Role of Intestinal Flora on the Metabolism, Absorption, and Biological Activity of Dietary Flavonoids

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Much attention has been focused on flavonoids because of their beneficial effects on human health. Flavonoids are the most abundant dietary polyphenols. Quercetin is one of the major flavonoids and is contained in many foods. Soybean and soy foods are rich sources of isoflavones. Recent research has shown that they are beneficial to human health. The two major sites of flavonoid metabolism are the liver and the intestinal flora. Intestinal flora play an important role in the absorption and metabolism of flavonoids. Many of the flavonols including quercetin occur in food in the form of *O*-glycosides, with α -glucose as the most common sugar residue. With respect to the bioavailability of flavonoid glycosides, intestinal flora are known to have an important role in hydrolysis. Colonic flora are known to catalyze the breakdown of flavonoids. It was also found that suppressing the breakdown of quercetin by intestinal flora is important for achieving higher concentrations of quercetin in the plasma. Soy isoflavone aglycone is absorbed faster and in higher amounts than glucosides in humans. Some dietary components are also known to affect the absorption of isoflavones. Human metabolism and excretion of isoflavones following the consumption of soy products show considerable variation. The bioavailability of soybean isoflavones to women is dependant on gut microflora. Equol is a metabolite of daidzein produced by intestinal flora. Equol has many biological activities relates to human health, and its production might be affected by dietary composition and intestinal floral composition. To achieve higher production of equol from daidzein in the gut, control of the metabolic activity of intestinal flora might be of importance.

Key words: flavonoid; quercetin; rutin; daidzein; genistein; equol; intestinal flora; metabolism; absorption

INTRODUCTION

Epidemiological studies suggest a relationship between the daily consumption of fruits and vegetables and prevention of various pathologies such as cancer and cardiovascular disease. Fruits and vegetables are the major dietary source of flavonoids (35, 38, 65). These compounds comprise one of the largest groups of secondary plant metabolites and are known to occur widely throughout the plant kingdom. In recent years, much attention has been focused on flavonoids because of their beneficial effects on human health. Flavonoids are the most abundant dietary polyphenols. The structures of some flavonoid families (flavone, isoflavone, flavonol, flavanone, flavanonol, flavanol, anthocyanidin) are shown in Fig. 1. These compounds are potent radical scavengers and metal chelators due to their polyphenolic structure. The electron-donating properties of flavonoids have been shown to be the basis of their antioxidant action (30, 50, 63). Quercetin is one

of the major flavonoids and is contained in many foods. Soybean and soy foods are rich sources of isoflavones. Recent research has shown that they are beneficial to health. Many of the beneficial properties of quercetin (17, 27, 45, 52, 64, 73) and isoflavones (3, 4, 8, 9, 44, 48) have been reported. In this review, we describe some of the main members of the flavonoid genre, namely, quercetin, rutin and isoflavones. The two major sites of flavonoid metabolism are the liver and the intestinal flora. The intestinal wall and kidney may play a role. Absorbed flavonoids and their related colonic metabolites are glucuronidated and sulfated by the liver in humans and rodents (15, 25, 46, 71). Unabsorbed flavonoids and flavonoid conjugates are secreted with bile into the gut and are degraded by intestinal flora in the lower intestine. The hydrolysis of conjugates and glycosides and ring fission of the aglycones to phenolic acid are the main actions of intestinal flora (20, 21). Thus, intestinal flora play an important role in the absorption and metabolism of flavonoids.

