Antioxidant Effects of Phenolic Rye (*Secale cereale* L.) Extracts, Monomeric Hydroxycinnamates, and Ferulic Acid Dehydrodimers on Human Low-Density Lipoproteins

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Dietary antioxidants that protect low-density lipoprotein (LDL) from oxidation may help to prevent atherosclerosis and coronary heart disease. The antioxidant activities of purified monomeric and dimeric hydroxycinnamates and of phenolic extracts from rye (whole grain, bran, and flour) were investigated using an in vitro copper-catalyzed human LDL oxidation assay. The most abundant ferulic acid dehydrodimer (diFA) found in rye, 8-*O*-4-diFA, was a slightly better antioxidant than ferulic acid and *p*-coumaric acid. The antioxidant activity of the 8-5-diFA was comparable to that of ferulic acid, but neither 5-5-diFA nor 8-5-benzofuran-diFA inhibited LDL oxidation when added at 10−40 μM. The antioxidant activity of the monomeric hydroxycinnamates decreased in the following order: caffeic acid > sinapic acid > ferulic acid > *p*-coumaric acid. The antioxidant activity of rye extracts was significantly correlated with their total content of monomeric and dimeric hydroxycinnamates, and the rye bran extract was the most potent. The data suggest that especially rye bran provides a source of dietary phenolic antioxidants that may have potential health effects.

Keywords: *Antioxidant; LDL; ferulic acid; ferulic acid dehydrodimers; hydroxycinnamates; phenolic extracts; rye; Secale cereale L.*

INTRODUCTION

Epidemiological studies indicate that a high consumption of whole grain products may reduce the risk of chronic diseases such as cardiovascular disease and certain types of cancer (*1*). These positive physiological properties have mainly been ascribed to dietary fiber, but other constituents, including phenolic acids, may contribute to the beneficial effects (*1*). LDL is the major cholesterol carrier in the blood, and it is well established that an elevated plasma level of LDL is correlated with an increased risk of atherosclerosis and cardiovascular disease (*2*). LDL does not form atherosclerotic plaques in its native form, but oxidative modification of LDL is now recognized as a key event in the pathogenesis of atherosclerosis leading to plaque buildup in arteries and consequently coronary heart disease (*3*, *4*). Dietary antioxidants that inhibit LDL oxidation may therefore be important in protection against these diseases (*3*).

Cereals such as rye, wheat, and barley are rich sources of phenolic acids, especially the hydroxycinnamates and the ferulic acid dehydrodimers (*5*−*8*). In grasses (Gramineae), in which ferulic acid linkages in plant cell walls have been investigated, such as wheat bran, maize bran, and barley (aleurone layer), the main portion of the feruloyl groups is attached to the cell wall arabinoxylan via the acid group acetylating the primary hydroxyl at the C5 position of α-L-arabinofuranosyl residues (*9*, *10*). It has been shown that ferulates can form dimers through oxidative cross-linking (*11*). This cross-linking between esterified feruloyl groups may serve to cross-link cell-wall polymers (*10*). The main ferulic acid dehydrodimers (diFA) hitherto identified in plant material are 8-*O*-4-diFA, 8-5-diFA, 8-5-benzofuran-diFA, 5-5-diFA, and 8-8-diFA non cyclic form, with 8-*O*-4-diFA often being the most prominent (*7*, *11*−*13*). We recently isolated 8-*O*-4-diFA, 8-5-diFA, 8-5-benzofuran-diFA, and 5-5-diFA from rye bran (*14*). The total diFA content in whole rye kernels is ∼300−400 μg/g of dry matter depending on the rye variety, but the concentration in the bran fraction is 10−20 times higher than in the endosperm, and rye bran is thus a rich source of diFA (*5*, *14*).

The antioxidant activities of the simple phenolic acids, the derivatives of benzoic acid and hydroxycinnamic acids, have during the past few years been studied using many different model systems. Despite the different test systems used in determining the antioxidant activity of phenolic acids, the studies, including those involving human LDL as the oxidizing substrate (*15*), rather consistently show that the hydroxycinnamic acids have a higher antioxidant activity compared to the corresponding hydroxybenzoic acids. On in vitro human LDL oxidation, the antioxidant activity improves as the number of hydroxyl and methoxyl groups increases, and particularly the presence of the *o*-dihydroxy group in the phenolic ring, as in caffeic acid, consistently enhances antioxidant activity (*15*, *16*). Although both

