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REVIEW

Effects of Intestinal Flora on the Metabolism and Absorption of Isoflavones

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Abstract

Soybean and soy foods are rich sources of isoflavones. Much attention has focused on isoflavonoids because of their beneficial effects on human health. Isoflavones belong to the group of phytoestrogens. Phytoestrogens are estrogenic compounds found in plants. Intestinal flora plays an important role in the absorption and metabolism of isoflavones. Some dietary components and chemical composition of isoflavones 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 dependent on gut microflora. Equol is a metabolite of daidzein produced by intestinal flora. Equol has many biological activities related to human health, and its production might be affected by compositions of diets and intestinal flora. To achieve higher production of equol from daidzein in the gut, control of the metabolic activity of intestinal flora might be of importance.

Discipline: Food
Additional key words: daidzein, equol, genistein

Introduction

Many studies have suggested that soy food consumption will reduce the risk of heart disease. These effects have been attributed to soy protein. Recently, in a cross section study of 1,033 pre- and postmenopausal women, it has been demonstrated that moderate intakes of soy foods are associated with the reduction of blood cholesterol concentrations[21]. Isoflavones contained in soy beans can account for the LDL cholesterol lowering effects of soy protein. Cassidy et al.[10] have reported that 45 mg of isoflavonoids, but not 23 mg of isoflavonoids, resulted in significant reductions in total and LDL cholesterol concentrations in young females. Anthony et al.[7] reported that soy protein containing phytoestrogens resulted in lower total and LDL cholesterol and higher HDL cholesterol concentrations than either a casein and lactalbumin mixture or an alcohol-extracted soy protein low in phytoestrogens in male cynomolgus monkeys. In a human experiment, high isoflavone intake led to significantly greater decreases in serum LDL cholesterol concentration than did the low isoflavone intake, demonstrating that isoflavones have LDL cholesterol-lowering effects independent of soy protein[59]. It has been demonstrated that intake of soy protein and isoflavone for 6 months may decrease the risk factors associated with cardiovascular disease in postmenopausal women[34]. By contrast, it has been demonstrated that soy isoflavone extracts given to human subjects do not result in cardiovascular benefits except for improvements in systemic arterial compliance[11]. However, isoflavone seems to play an important role in the biological activity of soy foods. The main dietary source of isoflavone is soybean and soy foods[12,32]. Soy foods contain isoflavones as the aglycone form, glycoside form or both. Asian fermented soy foods contain predominantly isoflavone aglycones, whereas in nonfermented soy foods of both American and Asian origins, isoflavones are present mainly as β-glycoside conjugates[12]. The main components of isoflavones are daidzin, genistin, daidzein (aglycone of daidzin), and genistein (aglycone of genistin). Glycitein, biochanin A, and formononetin also belong to the isoflavones. Equol is a metabolic product of daidzein produced by intestinal flora[9]. The structures of these isoflavonoids are shown in Fig. 1. These isoflavonoids belong to the group of phytoestrogens. Phytoestrogens are estrogenic compounds found in plants. The phytoestrogens are defined as com-

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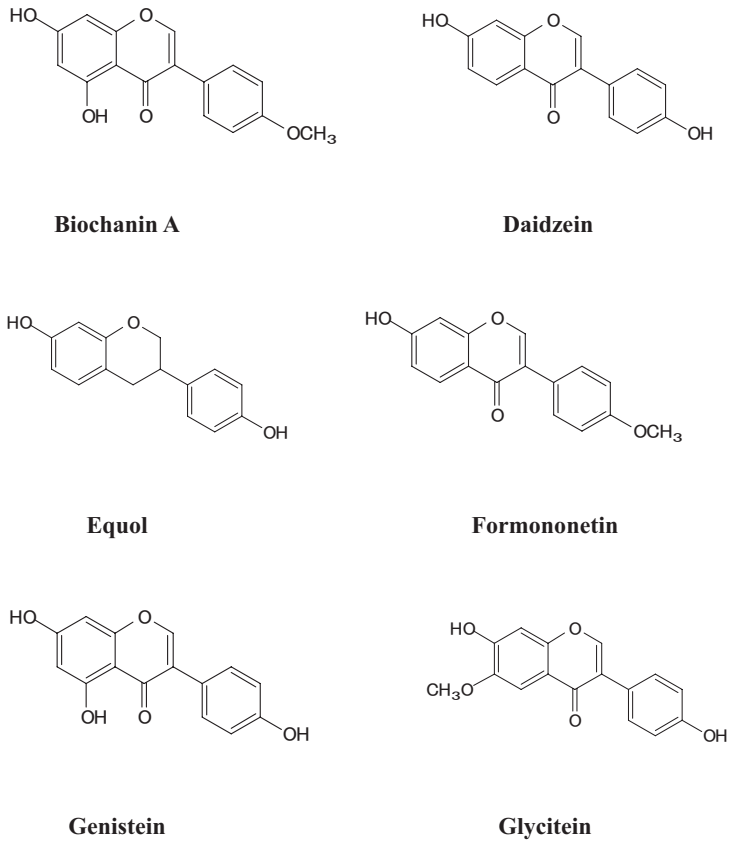

