Lack of correlation between the α-tocopherol content of plasma and LDL, but high correlations for γ-tocopherol and carotenoids

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Abstract In 59 healthy human subjects (37 male and 22 female) the concentrations of the lipid-soluble antioxidants α- and γ-tocopherol, α- and β-carotene, lycopene, cryptoxanthin, canthaxanthin, and lutein + zeaxanthin were determined in plasma (μmol/L) and in isolated low density lipoproteins (LDL) (μmol/mmol cholesterol). Plasma α-tocopherol concentrations were significantly correlated with plasma total cholesterol concentrations (r^2 = 0.51, P < 0.0001) yet not with the LDL α-tocopherol content (r^2 = 0.05, ns). Plasma γ-tocopherol concentrations were weakly correlated with plasma total cholesterol (r^2 = 0.12, P < 0.003) and both absolute and cholesterol standardized plasma γ-tocopherol concentrations correlated strongly with the LDL γ-tocopherol content (r^2 = 0.58 and r^2 = 0.72, respectively). In contrast, carotenoid concentrations did not correlate with cholesterol concentrations, but their LDL content correlated significantly with the respective plasma concentrations (r^2 = 0.67 to 0.92, all P < 0.0001). In a subgroup of study subjects (n = 13) the distribution of vitamin E and carotenoids among LDL was calculated. The proportion of plasma α- and γ-tocopherol found in LDL was 48 ± 7 (range, 36–61%) and 41 ± 7%, respectively, suggesting that LDL was in most of these subjects not the main carrier for these antioxidants. The lipophilic carotenoids, however, were predominantly carried by LDL (e.g., β-carotene: 87 ± 10%), whereas the proportion of the more polar ones carried by LDL was much smaller (e.g., lutein + zeaxanthin: 36 ± 6%). The results of this study show that plasma α-tocopherol concentrations are not predictive for the α-tocopherol content of LDL in nonsupplemented individuals. This finding could have implications in interpreting the cause of the inverse relationship between plasma α-tocopherol and risk of atherosclerosis.—**Ziouzenkova, O., B. M. Winklhofer-Roob, H. Puhl, J. M. Roob, and H. Esterbauer.** Lack of correlation between the α-tocopherol content of plasma and LDL, but high correlations for γ-tocopherol and carotenoids. *J. Lipid Res.* 1996. **37:** 1936–1946.

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Plasma concentrations of the lipid-soluble antioxidants vitamin E and carotenoids can vary considerably among healthy individuals, probably due to differences in the dietary of antioxidants and fat, their absorption, and other factors (1–4). Similarly, the LDL content of vitamin E and carotenoids shows a substantial inter-individual variation (5–7). For instance, the LDL α-tocopherol content may range from about 3 to 16 molecules per LDL particle and that of β-carotene can range from 0.03 to about 2 molecules per LDL (5). It is widely believed that the variability in the antioxidant content of LDL is related to that in plasma antioxidant levels. This assumption is supported by several supplementation studies with vitamin E (7–9) or β-carotene (10, 11), and the reported ease of spontaneous exchange of α-tocopherol or β-carotene between different lipoprotein classes observed in in vitro experiments (12–15). However, in healthy individuals not taking antioxidant supplements, the putative relationship between the antioxidant content of LDL and plasma has so far not been examined. The question whether plasma levels of antioxidants indeed predict the antioxidant content of LDL is of importance in the context of the LDL-oxidation hypothesis (16–18) and the epidemiologic observations of an inverse relationship between the risk of atherosclerosis and plasma levels or dietary intake of vitamin E or β-carotene measured directly or estimated from dietary questionnaires (19–22). We have tested the hypothesis that the variability of the LDL antioxidant content is related to differences in plasma levels using a large number of data collected in the course of our studies on factors affecting the resistance to oxidation

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LP, lipoproteins; EDTA, ethylenediaminetetraacetic acid; BHT, butylated hydroxytoluene; HPLC, high performance liquid chromatography; ns, not significant; SD, standard deviation.

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of LDL. The unexpected result, which we report here, is that in healthy subjects not taking supplements, the α-tocopherol content of LDL does not correlate with plasma α-tocopherol concentrations, whereas those of γ-tocopherol and major carotenoids show a very high correlation.

METHODS

Subjects

The study subjects comprised 59 healthy, randomly selected individuals (37 males and 22 females, aged 42 ± 17 years) living in the area of the city of Graz, Austria, who volunteered for the determination of all antioxidants. Additional 43 healthy, randomly selected subjects (17 males and 26 females, aged 31 ± 9 years) living in the area of the city of Zürich, Switzerland, who were previously enrolled as control subjects in either one or two intervention studies (23, 24), were included for the analysis of the relationship between plasma and LDL α-tocopherol concentrations. None of the 102 study subjects took antioxidant supplements and all had total cholesterol concentrations within the normal range. In a subgroup of 13 Austrian subjects (8 males and 5 females) the lipid status was assessed not only by plasma cholesterol, but also by triglyceride and LDL and HDL cholesterol concentrations, and the composition of their LDL was determined. These values were within the normal range. For the subgroup, the distribution of antioxidants among LDL and other lipoproteins was calculated.

