (individually or in mixtures) against the health diseases relevant to oxidative stress.

Abbreviations: DNA-DeoxyriboNucleic Acid, 4-HNE (4-hydroxyynonalen), LDL (low-density lipoproteins), MDA (Malondialdehyde), 8-OH-dG (8-hydroxy-2’-deoxyguanosine), PUFAs (polyunsaturated fatty acids), ROS (Reactive oxygen species), TBARS (thiobarbituric acid reacting substances).

Free radicals and their health detrimental effects

Reactive oxygen species

Reactive oxygen species (ROS) in vivo possess important roles in living organisms through their beneficial and detrimental effects [1,2]. Free radicals are formed in tissue cells by many endogenous and exogenous causes [3]. They are produced either (i) from normal cell metabolisms in situ (normal aerobic respiration i.e. mitochondria, stimulated polymorphulated leucocytes and macrophages) [4] or (ii) from external sources (pollution, cigarette smoke, radiation, medication) [5]. Oxygen free radicals (such as hydroxyl radicals; superoxide radicals and other active oxygen species such as singlet oxygen) adversely alter lipids, proteins, and DNA and trigger a number of human diseases [6]. A role of lipid peroxidation and oxidative stress in the association between thyroid diseases and breast cancer has been claimed by Dominguez and Castelao (2008) [7]. The ability of ROS to structurally modify cellular components, gene expression and protein production has led to the implication of their involvement in a variety of health diseases [8]. More specifically, ROS generate DNA oxidative damage and adversely affect biological membranes (e.g. LDL) of which pathological consequence, including cancer and cardiovascular diseases are well established [9]. Several of the most common in vivo ROS are shown in (Table 1), together with their major sources and target of damage [10].

LDL oxidative modification and atherosclerosis

Over the last few years, extensive clinical and epidemiologic research evidence has been gathered on the role of oxidized LDL (low-density lipoproteins) in the progression of atherosclerosis, as a risk factor for the development of coronary artery disease [11,12]. Atherosclerosis is a progressive disease of the arterial tree that involves deposition of lipid, mostly oxidized LDL, in the arterial intima leading finally to a thickening of the arterial wall and reduced luminal blood flow [13]. Oxidative modification of LDL, a lipid peroxidation reaction driven by free radicals is therefore a key step in the early stages of atherosclerosis [14,15]. The relationship between circulating ox-LDL and subclinical atherosclerosis has been recently explored and confirmed in one case control study performed by Fang, et al. (2011) [16] and two community-based cohort studies [17,18].

DNA oxidative damage and carcinogenesis

The development of cancer is a multistep process that involves a complex series of cellular and molecular changes mediated by a diversity of endogenous and exogenous stimuli [19]. It has recently become apparent that ROS generation from mitochondria first as the cellular response to oxidative stress and DNA damage is closely linked to carcinogenesis [20]. A body of research has in depth investigated into mechanisms of oxidative DNA damage trying to clarify how the components of the repair pathways may influence the cancer transformation. [21,22]. DNA damage can take many forms, ranging from specifically oxidised purine and pyrimidine bases (more than

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20 such oxidative lesions have been identified) to gross DNA changes such as strand breaks, sister chromatid exchange, and the formation of micronuclei [23]. According to one of the proposed oxidative mechanisms, hydrogen peroxide can cause DNA strand breakage, by generation of the hydroxyl radical (OH) close to the DNA molecule, via the Fenton reaction.

$$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH} + \text{OH} + \text{Fe}^{3+}$$

This may result in DNA instability, mutagenesis and ultimately carcinogenesis [24]. Specific DNA oxidation products accumulate depending on the ROS involved, its rate of production, and the cell's ability to protect or repair its DNA insult. Research efforts are intense to further elucidate DNA base excision repair, the primary mechanism to protect cells from genotoxicity caused by ROS [25].

