

# Dietary flavonoids as antioxidants *in vivo* : Conjugated metabolites of (-)-epicatechin and quercetin participate in antioxidative defense in blood plasma

Junji Terao

*Department of Nutrition, The University of Tokushima School of Medicine, Tokushima, Japan*

**Abstract :** Flavonoids are present in mainly plant foods and have attracted much attention in relation to disease prevention. Their antioxidant activity at least partly accounts for their potential health effect, because oxidative stress leads to a variety of pathophysiological events. It is essential to know the bioavailability of flavonoids involving intestinal absorption, metabolic conversion and urinary excretion, in order to evaluate their *in vivo* antioxidant activity after intake. Here (-)-epicatechin and quercetin were selected as typical flavanol- and flavonol-flavonoids present in vegetables, fruits and tea. Our rat study suggests that their metabolic conversion begins in the intestinal mucosa where the activity of uridine-5'-diphosphoglucuronosyltransferase (UGT) is at its highest. Both flavonoids accumulated mostly as glucuronide and sulfate conjugates in blood plasma after oral administration. No intact quercetin was found in the circulation. However, on the oral administration of these flavonoids, the antioxidative ability of rat plasma was enhanced indicating that conjugated metabolites participate in the antioxidant defense in blood plasma. Therefore, the intake of vegetables, fruits and tea rich in flavonoids may help to prevent oxidative damages in the blood. *J. Med. Invest.* **46** : 159-168, 1999

**Key words :** flavonoid, quercetin, (-)-epicatechin, antioxidant, glucuronidation

## INTRODUCTION

Flavonoids are polyphenol compounds containing a unique C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> structure (diphenyl propane structure) and more than 4,000 varieties of flavonoids are distributed in the plant kingdom. They are mostly present as glycosides in which phenolic hydrogen or hydrogens are substituted for the sugar moiety. Flavonoids can be classified as calcones, flavones, flavanones, flavanols and flavonols. Quercetin, a typical flavonol, possesses additional phenolic OH groups at the 5- and 7-position of the A ring and 3'- and 4'-position of the B-ring (Fig.1). Quercetin glycosides, such as rutin, quercitrin and quercimeritrin, are common flavonoids present in fruits and vegetables. On the other hand, tea catechins consist of

four flavanol-type compounds containing additional phenolic OH groups at the 5 and 7 position. Furthermore, (-)-epicatechin and (-)-epicatechin gallate contain OH groups at the 3' and 4' position of the B ring, and (-)-epigallocatechin and (-)-epigallocatechin, at the 3', 4' and 5' position, respectively. Interestingly, epicatechin gallate and epigallocatechin gallate are the derivatives of epicatechin and epigallocatechin in which the gallate group is esterified to an OH group at the 3-position of the flavanol structure. Thus, tea catechins are composed of free flavanols and their gallate esters.

Daily intake of flavonoids by humans is estimated at 25 mg (1). However, this value only covers five aglycones including quercetin and the total intake of flavonoids from plant food may reach several hundred mg/day (2). This level is not low as compared with that of vitamin E or vitamin C. In 1936, Szent-Györgyi (3) claimed that two flavonoids from citrus fruits reduced capillary fragility and permeability in humans and he named them vitamin P. At present, flavonoids are not involved in the

---

Received for publication June 1, 1999 ; accepted July 29, 1999.

Address correspondence and reprint requests to Junji Terao, Ph.D., Department of Nutrition, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-633-7087.

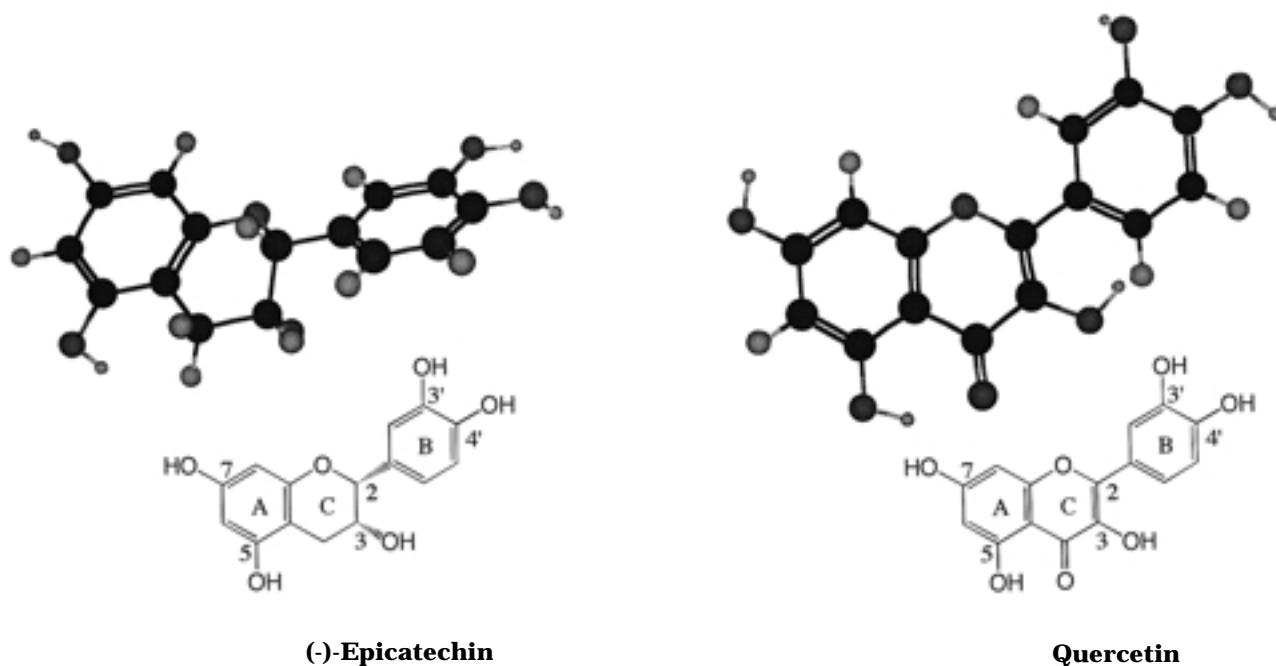


Fig.1. Structures of (-)-epicatechin and quercetin

category of vitamins. Nevertheless, they are recognized as having a potential beneficial effect in disease prevention (4-9). Epidemiological studies strongly suggest that consumption of fruits, vegetables and teas lowers the risk of coronary heart disease (10). It should be noted that an inverse relationship between the intake of flavonoids and coronary heart disease risk was also reported (11, 12). The so-called French paradox, the lack of a positive correlation between a high intake of saturated fat and the occurrence of coronary heart disease is related at least partly to the consumption of red wine (13), which is rich in flavonoids including epicatechin and quercetin.