Preparation of plasma and LDL

Fasting blood samples were obtained by venipuncture and collected by free flow into plastic tubes containing an aqueous solution of 10% EDTA. The final concentration of EDTA was 1 mg/ml blood. The blood was centrifuged within 1 h at 10°C at 1000 *g* for 10 min followed by 3000 *g* for 15 min. For measurement of plasma content of vitamin E, carotenoids and lipids, an aliquot of the EDTA plasma was frozen immediately and stored at -70°C for not longer than 1 week. For preparation of LDL, the freshly prepared plasma was used and LDL was isolated by ultracentrifugation as previously described (25). LDL samples were filtered through a 0.45-μm filter into sterile evacuated glass vials and stored under argon at 4°C. The antioxidant content of LDL was determined within 1 week. Plasma and LDL containing EDTA can be stored under such condition for up to 2 weeks without a measurable loss of antioxidants (25).

Measurement of the vitamin E and carotenoid content of plasma and LDL

Plasma was thawed and 0.2 ml was diluted with 0.2 ml aqueous EDTA solution (1 mg/ml) followed by 0.4 ml ice-cold ethanol containing 0.05 mg/ml butylated hydroxy toluene (BHT). Then 1 ml n-hexane was added and the mixture was vortexed for 1 min. After centrifugation (1000 *g*, 5 min) 0.8 ml of the upper hexane phase was collected and dried under nitrogen. The residue was dissolved in 0.1 ml ethanol–ethyl acetate 10:1 (v/v) and separated by HPLC on a Lichrospher 100 RP-18 (5 μm) column. Isocratic separation was performed with a mixture of methanol–acetonitrile–ethanol–water 60:50:20:2 (v/v) containing 0.01% ammonium acetate; flow rate was 1.2 ml/min. The effluent was monitored with two detectors in series; α- and γ-tocopherol were detected with a fluorescence detector set at 292/335 nm; carotenoids were detected by an UV-Vis detector at 450 nm. Peak quantification was done with a standard mixture separated under identical conditions. Lutein and zeaxanthin coeluted as a single peak. Compared to the mobile phase used previously (8, 26), the mobile phase described above permits separation of tocopherols and carotenoids in a single run and α-carotene and hydroxycarotenoids become better separated from neighboring peaks. For measurement of the vitamin E and carotenoid content of LDL, the total cholesterol content of the LDL sample prepared by ultracentrifugation was first determined. Then a volume containing about 100 μg cholesterol was filled up to 0.5 ml with an aqueous EDTA solution (1 mg/ml). The sample was mixed with 0.5 ml ice-cold ethanol containing 0.05 mg/ml BHT and extracted with 1 ml n-hexane as described above for plasma. All further steps including HPLC separation were the same as described for plasma antioxidants. The vitamin E and carotenoid content of LDL samples is expressed in relation to the total cholesterol content estimated in parallel in the same sample, i.e., μmol antioxidant/mmol cholesterol. The antioxidant content in plasma was expressed both absolute (μmol/L) and per mmol of plasma cholesterol (27–29).

Other analytical methods

Total plasma cholesterol and total cholesterol of the isolated LDL were determined enzymatically with the CHOD-PAP test kit (Boehringer-Mannheim, Germany). Standard clinical laboratory protocols were used for measurement of plasma triglycerides and plasma HDL-cholesterol and for the LDL-cholesterol calculation (30). The composition of isolated LDL (phospholipids, triglycerides, free and esterified cholesterol) was determined with test kits from Boehringer-Mannheim (Ger-

many). LDL-protein was measured with the BCA test kit (Pierce, Rockford, IL) using bovine serum albumin for calibration.

Statistical analysis

Comparisons between the cholesterol standardized antioxidant content of LDL and plasma were made by the paired *t*-test. Normal distribution of all variables was tested with the Kolmogorov-Smirnov test. SPSS Rel. 6.1.2. was used for all statistical procedures. A P value < 0.05 was considered significant. All values are expressed as mean ± SD.

RESULTS

Antioxidant content of plasma and LDL

Plasma concentrations of vitamin E, carotenoids, and total cholesterol are shown in **Table 1** and **Table 2.** Plasma total cholesterol concentrations correlated with plasma α-tocopherol (r^2 = 0.51, P = 0.0001) and γ-tocopherol concentrations (r^2 = 0.12, P = 0.003) but not with any of the carotenoids. Additionally, LDL was isolated by ultracentrifugation from each of the 59 plasma samples and the antioxidant and cholesterol content was determined and expressed both on a molar basis and standardized for the LDL cholesterol content (27) (**Table 3**). Comparison of the antioxidant content of LDL and the cholesterol standardized plasma antioxidant concentrations showed a significantly lower content of α-tocopherol (-23%), γ-tocopherol (-35%), cryptoxanthin (-21%), and lutein + zeaxanthin (-50%) in LDL compared with plasma, but a significantly higher content of β-carotene (+39%), lycopene (+25%) and α-carotene (+19%) (paired *t* test, all P < 0.001).

In the subgroup of 13 subjects we also estimated the LDL-cholesterol concentration of plasma. Based on the antioxidant content of the isolated LDL and the corresponding plasma concentrations of LDL cholesterol of each subject, the average proportion of the antioxidants that was carried by LDL was calculated (**Table 4**). For α-tocopherol this proportion varied substantially, depending on the donor, from 36 to 61%, with a mean ± SD of 47.9 ± 7.3%. Consistent with another report (31) there was a trend to higher proportion carried by LDL in men, but the difference between men and women did not reach statistical significance (data not shown). Even though the average plasma concentration of γ-tocopherol was about 10-fold lower than that of α-tocopherol (23.4 vs. 2.4 μmol/L, Table 1), the average proportion of γ-tocopherol carried by LDL (41.4%) was similar to that of α-tocopherol (47.9%). In the subgroup only the plasma α-tocopherol correlated with plasma LDL-cholesterol concentrations (r^2 = 0.24, P = 0.015). The distribution of the plasma carotenoids among LDL appeared to be dependent on their structure: LDL contained 64–99% of the lipophilic carotenoids (α- and β-carotene and lycopene) but only about 25–70% of the more polar carotenoids (cryptoxanthin, canthaxanthin, lutein + zeaxanthin) (Table 4).