### Most commonly used biomarkers of oxidative stress

A number of oxidative biomarkers have linked oxidative stress and the development of health diseases. An overview of the most commonly used is given below:

#### Estimation of plasma levels of oxidized LDL via formation of conjugated dienes (c.d)

The formation of conjugated dienes is generally accepted as evidence of lipid peroxidation and is due to re-arrangement of the double bonds which in presence of oxygen can form hydroperoxides [26]. A convenient and very frequently used method for monitoring the level of plasma oxidized LDL is the process of copper-induced LDL oxidation continuously through the spectrophotometric measurement of diene absorption at 233 nm [27]. The chronology of LDL oxidation by Cu$^{2+}$ ions can be divided into three consecutive time phases: lag phase, propagation phase and decomposition phase [28]. During the lag phase, LDL becomes progressively depleted of its endogenous antioxidants, with α-tocopherol as the first to be lost and β-carotene as the last to remain. Depleted of its antioxidants the LDL particle enters the propagation phase in which the polyunsaturated fatty acids (PUFAs) are rapidly converted to conjugated lipid hydroperoxides indicated by the increase in absorbance at 233 nm [29]. Secondary reactions of LDL oxidation leading to aldehydes (malondialdehyde, hexanal, 4-hydroxynonenal etc.), are accelerated by transition metal ions, such as Fe$^{2+}$, which may catalyse decomposition of lipid hydroperoxides to alkoxyl radicals in a Fenton-type reaction [30]:

$$\text{LOOH} + \text{Fe}^{2+} \rightarrow \text{LO} + \text{OH} + \text{Fe}^{3+}$$

### Lipid hydroperoxides

Lipid hydroperoxides are the primary products of lipid peroxidation and can be measured in several different ways [10,37]:

- (i) by HPLC coupled with chemiluminescence. This method is very sensitive and interference by biological antioxidants is avoided.
- (ii) by GC-MS after reduction to alcohols
- (iii) by the iodometric method, which is a sensitive method involving the reaction of hydroperoxides with iodide in acid to form iodine as shown below:

$$\text{ROOH} + 2\text{H} + 2\text{I}^- \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{I}_2$$

- (iv) by the FOX (Ferric oxidation of Xylenol). This a highly reproducible method for biological samples, based on the fact that hydroperoxides oxidise ferrous ion (Fe$^{2+}$) to ferric ion (Fe$^{3+}$) that can be detected by use of ferric sensitive dyes [38].

### Measurement of thiobarbituric acid reacting substances (TBARS)

The TBARS test is still the most frequently used test to assess secondary products of lipid peroxidation [39]. Among these substances, malondialdehyde (MDA), which is formed in vivo from trienes via

<table>
<thead>
<tr>
<th>Radical</th>
<th>Formation</th>
<th>Target or type of damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl (OH)</td>
<td>Production from O$_2$ or H$_2$O$_2$ in presence of transition metals</td>
<td>Damage to DNA and proteins</td>
</tr>
<tr>
<td>Superoxide (O$_2^-$)</td>
<td>Produced enzymatically by e$^-$ addition to O$_2$ by SOD, or non-enzymatically from H$_2$O$_2$</td>
<td>Attack biological membranes, sugars (oxidative damage)</td>
</tr>
<tr>
<td>Peroxyl or alkony (ROO, RO)</td>
<td>Formation through the breakdown of organic peroxides</td>
<td>Lipid peroxidation reaction and initiation of atherosclerosis</td>
</tr>
<tr>
<td>Singlet oxygen (O$_2$)</td>
<td>Photochemical activation of O$_2^-$ in presence of sensitizers</td>
<td>Lipid photodioxidative damage skin-damage, carcinogenesis</td>
</tr>
<tr>
<td>Nitric oxide (NO$^+$)</td>
<td>In vivo production from L-arginine</td>
<td>Pathogenetic in overproduction</td>
</tr>
</tbody>
</table>

**Table 1. Range of the most common *in vivo* reactive oxygen species (Kiokias 2002) [10]**

<table>
<thead>
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unsaturated peroxides, reflects unsaturated fatty acid composition as much as extent of lipid peroxidation [40]. MDA reacts with TBA reagent (in a 1:2 molar ratio) on heating under acidic conditions to give a red chromophore, which is measured by UV at 532 nm or by fluorescence at 553 nm [41]. TBARS test is easy to perform and inexpensive. However, it lacks specificity as chromogens are formed with many aldehydes other than MDA, and with carbohydrates, amino acids etc [41].

**Determination of Isoprostanes**

Isoprostanes are prostaglandine (PG) like compounds that are produced independently of the cyclooxygenase enzyme by free radical catalysed-peroxidation of arachidonic acid, and similar products are also formed during oxidation of EPA and DHA [9]. A substantial body of evidence indicates that measurement of F2–Isop levels (esterified in human tissues) provides a direct and reliable approach to assess oxidative damage in vivo compared with other methods [TBARS].