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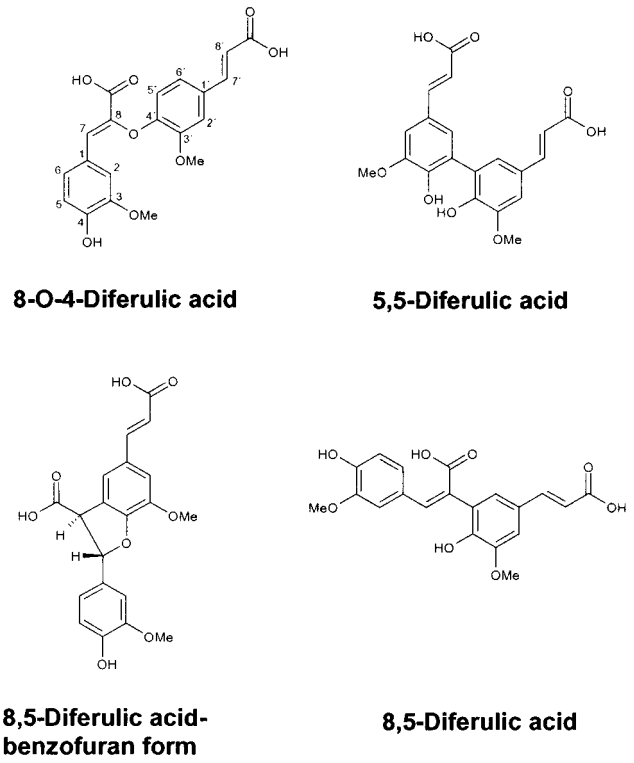

Figure 1. Chemical structures of the four ferulic acid dehydrodimers (diFA) used in this study.

caffeic acid and sinapic acid thus structurally may have higher antioxidant potency than ferulic and *p*-coumaric acid, ferulic acid is the dominant hydroxycinnamic acid occurring in cereal grains (*6*, *8*, *17*). This is also the case in rye, in which the concentration of free and bound ferulic acid in different varieties was recently found to range from 900 to 1170 μg/g of dry matter (*14*). Previously, ferulic acid was recognized as a possible potent antioxidant with potential applications in the pharmaceutical and food industries (*18*). Comparatively little is known about the antioxidant and potential biological activities of the ferulic acid dehydrodimers. Recently, Garcia-Conesa et al. (*19, 20*) showed that the dimers 8-*O*-4-diFA, 8-5-benzofuran-diFA, 5-5-diFA, and the 8-8-diFA noncyclic form exerted antioxidant activity in two in vitro assays: a lipid-based assay measuring the inhibition of peroxidation of phospholipid liposomes and an aqueous assay system measuring the scavenging of ABTS radicals. However, there are no reports on the antioxidant potency of ferulic acid dehydrodimers on LDL.

In the present study we investigated the potential antioxidant properties of phenolic acids found in rye and other cereals. The antioxidant activities of phenolic extracts from rye bran, endosperm, and whole grain as well as of four monomeric hydroxycinnamates and four ferulic acid dehydrodimers (Figure 1) were evaluated using an in vitro oxidation assay based on inhibition of the copper-catalyzed oxidation of human LDL.

MATERIALS AND METHODS

Materials. *trans*-Ferulic, *trans*-*p*-coumaric, *trans*-sinapic, *trans*-caffeic, and gallic acid were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 8-*O*-4-diFA was purified from rye bran, and 8-5-diFA, 8-5-benzofuran diFA and 5-5-diFA were synthesized from ethyl ferulate using peroxidase according to the method of Andreasen et al. (*14*). Human LDL was obtained from Sigma-Aldrich Chemie GmbH. This LDL had been prepared by sequential density ultracentrifugation in the presence of 0.01% w/v EDTA. The LDL was stored and shipped in vials in 0.01% EDTA, 0.15 M NaCl phosphate buffer at pH 7.4−7.5 flushed with argon and always kept at 2−8 °C. All other chemicals were of analytical or HPLC grade purity.

Preparation of Phenolic Extracts from Rye Bran, Flour, and Whole Grain. Whole rye grain (cv. Esprit, harvested in 1997) was milled on a Laboratory Mill (Barbender, Quadrumat Junior). A bran fraction (35% w/w) and a flour fraction (65% w/w) were obtained. The bran fraction was further sieved through a 0.71 mw sieve (dr. st. 0.45), and particles retained on the sieve were used. Bran, flour, and whole grain samples were ground before analysis to a maximum particle size of 0.5 mm. Rye extracts were prepared by treating rye samples with α-amylase and NaOH and then extracting the released phenolics with ethyl acetate as described previously (*5*). The content of phenolic acids in the extracts was analyzed by HPLC according to the method of Andreasen et al. (*5*).

