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Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women

JAE-HAK MOON,[1] RITSUKO NAKATA,[2] SYUNJI OSHIMA,[3]
TAKAHIRO INAKUMA,[3] AND JUNJI TERAO[1]
[1]*Department of Nutrition, School of Medicine, The University of Tokushima, Tokushima 770-8503;*
[2]*Department of Nursing, School of Medical Sciences, The University of Tokushima, Tokushima 770-8509; and* [3]*Kagome Research Institute, Kagome Company, Nishinasuno, Tochigi 329-2762, Japan*

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Moon, Jae-Hak, Ritsuko Nakata, Syunji Oshima, Takahiro Inakuma, and Junji Terao. Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R461–R467, 2000.—Quercetin is a typical flavonoid present mostly as glycosides in plant foods; it has attracted much attention for its potential beneficial effects in disease prevention. In this study, we examined human volunteers after the short-term ingestion of onion, a vegetable rich in quercetin glucosides. The subjects were served diets containing onion slices (quercetin equivalent: 67.6–93.6 mg/day) with meals for 1 wk. Quercetin was only found in glucuronidase-sulfatase-treated plasma, and its concentration after 10 h of fasting increased from 0.04 ± 0.04 μM before the trial to 0.63 ± 0.72 μM after the 1-wk trial. The quercetin content in low-density lipoprotein (LDL) after glucuronidase-sulfatase treatment corresponded to <1% of the α-tocopherol content. Human LDL isolated from the plasma after the trial showed little improvement of its resistance to copper ion-induced oxidation. It is therefore concluded that conjugated metabolites of quercetin accumulate exclusively in human blood plasma in the concentration range of $10^{-7} \sim 10^{-6}$ M after the short-term ingestion of vegetables rich in quercetin glucosides, although these metabolites are hardly incorporated into plasma LDL.

quercetin metabolites; quercetin glycosides; low-density lipoprotein oxidation; glucuronidation

QUERCETIN IS ONE OF THE ABUNDANT flavonol-type flavonoids, commonly found in vegetables and fruits (12, 15). The average daily intake of flavonoids, including three flavonol-type flavonoids (quercetin, myricetin, kaempferol) and two flavone-type flavonoids (luteolin, apigenin), was estimated to be 25 mg/person, with quercetin as the mostly consumed of these five flavonoids (16). Interest in quercetin was increased by the finding that quercetin was mutagenic in the Ames and other short-term tests (2, 36). However, in recent years, quercetin and other flavonoids have attracted renewed attention for their potential beneficial effects in disease prevention (8, 13, 34). In particular, epidemiological studies have demonstrated an inverse relationship between the intake of flavonoids, including quercetin, and coronary heart disease risk (14, 22). The antioxidant activity of flavonoids has frequently been mentioned in connection with their physiological function in the cardiovascular system (24), because oxidative modification of plasma low-density lipoprotein (LDL) is strongly suggested to participate in the initial event of atherosclerosis leading to coronary heart disease (35). Considerable studies (6, 11, 26, 33, 43) have shown that quercetin and its related flavonoids can prevent oxidation of LDL by scavenging reactive oxygen radicals (ROS), chelating iron, which is responsible for the generation of ROS, or inhibiting lipoxygenase. However, the in vivo function of dietary flavonoids cannot be determined without an understanding of their absorption and metabolic fate.

Gugler et al. (10) found that <1% of quercetin was absorbed into the human body after the oral administration of quercetin aglycone. Ueno et al. (41) demonstrated that 20% of quercetin was absorbed from the digestive tract and detected in bile and urine as glucuronide and sulfate conjugates within 48 h after oral administration to rats. We previously suggested that the efficiency of intestinal absorption of quercetin in rats is strongly affected by its solubility in vehicles (31). Quercetin is mostly present in the form of glycosides in vegetables and fruits, and dietary glycosides were believed to be converted to the respective aglycones in the large intestine by the glycosidase activity of intestinal bacteria (37). It was recently reported that the human small intestine possesses an ability to liberate the aglycone from quercetin glycosides (7). Nevertheless, Paganga and Rice-Evans (29) and Aziz et al. (1) reported that quercetin glucosides are present in human plasma without metabolic conversion. Hollman et al. (17, 19) studied the absorption and accumulation of quercetin glucosides in humans using onion as a dietary source of quercetin glycosides, and they claimed that quercetin glucosides are absorbed more easily than quercetin aglycone. However, Manach et al. (25) suspected that intact quercetin glucosides are

Address for reprint requests and other correspondence: J. Terao, Dept. of Nutrition, School of Medicine, The Univ. of Tokushima, Kuramoto-cho 3, Tokushima 770-8503, Japan (E-mail: terao@nutr.med.tokushima-u.ac.jp).