Fig. 1. The structures of some isoflavonoids

pounds that exert estrogenic effects on the central nervous system, induce estrus, and stimulate growth of the genital tract of female animals[27]. To estimate the biological activity of these isoflavonoids, elucidation of absorption and metabolism of isoflavonoids are deeply important. The available evidence suggests that human intestinal microflora plays an important role in the metabolism of isoflavones. It is also important to estimate the role of intestinal flora on the metabolism and absorption of isoflavones.

Absorption of isoflavones

King and Bursill showed that genistein and daidzein are absorbed by humans[24]. Human metabolism and excretion of isoflavones following the consumption of soy products show considerable variation[19,23,58]. It has been shown that Japanese men and women have significantly higher concentrations of daidzein, genistein and equol than individuals from the UK[31]. However, there were no differences in the pharmacokinetics of daidzein and genistein between pre- and postmenopausal women, indicating absorption and disposition of isoflavones to be independent of age or menopauseal status[45]. The bioavailability of soybean isoflavones in women is dependent on gut microflora[58]. 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[19]. However, urinary recovery of daidzein and genistein were higher in subjects that consumed the tempeh diet than in those who consumed unfermented soybean. The data suggest that the isoflavone aglycones in fermented food are more bioavailable than their glucosides. Soy isoflavone aglycone is absorbed faster and in higher amounts than glucosides in humans[20]. Setchell et al. demonstrated that isoflavone glycosides were not intact when absorbed across the enterocyte of healthy adults, and initial hydrolysis of the sugar moiety by intestinal β-glucosidases is required to be absorbed in the intestine[44]. Besides, food matrix and chemical composition may affect the genistein absorption. It has been demonstrated that the fractional absorption of genistein is potentially different in men and women and is influenced by the food matrix and chemical composition[15]. In contrast, Richelle et al. investigated

the hydrolysis of isoflavone glycosides to form aglycone by β-glucosidase[35]. It was found that the 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[35]. It has been demonstrated that the apparent bioavailability of genistein and daidzein is not different when consumed either as aglycone or glucoside by American women[60].

The pharmacokinetics of methoxylated isoflavones show distinct differences depending on the position of the methoxyl group in the molecule. Glycitin, found in two phytoestrogen supplements, underwent hydrolysis of the β-glycoside moiety but little further biotransformation, and the ingestion of these supplements led to an increase in plasma glycitein concentrations. Biochanin A and formononetin, two isoflavones found in one phytoestrogen supplement, were rapidly and efficiently demethylated, and the ingestion of the supplement resulted in an increase in plasma genistein and daidzein concentrations as has typically been observed after the ingestion of soy-containing foods[43].

Dietary components are also known to affect the absorption of isoflavones. Fiber-rich diets lowered plasma genistein concentration by 55% and also reduced urinary genistein excretion by 20% at 24 h after soy dosing ($p<0.05$)[55].

