JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 3017−3022 **3017**

Polyphenol-Rich Phloem Enhances the Resistance of Total Serum Lipids to Oxidation in Men

JAAKKO MURSU,*,† SARI VOUTILAINEN,† TARJA NURMI,† MERI HELLERANTA,† TIINA H. RISSANEN,†,‡ ANNA NURMI,§ JARI KAIKKONEN,† ELINA PORKKALA-SARATAHO,∥ KRISTIINA NYYSSÖNEN,† JYRKI K. VIRTANEN,† RIITTA SALONEN,†,⊥ AND JUKKA T. SALONEN†,#

Research Institute of Public Health, University of Kuopio, Kuopio, Finland, Department of Public Health and General Practice, University of Kuopio, Kuopio, Finland, Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, Finland, Department of Clinical Chemistry, University Hospital of Kuopio, Kuopio, Finland, Atherosclerosis Research Unit Clinical Research Centre, Mediteknia, University of Kuopio, Kuopio, Finland, and Oy Jurilab Limited, Kuopio, Finland

In humans, polyphenol supplementation studies have resulted in inconsistent findings in lipid peroxidation. Our aim was to investigate the effects of a 4-week consumption of polyphenol-rich phloem on serum lipids and lipid peroxidation in the hydrophilic fraction of serum and on isolated lipoproteins. We conducted a randomized double-blind supplementation study consisting of 75 nonsmoking hypercholesterolemic men. Participants consumed 70 g daily of either rye bread (placebo) or phloem-fortified rye bread containing 31 mg (low polyphenol, LP) or 62 mg (high polyphenol, HP) of catechins. The *ex vivo* susceptibility of total serum lipids and VLDL and LDL to oxidation after copper induction was measured as a lag time to the maximal oxidation rate at the baseline and after the supplementation. In the HP group, an increase in the oxidation resistance of total serum lipids was observed (11.4%), while no effect was seen in the LP group (−0.8%) or in the placebo group (−1.0%) ($p = 0.007$). No differences were observed in the oxidation resistance of VLDL and LDL between the study groups. The phloem also increased *in vitro* oxidation resistance of serum lipids and radical scavenging activity (DPPH•) in a dose-dependent manner. Our results suggest that polyphenols may inhibit lipid peroxidation in the hydrophilic fraction of serum.

KEYWORDS: Lipid peroxidation; antioxidants; polyphenols; phloem; healthy men; randomized double-blind supplementation study

INTRODUCTION

Oxidative modification of low-density lipoprotein (LDL) plays an important role in atherogenesis (*1*−*3*), and it is plausible that agents that prevent oxidation of LDL in the arterial wall also attenuate the development of atherosclerosis (*4*). Among the most potent diet-derived antioxidants are flavonoids and other polyphenols. Flavonoids are widely distributed in vegetables, fruits, and beverages such as tea and wine and are thus consumed daily by most people (*4*−*6*). Evidence from epidemiological studies suggests that high intake of flavonoids may decrease the risk of coronary heart disease (CHD) (*7*). Flavonoids and other polyphenolic compounds have been demonstrated to possess relatively high antioxidant capacity *in vitro* (*8*−*10*). Additionally, evidence from animal studies suggests that ingestion of polyphenols may decrease the oxidation of LDL and retard the progression of atherosclerosis (*11*−*14*). In humans, controlled long-term polyphenol supplementation studies have resulted in inconsistent findings concerning the effects on the resistance of LDL to oxidation (*9*, *15*−*21*). The use of different flavonoids and different methods in assessing the oxidizability of LDL could partly explain the inconsistency of the results. In the supplementation studies, the oxidizability has been determined usually after isolation of LDL from the aqueous phase of serum (*22*). However, it has been speculated that isolation of LDL may not be appropriate for the studies of the effects of polyphenolic compounds on lipoprotein oxidation (*23*). Because of the hydrophilic nature of flavonoids, they may not accumulate sufficiently to LDL to inhibit the oxidation, while they may act in the hydrophilic fraction, e.g., on the surface of

* To whom correspondence should be addressed: Research Institute of Public Health, University of Kuopio, P. O. Box 1627, 70211 Kuopio, Finland. Fax: +358-17-162936. E-mail: jaakko.mursu@uku.fi.
† Research Institute of Public Health, University of Kuopio.
‡ Department of Public Health and General Practice, University of Kuopio.
§ University of Helsinki.
∥ University Hospital of Kuopio.
⊥ Atherosclerosis Research Unit Clinical Research Centre, Mediteknia, University of Kuopio.
Oy Jurilab Ltd.

10.1021/jf048448x CCC: $30.25 © 2005 American Chemical Society
Published on Web 03/18/2005

lipoprotein particles (*12*, *14*, *16*, *23*, *24*). Also, it is possible that a part of the antioxidative effect of flavonoids is due to their ability to chelate oxidizing metals (*10*). The studies of the effect of flavonoids on the oxidation of serum lipoproteins in humans are scarce compared to those dealing with the LDL fraction, but in a recent study, ingestion of a single bolus of green tea resulted in a small decrease in the oxidation susceptibility of lipoproteins (*23*).