Antioxidant Activity upon LDL Oxidation. Lipid oxidation of human LDL was assessed by spectrophotometric monitoring of conjugated diene lipid hydroperoxide formation at 234 nm during copper-induced oxidation (5 μM copper, 37 °C, pH 7.4) according to a method slightly modified from that of Esterbauer et al. (*21*). The antioxidant activities of the samples were then measured as the inhibition of the formation of the conjugated dienes relative to a control calculated as explained below. To evaluate their antioxidant activity, pure compounds were tested in random order at concentrations of 5−40 μM in the LDL assay. Rye extracts were evaluated at 3−40 μM in total phenolics and compared at equal amounts of sample addition levels of 30 μL. For the LDL oxidation assay human LDL was diluted to a standard protein concentration of 0.2 mg/mL with 0.01 M PBS (0.15 M NaCl) (pH 7.4). Protein was measured according to the method of Lowry et al. (*22*), using bovine serum albumin as a standard. For the assays 450 μL of diluted human LDL solution was diluted in a quartz cuvette with (1340 − x) μL of PBS buffer and x μL of sample; $x = 10$ μL for single phenolic components dissolved in ethanol/water (50:50); $x = 10-30$ μL for rye extracts dissolved in methanol/water (50:50). For control samples additions of x μL of solvent/water (50:50) was used. The oxidation reaction was initiated by the addition of 10 μL of 0.9 mM cupric sulfate solution dissolved in PBS buffer (pH 7.4) resulting in a final concentration of 5 μM copper in the assay mixture. Antioxidant activities of the individual, pure compounds were estimated from the absorbance−time curves (Figure 1) by two different calculation methods: % inhibition $= [(C - S)/C] \times 100$, where C was the maximum absorbance of the control sample and S was the corresponding absorbance of the sample at the time when the control had maximum absorbance (both C and S were corrected for absorbance differences at time 0). If the % inhibition was >0, the tested sample had antioxidant activity to retard LDL oxidation. The 50-factor was calculated from the time it took to attain 50% of maximum absorbance ($t_{50\%}$) in samples versus controls: [$t_{50\%}$ of sample]/[$t_{50\%}$ of control]. If the 50-factor was >1, the tested sample had antioxidant activity to retard LDL oxidation.

Statistics. Samples were analyzed in duplicate for pure phenolic compounds and in triplicate for concentrated rye extracts. Correlation coefficients of phenolic content versus antioxidant activity for rye extracts were determined by linear regression analysis. The statistical significance of the correlations was tested by the dose response F test (*23*). The SAS 6.12 software package (1989−1996, SAS Institute Inc., Cary, NC) was used for statistical analysis.

RESULTS AND DISCUSSION

Antioxidant Activity of Monomeric Hydroxycinnamates on Copper-Induced LDL Oxidation. The antioxidant activities of four hydroxycinnamates, caffeic, ferulic, sinapic, and *p*-coumaric acid, differing