Effects of intestinal flora on metabolism of isoflavones

Intestinal flora plays a key role in the metabolism and bioavailability of isoflavones[41]. Both equol and *o*-desmethylangolensin are metabolic products of daidzein produced by intestinal flora[1,8]. Human intestinal flora plays an important role in the production of equol from daidzein. Recently, Tamura et al.[54] demonstrated that equol was detected in the plasma of human flora-associated (HFA) mice that were administered isoflavones. However, equol was not detected in the plasma of germ-free mice that were administered isoflavones[54]. Equol is considerably more estrogenic than daidzein or *o*-desmethylangolensin[47], and other major bacterial metabolites of daidzein. ρ-Ethylphenol is the most predominant metabolite of genistein in sheep. In humans, dihydrogenistein and 6'-hydroxy-*o*-desmethylangolensin are isolated and characterized as metabolites of genistein[22]. There are some reports of effects of intestinal bacteria on metabolism of isoflavones. Two strains of bacteria capable of producing primary and secondary metabolites from the natural isoflavone glycosides, daidzin and genistin, were detected from a healthy individual. Both *Escherichia coli* HGH21 and the gram-positive strain HGH6 converted daidzin and genistin to their respective aglycones, daidzein and genistein. Under anoxic conditions, strain HGH6 further metabolized daidzein and genistein to dihydrodaidzein and dihydrogenistein, respectively[17]. *Eubacterium ramulus*, a flavonoid-degrading anaerobic bacterium from the human gastrointestinal tract, was tested for its ability to transform the isoflavonoids including genistein-7-O-glucoside (genistin), genistein and daidzein. Genistein was completely degraded by *E. ramulus* via 6'-hydroxy-*o*-desmethylangolensin to 2-(4-hydroxyphenyl)-propionic acid[37]. *Eubacterium limosum* (ATCC 8486) is a strict anaerobe from the human intestinal tract that is capable of *o*-demethylation of several compounds and was tested for the ability to metabolize three methoxylated isoflavonoids including biochanin A, formononetin and glycitein. The metabolites were identified, using an on line liquid chromatography-electrospray mass spectrometer. *E. limosum* produced genistein from biochanin A and also produced daidzein from formononetin, after 26 days incubation[18]. Dietary isoflavones undergo enterohepatic circulation[57]. 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[46]. Intestinal perfusion studies have indicated that some of the genistein glucuronide produced in the rat small intestine is returned to the lumen[4–6]. Intestinal flora appears to be the major source of β-glucuronidase in the gut[26]. Tamura et al. reported that cecal β-glucuronidase activities were affected by dietary fiber in both young and aged mice[48]. It has also been demonstrated that fecal β-glucuronidase activities were significantly higher in mice fed a diet containing both 5% soy oligosaccharides-0.2% isoflavone (SOI diet) than in those fed a diet containing 5% cellulose-0.2% isoflavone (CEI diet). Plasma genistein concentrations were significantly higher in the SOI diet group than in the CEI diet group[49]. Higher floral β-glucuronidase activity may lead to efficient re-absorption of the genistein by hydrolyzing the glucuronide form of genistein that is excreted in the bile or is formed in the small intestine and returned to the lumen. Thus, intestinal flora seems to have an important influence on the metabolism and absorption of isoflavones. Studies have shown that only about 30–40% of subjects produce significant quantities of equol after isoflavone consumption[25,41]. Infants fed soy-containing infant formula in the first 4 months of life (when gut microflora is underdeveloped) can not form large quantities of equol[13,42]. It was demonstrated that the floral composi-

tions of the mice fed a soy protein diet were significantly different from those fed a casein diet group[52]. In an experiment investigating the *in vitro* incubation of daidzein with fecal flora of mice, it was found that equol production was significantly higher in mice fed the soy protein diet as compared with those fed the casein diet[53]. Tamura et al. demonstrated that plasma equol concentrations were significantly higher in mice fed a 20% soy protein-0.25% isoflavone diet than in those fed a 20% casein-0.25% isoflavone diet[53]. In this experiment, the composition of intestinal flora differed between the two dietary groups. Lactobacilli were 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. Changes in the floral composition induced by different protein sources may increase the rate of conversion of daidzein to equol. 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 conversion of daidzein to equol is increased[38,40]. Rowland et al. demonstrated that good equol producers consumed less fat and more carbohydrate as energy sources than did the poor excretors[36]. Rowland et al. also suggested that dietary fat intake decreases the capacity of intestinal flora to synthesize equol[36]. These reports suggest that dietary composition exerts an important influence in the conversion of daidzein to equol. Tamura et al. also demonstrated that plasma equol concentrations were significantly higher in the mice fed a potato starch diet supplemented with 0.25% isoflavone than in those fed a rice starch diet supplemented with 0.25% isoflavone[51]. The number of bifidobacteria in the potato starch diet supplemented with 0.25% isoflavone group was significantly higher than that of the rice diet supplemented with 0.25% isoflavone group. These results suggest that not only the amount but also the type of carbohydrate 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 diet supplemented with isoflavone group should be studied in more detail. Ohta et al. showed that dietary fructooligosaccharides increase the equol production from daidzein in ovariectomized mice[33]. Therefore, it is apparent that prebiotics also affect equol production. Probiotics also affect the composition of intestinal flora[16]. Recently Tamura et al. firstly demonstrated that the numbers of lactobacilli present in the feces of mice were significantly higher and plasma equol concentrations were significantly less in the *Lactobacillus gasseri* administerated group than in the control group[50]. Therefore, probiotics may also affect the equol production from daidzein.