The phloem powder, which is manufactured from the bark layer of pine tree, has a very high content of insoluble fiber and different polyphenols such as lignans and different forms of catechins, and thus, it has aroused interest, e.g., in the Nordic countries as a potential part of the healthy diet. In addition to catechins and lignans, phloem contains lignin (*25*), which is a polymer of different phenolic compounds (*26*). It is very likely that part of those phenolic compounds are not completely bound to an insoluble fiber fraction, and therefore, they may be absorbed from the gut like catechins and lignans. Previously, phloem powder has been widely used in Finland as a flour substitute in bread during the times of famine, for example, in the beginning of 20th century. We have shown that lignans in phloem are at least partly bioavailable (*27*), and in addition, French maritime pine bark (*Pinus pinaster*), which resembles phloem in nutritional composition, has been shown to increase antioxidant capacity in humans (*28*). The aim of this randomized double-blind supplementation study was to study the long-term effects of polyphenol-rich phloem on serum lipids and the oxidation of lipoproteins in hypecholesterolemic men.

MATERIALS AND METHODS

Subjects. A total of 75 nonsmoking male volunteers 31−70 years of age were recruited from the Kuopio area in eastern Finland through newspaper advertisements. Potential participants were screened in an initial telephone interview using the following inclusion criteria by a public health nurse: (1) no severe obesity [body mass index (BMI) < 32 kg/m^2], (2) elevated serum cholesterol concentration (total cholesterol of 6−9 mmol/L), (3) no regular use of any drug or supplement with antioxidant (β-carotene or vitamins C or E) or lipid-lowering properties, (4) no chronic diseases such as diabetes, CHD, or other major illness, (5) willingness to consume 70 g of dried rye bread per day for 4 weeks. All criteria were ascertained prior to entering the study by a physician. A written informed consent was obtained from all participants. The study protocol was approved by the Research Ethics Committee, Hospital District of Northern Savo, Kuopio, Finland.

Study Design. The study was a 4-week randomized double-blind supplementation study. Subjects were randomly assigned to consume daily 70 g of normal dried rye bread (placebo group, $n = 30$), rye bread in which 7% of the rye flour was substituted with phloem powder (low polyphenol, LP group, $n = 30$), or bread in which 14% of the rye flour was substituted with phloem powder (high polyphenol, HP group, $n = 15$). The study was conducted in two parts: for the first part, 15 + 15 men were recruited for the placebo and LP groups. To test the effects of a higher amount of polyphenols, in the second part, 15 + 15 + 15 men were recruited for the placebo, LP, and HP groups. The nutrient content of phloem powder and study breads is presented in **Table 1**. The placebo group received 0.6 mg; LP group, 30.8 mg; and HP group, 62.0 mg of catechins daily from the study bread.

The breads used in this study were packed in 30 1-day servings, and subjects were instructed to consume the breads throughout the day. The subjects were advised to discontinue the use of tea, red wine, cocoa, and chocolate 1 week prior to the study and to avoid the use of alcohol and analgesics 3 days and vigorous physical activity 1 day before the study visits. A 4-day food recording was required before and during the last week of the intervention period to control possible confounding factors and to check the compliance with the given instructions. Food records were checked by a nutritionist together with the subjects and then analyzed by using the Nutrica software (version 2.5). The compliance was also checked with a questionnaire designed to assess the amount of breads eaten. Blood samples were drawn with Venoject vacuum tubes (Terumo) after an overnight fast (10 h). All measurements were done at the baseline and after the 4-week supplementation period.

Table 1. Nutrient Content of Phloem Powder and Study Breads per 100 g[a]

nutrient	phloem powder	placebo bread	LP bread	HP bread
energy (kcal)	140.0	243.3	233.5	224.9
protein (g)	2.5	9.0	8.5	8.0
carbohydrates (g)	26.9	46.3	44.4	42.8
fat (g)	2.3	2.1	2.1	2.0
total fiber (g)	57.5	8.4	12.0	14.7
total amount of catechins (mg)	756.0	0.6	44.0	88.5
catechin (mg)	296.9	0.2	24.2	48.9
epicatechin (mg)	9.1	0.4	1.1	2.3
procyanidins (mg)[b]	450.0	0.0	18.7	37.3

[a] LP, low polyphenol bread; HP, high polyphenol bread. [b] Epicatechin-(4β→8)-catechin (B1), epicatechin-(4β→8)-epicatechin (B2), and catechin-(4α→8)-epicatechin (B4).

Resistance of Total Serum Lipids and Isolated VLDL and LDL to Oxidation. The resistance of total serum lipids to oxidation was measured as described previously (*29*). Briefly, serum was diluted to a concentration of 0.67% in 0.02 mol/L phosphate-buffered saline (PBS) at pH 7.4. Oxidation was initiated by addition of 100 μL of 1 mmol/L $CuCl_2$ into 2 mL of diluted, prewarmed (30 °C) serum. The formation of conjugated dienes was followed by monitoring the change in 234 nm absorbance at 30 °C on a spectrophotometer (Beckman DU-6401, Fullerton, CA) equipped with a six-position automatic sample changer. The change in absorbance was recorded every 5 min for 4 h. The time required from the start to the maximal rate of the reaction (lag time) was determined.

