Interactive effects of polyphenols, tocopherol and ascorbic acid on the Cu2+–mediated oxidative modification of human low density lipoproteins

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quercetin, caffeic acid, epicatechin, hesperetin and phloretin as well as α-tocopherol and ascorbic acid through their ability to interact with copper ions. *Methods* Isolated human LDL were incubated with single antioxidants or a combination of two and the kinetics of lipid peroxidation were assessed by measurement of conjugated diene formation (lag phase) via monitoring the absorbance at 234 nm after addition of copper ions. In addition, the degree of oxidation of the LDL protein moiety was followed by tryptophan fluorescence and carbonyl content measurements. *Results* α-Tocopherol and ascorbic acid showed a lower antioxidant activity in all test systems as compared to polyphenols at equimolar concentrations. Quercetin was the most effective compound in all three systems ($p < 0.001$ for lag phase and carbonyl content determination). A significant ($p < 0.001$) prolongation of the lag phase was found when combinations of ascorbic acid/quercetin, ascorbic acid/epicatechin, epicatechin/caffeic acid, and quercetin/epicatechin were tested as compared to the sum of the individual effects. Concerning the effects on LDL protein oxidation, the results from carbonyl content and the tryptophan fluorescence measurements showed that the combination of quercetin and caffeic acid revealed the strongest inhibitory effect ($p < 0.001$ carbonyl content; $p \leq 0.002$ tryptohan fluorescence) on protein oxidation which was higher than the effect of the single compounds. *Conclusions* The results of the present study indicate that a combination of different antioxidants can be superior to the action of single antioxidants in protecting LDL lipid and protein moiety against oxidation. However, the substances may act by different antioxidative mechanisms, which are not necessarily complementary.

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Introduction

Free radicals can be defined as any atom or molecule that contains one or more unpaired electrons. Free radical formation occurs in the human body, and the rates of free radical generation are probably increased in diseases like cancer, atherosclerosis and cataract [1, 2]. When a free radical reacts with a non-radical, a free radical chain reaction is initiated [3]. Oxidation of LDL is a lipid peroxidation chain reaction driven by free radicals and starts when reactive radicals abstract hydrogen from polyunsaturated fatty acids. One of the most commonly used *in vitro* models is the copper-mediated per-

oxidation of LDL [4, 5]. In the healthy human body, metal ions appear largely sequestered in forms unable to catalyze free radical reactions [6, 7]. However injury to tissues may release copper and copper ions have been determined in atherosclerotic lesions [8, 9]. Antioxidants can prevent copper-induced LDL oxidation by peroxyl radical scavenging and metal ion chelation. In general, antioxidants are defined as substances that, when present at low concentrations compared to those of an oxidizable substrate, delay or inhibit oxidation of that substrate [10].

Only limited knowledge is available about any interactions between flavonoids and hydroxycinnamates in inhibiting LDL oxidation. Information on antioxidant interactions of different plant polyphenols would provide a better understanding of the antioxidant effects of phytochemicals in complex mixtures such as fruits. Many foods and beverages contain high levels of phenolic compounds; therefore, these compounds should not be considered in isolation from each other. This study aimed at establishing the dynamic interactions among quercetin, caffeic acid and structurally related compounds (see Fig. 3) as well as α-tocopherol and ascorbic acid and examining the structure-antioxidant activity relationship through their abilities to interact with copper ions.

Material and methods

LDL preparation

Blood samples were drawn from five healthy, non-smoking volunteers with plasma cholesterol levels within the normal range. The plasma was obtained by centrifuging (4000 rpm, 15 min) the blood samples. LDL (d = 1.019–1.063) was isolated by density gradient ultracentrifugation of the plasma according to the method of Havel et al. [11] and stored under nitrogen at 4 °C until use.

LDL were dialyzed in the dark for 16 h at 4 °C against PBS (pH 7.4). The protein concentration of each sample was determined using the Lowry assay [12]. For the measurements of conjugated dienes and tryptophan fluorescence, LDL were diluted to 0.1 mg protein/ml and for the measurement of the carbonyl content to 0.3 mg protein/ml.

LDL were incubated with different compounds either alone or in different combinations for 30 min at 37 °C. In every experiment a control (LDL incubated with the same amount of methanol instead of antioxidant solution) was used. The oxidation reaction was started by addition of copper ions (16.6 μM) and the oxidative modifications of LDL were evaluated through the measurement of conjugated diene formation, tryptophan residue destruction and carbonyl formation.

