Title: Enhancing effect of the coexisting alpha-tocopherol on quercetin absorption and metabolism.

Rikito Mitsuzane[1], Reiko Okubo[1], Miyu Nishikawa[2], Shinichi Ikushiro[2], Shintaro Munemasa[1], Yoshiyuki Murata[1], Yoshimasa Nakamura[1], Toshiyuki Nakamura[1,*]

[1] Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan
[2] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, Toyama, Japan

[*]Corresponding author: Toshiyuki Nakamura, Ph. D, Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan. Fax: +81-86-251-8310. E-mail address: t-nakamura@okayama-u.ac.jp.

Abbreviations:
Q, quercetin; IR, isorhamnetin; GA, glucuronide; S, sulfate; αT, alpha-tocopherol; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ORAC, oxygen radical absorbance capacity; HO-1, heme oxygenase-1.

Keywords: Quercetin; metabolite; absorption; metabolism; antioxidant activity

Abstract

The aim of this study is to investigate the modulating effect of coexisting food components on the absorption and metabolism of quercetin and blood plasma antioxidant potentials. The combination of quercetin with α-tocopherol (αT), cellulose, or a commercially-available vegetable beverage containing αT and dietary fiber was orally administered to mice. Compared to the single administration of quercetin aglycone, the co-administration of αT with quercetin significantly increased the plasma quercetin concentration at 0.5 h, whereas the combination of quercetin and cellulose decreased it. Interestingly, the administration of quercetin mixed with the vegetable beverage showed no significant change of the quercetin concentration in the mice plasma. The treatment of the cells with the blood plasma after the co-administration of αT with quercetin significantly upregulated the gene expression of the antioxidant enzyme (heme oxygenase-1), whereas the quercetin and cellulose combination did not. In the plasma of the quercetin-administered mice, eight types of quercetin metabolites were detected and their quantities were affected by the combination with αT. The potentials of the heme oxygenase-1 gene expression by these metabolites were very limited, although several metabolites showed radical scavenging activities comparable to aglycone in the *in vitro* assays. These results suggested that the combination of αT potentiates the quercetin absorption and metabolism and thus the plasma antioxidant potentials, at least in part, by the quantitative changes in the quercetin metabolites.

Introduction

Quercetin (Q) is one of major dietary flavonoid aglycones in fruits and vegetables. Since Q has various biological activities, it is expected to contribute to health promotion and disease prevention in humans [1]. The absorption of Q into the body is considered one of the prerequisites for the expression of physiological activities. Q is mainly metabolized by some drug-metabolizing enzymes such as uridine 5′-diphosphate-glucuronosyltransferase, sulfotransferase, and/or catechol *O*-methyl transferase. The metabolic profiles of Q in the human plasma were shown as its glucuronides, sulfates, and sulfo-glucuronides with or without methylation [2-4]. Especially, the glucuronides and sulfates of Q are known to be major Q metabolites in the blood plasma [5-7], whereas Q aglycone was hardly detected in the human plasma [8,9].

Several reports have shown the modulatory effects of the co-administered food ingredients on the bioavailability of flavonoids [10]. For example, the plasma concentrations of Q and methylated Q (isorhamnetin, IR) were significantly higher in the rutin diet with pectin in comparison to the diet with cellulose [11]. The Q concentration in the brain of the mice co-administered with three polyphenols and α-tocopherol (αT) for 3 months was significantly higher than in those administered with polyphenols alone, whereas the plasma concentration of Q is slightly, but not significantly, higher in the co-administered mice [12]. As for the biological effects, the combined treatment with Q and αT has been shown to normalize some biochemical parameters in cadmium-treated rats more clearly than the individual treatments [13]. The combined treatment with Q and luteolin, which is also a major flavonoid, has been reported to enhance the antiproliferative effects in the nicotine-treated human breast cancer cell line [14].

Q has been widely known to have antioxidant activities by *in vitro* assays, such as the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and ORAC (oxygen radical absorbance capacity) assay [15-18]. In addition, Q has been reported to modulate the expression of various types of the cytoprotective genes [19-23]. Regarding the Q metabolites, Q 3-glucuronide (Q3GA) shows the DPPH radical scavenging activity and inhibition of copper ion-induced lipid peroxidation of human plasma LDL *in vitro* [5]. In addition, Q3GA, Q3'GA and Q7GA, but not Q4'GA, exerted inhibitory effect of lipopolysaccharide-dependent induction of the interleukin-6 gene expression in RAW 264.7 cells [24]. On the other hand, Q4'GA exhibited the strongest inhibitory effect of the xanthine oxidase activity, which was comparable to that by Q aglycone [25]. IR 3-glucuronide (IR3GA) suppressed the protein expression of certain signaling molecules and increased the heme oxygenase-1 (HO-1) protein expression in lipopolysaccharide-treated RAW264.7 cells [26]. In addition, not only the blood

plasma of the mice administered with Q, but also Q3GA and Q 3'-sulfate (Q3'S) upregulated the peroxisome proliferator-activated receptor gamma expression in A549 lung cancer cells [27,28].

Although there have already been many reports on the absorption and biological activities of Q, the modulating effects of the coexisting food components not only on the metabolic properties of Q, but also on the contribution of the Q metabolites to the Q-induced biological activity *in vivo* remain unclear. In this study, we investigated the modulating effect of the co-administration of food components, such as αT or cellulose, with Q on the absorption and metabolism of Q, and estimated the contribution of the Q metabolites to the *in vivo* antioxidant ability.

Materials and Methods

Chemicals

Q, IR and D-αT were obtained from Tokyo Chemical Industry (Tokyo, Japan). Cellulose was obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). A commercial vegetable beverage was purchased from a local supermarket in Okayama. Standards of the Q metabolites were synthesized according to a previously reported procedure using conjugate-producing budding yeast [29]. Glucuronidase/sulfatase derived from *Helix pomatia* (Sulfatase type H-1) was purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were of analytical grade.

Animals and study design

The study protocol was approved by the Institutional Animal Care and Use Committee of Okayama University (permission number OKU-2019020). ICR mice (male, 7-9 weeks old) housed by a previously described method were used [30]. After overnight fasting, except for free access to tap water, Q solubilized with 50% propylene glycol was administered into the intragastric administration as 5 mg Q aglycone/kg body weight with or without αT (250 μg/kg body weight), cellulose (60 mg/kg body weight), mixture of αT and cellulose, or a commercial vegetable beverage (5 mL/kg body weight), which contains protein 1.2 g, lipid 0 g, carbohydrate 7.8 g, sodium chloride equivalent 0–0.3 g, vitamin A 0.1–0.7 mg, lycopene 16 mg, β-carotene 1.5–6.5 mg per 100 mL. Peripheral blood was collected from the tail vein using heparinized capillaries before and at 0.5, 1, 3, and 6 h after administration. The samples were stored at −20°C until use. Q in the plasma was below the detection limits after administration of only the commercial vegetable beverage.