ABSORPTION OF QUERCETIN AND QUERCETIN-3-RUTINOSIDE (RUTIN)

Quercetin, the main flavonol in our diet, is present in

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Fig. 1. Structures of some flavonoid families.

many fruits, vegetables, and beverages. It is particularly abundant in onions (22) and tea (23). There are some reports on the metabolism and absorption of quercetin and rutin after feeding with supplemented diets (37). Manach et al. reported the bioavailability of rutin and quercetin in rats. Conjugated derivatives of quercetin and its methylated forms (isorhamnetin and tamarixetin) were recovered in plasma, although after 10 days, no tamarixetin was detected. Rutin was shown to be absorbed more slowly than quercetin. Manach et al. suggested that rutin might not be absorbed from the small intestine because of its sugar moiety. It is now

known that rutin must be hydrolyzed by cecal microflora. Evidence suggests that quercetin is absorbed faster than rutin. However, in healthy ileostomy volunteers, quercetin glucosides from onions were more readily absorbed than the aglycone form (25). The bioavailability of pure rutin was 30% relative to the quercetin from onions, which contain only quercetin-glucosides (26). These reports indicate the important role of sugar moiety in the bioavailability and absorption of dietary quercetin in the human body. Many of the flavonols including quercetin occur in food in the form of *O*-glycosides, with D-glucose as the most common sugar residue. Other

sugar residues are D-galactose, L-rhamnose, L-arabinose, D-xylose, and D-glucuronic acid (24). Glycosylation influences the chemical, physiological and biological properties of flavonoids.

THE EFFECTS OF INTESTINAL FLORA ON THE METABOLISM OF QUERCETIN AND RUTIN

The chemical structures of flavonoids influence their rate and extent of intestinal absorption and also the types of flavonoid metabolites circulating in the plasma. Rutin is a rhamnoglucoside of quercetin that requires deglycosylation by the intestinal flora prior to absorption in the colon (26). Flavonoids including quercetin are usually glycosylated. It has been reported that the rate of deglycosylation of quercetin 3-glucoside with cell-free extracts of small intestine was low and that rutin was not hydrolyzed (16). However, rutin was shown to be hydrolyzed by the colonic flora. With respect to the bioavailability of flavonoid glycosides, intestinal flora are known to have an important role in hydrolysis. Rutin and quercetin-3-*O*-rhamnoside are not hydrolyzed by endogenous human enzymes. But they are hydrolyzed by intestinal flora to quercetin. Intestinal bacteria such as *Bacteroides distasonis* (α -rhamnosidase and β -glucosidase), *B. uniformis* (β -glucosidase), and *Bacteroides ovatus* (β -glucosidase) were able to catalyze this reaction (12). *Enterococcus casseliflavus* is able to hydrolyze quercetin-3-*O*-glucoside but does not metabolize aglycone. Quercetin-3-*O*-glucoside is known to be transformed to 3,4-dihydroxyphenylacetic acid, acetate, and butyrate by *Eubacterium ramulus* (54). Tamura et al. studied the effects of intestinal flora on plasma quercetin concentrations (69). Tamura et al. demonstrated that intestinal flora decompose much of the dietary quercetin in the cecum. It was also found that suppressing the breakdown of quercetin by intestinal flora is important for achieving higher concentrations of quercetin in the plasma. Colonic flora are known to catalyze the breakdown of flavonoids. Following the incubation of quercetin-3-*O*-rhamnoside with intestinal bacteria, quercetin, 3,4-dihydroxyphenylacetic acid and 4-hydroxybenzoic acid were found to be the metabolites. When rutin and quercetin are administered to humans, the metabolites produced by the breakdown caused by colonic flora are 3-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxytoluene, and β -*m*-hydroxyphenylhydracrylic acid. These compounds have been detected in human urine, while the parent compound, rutin, was not (10).