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present in blood circulation without metabolic conversion. Their suggestions were based on a human volunteer study that found that quercetin conjugates, not glucosides, accumulated in the plasma after a single ingestion of quercetin glucoside-rich food (23).

The aim of the present study was to clarify whether quercetin glucosides accumulate in human plasma after periodic ingestion of vegetables rich in quercetin glucosides. Onion was used as a dietary source rich in quercetin. The accumulation of quercetin in blood plasma and its distribution to plasma LDL were investigated after short-term ingestion of onion in female volunteers. By using HPLC analysis of nonhydrolyzed extracts, we show that quercetin conjugates, not glucosides, accumulated in blood plasma after short-term ingestion of quercetin glucosides from the diet.

MATERIALS AND METHODS

Chemicals. Quercetin and sulfatase type H-5 (from *Helix pomatia*, EC 3.1.6.1) were supplied by Sigma Chemical (St. Louis, MO). Quercetin 3,4′-di-*O*-β-glucoside (Q3,4′G) and quercetin 4′-*O*-β-glucoside (Q4′G) were kindly supplied by Dr. T. Tsushida of the National Food Research Institute, Japan. Quercetin 3-*O*-β-glucoside (Q3G) was purchased from Funakoshi (Tokyo, Japan). Diethylenetriaminepentaacetic acid (DTPA), EDTA, and 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) were obtained from Nacalai Tesque (Kyoto, Japan). *N*,*O*-bis(trimethylsilyl)acetamide was purchased from Tokyo Kasei (Tokyo, Japan). All other chemicals and solvents were of analytic grade.

Human study. Seven volunteers (women, 20 ~ 21 yr of age) participated in the study. They were healthy, not on any medication, and provided informed consent. Cooked onion slices were obtained from a local market. The subjects were freely served diet containing onion slices three times a day (260 ~ 360 g/day) for 1 wk. Before and after the trial, blood was collected from each subject after fasting for 10 h. Plasma was obtained by centrifuging heparinized blood at 1,600 *g* for 20 min at 4°C. LDL was isolated from the plasma by density-gradient ultracentrifugation according to the method described previously (6). The concentration of cholesterol in plasma lipoproteins was determined enzymatically using Cholesterol E Test-Wako (Wako Pure Chemicals, Tokyo, Japan).

Measurement of quercetin glucosides in onion. Twenty grams of onion slices were homogenized with 80 ml of methanol using a Polytron homogenizer at room temperature (41). The homogenate was filtered through no. 2 filter paper (Advantec, Toyo, Tokyo, Japan). The residue retained on the paper was mixed with 100 ml of 80% methanol and then filtered again. Both filtrates were poured into a 200-ml volumetric flask and filled to volume with 80% methanol. Subsequently, the extract was filtered through a 0.45-μm filter (GL Sciences, Tokyo, Japan). Ten milliliters of the filtrate were then injected directly in an HPLC apparatus equipped with an octadecylsilane (ODS) column (TSK gel ODS-80TS, 5 μm, 150 × 4.6 mm, TOSOH). Isocratic elution was carried out with water-methanol-acetic acid (68:30:2, vol/vol/vol) in 50 mM lithium acetate at a flow rate of 1.0 ml/min. The quercetin glucosides eluted from the column were monitored with an amperometric electrochemical detector (+800 mV; ICA-5212, TOA Electronics, Tokyo, Japan) and identified by the coincidence of their retention times with those of respective standard compounds. Their concentrations were calculated using standard curves for each quercetin glucoside.

