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LDL Susceptibility to Copper-Induced Oxidation after Administration of a Single Dose of Free or Esterified Beta-Cryptoxanthin

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Key Words

Beta-cryptoxanthin · Beta-cryptoxanthin ester · Low-density lipoprotein · Low-density lipoprotein oxidation

Abstract

Background: The oxidative modification of LDL is believed to be an initial step in atherosclerosis. Thus, antioxidative substances such as carotenoids may have a role in the prevention of coronary heart disease. We examined the susceptibility of LDL to Cu^{2+} oxidation in young adults before and after a single dose of β-cryptoxanthin. ***Methods:*** 1.3 mg of β-cryptoxanthin was administered to 12 apparently healthy young volunteers. Six of the volunteers received esters, the other six free β-cryptoxanthin. The plasma concentration of β-cryptoxanthin and the susceptibility of LDL to copper-induced oxidation ex vivo in terms of the duration of lag time were measured before and 12 h after β-cryptoxanthin ingestion. ***Results:*** A single dose of β-cryptoxanthin significantly increased the mean plasma β-cryptoxanthin concentration and the mean cholesterol adjusted β-cryptoxanthin concentration by 117 and 133%, respectively. No effect on the length of lag time was assessed. However, in LDL isolated from plasma 12 h after β-cryptoxanthin administration the lengths of lag time correlated significantly with the plasma β-cryptoxanthin concentration and with the cholesterol adjusted β-cryptoxanthin levels. The lag time did not differ significantly between volunteers who received esters and those who received the same dosage as free β-cryptoxanthin. At both measuring points, smokers, male volunteers and women using oral contraceptives tended to exhibit lower β-cryptoxanthin concentrations and lower cholesterol adjusted β-cryptoxanthin concentrations as well as increased LDL oxidizability compared to nonsmokers and women not using oral contraceptives. ***Conclusion:*** A single dose of β-cryptoxanthin does not enhance the duration of LDL lag time ex vivo in healthy young subjects.

Introduction

Oxidized lipoproteins may play an important role in the pathogenesis of atherosclerosis. Chemically modified LDL, but not native LDL, is able to induce the formation of foam cells, initiating the process of plaque formation. LDL oxidation is likely to be the most important form of LDL modification in humans and is connected with the conversion of polyunsaturated fatty acids to lipid peroxides, which easily decompose to products such as biologically active aldehydes [1, 2].

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Antioxidants have been shown to prevent LDL oxidation in vitro and may have a protective effect against the progression of atherosclerosis. The predominant antioxidant in LDL is α-tocopherol, other substances with potential antioxidant activity are γ-tocopherol, ubiquinol-10, phytofluene and the carotenoids β-carotene, α-carotene, lycopene, β-cryptoxanthin and canthaxanthin [3]. Epidemiologic data indicate that a high fruit and vegetable consumption and a high intake of carotenoids may prevent atherosclerosis [4–7] as well as cancer [8–10]. Furthermore, results of studies have shown protective effects of β-cryptoxanthin, a xanthophyll that occurs mainly in papaya, apricot, tangerine, peach and red pepper. Higher dietary intake or higher plasma levels of β-cryptoxanthin were associated with a lower risk of angina pectoris [11, 12], cancer [13–17] and hyperglycemia [18] or revealed an inverse association with the total mortality [19]. Since a comparative study of the serum concentrations of carotenoids in five European countries has shown that the concentrations of the sum of xanthophylls (lutein, zeaxanthin, β-cryptoxanthin) and of β-cryptoxanthin alone were higher in southern (Spain) compared to the northern areas, these serum levels have been suggested as important markers related to the healthy or protective effects of the Mediterranean diet [20].

Effects of supplemental carotenoids on ex vivo LDL oxidation are contradictory. Some authors found reduced LDL oxidation after β-carotene administration [21, 22], whereas other studies reported no effect on LDL oxidation [23, 24]. Less information is available on the effect of carotenoids other than β-carotene on the susceptibility of LDL to oxidation. In a study with lycopene, 4 healthy subjects received a single dose of 30 mg of lycopene. Postprandial LDL isolated 5 h after lycopene administration exhibited a significantly reduced susceptibility to oxidation by 21% [25]. However in elderly Irish adults, supplementation with a carotene mixture or lycopene for 12 weeks had no effect on oxidative modification of LDL in vitro [26]. In a short-term intervention study, a dietary intake of >40 mg/day of lycopene by a group of nonsmoking individuals significantly reduced the susceptibility of LDL to oxidation, whereas an equivalent increase in lycopene by a group of smokers showed no such effect [27]. Different dosages of astaxanthin in the diet led to a reduction of the LDL oxidation [28].