ABSORPTION OF DAIDZEIN, GENISTEIN, DAIZIN, AND GENISTIN

The main dietary source of isoflavone is soybean and soy foods (13, 43). The main components of isoflavones are daidzin, genistin, daizein (aglycone of daidzin), and genistein (aglycone of genistin). Isoflavones belong to the group of phytoestrogens. Phytoestrogens are estrogenic compounds found in plants. The phytoestrogens are defined as compounds that exert estrogenic effects on the central nervous system, induce estrus, and stimulate the growth of the genital tract of female animals (36). King and Bursill showed that genistein and daidzein are absorbed by humans (32). Human metabolism and excretion of isoflavones following the consumption of soy products show considerable variation (28, 31, 75). It has been shown that Japanese men and women have significantly higher concentrations of daidzein, genistein, and equol than individuals from the UK (41). The bioavailability of soybean isoflavones to women is dependant on gut microflora (75). The average plasma concentration of genistein at 24 hr after a breakfast-based isoflavone dose in subjects excreting large amounts of fecal isoflavones was significantly greater than in subjects who excreted small amounts of fecal isoflavones (2.5-fold, $p < 0.05$). Soy food processing also influences isoflavone bioavailability. Urinary isoflavone excretion was found to be similar in 17 male subjects who consumed either 112 g of fermented soy tempeh or 125 g of unfermented soybean for nine days (28). However, urinary recovery of daidzein and genistein was higher in subjects that consumed the tempeh diet. The data suggest that the isoflavone aglycones in fermented food may be more bioavailable than their glucosides. Soy isoflavone aglycone is absorbed faster and in higher amounts than glucosides in humans (29). Setchell et al. demonstrated that isoflavone glycosides were not intact when absorbed across the enterocyte of healthy adults, and their bioavailability required initial hydrolysis of the sugar moiety by intestinal β -glucosidases prior to uptake (58). In contrast, Richelle et al. investigated the hydrolysis of isoflavone glycosides to aglycone by β -glucosidase (51). It was found that hydrolysis does not alter the plasma and urinary isoflavone pharmacokinetics in postmenopausal women. Richelle et al. explained that the difference could be due to the food matrix in which the isoflavones were incorporated and whether they were given as pure isoflavones, as soy protein isolates, or as enriched isoflavone extracts (51). Other dietary components are also known to affect the absorption of isoflavones.

Fiber-rich diets have been shown to lead to a 55% lower plasma genistein concentration and to reduced urinary genistein by 20%, 24 hr after soy dosing ($p < 0.05$) (70). Dietary isoflavones undergo enterohepatic circulation (74). When 4- ^{14}C -genistein is infused into the duodenum of rats, it is rapidly absorbed from the intestine, taken up by the liver, and excreted into the bile as its 7- O - β -glucuronide conjugate (61). Intestinal perfusion studies have indicated that some of the genistein glucuronide produced in the rat small intestine is returned to the lumen (5–7). Intestinal flora appear to be the major source of β -glucuronidase in the gut (34). Higher floral β -glucuronidase activity may lead to efficient re-absorption of the genistein by hydrolyzing the bile excretory genistein-glucuronide and genistein-glucuronide formed in the small intestine and returned to the lumen.

EFFECTS OF INTESTINAL FLORA ON METABOLISM OF DAIDZEIN AND GENISTEIN

Intestinal flora play a key role in the metabolism and bioavailability of isoflavones (57). Both equol and o -desmethylangolensin are metabolic products of daidzein produced by intestinal flora (1, 11). p -Ethylphenol is a metabolite of genistein produced by intestinal flora (20). Equol is considerably more estrogenic than daidzein or o -desmethylangolensin (62), another major bacterial metabolite of daidzein. p -Ethylphenol is an inactive metabolite. Intestinal flora seem to have an important influence on the effects of isoflavones on the host. Studies have shown that only about 30–40% of the subjects excreted significant quantities of equol after isoflavone consumption (33, 57). Infants fed infant formula containing soy in the first 4 months of life (when gut microflora are underdeveloped) cannot form large quantities of equol (14, 60). It was demonstrated that the floral composition of the soy protein diet group was significantly different from that of the casein diet group (67). In an experiment investigating the *in vitro* incubation of daidzein with the fecal flora of mice, it was found that equol concentrations were significantly higher in mice fed a soy protein diet as compared with the casein diet group (68). Tamura et al. demonstrated that, in mice, plasma equol concentrations were significantly higher in the soy protein-isoflavone diet group than in the casein-isoflavone diet group (68). In this experiment, the composition of intestinal flora differed between the two dietary groups. Lactobacilli was much more abundant in the soy protein-isoflavone diet group than in the casein-isoflavone diet group. Fusiform-shaped bacteria were significantly fewer in the