Biological activity of equol

Shutt and Cox[47] reported that equol displays higher estrogenic activity than daidzein. The binding affinity for human ER (estrogen receptor) α and ER (estrogen receptor) β was found to be similar between equol and genistein. Daidzein was shown to have a poor affinity in these experiments[30]. Equol is a more effective antioxidant than daidzein or genistein[29,56]. The higher antioxidation activity of equol would lead to greater inhibition of lipid peroxidation and contributes 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[34]. Ohta et al. demonstrated that a diet containing both fructooligosaccharides and isoflavone increased concentrations of equol in the plasma of mice accompanying the reduction in the incidence of distal and trabecular bone loss[33]. Several studies support the hypothesis that adequate dietary isoflavone intake reduces the risk of cancer[2,39]. It has been demonstrated that the association of equol excretion and lowered breast cancer risk may largely reflect the tendency of equol excretors to have more favorable hormonal profiles, as opposed to merely reflecting increased isoflavone intake[14]. Prostatic cancer is known to be responsive to estrogen therapy. Akaza et al. demonstrated that the poorly differentiated cancer patient group included a significantly lower percentage of daidzein metabolizers[3]. This author concluded that there is some correlation between daidzein non-metabolizers and prostate cancer patients. It has been demonstrated that regular consumption of whole soybean milk and yogurt products had no effect on plasma lipids at cardiovascular risk subjects, despite substantial high isoflavone levels in blood and urine. However, retrospective analysis revealed that the soy diet significantly reduced in plasma concentrations of total cholesterol (8.5%), LDL cholesterol (10%), LDL:HDL ratio (13.5%), triglycerides (21%) and lipoprotein(a) (11%) among 8 equol-positive subjects in whom equol was detected in either plasma or urine[28].

Conclusion

Some of the intestinal bacteria have an ability to metabolize isoflavones. The bioavailability of isofla-

vones is dependent on intestinal flora. Chemical composition of isoflavones and some dietary components affect the bioavailability of isoflavones. Equol is a metabolite of daidzein produced by intestinal flora. Equol has many biological activities related to human health, and its production might be affected by the compositions of diets and intestinal flora. Both prebiotics and probiotics seem to affect the equol production. To achieve higher production of equol from daidzein in the gut, control of the metabolic activity of intestinal flora might be of importance.