Correlations between LDL and plasma tocopherols

The α-tocopherol content of LDL of the 59 subjects did not show a correlation with the absolute plasma α-tocopherol concentrations (r^2 = 0.05, P = 0.09) (Table

TABLE 1. Plasma concentrations of antioxidants in the whole group of 59 Austrian subjects

	Absolute	Standardized	Correlation[a]
	μmol/L	*μmol/mmol cholesterol*	r^2
α-Tocopherol	23.4 ± 7.10	4.80 ± 1.01	0.51
γ-Tocopherol	2.42 ± 1.12	0.50 ± 0.21	0.12
β-Carotene	0.49 ± 0.36	0.10 ± 0.08	(-0.04)
Lycopene	0.23 ± 0.16	0.05 ± 0.03	(-0.06)
α-Carotene[b]	0.06 ± 0.04	0.01 ± 0.01	(-0.12)
Cryptoxanthin	0.29 ± 0.19	0.06 ± 0.04	(-0.02)
Canthaxanthin	0.07 ± 0.06	0.01 ± 0.01	(0.01)
Lutein + zeaxanthin	0.26 ± 0.19	0.05 ± 0.04	(0.06)
Swiss	n = 43		
α-Tocopherol	26.6 ± 4.74	5.58 ± 0.83	0.43

Additional α-tocopherol concentrations are shown for 43 Swiss subjects. Values are expressed as mean ± SD.

[a]Linear correlation between plasma cholesterol and absolute concentrations of antioxidants; values in parentheses are not significant.

[b]n = 32.

TABLE 2. Plasma lipids and composition of isolated LDL in the subgroup (n = 13)

	Mean ± SD	Range
Plasma lipids (mmol/L)		
Cholesterol	5.18 ± 0.81	4.10–6.63
Triglycerides	1.45 ± 0.94	0.55–3.45
LDL-cholesterol	3.42 ± 0.66	2.20–4.50
HDL-cholesterol	1.24 ± 0.30	0.75–1.78
Isolated LDL (weight %)		
Phospholipids	19.1 ± 0.9	17.3–20.8
Triglycerides	5.14 ± 1.0	4.0–7.5
Free cholesterol	7.71 ± 0.7	6.3–8.5
Cholesteryl ester	39.6 ± 2.4	36.1–44.3
Total cholesterol	31.3 ± 1.3	28.7–32.8
Protein[a]	28.4 ± 2.0	25.2–31.3

Plasma cholesterol concentrations were 4.88 ± 1.14 in the whole Austrian group (n = 59) and 4.79 ± 0.74 in the Swiss group (n = 43).
[a]Calibrated with bovine serum albumin, fraction V.

3). To further rule out that this finding was due to small sample size, data from additional 43 healthy Swiss subjects who had been investigated previously in our laboratory (23, 24) were included in the analysis. The lack of a correlation between plasma and LDL α-tocopherol concentrations was confirmed both when this group of 43 Swiss subjects was analyzed separately (r^2 = 0.002, P = 0.8) and when the Swiss subjects were combined with the Austrian subjects, giving a total number of 102 (r^2 = 0.001, P = 0.8) (**Fig. 1**). Whereas a weak correlation existed between the α-tocopherol content of LDL and the cholesterol standardized plasma α-tocopherol concentrations in the Austrian and Swiss group individually (r^2 = 0.13, P = 0.005 and r^2 = 0.16, P = 0.009), no correlation was observed when both groups were combined (r^2 = 0.015, P = 0.2). In contrast, the γ-tocopherol content of LDL in the group of 59 subjects was strongly correlated with the absolute plasma γ-tocopherol concentrations (r^2 = 0.58, P = 0.0001) (Fig. 1) and even stronger with the cholesterol standardized plasma γ-tocopherol concentrations (r^2 = 0.72, P = 0.0001).

The lack of a correlation between the α-tocopherol content of LDL and plasma α-tocopherol concentrations was very striking, because previous studies (7–9) on vitamin E-supplemented subjects had shown a high correlation. Therefore, the relationship between the α-tocopherol concentrations of plasma and LDL was further investigated in a subgroup of 13 subjects who had LDL-cholesterol determinations as described above. Plasma total cholesterol concentrations and the antioxidant content of plasma and LDL in these subjects were not significantly different from those in the whole study group (Tables 1–4). As can be seen from Table 2, the composition of the LDL samples prepared from the individuals of the subgroup was fully consistent with previously reported values (26, 32). In agreement with the finding in the whole group, this subgroup did not show a correlation between the α-tocopherol content of LDL and plasma α-tocopherol concentrations (r^2 = 0.01, P = 0.5). However, when the total amount of α-tocopherol carried by the sum of all LDL particles in plasma was calculated as: [α-tocopherol concentration in LDL expressed as μmol/mmol cholesterol] multiplied by [total plasma LDL concentration expressed as mmol/L], the proportion of plasma α-tocopherol carried by LDL (μmol LDL α-tocopherol/L plasma) correlated strongly with the total plasma α-tocopherol concentrations (r^2 = 0.43, P < 0.001); for γ-tocopherol this correlation was very strong (r^2 = 0.86, P < 0.001). Despite the fact that an approximately equal amount of α-tocopherol was carried by the sum of other lipoproteins (52%) compared to LDL alone (48%) (Table 4), a correlation was not observed between the amount of α-tocopherol carried by LDL and the other lipoproteins (**Fig. 2,** left panel). In contrast, there was a very strong correlation for γ-tocopherol (r^2 = 0.83, P < 0.001) (Fig. 2, right panel).

