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Absorption of Flavonols Derived from Sea Buckthorn (*Hippophaë rhamnoides* L.) and Their Effect on Emerging Risk Factors for Cardiovascular Disease in Humans

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Sea buckthorn (*Hippophaë rhamnoides* L.) is a rich source of flavonols, especially isorhamnetin. Most prospective cohort studies have indicated some degree of inverse association between flavonoid intake and coronary heart disease. Animal and human studies suggest that sea buckthorn flavonoids may scavenge free radicals, lower blood viscosity, and enhance cardiac function. The effects of flavonol aglycones derived from sea buckthorn on the risk factors of cardiovascular disease as well as their absorption were studied in humans. The flavonols, ingested with oatmeal porridge, did not have a significant effect on the levels of oxidized low-density lipoprotein, C-reactive protein, and homocysteine, on the plasma antioxidant potential, or on the paraoxonase activity. Flavonols at two dosages in oatmeal porridge were rapidly absorbed, and a relatively small amount of sea buckthorn oil added to the porridge seemed to have increased the bioavailability of sea buckthorn flavonols consumed at the higher dose.

KEYWORDS: Flavonols; flavonol absorption; oxidized LDL; lipid oxidation; high-performance liquid chromatography; baseline diene conjugation; cardiovascular disease

INTRODUCTION

Experimental studies on animals and cultured human cell lines support the role of polyphenols in the prevention of cardiovascular disease. It is, however, difficult to predict from these results the effects of polyphenol intake on disease prevention in humans (*1*). Epidemiological data on the effect of flavonoids, a major group of polyphenols, on health is conflicting, but most prospective cohort studies have indicated some degree (from weak to modest) of inverse association between flavonoid intake and coronary heart disease (*2*). However, the epidemiological evidence is difficult to interpret because of potential confounding factors. Dietary intake data do not provide specific information on individual compounds that are ingested. In addition to dietary intake data, it is important to use serum biomarkers in the epidemiological studies on associations between flavonoids and risk of diseases. The few clinical studies on biomarkers of oxidative stress and cardiovascular disease risk factors have given contradictory and inconclusive results (*1*).

Sea buckthorn (*Hippophaë rhamnoides* L.) is a yellow-orange berry rich in oil, vitamin C, tocopherols, carotenoids, and flavonoids. The flavonoids in sea buckthorn fruit pulp are mainly flavonols, of which isorhamnetin is typically found in the largest amounts (**Figure 1**). Quercetin is also present, as well as small amounts of kaempferol. In the berries, flavonols are present as glycosides and aglycones. The most important flavonol glycosides in sea buckthorn are isorhamnetin-3-O-sophoroside-7-O-rhamnoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, quercetin-3-O-rutinoside, and quercetin-3-O-glucoside (*3*, *4*). Some studies carried out earlier suggest that sea buckthorn flavonoids may scavenge free radicals, lower blood viscosity, and enhance cardiac function (*5*−*11*). Dietary sea buckthorn oil has been shown to have beneficial effects on skin and to inhibit platelet aggregation, and it may have synergistic effects with sea buckthorn flavonols (*12*, *13*).

A substantial proportion of cardiovascular events occurs in individuals without the established risk factors such as elevated blood pressure, dyslipidemia, smoking, or diabetes mellitus (*14*). In recent years, a number of new potential risk factors or markers have been proposed as predictors of atherosclerosis and its complications. C-reactive protein (CRP) is a circulating acute-phase reactant that is increased many-fold during the inflammatory response to tissue injury or infection (*15*). An evolving body of work suggests that even small increases in CRP within