The antioxidant activity of flavonoids has been frequently mentioned in connection with their physiological function *in vivo*, because oxidative stress is known to participate in the initial process of atherosclerosis leading to coronary heart disease (6). A number of studies have revealed that flavonoids act as antioxidants by scavenging reactive oxygen species (ROS) and/or chelating metal ion responsible for the generation of ROS. The structure-activity relationship of flavonoids and their antioxidant activity is well documented (14). We have already carried out a kinetic study of the inhibitory effect of several flavonoids on lipid peroxidation in solution and in liposomal membranes (15, 16). The results implied that flavonoids act as interfacial

antioxidants in the lipid/water biphasic system, because the hydrophilic property of the flavonoids facilitates their localization at the interface of the lipid bilayers resulting in an effective inhibition of the initial attack by aqueous radicals (17). However, the *in vivo* function of dietary flavonoids cannot be estimated without a knowledge of their absorption and metabolic fate. Thus, much study has been done on the absorption and metabolism of flavonoids in recent years. We are also investigating the absorption rate and metabolic process of flavonoids, in particular, (-)-epicatechin and quercetin, in rat and human. Here we review recent studies on the absorption and metabolism of these two flavonoids.

#### ABSORPTION AND METABOLIC PATHWAY OF DIETARY (-)-EPICATECHIN

In 1971, Das *et al.* (18) detected (+)-catechin and its metabolites in human urine after oral administration of (+)-catechin. This was the first evidence that catechins are absorbed into the human body. It was recently confirmed that tea catechins are absorbed into human body and accumulated in the blood plasma by the intake of tea catechin concentrate (19, 20). A rat study (21) demonstrated that a main component of tea catechin, (-)-epigallocatechin gallate, was widely distributed in several tissues

including the liver and kidney. On the other hand, Hackett (22) showed that oral administration of epicatechin results in urinary excretion of glucuronide and sulfate conjugates of epicatechin and 3-*O*-methyl epicatechin, indicating that absorbed catechins are mostly subject to metabolic conversion into conjugates. It is also suggested that conjugated metabolites come to bile from liver and are then reabsorbed into the body after hydrolysis and ring-cleavage (enterohepatic circulation) (23). It is therefore likely that tea catechins accumulate in human plasma through enterohepatic circulation.

In general, liver is a main tissue for the metabolism of xenobiotic substances. However, intestinal mucosa, kidney and other tissues also possess enzymatic activity for metabolism such as glucuronidation, *O*-methylation, and hydroxylation (P-450). It is unclear how and where catechins are metabolized after intestinal absorption. Here we used (-)-epicatechin and measured the enzymatic activities for its metabolic conversion in several rat tissues (24). The enzymes are uridine-5'-diphosphoglucuronosyl transferase (UGT) for glucuronidation, phenolsulfotransferase (PST) for sulfation and catechol-*O*-methyltransferase (COMT) for methylation. Figure 2 shows that the UGT activity was strongest for the preparation from the intestine. The only organ to present activity of PST was the liver. The liver is the main organ for COMT and the activity in the kidney was lower than that in the liver but was higher than in the other tissues. Thus, we propose a metabolic pathway of orally administered (-)-epicatechin in rats as shown in Fig. 3. Absorbed epicatechin is likely to be immediately conjugated with glucuronic acid in the intestinal mucosa. Conjugation in the intestinal mucosa is plausible because the glucuronidation of phenolic compounds in the intestinal mucosa has been reported elsewhere (25). The second step for metabolic conversion is conjugation with sulfate. The final step is methylation resulting in *O*-methylated catechin, which seems to be the final product of absorbed EC. We therefore postulate that epicatechin is metabolized to glucuronyl conjugates in the intestinal mucosa and these enter the portal vein and are metabolized further in liver and other tissues. Finally, they are excreted from the body *via* bile or urine. The antioxidant activity of catechins has been often discussed based on the results of *in vitro* studies. However, the activity of their metabolites should be taken into account when estimating their *in vivo* effectiveness.