Multiple regression analysis including plasma total cholesterol and plasma and LDL α-tocopherol concentrations of the combined Austrian and Swiss group showed that the variable that contributed strongest to the variability of the α-tocopherol of LDL was the "interindividual variability" among the different subjects (T

TABLE 3. Antioxidant content of LDL in whole group of 59 Austrian subjects

	Standardized μmol/mmol cholesterol	mol/mol[a]	Correlation[b] r^2
α-Tocopherol	3.58 ± 0.86	7.32	(0.05)/0.13
γ-Tocopherol	0.32 ± 0.12	0.65	0.58/0.72
β-Carotene	0.12 ± 0.25	0.25	0.88/0.85
Lycopene	0.06 ± 0.05	0.14	0.69/0.71
α-Carotene[c]	0.01 ± 0.01	0.02	0.83/0.67
Cryptoxanthin	0.05 ± 0.04	0.1	0.90/0.92
Canthaxanthin	0.01 ± 0.01	0.03	0.92/0.85
Lutein + zeaxanthin	0.03 ± 0.02	0.06	0.90/0.92
Swiss n = 43			
α-Tocopherol	2.77 ± 0.61	6.62	(0.002)/0.16

Additional α-tocopherol concentrations are shown for 43 Swiss subjects.
[a]For comparison with other studies, the average molecules of antioxidants per LDL particle is given.
[b]Linear correlation between antioxidant content of LDL (μmol/mmol cholesterol) and the absolute/or the standardized plasma concentrations (Table 1); values in parentheses are not significant.
[c]n = 32.

TABLE 4. Antioxidant concentrations in subgroup (n = 13)

	Plasma, Absolute	LDL, Standardized	LDL, Absolute[a]	Other LP Absolute[b]	LDL[c]	Correlation[d]
	μmol/L	μmol/mmol cholesterol	μmol/L	μmol/L	%	r^2
α-Tocopherol	25.4 ± 4.50	3.55 ± 0.44	12.1 ± 2.30	13.2 ± 2.94	47.9 ± 7.30	0.43/(0.01)
γ-Tocopherol	2.80 ± 1.60	0.34 ± 0.15	1.13 ± 0.56	1.75 ± 1.18	41.4 ± 6.70	0.92/0.83
β-Carotene	0.53 ± 0.28	0.14 ± 0.07	0.47 ± 0.27	0.06 ± 0.05	87.4 ± 10.1	0.97/(0.05)
Lycopene	0.32 ± 0.20	0.08 ± 0.05	0.29 ± 0.20	0.03 ± 0.02	87.4 ± 10.5	0.99/(0.01)
α-Carotene	0.08 ± 0.05	0.02 ± 0.01	0.06 ± 0.04	0.02 ± 0.02	77.9 ± 24.5	0.72/(0.04)
Cryptoxanthin	0.32 ± 0.17	0.05 ± 0.03	0.18 ± 0.10	0.14 ± 0.08	58.3 ± 7.40	0.93/0.66
Canthaxanthin	0.12 ± 0.05	0.02 ± 0.01	0.07 ± 0.03	0.05 ± 0.03	55.2 ± 10.3	0.73/(0.25)
Lutein + zeaxanthin	0.47 ± 0.17	0.05 ± 0.02	0.17 ± 0.07	0.30 ± 0.11	36.4 ± 5.60	0.86/0.66

[a]Vitamin E and carotenoids carried by LDL were determined according to: μmol/L = a × b, where a is LDL - cholesterol (mmol/L), b is the antioxidant content of the isolated LDL (μmol/mmol cholesterol).
[b]Vitamin E and carotenoids carried by other lipoproteins (LP) in μmol/L. Values were determined according to: μmol/L = plasma absolute - LDL absolute.
[c]Percentage of plasma antioxidants carried by LDL.
[d]Linear correlation between antioxidants in LDL absolute and plasma absolute/or in other LP absolute.

= 7.7), whereas plasma α-tocopherol and total cholesterol concentrations did not contribute significantly (T = 1.02 and T = -1.18, respectively).

Correlations between LDL carotenoids and plasma carotenoids

All carotenoids investigated in this study (α- and β-carotene, lycopene, cryptoxanthin, canthaxanthin, lutein + zeaxanthin) showed a strong correlation ($P < 0.0001$) between the content in LDL on one hand and the absolute plasma antioxidant concentrations (r^2 from 0.69 to 0.92) and the cholesterol standardized plasma antioxidants concentrations (r^2 from 0.71 to 0.92) on the other hand (Table 3). **Figure 3** shows the relationship for β-carotene as an example for an unpolar, lipophilic carotenoid and lutein + zeaxanthin as an example for polar, less lipophilic carotenoids.