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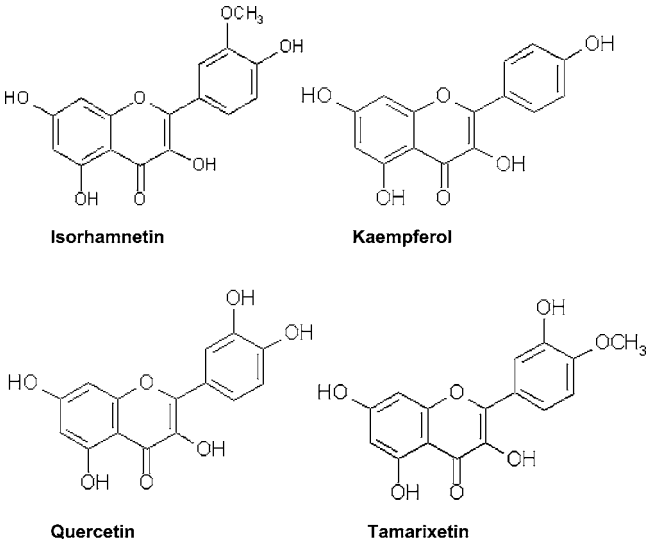

Figure 1. Molecular structures of some flavonol aglycones.

the normal range are predictive of future vascular events (*14*). Homocysteine is a highly reactive amino acid formed as a byproduct of the metabolism of the essential amino acid methionine (*16*). Numerous observational studies have reported on the association between homocysteine levels and vascular risk (*14*). The role of low-density lipoprotein (LDL) in atherogenesis has been known for several years. Studies suggest that the atherogenic LDLs are modified, mainly oxidized, LDL particles (*17*−*19*). Paraoxonase is an antioxidative enzyme that has a role in removing oxidized lipids from LDL and high-density lipoprotein (HDL). Studies suggest that low paraoxonase activity is a risk factor of cardiovascular disease (*20*).

Flavonoids and their in vivo metabolites may affect disease states by their antioxidative properties, by their ability to regulate cell signaling, and also by currently unknown mechanisms (*21*). As opposed to the antioxidative effects, the effects on the modulation of cell signaling are not necessarily changed even if the metabolite would be a weaker antioxidant than the original molecule (*22*).

In the present research project, the effects of a nonglycosidic sea buckthorn flavonol extract (contains 19.4% flavonol aglycones as well as 0.25% proanthocyanidins) on the potential risk factors of cardiovascular disease were studied in human subjects (study 1). The extract was mixed in oatmeal porridge, which was regarded as a good matrix. The absorption and plasma concentrations of the flavonol aglycones were investigated. Also, the effect of added sea buckthorn oil on the absorption and bioavailability of the flavonols was studied (study 2).

MATERIALS AND METHODS

Safety. Volunteers were asked about their health status at a prestudy examination and with a questionnaire. The subjects were healthy. Each subject provided an informed written consent. The study plan was approved by the Ethics Committee of the Hospital District of Southwest Finland.

Chemicals and Reagents. Type HP-2 *Helix pomatia* extract with glucuronidase and sulfatase activity and O,O-diethyl-O-*p*-nitrophenylphosphate were obtained from Sigma (St. Louis, MO), and $Al(NO_3)_3 \cdot 9H_20$ was obtained from Fluka (Buchs, Switzerland). Quercetin, kaempferol, and isorhamnetin were purchased from Extrasynthese (Genay, France). All solvents were of chromatography or analytical grade and were purchased from local suppliers. The oxidized derivatives of synthetic triacylglycerols (TAG) were prepared as described earlier (*23*, *24*).

Table 1. Information on the Subjects[a]

	study 1[b]	study 2[c]
age (years)	46.6 ± 5.6	47.9 ± 7.9
weight (kg)	83.6 ± 9.7	83.6 ± 9.8
height (cm)	180.1 ± 4.7	181.4 ± 6.5

[a] Results expressed as means ± SD. [b] Total amount of subjects: 14. [c] Total amount of subjects: 22.