According to Barocas, et al. (2011) [42] the oxidative stress measured by urine F2-isoprostane level is strongly associated with prostate cancer.

**Other bio-indicators**

During the last decade, the development of immunochemical detection of HNE-histidine cytotoxic adducts (4-hydroxynonenal (4-HNE)), has opened more advanced methodological possibilities for qualitative and quantitative detection of lipid peroxidation in various human and animal tissues [43]. In addition, short chain hydrocarbon gases, e.g. ethane and pentane, are produced in vivo by thermal or ion catalysed decomposition of lipid hydroperoxides. Measurement of these exhaled gases by GC has therefore been largely applied to assess lipid peroxidation [44].

**Protective effects of vitamins against oxidative stress**

**Biological antioxidants**

The term biological antioxidants refer to compounds that protect biological systems against the potentially harmful effect of reactions that cause extensive oxidation [45]. They can act at several different stages in an oxidative sequence by:

- Removing oxygen or decreasing local \( \text{O}_2 \) concentrations
- Removing catalytic metal ions or reactive oxygen species such as \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \)
- Scavenging initiating radicals such as \( \text{OH}^- \), \( \text{RO}^- \), \( \text{RO}_2^- \)

Broadly, we distinguish between two types of biological antioxidants:

(a) **Endogenous (intracellular) antioxidants**: Oxygen metabolism occurs within cells where a variety of enzymes and proteins are acting specifically to remove oxygen intermediates. Such substances include catalase, selenium dependent glutathione peroxidase, copper and zinc-dependent superoxide dismutase, uric acid, and the transition metal-binding proteins, such as transferrin and caeruloplasmin [9]. These compounds are called endogenous antioxidants and offer protection at several different levels within the cells for example by preventing radical formation, repairing oxidative damage and increasing elimination of damaged molecules [38].

(b) **Dietary antioxidants / focus of the current analysis**: In addition to the endogenous antioxidants, nature has offered a wide range of nutritional compounds with strong antioxidant activities. Among these certain fat- or water-soluble vitamins can act as radical scavengers in model biological systems and in the human organism [46]. This paper focuses on selected compounds with vitamin or provitamin A activity given that tissues deficient in such nutrients may be prone to harmful peroxidation reactions and more specifically:

(i) **Vitamin E**: It comprises a category of eight monophenolic compounds (known as Tocopherols and tocotrienols) with strong reported antioxidant activities in food and biological systems, mainly acting as chain breaking antioxidants that inactivate free radicals via their hydrogen donating character [10]

(ii) **Vitamin C**: A widespread vitamin in nature -in many fruits and vegetables- well known as antioxidant with multi-functional effects (incl. metal chelating properties) that can act synergistically with chain breaking antioxidants (e.g. tocopherols, flavonoids) resulting into synergistic effects [9]

(iii) **Provitamin A**: (a, \( \beta \)-carotenes and \( \beta \)-cryptoxanthin): 40-carbon terpenoids widely available in nature with well-known scavenging activities against free radicals that are trapped in their conjugated structure. [5].

(iv) Since the above-mentioned antioxidants are exogenous in nature, their levels can be manipulated by supplements and dietary modifications Natural occurrence, chemical structure and mechanism of antioxidant action of the above dietary antioxidants have been detailed by various researchers including Vance, et al. 2013 [47] and Kiokias, et al. 2009 [48]. Arvanitoyannis, et al. (2009) [49] reviewed the various available methods for the determination of chain-breaking antioxidant activity in food and biological systems. The total peroxyl radical trapping parameter (TRAP) and the oxygen radical absorbance capacity assay (ORAC) have served as the most commonly used methods of antioxidant capacity in food and biological systems during the last two decades [50,51]. Section 3 provides more details about the in vivo activity of antioxidant vitamins against LDL and DNA oxidative damage.
Human studies on the effect of natural antioxidant vitamins against LDL damage/atherosclerosis

(a) Effect of vitamins as individual compounds against LDL oxidation: A large number of studies have examined the effects of antioxidant vitamins either individually or in combination, on ex vivo LDL oxidation with most information being available for the effects of provitamins A, vitamins C and vitamin E [38].