DISCUSSION

Plasma concentrations of vitamin E (α- and γ-tocopherol) and carotenoids (α- and β-carotene, lycopene, canthaxanthin, β-cryptoxanthin, and lutein + zeaxanthin) determined in this study (Table 1) were within the range reported for other cohorts (1–3, 33). Plasma vitamin E concentrations vary with the concentrations of plasma lipoproteins (13) and, consistent with other reports (23, 28), a high correlation was found between plasma concentrations of α-tocopherol and total cholesterol. The corresponding correlation for γ-tocopherol was weaker. To take this lipid dependent variability into account, the vitamin E status of humans is generally expressed relative to either total plasma lipids or total cholesterol (27, 28). In relation to the risk of cardiovascular disease, the optimum level is estimated to be ≥5.2 μmol vitamin E/mmol cholesterol (29). The mean value (α- plus γ-tocopherol) found in our study group was 5.3 μmol vitamin E/mmol cholesterol (Table 2). In contrast, neither plasma concentration of β-carotene nor that of any of the other carotenoids correlated with plasma total cholesterol (Table 1) or with LDL cholesterol concentrations, indicating that their variability is independent of the plasma cholesterol content. A possible explanation is that plasma concentrations of carotenoids are to a higher extent determined by dietary intake and absorption rather than by metabolic factors associated with cholesterol and lipoprotein metabolism.

The antioxidant content of LDL is generally expressed in relation to the protein or cholesterol content determined in the same LDL sample. Assuming a "typical" LDL composition and an average LDL molecular weight of 2500 kDa (26), the antioxidant content may also be expressed on a molar basis in molecules per LDL particle. This makes data on the antioxidant content better understandable and is particularly useful for a mechanistic interpretation of elementary reactions occurring in the LDL particle itself during oxidation (26, 34). In order to facilitate a comparison with previous reports, we included the LDL antioxidant content expressed as mol/mol LDL in Table 3. The 7.32 mol α-tocopherol/mol LDL found in this study is in excellent agreement with the 7.26 estimated previously as the average value in a group of 149 individuals (5). Moreover, the mol vitamin E (α- plus γ-tocopherol) per mol LDL is close to 8.5 ± 1.6 (mean ± SD) reported by Frei and Gaziano (6) for a group of 62 U.S. individuals (The

value in ref. 6 is: 15.5 ± 2.9 nmol vitamin E/mg protein; the mol/mol value was calculated based on the protein content of 22%, according to ref. 26.) With the exception of α-carotene, LDL concentrations of all carotenoids are also close to values reported previously by us (5, 8) and others (10, 11). The significantly lower α-carotene content of 0.02 mol/mol in refs. 5 and 8 compared with 0.12 mol/mol LDL in this study is likely due to an improved resolution of the HPLC separation that eliminated previously interfering peaks. However, to avoid any misinterpretation that could be introduced by parameters other than those actually measured, we used for the statistical analysis the cholesterol standardized antioxidant content of LDL, i.e., μmol antioxidant/mmol cholesterol (Table 3).

Only a few previous studies comprising low numbers of subjects have investigated the distribution of plasma α-tocopherol and β-carotene among the different lipoprotein fractions (31, 35–40). Traber, Cohn, and Muller (35) showed in a recent publication a figure representing α-tocopherol distribution in a single normolipidemic fasting human subject. From this figure it can be estimated that ~47% of α-tocopherol was present in LDL and roughly 27, 15 and 11% in HDL, IDL and VLDL, respectively. Ogihara et al. (36) reported, for 11 men and 8 women, a distribution in HDL, IDL, and VLDL of 45, 41, 14% for men and 41, 50, 9% for women, respectively. Behrens, Thompson, and Madére (31) investigated 12 subjects (6 males, 6 females) and found 50, 44, and 5% of α-tocopherol in LDL, HDL, and VLDL, respectively, as well as a higher proportion of α-tocopherol in HDL (56%) than in LDL (42%) in females in contrast to 33% and 59% in males. A recent study by Romanchik, Morel, and Harrison (37) including 7 subjects found 44 ± 11% of total plasma α-tocopherol in LDL. Krinsky, Cornwell, and Oncley (38) reported in 1958 that plasma carotenoids were transported with lipoproteins, but quantitative data on carotenoid distribution and inter-individual variance were scarce. Only two studies, one including 7 subjects (37) and another one 22 subjects (39) reported other cartenoids in addition to β-carotene. All studies found that β-carotene contained in LDL accounts for the major proportion of plasma β-carotene, the reported values are 63 to 78% (40), 72 ± 6% (41), 72 ± 10% (37), and 67% (39). For other carotenoids the following values can be found in the literature: lycopene 79 ± 9% (37) and 73% (39), cryptoxanthin 68 ± 9% (37) and 42% (39), α-carotene 58% (39), lutein 44 ± 11% (37), and lutein + zeaxanthin 31% (39). To the best of our knowledge, values for γ-tocopherol or canthaxanthin have so far not been reported. Our study did not show a sex-related difference for α-tocopherol, and the 48 ± 7% of α-tocopherol found in LDL (Table 4) agrees well with the reports cited above. The proportion of γ-tocopherol carried by LDL was 41 ± 7%. We found 87 ± 10% of plasma β-carotene in LDL, which is slightly more than 63 to 78% reported by others. The LDL content of carotenoids (Table 4) suggest that, consistent with other reports (37, 39) their distribution is strongly dependent on their structure.