Human Study 1 (Crossover Study). The double-blind, placebo-controlled, crossover study was designed to investigate the effects of sea buckthorn flavonol extract on biomarkers/risk factors of cardiovascular disease. Fourteen healthy, nonsmoking males (aged 35−53) with a slightly elevated total cholesterol level (5.3−7.2 mmol/L) were recruited for the study (**Table 1**). The effects of oatmeal porridge (portion size, 185 g; contained peeled apple and cinnamon as flavorings) supplemented with a sea buckthorn flavonol extract were studied against a control porridge that did not contain the flavonols. The extract (0.4 g) added to the porridge contained a total of 78 mg of flavonol aglycones, of which 54.1 mg of isorhamnetin, 20.2 mg of quercetin, and 3.4 mg of kaempferol were present. Flavonols were extracted from fruit pulps of sea buckthorn with an ethanol−water solution. The extract was purified by extraction of oil with hexane and removal of sugars with water.

Exclusion criteria were hormonal, renal, hematological, or hepatic dysfunction, a myocardial infarction, treatment with lipid-lowering or nonsteroidal inflammatory drugs, cardiovascular medication, and an ongoing inflammatory state or disease. Each subject provided an informed written consent, and they were free to discontinue their participation in the study at any point without explanation. The study plan was approved by the Ethics Committee of the Hospital District of Southwest Finland.

During two study periods of 4 weeks, the subjects consumed daily a portion of oatmeal porridge with or without the sea buckthorn flavonol extract. The order of the study periods was randomized. The study periods were separated by a washout period of 4 weeks. Before and after each study period, after a 12 h fast, blood samples were collected by venipuncture for the determination of CRP, homocysteine, paraoxonase activity, and conjugated dienes and oxidized TAG in LDL.

Human Study 2 (Absorption Study). The study was designed to investigate the absorption of sea buckthorn flavonols and the influence of sea buckthorn oil on the absorption efficiency. Twenty-two healthy, nonsmoking males (aged 34−59) were recruited for the study on the absorption of the nonglycosidic flavonol extract (**Table 1**). Exclusion criteria were the same as in the crossover study. The subjects provided an informed written consent before the study. The study plan (a continuation of the earlier study) was approved by the Ethics Committee of the Hospital District of Southwest Finland. The subjects were divided randomly into two groups (see footnotes of **Table 3**). During each of two separate visits, after an overnight fast, the subjects were given a single dose of oatmeal porridge (portion size, 185 g; unflavored) containing the flavonol extract (group 1, 78 mg of total flavonols; group 2, 39 mg of total flavonols). Each subject was also given 3 g of sea buckthorn oil mixed in one of the two meals. The oil (donated by Aromtech Ltd., Tornio, Finland) had a standardized composition of a mixture of sea buckthorn seed oil and berry oil extracted with supercritical CO_2. The oil did not contain any flavonoids. The order of the meals (with or without oil) was randomized. The subjects were asked to restrain from flavonol-rich foods for 2 days preceding the meals. Blood samples were collected before and 1, 2, and 4 h after the meals for the determination of the levels of flavonols.

Measurement of Blood Parameters. The blood samples were centrifuged to yield plasma and serum, which were frozen and stored in −70 °C. Plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and glucose were measured by standard spectrophotometric methods. Paraoxonase activity was determined using paraoxon (O,O-diethyl-O-*p*-nitrophenylphosphate) as the substrate and measuring the formation of 4-nitrophenol by increase of absorbance at 412 nm (*25*). Determination of high-sensitivity CRP concentrations was performed from the serum using an ultrasensitive immunoassay (*26*). The

Table 2. Blood Parameters Measured Before and After Study Periods of the Human Study 1[a]