Provitamin A carotenoids. Major non-provitamin A carotenoids (lycopene, lutein, and zeaxanthin) and provitamin A compounds carotenoids (Figure 2) have different biological activities and efficacy, depending on their food content, dietary intake, bioavailability, and bioconversion [52]. Epidemiologic studies have shown that diets rich in provitamin A-containing foods are associated with a decreased risk of conducting cardiovascular problems and other pathologic conditions [53]. The disease-preventing activity of β-carotene and other provitamin A carotenoids could be ascribed either to their conversion into retinoid or to their activity as intact molecules. The results of several human intervention studies, however, indicate that a high-dose supplementation with β-carotene, not only does not significantly decrease the risk for development for atherosclerosis, but could even be harmful to smokers or former asbestos workers [54]. Thus, it may be that β-carotene and other carotenoids (e.g. astaxanthin) promote health when supplemented at physiologic amounts in foods but could even present adverse prooxidant harmful activities when given in high doses and under highly oxidative conditions [44].

Vitamin C. Ascorbic acid is a water-soluble antioxidant, and as such is expected to be removed from LDL during isolation. In an earlier study, Wen, et al. (1997) [55] did not find any significant effect of dietary supplementation with vitamin C on LDL oxidation in smoking volunteers. On the contrary other researchers have reported beneficial effects of dietary supplementation with 1000 mg vitamin C for 4 weeks [56] or 500 mg/d for 2 months [57] against LDL oxidative deterioration. In addition, McRae (2008) [58] noted that Vitamin C supplementation reduced serum LDL and triglycerides levels.

Vitamin E: Hodis, et al. (2012) [59] reported that α-tocopherol supplementation (400 IU/day) significantly raised plasma vitamin E levels (P < 0.0001) whereas reduced circulating oxidised-LDL (P = 0.03) and LDL oxidative susceptibility (P < 0.01). Studies that included vitamin E supplementation generally support a significant protection of LDL usually at doses higher than 400 IU/day [60]. As noted by Rizvi, et al. (2014) [61] Vitamin E is the major lipid-soluble component in the cell antioxidant defense system with numerous important roles within the body because of its antioxidant activity. However, it has also been suggested that when accompanying antioxidants such as ubiquinone and vitamin C are not available (to react and quench the vitamin E radicals) a prooxidant tocopherol effect could be induced [44]. Actually Nikiforuk (2010) [62] noted that recent human clinical trials with vitamin E have not yielded positive results against LDL oxidative deterioration on the contrary to in vitro experiments.

(b) Effect of vitamins’ antioxidant mixtures against LDL oxidation

A growing body of human clinical investigation has focused into combination of various antioxidants in order to explore the potential of interactions because of varying modes and mechanisms of antioxidant action [9]. Interestingly, a few studies that were designed to supplement subjects with mixtures of antioxidant vitamins in generally observed an enhanced beneficial effect on oxidative stability of LDL, presumably resulting from a synergistic action between the vitamins [44,54]. Cocate, et al. (2015) [63] have recently conducted a cross-sectional study and observed that the total daily carotenoid intake of provitamin A carotenoids (β-cryptoxanthin, β and α-carotene lycopene) mixedtured with xanthophylls (lutein plus zeaxanthin,) was inversely associated (p<0.05) with the plasma oxidised-LDL concentrations. Kiokias and Gordon (2003) [27] supplemented for 3 weeks 30 healthy volunteers with a carotenoid mixture (palm oil carotenes, lycopine, paprika, lutein, bixin in a total amount of 30 mg active carotenoid /day) and reported an increased resistance of LDL to oxidation, compared with placebo (monitored by CD at 233 nm).

Boushehri, et al. (2012) [64] examined the effect antioxidant vitamins on serum oxidized LDL levels in male subjects with cardiovascular disease risk factors. They reported that a diet enriched with a combination of vitamin C (500 mg), vitamin E (400 IU), β-carotene (15 mg), was strongly associated with lower serum oxidized LDL levels. Similarly, in an earlier study [65] Nyssonen, et al. (1994). a daily supplementation for 2 months with a mixture of vitamin E (200 mg) together with vitamin C (400 mg) and β-carotene (20 mg) decreased significantly the susceptibility of LDL to oxidative deterioration.