Very low-density lipoprotein (VLDL) and LDL were isolated in a combined fraction from fresh ethylenediamine tetraacetic acid (EDTA) plasma by ultracentrifugation. EDTA and gradient salts were removed by gel-permeation columns. VLDL and LDL was exposed to copper-induced oxidation, and the lag time was determined as previously described (*30*).

Other Measurements. Serum cholesterol (Konelab, Espoo, Finland) and triglycerides (Roche Diagnostics, Mannheim, Germany) were determined with enzymatic colorimetric tests. Serum high-density lipoprotein (HDL) cholesterol was measured from supernatant after magnesium chloride dextran sulfate precipitation. The blood cell count, including erythrocyte, leukocyte, and thrombocyte counts and hemoglobin, was measured by a blood cell counter (Cell Dyn 610, Sequoia Turner, Mountain View, CA), and serum aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and creatinine were measured with a clinical chemistry analyzer (Konelab, Espoo, Finland). Serum fatty acids were analyzed after an extraction with chloroform−methanol and methylation with sulfuric acid−methanol by a gas chromatograph (Hewlett−Packard 5890, Avondale, PA) equipped with a flame ionization detector and a NB-351 Capillary column (HNU-Nordion, Helsinki, Finland).

Catechin and Procyanidin Contents of the Phloem Powder and Study Breads. Catechins occur in nature as aglycones, and therefore, the analytes can be directly extracted with a solvent from the sample matrix. Catechins and procyanidins present in the rye bread, phloem bread, and phloem powder were extracted in 2 h at 50 °C with 50% methanol (MeOH) containing 0.1 M hydrochloric acid (*31*). Catechin and procyanidin analyses were carried out with HPLC using a coulometric electrode array detector (ESA, Inc., Chelmsford, MA) as described earlier (*31*). Six different catechins: (+)-catechin, (−)-catechin gallate, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate and three different procyanidins: epicatechin-(4β→8)-catechin (B1), epicatechin-(4β→8)-

epicatechin (B2), and catechin-(4α→8)-epicatechin (B4) were analyzed. Catechin and procyanidin contents of the study breads are presented in **Table 1**.

Both LP and HP breads contained 16% less total catechins than was expected according to the amount of catechins quantified in phloem powder. Heating the phloem powder at 150 °C caused a loss of catechins, and higher temperature increased and speeded up the loss. A decomposition of catechins above the temperature of 130 °C was recently reported also by Pineiro and co-workers (*32*). In our study breads, the loss of procyanidins was 40%, while the loss of total catechins was only 16%. When the amount of catechin monomers and procyanidins in the phloem and phloem breads was determined, it was not possible to distinguish whether the catechin monomers are those, detected in unprocessed phloem, or whether they were cleavage products of procyanidins. Carbon−carbon bonds in procyanidins are relatively stable, but the processing of rye bread dough may have effected procyanidins. Therefore, part of the observed catechin monomers may originate from procyanidins, and a loss of phloem originating catechin monomers may be more intensive than can be shown with the analytical results.

Effects of the Phloem Breads and Catechin on Serum Lag Time *in Vitro*. To study the effects of phloem polyphenols on the oxidation resistance of total serum lipids *in vitro*, the breads were grinded and 100 mg were extracted 3 times with 3 mL of 80% MeOH. Supernatants were taken into 10 mL volumetric flask after the centrifugation, and the flask was filled with 80% MeOH. Into five different vials were taken 0, 10, 50, 100, and 200 μL of phloem bread extract, which was then evaporated under N_2 flow. The dry residue was dissolved in control serum, which was diluted to a concentration of 0.67% with PBS buffer at 30 °C. The oxidation was initiated by the addition of 1 mmol/L $CuCl_2$. The formation of conjugated dienes was followed as described above. The effect of catechin was studied with a pure compound in a separate assay. Different serum was used in the bread assays and catechin assay, and serum in the catechin assay had a lower baseline lag-time value. The *in vitro* concentrations of phloem-derived catechins in serum ranged from 0.01 to 0.24 μg/mL for placebo bread, from 0.88 to 17.6 μg/mL for LP bread, and from 1.77 to 35.4 μg/mL for HP bread. The *in vitro* concentration of the catechin standard in serum ranged from 2.0 to 39.9 μg/mL.