	flavonol period		placebo period	
	before	after	before	after
tot.chol[b]	5.8 ± 0.7	5.6 ± 0.5	5.8 ± 0.6	5.6 ± 0.6
HDL-chol[b]	1.4 ± 0.4	1.4 ± 0.3	1.5 ± 0.4[A]	1.4 ± 0.4[B]
LDL-chol[b]	3.3 ± 0.5	3.4 ± 0.4	3.4 ± 0.3	3.4 ± 0.6
glucose[b]	5.2 ± 0.5	5.3 ± 0.8	5.3 ± 0.6	5.3 ± 0.6
trigly[b]	1.5 ± 0.8	1.6 ± 1.1	1.5 ± 0.6	1.6 ± 0.8
homocysteine[c]	10.1 ± 2.2	9.9 ± 2.3	10.1 ± 2.4	10.0 ± 2.3
CRP[d]	0.8 ± 0.6	0.8 ± 0.6	0.7 ± 0.4	0.9 ± 0.7
BDC[c]	32.0 ± 8.9	32.1 ± 6.4	32.6 ± 10.4	32.1 ± 10.1
TRAP[c]	838 ± 137	793 ± 152	863 ± 176	863 ± 134
paraoxonase[e]	80.9 ± 58.3	76.6 ± 49.8	77.1 ± 51.1	79.5 ± 54.4

[a] Results expressed as means ± SD, $n = 14$. Different letters in a row indicate significant differences between the four time points. Tot.chol, total cholesterol; HDL-chol, HDL-cholesterol; LDL-chol, LDL-cholesterol; trigly, triglycerides; CRP, C-reactive protein; BDC, baseline diene conjugation in 234 nm; TRAP, antioxidant potential masured by resistance of 2,2′-azobis(2-amidinopropane)dihydrochloride-induced peroxidation. [b] mmol/L. [c] μmol/L. [d] mg/L, $n = 12$. [e] units/L.

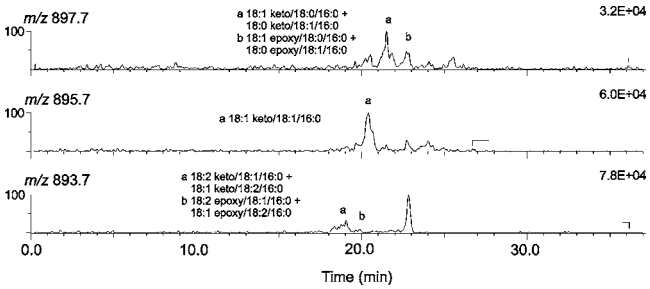

Figure 2. Ion chromatograms showing oxidized LDL TAGs of 52 acyl carbons and their postulated molecular structures (not regioisomers) after a study period with flavonol-containing porridge (pooled sample). Molecules were detected as $[M + Na]^+$ ions.

plasma homocysteine level was analyzed by fluorescence polarization immunoassay (*27*). The antioxidant potential of the plasma samples was measured by their potency in resisting 2,2′-azobis(2-amidinopropane)dihydrochloride (ABAP)-induced peroxidation (TRAP method) (*28*).

Determination of Oxidized Lipids. LDL was isolated by a precipitation method (*29*). The precipitation buffer consisted of 0.064 M trisodium citrate adjusted to pH 5.05 with 5 N HCl and contained 50000 IU/L heparin. Before precipitation of LDL, plasma samples [to which 1 mg/mL of ethylenediaminetetraacetic acid (EDTA) were added] and precipitation reagents were allowed to equilibrate to room temperature. One milliliter of the sample was added to 7 mL of the heparin−citrate buffer. After it was mixed with a Vortex mixer, the suspension was allowed to stand for 10 min at room temperature. The insoluble lipoproteins were then sedimented by centrifugation at 1000*g* for 10 min. The pellet was resuspended in 1 mL of Na−phosphate buffer, pH 7.4, containing 0.9% of NaCl. Total lipids were extracted from LDL using chloroform−methanol (2:1, by vol). For the estimation of oxidation by the baseline level of diene conjugation (BDC) in the lipoproteins (LDL−BDC method), the extracted lipids were dissolved in cyclohexane and analyzed spectrophotometrically (*28*).