Determination of quercetin metabolites in human plasma. Fifty microliters of human plasma were mixed with 50 μl of sulfatase type H-5 (25 units, from *H. pomatia*) solution in 0.1 M sodium acetate buffer (pH 5.0) (5). The mixture was incubated at 37°C in a shaking water bath for 50 min to liberate quercetin aglycone from its conjugates. Then the mixture was added to 900 μl of methanol-acetic acid (100:5, vol/vol) followed by sonication for 30 s. The mixture was vortexed for 30 s and then centrifuged (5,000 *g*) for 5 min at 4°C. The supernatant was concentrated by evaporation with nitrogen gas and dissolved in methanol. A portion of the resulting solution was injected onto the HPLC column (TSK gel ODS-80TS, 5 μm, 150 × 4.6 mm, TOSOH). The mobile phase was composed of water-methanol-acetic acid (57:41:2, vol/vol/vol) containing 50 mM lithium acetate. The flow rate was 1.0 ml/min. Elution was monitored with an amperometric detector (ICA-5212, TOA) with a working potential of +800 mV. Experiments with quercetin-spiked plasma showed that this procedure ensured a nearly 95% recovery. Determination of quercetin was performed using an external standard curve. The detection limit for quercetin was 1 nM with linear detector response up to 20 μM.

Isolation of quercetin from plasma and gas chromatography-mass spectrometry analysis. Plasma (1.0 ml) obtained from seven volunteers after the trial was subjected to enzymatic hydrolysis using sulfatase H-5, and the resultant methanolic extract was injected onto the HPLC column as described above. A fraction corresponding to quercetin was obtained and applied to a Sep-Pak C_{18} cartridge (Millipore, Waters, MA) for the elimination of lithium acetate from the HPLC mobile phase. After the cartridge was washed with methanol (10 ml) and water (10 ml), an aqueous solution of the isolated compound was used to charge the cartridge, which was then eluted with water (10 ml) followed by methanol (10 ml). The methanol eluate was evaporated to dryness, and the residue was trimethylsilyated using *N,O*-bis(trimethylsilyl)acetamide for gas chromatography-mass spectrometry analysis. A QP-5050 mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an SPD-1-fused silica capillary column (0.25 mm ID × 30 m, 10-μm film thickness; Supelco) in the electron impact mode (70 electron volt) was used. The carrier gas, helium, was applied at a flow rate of 1.0 ml/min. The column oven temperature was held at 120°C for 5 min before being elevated to 290°C at 20°C/min and then kept constant for 10 min.

Isolation of LDL from human plasma and its copper ion-induced lipid oxidation. The LDL fraction was isolated from heparinized plasma of volunteers before and after trials by differential density-gradient ultracentrifugation according to the method described previously (6). The isolated LDL solution was used immediately for experiments or was stored at 4°C for a maximum of 1 wk under a nitrogen atmosphere until used. The oxidation of the LDL solution [0.2 mg protein/ml PBS buffer (pH 7.4)] was initiated by the addition of cupric sulfate (final concentration of 5 μM). Cholesteryl ester hydroperoxides (CE-OOH) were determined by reversed-phase HPLC with ultraviolet detection at 235 nm as described previously (27). The concentration of CE-OOH was tentatively calculated from the standard curve of the hydroperoxy derivative of cholesteryl linoleate.

Contents of quercetin, α-tocopherol, and α-/β-carotene in LDL. To determine the quercetin content in LDL, the LDL solution was diluted with 0.1 M sodium acetate buffer (pH 5.0) to adjust it to the desired concentration of protein (mg protein LDL/ml), and the diluted solution (100 μl) was mixed with sulfatase type H-5 (25 units, 50 μl) solution in 0.1 M sodium acetate buffer (pH 5.0). The mixture was incubated at

37°C in a shaking water bath for 50 min. After the addition of 0.9 ml of methanol, the mixture was sonicated for 30 s and subjected to centrifugation at 4°C (5,000 *g*, 5 min). The supernatant was evaporated under a stream of nitrogen gas, and the residue was dissolved in methanol. The resulting solution was subjected to HPLC analysis for quercetin determination. The HPLC conditions were the same as those described above.

