Original Communication

Inverse Correlation Between Plasma β-Carotene and Interleukin-6 in Patients with Advanced Coronary Artery Disease

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Abstract: The interrelationships between plasma β-carotene, α-tocopherol, and the level of systemic inflammation and oxidative stress were investigated in patients with advanced coronary artery disease (CAD). Plasma β-carotene, α-tocopherol, malondialdehyde, free radicals, interleukin-6, high sensitive C-reactive protein levels, and other risk factors of CAD were determined in a group of patients with advanced CAD [significant stenosis according to coronarographic examination (n = 91) and a control group of examined patients with coronary arteries with no stenosis (n = 49)]. Between-group differences in continuous variables were analyzed with the Hotelling T^2-test (software NCSS2000), analyses of correlation matrix with the software STATISTICA. Advanced CAD coincided with significantly lower plasma concentrations of high-density lipoprotein (HDL)-cholesterol and β-carotene as well as with elevated levels of all inflammatory markers, but only with mild increase of oxidative stress. β-Carotene significantly inversely correlated with interleukin-6. This inverse correlation could suggest potential protective effect of β-carotene on atherosclerosis due to the inhibition of inflammatory processes.

Key words: β-carotene, interleukin-6, coronary artery disease, inflammation; oxidative stress.

Introduction

As oxidative stress and inflammation have been suggested to play a central role in the pathogenesis of coronary heart disease, the role of various antioxidants in protection against atherosclerosis has been extensively studied. Inverse correlation between low intake of vegetables and fruits and/or low antioxidant concentrations in plasma and risk of cardiovascular disease was reported in several epidemiological studies [1, 2]. Recently, a Dutch study [3] found inverse correlation between α- and β-carotene intake (but not of tocopherols and vitamin C) and 15-year cardiovascular mortality in Dutch elderly men. Association between high plasma levels of α- and β-carotene and lower risk of atherosclerosis was also described in a study by Bruneck [4].

Atherosclerosis and cardiovascular disease (CVD) are known to be associated with inflammation, oxidative stress, and endothelial dysfunction. The role of β-carotene as a chain-breaking antioxidant in lipid peroxidation has been described [5]. Recently, attention has been paid also to additional β-carotene effects.

The study presented was designed to investigate the association between plasma concentrations of β-carotene and α-tocopherol, the level of systemic inflammation, and oxidative stress in patients with and without advanced coronary artery disease (CAD). Recently, some authors have investigated these relations, focusing on the general population. Il'yasova *et al.* [6] extended these studies by analysis of the situation in patients with increased risk of CVD. A novelty of the presented study is the evaluation of the relations between β-carotene and α-tocopherol and the level of inflammatory and/or oxidative stress markers in patients with clearly proven, advanced CAD and those without coronary atherosclerosis.

Subjects and methods

Study subjects

This cross-sectional study involved 140 patients diagnosed with non-acute coronary angiography for chest pain, aged 45–69 years, fulfilling the following criteria: (1) clinically significant coronary stenosis without subsequent percutaneous coronary intervention treatment (PCI) [coronary stenosis of >50 % of the left main coronary artery or >70 % of the epicardial coronary arteries without PCI treatment because of indication for the coronary artery bypass graft (CABG) or conservative treatment as a result of contraindications to PCI or CABG]; (2) stenosis + PCI [stenosis of the same grade with subsequent PCI]; (3) patients without any stenosis [symptoms imitating CAD with normal angiographic finding and no stenosis (patients with musculoskeletal pain, arrhythmia, dilated cardiomyopathy, hypertension)]. All the participants underwent angiography, completed a questionnaire, and provided their blood samples. Patients who had any serious health complications and patients with high-sensitivity C-reactive protein (hsCRP) >10 mg/L were excluded. None of the studied subjects suffered from renal, hepatic, or oncologic disease. No participant was taking vitamin supplements. A written informed consent was obtained from all the participants before starting the protocol, and the study was approved by the Hospital Ethical Committee on Human Research.

Blood samples

Venous blood was obtained under standard conditions, from 7 to 8 a.m. after fasting for at least 12 hours the day after coronarographic examination. For the determination of β-carotene, α-tocopherol, and malondialdehyde concentrations, blood was collected in tubes with EDTA covered with aluminum foil to avoid carotenoid oxidation and polymerization by oxygen and light. Plasma was obtained by centrifugation of the blood samples at 1500 × g for 20 minutes and immediately stored at –80 °C in 1.5-mL amber polypropylene tubes.