To date, data on the effect of β-cryptoxanthin on LDL susceptibility to oxidation is scarce. In one study no association between the LDL lag time and the LDL β-cryptoxanthin concentrations was found in middle-aged and elderly female and male subjects fed different diets [29]. Studies have shown that atherosclerosis often is associated with the occurrence of small, dense LDL. Particle contents of β-cryptoxanthin and other carotenoids were markedly reduced in this type of LDL (LDL5, d = 1.050 to 1.065 g/ml) [30]. Furthermore, significantly lower plasma levels of β-cryptoxanthin were shown in subjects with elevated inflammatory markers, especially C-reactive protein which is associated to cardiovascular disease [31]. Reduced antioxidant levels may contribute to a lower resistance of LDL to oxidation and thus enhance oxidative LDL modification and atherosclerosis.

We originally designed a single-blind comparative study in order to compare the biokinetics of esters and free β-cryptoxanthin, which are both found in natural sources of β-cryptoxanthin. The results of the original study showed no significant difference in the biokinetics of esters and free β-cryptoxanthin. This indicates comparative bioavailability of both free β-cryptoxanthin and a mixture of different β-cryptoxanthin esters [32]. In this study, we evaluated the effect of a single dose of 1.3 mg of β-cryptoxanthin on the copper induced LDL oxidation ex vivo.

Subjects, Methods and Materials

Subjects

Twelve subjects, 6 females, 6 males, were randomly assigned to two groups. Among them 4 were smokers, the others were non-smokers. Three of the females used oral contraceptives. None of the persons suffered from gastrointestinal diseases, or took laxatives or drugs lowering plasma triacylglycerol or cholesterol levels. They were advised to take no supplements containing carotenoids or vitamins in the 8 weeks before the study and during the intervention. The volunteers were requested to keep a normal diet but to avoid β-cryptoxanthin-rich fruits and vegetables [32]. The study protocol was approved by the Medical Ethics Committee of the Medizinische Hochschule, Hannover, and the participants provided written informed consent.

Study Design

We investigated the effect of a single dose of esters and free β-cryptoxanthin on LDL susceptibility to oxidation ex vivo before (t0) and 12 h after application of β-cryptoxanthin (t1). The absolute amount present in the diet was set to 1.3 mg β-cryptoxanthin, corresponding to a serving size of about 100 g native papaya. Thus, a physiological dose of total β-cryptoxanthin was administered to the volunteers. The volunteers were instructed not to consume any fruits, vegetables or products made from fruits and vegetables including juices on the 24-hour intervention period. As previously described [32], carotenoid application was performed by mixing a serving of 150 g yogurt with 4 g of spiked sunflower oil, resulting in a total amount of 1.3 mg β-cryptoxanthin, independent of the free or esterified form. The yogurt was consumed together with a standardized breakfast with a total of approximately 24 g fat. Blood samples were

collected before (0 h) and 12 h after β-cryptoxanthin application. We used the plasma samples taken 12 h after the ingestion of the β-cryptoxanthin dose, because an elevated LDL β-cryptoxanthin concentration could be expected at that time. Wingerath et al. [33] found the peak levels of β-cryptoxanthin in chylomicrons 6 h after ingestion of a tangerine concentrate and our biokinetic study showed the highest plasma concentrations at the measure points 9 and 12 h after the β-cryptoxanthin enriched breakfast [32]. Free and esterified β-cryptoxanthin were prepared as previously described [32].