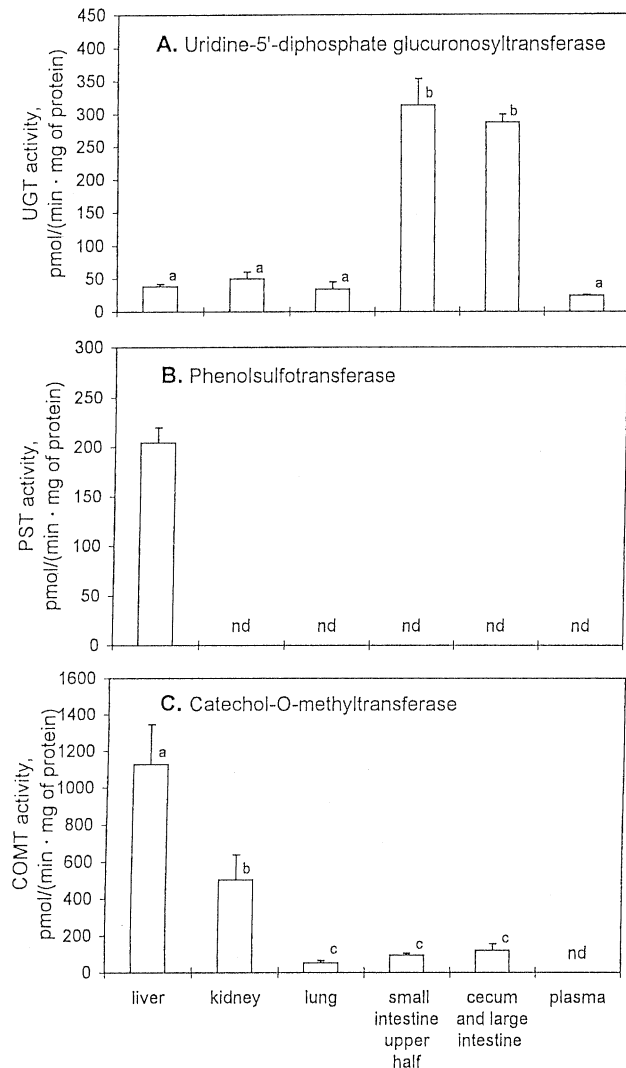


Fig. 2. Tissue distribution of conjugative enzyme activities in rats. Panels represent enzyme activities measured at tissue preparation, of UGT (panel A), PST (Panel B), and COMT (panel C). Activities were determined using (-)-epicatechin as substrate. Values are the means  $\pm$  SEM. Means with different letters are significantly different,  $P < 0.05$ . (ref. 24)

## EX VIVO EFFECT OF (-)-EPICATECHIN ON THE ANTIOXIDATIVE DEFENSE IN RAT BLOOD PLASMA

Blood plasma is a well-organized defense system which utilizing antioxidant enzymes such as extracellular superoxide dismutase and glutathione peroxidase, and low-molecular weight antioxidants such as vitamin E, vitamin C, uric acid and so on. It is of interest to know the role of dietary catechins in antioxidative defense in blood plasma, because catechins are found to be mainly present as conjugated and methylated metabolites. It remains unclear whether or not catechins possess any antioxidant activity after intestinal absorption and metabolic

conversion. Thus, we measured the changes in the oxidizability of rat plasma after oral administration of (-)-epicatechin (26). Male Wistar rats were fasted for 12-15 hr and then administered (-)-epicatechin (10 or 50 mg/200 g body weight) dissolved in 2.0 ml of water intragastrically by direct stomach intubation. The rats were anesthetized with diethylether at 1 hr

and 6 hr after administration. Rat plasma was obtained and the concentration of (-)-epicatechin and its metabolites were determined by HPLC analysis and a method of enzymatic hydrolysis which we developed. Table 1 shows the profiles of metabolites in rat plasma after (-)-epicatechin administration. One hr after an intragastric administration of 20 mg

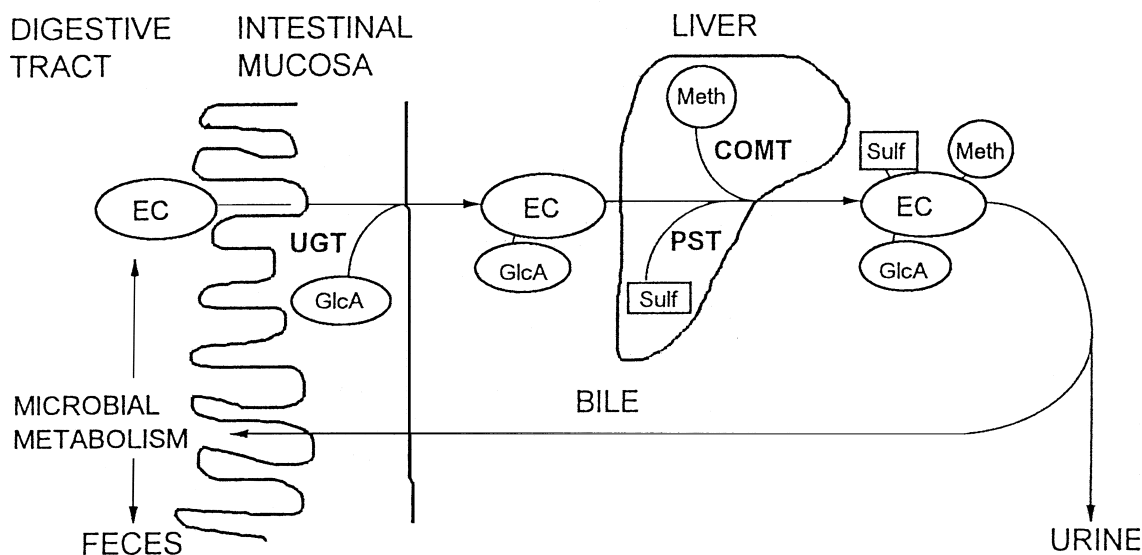


Fig. 3. Proposed scheme of the metabolic fate of orally administered (-)-epicatechin in rats. COMT, catechol-*O*-methyltransferase; EC, (-)-epicatechin; GlcA, glucuronide moiety; Meth, methyl moiety; PST, phenolsulfotransferase; Sulf, sulfate moiety; UGT, uridine 5-diphosphoglucuronosyltransferase (Ref. 24)

Table 1. Micromolar concentrations of (-)-epicatechin metabolites in rat plasma after intragastric administration of quercetin at two dose levels.

conjugates	Time after administration			
	1 h		6 h	
<b>Dose 10 mg*</b>	EC ( $\mu\text{M}$ )	Methyl-EC ( $\mu\text{M}$ )	EC ( $\mu\text{M}$ )	Methyl-EC ( $\mu\text{M}$ )
Free	3.0	0	0	0
Sulfate	1.0	1.3	0	0.9
Glucuronide	25.2	7.4	0.9	2.8
Sulfoglucuronide	0	4.4	0	2.2
Total	29.2	13.1	0.9	5.9
<b>Dose 50 mg*</b>	EC ( $\mu\text{M}$ )	Methyl-EC ( $\mu\text{M}$ )	EC ( $\mu\text{M}$ )	Methyl-EC ( $\mu\text{M}$ )
Free	13.3	0.9	2.6	0.3
Sulfate	0	7.6	3.6	9.3
Glucuronide	52.1	5.9	11.9	11.5
Sulfoglucuronide	0	23.2	5.4	28.7
Total	65.4	37.6	23.5	49.8

\* Dose : mg of free quercetin/200 g of rat body weight.