Determination of α-tocopherol and β-carotene in human plasma by HPLC with UV/VIS detection

α-Tocopherol and β-carotene were analyzed by high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan). The plasma samples or standards (0.5 mL) were placed in 15-mL disposable amber polypropylene test tubes and spiked with α-tocopherol acetate (internal standard). After incubation for 30 minutes at 4 °C, 0.5 mL ethanol was added and the tubes were vortex-mixed for 10 seconds. A 1.5-mL aliquot of n-hexane was added and the tubes were vortex-mixed for 10 minutes and centrifuged at 1600 × g for 10 minutes. The n-hexane layer was carefully transferred to a disposable amber glass test tube. Fat-soluble vitamins were extracted three times and the combined extracts were evaporated to dryness under nitrogen. The residue was dissolved in 0.2 mL

mobile phase, and the samples were transferred into 0.2-mL cramped amber vials.

The chromatography of fat-soluble vitamins was performed using isocratic elution on a LiChroCART 125 × 4 mm i.d., Purospher STAR RP-18e, 5-μm analytical column fitted with LiChroCART 4 × 4 mm i.d., Purospher STAR RP-18e, 5-μm guard column (Merck KgaA, Darmstadt, Germany) at 40 °C. The mobile phase consisted of 25 % ethanol in methanol (v/v). The flow rate was 0.7 mL/minute. The eluate was monitored photometrically with an ultraviolet/visible (UV/VIS) detector (292 nm for α-tocopherol and α-tocopherol acetate, 450 nm for β-carotene).

Determination of malondialdehyde in human plasma by HPLC with UV/VIS detection

Total malondialdehyde (MDA) in human plasma was measured as MDA-thiobarbituric acid complex using HPLC (Shimadzu). The 1,1,3,3-tetramethoxypropan (TMP) was used as a standard. MDA-thiobarbituric acid complex was extracted with n-butanol as an antioxidant. For HPLC separation, a reverse-phase column MAC 4 × 250 mm, Biospher SI 120 PSI C_{18}, particle size 7 μm; (Labio, Prague, Czech Republic) was used. The mixture of methanol and 8.3 mmol/L phosphate buffer, pH 7.2 (35:65, v/v) was used as a mobile phase. The MDA was detected at 532 nm. Retention time of MDA-thiobarbituric acid complex was 4.9 ± 0.1 minute at the flow rate of 0.7 mL/minute [7].

Total amount of free radicals in serum

Total amount of free radicals in serum was measured by direct spectrophotometric method based on the ability of chlorophyllin (semi-synthetic sodium/copper derivative of chlorophyll) to scavenge free electrons with simultaneous shift of absorbance maximum (kit Free Radicals, Sevapharma, Prague, Czech Republic) [8]. This method is suitable for measurement of the total amount all of types of free radicals, both reactive oxygen and reactive nitrogen species [9]. The method was validated by comparison with the basic method of electron paramagnetic resonance [8] with excellent correlation (r = 0.997).

Classical biochemical parameters

The fasting plasma levels of glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), high sensitivity C-reactive protein (hsCRP), albumin, transferrin, ceruloplasmin, and uric acid were determined by standard procedures using an automatic biochemistry analyzer Dimension (Dade-Behring, Deerfield, IL, USA); interleukin-6 was determined by means of an immunochemistry analyzer Immulite (DPC, Los Angeles, CA, USA); fibrinogen by an immunochemistry analyzer BN Prospec (Dade-Behring, Deerfield, IL, USA).

Clinical examination

Blood pressure and physical data (weight, height, waist circumference) were determined during the complete clinical examination. The detailed questionnaire concerning dietary habits and lifestyle was filled out by each of the patients in the study. The body mass index (BMI) was calculated as weight (kg) divided by height (m^2). All the patients underwent coronary angiography at a high-volume catheterization laboratory using standard percutaneous techniques; each angiogram was reviewed by two cardiologists.

Statistical analysis

Between-group differences in continuous variables were analyzed with the use of the Hotelling T^2-test for independent groups using the software NCSS2000 (Dr. Jerry L. Hintze, Kaysville, Utah, USA). Analyses of Correlation Matrix were carried out using the software STATISTICA (Statsoft, Tulsa, OK, USA).