Analysis of quercetin and its metabolites in the blood plasma

To determine the apparent total levels of Q before and after administration of the sample, the obtained blood plasma was analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS, Xevo TQD, Waters) with deconjugation treatment using glucuronidase/sulfatase (Sulfatase type H-1). After the enzyme treatment, Q was extracted with ethyl acetate as previously described [31]. HPLC separation was done by a gradient system using solvent A (0.1% formic acid) and solvent B (acetonitrile) using a ACQUITY UPLC BEH C18 column (1.7 μm, Waters) at the flow rate of 0.4 mL/min. The gradient program was 0 min (A 95%), 0.5 min (A 95%), 5 min (A 5%), 5.5 min (A 5%), 5.6 min (A 95%), and 6.5 min (A 95%). The detection limit of Q was 10 nM, and that of IR was 1 nM.

Regarding the metabolites, the blood plasma samples were extracted with methanol and acetonitrile and concentrated using a centrifugal evaporator. Extracts of the Q metabolites were analyzed by LC-MS/MS as previously described [32] with some modification. Briefly, the HPLC separation was done by a gradient system using solvent A (0.1% formic acid) and solvent B (acetonitrile) and a Develosil C30-UG-5 (2.0 × 150 mm) column (Nomura Chemical Company, Limited, Aichi, Japan) at the flow rate of 0.2 mL/min. The gradient program was 0 min (A 95%), 40 min (A 50%), 45 min (A 20%), 46 min (A 95%), and 55 min (A 95%). The peak area of these metabolites was corrected by the area of the internal standard, chalcone.

DPPH radical scavenging assay

The DPPH radical scavenging activity was assayed by a previously described method [33]. Briefly, 0.1 mL of various concentrations of the Q metabolites in ethanol or the blood plasma diluted by MES buffer (10, 20 and 40-folds) were mixed with 0.1 mL of a 0.2 mM DPPH ethanol solution, then the mixture was stored for 30 min at room temperature in the dark. The absorbance of the reaction solution was recorded at 520 nm. Trolox was used as a positive control, and the radical scavenging capacity of the metabolites was determined as % DPPH inhibition = [(DPPH Abs – blank Abs) – (sample Abs – blank Abs)]/(DPPH Abs – blank Abs) × 100.

ORAC assay

The ORAC assay was performed according to the protocol by Wu *et al*. [33]. Briefly, 35 μL of various concentrations (0.8-100 μM) of the Q metabolites, Trolox standard or blood plasma were mixed with 115 μL of a fluorescein working solution (77.5 nM) in a 96-well plate. The initial fluorescence was recorded as ($f_{0\,min}$). During the following 120 min, the fluorescence

was recorded every 2 min after the addition of 50 μL of 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 82.4 mM). The area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated by subtracting the AUC of the blank. The results were converted as relative to Trolox.

RT-PCR

Hepa1c1c7 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in MEMα supplemented with 10% FBS, 4 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were grown at 37 °C in 5% CO_2. Hepa1c1c7 cells were treated with media containing 10% blood plasma (instead of 10% FBS) or the Q metabolites at the indicated concentrations for 6 h. The cells were treated with DMSO as the control. The total RNA was extracted with TRIzol reagent according to the manufacturer's manual. The total RNA (5 μg) was reverse transcribed to cDNA using ReverTra Ace. The cDNA was used for PCR amplification with Taq polymerase. Primers used in the PCR amplification were as follows: mHO-1, (F) 5'-ACATCGACAGCCCCACCAAGTTCAA -3' and (R) 5'-CTGACGAAGTGACGCCATCTGTGAG-3'; β-actin, (F) 5'-GTCACCCACACTGTGCCCATCTA-3' and (R) 5'-GCAATGCCAGGGTACATGGTGGT-3'. The PCR conditions, including the cycles and annealing temperatures, were optimized as follows: mHO-1, 23 cycles, 60 °C; β-actin, 16 cycles, 65 °C. The amplified PCR products were separated on an agarose gel (1%), stained with ethidium bromide, and visualized using an image analyzer (ImageQuant LAS500, GE Healthcare). The relative densities of the bands were measured using Image J Software Program.

Statistical analyses

Results of the blood plasma are presented as means ± standard error (S.E.)., and all the other values are presented as means ± standard deviation (S.D.) ($n > 3$). Statistical significance was determined by Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey. Data were considered significant at $P < 0.05$.

Results

Effects of coexisting food components on the total plasma concentration of quercetin in the quercetin-administered mice

We first quantified the total plasma levels of the Q metabolites as free flavonoids using LC-MS/MS. After deconjugation treatment, Q and its methylated metabolites, IR, were

detected in the blood plasma of the mice after the administration of Q, Q with αT, Q with cellulose, and Q with αT and cellulose (Fig. 1A). The maximum concentration (C_{max}) of the deconjugated Q in the plasma was achieved 0.5 h after administration in all cases, whereas the C_{max} values of IR were observed later (1 to 6 h after administration). The total plasma concentration of Q and IR in the Q and αT-administered mice was significantly higher than that in the Q alone-administered mice, whereas that in the Q and cellulose-administered mice and Q, αT and cellulose-administrated mice were lower. Interestingly, the co-administration of a commercial vegetable beverage with Q showed no significant change of the Q concentration in the blood plasma compared to that in the Q alone-administered mice (Fig. 1B). These results implied that the Q absorption in the small intestine was influenced by the co-administered food components.

Effects of coexisting food components on the antioxidative phenomena induced by the blood plasma of the quercetin-administered mice

Using the blood plasma 0.5 h after the administration of Q with or without the food components, their antioxidative potentials were evaluated using both the DPPH and ORAC assays. As shown in Figure 2, all the blood plasma samples showed the same antioxidant activities as that in the control mice at every dilution ratio. We next examined the possibility that the plasma Q metabolites regulate the gene expression of the antioxidant enzyme, HO-1. The gene expression of HO-1 in mouse hepatoma Hepa1c1c7 cells tended to be enhanced by the blood plasma of the Q alone-administered mice. Furthermore, the treatment of the blood plasma of the Q and αT-administered mice significantly enhanced the HO-1 expression compared to that of the control (Fig. 3), whereas that of the Q and cellulose-administered mice did not change it at all.

Effects of coexisting food components on the concentration of each quercetin metabolite in the blood plasma of the quercetin-administered mice

The plasma levels of the individual metabolites detected by the LC-MS/MS analysis are shown in Figure 4. The Q glucuronides with or without methylation and Q sulfate were detected in the plasma 0.5 h after the administration. The composition pattern of the plasma Q metabolites was the same among all the groups, but the plasma concentration of each Q metabolite was changed by the addition of food components with Q. All the metabolites in the blood plasma of the Q and αT-administered mice tended to be higher than those after the administration of Q or Q with cellulose, in accordance with the total level (Fig. 1). The levels of Q7GA and Q3'S were quite higher than those of the other metabolites in the blood plasma

of all the Q-administered mice.

Effects of each quercetin metabolite on the antioxidative phenomena in the *in vitro* assays and cultured cell assay

To check the chemical antioxidant activities of the Q metabolites, the DPPH and ORAC assays were performed. The DPPH radical scavenging activities of Q and IR were comparable to that of Trolox (Fig. 5A and Table 1). The Q glucuronides, except for Q4'GA, were also highly effective in this assay. Meanwhile, the Q sulfate and IR glucuronides did not exert the significant free radical scavenging activity. Interestingly, not only the Q metabolites that can quench DPPH radicals, but also Q4'GA and the IR glucuronides showed a stronger antioxidant activity than Trolox in the ORAC assay (Fig. 5B).