EC : non methylated (-)-epicatechin.

Methyl-EC : methylated (-)-epicatechin (tentatively identified as (-)-epicatechin derivatives in which the hydroxyl group at the 3-position was converted to a methoxyl group).

(-)-epicatechin, the concentration of total (-)-epicatechin metabolites that had accumulated was 42.3  $\mu\text{M}$ . 70% and 30% were nonmethylated and *O*-methylated epicatechins, respectively. As little as 7% of the metabolites were in a non-conjugated form. After 6 hr administration, approx. 84% of metabolites were cleared from plasma. Administration of 50 mg (-)-epicatechin increased the level of total metabolites in plasma to 103.0  $\mu\text{M}$  and 73.3  $\mu\text{M}$  at 1 hr and 6 hr, respectively. 13% free (-)-epicatechin was detected in the plasma indicating saturation of the conjugation reaction.

Fig.4 shows the accumulation of cholesteryl ester

hydroperoxides (CE-OOH) and consumption of  $\alpha$ -tocopherol in copper ion-induced oxidation of diluted rat plasma. This figure clearly shows that oral administration of (-)-epicatechin hindered the accumulation of CE-OOH and retarded the consumption of  $\alpha$ -tocopherol. It is therefore likely that oral administration of (-)-epicatechin expands the antioxidative capacity of rat blood plasma, although (-)-epicatechin is mostly present as its metabolites. This implies that some (-)-epicatechin metabolites act as antioxidants in plasma by scavenging radicals and/or chelating metal ion.

Bors (27) suggested that the *O*-dihydroxyl structure in the B-ring (catechol structure) is essential to the free radical-scavenging activity for flavanol-type flavonoids. Metabolites possessing a *O*-dihydroxyl structure seem to be responsible for the antioxidant activity of orally administered (-)-epicatechin. A human study (28) demonstrated that ingestion of tea improves the antioxidant capacity of blood plasma. It is therefore likely that conjugated metabolites with a catechol structure are responsible for the *in vivo* antioxidant activity of tea catechins. Recently Okusio *et al.* (29) and Harada *et al.* (30) detected (-)-epicatechin-5-*O*-glucuronide as a (-)-epicatechin metabolite in rat blood plasma. This conjugated metabolite contains a catechol structure and thus may be responsible for the antioxidant activity of dietary catechins.

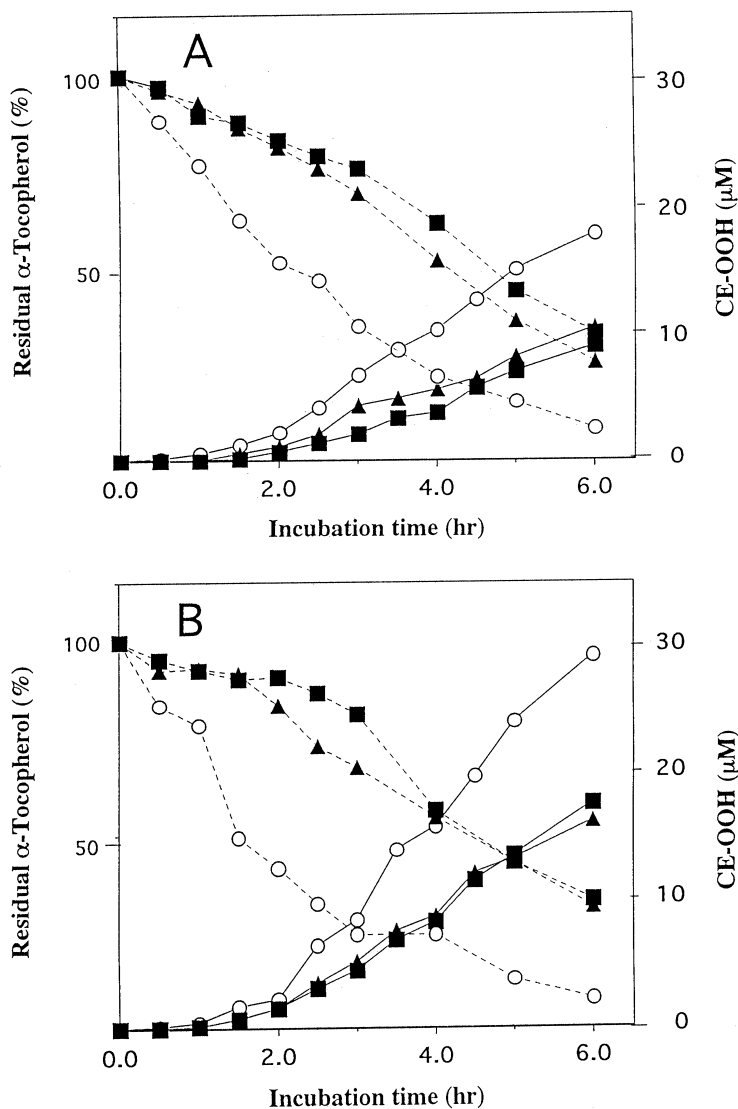


Fig. 4. Accumulation of cholesteryl ester hydroperoxides (CE-OOH) and consumption of  $\alpha$ -tocopherol in  $\text{CuSO}_4$ -induced oxidation of diluted rat plasma. (A) Diluted plasma from 1hr after the administration of water and epicatechin. (B) Diluted plasma from 6hr after the administration of water and epicatechin. Pooled plasma from four rats of each group was diluted four times with PBS and incubated with  $100\mu\text{M}$   $\text{CuSO}_4$  at  $37^\circ\text{C}$  for 6hr. ( ) control rat ( ) EC-administered rat (10mg/200g b.wt), ( ) EC-administered rat (50mg/200g b.wt). Solid line; the amount of CE-OOH, Dotted line; the amount of  $\alpha$ -tocopherol (ref. 26).