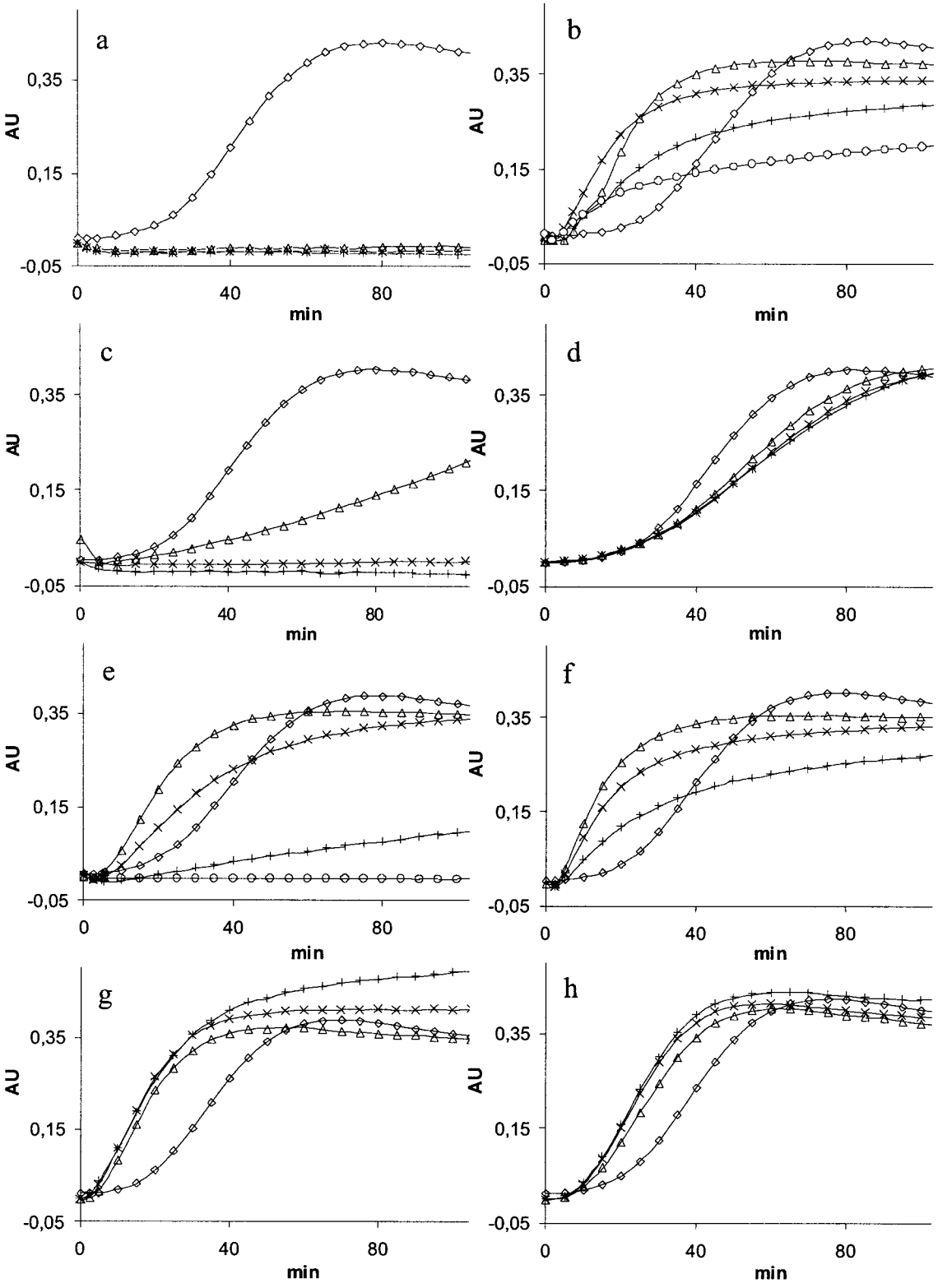

Figure 2. Inhibition of Cu(II)-catalyzed LDL oxidation by hydroxycinnamic acids and diFA, and kinetics of conjugated diene formation in the presence of phenolic compound. LDL (50 μg of protein/mL) was oxidized in PBS (pH 7.4) at 37 °C with 5 μM Cu(II), and absorbance was continuously monitored at 234 nm. The panels show the individual changes in absorption during the assay: a, caffeic acid; b, ferulic acid; c, sinapic acid; d, *p*-coumaric acid; e, 8-*O*-4-diFA; f, 8-5-diFA; g, 5-5-diFA; h, 8-5-benzofuran-diFA; ◇, control; △, 10 μM compound; ×, 20 μM compound; +, 40 μM compound; ○, 60 μM compound.

in hydroxyl and methoxyl substitution in the aromatic ring were compared using the Esterbauer LDL in vitro oxidation assay with Cu^{2+}-catalyzed oxidation and spectroscopic monitoring of conjugated diene hydroperoxides development (*21*). Caffeic acid was the best inhibitor of LDL lipid oxidation and completely blocked formation of conjugated dienes in LDL at all concentrations tested (5, 10, 20, and 40 μM) (Figure 2a; Table 1). In contrast, ferulic acid did not inhibit LDL oxidation by prolonging the induction time, but rather initially promoted conjugated dienes development at the tested concentrations of 10−40 μM (Figure 2b; Table 1). However, at all of the tested concentrations of ferulic acid, the maximum level of conjugated peroxides were