To determine α-tocopherol and α-/β-carotene contents, the LDL solution was diluted with 10 mM Tris · HCl buffer (25 μg protein/100 μl of final volume, pH 7.4) containing 0.5 mM DTPA. Ten microliters of water containing 1 mM EDTA were added to the LDL solution. δ-Tocopherol (0.5 nmol) and β-apo-8′-carotenal (50 pmol) were added as internal standards for the determination of α-tocopherol and carotenes, respectively. The resulting LDL solution was mixed vigorously with 500 μl of ethanol and 500 μl of *n*-hexane. Both the ethanol and *n*-hexane contained 1 mM BHT. After 1 min of sonication followed by centrifugation (3,000 *g*, 5 min, 4°C), the upper layer was collected and the solvent was subsequently evaporated under a stream of nitrogen gas and the residue was dissolved in chloroform. Portions of the chloroform solution were analyzed by HPLC for determination of α-tocopherol and α-/β-carotene, respectively. α-Tocopherol was analyzed by HPLC on a TSK gel Octyl-80TS column (5 μm, 4.6 × 150 mm, TOSOH) using 93% methanol as the eluting solvent at a flow rate of 1.0 ml/min. The eluate was monitored by fluorescence (excitation 295 nm; emission 325 nm). HPLC analysis of α- and β-carotene was carried out with a TSK gel ODS-80TS column (5 μm, 4.6 × 250 mm, TOSOH) with a mobile phase of methanol-acetonitrile-dichloromethane-water (7:7:2:0.16, vol/vol/vol/vol) at a flow rate of 1.0 ml/min. α- and β-Carotene were detected by measuring the absorbance at 450 nm.

Statistical analysis. Reported values were presented as means ± SD. Statistical analysis was evaluated by Wilcoxon signed-ranks test to identify significant differences using StatView J-4.5 (Abacus Concepts, Berkeley, CA) with Macintosh software. The level of significance was set at $P < 0.05$.

RESULTS

Content of quercetin glucosides in onion slices. Onion slices used in this trial contained two major glucosides (Q3,4′G and Q4′G) and one minor glucoside (Q3G). The contents of Q3,4′G, Q4′G, and Q3G were 40.5, 9.7, and 0.3 mg/100 g, respectively, on a fresh weight basis. The quercetin equivalent ingested by the subjects was 67.6 ~ 93.6 mg · day^{-1} · person^{-1} in the experiment. Quercetin in the free form was not detected in this preparation.

Plasma and lipoprotein cholesterol levels before and after short-term ingestion of onion slices. The cholesterol levels before and after short-term ingestion of onion slices were as follows: total plasma cholesterol, 169.3 ± 19.4 (before trial) and 161.6 ± 19.6 mg/dl (after trial); high-density lipoprotein (HDL) cholesterol, 58.7 ± 9.4 (before trial) and 59.4 ± 9.2 mg/dl (after trial); LDL cholesterol, 98.6 ± 12.6 (before trial) and 91.6 ± 13.4 mg/dl (after trial). The plasma and HDL cholesterol levels after the trial did not differ significantly from the respective levels before the trial. However, the LDL cholesterol level after the trial was significantly lower than that before the trial ($P < 0.05$).

Detection of quercetin in human plasma before and after short-term ingestion of onion slices. We performed HPLC analysis of methanol extracts of the plasma to know whether quercetin in the free form was present in human plasma before and after short-term ingestion of onion (Fig. 1, *A* and *B*). No obvious peak corresponding to free quercetin appeared in the chromatogram from either plasma extract as shown in Fig. 1, *A* and *B*. However, after sulfatase H-5 treatment, a prominent peak emerged with a retention time of 19.7 min in the extract of human plasma after short-term ingestion of onion slices (Fig. 1*C*). This peak was isolated and subjected to GC-MS after trimethylsilylation. Molecular ion (M^+) was observed at mass-to-charge ratio (*m/z*; relative intensity) 662 (1.5%). Other ions were formed in the fragmentation [M-CH_3^+] and [M-$OSiMe_3^+$] at *m/z* 647 (100%) and 573 (1.4%), respectively. This spectrum was in good agreement with that obtained from the trimethylsilylated derivative of standard quercetin.