Results

The patients were grouped according to the results of angiographic examination and subsequent treatment into 3 groups. The first group consisted of patients with substantial stenosis of their coronary arteries without PCI treatment, the second one included patients with stenosis of the same grade with subsequent PCI. The third evaluated group consisted of patients with CAD-like symptoms, but with normal angiographic finding without stenosis. Blood samples were collected the day after coronarographic examination. Mean values (± S.D.) of selected vascular risk factors are given in Table I, and suitable representative values (geometric, re-transformed, or arithmetic means) of oxidative stress and inflammation markers are given in Table II.

No significant differences were found between the first two groups of patients with coronary stenosis, except of higher occurrence of diabetes mellitus in the first group. These findings indicate that PCI has no significant influence on either oxidative stress or inflammation. Therefore, we evaluated both groups together as one group of patients with coronary stenosis.

In patients with stenosis of coronary arteries (S group), significantly more males and higher diabetes mellitus occurrence were found in comparison with the group of patients with no stenosis (N group). The S group exhibited significantly lower levels of HDL-C and higher levels of inflammatory markers IL-6, high-sensitivity CRP, and fibrinogen. Markers of oxidative stress were elevated only mildly in comparison with the N group; the increase did not reach statistical significance. Levels of both β-carotene and α-tocopherol were markedly below the recommended plasma concentrations in all groups (recommended levels: β-carotene >0.4 μmol/L, α-tocopherol >30 μmol/L) [10]; especially low levels of β-carotene were seen in both stenosis groups. Advanced coronary atherosclerosis has been found to be associated with higher systemic inflammation and lower levels of β-carotene and HDL-C.

The data were subjected to multidimensional statistical analysis, which was based on the set of Principal Component Analysis loadings plots. The levels of inflammatory markers IL-6, high-sensitivity CRP, and fibrinogen correlated with each other in the S group. Correlation between inflammatory and oxidative stress markers did not reach statistical significance. IL-6 exhibited significant inverse correlation with the level of β-carotene (Pearson correlation –0.29, Table III). There was no notable correlation between plasma levels of α-tocopherol, or α-tocopherol/cholesterol ratio and plasma IL-6, or other inflammatory markers in the S group. As to oxidative stress, we did not

Table I: Group characteristics (PCI, percutaneous coronary intervention).

	Coronary stenosis without PCI (1; n = 48)	1 vs. 2	Coronary stenosis with PCI (2; n = 43)	2 vs. 3	No stenosis (3; n = 49)	1 vs. 3
Age (y)	61.00 (5.68)	–	59.00 (5.79)	–	57.50 (6.06)	–
Male/Female (%)	80/20	–	78/22	*	46/54	*
BMI (kg/m^2)	30.70 (5.54)	–	30.10 (4.11)	–	30.40 (4.52)	–
Waist circumference (cm)	105.00 (17.25)	–	104.00 (12.65)	–	103.00 (11.26)	–
Smoking (%)	24	–	24	–	22	–
Hypertension (%)	68	–	72	–	67	–
Diabetes mellitus (%)	44	*	22	*	14	**
Systolic blood pressure (mm Hg)	152.50 (25.09)	–	160.00 (20.23)	–	155.00 (19.20)	–
Diastolic blood pressure (mm Hg)	80.00 (14.05)	–	90.00 (8.94)	–	90.00 (11.50)	–
Glucose (mmol/L)	6.50 (2.89)	–	6.35 (2.38)	–	5.80 (0.90)	*
Total cholesterol (mmol/L)	4.79 (1.28)	–	4.72 (1.00)	–	5.20 (1.01)	–
HDL-cholesterol (mmol/L)	1.01 (0.29)	–	1.04 (0.22)	**	1.20 (0.25)	*
LDL-cholesterol (mmol/L)	2.66 (1.12)	–	2.81 (0.92)	*	3.52 (0.91)	–
Triglycerides (mmol/L)	1.65 (0.85)	–	1.81 (0.86)	–	1.61 (1.03)	–
Fibrinogen (g/L)	4.03 (0.94)	–	3.61 (0.65)	–	3.44 (0.63)	*
Albumin (g/L)	40.00 (3.56)	–	40.00 (3.29)	–	41.00 (2.30)	–
Transferrin (g/L)	2.63 (0.38)	–	2.60 (0.29)	–	2.46 (0.35)	–
Ceruloplasmin (g/L)	0.24 (0.05)	–	0.22 (0.04)	–	0.22 (0.04)	–
Uric acid (μmol/L)	347.00 (83.77)	–	328.50 (80.14)	–	327.00 (92.29)	–
Hemoglobin (g/L)	154.00 (17.10)	–	152.00 (12.26)	–	147.00 (26.58)	–