Table 1. Antioxidant Activity of Phenolic Compounds toward Human LDL Oxidation[a]

component group	component	concn (μM)	antiox act. % inhibition[b]	antiox act. 50-factor[c]
hydroxycinnamate acids	caffeic	5	~100	>5
		10	~100	>5
		20	~100	>5
		40	~100	>5
	ferulic	10	11 ± 4	0.4 ± 0.1
		20	20 ± 1	0.4 ± 0.1
		40	34 ± 3	0.9 ± 0.2
		60	55 ± 6	~3
	sinapic	10	64 ± 3	2.4 ± 0.1
		20	~100	>5
		40	~100	>5
	p-coumaric	10	10 ± 1	1.2 ± 0.1
		20	16 ± 1	1.2 ± 0.1
		40	18 ± 2	1.2 ± 0.1
benzoic acids	gallic[d]	5	~100	>5
		15	~100	>5
		20	~100	>5
		40	~100	>5
ferulic acid dehydrodimers	8-*O*-4-diFA	10	9 ± 5	0.5 ± 0.1
		20	18 ± 5	0.7 ± 0.1
		40	81 ± 9	4.1 ± 0.2
		60	~100	>5
	8-5-diFA	10	12 ± 1	0.4 ± 0.1
		20	20 ± 2	0.5 ± 0.1
		40	37 ± 6	1.1 ± 0.4
	5-5-diFA	10	6 ± 7	0.5 ± 0.1
		20	<0[e]	0.5 ± 0.1
		40	<0[e]	0.5 ± 0.1
	8-5-benzofuran-diFA	10	7 ± 2	0.7 ± 0.2
		20	4 ± 2	0.6 ± 0.2
		40	<0[e]	0.6 ± 0.1

[a] Antioxidant activities are expressed as the percent inhibition and the 50-factor. Results are expressed as average ± SD ($n = 2$). [b] Percent inhibition measures the percent inhibition of formation of conjugated diene of a sample relative to the control at the time of maximum absorbance at 234 nm; see the text for details. If the percent inhibition is >0, the tested sample has antioxidant activity to retard LDL oxidation. [c] The 50-factor is calculated as the time it takes to attain 50% of maximum absorbance ($t_{50\%}$) in samples vs controls; see text for details. If the 50-factor is >1, the tested sample has antioxidant activity to retard LDL oxidation. [d] Antioxidant control compound. [e] The extract showed no inhibition; instead, it had a pro-oxidant effect in this LDL oxidation assay.

less than the control's (% inhibition > 0, Table 1), indicating that ferulic acid decreased the maximum concentration of oxidized lipids in LDL. More importantly, ferulic acid addition appeared to retard the rate of conjugated dienes formation at test levels of 40 and 60 μM, as the slopes of these curves were lower than those of the control (Figure 2b). If the antioxidant effect of ferulic acid was calculated according to the 50-factor method, a significant antioxidant activity was seen only at the highest test level (Table 1), and ferulic acid was clearly a poorer antioxidant than caffeic acid in the LDL system. Sinapic acid was a better antioxidant than ferulic acid and significantly retarded the rate of LDL oxidation even at the lowest addition level of 10 μM and completely blocked oxidation at 20 and 40 μM (Figure 2c; Table 1). *p*-Coumaric acid did not prolong the induction time of LDL oxidation at any of the tested doses but showed a weak inhibitory activity on the oxidation by diminishing the rate of LDL oxidation (Figure 2d). This lowering of the oxidation rate was similar at all test concentrations of *p*-coumaric acid. Gallic acid having three hydroxyl groups was used as an antioxidant control compound, and at all tested concentrations gallic acid inhibited the oxidation of LDL by 100% (Table 1).

The overall order of antioxidative activity among the hydroxycinnamates was caffeic acid > sinapic acid ≫ ferulic acid > *p*-coumaric acid. This relative order of antioxidant activity among the hydroxycinnamates is in agreement with the results reported by other investigators (*15*, *16*, *24*−*26*). The observed high antioxidant efficiencies of caffeic acid and sinapic acid are likely related to their capacity to complex copper ions (*15*, *24*). Thus, their inhibitory activity in this LDL system could be entirely due to metal chelation, because the tested levels of the hydroxycinnamic acids exceeded the added concentration of copper. In contrast, *p*-coumaric and ferulic acid may be unlikely to chelate metals (*18*), for which reason these compounds may not exert antioxidant activity via metal chelation. The observed structure−activity relationships are also consistent with the radical-scavenging antioxidant mechanism recognized for other phenolic antioxidants, including flavonoids (*27*). Good free radical scavenging activity thus involves the ability to donate a hydrogen and stabilization of the resulting antioxidant radical by electron delocalization. The high antioxidant activity of caffeic acid may also be due to its two adjacent hydroxy groups being able to form a transient aryloxyl radical having high antioxidant activity: Thus, on the basis of the results obtained in studies of solvent effects on phenolic antioxidants (*28*), it was recently proposed that the strong antioxidant activity of catechol structures could be explained from the presumed low activation energy of the transfer of the second phenolic H atom in the reaction between the antioxidant hydroquinone radical and a lipid peroxyl group to yield the *o*-quinone (*28*). Clearly, hydroxycinnamic acids may inhibit LDL oxidation by several different mechanisms as also discussed elsewhere (*29*).