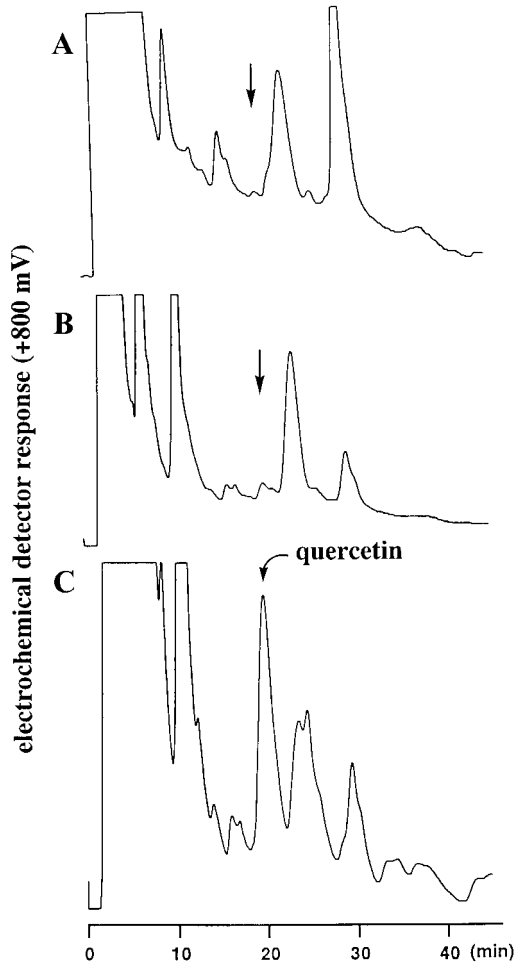

Fig. 1. HPLC chromatograms of methanol extracts of human plasma before and after short-term ingestion of onion slices. *A*: extract of human plasma before the trial; *B*: extract of human plasma after the trial; *C*: extract of human plasma after the trial after treatment with sulfatase H-5 (from *Helix pomatia*). The eluting solvent for the HPLC analysis was composed of methanol-water-acetic acid (41:57:2, vol/vol/vol) containing 50 mM lithium acetate. Other analytic conditions were the same as those described in MATERIALS AND METHODS.

Consequently, the isolated compound was identified as quercetin. These results strongly indicate that quercetin was present exclusively as the conjugated form in human plasma; after short-term ingestion of onion, the quercetin aglycone was liberated by the action of sulfatase H-5. Sulfatase H-5 possesses both glucuronidase activity and sulfatase activity (31). However, this enzyme preparation can also react with quercetin glucosides liberating quercetin, similar to the reaction with the conjugates of the blood extracts. Therefore, to confirm the presence or absence of quercetin glucosides in blood plasma, HPLC was carried out for the detection of glucosides using the eluting solvent with higher polarity without sulfatase H-5 treatment. Figure 2 shows a representative chromatogram of a methanol extract of human blood plasma after short-term ingestion of onion (Fig. 2*B*) and of cochromatography of this extract with the quercetin glucosides present in onion slices (Fig. 2*A*). Two peaks (retention time 12.1 and 23.9 min) were observed in the HPLC chromatogram (Fig. 2*B*) of the plasma extract after onion ingestion. However, these peaks did not correspond to Q3,4′G, Q3G, or Q4′G. The detection limits for quercetin glucosides were 20, 2, and 10 nM for Q3,4′G, Q3G, and Q4′G, respectively. It is therefore suggested that the level of quercetin glucoside accumulation in the plasma is at least <20 nM.

Quantitative change of quercetin in human plasma by short-term ingestion of onion slices. Table 1 shows the concentrations of quercetin in human plasma from seven volunteers before and after short-term ingestion of onion slices. Here, quercetin was quantified in methanol extracts after hydrolysis with sulfatase H-5. The average concentrations of quercetin in human plasma before and after short-term ingestion of onion were 0.04 ± 0.04 and 0.69 ± 0.72 μM, respectively. Although the concentration of quercetin in human plasma varied widely (0.08 ~ 1.88 μM), after the trial, every volunteer showed elevation of the concentration of quercetin after short-term ingestion of onion slices.