### ABSORPTION OF QUERCETIN AND QUERCETIN GLYCOSIDES

In 1975, Gugler *et al.* (31) reported that less than 1% of quercetin was absorbed into the human body following oral administration of quercetin aglycone. Ueno (32) *et al.* demonstrated using  $\text{C}^{14}$ -labeled quercetin that 20% of quercetin was absorbed from the digestive tract and present in bile and urine as glucuronide and sulfate conjugates within 48 hrs. We are interested in the effects of the vehicles for quercetin in oral administration on the efficiency of intestinal absorption and accumulation in blood (33). Quercetin's solubility in the vehicles used for its administration was compared with respective absorption

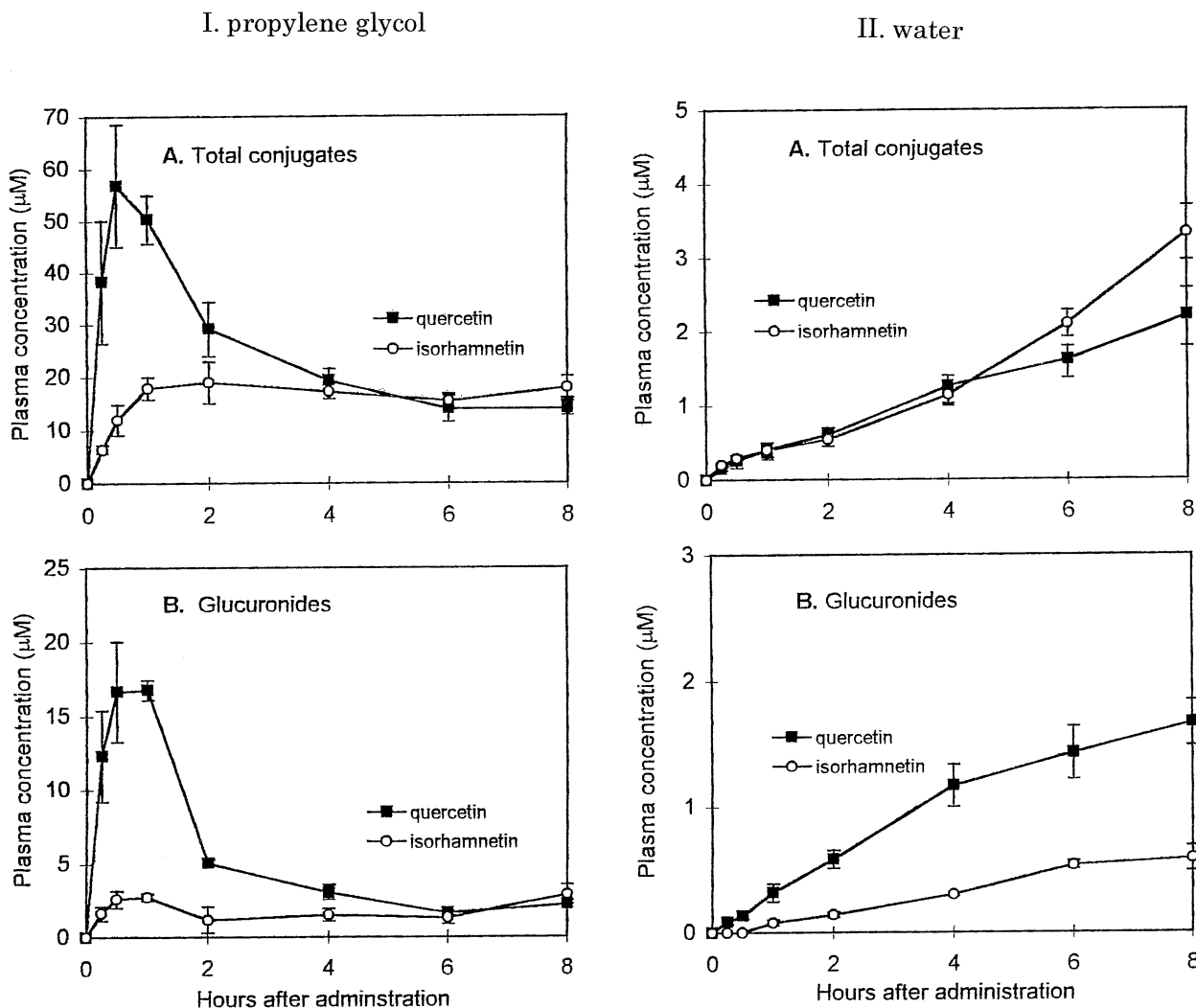


Fig. 5. Quercetin metabolite concentrations in rat plasma after oral administration of 50 mg/kg quercetin in 2 ml of propylene glycol (I) or water (II). Values are the mean  $\pm$  SEM ( $n=3$ ) (ref.33).

profiles. We compared water and propylene glycol as vehicles for the administration in the rat. If quercetin's solubility in propylene glycol is taken as 1 (complete solubilization), the relative solubility in water is  $1.6 \times 10^{-5}$ . The results shown in Fig.5 clearly reveal that the extent of quercetin absorption depends on the solubility in the vehicle used for the administration. It should be emphasized that the vehicles substantially affect the efficiency of the absorption. Alcohol as a vehicle may elevate the absorption of quercetin from the digestive tract because of its high solubility.