Measurement of 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) Radical Scavenging Capacity of the Study Breads. Extracts of the study breads were prepared by an ultrasonication-assisted extraction with 50% MeOH. The concentration of the extracts obtained was 250 mg of raw material/mL of solvent. For the DPPH assay, these extracts were diluted in MeOH into a range of concentrations to enable determination of IC_{50} value. A total of 600 μL of a DPPH· solution (60 μM in MeOH) was added to 600 μL of each diluted sample, and the resulting solution was allowed to react for 30 min in the dark at ambient temperature. The absorbance caused by the DPPH· radical at 517 nm was determined by a Unicam UV 500 spectrophotometer (Unicam, U.K.) as described earlier (*33*, *34*). Radical scavenging capacity is expressed as $1/IC_{50}$, and values are the means of three replicates.

Statistics. Results are displayed as means and standard deviations (SDs). Means between the study groups were compared by the analysis of variance (ANOVA), and the post hoc Tukey's test was used whenever a statistically significant heterogeneity between the groups was shown by ANOVA. Differences with two-sided *p* value of 0.05 or less were considered significant. The SPSS for Windows, software version 10.0 was used.

RESULTS

All of the 75 recruited men completed the study. Two participants were excluded from the study, one in the LP group because of a pathological concentration of serum triglycerides (8.8 mmol/L) and one in the placebo group because of an insufficient dietary compliance. The physical characteristics or the dietary intake of various nutrients did not differ significantly between the study groups at the entry (**Table 2**) or during the study (data not shown). The activity of ASAT and ALAT enzymes was significantly different between the study groups ($p = 0.050$ and 0.045, respectively) at the study baseline (**Table 3**). According to two 4-day food records and the questionnaire, the compliance of the 73 remaining volunteers to the given dietary and lifestyle instructions was good during the experiment and no adverse effects were reported by the subjects during the study.

Table 2. Baseline Characteristics and Estimated Nutrient Intakes at Baseline Based on a 4-Day Food Recording[a]

	placebo group ($n = 29$)	LP ($n = 29$)	HP ($n = 15$)
age (years)	51.9 ± 12.4	47.5 ± 9.4	54.5 ± 9.2
BMI (kg/m^2)	25.8 ± 2.7	25.3 ± 2.3	26.6 ± 3.4
diastolic blood pressure (mmHg)	81.5 ± 7.7	81.8 ± 8.3	87.2 ± 9.7
energy intake (MJ/day)	9.2 ± 2.3	9.4 ± 2.0	9.2 ± 1.9
total fat (E%)[b]	31.9 ± 5.1	32.8 ± 4.4	32.2 ± 4.4
SAFAs (E%)	12.8 ± 3.2	13.6 ± 3.2	12.8 ± 2.4
MUFAs (E%)	10.7 ± 2.0	10.6 ± 1.6	10.8 ± 2.2
PUFAs (E%)	5.1 ± 1.4	5.3 ± 1.0	5.2 ± 1.7
fiber (g/d)	25.5 ± 8.6	27.3 ± 10.7	26.8 ± 9.2
vitamin E intake (mg/day)	9.2 ± 2.9	10.2 ± 3.5	9.8 ± 3.2
vitamin C intake (mg/day)	76.6 ± 51.2	103.1 ± 70.0	92.4 ± 56.5
β-carotene intake (mg/day)	2.1 ± 2.0	2.8 ± 1.7	2.6 ± 2.0
folate intake (μg/day)	251 ± 66	321 ± 78	268 ± 69

[a] Mean ± SD. LP, low polyphenol group; HP, high polyphenol group. [b] Percentage of total daily energy intake.

The consumption of placebo, LP, or HP bread did not significantly alter the serum total, LDL or HDL cholesterol, or triglyceride concentrations (**Table 3**). An increase in the oxidation resistance of total serum lipids measured as a lag time to the maximal oxidation rate was observed in the HP group (11.4 ± 13.8%), while no effect was seen in the LP group (−0.8 ± 12.8%) or in the placebo group (−1.0 ± 10.8%). According to the Tukey's post hoc analysis, the increase in the oxidation resistance was significantly greater in the HP group as compared with the LP ($p = 0.005$) and with the placebo group ($p = 0.004$). There were no significant differences in the changes of the oxidation resistance of VLDL and LDL between the study groups.

There was an increase in the concentration of serum creatinine in the HP group (7.8 ± 10.1%), while there was no change either in the LP group (0.3 ± 8.1%) or in the placebo group (−1.5 ± 13.7%). In the post hoc analysis, the increase of serum creatinine concentration was significantly greater in the HP group as compared with the LP ($p = 0.04$) and placebo ($p = 0.01$) groups. This significant change observed in the HP group was mostly due to a large increase in the concentration of serum creatinine in a single subject, rising from 84 to 111 μmol/L. We tested that, if this subject would have been excluded from the analysis, the differences between the study groups in the change of serum creatinine were not statistically significant ($p = 0.100$). There were no differences in the other safety measurements, changes of the blood cell count, hemoglobin, activity of serum liver enzymes, or concentrations of serum fatty acids between the study groups.