Results are expressed as the mean value with the estimated standard deviation (S.D.), the statistical significance of a difference: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table II: Inflammatory and oxidative stress markers in studied groups.

	Coronary stenosis without PCI (1; n = 48)	Coronary stenosis with PCI (2; n = 43)	(1+2) vs 3	No stenosis (3; n = 49)
High-sensitivity CRP (mg/L)	4.55 ± 0.99	3.44 ± 0.79	*	2.95 ± 0.53
IL-6 (ng/L)	4.42 ± 1.00	4.83 ± 0.88	*	2.99 ± 0.65
β-Carotene (μmol/L)	0.063 ± 0.012	0.061 ± 0.011	*	0.112 ± 0.020
α-Tocopherol (μmol/L)	22.44 ± 2.54	21.57 ± 1.39	–	22.87 ± 1.68
α-Tocopherol/ cholesterol+triglycerides	3.25 ± 0.20	3.18 ± 0.16	–	3.18 ± 0.15
Malondialdehyde (μmol/L)	3.10 ± 0.19	2.98 ± 0.15	–	2.96 ± 0.14
Free radicals (mmol/L)	3.75 ± 0.28	3.58 ± 0.25	–	3.53 ± 0.24

Due to the logarithmic distribution, the results are expressed as the point and interval estimate of the geometric mean (IL-6, α-Tocopherol, α-Tocopherol/cholesterol+triglycerides) or the re-transformed mean (β-Carotene and hsCRP), and the arithmetic mean for normal distribution (Malondialdehyde, Free radicals), statistical significance of a difference between means of both groups: * $p < 0.05$.

Table III: Correlation matrix of plasma β-carotene and α-tocopherol with selected variables.

Variables	Stenosis group		No stenosis group	
	β-carotene	α-tocopherol	β-carotene	α-tocopherol
IL-6	–0.29*	–0.03	–0.01	–0.25
hsCRP	–0.12	–0.00	–0.17	–0.19
Fibrinogen	–0.09	0.04	0.27	–0.15
Albumin	0.05	0.14	0.12	0.13
Transferrin	0.09	0.30*	0.04	0.13
Ceruloplasmin	0.15	–0.00	–0.24	0.20
Free radicals	–0.15	0.12	–0.08	0.09
Malondialdehyde	–0.06	0.28*	–0.35*	–0.12
Total cholesterol	0.34*	0.55*	0.07	0.74*
Triglycerides	–0.05	0.55*	–0.38*	0.67*
HDL-C	–0.07	–0.08	0.11	0.11
LDL-C	0.29*	0.57*	0.15	0.63*
Glucose	0.02	0.11	0.03	–0.22
BMI	–0.18	–0.06	–0.08	–0.01
Age	0.16	–0.03	0.07	–0.14

The star * signifies that the correlation coefficient is significant at the level $p < 0.05$.

find any negative correlation between β-carotene or α-tocopherol and free radicals, as well as MDA, in the S group. Surprisingly, a positive correlation between α-tocopherol and MDA was observed in the S group. The reason might be that α-tocopherol occurs in the blood bound to lipoproteins; thus in case of increased amount of lipids, a higher amount of α-tocopherol can be retained in the organism. Simultaneously, the elevated lipids may be associated with stimulation of lipid peroxidation, evaluated via its product, MDA. α-Tocopherol could act as an antioxidant, but its protective capacity may not be sufficient to counteract the elevated radicals in a higher inflammatory state. The plasma α-tocopherol levels correlated with the

total cholesterol, LDL-C, and triglycerides, while the plasma β-carotene correlated only with the total cholesterol and LDL-C, as anticipated from the lipophilic character of these compounds. Other statistically significant correlations in the S group are mentioned below (the whole correlation matrix is not shown). Levels of MDA were significantly related to plasma levels of glucose and triglycerides. Furthermore, the S group showed a negative correlation of all reactants of the acute inflammatory phase with albumin and transferrin.