Formation of conjugated dienes

For the measurement of conjugated diene formation, LDL was incubated with α-tocopherol (5 μM), ascorbic acid (3 μM), quercetin (1 μM), caffeic acid (1 μM), epicatechin (1 μM), hesperetin (1 μM) or phloretin (1 μM) either alone or in different combinations. The kinetic of lipid peroxidation (assessed by conjugated diene formation) was determined spectrophotometrically by continuously monitoring the absorbance at 234 nm [14]. Results from the conjugated diene measurements are expressed in terms of the lag phase. The lag phase is used to measure the resistance of LDL to oxidation and was determined by drawing a tangent to the propagation phase of the curve and extrapolating this line through the horizontal axis. The interval between copper addition and the intersection point on this axis was defined as lag phase. Results are expressed in % increase of the lag-phase compared to control LDL, i. e. LDL from the same person but without addition of antioxidants.

Carbonyl formation

For the measurements of the LDL carbonyl content, LDL was incubated with α-tocopherol (5 μM), ascorbic acid (3 μM), quercetin (1 μM) or caffeic acid (1 μM) either alone or in different combinations. Apo B carbonyls were measured spectrophotometrically with the use of the carbonyl-specific reagent DNPH as previously described [15]. A total of 0.2 ml DNPH in 2 M HCl was added to native or oxidized LDL (300 protein/ml). After incubation at room temperature for 1 h, 0.6 ml denaturating buffer (0.15 M sodium phosphate buffer containing 3 % SDS) was added and mixed. Then ethanol and heptane (1.8 ml of each) were added, mixed for 1 minute, and centrifuged, and the precipitated protein was isolated from the interface. The protein was washed three times with 1.5 ml ethylacetate/ethanol (1/1, v/v) and dissolved in 1 ml denaturating buffer. Each sample was measured spectrophotometrically (360 nm) against denaturating buffer. The carbonyl content was calculated from the absorbance (360 nm) using an absorption coefficient ε of 22.000 M^{-1} cm^{-1} [15–17].

Measurement of LDL-tryptophan fluorescence

For the determination of LDL tryptophan fluorescence intensity, LDL from two volunteers was incubated with α-tocopherol (2.5 μM), ascorbic acid (1.5 μM), quercetin (0.5 μM) or caffeic acid (0.5 μM) either alone or in different combinations. The concentrations used in these experiments were lower than the concentrations for the conjugated dienes and carbonyl measurements. With higher concentrations a measurement would not have

been possible due to timely and organizational reasons. All fluorescence measurements were performed with a fluorescence spectrophotometer F-4500 (Hitachi, Tokyo, Japan). Before and at various time points after addition of copper ions the decrease of the LDL-tryptophan fluorescence in the presence of Cu^{2+} ions was measured at an emission wavelength of 331 nm, with excitation set at 282 nm [18, 19]. The tryptophan fluorescence was measured at intervals of 10 min. The results are displayed in fluorescence intensity (% of the highest measured value).

Statistical analysis

The results are given in terms of mean and standard deviation. A statistical analysis of the results was performed by using SPSS 11.0 for Windows. A one-way ANOVA was used to compare the means followed by Duncan multiple comparison test.

Results

Formation of conjugated dienes

The formation of conjugated dienes in the lipid part of the LDL gives information about the susceptibility of the LDL samples towards oxidation. A prolonged lag phase means an increased resistance of the LDL towards oxidation. To evaluate the influence of the selected antioxidants on LDL oxidation, these compounds were studied systematically alone or in combinations of two compounds. Results are expressed in % above 100 % control LDL.

The results showed that α-tocopherol (5 μM) and ascorbic acid (3 μM) had lower antioxidant activities than the polyphenols tested (Table 1). LDL incubated with α-tocopherol showed a prolongation of the lag phase by 74 ± 24.2 % (Mean ± SD, n = 5) and ascorbic acid by 47 ± 14.1 %, in comparison to control LDL (LDL without antioxidants). Compared to the other antioxidants tested, quercetin (1 μM) was the most effective compound, prolonging the lag phase of LDL during copper-mediated oxidation by 108 ± 15.5 % ($p < 0.001$). Caffeic acid (1 μM) (80 ± 24 %) and epicatechin (1μM) (74 ± 20.9 %) showed a strong inhibition of the lag phase. Hesperetin (1 μM) and phloretin (1 μM) were the weakest of the polyphenols prolonging the lag phase by 38 ± 14.2 and 31 ± 14.7 %, respectively.