Plasma Sample Preparation and LDL Oxidation Measurement

At both blood withdrawals, a volume of 9 ml was sampled from each participant. Immediately after collection, the plasma was separated from the erythrocytes by centrifugation (5,000 rpm, 10 min) and stored at –70°C in plastic caps. From half of the plasma sample the plasma β-cryptoxanthin concentrations had been measured by HPLC as described previously [32, 34, 35]. Total plasma triacylglycerol and total cholesterol were measured manually by using commercial in vitro enzymatic test kits. From the other half of the plasma, LDL was isolated by an ultracentrifugation method based on non-equilibrium density-gradient ultracentrifugation. For centrifugation a 70Ti rotor (Beckman Optima LE-80K, Beckman Instruments, Palo Alto, Calif., USA) was used. Centrifugation was carried out in polycarbonate centrifuge tubes at 288,000 *g* for 2 h at 15°C. The LDL containing fraction was collected by aspiration. Salts and EDTA were removed from LDL by gel filtration on Pharmacia PD 10 disposable columns (Amersham Pharmacia Biotech, Freiburg, Germany). Once the LDL was isolated, it was used immediately with no storage.

The ex vivo oxidation of LDL was performed after adding $CuCl_2$ to a final concentration of 20 μmol/L based on a method previously described [36]. Conjugated diene absorption was measured spectrophotometrically at 234 nm every 5 min for 4 h. As a measure of the susceptibility of LDL to oxidation the lag time was used. This has been assessed from the time-absorption curve and is the time interval between the intercept of the linear last square slope of the curve with the initial-absorbance axis [36, 37].

Statistics

Results are expressed as mean values ± SDs. Normal distribution of data was checked using the Kolmogorov-Smirnov test. Since the data showed a normal distribution, the t test for paired samples was used to evaluate for differences between values of the same sample at two time points, before (t0) and after (t1) application of β-cryptoxanthin. The independent-sample t-test was used to reveal significant differences in the plasma concentrations and the lag times between the two groups (receiving free or esters of β-cryptoxanthin) and between smokers and non-smokers and male and female volunteers. Pearson correlation coefficients were calculated in order to evaluate an association between the lag time and the plasma concentration of β-cryptoxanthin. $p < 0.05$ was considered statistically significant. All statistics were performed with SPSS 11.0 (SPSS Inc., Chicago, Ill., USA).

Results

The characteristics of the study population are shown in table 1. Since carotenoids are transported in association with lipoproteins, plasma triacylglycerol and cholesterol concentrations are highly correlated with circulating carotenoid concentrations [38]. In fasting plasma, total cholesterol correlated significantly with β-cryptoxanthin ($r = 0.656$, $p = 0.021$) in our sample, but there was no significant association with plasma triacylglycerol. Thus, plasma β-cryptoxanthin concentrations are also indicated as adjusted values for total cholesterol (μmol β-cryptoxanthin/mmol total cholesterol).

Table 1. Characteristics of the volunteers (mean ± SD)

	Value (n = 12)
Age, years	27.3 ± 5.7
BMI, kg/m^2	24.5 ± 3.0
Fasting plasma concentrations	
Total cholesterol, mmol/L	4.51 ± 0.87
Triacylglycerol, mmol/L	1.09 ± 0.58

The mean lag time measured in LDL from fasting plasma before application of β-cryptoxanthin was lower than that in LDL from plasma taken 12 h after a single dose of β-cryptoxanthin, indicating a higher resistance to oxidation after 12 h (table 2). However, the difference was small and not statistically significant. No significant differences in lag time, β-cryptoxanthin concentration, cholesterol adjusted β-cryptoxanthin concentration at any time point were seen between the volunteers who received free β-cryptoxanthin and those who received the esters.

At the measurement 12 h after β-cryptoxanthin intake, the LDL lag time of the total sample correlated significantly with the β-cryptoxanthin plasma concentration ($r = 0.654$, $p = 0.021$) and with the cholesterol adjusted β-cryptoxanthin plasma concentration ($r = 0.780$, $p = 0.003$) (fig. 1). This association was not seen if only the volunteers who received the ester were considered. However, in volunteers who received free β-cryptoxanthin, cholesterol adjusted β-cryptoxanthin plasma concentration correlated significantly with the lag time, both measured 12 h after β-cryptoxanthin ingestion (t1) ($r = 0.865$, $p = 0.026$).

At both time points, men, smokers and women who used oral contraceptives had lower mean β-cryptoxanthin concentrations and a lower mean lag-time than women, nonsmokers, and women not using oral contraceptives, respectively. However, these differences were not statistically significant (table 2).