In the group of patients with symptoms of CAD but normal angiographic findings, with no stenosis of coronary arteries (the N group), neither of these significant correlations was detected except the correlation of α-tocopherol with the total cholesterol, LDL-C, and triglycerides plasma concentrations, as well as correlation of fibrinogen with high-sensitivity CRP and IL-6. In the N group we found a small significant negative correlation between β-carotene and MDA and triglyceride levels (Table III) and positive correlation between MDA and smoking status.

Discussion

In our study we compared several markers of oxidative stress, inflammation, and well-established risk factors of atherosclerosis in patients with advanced coronary stenosis and a group of patients with symptoms imitating CAD with normal angiographic findings, without stenosis.

Significant elevation of concentrations of all studied inflammatory markers (IL-6, hsCRP, fibrinogen) was found in the group of patients with coronary stenosis (S). This finding reflects the association of atherosclerosis with low-grade systemic inflammation [11]. Contrary to expectations, elevation of plasma oxidative stress markers in the S group did not reach statistical significance in comparison with the N group. This might be due to the fact that the N group consisted of patients with musculoskeletal pain, arrhythmia, dilated cardiomyopathy, and hypertension, which are also associated with moderate elevation of oxidative stress. As oxidative stress has been reported to induce inflammation pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins [12, 13], higher oxidative stress should correlate with the presence of inflammation [14]. Oxidative stress and inflammation have been reported to create a self-perpetuating cycle of oxidation leading to inflammation and further oxidation, but our findings indicate a prevailing impact of inflammation in the progression of atherogenesis.

Our results revealed a significant correlation of CAD occurrence and low β-carotene levels. β-Carotene is one of the most abundant carotenoids in human plasma. Its role in both physiological and pathological processes has been widely discussed. Many of the biological actions of carotenoids have been attributed to their antioxidant properties, given by the antioxidant capacity of the carotenoid molecule *per se*, as well as by their potential effect on intracellular redox status. At high concentrations, however, carotenoids may have pro-oxidant effects [15]. Other functions of carotenoids include enhancement of gap junctional communication, protection of DNA against peroxidation, immunomodulation, and tumor-suppressive activity, but also carcinogenesis [16, 17]. Increasing evidence shows that carotenoids may modulate molecular pathways involved in cell proliferation, acting on tyrosine kinases, MAP kinase, and growth factor-signaling cascades [18, 19]. The exact mechanisms by which carotenoids may exert their beneficial effects in atherosclerosis are still under debate.

The protective role of β-carotene in atherosclerosis has been usually attributed to its antioxidant function. Several studies [4, 20–22] showed an inverse association between β-carotene plasma levels and atherosclerotic progression in arteries. Our results indicate that the positive effect of β-carotene on atherogenesis might include, apart from inhibition of LDL oxidation, also modulation of the systemic inflammation response. The potentially protective effect of carotenoids on atherosclerosis via the influence on inflammatory processes and endothelial function was suggested also by Hozawa *et al.* [23] and van Herpen-Broekmans *et al.* [24], who observed inverse relationships between carotenoids, vitamin C, and soluble intercellular adhesion molecule-1, CRP, and leukocytes. Also, other studies have reported an inverse association between β-carotene and inflammatory markers, mainly CRP, IL-6, or leukocyte count [6, 25–28]. The above-mentioned studies, however, described the situation in the general population. Only Il'yasova *et al.* [6] analyzed the relationships in patients with moderate risk of cardiovascular disease (CRP 2.26 mg/L). They found no correlation between plasma α-tocopherol and inflammatory markers, significant inverse correlation of β-carotene with IL-6 and CRP, and positive correlation of oxidative stress marker urinary F_2-isoprostane with CRP, but not with IL-6 in patients with a median level of CRP 2.26 mg/L. Patients involved in our study that were diagnosed with

clinically significant coronary atherosclerosis demonstrated much more expressed inflammatory process (the median level of CRP in the whole S group was 4.06 mg/L), which influenced interrelationships and prevailed over the impact of oxidative stress.