The incubation of LDL with α-tocopherol and ascorbic acid or with α-tocopherol and phloretin resulted in lower antioxidant activity than the individual effects (Table 1). Incubation of LDL with combinations of α-tocopherol and a polyphenol resulted in lower antioxidant activities than expected by summing up the activities of the individual substances. The combination of ascorbic acid with a polyphenol showed a stronger protection against LDL oxidation than the corresponding polyphenol/α-tocopherol mixture (Table 1), although ascorbic acid had a lower individual antioxidant activity than α-tocopherol. Incubation of LDL with ascorbic acid in combination with a polyphenol showed additive antioxidant effects. Only when LDL was incubated with ascorbic acid and phloretin, the antioxidant activity was lower than the sum of the individual effects.

The combinations epicatechin/hesperetin, epicatechin/phloretin, caffeic acid/hesperetin or hesperetin/phloretin showed an additive protection against LDL lipid oxidation. Synergistic interactions were found when combinations of caffeic acid/phloretin, caffeic

Table 1 Prolongation of the lag-phase of LDL (% above controls) after incubation with 5 μM α-tocopherol, 3 μM ascorbic acid and different polyphenols (1 μM) in comparison to control LDL (without additional antioxidants) (Mean ± SD, n = 5) and the expected effect (sum of the individual effects)

Antioxidants	Mean ± SD	Expected effect (sum of individual effects)
α-Tocopherol	73.5 ± 24.2	
Ascorbic acid	46.6 ± 14.1	
Quercetin[a]	107.6 ± 15.5	
Epicatechin	73.7 ± 20.9	
Hesperetin	38.1 ± 14.2	
Caffeic acid	80.1 ± 24.0	
Phloretin	31.3 ± 14.7	
α-Tocopherol and ascorbic acid	59.5 ± 16.1	120
α-Tocopherol and quercetin[c]	137.0 ± 40.2	181
α-Tocopherol and epicatechin	126.1 ± 35.8	147
α-Tocopherol and hesperetin	92.0 ± 9.3	112
α-Tocopherol and caffeic acid[b, c]	103.5 ± 34.5	154
α-Tocopherol and phloretin	58.4 ± 11.3	105
Ascorbic acid and quercetin[b, c]	163.7 ± 45.1	154
Ascorbic acid and epicatechin[b, c]	171.9 ± 82.6	120
Ascorbic acid and hesperetin	91.5 ± 59.2	85
Ascorbic acid and caffeic acid[b]	153.6 ± 38.0	127
Ascorbic acid and phloretin	68.9 ± 20.0	78
Quercetin and epicatechin[b, c]	242.6 ± 122.3	181
Quercetin and hesperetin	156.6 ± 23.1	146
Quercetin and caffeic acid[b]	244.0 ± 111.3	188
Quercetin and phloretin	163.6 ± 61.5	139
Epicatechin and hesperetin	117.6 ± 33.0	112
Epicatechin and caffeic acid[b, c]	223.2 ± 51.8	154
Epicatechin and phloretin	113.3 ± 23.5	105
Hesperetin and caffeic acid[c]	126.5 ± 26.3	118
Hesperetin and phloretin	62.1 ± 26.7	69
Caffeic acid and phloretin[c]	139.6 ± 25.4	111

[a] Mean (of the individual compound) significantly different from other individual compounds, $p < 0.001$, Dunkan test
[b] Mean (of the combination) significantly different from the individual effects, $p < 0.001$, Dunkan test
[c] Mean (of the combination) significantly different from the sum of the individual effects, $p < 0.001$, Dunkan test

acid/epicatechin ($p < 0.001$, significantly different from the individual effects), quercetin/phloretin, quercetin/caffeic acid ($p < 0.001$), quercetin/epicatechin ($p < 0.001$), ascorbic acid/caffeic acid ($p < 0.001$) and ascorbic acid/epicatechin ($p < 0.001$) were tested (Table 1). These effects were higher than the sum of the individual effects, i. e., the theoretically expected activity.