In our *in vitro* study, the phloem increased the oxidation resistance of serum in a dose-dependent manner (**Figure 1**). After incubation of the placebo, LP, and HP breads, the

Table 3. Serum ASAT, ALAT, Creatinine, Serum Lipoproteins, and Oxidation Kinetics before and after a 4-Week Consumption of Study Breads[a]

	placebo group ($n = 29$)		LP ($n = 29$)		HP ($n = 15$)		
	baseline	change	baseline	change	baseline	change	p^b
ASAT (units/L)	26 ± 6	0 ± 5	27 ± 7	−0 ± 6	31 ± 9	−3 ± 12	0.318
ALAT (units/L)	31 ± 11	3 ± 11	34 ± 18	−1 ± 15	44 ± 20	−5 ± 11	0.139
creatinine (μmol/L)	92 ± 15	−2 ± 14	94 ± 11	−0 ± 7	93 ± 10	7 ± 8	0.037
LDL cholesterol (mmol/L)	4.82 ± 1.15	−0.20 ± 0.59	4.96 ± 0.92	−0.11 ± 0.78	4.77 ± 0.90	−0.12 ± 0.58	0.886
HDL cholesterol (mmol/L)	1.38 ± 0.34	−0.02 ± 0.15	1.26 ± 0.24	0.00 ± 0.15	1.19 ± 0.23	0.02 ± 0.24	0.776
triglycerides (mmol/L)	1.72 ± 0.85	−0.18 ± 0.75	1.74 ± 0.92	0.24 ± 0.68	2.23 ± 1.37	−0.01 ± 1.09	0.152
serum lipid oxidation resistance (lagtime, min)	165 ± 25	−3 ± 17 (28)	180 ± 22	−2 ± 24	175 ± 27	20 ± 23 (12)	0.007
VLDL and LDL oxidation resistance (lagtime, min)	64 ± 5	3 ± 7	66 ± 6	2 ± 9	66 ± 6	−1 ± 3 (14)	0.341

[a] Mean ± SD. $n = 29$ in placebo and LP group and 15 in HP group, except where otherwise indicated in brackets. LP, low polyphenol group; HP, high polyphenol group. [b] p for the differences in changes between the study groups (one-way ANOVA).

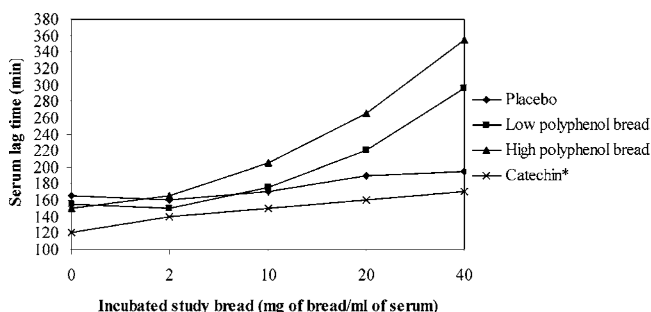

Figure 1. Effects of study breads and catechin on the oxidation resistance of serum. An asterisk indicates that the tests with catechin were conducted in a separate assay with different serum.

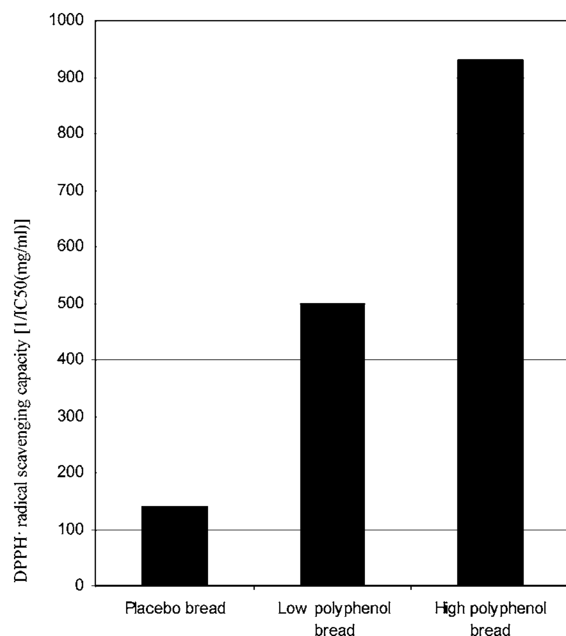

Figure 2. DPPH· radical scavenging capacity [1/IC_{50} (mg/mL)] of the study bread extracts.

oxidation resistance increased 18, 90, and 137%, respectively. The effect of pure (+)-catechin on the oxidation resistance was 42% in comparison to the baseline value in the catechin assay. Radical scavenging capacity (DPPH·) also increased linearly with the amount of phloem in the study breads (**Figure 2**). The radical scavenging capacity of LP and HP bread was 257 and 564% higher, respectively, when compared with the placebo bread.