References

1. Adlercreutz, H. et al. (1982) Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. *Lancet,* **320**, 1295–1299.
2. Adlercreutz, H. et al. (1995) Soybean phytoestrogen intake and cancer risk. *J. Nutr.*, **125**, 757S–770S.
3. Akaza, H. et al. (2002) Is daidzein non-metabolizer a high risk for prostate cancer? A case-controlled study of serum soybean isoflavone concentration. *Jpn. J. Clin. Oncol*., **32**, 296–300.
4. Andlauer, W., Kolb, J. & Fürst, P. (2000a) Absorption and metabolism of genistin in the isolated rat small intestine. *FEBS Lett*., **475**, 127–130.
5. Andlauer, W., Kolb, J. & Fürst, P. (2000b) Isoflavones from tofu are absorbed and metabolized in the isolated rat small intestine. *J. Nutr.,* **130**, 3021–3027.
6. Andlauer, W. et al. (2000) Absorption and metabolism of genistein in isolated rat small intestine. *J. Nutr*., **130**, 843–846.
7. Anthony, M. S. et al. (1997) Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. *Arterioscler. Thromb. Vasc. Biol*., **17**, 2524–2531.
8. Bannwart, C. et al. (1984) *o*-Desmethylangolensin, a metabolite of daidzein and of matairesinol, one likely plant precursor of the animal lignan enterolactone in human urine. *Finn. Chem. Lett*., **4-5**, 120–125.
9. Bowey, E., Adlercreutz, H. & Rowland, I. (2003) Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. *Food Chem. Toxicol*., **41**, 631–636.
10. Cassidy, A., Bingham, S. & Setchell, K. (1995) Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *Br. J. Nutr.*, **74**, 587–601.
11. Clarkson, T. B. (2002) Soy, soy phytoestrogens and cardiovascular disease. *J. Nutr.*, **132**, 566S–569S.
12. Coward, L. et al. (1993) Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *J. Agric. Food Chem*., **41**, 1961–1967.
13. Cruz, M. L. A. et al. (1994) Effects of infant nutrition on cholesterol synthesis rates. *Pediatr. Res.,* **35**, 135–140.
14. Duncan, A. M. et al. (2000) Premenopausal equol excretors show plasma hormone profiles associated with lowered risk of breast cancer. *Cancer Epidemiol. Biomarkers Preview*, **9**, 581–586.
15. Faughnan, M. S. et al. (2004) Urinary isoflavone kinetics: the effect of age, gender, food matrix and chemical composition. *Br. J. Nutr.*, **91**, 567–574.
16. Fujiwara, S. (2002) *Bifidobacterium longum* SBT2928 and its biological significance. *Biosci. Microflora*, **21**, 225–238.
17. Hur, H. -G. et al. (2000) Isolation of human intestinal bacteria metabolizing the natural isoflavone glycosides daidzin and genistin. *Arch. Microbiol*., **174**, 422–428.
18. Hur, H. -G. & Rafii, F. (2000) Biotransformation of the isoflavonoids biochanin A, formononetin, and glycitein by *Eubacterium limosum*. *FEMS Microbiol. Lett*., **192**, 21–25.
19. Hutchins, A. M., Slavin, J. L. & Lampe, J. W. (1995) Urinary isoflavonoid phytoestrogen and lignan excretion after consumption of fermented and unfermented soy products. *J. Am. Diet. Assoc.,* **95**, 545–551.
20. Izumi, T. et al. (2000) Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr*., **130**, 1695–1699.
21. Jenkins, D. J. A. et al. (2002) Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am. J. Clin. Nutr*., **76**, 365–372.
22. Joannou, G. E. et al. (1995) A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. *J. Steroid Biochem. Mol. Biol*., **54**, 167–184.
23. Kelly, G. E. et al. (1995) The variable metabolic response to dietary isoflavones in humans. *Proc. Soc. Exp. Biol. Med.,* **208**, 40–43.
24. King, R. A. & Bursill, D. B. (1998) Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. *Am. J. Clin. Nutr.,* **67**, 867–872.
25. Lampe, J. W. et al. (1998) Urinary equol excretion with a soy challenge: influence of habitual diet. *Proc. Soc. Exp. Biol. Med*., **217**, 335–339.
26. Larsen, G. L. (1988) Deconjugation of biliary metabolites by microfloral β-glucuronidases, sulphatases and cysteine conjugate β-lyases and their subsequent enterohepatic circulation. *In* Role of the gut flora in toxicity and cancer, ed. Rowland, I. R., Academic Press, London, 79–107.
27. Lieberman, S. (1996) Are the differences between estradiol and other estrogens, naturally occurring or synthetic, merely semantical? *J. Clin. Endocrinol. Metab*., **81**, 850–851.
28. Meyer, B. J. et al. (2004) Limited lipid-lowering effects of regular consumption of whole soybean foods. *Ann. Nutr. Metab*., **48**, 67–78.
29. Mitchell, J. H. et al. (1998) Antioxidant efficacy of phytoestrogens in chemical and biological model systems. *Arch. Biochem. Biophys*., **360**, 142–148.
30. Morito, K. et al. (2001) Interaction of phytoestrogens with estrogen receptors α and β. *Biol. Pharm. Bull*., **24**, 351–356.
31. Morton, M. S. et al. (2002) Phytoestrogen concentrations in serum from Japanese men and woman over forty years of age. *J. Nutr*., **132**, 3168–3171.