To further investigate the cellular antioxidant activity of the Q metabolites, the gene expression of HO-1 was evaluated using the mouse hepatoma cell line. The 5 μM treatment of Q or IR with Hepa1c1c7 cells resulted in the significant upregulation of the HO-1 gene expression (Fig. 6). Regarding the Q metabolites, 50 μM of Q3GA, Q3'GA and Q7GA significantly, but to a lesser extent, enhanced it. IR3GA and IR7GA also upregulated the HO-1 gene expression, even though both compounds did not scavenge the DPPH radicals (Fig. 5). Q3'S did not show any antioxidant activities in the cellular assay as well as the *in vitro* experiments of this study.

Discussion

In the present study, we demonstrated the modulating effect of the coexisting food components, αT and fiber, on the Q absorption and metabolism. In most previous studies, the bioavailability of flavonoids was investigated either by the individual administration of a pure compound or by the dosing of food matrices, such as juices and vegetables, but not by the co-ingestion of flavonoids with certain food ingredients [34-36]. Although there are a few reports showing the bioavailability of flavonoids in the co-administration [10], information on the behavior of flavonoids when administered in combination is very limited. This is, to the best of our knowledge, the first report to reveal the enhancing effect of the coexisting αT on the Q absorption in the blood plasma (Fig. 1A). These data are similar to a previous report showing that the combined administration of Q with long-chain fatty acids increased the plasma C_{max} of the Q metabolites [37], suggesting that certain lipophilic compounds are capable of enhancing the stability of Q during digestion and absorption.

On the other hand, the plasma levels of the Q metabolites in the cellulose-co-administered

mice were significantly lower than that of the control group (Fig. 1A). In addition, it was not improved by the combination with αT. The interaction of flavonoids with various dietary fibers has been widely studied. A molecular simulation study revealed that tea catechin interacts with cellulose by intermolecular forces [38]. A fiber-rich diet actually reduced the absorption of genistein, one of the major isoflavones, in a single meal ingestion [39]. It has been considered that the flavonoids that interacted with the dietary fibers are not bioaccessible for absorption in the small intestine [40]. On the other hand, the co-administration of Q with a commercial vegetable beverage did not significantly change the plasma Q concentration compared to the control (Fig. 1B). Five mL of the vegetable beverage contains approximately 250 μg αT and 60 mg fiber, both of which were the same doses used in this study. In addition, the vegetable beverage contains many chemicals such as other vitamins and carotenoids. It is suggested that these chemicals contribute to the absorption of Q in the body in a coordinated manner.

The blood plasma after co-administration of Q and αT did not enhance the radical scavenging activity compared to the control (Fig. 2). A previous report showed that the administration of a diet containing green tea extract did not enhance the antioxidant capacity of the mouse plasma determined by the ferric reducing antioxidant power assay [41]. In addition, the administration of a high fat diet with or without resveratrol showed no difference in the antioxidant activities of the mouse plasma evaluated by the DPPH and ORAC assays [42]. The reason why the potential of the Q-administered mouse plasma to scavenge free radicals was not influenced by the coexisting food components is that the mice plasma contains considerable amounts of antioxidants, such as glutathione and ascorbic acid [43,44]. In contrast to the non-enzymatic antioxidant activity, the administration of a diet containing resveratrol could enhance the enzyme activity of glutathione peroxidase [42]. The present study also showed that the blood plasma of the Q and αT-administered mice significantly enhanced the HO-1 gene expression in mouse hepatocytes (Fig. 3). The characteristic of *in vivo* antioxidant activity by Q metabolites may be mediated by HO-1 gene expression rather than by radical scavenging effects (Fig. 2 and 3), although Q metabolites themselves have the potency of radical scavenging effects (Fig. 5). We have recently observed that αT could enhance the HO-1 gene expression [33]. Since αT is slowly absorbed through the lymphatic pathway [45], it might not be absorbed into the blood plasma at 0.5 h after administration. Thus, the Q metabolites, but not αT itself, in the blood plasma might be involved in the promotion of the HO-1 gene expressions.

Eight types of Q metabolites were detected in the blood plasma of the Q-administered mice (Fig. 4). Among them, Q7GA and Q3'S were predominant in all the Q-administered mice. Our previous study using Wistar/ST rats also showed that Q7GA was the most abundant metabolite

among the four Q glucuronides [29]. The amount of total deconjugated Q was higher than the sum of Q metabolites (Fig. 1A and Fig. 4). There are several possibilities for this difference. Quercetin is metabolized other types of metabolites such as di-glucuronide and glucuronide-sulfate, although this study focused on major 8 types of quercetin metabolites. Also, it may be due to extraction efficiency, because polyphenols easily interact with plasma components and co-precipitate with them in the extraction process.

Q3GA, Q3'GA and Q7GA, but not Q4'GA, had the significant ability to capture the DPPH radicals (Fig. 5). Moon *et al.* reported that the antioxidant ability of Q3GA was much higher than that of Q4'GA [5], partially consistent with our data. Interestingly, the antioxidant activities of the Q metabolites, especially the IR glucuronides, were different between the DPPH and ORAC assays (Fig. 5). Because the ORAC assay uses a peroxyl radical-producing compound, it is regarded to reflect the chemical reactions under actual *in vivo* conditions better than the DPPH radical scavenging assay. Actually, the potencies of the Q metabolites to enhance the HO-1 gene expression is consistent with their antioxidative activities evaluated by the ORAC assay (Figs. 5 and 6). Although the gene expression of HO-1 was induced by 50 μM of the Q metabolites (Fig. 6), they are present in the blood plasma only in the nanomolar range (Fig. 4). It has been reported that the combination of Q glucuronides and Q3S inhibited the oxidation of human LDL comparable to that of the Q aglycone [46]. Thus, the Q metabolites might exert the *in vivo* antioxidant effects in a coordinated manner. In addition, the IR glucuronides might be maintained at a high level for a relatively longer time (~6 h) after administration than the Q glucuronides. Therefore, the IR glucuronides might be involved in the continuous induction of the antioxidant enzyme gene expression.

In our previous study, it was revealed that Q metabolism was influenced by co-administration of isoflavone-rich food, altering not only the amount of Q absorbed but also the profile of Q metabolites [32]. On the other hand, co-administration with αT did not change the profile of Q metabolites in this study (Fig. 4). Considering the activity of Q metabolites, changes in quercetin absorption and its metabolic profile by the co-administered food components would be important information. This study focused on anti-oxidative effects (DPPH and ORAC) and oxidative stress-mediated anti-oxidative effects (induction of phase II enzyme) by quercetin metabolites because quercetin itself, due to its unique chemical structure, has the potential not only to scavenge free radical but also to induce some signal transductions. Generally, it is known that the phytochemicals including Q are xenobiotic substances. However, appropriate doses of the phytochemicals can be beneficial effects to our body. Thus, multifaceted approaches would provide useful information for actual effects of Q in our body.