DISCUSSION

The phloem powder, which is manufactured from the bark layer of pine tree, has a very high content of insoluble fiber and different polyphenols such as lignans and different forms of catechins and thus may have an effect on serum lipids and lipid oxidation. Our aim was to study the long-term effects of phloem on serum lipids and the oxidation of lipoproteins in hypercholesterolemic but otherwise healthy nonsmoking men. The main finding of this study was that, after a 4-week consumption of phloem-fortified bread, the resistance of total serum lipids to oxidation was enhanced in the group receiving the highest amount of polyphenols (62 mg of catechins per day). No effect was seen in the groups that received a lower amount of polyphenols (31 mg of catechins per day) or rye bread. Neither the phloem-powder-enriched rye breads nor the normal rye bread has any detectable effects on the susceptibility of VLDL and LDL to oxidation *ex vivo* or serum lipids. In addition, the phloem increased *in vitro* the resistance of total serum lipids to oxidation and the ability to scavenge radicals in a dose-dependent manner. Our results suggest that phloem polyphenols may increase the oxidation resistance in the hydrophilic fraction of serum but not isolated lipoproteins. In addition, there might be a threshold concentration of phloem polyphenols, which must be achieved, before any effects on lipid peroxidation can be observed, because no effect was seen with the lower amount.

One of the drawbacks of this study was that we did not measure the bioavailability of catechins and procyanidins. However, we have published results from this same study showing that other polyphenolic compounds of phloem, lignans, are bioavailable as phloem supplementation increased the serum concentration of enterolactone (*27*), which is a metabolite of plant lignans present in the phloem. In addition, catechins and procyanidins have been shown to be at least partly bioavailable, and thus, we assume that those compounds were, at least to some extent, absorbed from phloem (*35*). The change in the serum concentration of enterolactone did not correlate with the change in the oxidation resistance of serum (data not shown). Thus, other polyphenols such as catechins are most likely responsible for the increase in oxidation resistance. We also analyzed the amount of different phenolic acids (cinnamic and benzoic acid derivates), flavonols, flavones, and flavonones in the phloem. The amount of phenolic acids was 1.8 mg/g in the phloem itself, while the other compounds were not detected. The amount of terpenes was not determined, and they may have an effect on the lipid peroxidation in humans when present in high concentrations (*36*, *37*).

Oxidative modification of LDL plays an important role in the atherogenesis, and polyphenols with antioxidant properties are candidates for preventing the oxidation of LDL (*1−4*). Even though the results of *in vitro* (*8−10*) and animal (*11−14*) studies are promising, in humans, the findings of polyphenol supplementation on lipid peroxidation studies are inconsistent (*9*, *15−21*). This inconsistency of the effects on LDL oxidation is suggested to be partly related to the method used to assess LDL oxidizability (*23*). The oxidizability of lipoproteins has usually been determined after isolation of LDL. However, it has been suggested that this might not be suitable, because flavonoids as hydrophilic compounds may not accumulate on LDL sufficiently to inhibit the oxidation (*14*, *23*, *24*). van het Hof et al. (1999) found in their study that after a 3-day consumption of tea less than 10% of the catechins found in plasma were distributed in LDL and more than 50% were recovered in the plasma protein fraction (*24*). Furthermore, Lotito and Fraga (*10*) showed that catechins have a stronger antioxidant capacity in the aqueous than the lipid phase. Recently, Hodgson et al. (*23*) studied the effects of short-term tea ingestion on the oxidation of total serum lipids. In the study, the ingestion of a single 400 mL serving of green tea, containing 1.44 g of total polyphenols, resulted in a 4%, even though nonsignificant ($p = 0.17$), increase in the lag time of serum lipid peroxidation, assessed 60 min after the tea consumption (*23*). These observations support the hypothesis that flavonoids may act in the aqueous phase, perhaps on the surface of the lipoprotein particles (*12*, *16*).

The amount of polyphenols ingested daily in the present study was rather low when compared with the previous studies. In the previous similar studies, the amount of catechins and procyanidins ingested per day has been considerably larger, varying from 80 mg to as high as 2500 mg (*9*, *15−21*). In future studies, it would be interesting to test whether the oxidation of total serum lipids could be further attenuated by increasing the amount of polyphenols ingested and whether the increased amount would lead to a sufficient accumulation on LDL and to inhibition of lipid peroxidation also *in vivo*.

In conclusion, polyphenol-rich phloem increased the oxidation resistance of total serum lipids, while no effect was seen in the isolated lipoproteins (LDL and VLDL). In addition, phloem increased the oxidation resistance of serum lipoproteins *in vitro* as well as the ability to scavenge free radicals in a dose-dependent manner. However, further studies are needed to confirm our results and to make a final conclusion about the antioxidant effect of polyphenols *in vivo*.

ABBREVIATIONS USED

BMI, body mass index; CHD, coronary heart disease; HP, high catechin group; HDL, high-density cholesterol; LP, low catechin group; LDL, low-density cholesterol; PBS, phosphate-buffered saline; VLDL, very low-density lipoprotein; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; MeOH, methanol; DPPH•, 1,1-diphenyl-2-picrylhydrazyl.

ACKNOWLEDGMENT

We would like to thank the volunteers for their participation, Tomi-Pekka Tuomainen for valuable discussions during the preparation of this manuscript, public health nurse Pirkko Kanerva for subject recruitment and management, our lab staff at the Research Institute of Public Health for performing blood analyses, Finnpettu Oy for delivering the phloem powder, and Linkosuo Oy for preparing the study breads.