To study oxidized TAG molecular structures in LDL, oxidized TAGs were separated from total lipids of LDL by thin-layer chromatography (TLC) and analyzed by high-performance liquid chromatography−electrospray ionization−mass spectrometry (HPLC-ESI-MS) (*23*, *30*, *31*). Heptane/di-isopropyl ether/acetic acid (60:40:4, by vol) solution was used as the mobile eluent in TLC performed with silica G-plates. The HPLC system consisted of a Hitachi (Tokyo, Japan) L-6200 Intelligent Pump with a Discovery HS C18 column (250 mm × 4.6 mm i.d.; Supelco Inc., Bellefonte, PA). The column was eluted at 0.85 mL/min, and a linear gradient was used as follows: 20% 2-propanol in methanol was changed to 80% 2-propanol in 20 min. The final

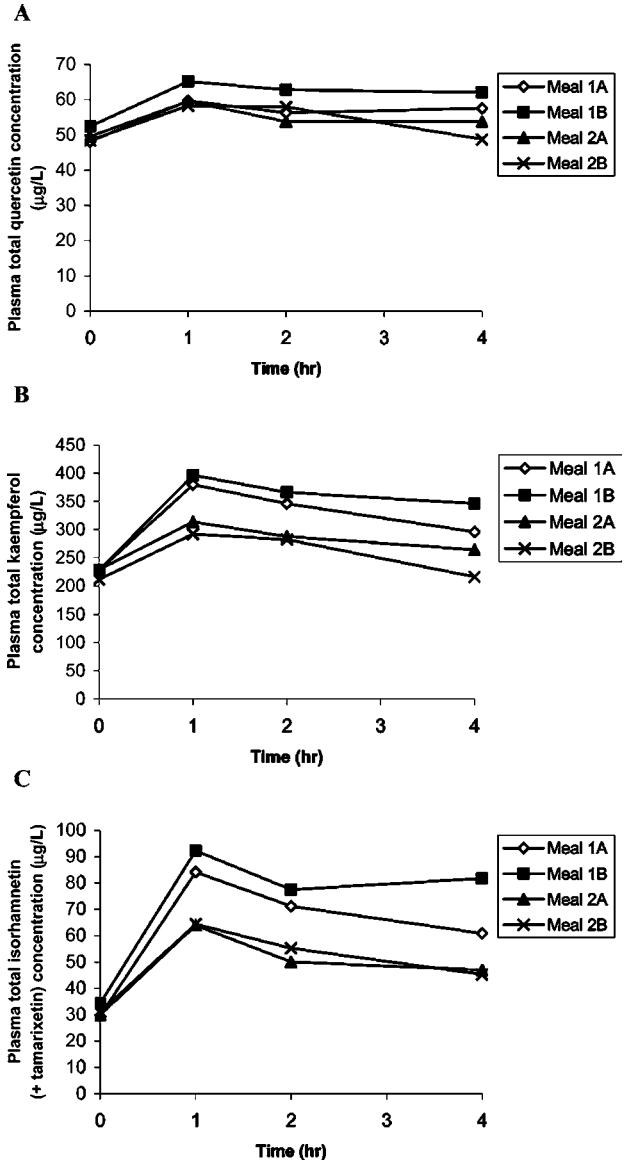

Figure 3. Plasma concentrations (μg/L) of quercetin (**A**), kaempferol (**B**), and isorhamnetin/tamarixetin (**C**) before and 1, 2, and 4 h after different meals (quercetin results are close to the limit of quantitation). Results are expressed as means, $n = 11$. See **Table 3** for the descriptions of the meals.

composition was held for 10 min. Eighty-five percent of the effluent (0.72 mL/min) was led to a Sedex 75 (S.E.D.E.R.E., Alfortville, France) evaporative light scattering detector. An evaporation temperature of 70 °C and a nebulizer gas (air) pressure of 2.7 bar were used in the detector. Fifteen percent of the effluent (0.13 mL/min) was led to a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer (Finnigan, San Jose, CA) equipped with a nebulizer-assisted electrospray interface. Full-scan MS spectra were collected in positive (*m*/*z* 450−1100) ionization mode. The electrospray voltage used was +4.5 kV.