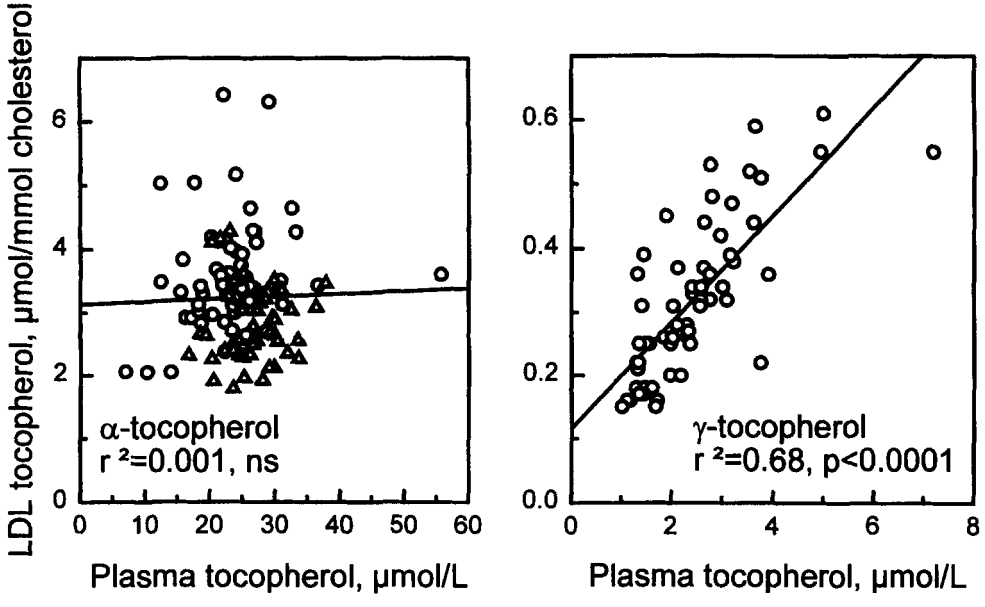

Fig. 1. Correlation between the plasma tocopherol concentration and the tocopherol content of LDL. The left panel shows α-tocopherol (circle, Austrian subjects, triangle, Swiss subjects); the right panel shows γ-tocopherol. The equations for the regression line for α- and γ-tocopherol are y = 0.004 × + 3.1 (n = 102) and y = 0.08 × + 0.12 (n = 59), respectively.

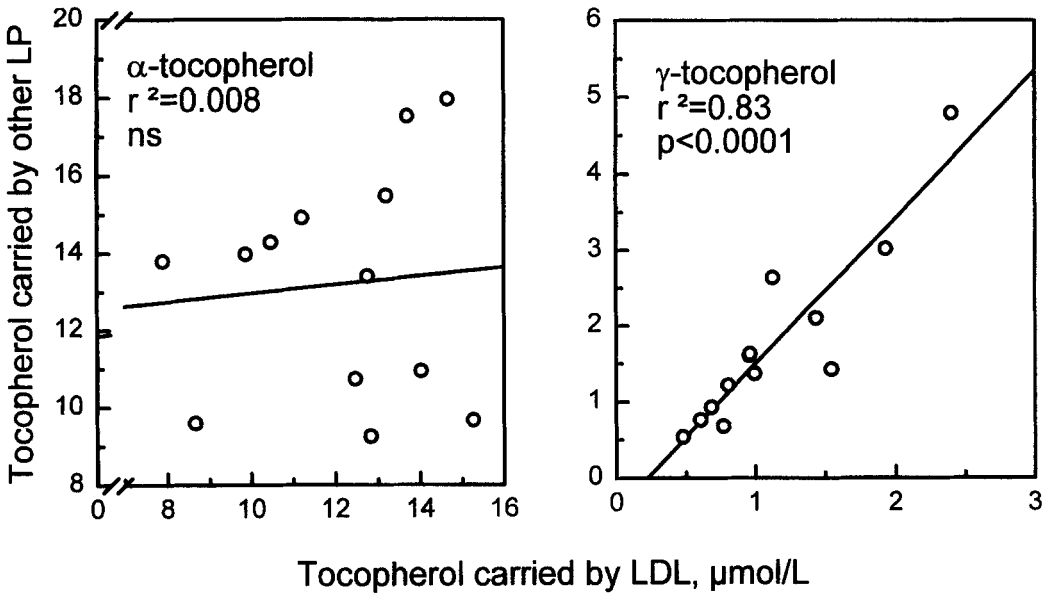

Fig. 2. Correlation between the plasma concentration of tocopherol carried by LDL and the plasma concentration of tocopherol carried by the other lipoproteins. The left panel shows α-tocopherol; the right panel shows γ-tocopherol. The equation for the regression line for γ-tocopherol is y = 1.9 × - 0.43.

The lipophilic carotenoids α- and β-carotene and lycopene are predominantly carried by LDL, whereas the more polar carotenoids (cryptoxanthin, canthaxanthin, lutein + zeaxanthin) are more equally distributed between lipoproteins with 36% (lutein + zeaxanthin), 55% (canthaxanthin) and 58% (cryptoxanthin) in LDL.

The antioxidant content of LDL differs substantially among individuals (5–7) and it is generally assumed that these differences are related to a large extent to differences in plasma antioxidant levels (1–4). Indeed, an increase in plasma α-tocopherol levels by oral intake of vitamin E supplements is accompanied by a more or less linear increase in the LDL α-tocopherol content (7–9). Similar observations were made during β-carotene supplementation (10, 11). In an attempt to show such a relationship for supplemented individuals, we have combined data from our own vitamin E supplementation studies (8, 42) into one plot (**Fig. 4**). It can be seen that the α-tocopherol content of LDL strongly correlated with plasma α-tocopherol (r^2 = 0.56) in this group of individuals. When plasma α-tocopherol concentrations are doubled from 25 to 50 µmol/L, the α-tocopherol content of LDL increases by about 50% (7.7 vs. 11.8 mol α-tocopherol/mol LDL). These and similar findings (7, 9), taken together with the fact that a large proportion of plasma vitamin E and carotenoids is carried by LDL, are likely to have led to the assumption that a similar strong relationship exists in "normal" nonsupplemented individuals. Our study is the first one that has tested this hypothesis. We found that the γ-tocopherol content of LDL is strongly correlated with plasma γ-tocopherol concentrations (Fig. 1). The correlation between the LDL and plasma content of all carotenoids was even stronger (Fig. 3). But rather surprisingly, we did not find a correlation between the α-tocopherol content of LDL and the plasma concentration of α-tocopherol (r^2 = 0.001, ns) nor between the α-tocopherol content of LDL and the cholesterol standardized plasma α-tocopherol concentrations (r^2 = 0.02). Multiple regression analysis revealed that the α-tocopherol content of LDL is to a large extent due to unexplained interindividual variability, whereas plasma concentrations of α-tocopherol have only a weak positive effect and plasma cholesterol concentrations have a weak negative effect.