Antioxidant Activity of Ferulic Acid Dehydrodimers on Copper-Induced LDL Oxidation. The antioxidant activities of four ferulic acid dehydrodimers, 8-*O*-4-diFA, 8-5-diFA, 8-5-benzofuran-diFA, and 5-5-diFA (Figure 1), were compared at equimolar concentrations of 10, 20, and 40 μM dimer, and the 8-*O*-4-diFA was also tested at 60 μM. At low test concentrations, the 8-*O*-4-diFA was unable to inhibit oxidation and tended to exert a prooxidant activity in the test system (Figure 2e). However, when evaluated at addition levels of 40 and 60 μM, the 8-*O*-4-dimer was a good inhibitor of LDL oxidation and more efficient than ferulic acid at equimolar concentrations (Table 1; Figure 2b,e). At 60 μM 8-*O*-4-diFA completely inhibited LDL lipid oxidation (Table 1; Figure 2e). 8-5-diFA had antioxidant activities similar to those of ferulic acid at equimolar concentrations (Figure 2f; Table 1). 5-5-diFA and 8-5-benzofuran-diFA were not able to inhibit LDL oxidation at any of the tested concentrations (10, 20, and 40 μM): For both of these dimers, the induction time was decreased at all tested addition levels, and in effect both acted as prooxidants by promoting conjugated hydroperoxides formation in LDL (Figure 2g,h).

Measurements of the antioxidant capacity of ferulic acid dimers in an aqueous system by measuring the scavenging of ABTS radicals showed that 8-*O*-4-diFA was a better antioxidant than 8-5-benzofuran-diFA and 5-5-diFA (*20*). If the chemical structures of the three

Table 2. Total Phenolics (Micromolar) in Rye Extract[a]

extract from rye	ferulic acid	*p*-coumaric acid	sinapic acid	8-5-diFA	5-5-diFA	8-*O*-4-diFA	8-5-benzofuran-diFA	total phenolics
flour	112	4.3	1.3	1.0	4.7	16	13	152
whole grain	630	38	64	11	30	62	26	862
bran	1882	96	193	27	54	129	67	2248

[a] Rye extracts were prepared from 2 g of rye sample pretreated with α-amylase and saponified with NaOH and then extracted with ethyl acetate (*5*). Evaporated samples were dissolved in 10 mL of methanol/H_2O (50:50) and analyzed by HPLC for content of phenolics. Results are expressed as average of triplicate determination, coefficient of variation on mean < 10%.

dimers are compared, it would be expected that 5-5-diFA would be the best antioxidant due to the fact that it has two free hydroxyl groups per molecule compared to only one free hydroxyl group per molecule for 8-*O*-4-diFA and 8-5-benzofuran-diFA. Furthermore, the dissociation energy of the OH bonds in 5-5-diFA should be lower than that of the OH bond in 8-*O*-4-diFA and 8-5-benzofuran-diFA because more resonance stabilization forms can be formed in the 5-5-diFA molecule (*20*). On the other hand, if the biphenyl moiety of the 5-5-diFA structure is not planar, the conjugation of the aryloxyl radical with the other phenyl ring in the 5-5-diFA biphenyl may be reduced. Nevertheless, both the present study and the study by Garcia-Conesa et al. (*20*) showed that the way the ferulic acid dimers prevent oxidation may be far more complex than what can be predicted from the structural traits of the dimers. Thus, the hydroxycinnamates and the diFAs may exert antioxidant activity to inhibit LDL oxidation by several different mechanisms. These antioxidant mechanisms include free radical scavenging, metal chelation, regeneration of endogenous LDL antioxidants, and perhaps binding to apolipoprotein B in the LDL particle (*29*). The latter may be a unique antioxidant mechanism for LDL oxidation because copper-mediated oxidation of tryptophans in apolipoprotein B initiates lipid oxidation of LDL (*30*).