Quercetin is, in general, present in the form of glycosides in plant foods and thus the absorption of quercetin glycosides need to be clarified to estimate the physiological function of dietary quercetin. Water-soluble glycosides seem to be little absorbed because of their poor solubility in bile acid micelles in the intestinal tract. However, in the large intestine,

glycosides are hydrolyzed to release aglycone by the action of  $\beta$ -glycosidase in anaerobic enterobacteria (34). Furthermore, a part of the aglycone is subjected to ring scission (35). Thus, flavonoids improve their lipophilicity resulting in high solubility in bile acid micelles. Nevertheless, there have been contradictory results on the intestinal absorption of quercetin glycosides. Manach *et al.* (36) suggested that the absorption of rutin (quercetin-3-rutinoside) in rats is slower than that of the respective aglycone because hydrolysis in the large intestine is required. On the other hand, Holmann (37, 38) *et al.* claimed that quercetin glucosides are absorbed more easily than quercetin aglycone in humans. Papanga *et al.* (39) and Aziz *et al.* (40) reported that quercetin glucosides are present in human plasma without metabolic conversion. However, Manach *et al.* (41) suspected that intact quercetin glucosides are present in blood

circulation without metabolic conversion. They suggested that conjugated metabolites including sulfates and glucuronides exclusively accumulate in the plasma after intake of quercetin glucosides from plant food (42). It is still obscure whether or not quercetin glucosides are absorbed directly or absorbed after hydrolysis in the tract. We pointed out that  $\beta$ -glucosidase activity is present in rat intestinal mucosa homogenate (43). Thus we suggest that quercetin glucosides are hydrolyzed in the intestinal mucosa and incorporated into the cell in which glucuronidation occurs. The participation of the glucose transporter in the cellular intake of quercetin glucosides from diet has been reported (44, 45). However, there is no direct evidence that this transport system is responsible for the absorption of quercetin glucosides (46). Our recent results suggest that not glucosides but glucuronide conjugates accumulated in human plasma after intake of onion rich in quercetin glucosides (unpublished data).

**QUERCETIN METABOLITES ACT AS ANTI-OXIDANTS ON LIPID PEROXIDATION IN RAT PLASMA**

Absorbed quercetin seems to be metabolized *via* the same pathway as (-)-epicatechin, in which glucuronidation occurs at first (Fig 6). It was reported that in a rat experiment isorhamnetin (3-O-methylated quercetin) and 4-O-methylated quercetin

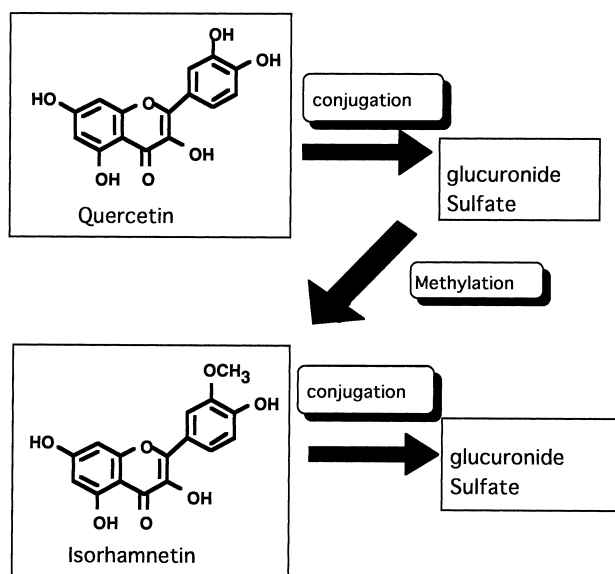


Fig. 6. Proposed scheme of the metabolic pathway of orally administered quercetin in rats.

accumulated after oral administration of quercetin (47). However, we found that glucuronide and sulfate conjugates without methylation also accumulated in blood plasma after oral administration (33) and thus supposed that some conjugates in circulation act as antioxidants. For example, in metabolic profiles of quercetin in the rat obtained after oral administration of 2 mg and 5 mg of quercetin aglycone in propylene glycol, respectively (Table 2) (48), at 1 hr and 6 hr after the administration neither free quercetin nor free isorhamnetin was found in the plasma, indicating that all of the absorbed quercetin is present as conjugated metabolite in the circulation. At 2.0 mg, 13  $\mu$ M of quercetin accumulated at

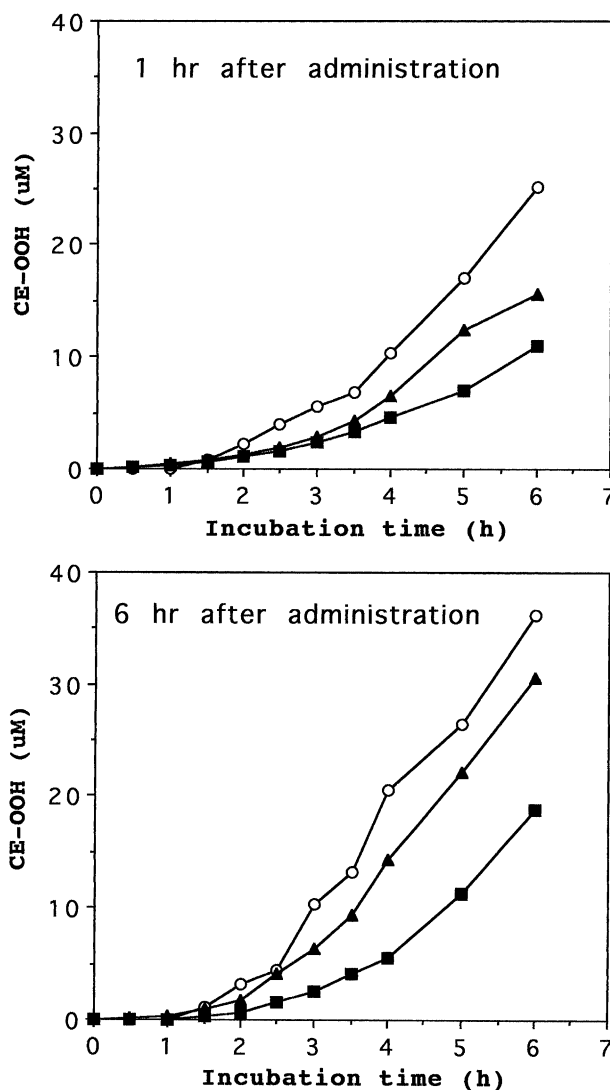


Fig. 7. Copper ion-induced lipid peroxidation of rat plasma obtained 1 hr after the administration of propylene glycol (control; open symbols) or quercetin solution (closed symbols), 2 mg ( ) or 10 mg ( )/200 g b. wt. Plasma from rats of each group was pooled, diluted four times with PBS and incubated with 100  $\mu$ M CuSO<sub>4</sub> at 37 °C for up to 6 hr. (ref. 48).