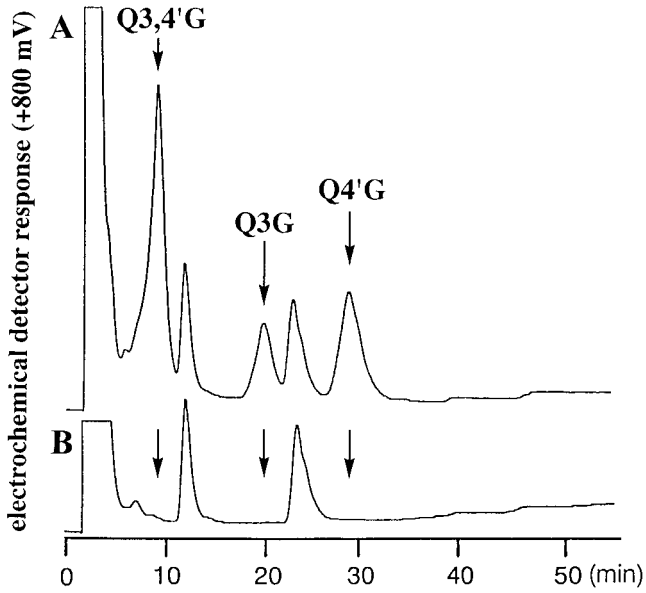

Fig. 2. HPLC chromatograms of methanol extracts of human plasma after short-term ingestion of onion slices. *A*: cochromatography of extract of human plasma after ingestion of onion and standard compounds of quercetin glucosides: quercetin 3,4′-di-*O*-β-glucoside (Q3,4′G); quercetin 3-*O*-β-glucoside (Q3G); quercetin 4′-*O*-β-glucoside (Q4′G); *B*: extract of human plasma after ingestion of onion;. The eluting solvent for the HPLC analysis was composed of methanol-water-acetic acid (30:68:2, vol/vol/vol) containing 50 mM lithium acetate. Other analytic conditions were the same as those described in MATERIALS AND METHODS.

Table 1. *Concentration of quercetin in human plasma from 7 volunteers before and after trial*

Volunteer	Before Trial	After Trial
A	0.09	1.52
B	0.12	0.40
C	0.07	1.88
D	0.01	0.13
E	0.01	0.65
F	0.03	0.15
G	<0.01	0.08

Values are in μM. Human plasma was hydrolyzed by sulfatase H-5 (from *Helix pomatia*), and the methanol extract was analyzed.

Content of quercetin, α-tocopherol, and carotene in LDL isolated from human plasma. Table 2 shows the contents of quercetin, α-tocopherol, and α-/β-carotene in LDL isolated from human plasma before and after short-term ingestion of onion slices. Quercetin was analyzed after hydrolysis with sulfatase H-5. The α- and β-carotene levels were not changed by the trials. The α-tocopherol level in human plasma after onion ingestion (16.6 ± 2.6 nmol/mg protein LDL) was significantly higher than that before onion ingestion (12.6 ± 1.7 nmol/mg protein LDL). Quercetin was not detected in human plasma LDL before onion ingestion. Quercetin at the level of 0.03 nmol/mg protein LDL was found in plasma LDL after short-term ingestion of onion. This concentration is quite low compared with the α-tocopherol and carotene levels in human plasma LDL. The quercetin content in LDL (0.03 nmol/mg protein) corresponded to 1.8 ng/1.0 ml plasma, because the LDL concentration in plasma was 0.2 mg protein/ml. The average concentration of quercetin in human plasma after the trial (0.7 μM) was estimated to be 210 ng/ml. It is therefore estimated that the quercetin in LDL represents <1% of the quercetin present in human plasma after onion ingestion.

Table 2. *Contents of quercetin, α-tocopherol, and α-/β-carotenes in LDL isolated from human plasma before and after short-term ingestion of onion slices*

	Content			
	Quercetin	α-Tocopherol	α-Carotene	β-Carotene
Before trial	ND	12.6 ± 1.7*	0.19 ± 0.10	0.71 ± 0.42
After trial	0.03	16.6 ± 2.6*	0.18 ± 0.08	0.67 ± 0.34

Values are means ± SE in nmol/mg protein low-density lipoprotein (LDL). Quercetin was determined after hydrolysis with sulfatase type H-5 (from *Helix pomatia*). *Significantly different before and after the trial ($P < 0.05$); ND, not determined.

Effect of short-term onion ingestion on copper ion-induced oxidation of LDL isolated from human plasma. The LDL isolated from the plasma of the volunteers before and after onion ingestion was oxidized with copper ion at 37°C. Lipid peroxidation was monitored by the increase of CE-OOH concentration (Fig. 3). CE-OOH levels produced by 0.5, 1, and 2 h of oxidation did not differ significantly before and after onion ingestion. Thus short-term ingestion of onion had no influence on the susceptibility of isolated LDL to copper ion-induced oxidation.