Human studies on the effects of antioxidant vitamins against DNA damage/carcinogenesis

(a) Effect of vitamins as individual compounds: Steady state estimates of cellular DNA oxidation, in general have provided support for a beneficial role of antioxidant vitamins in DNA protection [66].

Provitamin A compounds: Lorenzo, et al. (2009) [67] have investigated the biological properties of beta-cryptoxanthin, in cell culture model systems, using the comet assay to measure DNA damage. They reported that at low concentrations, close to those found in plasma, beta-cryptoxanthin does not itself cause damage, but rather protects transformed human cells from damage induced by H(2)O(2) or by visible light in the presence of a photosensitizer.

Astley, et al. (2004) [68] supplemented healthy male volunteers with lutein, beta-carotene or lycopene (natural isolate capsules, 15 mg/d, 4 weeks) and observed that both beta carotene and non-provitamin A carotenoids exerted an antioxidant protection by scavenging DNA-damaging free radicals and modulation of DNA repair mechanisms. On the other side, Collins and Azqueta and (2012) [34] stated that studying reports from the last 5 years, revealed a clear distinction between effects of pro-vitamin A carotenoids (carotenens and β-cryptoxanthin) and the effects of non-vitamin A carotenoids (lycopene, lutein, astaxanthin and zeaxanthin). Whereas the compounds of the latter group are almost invariably reported to protect against DNA damage, the provitamin A carotenoids show a more varied spectrum of effects, sometimes protected and sometimes enhancing DNA damage.

Vitamin E: Makpol, et al. (2010) [69] observed that alphatocopherol protected against H(2)O(2)-induced DNA damage and...
this modulation was affected by donor age. Ragin, et al. (2010) [70] conducted a human trial showing that an intake of food rich in α-tocopherol could decrease levels of DNA oxidative adducts. Asgard (2014) [71] reported a significant decrease of catechol-induced (1 mM) general DNA damage in the presence of 20 μM of α-tocopherol. On the contrary, Barcelos, et al. (2011) [72] supported that dietary supplementation with α-tocopherol can even induce DNA oxidative stress.

**Vitamin C:** A protective effect of vitamin C supplementation in human with plasma levels>50μmol/l was observed by Sram, et al. (2012) [73] in terms of 8-oxodG levels. Similarly, in an earlier study, Kadirov, et al. (2007) [74] reported that supplementation with ascorbic acid significantly prevents the arsenic-induced protein oxidation and DNA damage in rats. More recently, Kontek, et al. (2013) [75] noted that vitamin C (in a concentration range 10-100 μm) caused a clear protecting effect against DNA damage. More specifically, they concluded that vitamin C modulates DNA damage induced by hydrogen peroxide in human colorectal adenocarcinoma cell lines (HT29), estimated by COMET assay in vitro (decrease ~ 30%). Asgard (2014) [71] reported that high plasma levels of ascorbate reduced the levels of oxidative DNA damage (8-oxo-dG) in mononuclear white blood cells. Overall, Konopacka (2004) [76] highlighted that data concerning the influence of vitamin C on oxidative DNA damage are conflicting and some of the discrepancies can be explained by the different experimental methodologies employed.

**(b) Effect of vitamins in antioxidant mixtures:** In an earlier study, Sweetman, et al. (1997) [77] had examined the effect of antioxidant vitamin supplementation on DNA damage and repair in human lymphoblastoid cells. After 24-hour supplementation period with a mixture ascorbic acid + α-tocopherol, (60 microM in total) the level of endogenous DNA damage was significantly lower than in the nonsupplemented culture, as assessed by the comet assay. In addition, a human clinical trial [78] by Duthie, et al. (2015) [63] conducted a human trial with the participation of 296 apparently healthy middle-aged men to assess the potential relationships of carotenoid intake with lipid and oxidative stress markers.

In conclusion, the total daily carotenoid intake based on five investigated carotenoid types (β-cryptoxanthin, lycopene, lutein plus zeaxanthin, β-carotene and α-carotene) was inversely associated with the production of urinary 8-OH-dG as oxidative stress biomarker (p<0.05). On the contrary, Asgard (2014) [72] reported that supplementation of 47 type-2 diabetes subjects for 12 weeks with 16 capsules/day (mixture of β-carotene and α-tocopherol) did not exert any inhibitory effect against DNA oxidative stress.