In conclusion, the bioavailability of Q was enhanced by the co-administration of αT via

the increase of absorption from small intestine, although the pattern of the Q metabolites in the blood plasma was similar for any administration. The enhanced Q absorption could influence the antioxidant capacity in the blood plasma through the modulation of antioxidant enzyme gene expression. Therefore, it is important to note once again that coexisting food components have a significant impact on the bioavailability of flavonoids. Some of the Q metabolites actually have a potential to elicit antioxidative phenomena through the enhanced gene expression of antioxidant enzymes as well as the direct chemical reaction, even though the antioxidant ability of each metabolite is very limited. On the other hand, the rodent model as well as the cultured mouse hepatocyte model has some limitations, such as not reflecting the characteristics of human ones. Additionally, the Hepa1c1c7 cells do not reflect the characteristics of intact hepatocytes because this cell line originated from hepatocellular carcinoma. In addition, the effective concentrations of each Q metabolite required for its antioxidant activity might be supraphysiological. Future efforts will be related to clarify the precise mechanism of how αT enhances the Q absorption and the synergistic effects of the metabolites or other biological factors in human hepatocyte models. In addition, the enhancement of Q bioavailability by the co-administration of αT in human would need to be clarified.

References

1. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochem Pharmacol. 2012, 83, 6-15.
2. Berger LM, Wein S, Blank R, Metges CC, Wolffram S. Bioavailability of the flavonol quercetin in cows after intraruminal application of quercetin aglycone and rutin. J Dairy Sci. 2012, 95, 5047-5055.
3. Ader P, Wessmann A, Wolffram S. Bioavailability and metabolism of the flavonol quercetin in the pig. Free Radic Biol Med. 2000, 28, 1056-1067.
4. Mullen W, Edwards CA, Crozier A. Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. Br J Nutr. 2006, 96, 107-116.
5. Moon JH, Tsushida T, Nakahara K, Terao J. Identification of quercetin 3-O-beta-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. Free Radic Biol Med. 2001, 30, 1274-1285.
6. Day AJ, Mellon F, Barron D, Sarrazin G, Morgan MR, Williamson G. Human metabolism of dietary flavonoids: Identification of plasma metabolites of quercetin. Free Radic Res.

2001, 35, 941-952.
7. Tanaka S, Trakooncharoenvit A, Nishikawa M, Ikushiro S, Hara H. Comprehensive analyses of quercetin conjugates by lc/ms/ms revealed that isorhamnetin-7-O-glucuronide-4'-O-sulfate is a major metabolite in plasma of rats fed with quercetin glucosides. J Agric Food Chem. 2019, 67, 4240-4249.
8. Moon JH, Nakata R, Oshima S, Inakuma T, Terao J. Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. Am J Physiol Regul Integr Comp Physiol. 2000, 279, R461-467.
9. Sesink AL, O'Leary KA, Hollman PC. Quercetin glucuronides but not glucosides are present in human plasma after consumption of quercetin-3-glucoside or quercetin-4'-glucoside, J. Nutr. 2001, 131, 1938-1941.
10. Kamiloglu S, Tomas M, Ozdal T, Capanoglu E. Effect of food matrix on the content and bioavailability of flavonoids. Trends Food Sci Technol. 2021, 117, 15–33.
11. Tamura M, Nakagawa H, Tsushida T, Hirayama K, Itoh K. Effect of pectin enhancement on plasma quercetin and fecal flora in rutin-supplemented mice. J Food Sci. 2007, 72, S648-651.
12. Ferri P, Angelino D, Gennari L, Benedetti S, Ambrogini P, Del Grande P, Ninfali P. Enhancement of flavonoid ability to cross the blood-brain barrier of rats by co-administration with α-tocopherol. Food Funct. 2015, 6, 394-400.
13. Prabu SM, Shagirtha K, Renugadevi J. Amelioration of cadmium-induced oxidative stress, impairment in lipids and plasma lipoproteins by the combined treatment with quercetin and α-tocopherol in rats. J Food Sci. 2010, 75, T132-140.
14. Shih YL, Liu HC, Chen CS, Hsu CH, Pan MH, Chang HW, Chang CH, Chen FC, Ho CT, Yang YY, Ho YS. Combination treatment with luteolin and quercetin enhances antiproliferative effects in nicotine-treated MDA-MB-231 cells by down-regulating nicotinic acetylcholine receptors. J Agric Food Chem. 2010, 58, 235-241.
15. Foti MC, Daquino C, DiLabio GA, Ingold KU. Kinetics of the oxidation of quercetin by 2,2-diphenyl-1-picrylhydrazyl (dpph•). Org Lett. 2011, 13, 4826-4829.
16. Tsimogiannis DI, Oreopoulou V. The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3′,4′-hydroxy substituted members. Innov Food Sci Emerg Technol. 2006, 7, 140-146.
17. Atala E, Fuentes J, Wehrhahn MJ, Speisky H. Quercetin and related flavonoids conserve their antioxidant properties despite undergoing chemical or enzymatic oxidation, Food Chem. 2017, 234, 479-485.
18. Aaby K, Hvattum E, Skrede G. Analysis of flavonoids and other phenolic compounds

using high-performance liquid chromatography with coulometric array detection: relationship to antioxidant activity. J Agric Food Chem. 2004, 52, 4595-4603.
19. Rather RA, Bhagat M. Quercetin as an innovative therapeutic tool for cancer chemoprevention: Molecular mechanisms and implications in human health, Cancer Med. 2020, 9, 9181-9192.
20. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H, Yin Y. Quercetin, inflammation and immunity. Nutrients. 2016, 8, 167.
21. Moskaug JØ, Carlsen H, Myhrstad M, Blomhoff R. Molecular imaging of the biological effects of quercetin and quercetin-rich foods. Mech Ageing Dev. 2004, 125, 315-324.
22. Boesch-Saadatmandi C, Loboda A, Wagner AE, Stachurska A, Jozkowicz A, Dulak J, Döring F, Wolffram S, Rimbach G. Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. J Nutr Biochem. 2011, 22, 293-299.
23. Chow JM, Shen SC, Huan SK, Lin HY, Chen YC. Quercetin, but not rutin and quercitrin, prevention of H2O2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. Biochem Pharmacol. 2005, 69, 1839-1851.
24. Nishikawa M, Kada Y, Kimata M, Sakaki T, Ikushiro S. Comparison of metabolism and biological properties among positional isomers of quercetin glucuronide in LPS- and RANKL-challenged RAW264.7 cells. Biosci Biotechnol Biochem. 2022, 86, 1670-1679.
25. Day AJ, Bao Y, Morgan MR, Williamson G. Conjugation position of quercetin glucuronides and effect on biological activity. Free Radic Biol Med. 2000, 29, 1234-1243.
26. Park JY, Kim SI, Lee HJ, Kim SS, Kwon YS, Chun W. Isorhamnetin-3-O-glucuronide suppresses JNK and p38 activation and increases heme-oxygenase-1 in lipopolysaccharide-challenged RAW264.7 cells. Drug Dev Res. 2016, 77, 143-151.
27. Yeh SL, Yeh CL, Chan ST, Chuang CH. Plasma rich in quercetin metabolites induces G2/M arrest by upregulating PPAR-γ expression in human A549 lung cancer cells. Planta Med. 2011, 77, 992-998.
28. Chuang CH, Yeh CL, Yeh SL, Lin ES, Wang LY, Wang YH. Quercetin metabolites inhibit MMP-2 expression in A549 lung cancer cells by PPAR-γ associated mechanisms. J Nutr Biochem. 2016, 33, 45-53.
29. Nakamura T, Kinjo C, Nakamura Y, Kato Y, Nishikawa M, Hamada M, Nakajima N, Ikushiro S, Murota K. Lymphatic metabolites of quercetin after intestinal administration of quercetin-3-glucoside and its aglycone in rats. Arch Biochem Biophys. 2018, 645, 126-136.