Carbonyl formation and measurement of LDL-tryptophan fluorescence

For the measurement of the LDL carbonyl content and the tryptophan fluorescence intensity, LDL was only incubated with α-tocopherol, ascorbic acid, quercetin or caffeic acid either alone or in different combinations. Quercetin had the strongest antioxidant effect in these experiments followed by caffeic acid (carbonyl content: at 3h, $p < 0.001$) (Table 2, Fig. 1). When combinations of two antioxidants were tested, the combination of quercetin and caffeic acid showed the strongest effect (carbonyl content at 4 h, $p < 0.001$) (tryptophan fluorescence: after 420 min the mean was different from all other compounds and combinations with $p \leq 0.002$) on the inhibition of the LDL oxidation (Table 2, Fig. 2). When ascorbic acid was paired with quercetin or caffeic acid, a higher antioxidant capacity was obtained as compared to combinations of α-tocopherol with quercetin or caffeic acid, although ascorbic acid alone showed lower antioxidative activity than α-tocopherol.

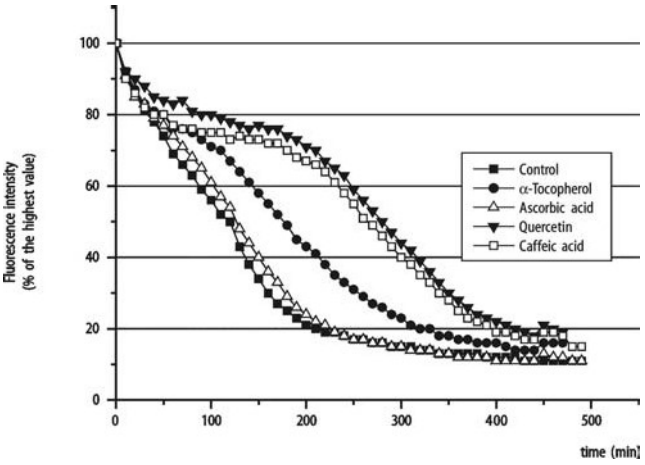

Fig. 1 Tryptophan fluorescence of LDL (Mean, % of the highest measured value, n = 2) after incubation with 2.5 µM α-tocopherol, 1.5 µM ascorbic acid, 0.5 µM quercetin and 0.5 µM caffeic acid at specific time points during copper-induced oxidation

Discussion

In nature, antioxidants exist in combination and these antioxidants may act additively or even synergistically against oxidation. To investigate the influence of different combinations of antioxidants on the copper-mediated LDL oxidation, the individual effect of the different antioxidants had to be tested first. The results showed that α-tocopherol and ascorbic acid had a lower efficacy in inhibiting copper-mediated LDL oxidation compared to the tested polyphenols in relation to their concentrations. This is in agreement with Cao et al. [20] and Wang et al. [21]. They suggested that polyphenols have a higher antioxidant activity than the 'common' antioxidants. They assumed that more than 80 % of the total antioxidant activity of fruits is derived from ingredients other than vitamin E, C and β-carotene.

Since the phenolics occur ubiquitously in the plants, they are consumed in human nutrition in various amounts and reach the plasma as a function of diet.

Table 2 Carbonyl content (nmol carbonly/mg protein) of LDL (Mean ± SD, n = 5) after incubation with 5 µM α-tocopherol, 3 µM ascorbic acid, 1 µM quercetin and 1 µM caffeic acid at specific time points during copper-induced oxidation

	Mean ± SD				
	0 h	1 h	2 h	3 h	4 h
Control	1.3±0.3	7.7±2.8	19.8±4.1	27.0±4.6	32.9±4.7
α-Tocopherol	1.2±0.6	3.2±1.2	12.9±6.7[a]	21.6±7.8	28.8±8.6
Ascorbic acid	1.5±0.3	5.4±2.4	16.3±6.2[a]	24.0±7.7	32.6±9.5
Quercetin	1.5±0.4	4.0±0.6	6.6±1.7	12.6±5.3[a]	21.0±6.9[a]
Caffeic acid	1.4±0.3	3.7±1.0	5.7±0.9	17.7±5.8[a]	29.6±9.5
α-Tocopherol and ascorbic acid	1.5±0.3	3.7±0.9	9.5±4.4	19.5±7.9	29.4±10.7[b]
α-Tocopherol and quercetin	1.6±0.2	3.7±1.0	5.3±1.0	8.0±1.9	14.2±6.2
α-Tocopherol and caffeic acid	1.5±0.3	3.7±1.1	5.0±1.3	10.7±2.8[b]	22.2±6.5[b]
Ascorbic acid and quercetin	1.6±0.6	3.5±1.1	4.8±1.4	6.2±1.8	10.1±6.5[b]
Ascorbic acid and caffeic acid	1.5±0.4	3.8±0.9	5.4±1.2	8.0±2.8[b]	18.5±4.8[b]
Quercetin und caffeic acid	1.4±0.4	4.2±0.9	6.0±2.3	7.7±3.1	10.0±4.2[b]