Antioxidant Activity of Phenolic Extracts From Rye Samples. Phenolic extracts made from rye flour, rye bran, and whole rye grain were also tested for their ability to inhibit LDL oxidation. The three extracts were all made from the same amount of sample and diluted in the same volume, so that the antioxidant potency of the extracts could be compared directly. The contents of phenolic compounds in the three rye extracts are shown in Table 2. As expected, we found a high content of the monomeric and dimeric hydroxycinnamates in the rye bran, a lower content in the extract made from whole rye grain, and a very low content in the extracts made from rye flour (Table 2). The rye bran extracts inhibited LDL lipid oxidation in a dose-dependent manner (Table 3), and addition of 30 μL of the rye bran extract, equivalent to 40 μM total phenolic acids, completely inhibited the lipid oxidation of LDL, whereas 30 μL of the whole flour extract was a weak antioxidant and the same amount of the flour extract seemed to initially act as a weak prooxidant in the assay, but with prolonged contact with the LDL had no effect as its conjugated dienes absorbance curve overlapped the control curve (Table 3; Figure 3).

There was a fine correlation between content of phenolic acids and dimers in the extracts and the antioxidant activity evaluated as percent inhibition; the linear regression equation was $y = 2.6x - 5.3$, with a correlation coefficient $r = 0.97$ ($n = 15$, $P = 1.17 \times 10^{-9}$).

The content of all four diFAs was higher in the bran extract than in the two other rye extracts. However, considering the relatively poor antioxidant activity of the diFAs on LDL oxidation, the high antioxidant activity of the bran extract is presumably not ascribable to the high diFA content: The content of 8-*O*-4-diFA in the rye bran extract made up 9% of the total analyzed phenolics (Table 2), and this level corresponded to an assay concentration of only 2 μM for 8-*O*-4-diFA in the antioxidant evaluation of 30 μL of bran extract. The main phenolic compound in the rye bran extract, ferulic acid, inhibited LDL oxidation by only ~34% at 40 μM, so compounds other than ferulic acid and the diFAs presumably contributed to the high antioxidant activity of the rye bran extract: Sinapic acid could contribute to the antioxidant activity, but this compound made up

Table 3. Antioxidant Activity of Phenolic Extracts from Rye[a]

extract from rye	HPLC total phenolics[d] (μM)	vol of extract added to the assay[e] (μL)	antiox act. % inhibition[b]	antiox act. 50-factor[c]
bran	**15**	11	35 ± 4	1.1 ± 0.1
	20	15	48 ± 6	1.5 ± 0.1
	40	**30**	97 ± 16	>5
whole grain	14	**30**	33 ± 10	1.1 ± 0.1
flour	3	**30**	≤0	0.9 ± 0.1

[a] Inhibition of Cu(II)-catalyzed LDL oxidation by rye extracts was measured as the percent inhibition of formation of conjugated diene hydroperoxide relative to an LDL control sample and as a 50 factor. Results are expressed as average ± SD ($n = 3$). [b] The percent inhibition measures the percent inhibition of formation of conjugated diene of a sample relative to the control at the time where there is maximum absorbance at 234 nm; see the text for details. [c] The 50-factor is calculated as the time it takes to attain 50% of maximum absorbance ($t_{50\%}$) in samples vs controls; see text for details. [d] The total phenolic content in the rye extract samples was calculated from the individual phenolics measured by HPLC; see Table 2. [e] The three rye extracts made from the different parts of rye were made in identical ways, so that the extracts could be directly compared.

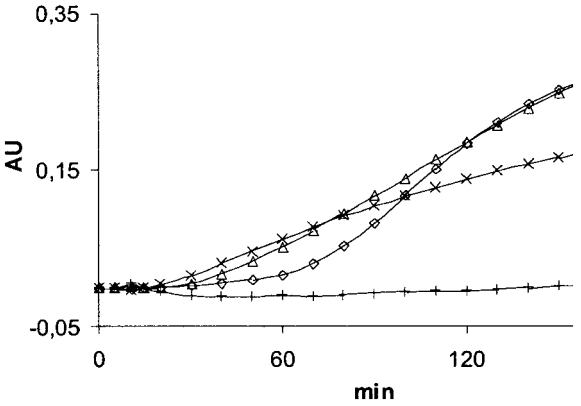

Figure 3. Inhibition of Cu(II)-catalyzed LDL oxidation by rye extracts and kinetics of conjugated diene formation. LDL (50 μg of protein/mL) was oxidized in PBS (pH 7.4) at 37 °C with 5 μM Cu(II), and absorbance was continuously monitored at 234 nm. Individual changes in absorption during the assay are shown: ◇, control; △, 30 μL of rye flour extract; ×, 30 μL of whole rye grain extract; +, 30 μL of rye bran extract.