Table 2. Micromolar concentrations of quercetin metabolites in rat plasma after intragastric administration of quercetin at two dose levels.

conjugates	Time after administration			
	1 h		6 h	
<b>Dose 2 mg*</b>	Quercetin ( $\mu\text{M}$ )	Isorhamnetin ( $\mu\text{M}$ )	Quercetin ( $\mu\text{M}$ )	Isorhamnetin ( $\mu\text{M}$ )
Free	0	0	0	0
Sulfate	0.38	0	0	0
Glucuronide	6.14	0	1.33	0
Sulfoglucuronide	3.13	4.22	2.88	4.88
Total	9.64	4.22	4.21	4.88
<b>Dose 10 mg*</b>	Quercetin ( $\mu\text{M}$ )	Isorhamnetin ( $\mu\text{M}$ )	Quercetin ( $\mu\text{M}$ )	Isorhamnetin ( $\mu\text{M}$ )
Free	0	0	0	0
Sulfate	10.4	0	0.4	0
Glucuronide	14.0	0	1.03	2.44
Sulfoglucuronide	44.6	20.4	18.0	13.56
Total	69.0	20.4	19.3	16.00

\*Dose : mg of free quercetin/200 g of rat body weight.

1 hr and 9  $\mu\text{M}$  after 6 hr. When the dose was elevated to 10 mg, the concentration of quercetin was 89.4 and 35.3  $\mu\text{M}$ , after 1 and 6 hr, respectively. The antioxidant activity of quercetin-treated plasma was determined after dilution by measuring the accumulation of CE-OOH during the copper ion-induced lipid peroxidation of rat plasma (Fig.7). Plasma after administration of quercetin enhanced the resistance against CE-OOH accumulation indicating that quercetin metabolites participate in the antioxidative defense in blood plasma. It can be concluded that some conjugated metabolites of quercetin inhibit copper ion-induced plasma oxidation. Thus, quercetin possesses antioxidative activity for copper-ion induced oxidation of plasma even after its metabolic conversion. We are now trying to clarify the metabolites responsible for the antioxidant defense in blood.

## CONCLUSION

(-)-Epicatechin and quercetin are obtained by the daily intake of vegetables, fruits and tea. Intestinal absorption and the subsequent metabolic conversion should be taken into account when elucidating their physiological functions. Although little is clear as to their absorption and metabolism in humans, studies indicate that both flavonoids are partly absorbed into the body and largely accumulated as

glucuronide and sulfate conjugates. Conjugated metabolites containing a catechol group are likely to be responsible for the increase of plasma antioxidative capacity. Although the conjugation with glucuronide and sulfate is a step in the detoxification to lose the physicochemical activity, intermediate products should retain their activity. Metabolites may be at least partly responsible for the physiological function of dietary flavonoids.

## REFERENCES

- Hertog MGL, Holmann PCH, Katan MN : Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Nutr Cancer* 20: 21-29, 1993
- Kuhn J : Flavonoids ; A class of semi-essential food components : their role in human nutrition. *World Rev Nutr Diet* 24 : 117-191, 1976.
- Rusznyak S, Szent-Györgyi : Vitamin nature of flavones. *Nature* 138 : 798, 1936
- Hertog MGL, Holmann PCH : Potential health effects of the dietary flavonol quercetin. *Eur J Clin Nutr* 50 : 63-71, 1996
- Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. *Fd Chem Toxic* 33 : 1061-1080, 1995
- Katan M : Flavonoids and heart disease. *Am J*