[a] Mean (of the individual compound) significantly different from other individual compounds, $p < 0.001$, Dunkan test

[b] Mean (of the combination) significantly different from the sum of the individual effects, $p < 0.001$, Dunkan test

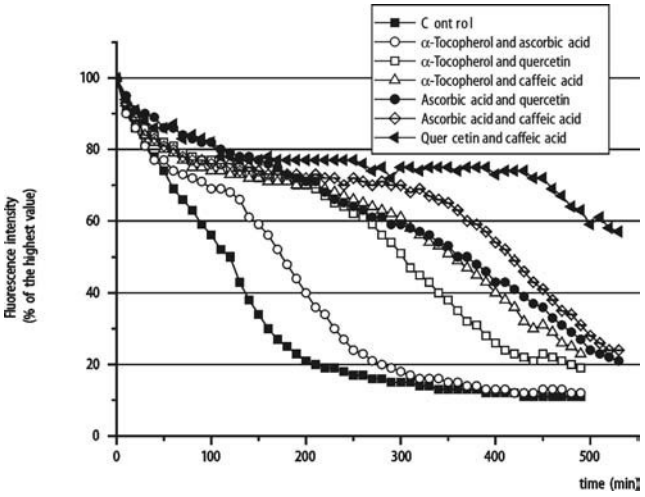

Fig. 2 Tryptophan fluorescence of LDL (Mean, % of the highest measured value, n = 2) incubated with different combinations of 2.5 µM α-tocopherol, 1.5 µM ascorbic acid, 0.5 µM quercetin and 0.5 µM caffeic acid at specific time points during copper-induced oxidation

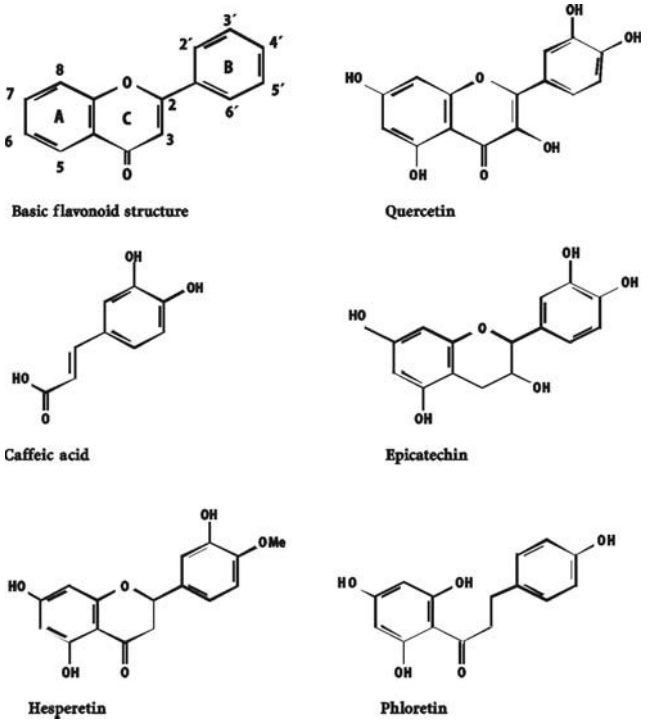

Fig. 3 Basic flavonoid structure and chemical structure of selected polyphenols

Manach et al. [22] measured quercetin plasma concentrations at baseline ranging from 28 to 142 nM and a marked increase was observed 3 h after a quercetin reach meal with a mean plasma concentration of 373 nM. Radtke et al. [23] measured mean plasma concentrations of 23 nmol/l quercetin and 22 nmol/l hesperetin after a mean intake (7-d period) of quercetin and hesperetin of 17.9 and 17.4 mg/d, respectively. After ingestion of orange juice or grapefruit juice a mean plasma hesperetin concentration of 2.2 µmol/L was reported [24]. Rein et al. [25] found epicatechin plasma concentrations ranging from 22 to 257 nmol/l after consumption of chocolate and Lee et al. [26] determined maximum plasma concentrations of epicaetchin after ingestion of green tea of 124.03 ng/ml. Concerning phloretin, Paganga et al. [27] was unable to detect it in plasma but 21 % of the ingested dose was found in the urine.