30. Nakamura T, Tokushima T, Kawabata K, Yamamoto N, Miyamoto M, Ashida H. Absorption and metabolism of 4-hydroxyderricin and xanthoangelol after oral administration of Angelica keiskei (Ashitaba) extract in mice. Arch Biochem Biophys. 2012, 521, 71-76.
31. Murota K, Matsuda N, Kashino Y, Fujikura Y, Nakamura T, Kato Y, Shimizu R, Okuyama S, Tanaka H, Koda T, Sekido K, Terao J. alpha-Oligoglucosylation of a sugar moiety enhances the bioavailability of quercetin glucosides in humans. Arch Biochem Biophys. 2010, 501, 91-7.
32. Nakamura T, Murota K, Kumamoto S, Misumi K, Bando N, Ikushiro S, Takahashi N, Sekido K, Kato Y, Terao J. Plasma metabolites of dietary flavonoids after combination meal consumption with onion and tofu in humans. Mol Nutr Food Res. 2014, 58, 310-317.
33. Wu H, Nakamura T, Guo Y, Hirooka M, Zhang G, Munemasa S, Murata Y, Fujita A, Nakamura Y. White rice ethanol extract is qualitatively, but not quantitatively, equivalent to that of brown rice as an antioxidant source. Biosci Biotechnol Biochem. 2021, 85, 2161-2168.
34. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005, 81, 230S-242S.
35. Di Lorenzo C, Colombo F, Biella S, Stockley C, Restani P. Polyphenols and human health: The role of bioavailability. Nutrients. 2021, 13, 273.
36. Dabeek WM, Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients. 2019, 11, 2288.
37. Murota K, Cermak R, Terao J, Wolffram S. Influence of fatty acid patterns on the intestinal absorption pathway of quercetin in thoracic lymph duct-cannulated rats, Br J Nutr. 2013 , 109, 2147-2153.
38. Liu Y, Ying D, Sanguansri L, Cai Y, Le X. Adsorption of catechin onto cellulose and its mechanism study: Kinetic models, characterization and molecular simulation. Food Res Int. 2018, 112, 225-232.
39. Tew BY, Xu X, Wang HJ, Murphy PA, Hendrich S. A diet high in wheat fiber decreases the bioavailability of soybean isoflavones in a single meal fed to women. J Nutr. 1996 , 126, 871-877.
40. Palafox-Carlos H, Ayala-Zavala JF, González-Aguilar GA. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. J Food Sci. 2011, 76, R6-R15.
41. Koutelidakis AE, Kizis D, Argyri K, Kyriakou A, Komaitis M, Kapsokefalou M. The effect

of iron and fat in a diet containing green tea extract (camellia sinensis) on the antioxidant capacity of some organs and the mrna expression of specific genes in mice. J Med Food. 2014, 17, 1232-1238.
42. Ribeiro VM, Bede TP, Rocha GS, Barroso S, Valença S, Azeredo VB. High fat diet and high polyphenols beverages effects in enzymatic and non-enzymatic antioxidant activity. Nutr Hosp. 2017, 35, 169-175.
43. Igarashi T, Satoh T, Ueno K, Kitagawa H. Species difference in glutathione level and glutathione related enzyme activities in rats, mice, guinea pigs and hamsters. J Pharmacobiodyn. 1983, 6, 941-949.
44. Iwama M, Amano A, Shimokado K, Maruyama N, Ishigami A. Ascorbic acid levels in various tissues, plasma and urine of mice during aging. J Nutr Sci Vitaminol (Tokyo). 2012, 58, 169-174.
45. Traber MG, Leonard SW, Ebenuwa I, Violet PC, Wang Y, Niyyati M, Padayatty S, Tu H, Courville A, Bernstein S, Choi J, Shamburek R, Smith S, Head B, Bobe G, Ramakrishnan R, Levine M. Vitamin E absorption and kinetics in healthy women, as modulated by food and by fat, studied using 2 deuterium-labeled α-tocopherols in a 3-phase crossover design. Am J Clin Nutr. 2019, 110, 1148-1167.
46. Manach C, Morand C, Crespy V, Demigné C, Texier O, Régérat F, Rémésy C. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett. 1998, 426, 331-336.

CRediT authorship contribution statement

Conceptualization, R.M. and T.N.; Methodology, R.M., R.O., M.N. and S.I.; Investigation, R.M., R.O., M.N. and S.I.; Formal analysis, R.M., R.O., M.N. and S.I.; Data Curation, R.M. Y.N. and T.N.; Validation, S.M., Y.M., Y.N. and T.N.; Resources, M.N. and S.I.; Supervision, Y.N. and T.N.; Writing - Original Draft. R.M., Y.N. and Y.N.; Writing -Review & Editing, R.M. Y.N. and T.N. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was supported in part by MEXT KAKENHI [grant numbers 20H02933 and 23H02161 (Y.N.), 17H04725 and 21K11676 (T.N.)].

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure captions

Figure 1. Time-dependent changes of the Q and IR levels in the plasma, (A) after administration of Q with or without αT or fiber. (B) after administration of Q with the commercial vegetable beverage. Peripheral venous blood was collected at the desired time points before and at 0.5, 1, 3, and 6 h after administration. The collected samples were treated with deconjugation enzymes before analysis. (A) Open circles denote administration of Q, grey circles denote co-administration of Q and αT, and filled circles denote co-administration of Q and cellulose. (B) Open circles denote administration of Q, and filled circles denote co-administration of Q and the vegetable beverage. Values are means ± S.E. (n = 4–5). *P< 0.05 vs Q administration.

Figure 2. (A) DPPH radical scavenging capacities of blood plasma after administration of Q with or without food components. The plasma samples were incubated with DPPH for 30 min at room temperature in the dark. The DPPH radical scavenging activity was evaluated by the absorbance (520 nm). The final dilution ratio of the plasma was 1/20, 1/40 and 1/80. Values are means ± S.D. (n = 4–5). (B) ORAC values of blood plasma after administration of Q with or without food components. The plasma samples were mixed with fluorescein and AAPH. The ORAC values were evaluated by the fluorescence (Ex 485 nm/Em 525 nm). The results were converted as relative to Trolox using the net AUC values. Values are means ± S.D. (n = 4–5). There were no significant differences in all the blood plasma samples at any dilution ratio. P, the plasma of the 50% propylene glycol-administrated mice; QP, the plasma of the Q-administered mice; αTP, the plasma of the Q and αT-administered mice; CP, the plasma of the Q and cellulose-administered mice.