only 6% of the phenolic content in the rye bran extract (Table 2), a level corresponding to an assay concentration of 3 μM sinapic acid when 30 μL of rye bran was tested. The bran extract thus seemed to have a much higher antioxidant activity than what can be immediately explained from the individual phenolic acids' contribution. The phenolic acids in the rye bran extracts may therefore act synergistically or there may be other components in the extract that contribute to the antioxidant effect. Further work is required to clarify the putative antioxidant substances in rye bran.

Recently, much work has focused on the health-related properties of antioxidants from natural sources. Rye and other cereals have, as mentioned above, a high content of hydroxycinnamates that may have potential health effects. Although hydroxycinnamates and their dimers exist in cereals principally bound as esters to arabinoxylan, the potential phenolic antioxidants were tested in their free forms. To better understand the antioxidant activity of hydroxycinnamates and ferulic acid dehydrodimers in cereals, additional data are needed on the activity of phenolic compounds as they occur naturally in bound forms. The release in the gut of monomeric hydroxycinnamates by microbial esterases and absorption of the free monomeric acids into the circulatory system has been demonstrated in both rats and humans (*31*−*35*). Some of the absorbed hydroxycinnamic acids may be conjugated to form the more soluble glucuronide and/or sulfate conjugates (*36*); however, the structures of these conjugates have not yet been determined. The effect of these conjugated compounds in vivo is still uncertain, and they might have a different effect on the inhibition of LDL oxidation than the free acids. Even if only a small portion of the hydroxycinnamates in rye is absorbed in the human gut, the bound hydroxycinnamates may have an effect in the human colon through the passage. Esterified hydroxycinnamates that are covalently bound to an insoluble rye bran matrix may play an antioxidant role different from that of soluble forms of esterified ferulic acid and the unconjugated free acid (*31*).

Conclusion. The present study shows that the most abundant phenolic acids in rye, ferulic acid, sinapic acid, and the dimer 8-*O*-4-diFA, exert antioxidant activity to inhibit LDL oxidation in vitro. However, the effect of these compounds in vivo is still uncertain as there is a gap in knowledge about the mechanism of absorption and their bioavailability. It also remains unknown whether the compounds are mainly present in the plasma as glucuronide and/or sulfate conjugates or occur in their free form after their eventual absorption. The conjugated compounds may have an effect on the inhibition of LDL oxidation different from that shown by the pure unconjugated hydroxycinnamates. The overall order of antioxidative reactivity among the hydroxycinnamates to inhibit LDL oxidation was caffeic acid > sinapic acid > ferulic acid > *p*-coumaric acid. We found a correlation between the total content of monomeric and dimeric hydroxycinnamates in rye extracts and their antioxidant activity. The antioxidant activity of the rye bran extract was higher than the predicted antioxidant activity based on the calculated concentrations of the identified monomeric and dimeric hydroxycinnamates. This indicates that the phenolic compounds in the rye bran extract may act synergistically and/or the presence of other compounds with antioxidant activity in rye bran. The present study suggests that whole rye grain and especially the rye bran fraction provide a source of dietary phenolic antioxidants that may have potential health effects provided the antioxidant compounds are absorbed and remain in an active form.

ABBREVIATIONS USED

8-*O*-4-diFA, (*Z*)-*β*-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy}-4-hydroxy-3-methoxycinnamic acid; 8-5-benzofuran-diFA, *trans*-5-[(*E*)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid; 5-5-diFA, (*E*,*E*)-4,4′-dihydroxy-5,5′-dimethoxy-3,3′-bicinnamic acid; 8-5-diFA, (*E*,*E*)-4,4′-dihydroxy-3,5′-dimethoxy-*β*,3′-bicinnamic acid; dm, dry matter; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfhonate); EDTA, ethylenediaminetetraacetic acid; PBS, phosphate-buffered saline.

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