As compared to the concentrations used in the present study, polyphenol concentrations were higher than the concentration determined in plasma, and for ascorbic acid and tocopherol the concentrations used for the experiments were below usual plasma concentrations.

The most efficient antioxidant in the present study was quercetin followed by epicatechin and caffeic acid. To explain the observed variability in inhibition of LDL oxidation between the different compounds, differences in their structural features can be considered. The group of the phenolic antioxidants embraces a wide range of plant substances that possess an aromatic ring with one or more hydroxyl substituents (Fig. 3). The antioxidant activity of the polyphenols is defined principally by the presence of the orthodihydroxy substituents giving maximum radical stabilization or allowing metal chelation, and an unsaturated 2.3-double bond in combination with a 4-oxo function (responsible for electron delocalization from the B-ring) [7, 28] (Fig. 3). The phenolic compound caffeic acid also retains these features [29], the ortho-dihydroxy structure and a lateral double bond in conjunction with a carbonyl group and the phenol. Compared to the antioxidants quercetin, epicatechin and caffeic acid with an ortho-dihydroxy struture, hesperetin and phloretin had a low antioxidant activity in the present study.

Epicatechin belongs to the group of flavan-3-ols (catechins) which have a saturated heterocyclic ring. The missing conjugation of A- and B-rings as compared to the flavonols (Fig. 3) results in a lower antioxidant activity. The 4-oxo group is only effective in combination with a 2.3-double bond. However, due to the catechol structure epicatechin had a high activity in the present study compared to phloretin and hesperetin.

Flavanols with only one hydroxyl group in the B-ring like hesperetin possess lower antioxidant activity because the location of the hydroxyl group in the B-ring at the 3'-position results in minor electron-donating potential [30]. If the catechol structure is missing the rest of the flavonoid becomes more important and methylation on the 4'-position can activate the 3'-OH as in hesperetin [31]. The hydroxyl groups of the A-ring contribute only little to the antioxidant activity of a substance if the catechol structure is missing. Due to the missing 2.3-double bond in the heterocyclic ring in hes-

peretin no electron delocalization between A- and B-ring can occur [32].

In the present study phloretin showed a low antioxidant activity during copper-induced LDL oxidation due to the missing catechol structure, which contributes to the complexation of copper ions [33]. The 5-hydroxyl group in combination with the 4-oxo group is a binding site for trace metals, but the 2.3-double bond is missing. The double bond in the C-ring allows delocalization between the A and B rings stabilizing the aryloxyl radical after hydrogen donation [34]. Therefore, electron delocalization between A and B rings in phloretin during radical formation is not possible.

The interaction of flavonoids with ascorbic acid has already been shown by different authors, analogous to the synergism between ascorbic acid and α-tocopherol [7, 35, 36].

The ability of flavonoids to act as a defense agent by donating an electron to an oxidant critically depends on the reduction potentials of their radicals [37]. Those flavonoids with a lower redox potential than the ascorbate radical/ascorbate couple should be able to regenerate ascorbate [36]. A synergistic interaction of quercetin and ascorbic acid by the enhancement of the antiproliferative effect has been shown, possibly due to the ability of ascorbic acid to protect the polyphenol from oxidative degradation [38]. This synergistic effect between phenolic acids and ascorbic acid can be explained by recycling of the phenoxyl radical by ascorbic acid and thereby yielding a very stable ascorbyl radical [39, 40].

Flavonoids with a catechol structure in the B ring have a higher oxidation potential in comparison to ascorbic acid [35]. Both quercetin and caffeic acid contain this structural feature. In view of the reduction potentials of the redox couples involved, a regeneration of the caffeic acid radical by ascorbic acid seems to be possible [29, 35, 41]. The differences in antioxidant activities towards LDL oxidation could be ascribed to the differences in the structural features and to other factors, including differences in the solubility and partitioning behavior between the aqueous and lipid phases in the LDL system.

In the present study, incubation of LDL with α-tocopherol and ascorbic acid resulted in a decrease of the lag phase compared to the individual effect of α-tocopherol. This effect was also found when a combination of α-tocopherol and phloretin was tested. On the contrary, the incubation of LDL with α-tocopherol and quercetin, caffeic acid, epicatechin or hesperetin showed additive antioxidant effects. Combinations of ascorbic acid with a polyphenol or a phenolic acid, with the exception of phloretin, resulted in antioxidant effects that were higher than the sum of the individual effects. These effects were higher than those of the corresponding combinations with α-tocopherol, although the individual effect of α-tocopherol was higher compared to ascorbic acid. These results assume a synergism between ascorbic acid and polyphenols against LDL oxidation.