LITERATURE CITED

(1) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Witztum, J. L. Modifications of low-density lipoprotein that increase its atherogenecity. *N. Engl. J. Med.* **1989**, *320*, 915−924.

(2) Salonen, J. T.; Ylä-Herttuala, S.; Yamamoto, R.; Butler, S.; Korpela, H.; Salonen, R.; Nyyssönen, K.; Palinski, W.; Witztum, J. L. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* **1992**, *339*, 883−887.

(3) Salonen, J. T.; Nyyssönen, K.; Salonen, R.; Porkkala-Sarataho, E.; Tuomainen, T. P.; Diczfalusy, U.; Bjorkhem, I. Lipoprotein oxidation and progression of carotid atherosclerosis. *Circulation* **1997**, *95*, 840−845.

(4) Fuhrman, B.; Aviram, M. Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Curr. Opin. Lipidol.* **2001**, *12*, 41−48.

(5) Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(6) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S−2085S.

(7) Huxley, R. R.; Neil, H. A. The relation between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of prospective cohort studies. *Eur. J. Clin. Nutr.* **2003**, *57*, 904−908.

(8) Serafini, M.; Ghiselli, A.; Ferro-Luzzi, A. *In vivo* antioxidant effect of green and black tea in man. *Eur. J. Clin. Nutr.* **1996**, *50*, 28−32.

(9) McAnlis, G. T.; McEneny, J.; Pearce, J.; Young, I. S. Black tea consumption does not protect low-density lipoprotein from oxidative modification. *Eur. J. Clin. Nutr.* **1998**, *52*, 202−206.

(10) Lotito, S. B.; Fraga, C. G. (+)-Catechin prevents human plasma oxidation. *Free Radical Biol. Med.* **1998**, *24*, 435−441.

(11) Hayek, T.; Furhman, B.; Vaya, J.; Rosenblat, M.; Belinky, P.; Coleman, R.; Elis, A.; Aviram, M. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation aggregation. *Arterioscler., Thromb., Vasc. Biol.* **1997**, *17*, 2744−2752.

(12) Yamakoshi, J.; Ktaoka, S.; Koga, T.; Ariga, T. Proanthocyanidin-rich extract from grape seed attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* **1999**, *142*, 139−149.

(13) Xu, R.; Yokoyama, W. H.; Irving, D.; Rein, D.; Walzem, R. L.; German, J. B. Effect of dietary catechin and vitamin E on aortic fatty streak accumulation in hypercholesterolemic hamsters. *Atherosclerosis* **1998**, *137*, 29−36.

(14) Tijburg, L. B.; Wiseman, S. A.; Meijer, G. W.; Weststrate, J. A. Effects of green tea, black tea, and dietary lipophilic antioxidants on LDL oxidizability and atherosclerosis in hypercholesterolaemic rabbits. *Atherosclerosis* **1997**, *135*, 37−47.

(15) de Rijke, Y.; Demacker, P. N. M.; Assen, N. A.; Sloots, L. M.; Katan, M. B.; Stalenhoef, A. F. H. Red wine consumption does not affect oxidizability of low-density lipoprotein in volunteers. *Am. J. Clin. Nutr.* **1996**, *63*, 329−334.

(16) Carbonneau, M.-A.; Leger, C. L.; Monnier, L. Supplementation with wine phenolic compounds increases the antioxidant capacity of plasma and vitamin E of low-density lipoprotein without changing the lipoprotein Cu^{2+}-oxidizability: Possible explanation by phenolic location. *Eur. J. Clin. Nutr.* **1997**, *51*, 682−690.

(17) Nigdikar, S. V.; Williams, N. R.; Griffin, B. A.; Howard, A. N. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation *in vivo*. *Am. J. Clin. Nutr.* **1998**, *68*, 258−265.

(18) Chopra, M.; Fitzsimons, P. E. E.; Strain, J. J.; Thurnham, D. I.; Howard, A. N. Nonalcoholic red wine extract and quercetin inhibit LDL oxidation without affecting plasma antioxidant vitamin and carotenoid concentrations. *Clin. Chem.* **2000**, *46*, 1162−1170.

(19) van het Hof, K. H.; de Boer, H. S. M.; Wiseman, S. A.; Lien, N.; Weststrate, J. A.; Tijburg, L. B. M. Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans. *Am. J. Clin. Nutr.* **1997**, *66*, 1125−1132.

(20) Ishikawa, T.; Suzukawa, M.; Ito, T.; Yoshida, H.; Ayaori, M.; Nishiwaki, M.; Yonemura, A.; Hara, Y.; Nakamura, H. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. *Am. J. Clin. Nutr.* **1997**, *66*, 261−266.

(21) Princen, H. M.; van Duyvenvoorde, W.; Buytenhek, R.; Blonk, C.; Tijburg, L. B.; Langius, J. A.; Meinders, A. E.; Pijl, H. No effect of consumption of green and black tea on plasma lipid antioxidant level and on LDL oxidation in smokers. *Arterioscler., Thromb., Vasc. Biol.* **1998**, *18*, 833−841.