DISCUSSION

The aim of this study was to determine whether quercetin accumulates in human plasma after short-term ingestion of quercetin-rich vegetables. We used onion as the dietary source of quercetin. Onion samples contained only quercetin glucosides and no quercetin aglycone. Therefore, we tried to measure the plasma concentration of quercetin glucosides as well as quercetin in free form. It is apparent from the analysis with sulfatase H-5 treatment that the quercetin content in blood plasma after 10 h of fasting was increased by the short-term ingestion of onion for 1 wk (Table 1). However, quercetin in the free form was not detected in the plasma of volunteers before or after the trial (Fig. 1). Moreover, the quercetin glucosides present in the onion samples, Q4′G, Q3,4′G, and Q3G, were scarcely detectable in the plasma after the trial (Fig. 2). Therefore, two conclusions can be derived from these results. One is that periodic short-term ingestion of quercetin-rich vegetables elevates the concentration of quercetin in human plasma even after fasting. The other conclusion is that conjugated metabolites of quercetin accumulate exclusively in human plasma after quercetin glucosides are supplied in the diet.

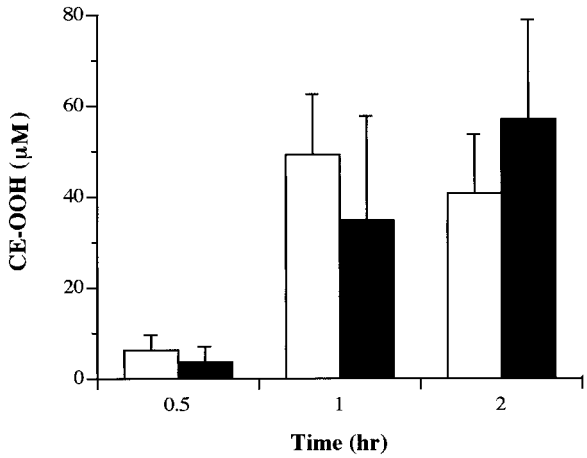

Fig. 3. Accumulation of cholesterylester hypdoperoxides (CE-OOH) in copper ion-induced lipid peroxidation of low-density lipoprotein (LDL) isolated from human plasma LDL. Oxidation of the LDL suspension (0.2 mg protein/ml) was initiated by the addition of cupric sulfate (final concentration 5 μM) dissolved in PBS buffer (pH 7.4). Open bars and filled bars show average CE-OOH concentrations before and after trials, respectively. Initial concentrations of quercetin and α-tocopherol in the LDL suspension before and after short-term ingestion of onion are shown in Table 1.

Hollman et al. (17) demonstrated by single ingestion experiments that the quercetin glucosides from onion are absorbed into human plasma and eliminated slowly throughout the day. Thus it is rational to assume that the plasma quercetin level is maintained by the periodic ingestion of quercetin-rich vegetables with meals. Our results strongly suggest that the plasma quercetin level can be maintained in the range of $10^{-7} \sim 10^{-6}$ M by the periodic ingestion of 100 ~ 200 g of onion/day, although the level of quercetin in the plasma may be transiently elevated a few hours after the intake. This study provides the first evidence that quercetin accumulates in human plasma after the periodic ingestion of quercetin-rich vegetables. Hollman et al. (18) also suggested that quercetin glucosides are absorbed more easily than other glycosides or aglycones. However, they did not distinguish between quercetin aglycone and its conjugates present in human plasma, because they measured the quercetin content after the hydrolysis of blood plasma by acid catalysis. Manach et al. (23) showed by single ingestion experiments that quercetin as conjugated metabolite forms, including sulfates and glucuronides, exclusively accumulated in human plasma after the intake of complex meals containing onion and other quercetin-rich foods. However, Paganga and Rice-Evans (29) demonstrated the presence of quercetin glucosides in human plasma using nonsupplemented diets. Aziz et al. (1) also reported the presence of Q4′G and isorhamnetin 4′-*O*-β-glucoside in human plasma 1.5 h after consuming onion. Therefore, the absorption and accumulation of quercetin glucosides in human plasma is still a subject of controversy in terms of the bioavailability of dietary flavonoids. Our results demonstrated at least that quercetin glucosides do not accumulate in human plasma in an intact form, and they are mostly subject to metabolic conversion before circulation.