It might be argued that this lack of a correlation is due to small and inconclusive variation of plasma and LDL α-tocopherol concentrations within a narrow band. However, healthy subjects not taking vitamin E supplements show plasma α-tocopherol concentrations within a range that rarely extends beyond about 10–15 µmol/L on the lower and about 40–50 µmol/L on the upper end (2, 4, 10, 19, 33). In an attempt to ensure that the results of this study are indeed representative for healthy subjects and a correlation is not being missed, we included two, randomly selected groups of healthy subjects from different mid-European populations. The lack of a correlation was observed in both groups when analyzed separately or when combined. γ-Tocopherol, which showed a very strong correlation (Fig. 4), had a coefficient of variation of 46% in plasma and 37% in LDL that

is only slightly larger than the 30 and 33% variation found for α-tocopherol. The inclusion of both males (62%) and females (38%) in the statistics can also be disregarded as having affected the results because separate analyses of males and females gave the same results (data not shown). Finally, limited accuracy and reproducibility of methods to assess the α-tocopherol content of plasma and LDL can also be excluded as a confounding factor because the methods used in this study are well established and have an inter-assay variation of less than 5% (25).

Taken together, we conclude that in nonsupplemented individuals the α-tocopherol content of LDL is determined to a large extent by factors other than the plasma α-tocopherol concentration. Essential for a correlation between plasma and LDL α-tocopherol concentrations may be a rapid exchange of α-tocopherol that leads to a similar distribution among lipoproteins in different subjects. However, if the rate of α-tocopherol incorporation into VLDL in the liver, and perhaps the rate of α-tocopherol uptake by cells and tissues, are more efficient, such a correlation may not be observed. Besides a spontaneous exchange of α-tocopherol among different lipoproteins observed in vitro (13), modulation of its exchange by the phospholipid transfer protein has been reported (14). Compared with the spontaneous exchange, the rate of exchange of α-tocopherol is 2- to 4-fold enhanced by this protein, and its activity seems to be dependent on plasma LDL-cholesterol concentrations (14). The distribution of α-tocopherol has also been shown to be affected by the HDL/LDL ratio (12). On the other hand, Traber et al. (12, 35, 41) described a preferential incorporation of α-versus γ-tocopherol and RRR- versus SRR-α-tocopherol into VLDL in the liver that is mediated by the α-tocopherol-binding protein. Even though α- and γ-tocopherol are equally well absorbed (35), these differences in the handling at the liver level lead to 10 times higher concentrations of α-tocopherol than γ-tocopherol in the blood. It seems also possible that the lipoprotein lipase activity, which is responsible for the catabolism of VLDL to LDL, may affect the proportion of α-tocopherol present in LDL. Therefore it is conceivable, but still needs to be proven, that all of the above factors may influence the correlation between plasma and LDL α-tocopherol concentrations by directly or indirectly affecting the concentration of α-tocopherol in the LDL particles.

If the vitamin E intake is increased by oral RRR-α-tocopherol supplements, a correlation between the α-tocopherol content of LDL and plasma α-tocopherol concentrations can be observed (Fig. 4, left panel) suggesting that the unexplained interindividual variability is to some extent alleviated by high vitamin E intake. A possible mechanism could be the saturation of the α-tocopherol transfer protein in the liver. However, quite large doses of about 10 to 50 times the normal dietary intake are needed and the relationship between LDL and plasma content (r^2 = 0.53) is still weaker than