Figure 3. Enhancing effect of the plasma at 0.5 h after administration of compounds on the gene expressions of HO-1. The confluent Hepa1c1c7 cells were treated with the media containing 10% blood plasma for 6 h. The gene expression levels of HO-1 were determined by RT-PCR. Values are means ± S.D. (n = 3–4). *P< 0.05 vs control. P, the plasma of the 50% propylene glycol-administrated mice; QP, the plasma of the Q-administered mice; αTP, the plasma of the Q and αT-administered mice; CP, the plasma of the Q and cellulose-administered mice.

Figure 4. Concentration of the metabolites in the plasma at 0.5 h after each administration. The blood plasma samples were extracted with methanol and acetonitrile. Extracts of the Q metabolites were analyzed by LC-MS/MS. Open bars denote administration of Q, grey bars

denote co-administration of Q and αT, and filled bars denote co-administration of Q and cellulose. Values are means ± S.E. (n = 4–5). *P< 0.05 vs Q administration.

Figure 5. (A) DPPH radical scavenging capacities of the Q metabolites. The Q metabolites were incubated with DPPH for 30 min at room temperature in the dark. The DPPH radical scavenging activity was evaluated by the absorbance (520 nm). Values are means ± S.D. (n = 4). The IC50 values and their statistical analyses were shown in Table 1. (B) ORAC values of the Q metabolites. The Q metabolites were mixed with fluorescein and AAPH. The ORAC values were evaluated by the fluorescence (Ex 485 nm/Em 525 nm). The results were converted as relative to Trolox using the net AUC values. Values are means ± S.D. (n = 3). Different letters above the bars indicate significant differences among the treatments (p<0.05).

Figure 6. The modulating effects of the Q metabolites on the HO-1 gene expression. The confluent Hepa1c1c7 cells were treated with the Q metabolites at the indicated concentrations for 6 h. The gene expression levels of HO-1 were determined by RT-PCR. Values are means ± S.D. (n = 3–4). *P< 0.05 vs control.

Fig. 1

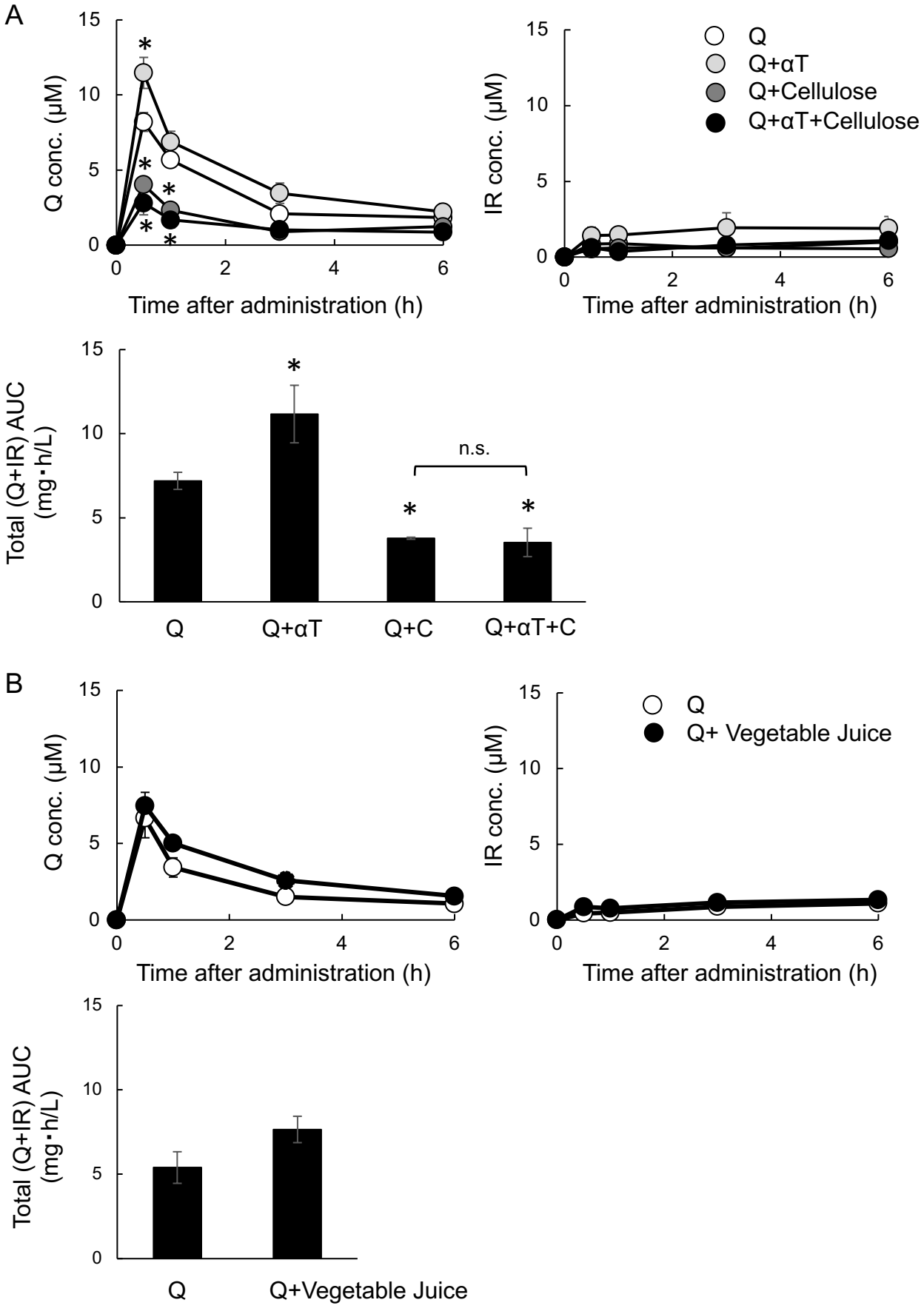

A
Q conc. (μM)
Time after administration (h)
IR conc. (μM)
Time after administration (h)
Q
Q+αT
Q+Cellulose
Q+αT+Cellulose
Total (Q+IR) AUC (mg・h/L)
n.s.
Q
Q+αT
Q+C
Q+αT+C
B
Q conc. (μM)
Time after administration (h)
IR conc. (μM)
Time after administration (h)
Q
Q+ Vegetable Juice
Total (Q+IR) AUC (mg・h/L)
Q
Q+Vegetable Juice