The inverse relation between β-carotene and IL-6 could suggest a potentially protective effect of β-carotene on atherosclerosis due to the inhibition of inflammatory processes. IL-6 is the central mediator of the acute-phase response; it is also a primary stimulant for hepatic production of acute-phase proteins, such as CRP. A fundamental role in regulating acute inflammation is played by nuclear factor kappa B (NF-κB), an important transcription factor complex, through activation of the cytokine cascade and production of other pro-inflammatory mediators [29]. On the basis of the *in vitro* experiment of Bai *et al.* [30], it can be deduced that the underlying mechanism of the observed inverse relationship might be β-carotene suppression of NF-κB activation, resulting in the inhibition of the expression of inflammation-associated genes and production of inflammatory cytokines, such as IL-6. Bai *et al.* observed that β-carotene is able to suppress IκB degradation and subsequent NF-κB activation by scavenging intracellular reactive oxygen species. Via the inhibition of redox-based NF-κB activation, β-carotene suppresses the NF-κB-dependent expression of inflammatory genes and production of inflammatory cytokines, such as IL-6. Similar results were obtained by Novoselova *et al.* [31], who found that dietary supplementation with coenzyme Q_9, α-tocopherol, and β-carotene suppressed phosphorylation of NF-κB, IκB, and SAPK/JNK proteins, thereby preventing the activation of the NF-κB and SAPK/JNK signaling pathways in lipopolysaccharide-treated mice.

Negative correlation between β-carotene and MDA levels found only in the N group could suggest that the protective role of β-carotene against oxidation of lipids might take place under physiological conditions, but is not sufficient to meet the elevated demands occurring within a higher inflammatory state.

Some studies have suggested, that apart from β-carotene, other carotenoids (lutein, zeaxanthin, β-cryptoxanthin) may play important physiological functions [32]. Moreover, other categories of dietary antioxidants (e.g., retinol, α-tocopherol, zinc, and selenium) may be effective in suppressing activation of these proinflammatory pathways through the quenching of free radical molecules [33, 34, 27] or induction of detoxifying antioxidant enzymes [35]. Our results did not indicate a correlation between circulating levels of α-tocopherol with elevated plasma concentrations of inflammatory markers in patients with advanced CAD. This finding is consistent with previously published results [6, 27, 28]. D'Odorico [4] as well as our study failed to obtain a significant relation between plasma concentrations of vitamin E and stage of atherosclerosis. A potential reason for the lack of correlation between vitamin E and CAD might be strict regulation of vitamin E homeostasis, maintained by the liver, which involves the uptake of chylomicron remnants with a variety of vitamin E forms, together with secretion of vitamin E into circulating lipoproteins.

Our study has had some limitations. First, our data were cross-sectional in nature. Therefore, we have been unable to clarify unequivocally the timing of the concentration changes of inflammatory markers and β-carotene, and we thus cannot rule out that the association was one in which inflammation led to decreased β-carotene. The second limitation was that the data were observational, so we cannot conclusively prove that the β-carotene was associated with IL-6 independently of confounding factors. Supplementation with β-carotene failed to demonstrate its general protective effect against cardiovascular disease in randomized trials [36–38]. A potential reason for this finding may be that in the intervention studies, quite often too-high doses of β-carotene were used, which could result in a pro-inflammatory effect. Another explanation may be the hypothesis that only lack of β-carotene, i.e. its concentration below the threshold, represents a risk factor for ischemic heart disease, and supplementation above this limit does not provide additional protective effect. The other possibility is that β-carotene levels can be a marker of favorable dietary or lifestyle factors, which are associated with reduced risk of CAD and lower levels of inflammation [39]. However, confirmation of such assumptions requires further studies of a wide range of substances, as well as evaluation of the link between their levels and pathogenic mechanisms of CAD.

In conclusion, we found that coronary atherosclerosis was associated with active inflammation and low levels of HDL-cholesterol and β-carotene; and plasma levels of β-carotene (but not those of α-tocopherol) were inversely correlated with the level of IL-6 in patients with advanced CAD. Taking into account that coincidence need not represent causation, we consider this correlation as evidence of interconnection, but not necessarily of a causal relationship, between β-carotene and inflammation. Clinical trials that assess the effect of β-carotene supplementation on markers of inflammation and cellular signaling pathways would shed more light under both physiological and pathological conditions.

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