soy protein-isoflavone diet group than in the casein-isoflavone diet group. Clearly, changes in the floral composition affected by different protein sources may increase the rate of conversion of daidzein to equol. The composition of intestinal flora might have an important influence on the production of equol from daidzein in the gut. Using an *in vitro* model of the human colonic fermentation system, it was shown that the conversion of daidzein to equol by cultured human fecal flora could be achieved. In the presence of large quantities of carbohydrates, the rate of the conversion of daidzein to equol is increased (55, 59). Rowland et al. demonstrated that good equol producers consumed less fat as a percentage of energy than poor excretors and more carbohydrate as a percentage of energy than poor excretors (53). Rowland et al. also suggested that dietary fat intake decreases the capacity of intestinal flora to synthesize equol (53). These reports suggest that dietary composition plays an important role in the conversion of daidzein to equol. Tamura et al. also demonstrated that plasma equol concentrations were significantly higher in a potato starch-isoflavone diet group than in the rice starch-isoflavone diet group (66). The number of bifidobacteria in the potato starch-isoflavone diet group was significantly higher than that of the rice-isoflavone diet group. These results suggest that not only a higher percentage of carbohydrate in the diet, but also the type of carbohydrate might influence the rate of conversion of daidzein to equol. The relationship between the higher number of bifidobacteria and the higher plasma equol concentrations in the potato starch-isoflavone diet group should be studied in greater detail. Prebiotics also affect equol production. Ohta et al. showed that dietary fructooligosaccharides increase equol production from daidzein in ovariectomized mice (47). Probiotics also affect the composition of intestinal flora (19). The use of probiotics might also affect equol production from daidzein.

BIOLOGICAL ACTIVITY OF EQUOL

Shutt and Cox (62) reported that equol displays higher estrogenic activity than daidzein. The binding affinity of equol for human ER α and ER β was found to be similar to that of genistein. Daidzein was shown to have poor affinity in these experiments (40). Equol is a more effective antioxidant than daidzein or genistein (39, 72). The higher antioxidant activity of equol would lead to greater inhibition of lipid peroxidation and contribute to a reduction in the risk of cardiovascular disease. It has been demonstrated that soybean protein and

isoflavone supplementation prevents postmenopausal bone loss in humans (49). Ohta et al. (47) demonstrated that increased concentrations of equol in the plasma of mice fed a diet containing fructooligosaccharide-isoflavone might have a lower incidence of distal and trabecular bone loss. Several studies support the hypothesis that adequate dietary isoflavone intake reduces the risk of cancer (2, 56). Prostatic cancer is known to be responsive to estrogen therapy. Asian men, who are considered to be at a lower risk of prostate cancer relative to European men, were found to have higher concentrations of isoflavones, equol, and daidzein in plasma and prostatic fluids (42). Duncan et al. demonstrated that the relationship between equol excretion and lowered breast cancer risk may reflect the tendency of equol excretors to have more favorable hormonal profiles, as opposed to merely reflecting increased isoflavone intake (18).

CONCLUSION

Intestinal flora influence the bioavailability of flavonoids. In order to obtain higher plasma quercetin, daidzein, and genistein concentrations, suppressing the breakdown of these flavonoids by intestinal flora is needed. Increasing the activity of β -glucosidase by intestinal flora to hydrolyze the flavonoid glucoside to aglycone is also advantageous. Equol is a metabolite of daidzein produced by intestinal flora. It has many biological activities related to human health, and its production might be affected by dietary composition and intestinal floral composition. To achieve higher production of equol from daidzein in the gut, control of the metabolic activity of intestinal flora might be of importance. To control floral composition by dietary intake, the use of prebiotics in order to increase the rate of production of equol from daidzein in the gut seems to increase the health effects of isoflavones on the host.

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