Fig. 2

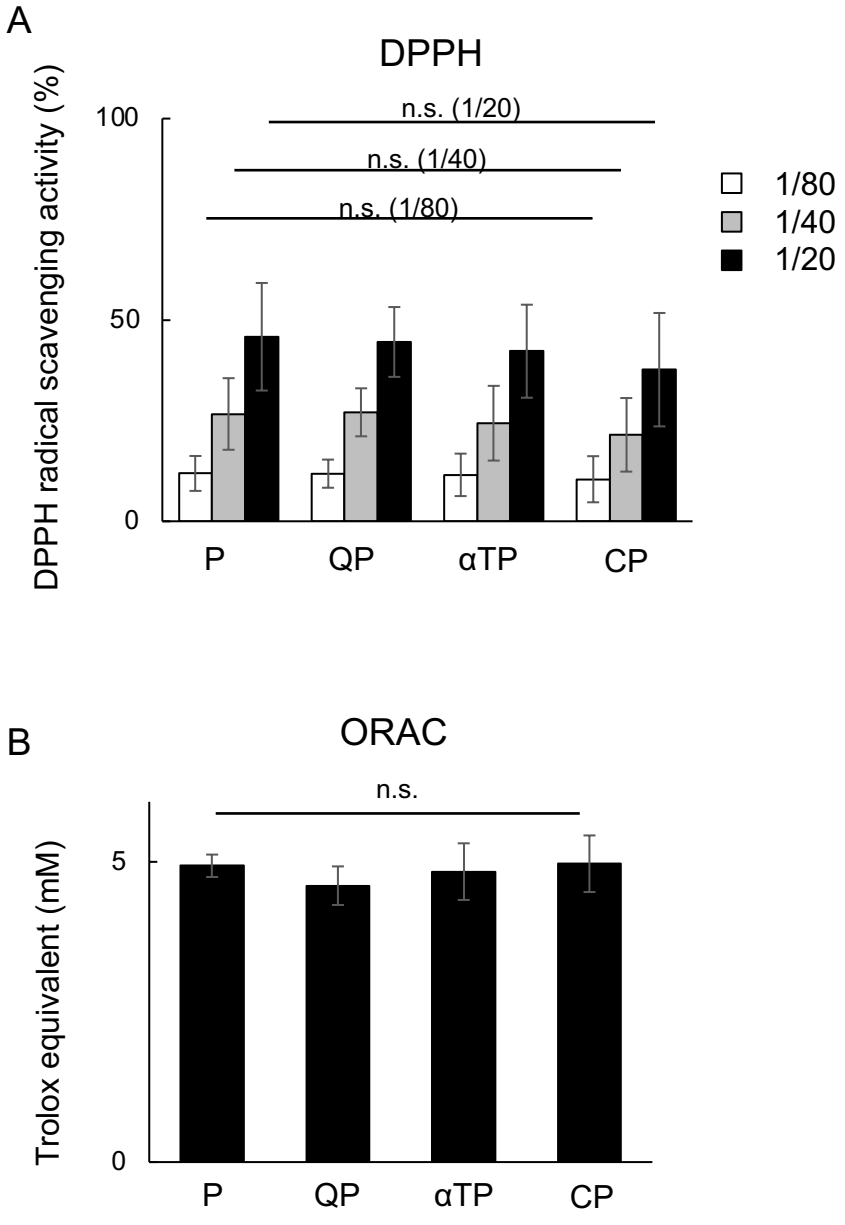

A
DPPH
DPPH radical scavenging activity (%)
100
50
0
n.s. (1/20)
n.s. (1/40)
n.s. (1/80)
1/80
1/40
1/20
P
QP
αTP
CP
B
ORAC
Trolox equivalent (mM)
5
0
n.s.
P
QP
αTP
CP

Fig. 3

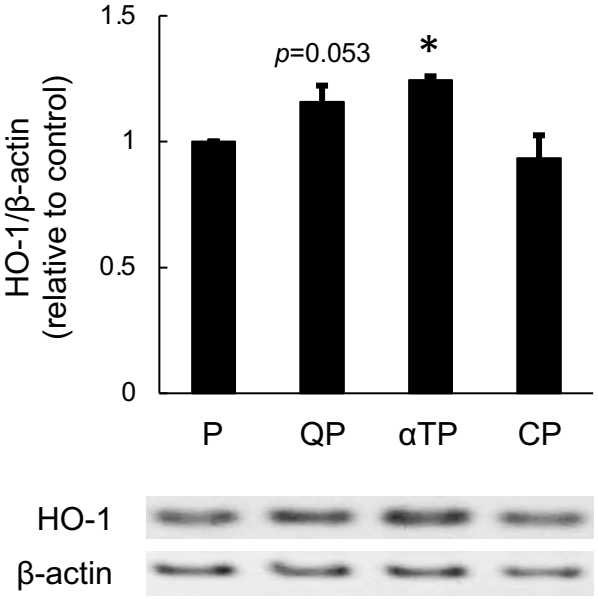

1.5
1
0.5
0
HO-1/β-actin
(relative to control)
p=0.053
*
P
QP
αTP
CP
HO-1
β-actin

Fig. 4

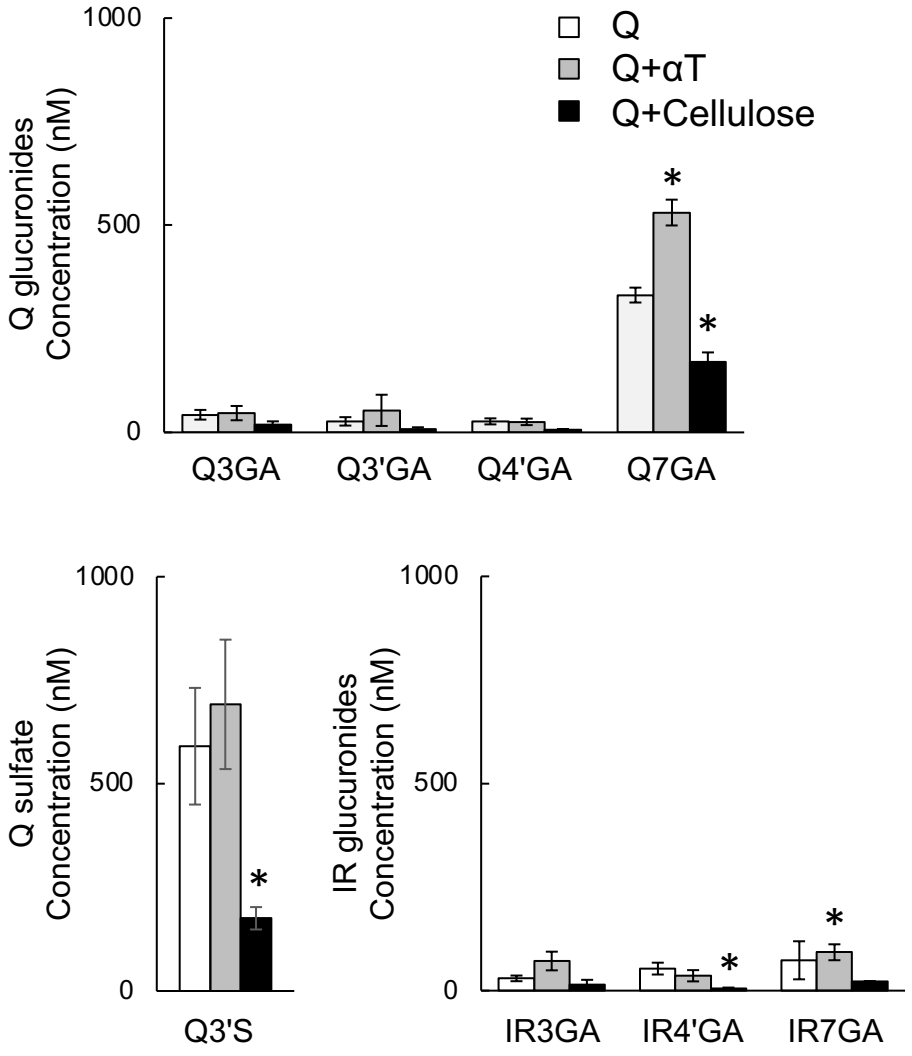

Q
Q+αT
Q+Cellulose
Q glucuronides Concentration (nM)
1000
500
0
*
*
Q3GA
Q3'GA
Q4'GA
Q7GA
Q sulfate Concentration (nM)
1000
500
0
*
Q3'S
IR glucuronides Concentration (nM)
1000
500
0
*
*
IR3GA
IR4'GA
IR7GA