(22) Rice-Evans, C.; Leake, D.; Bruckdorfer, K. R.; Diplock, A. T. Practical approaches to low-density lipoprotein oxidation: Whys, wherefores, and pitfalls. *Free Radical Res.* **1996**, *25*, 285−311.

(23) Hodgson, J. M.; Puddey, I. B.; Croft, K. D.; Burke, V.; Mori, T. A.; Caccetta, R. A.; Beilin, L. J. Acute effect of ingestion of black and green tea on lipoprotein oxidation. *Am. J. Clin. Nutr.* **2000**, *71*, 1103−1107.

(24) van het Hof, K. H.; Wiseman, S. A.; Yang, C. S.; Tijburg, L. B. M. Plasma and lipoprotein levels of tea catechins following repeated tea consumption. *Proc. Soc. Exp. Biol. Med.* **1999**, *220*, 203−209.

(25) Nagy, N. E.; Franceschi, V. R.; Solheim, H.; Krekling, T.; Christiansen, E. Wound-indiced traumatic resin duct development in stems of Norway spruce (*Pinaceae*): Anatomy and cytochemical traits. *Am. J. Bot.* **2000**, *87*, 302−313.

(26) Chen, Y.-R.; Sarkanen, S. Macromolecular lignin replication: A mechanistic working hypothesis. *Phytochem. Rev.* **2003**, *2*, 235−255.

(27) Vanharanta, M.; Mursu, J.; Voutilainen, S.; Rissanen, T. H.; Nurmi, T.; Salonen, R.; Adlercreutz, C. H.; Salonen, J. T. Phloem fortification in rye bread elevates serum enterolactone level. *Eur. J. Clin. Nutr.* **2002**, *56*, 952−957.

(28) Devaraj, S.; Vega-Lopez, S.; Kaul, N.; Schonlau, F.; Rohdewald, P.; Jialal, I. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. *Lipids* **2002**, *37*, 931−934.

(29) Nyyssönen, K.; Porkkala-Sarataho, E.; Kaikkonen, J.; Salonen, J. T. Ascorbate and urate are strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation in Finnish men. *Atherosclerosis* **1997**, *130*, 223−233.

(30) Porkkala-Sarataho, E. K.; Nyyssönen, K. M.; Kaikkonen, J. E.; Poulsen, H. E.; Hayn, E. M.; Salonen, R. M.; Salonen, J. T. A randomized, single-blind, placebo controlled trial of the effects of 200 mg α-tocopherol on the oxidation resistance of atherogenic lipoproteins. *Am. J. Clin. Nutr.* **1998**, *68*, 1034−1041.

(31) Mursu, J.; Voutilainen, S.; Nurmi, T.; Rissanen, T. H.; Virtanen, J. K.; Kaikkonen, J.; Nyyssönen, K.; Salonen, J. T. Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. *Free Radical Biol. Med.* **2004**, *37*, 1351−1359.

(32) Pineiro, Z.; Palma, M.; Barroso, C. G. Determination of catechins by means of extrcation with pressurized liquids. *J. Chromatogr., A* **2004**, *1026*, 19−23.

(33) Goupy, P.; Hughes, M.; Boivin, P.; Amiot, M. J. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts of isolated phenolic compounds. *J. Sci. Food Agr.* **1999**, *79*, 1625−1634.

(34) Ollanketo, M.; Peltoketo, A.; Hartonen, K.; Hiltunen, R.; Riekkola, M.-L. Extraction of sage (*Salvia officinalis* L.) by pressurized hot water and conventional methods: Antioxidant activity of the extracts. *Eur. Food Res. Technol.* **2002**, *215*, 158−163.

(35) Holt, R. R.; Lazarus, S. A.; Sullards, M. C.; Zhu, Q. Y.; Schramm, D. D.; Hammerstone, J. F.; Fraga, C. G.; Schmitz, H. H.; Keen, C. L. Procyanidin dimer B2 [epicatechin-(4*β*-8)-epicatechin] in human plasma after the consumption of a flavonol-rich cocoa. *Am. J. Clin. Nutr.* **2002**, *76*, 798−804.

(36) Grassman, J.; Hippeli, S.; Vollmann, R.; Elstner, E. F. Antioxidative properties of the essential oil from *Pinus mugo*. *J. Agric. Food Chem.* **2003**, *51*, 7576−7582.

(37) Karioti, A.; Hadjipavlou-Litina, D.; Mensah, M. L. K.; Fleischer, T. C.; Skaltsa, H. Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. Rich. (*Annonaceae*) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana. *J. Agric. Food Chem.* **2004**, *52*, 8094−8098.

Received for review September 16, 2004. Revised manuscript received February 10, 2005. Accepted February 14, 2005. This study was supported by grants from the Juho Vainio Foundation and Yrjö Jahnsson Foundation.

JF048448X