An overview on the human trial that investigated into antioxidant effects of vitamins combinations against LDL and/or DNA oxidative changes is given in (Table 2).

**Conclusion/future work in this scientific field**

The review of the earlier indicated studies on oxidative stress and effect of antioxidant vitamins has led to the following conclusions in the frame of the current analysis:

(a) Relatively low levels of LDL enrichment in provitamin A (<30 mg/day) can exert a better protective effect against oxidation of LDL ex vivo than higher doses of carotenoid supplements (60-100 mg of carotenoids/day) that fail to present any activity. An

### Table 2. Selection of human clinical trials designed to examine the effect of dietary supplementation with vitamins mixtures against LDL and/or DNA oxidative changes (current analysis).

<table>
<thead>
<tr>
<th>Authors/studies (listed in date order)</th>
<th>Condition of Human Clinical trials</th>
<th>Protective (or not) effect of the vitamin mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Effect against LDL oxidation</td>
</tr>
<tr>
<td><strong>Duthie, et al. (1997)</strong> [78]</td>
<td>Supplementation of smokers and non-smokers with an antioxidant mixture (vitamin C-100 mg +vitamin E-280 mg +and β-carotene-25 mg per day)</td>
<td></td>
</tr>
<tr>
<td><strong>Kioskias &amp; Gordon (2003)</strong> [27]</td>
<td>30 healthy volunteers were supplemented for 3 weeks with a mixture of 30 mg active carotenoid/day containing palm oil carotenes, lycopene, paprika, lutein, bixin.</td>
<td>A reduction of <em>ex vivo</em> LDL oxidative modification (monitored by conjugated dienes at 233 nm)</td>
</tr>
<tr>
<td><strong>Astley, et al. (2004)</strong> [68]</td>
<td>Healthy males were supplemented with 15 mg/d of lutein, β-carotene or lycopene (natural isolate capsules) for 4 weeks (3 independent clinical trials)</td>
<td></td>
</tr>
<tr>
<td><strong>Boushehrir, et al. (2012)</strong> [64]</td>
<td>Male subjects followed a diet enriched with a combination of vitamin C (500 mg), vitamin E (400 IU), β-carotene (15 mg).</td>
<td>The antioxidant treatment resulted into significantly lower serum oxidized LDL levels.</td>
</tr>
<tr>
<td><strong>Asgard (2014)</strong> [72]</td>
<td>47 type-2 diabetes subjects supplemented for 12 weeks with 16 capsules/day (mixture of β-carotene + α-tocopherol)</td>
<td></td>
</tr>
<tr>
<td><strong>Cocate, et al. (2015)</strong> [63]</td>
<td>296 healthy middle-aged subjects were supplemented with a carotenoid mixture (β-cryptoxanthin, lycopene, lutein plus zeaxanthin, β-carotene and α-carotene).</td>
<td>The carotenoid supplementation resulted into reduced plasma oxidised-LDL concentrations (p&lt;0.05)</td>
</tr>
</tbody>
</table>
explanation for this is that a dietary intervention resulting in high carotenoid enrichments may have excessively loaded LDL particles with carotene autooxidation metabolites that can generate reactive oxygen species (leading thereby to increased LDL oxidative susceptibility rather than to any protection effect).

(b) A recent body of clinical research evidence has concluded that dietary combination of vitamins (e.g. vitamin E and vitamin C) can be more effective against oxidative damage of either LDL or DNA than the supplementation of each individual vitamin. Such an enhanced effect of vitamins mixtures may relate to the different mode of activities of the individual compounds thereby allowing a synergistic effect when combined in the diet. In particular for carotenoids, a better antioxidant effect against oxidative damage has been reported when provitamin A compounds (mainly hydrophobic α- and β-carotene) are supplemented together with preparations of more polar xanthophylls (e.g. lutein or paprika) in recent human clinical studies.

(c) The association between plasma oxidized LDL and DNA oxidative adducts with the eventual risk of developing a cardiovascular disease or cancer respectively are not completely elucidated yet. Further investigation is required in this field to obtain more recent and valid clinical intervention data further to the existing epidemiological data. The development of optimal nutritional and future health strategies would certainly be facilitated by further investigation into the clinical effects of combined vitamins dietary supplementation against oxidative pathological conditions.

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