Analysis of Flavonol Absorption. In the absorption study, flavonol conjugates were hydrolyzed to determine plasma levels of quercetin, kaempferol, and isorhamnetin (*32*). A 0.8 mL amount of EDTA plasma was incubated with 88 μL of 0.78 M sodium acetate buffer (pH 4.8), 80 μL of 0.1 M ascorbic acid, and 32 μL of a crude preparation from *H. pomatia*, containing 3200 U of β-glucuronidase and 160 U of sulfatase activity, for 17 h at 37 °C.

After incubation, the sample was diluted with 2 mL of phosphate buffer (70 mM, pH 2.4) and added to a Bond Elut C18 solid-phase extraction column (Varian Inc., Palo Alto, CA), preconditioned with 6 mL of methanol and 6 mL of phosphate buffer. The column was washed with 9 mL of phosphate buffer and 0.5 mL of water. The column was

Table 3. Total Flavonol Concentrations in Plasma Samples (Human Study 2)[a]

	time			
meal[b]	0 h	1 h	2 h	4 h
		quercetin		
1A	48.1 ± 15.2 A; ψ	59.7 ± 19.1 A; ψ	56.3 ± 18.8 A; ψ	57.5 ± 23.0 A; ψ
1B[c]	52.4 ± 16.1 A; ψ	65.2 ± 20.1 A; ψ	62.8 ± 15.2 A; ψ	62.1 ± 23.4 A; ψ
2A	49.6 ± 13.5 A; ψ	59.6 ± 20.6 A; ψ	53.8 ± 13.9 A; ψ	53.8 ± 15.2 A; ψ
2B	48.5 ± 15.7 AB; ψ	58.2 ± 18.4 CD; ψ	57.9 ± 18.9 ACE; ψ	48.8 ± 16.0 BDF; ψ
		kaempferol		
1A	227.4 ± 95.8 A; ψ	379.5 ± 115.0 B; ψ	346.1 ± 103.1 C; ψ	296.0 ± 119.6 D;§ψ
1B[c]	227.4 ± 59.6 A; ψ	396.7 ± 119.9 B; ψ	366.0 ± 98.9 BC; ψ	346.1 ± 138.5 C;§
2A	228.9 ± 54.7 A; ψ	313.7 ± 63.5 BC; ψ	288.0 ± 50.7 CD; ψ	264.2 ± 72.0 AD;§ψ
2B	211.3 ± 47.1 A; ψ	292.1 ± 89.1 B; ψ	282.3 ± 88.1 B; ψ	216.1 ± 60.9 A; ψ
		isorhamnetin (+ tamarixetin)		
1A	29.6 ± 5.2 A; ψ	84.1 ± 26.8 B; §ψ	71.2 ± 22.2 C; §ψ	60.8 ± 21.8 C; §ψ
1B[c]	34.1 ± 14.0 A; ψ	92.3 ± 28.5 B; §	77.5 ± 21.2 C; §	81.7 ± 41.0 BC; §
2A	29.9 ± 3.6 A; ψ	63.9 ± 12.3 B; ψ	50.1 ± 7.7 C; Δ	47.0 ± 9.3 C; ψ
2B	31.0 ± 4.6 A; ψ	64.4 ± 20.6 B; ψ	55.2 ± 15.2 B; ψΔ	45.3 ± 10.0 C; ψ

[a] Results expressed as μg/L (means ± SD, $n = 11$). Means between different time points without a common capital letter differ ($P < 0.05$). Means between different meals within a time point without a common symbol differ ($P < 0.05$). [b] Meal 1A, 78 mg of total flavonols in porridge; 1B, 78 mg of total flavonols and 3 g of sea buckthorn oil in porridge; 2A, 39 mg of total flavonols in porridge; and 2B, 39 mg of total flavonols and 3 g of sea buckthorn oil in porridge. [c] $n = 10$.

dried by suction, and flavonols were eluted into a glass tube with 2 mL of methanol. The methanol eluate was dried with nitrogen and dissolved in a small amount of methanol. The samples were immediately frozen and stored in −70 °C.