- Clin Nutr 65 : 1542-1543, 1997
7. Manach C, Regerat F, Texier O, Agullo G, Demigne C, Remesy C : Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr. Res. 16 : 517-544, 1996
  8. Starvic B : Quercetin in our diet : from potent mutagen to probable anticarcinogen. Clin Biochem 27 : 245-248, 1994
  9. Cook, NC, Sammn S : Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 7 : 66-76, 1996
  10. World Cancer Research Fund : Food, nutrition and the prevention of cancer: A global perspective. American Institute for Cancer Research, New York, 1997
  11. Knekt P, Jarvinen, R, Reunanen A, Maatela J : Flavonoid intake and coronary mortality in Finland : a cohort study. Br Med J 312: 478-481, 1996
  12. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen, M, Simic BS, Toshoma H, Feskens EJM, Holmann PCH, Katan M : Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med. 155 : 381-386, 1995
  13. Renaud S, deLorgeli M : Wine, alcohol, platelets and the French paradox for coronary heart disease. Lancet 339 : 1523-1526, 1992
  14. Rice-Evans C, Miller NJ, Paganga G : Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol Med 20: 933-956, 1996
  15. Terao J, Piskula M, Yao Q : Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch Biochem Biophys 308 : 278-284, 1994
  16. Ioku K, Tsushida T, Takei Y, Nakatani N, Terao J : Antioxidative activity of quercetin and quercetin monoglucosides in solution and phospholipid bilayers. Biochim. Biophys Acta 1234 : 99-104, 1995
  17. Terao J, Piskula MK : Flavonoids as inhibitors of lipid peroxidation in membranes. In : Rice-Evans CA, Packer L, eds. Flavonoids in health and disease. Marcel Dekker Inc., New York, 1998, pp 277-293
  18. Das NP : Studies on flavonoid metabolism : absorption and metabolism of (+)-catechin in man. Biochem Pharmacol 20 : 3435-3445, 1971
  19. Okushio K, Matsumoto N, Suzuki M, Nanjo F, Hara Y : Absorption of (-)-epigallocatechin gallate into rat portal vein. Biol Pharm Bull 18 : 190-191, 1995
  20. Unno T, Takeo T : Absorption of (-)-epicatechin gallate into circulation system of rats. Biosci Biotech Biochem 59 : 1558-1559, 1995
  21. Nakagawa K, Miyazawa T : Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate, in the rat. J Nutr Sci Vitaminol 43 : 679-684 : 1997
  22. Hackett AM, Griffiths LA, Broilet A, Wermeille M : The metabolism and excretion of (+)-[C<sup>14</sup>] cyanidanol-3 in man following oral administration. Xenobiotica 5 : 279-286, 1983
  23. Hackett AM : the Metabolism of flavonoid compounds in mammals. In : Cody V, Middleton E, Hardorne JB, eds. Plant Flavonoids in biology and medicine : biochemical, pharmacological and structure-activity relationship. Aran Press New York, 1986, pp177-194
  24. Piskula MK, Terao J : Accumulation of (-)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. J Nutr 128 : 1172-1178, 1998
  25. Radomska-Pandya A, Little JM, Pandya JT, Tephly TR, King CD, Barone GW, Raufman J-P : UDP-glucuronosyltransferases in human intestinal mucosa. Biochim. Biophys Acta 1394: 199-208, 1998
  26. DaSilva EL, Piskula MK, Terao J : Enhancement of antioxidative ability of rat plasma by oral administration of (-)-epicatechin. Free Radical Biol Med 24 : 1209-1216, 1998
  27. Bors W, Heller W, Michel C : Flavonoids as antioxidants : determination of radical-scavenging efficiencies. Methods Enzymol 7 : 66-76, 1996
  28. 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 66 : 261-266, 1997
  29. Okushio K, Suzuki M, Matsumoto N, Nanjo F, Hara Y : Identification of (-)-epicatechin metabolites and their metabolic fate in the rat. Drug Metab Dispo 27 : 309-316, 1999
  30. Harada M, Kan Y, Naoki H, Fukui Y, Kegayama N, Nakai M, Miki W, Kiso Y : Identification of the major antioxidative metabolites in biological fluid of the rat with ingested (+)-catechin and (-)-epicatechin. Biosci Biotechnol Biochem 63:

- 973-977, 1999
31. Gugler R, Leschik M, Dengler HJ : Disposition of quercetin in man after single oral and intravenous doses. *Eur J Clin Pharmacol* 9: 223-234, 1975
  32. Ueno I, Nakamura N, Nironol, Metabolic fate of [<sup>14</sup>C] quercetin in the ACI rat. *Japan J Exp Med* 53 : 41-50, 1983
  33. Piskula M, Terao J : Quercetin's solubility affects its accumulation in rat plasma after oral administration. *J Agric Food Chem* 46: 4313-4317, 1998
  34. Tamura G, Gold C, Ferr-Luzi A, Ames BN : Fecalase :A model for activation of dietary glycosides to mutagens by intestinal flora. *Proc Natl Acad Sci USA* 77 : 4961, 1981
  35. Booth AN, Murray CW, Junes FT, DeEds F : the metabolic fate of rutin and quercetin in the animal body. *J Biol Chem* 233 : 251-257
  36. Manach C, Morand C, Taxier O, Farier MC, Agullo G, Demigne C, Regeat F, Remesy C : Quercetin metabolites in plasma of rats fed containing rutin and quercetin. *J Nutr* 125: 1911-1922, 1995
  37. Holmann PCH, Vries JHM, Van Leeuwen SD, Mengelers MJB, Katan MB : Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am J Clin Nutr* 62 : 1276-1282, 1995
  38. Holmann PCH, Garrg M, Mengelers MJB, van Trup JMP, de Vries JHM, Katan MB : Absorption and free disposition kinetics of the dietary antioxidant quercetin in man. *Free Radical Biol Med* 21 : 703-707, 1996
  39. Paganga G, Rice-Evans CA : The identification of flavonoids as glycosides in human plasma. *FEBS Lett* 401 : 78-82, 1997
  40. Aziz A, Edwards, CA, Lean MEJ, Crozier A : Absorption and excretion of conjugated flavonols, including quercetin-4'-O-β-glucoside and isorhametin-4'-O-β-glucoside by human volunteers after the consumption of onions. *Free Radical Res* 29 : 257-269, 1998
  41. Manach C, Morand C, Crespy V, Demigne CM, Texier O, Regeat F, Remesy C : Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Lett* 426 : 331-336, 1998
  42. Manach C, Texier O, Regeat F, Agullo, G, Demigne C, Remesy C : Dietary quercetin is recovered in rat plasma as conjugated derivatives of isorhamnetin and quercetin. *Nutr Biochem* 7 : 375-380, 1996
  43. Ioku K, Pongpiriyadacha Y, Konishi Y, Takei Y, Nakatani N, Terao J : β-Glucosidase activity in the rat small intestine toward quercetin monoglucosides. *Biosci Biotechnol Biochem* 62 : 1428-1431, 1998.
  44. Gee JM, Dupont S, Rhodes MJC, Johnson IT : Quercetin glucosides interact with the intestinal glucose transport pathway. *Free Radical Biol Med* 25 : 19-25, 1998
  45. Noteborn HPJM, Jansen E, Benito S, Mengelers MJB : Oral absorption and metabolism of quercetin and sugar-conjugated derivatives in specific transport systems. *Cancer Lett* 114: 175-177, 1997
  46. Walgren, RA, Walle UK, Walle T. Transport of quercetin and its glucosides across human intestinal epithelial Caco-II cells. *Biochem Pharmacol* 55 : 1721-1727, 1998
  47. Morand C, Crespy V, Manach C, Besson C, Demigne C, Remesy C : Plasma metabolites of quercetin and their antioxidant properties. *Am J Physiol* : R212-R219, 1998
  48. DaSilva EL, Piskula MK, Yamamoto N, Moon J-H, Terao J : Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. *FEBS Lett* 430 : 405-408, 1998