Table 2. Length of the lag phase and β-cryptoxanthin plasma concentrations before (t0) and after (t1) ingestion of 1.3 mg β-cryptoxanthin (mean values ± SD)

	Lag phase t0, min	Lag phase t1, min	β-Cryptoxanthin t0, μmol/L	β-Cryptoxanthin t1, μmol/L	β-Cryptoxanthin t0, μmol/mmol cholesterol	β-Cryptoxanthin t1, μmol/mmol cholesterol
Total sample (n = 12)	74.3 ± 10.3	75.5 ± 11.1	0.06 ± 0.05	0.13 ± 0.08[1]	0.012 ± 0.009	0.028 ± 0.016[1]
Group receiving free β-cryptoxanthin (n = 6)	76.9 ± 12.1	76.9 ± 13.0	0.05 ± 0.04	0.11 ± 0.01[2]	0.011 ± 0.008	0.027 ± 0.017[3]
Group receiving β-cryptoxanthin esters (n = 6)	71.6 ± 8.5	74.2 ± 9.9	0.07 ± 0.07	0.14 ± 0.10[4]	0.013 ± 0.011	0.029 ± 0.015[5]
Women (n = 6)	76.7 ± 14.0	78.7 ± 14.6	0.07 ± 0.07	0.16 ± 0.11[6]	0.014 ± 0.011	0.033 ± 0.020[7]
Men (n = 6)	71.9 ± 5.0	72.3 ± 6.02	0.05 ± 0.04	0.10 ± 0.04[8]	0.010 ± 0.008	0.024 ± 0.009[9]
Non-smokers (n = 8)	75.5 ± 11.2	77.3 ± 12.6	0.08 ± 0.06	0.16 ± 0.08[10]	0.015 ± 0.010	0.033 ± 0.015[11]
Smokers (n = 4)	71.9 ± 9.2	72.1 ± 7.7	0.03 ± 0.02	0.07 ± 0.05	0.006 ± 0.004	0.019 ± 0.013
Women not using oral contraceptives (n = 3)	84.3 ± 11.6	86.4 ± 15.9	0.11 ± 0.08	0.22 ± 0.09[2]	0.021 ± 0.011	0.045 ± 0.017
Women using oral contraceptives (n = 3)	69.0 ± 13.5	71.1 ± 10.1	0.03 ± 0.03	0.09 ± 0.08	0.007 ± 0.006	0.020 ± 0.016

[1] Significantly different from t0 ($p < 0.001$, paired t test).
[2] Significantly different from t0 ($p = 0.029$, paired t test).
[3] Significantly different from t0 ($p = 0.04$, paired t test).
[4] Significantly different from t0 ($p = 0.004$, paired t test).
[5] Significantly different from t0 ($p = 0.003$, paired t test).
[6] Significantly different from t0 ($p = 0.007$, paired t test).
[7] Significantly different from t0 ($p = 0.014$, paired t test).
[8] Significantly different from t0 ($p = 0.013$, paired t test).
[9] Significantly different from t0 ($p = 0.016$, paired t test).
[10] Significantly different from t0 ($p = 0.001$, paired t test).
[11] Significantly different from t0 ($p = 0.002$, paired t test).

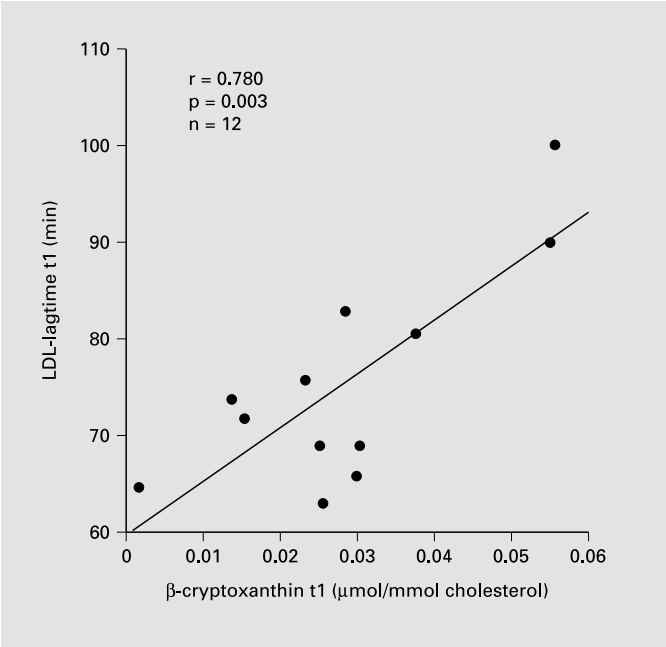

Fig. 1. Correlation between the duration of LDL lag time and the cholesterol adjusted β-cryptoxanthin concentrations 12 h after ingestion of 1.3 mg β-cryptoxanthin.