Fig. 5

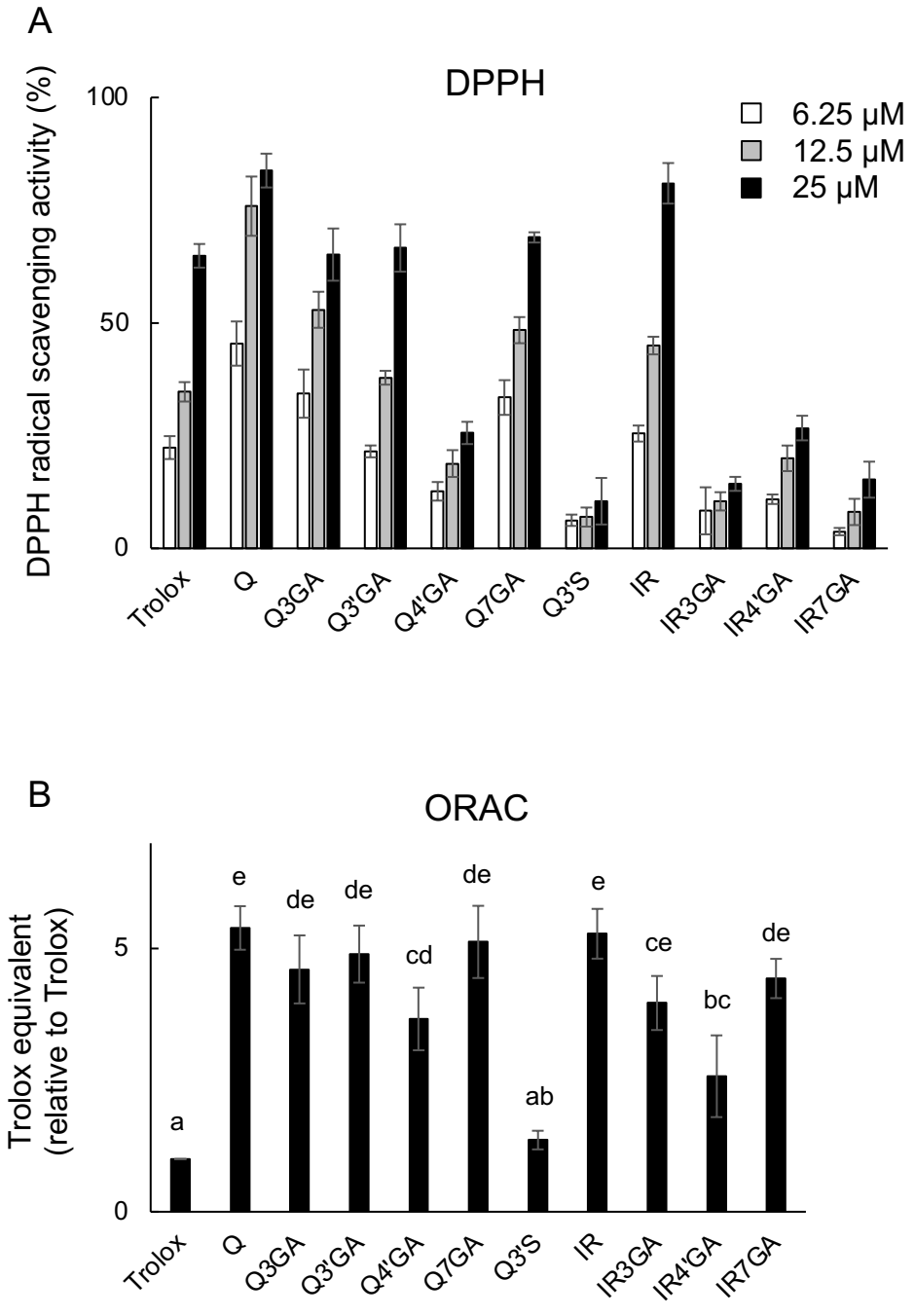
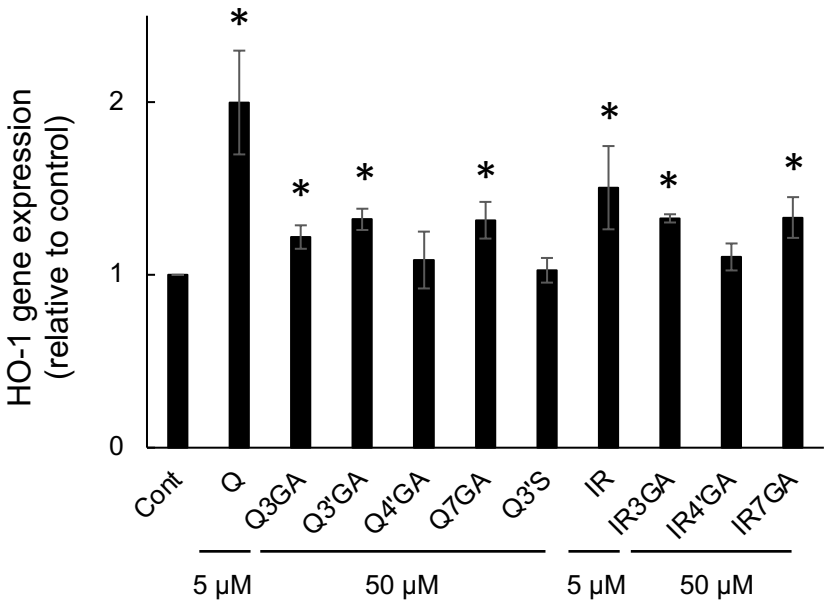

A
DPPH
DPPH radical scavenging activity (%)
100
50
0
6.25 μM
12.5 μM
25 μM
Trolox
Q
Q3GA
Q3'GA
Q4'GA
Q7GA
Q3'S
IR
IR3GA
IR4'GA
IR7GA
B
ORAC
Trolox equivalent (relative to Trolox)
5
0
a
e
de
de
cd
de
ab
e
ce
bc
de

Fig. 6


HO-1 gene expression
(relative to control)
0
1
2
*
Cont
Q
Q3GA
Q3'GA
Q4'GA
Q7GA
Q3'S
IR
IR3GA
IR4'GA
IR7GA
5 μM
50 μM
5 μM
50 μM