It is generally accepted that water-soluble quercetin glucosides seem to be poorly absorbed because of their poor solubility in bile acid micelles in the intestinal tract (38). However, in the large intestine, glycosides are hydrolyzed to release the aglycone by the action of β-glycosidase in anaerobic enterobacteria (37). This improves the lipophylicity of quercetin, resulting in high solubility in bile acid micelles. We recently reported that rat intestinal mucosal homogenates easily hydrolyze Q4′G to liberate quercetin aglycone (21). Furthermore, Day et al. (7) demonstrated that the human small intestine possesses cytosolic β-glucosidase activity with broad specificity. Therefore, most quercetin glucosides from onion seem to be hydrolyzed during intestinal absorption by β-glucosidase activity from enterobacteria and/or intestinal mucosa. We previously found that the activity of uridine-5′-diphosphoglucuronosyl transferase (UDP-GT), a typical glucuronidation enzyme, was the strongest in the preparation from the intestine among rat tissues (30). Glucuronidation of quercetin in Caco-2 cells, which have been used as a model for the small intestinal epithelial cells, was also found by our research group (20). The presence of UDP-GT in human intestinal

mucosa was also recently reported by Radominska-Pandya et al. (32). On the other hand, the participation of the glucose transport system in the cellular uptake of quercetin glucosides from the diet has been reported (9, 28), and this may support the idea that quercetin glucosides are absorbed into the intestinal tract and present in human plasma in an intact form. However, Walgren et al. (42) claimed, on the basis of studies using Caco-2 cells, that an active transport process for quercetin glucosides is doubtful. It is therefore likely that quercetin glucosides from onion are converted mostly to glucuronyl conjugates in intestinal epithelial cells by hydrolysis with β-glucosidase and conjugation by UDP-GT.

Our results clearly show that quercetin is scarcely incorporated within the LDL particles after short-term ingestion of quercetin-rich vegetables (Table 2). The level of quercetin in LDL was found to be <1% of the α-tocopherol level, indicating that the participation of quercetin in an antioxidant capacity in isolated LDL is not likely. The results of LDL oxidation with copper ion (Fig. 3) demonstrated that short-term ingestion of quercetin-rich vegetables does not improve the resistance of isolated LDL to oxidation. Blostein-Fujii et al. (3) showed that citrus flavonoid supplementation does not necessarily alter LDL susceptibility to oxidation assessed in vitro. We have assumed that flavonoids are efficient antioxidants at the interface between the lipid and water phases (39). It is unlikely that quercetin conjugates enter into LDL particles because of a rather high water solubility, although they may possess an affinity to outer phospholipid membranes in LDL particles. Quercetin is known to bind extensively to human serum albumin (4). However, no information is available on the interaction of quercetin conjugates with serum albumin. Thus, for the assessment of the antioxidant activity of quercetin conjugates in the circulation, further studies are required to demonstrate whether these conjugates are present free in the water phase or bound to specific proteins.

In conclusion, our volunteer study clarified that conjugated metabolites of quercetin accumulate in human plasma in the concentration range of $10^{-7} \sim 10^{-6}$ M after the periodic ingestion of onions with meals for 1 wk. This level corresponds to the concentrations of carotenoids and ubiquinol, well-known plasma antioxidants, in human plasma. Although the susceptibility of LDL to oxidation is not altered by the ingestion of onion because of scant incorporation into isolated LDL particles, quercetin metabolites in the plasma may contribute to the antioxidant defense in the circulation.

Perspectives

Quercetin glycosides are obtained by the daily intake of vegetables, fruits, and beverages. This study clearly indicates that they are accumulated as glucuronide and/or sulfate conjugates in circulation. Therefore, antiatherosclerotic and other vascular function of quercetin is undoubtedly originated from its conjugated metabolites. Although the conjugation with glucuronide and sulfate is a step in the detoxification to lose the physicochemical property, intermediate products should retain their activity and exert biological function. Then, localization of quercetin conjugates in the vascular system should be clarified to assess their efficacy as plasma antioxidants. Their diphenylpropane moiety may facilitate their localization near the surface of lipoproteins because of the interaction between this planar structure and outer phospholipid layers. It is unlikely that quercetin conjugates penetrate into cytosol through cellular membranes and exert their activity on redox regulation in vascular cells. However, they can modulate the redox state of cellular membranes by interacting with phospholipid bilayers. Physicochemical reaction of the metabolites toward biomembranes should be clarified to understand antiatherosclerotic and other physiological function of dietary flavonoids in the vascular system.

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