32. Murphy, P. A. (1982) Phytoestrogen content of processed soybean products. *Food Technol*., **36**(1), 60–64.
33. Ohta, A. et al. (2002) A combination of dietary fructo-oligosaccharides and isoflavone conjugates increases femoral bone mineral density and equol production in ovariectomized mice. *J. Nutr.,* **132**, 2048–2054.
34. Potter, S. M. et al. (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in post-menopausal women. *Am. J. Clin. Nutr.,* **68**, 1375S–1379S.
35. Richelle, M. et al. (2002) Hydrolysis of isoflavone glycosides to aglycones by β-glycosidase does not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. *J. Nutr*., **132**, 2587–2592.
36. Rowland, I. R. et al. (2000) Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. *Nutr. Cancer,* **36**, 27–32.
37. Schoefer, L. et al. (2002) Anaerobic C-ring cleavage of genistein and daidzein by *Eubacterium ramulus*. *FEMS Microbiol. Lett.,* **208**, 197–202.
38. Setchell, K. D. R. (1998) Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. *Am. J. Clin. Nutr.,* **68**, 1333S–1346S.
39. Setchell, K. D. R. & Adlercreutz, H. (1988) Mammalian lignans and phyto-oestrogens. Recent studies on their formation, metabolism and biological role in health and disease. *In* Role of the gut flora in toxicity and cancer, ed. Rowland, I. R., Academic Press, London, 315–345.
40. Setchell, K. D. R. & Cassidy, A. (1999) Dietary isoflavones: biological effects and relevance to human health. *J. Nutr*., **129**, 758S–767S.
41. Setchell, K. D. R. et al. (1984) Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am. J. Clin. Nutr.,* **40**, 569–578.
42. Setchell, K. D. R. et al. (1997) Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet,* **350**, 23–27.
43. Setchell, K. D. R. et al. (2001) Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J. Nutr*., **131**, 1362S–1375S.
44. Setchell, K. D. R. et al. (2002) Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am. J. Clin. Nutr*., **76**, 447–453.
45. Setchell, K. D. R. et al. (2003) Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *J. Nutr*., **133**,1027–1035.
46. Sfakianos, J. et al. (1997) Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *J. Nutr.,* **127**, 1260–1268.
47. Shutt, D. A. & Cox, R. I. (1972) Steroid and phyto-oestrogen binding to sheep uterine receptors in vitro. *J. Endocrinol.,* **52**, 299–310.
48. Tamura, M., Hirayama, K. & Itoh, K. (1999) Effects of guar gum and cellulose on cecal enzyme activity and cecal short-chain fatty acids in young and aged mice. *Ann. Nutr. Metab*., **43**, 60–65.
49. Tamura, M., Hirayama, K. & Itoh, K. (2003) Effects of soy oligosaccharides on plasma and cecal isoflavones, and cecal enzyme activities in mice. *J. Nutr. Sci. Vitaminol*., **49**, 168–171.
50. Tamura, M., Ohnishi-Kameyama, M. & Shinohara, K. (2004*) Lactobacillus gasseri*: effects on mouse intestinal flora enzyme activity and isoflavonoids in the caecum and plasma. *Br. J. Nutr.,* **92**, 771–776.
51. Tamura, M. et al. (2002a) Effects of rice starch-isoflavone diet or potato starch-isoflavone diet on plasma isoflavone, plasma lipids, cecal enzyme activity, and composition of fecal microflora in adult mice. *J. Nutr. Sci. Vitaminol.,* **48**, 225–229.
52. Tamura, M. et al. (2002b) Effects of soy protein diet on plasma equol and intestinal microflora in adult mice. *Biosci. Microflora,* **21**, 121–124.
53. Tamura, M. et al. (2002c) Effects of soy protein-isoflavone diet on plasma isoflavone and intestinal microflora in adult mice. *Nutr. Res*., **22**, 705–713.
54. Tamura, M. et al. (2004) Effects of human intestinal flora on plasma and caecal isoflavones, and effects of isoflavones on the composition and metabolism of flora in human flora-associated (HFA) mice. *Microbial. Ecol. Health Dis*., **16**, 18–22.
55. Tew, B. -Y. et al. (1996) A diet high in wheat fiber decreases the bioavailability of soybean isoflavones in a single meal fed to women. *J. Nutr.,* **126**, 871–877.
56. Vedavanam, K. et al. (1999) Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soyabean phytochemical extract (SPE). *Phytother. Res*., **13**, 601–608.
57. Watanabe, S. et al. (1998) Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (Kinako). *J. Nutr.,* **128**, 1710–1715.
58. Xu, X. et al. (1995) Bioavailability of soybean isoflavones depends upon gut microflora in women. *J. Nutr*., **125**, 2307–2315.
59. Zhuo, X. -G., Melby, M. K. & Watanabe, S. (2004) Soy isoflavone intake lowers serum LDL cholesterol: a meta-analysis of 8 randomized controlled trials in humans. *J. Nutr*., **134**, 2395–2400.
60. Zubik, L. & Meydani, M. (2003) Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. *Am. J. Clin. Nutr.,* **77**, 1459–1465.