Discussion

In this study we evaluated the effect of a single dose of free or esterified β-cryptoxanthin on the LDL susceptibility to ex vivo oxidation. All participants were instructed to consume a low-carotenoid diet two weeks before and during the intervention. The mean baseline concentration of β-cryptoxanthin was well below the usually observed concentration in human plasma and demonstrated the effect of the depletion phase before beginning the intervention. Both total triacylglycerol and cholesterol levels were in the normal range for all subjects of both groups [32].

Since subjects who received esters and those who received free β-cryptoxanthin did not differ either in β-cryptoxanthin plasma concentrations or in cholesterol adjusted β-cryptoxanthin plasma concentrations, or LDL lag times, the results could be considered for the total sample. After β-cryptoxanthin application the plasma β-cryptoxanthin concentration increased significantly, whereas the mean length of lag time was elevated marginally by only <2%, which was not significant.

The results of previous studies evaluating the effect of carotenoids on the susceptibility of LDL to oxidation are inconsistent. In a study with apparently healthy adults who either received a low-vegetable diet, a high-vegetable diet or a low-vegetable diet with a carotenoid supplement for 4 weeks, no significant effect on the ex vivo LDL oxidation lag time was observed in any of the three interven-

tion groups [39]. Other authors assessed the LDL oxidation by human endothelial cells in culture and observed no correlation with the carotenoid but with the tocopherol content of LDL [40].

In contrast, Lowe and coworkers reported a strong positive correlation between total carotenoid plasma concentrations and the lag time of Cu^{2+}-mediated oxidation [41]. This result had also been found earlier by an investigation showing significantly enhanced resistance of LDL to oxidation after 2 weeks of increased fruit and vegetable consumption in smokers and nonsmokers of 14 and 28%, respectively [42]. According to previous results [29], we observed no significant correlation between the LDL lag time and the plasma β-cryptoxanthin concentrations before β-cryptoxanthin administration. In contrast, the lag time 12 h after β-cryptoxanthin application correlated significantly with both the plasma β-cryptoxanthin concentrations as well as the cholesterol adjusted β-cryptoxanthin concentrations. This correlation was even stronger, if only volunteers who received free β-cryptoxanthin were considered. Since the participants were advised not to eat any vegetables or fruits or products from vegetables or fruits during the 24-hour intervention period, they had no intake of typical antioxidants from vegetable sources. Therefore β-cryptoxanthin might have exhibited an effect on the lag time. Though, we cannot exclude that other substances of the diet such as vitamin E had an influence on the lag time.

Hininger et al. [42] observed significantly lower plasma β-cryptoxanthin concentrations in smokers than in nonsmokers. This corresponds to recent results [43–45] and is confirmed by a tendency of lower β-cryptoxanthin concentrations in smokers than in nonsmokers in our sample. Hininger et al. [42] found 30% higher baseline β-cryptoxanthin concentrations in nonsmokers than in smokers. The smokers in our sample exhibited a 63% lower mean baseline concentration than nonsmokers, but probably due to the small number of subjects the difference was not significant. Furthermore, we observed a tendency of lower β-cryptoxanthin concentrations in the plasma of males compared to females. This agrees with recent results from a Japanese study. In this investigation plasma carotenoids had been investigated in 158 Japanese men and women in relationship to their smoking habits. Plasma levels of β-cryptoxanthin were significantly lower in males than in females and were significantly lower in males who were current smokers, compared to non-smokers [46].

Conclusion

A single dose of β-cryptoxanthin significantly increases the plasma β-cryptoxanthin concentration and leads to a significant correlation between the plasma concentration and the LDL lag time 12 h after β-cryptoxanthin ingestion. However, a physiological dose of β-cryptoxanthin does not induce a significant reduction of the susceptibility of LDL to copper-induced oxidation.

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