Plasma levels of flavonols (quercetin, kaempferol, and isorhamnetin/tamarixetin) were determined by HPLC with fluorescence detection, where $Al(NO_3)_3$ was used as a chelating agent (*33*). The sample was dried and dissolved in methanol−water (1:1, by vol) and was injected onto Phenomenex Prodigy ODS 5 μm (3) column (250 mm × 4.60 mm, 5 μm; Torrance, CA) using acetonitrile/0.025 M phosphate buffer (pH 2.4), 31:69 (by vol), as the mobile phase at a flow rate of 1 mL/min. The column was placed in a column oven set at 30 °C. The column effluent was mixed with 0.4 mL/min 1.5 M $Al(NO_3)_3$ in methanol containing 7.2% (by vol) acetic acid in a postcolumn reactor placed in the column oven. The reactor consisted of a 15 m (0.25 mm internal diameter) Teflon tubing connected to the HPLC column with a low dead volume tee. The fluorescence of the flavonol−metal complexes was measured at 485 nm using a Shimadzu RF-530 (Shimadzu Corporation, Kyoto, Japan) fluorescence detector with an excitation wavelength set at 422 nm. Areas under the concentration−time curve (AUC) were calculated using the trapezoidal rule. Areas under baseline concentration levels were ignored.

Statistical Methods. SPSS 12.0 (Chicago, IL) for Windows was used for data analysis. Analysis of variance for repeated measures and Friedman tests were used to compare the values between different time points. *T*-tests for independent and paired samples as well as corresponding nonparametric tests were used to compare individual pairs between groups and time points. Appropriate Bonferroni corrections were used.

RESULTS AND DISCUSSION

In human study 1, the porridge with the sea buckthorn flavonol extract did not have effects on most of the parameters measured (**Table 2**). However, HDL-cholesterol was slightly decreased during the control period. There seems to have been a minor decrease in total cholesterol level as well during both periods, although this decrease was not significant. The phenomenon could be explained by the daily use of oatmeal porridge by the subjects. Oats are a good source of soluble fibers that lower plasma total cholesterol (*34*). However, a decrease in HDL-cholesterol level is not an expected effect of the fibers.

In all subjects, some oxidized TAG molecules of 50 and 52 acyl carbons were detected in LDL after both study periods. The molecules were observed to contain keto and epoxy structures in their fatty acid residues (**Figure 2**). However, the consumption of test meals during the study periods did not have clear effects on the proportion of these structures in the LDL total lipids.

In human study 2, plasma flavonol concentrations were estimated by using standard compounds, which went through the same sample preparation procedures as the plasma samples. After this, a series of dilutions were made and standard curves were prepared. Limits of detection based on the standard curves were approximately 30 μg/L plasma for quercetin, 40 μg/L plasma for kaempferol, and 20 μg/L plasma for isorhamnetin. Sensitivity of the method was estimated to be 0.03 area units/(μg/L) for quercetin, 0.02 area units/(μg/L) for kaempferol, and 0.09 area units/(μg/L) for isorhamnetin.

In plasma, absorbed flavonols are mostly present as glucuronides and sulfates regardless of the form that they are ingested in (glycosidic or nonglycosidic) (*33*, *35*). In order to analyze the aglycones, the ester bonds were enzymatically cleaved. The flavonols added to porridge, both with and without added sea buckthorn oil, increased the plasma levels of isorhamnetin and kaempferol with both doses of the flavonoid extract (**Figure 3** and **Table 3**). Although the trend was similar after all meals, the increase in the plasma quercetin concentration was only significant after meal 2B. Quercetin concentrations were close to the limit of quantitation. The relatively high standard deviations and hence somewhat poor precision were not caused by HPLC separation or fluorescense detection but rather by sample preparation steps. Despite this, the results clearly demonstrate the absorption of the flavonol aglycones.

AUCs were also calculated, and both with and without added sea buckthorn oil, the results showed significantly ($P < 0.05$) higher plasma levels of kaempferol and isorhamnetin, when a higher dose of the extract was used (**Table 4**).