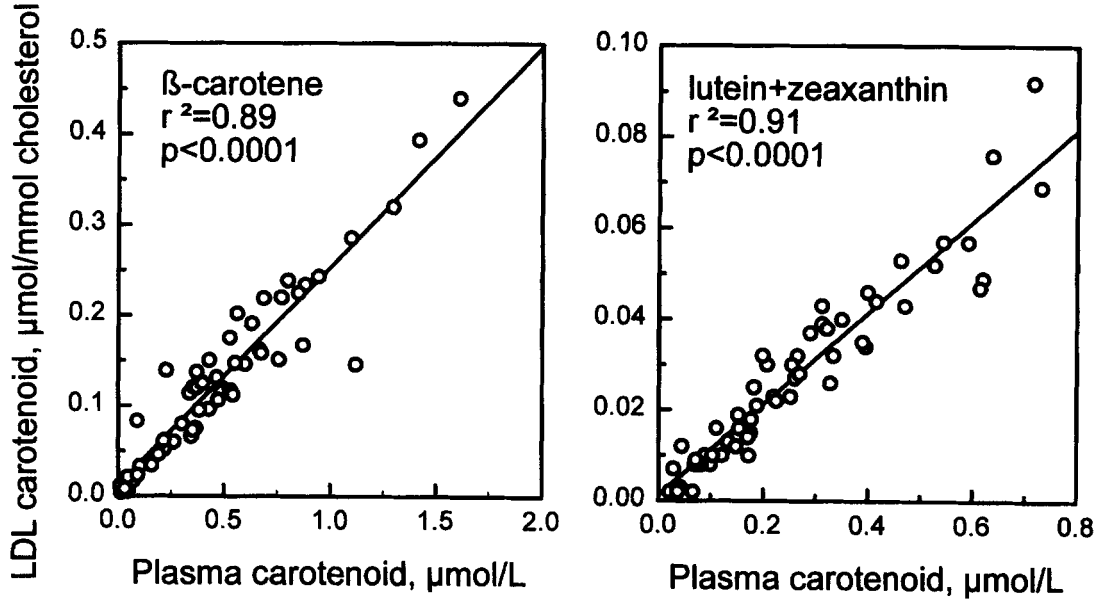

Fig. 3. Correlation between the plasma carotenoid concentration and the carotenoid content of LDL. The left panel shows β-carotene; the right panel shows lutein + zeaxanthin. The equation for the β-carotene regression line is: y = 0.24 × + 0.01; for lutein + zeaxanthin: y = 0.1 × + 0.001. There was also a high correlation for other carotenoids (not shown). The respective equations were: lycopene: y = 0.27 × + 0.001 (r^2 = 0.69); α-carotene: y = 0.20 × + 0.002 (r^2 = 0.83); cryptoxanthin: y = 0.18 × - 0.003 (r^2 = 0.90); canthaxanthin: y = 0.14 × + 0.002 (r^2 = 0.92).

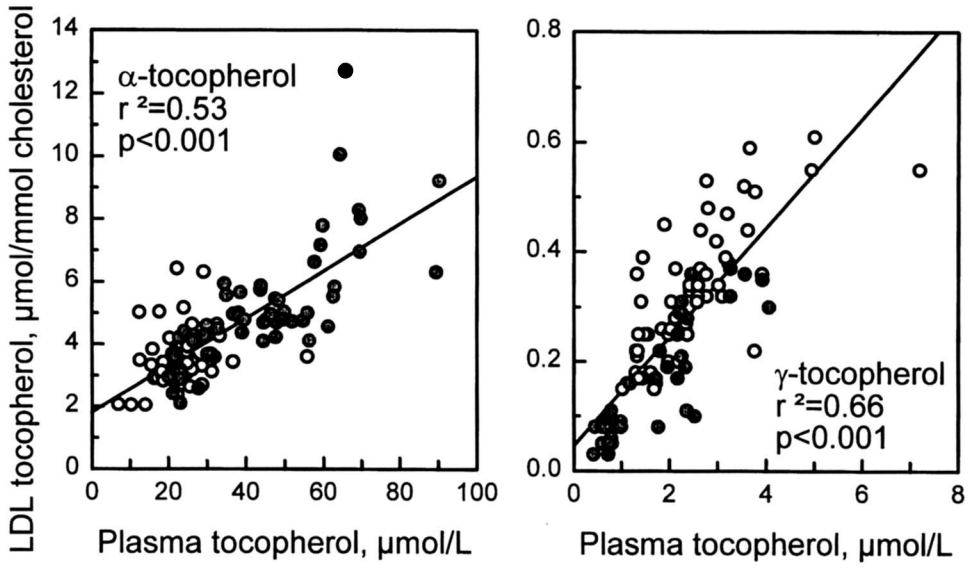

Fig. 4. Correlation between the plasma tocopherol concentration and the tocopherol content of LDL in subjects with and without RRR-α-tocopherol supplementation. The plot was compiled from data obtained in the study described in ref. 8. The left panel shows α-tocopherol; the right panel shows γ-tocopherol (open circles, nonsupplemented subjects; closed circles, subjects supplemented with RRR-α-tocopherol). The equations for the regression line for α- and γ-tocopherol are $y = 0.08 \times + 1.8$ (n = 102) and $y = 0.1 \times + 0.05$ (n = 96), respectively.

that of γ-tocopherol in subjects not taking supplements. In subjects receiving RRR-α-tocopherol supplements, plasma and LDL γ-tocopherol concentrations decrease (8, 9, 23), but the relationship between their plasma and LDL γ-tocopherol concentrations does not change (Fig. 4, right panel). The different handling of α-tocopherol compared with γ-tocopherol described above could eventually explain the differences in the correlation between plasma and LDL concentrations of α- and γ-tocopherol in nonsupplemented subjects.

Several epidemiological studies showed that the incidence of atherosclerosis is inversely related to the dietary intake of antioxidants (including supplements) (20–22) or plasma antioxidant concentrations (19). The evidence for a protective effect is particularly strong for vitamin E and somewhat less so for β-carotene. A plausible explanation for the protective effect of antioxidants is believed to be their ability to inhibit the formation of oxidized LDL (17, 19, 26), which possesses pro-atherogenic properties (17). The lack of a correlation between LDL and plasma α-tocopherol concentrations in nonsupplemented subjects suggests that the α-tocopherol content of LDL might not be the exclusive determinant for the inverse relationship between the risk of cardiovascular disease and the plasma α-tocopherol level. It has previously been shown that α-tocopherol decreases platelet adhesion (43), smooth muscle cell proliferation (44), and monocyte adhesion to endothelium (45), all of which could be major mechanisms by which α-tocopherol exerts its antiatherogenic effect. Studies relating the α-tocopherol content of LDL and HDL to the risk of cardiovascular disease are needed to prove a possible association with these lipoprotein classes.

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