α-Tocopherol is known to act as chain-breaking antioxidant scavenging lipid peroxyl radicals by donating hydrogen to a lipid peroxyl radical that otherwise would propagate the radical chain reaction of lipid peroxidation [29, 42] and is most likely located in the outer monolayer of LDL with the chromanol ring oriented to the aqueous phase [43]. This physical arrangement allows the reaction of the antioxidant-derived radical with water soluble antioxidants like ascorbic acid [29]. The hydrophilic properties of polyphenols and flavonoids facilitate their localization at the interface of the lipid bilayers in membranes and thereby inhibit the initial attack by aqueous radicals [44] and enable the repair of lipophilic free radicals [29]. The arising polypenol radical can be recovered by aqueous phase electron donors, such as ascorbic acid [7, 29, 34].

The antioxidative efficacy of combinations of different antioxidants seems to be based on their structure dependent physical and chemical properties and the related arrangement in the LDL model. The substances possess different antioxidative mechanisms, which are not always complementary. All together, the results of the present study indicate that a combination of different antioxidants may be superior to the action of a single antioxidant in protecting LDL against oxidation. The *in vitro* effects of the antioxidant combinations do of course not reproduce the situation *in vivo*, but can help to get a better understanding of the interactions of different antioxidants *in vivo*.

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References

1. Morton LW, Abu-Amsha CR, Puddey IB, Croft KD (2000) Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin Exp Pharmacol Physiol 27: 152–159
2. Noguchi N, Niki E (2000) Phenolic antioxidants: a rationale for design and evaluation of novel antioxidant drug for atherosclerosis. Free Radic Biol Med 28: 1538–1546
3. Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet 344: 721–724
4. Pinchuk I, Gal S, Lichtenberg D (2001) The dose-dependent effect of copper-chelating agents on the kinetics of peroxidation of low-density lipoprotein (LDL). Free Radic Res 34:349–362
5. Esterbauer H, Ramos P (1996) Chemistry and pathophysiology of oxidation of LDL. Rev Physiol Biochem Pharmacol 127:31–64