The chromatographic response of kaempferol was relatively low with the present approach. This could at least partly be explained by kaempferol degradation. It was shown that the compound was partly broken down during the sample preparation procedure involving the enzyme treatment. A relatively large peak eluting much earlier than kaempferol appeared in the UV chromatogram when reference compounds treated like samples were analyzed in 270 nm (data not shown). Because of the poor sensitivity of UV detection, the phenomenon could not be shown with the samples, although it probably also took

Table 4. Flavonol Absorption Data As AUC during a Period of 4 h Postprandially (Human Study 2)[a]

	quercetin		
meal[b]	quercetin	kaempferol	isorhamnetin (+ tamarixetin)
1A	38.9 ± 34.8	399.1 ± 131.5	148.3 ± 73.8
1B[c]	43.8 ± 25.9	497.6 ± 249.6	170.8 ± 82.1
2A	27.6 ± 31.2	215.1 ± 125.4	81.5 ± 27.3
2B	28.0 ± 18.7	200.5 ± 143.5	84.0 ± 41.0

[a] Results expressed as μg/L h (means ± SD, $n = 11$). Meals 1A and 1B led to significantly ($P < 0.05$) higher plasma levels of kaempferol and isorhamnetin than meals 2A and 2B. [b] See **Table 3** for the description of the meals. [c] $n = 10$.

place in them. The results suggest a very high plasma concentration for kaempferol (**Table 3**) even in the fasting samples. This is surprising and could be partly explained by possible traces of kaempferol in *H. pomatia* extract. Nevertheless, the kaempferol concentration increased considerably after the meals. In addition to the absorption of kaempferol itself, dehydroxylation of quercetin and demethoxylation of isorhamnetin can give rise to kaempferol and might partly explain the increased plasma concentration. However, to our knowledge, it is not known if these reactions take place in vivo.

Figure 3 demonstrates a rapid absorption for isorhamnetin. As with quercetin and kaempferol, the plasma concentration of isorhamnetin peaked 1 h after the meal and started to decrease after that. It is noteworthy that some of the isorhamnetin found in the plasma may originate from quercetin methylated after absorption (3′-methylation) (*36*). The degree of 3′-methylation in humans as compared to 4′-methylation is not known. However, it seems that most of the quercetin remains unmethylated (*2*, *37*, *38*). The HPLC method used does not efficiently differentiate between isorhamnetin (3′-methylquercetin) and tamarixetin (4′-methylquercetin) (**Figure 1**). Thus, some tamarixetin formed from quercetin after absorption may have eluted along with isorhamnetin.

The fat content of food seems to affect the absorption and bioavailability of flavonols. However, direct studies on these effects are scarce (*36*, *39*, *40*). In the present study, although the differences did not reach statistical significance because of the relatively high deviations, the addition of a small amount of sea buckthorn oil seemed to have enhanced the absorption and bioavailability (determined as AUC) of the flavonols in the group with a higher amount of dietary flavonols (**Figure 3** and **Table 4**). The enhanced absorption is in accordance with the study of Lesser et al. (*36*), in which a higher fat concentration increased the bioavailability of quercetin. With the lower dose of flavonol extract, the increase was not evident in the present study; in fact, 4 h after the meal, the kaempferol concentration seemed to be smaller when the oil was added.

The results give interesting new information on the absorption of different flavonol classes. The absorption of kaempferol and isorhamnetin aglycones, flavonols whose absorption and bioavailability have not been studied widely, was clearly shown.

More well-designed clinical studies using nutritional doses of flavonols in different matrices are needed to further add knowledge on the bioavailability and health effects of different flavonols.

ABBREVIATIONS USED

AUC, area under the concentration−time curve; BDC, baseline diene conjugation; CRP, C-reactive protein; EDTA, ethylenediaminetetraacetic acid; ESI, electrospray ionization; HDL, high-density lipoprotein; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; MS, mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography.

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