6. Halliwell B, Gutteridge JM (1990) The antioxidants of human extracellular fluids. Arch Biochem Biophys 280:1–8
7. Croft KD (1998) The chemistry and biological effects of flavonoids and phenolic acids. Ann N Y Acad Sci 20(854): 435–442
8. Evans PJ, Smith C, Mitchinson MJ, Halliwell B (1995) Metal ion release from mechanically-disrupted human arterial wall. Implications for the development of atherosclerosis. Free Radic Res 23:465–469
9. Smith C, Mitchinson MJ, Aruoma OI, Halliwell B (1992) Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. Biochem J 286: 901–905
10. Halliwell B, Aeschbach R, Loliger J, Aruoma OI (1995) The characterization of antioxidants. Food Chem Toxicol 33: 601–617
11. Havel RJ, Eder HA, Bragdon JH (1955) The distribution and chemical composition of ultracentrifugally seperated lipoproteins in human serum. J Clin Invest 34:1345–1353
12. Lowry OH, Rosebrough NJ, Farr L, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
13. Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W (1991) Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res 61:232–238
14. Esterbauer H, Striegl G, Puhl H, Rotheneder M (1989) Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 6:67–75
15. Yan LJ, Traber MG, Packer L (1995) Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. Anal Biochem 228:349–351
16. Reznick AZ, Packer L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 233:357–363
17. Yan LJ, Lodge JK, Traber MG, Packer L (1997) Apolipoprotein B carbonyl formation is enhanced by lipid peroxidation during copper-mediated oxidation of human low-density lipoproteins. Arch Biochem Biophys 339:165–171
18. Horakova L, Giessauf A, Raber G, Esterbauer H (1996) Effect of stobadine on Cu(++)-mediated oxidation of low-density lipoprotein. Biochem Pharmacol 51:1277–1282
19. Giessauf A, Steiner E, Esterbauer H (1995) Early destruction of tryptophan residues of apolipoprotein B is a vitamin E-independent process during copper-mediated oxidation of LDL. Biochim Biophys Acta 1256:221–232
20. Cao G, Sofic E, Prior RL (1996) Antioxidant capacity of tea and common vegetables. J Agric Food Chem 44: 3426–3431
21. Wang H, Cao G, Prior RL (1996) Total antioxidant capacity of fruits. J Agric Food Chem 44:701–705
22. Manach C, Morand C, Crespy V, Demigne C, Texier O, Regerat F, Rémésy C (1998) Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett 426:331–336
23. Radtke J, Linseisen J, Wolfram G (2002) Fasting plasma concentrations of selected flavonoids as markers of their ordinary dietary intake. Eur J Nutr 41(5):203–209
24. Erlund I, Meririnne E, Alfthan G, Aro A (2001) Plasma kinetics and urinary excretion of the flavanones naringenin and Hesperetin in humans after ingestion of orange juice and grapefruit juice. J Nutr 131:235–241
25. Rein D, Lotito S, Holt R, Keen CL, Schmitz HH, Fraga CG (2000) Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma oxidation status. J Nutr 130:2109S–2114S
26. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS (2002) Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 11(10 Pt 1):1025–1032
27. Paganga G, Rice-Evans CA (1997) The identification of flavonoids as glycosides in human plasma. FEBS Lett 13; 401(1):78–82
28. Böhm H, Boeing H, Hempel J, Raab B, Kroke A (1998) Flavonols, flavone and anthocyanins as natural antioxidants of food and their possible role in the prevention of chronic diseases. Z Ernährungswiss 37:147–163
29. Laranjinha J, Cadenas E (1999) Redox cycles of caffeic acid, alpha-tocopherol, and ascorbate: implications for protection of low-density lipoproteins against oxidation. IUBMB Life 48:57–65
30. Sekher PA, Chan TS, O'Brien PJ, Rice-Evans CA (2001) Flavonoid b-ring chemistry and antioxidant activity: fast reaction kinetics. Biochem Biophys Res Commun 282:1161–1168
31. van Acker SA, Bast A, van der Vijgh WJF (1998) Structural aspects of antioxidant activity of flavonoids. In: Rice-Evans CA, Packer L (eds) Flavonoids in health and disease. Marcel Dekker Inc., New York, pp 221–252
32. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20: 933–956
33. Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042
34. Rice-Evans C, Miller NJ (1998) Structure-Antioxidant Activity Relationships of Flavonoids and Isoflavonoids. In: Rice-Evans C, Packer L (eds) Flavonoids in Health and Disease. Marcel Dekker, Inc., New York, pp 199–219
35. Bors W, Michel C, Schikora S (1995) Interaction of flavonoids with ascorbate and determination of their univalent redox potentials: a pulse radiolysis study. Free Radic Biol Med 19:45–52
36. Cossins E, Lee R, Packer L (1998) ESR studies of vitamin C regeneration, order of reactivity of natural source phytochemical preparations. Biochem Mol Biol Int 45:583–597
37. Jovanovic SV, Steenken S, Simic MG, Hara Y (1998) Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoid radicals. In: Rice-Evans C, Packer JE (eds) Flavonoids in health and disease. Marcel Dekker, Inc, New York, pp 137–161
38. Kandaswami C, Perkins E, Soloniuk DS, Drzewiecki G, Middleton EJ (1993) Ascorbic acid-enhanced antiproliferative effect of flavonoids on squamous cell carcinoma in vitro. Anticancer Drugs 4:91–96
39. Jovanovic SV, Steenken S, Tosic M, Marjanovic B, Simic MG (1994) Flavonoids as antioxidants. J Am Chemical Society 116:4846–4851
40. Steenken S, Neta P (1982) One-electron redox potentials of phenols. Hydroxy- and aminophenols and related compounds of biological interest. J Physical Chemistry 86:3661–3667
41. Buettner GR, Jurkiewicz BA (1993) Ascorbate free radical as a marker of oxidative stress: an EPR study. Free Radic Biol Med 14:49–55
42. Frei B, Keaney JF, Retsky KL, Chen K (1996) Vitamins C and E and LDL oxidation. Vitam Horm 52:1–34
43. Esterbauer H, Dieber-Rotheneder M, Striegl G, Waeg G (1991) Role of vitamin E in preventing the oxidation of low-density lipoprotein. Am J Clin Nutr 53: 314S–321S
44. Terao J, Piskula M (1997) Flavonoids as inhibitors of lipid peroxidation in membranes. In: Rice-Evans C, Packer JE (eds) Flavonoids in health and disease. Marcel Dekker Inc, New York, pp 277–294