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Original Article

Pharmacokinetics of an Equol Supplement in Humans

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Abstract

Equol, a metabolite of daidzein, is considered to be the most effective estrogen modifier in the human body. The production of equol depends on an individual's intestinal flora, however, so an equol supplement has been developed for nonproducers. To examine the pharmacokinetics in humans of a newly developed equol supplement made from fermented soy germ by *Lactococcus sp.* Equol supplements were given to 18 adults (20–22 yr) in three doses (one 10-mg dose, one 30-mg dose, or three 10-mg doses per day) to investigate the pharmacokinetics and physiological effects. Equol reached a peak in plasma at 30 or 60 min after supplement intake, and the average plasma half-life was 83 min. Plasma concentrations of equol were always higher in females than in males. Less than 5% of the highest plasma concentration remained in the plasma after 2 days. Up to 56% of the orally administered equol was excreted in urine within 1 day. This short-term, high-dose equol exposure yielded no observable adverse effect in both feeling and biochemical markers. For equol nonproducers, this newly developed equol supplement may yield more beneficial effects of isoflavones.

KEY WORDS: Soy isoflavone, equol, pharmacokinetics, biomarker

Introduction

The beneficial effects of isoflavones are widely supported by both experimental and epidemiological data.[1,2] Equol, a metabolite of daidzein, is considered to be the most effective estrogen modifier in the body and has been shown to improve osteoporosis and climacteric syndrome.[3,4] Only those individuals who have particular intestinal bacteria produce equol, however, and the rate of equol producers is reported to be about 20–30% among Caucasians and 30–50% among Japanese.[5]

We previously isolated three strains of equol-producing bacteria, *Streptococcus bovis ssp., Bacteroides uniformis ssp.,* and *Corinebacteriaceae.*[6] These strains could not be used for food, however, because of difficulty in maintaining a stable equol yield. There has been no other report on the specific lactic acid bacteria capable of metabolizing daidzein to equol.

We recently developed an equol supplement by fermentation of soy germ using *Lactococcus sp.* strain Lc.G20-92.[7] Compared to soy seed or soy protein, soy germ contains five times more daidzein.[8] The biologically produced equol contains only the s-enantimer, thus it should be more effective compared to the chemically synthesized racemic equol, which is composed of both r- and s-enantimers due to the chiral center of the heterocyclic ring.[3,9] In this study, the pharmacokinetics of this equol supplement were investigated in humans. The health effects were also studied using various biomarkers.

Subjects and Methods

The equol supplement was made in dry granular form after fermentation of soy germ, as described previously.[7] On average, 1 g of the dry powder of the fermented broth contained 6.5 mg equol, 0.6 mg daidzein, 0.6 mg genistein, and 3.2 mg glycitein *(Table 1)*. Almost all equol are aglycon. For this study, one capsule contained 10.9 mg (41.7 μmole) equol; 66.7% of the powder was fermented soy material and 33.3% was erythrytol and other additives to improve the taste. These supplements were prepared at the Otsuka Pharmaceutical Company, Saga, Japan.

Table 1 ***Consumption of isoflavones by equol supplement in Eq10 and Eq30 groups***

	Eq10	Eq30
Equol	10.1 mg (10.1 mg)	30.3 mg (30.3 mg)
Daidzein	1.3 mg (0.8 mg)	3.9 mg (2.4 mg)
Genistein	5.1 mg (2.0 mg)	15.3 mg (6.0 mg)
Glycitein	2.1 mg (4.5 mg)	6.3 mg (13.5 mg)
Total	18.5 mg (17.4 mg)	55.5 mg (32.2 mg)

Amount of aglycone in parenthesis.

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Volunteers were 18 healthy students (6 males and 12 females) aged 20–22 yr. They were asked not to consume any soy products for 3 days prior to the experiment. They stayed in the dormitory of National Institute of Health and Nutrition (NIHN) for 3 days during the experiment and were fed standard test meals that did not contain any soy foods. Participants reported their dietary habits for the previous month using the semiquantitative diet history questionnaire, and nutrient intake was estimated based on their replies.[10] Isoflavone intake was separately calculated using the functional food factors database.[11] The protocol was approved by the NIHN Ethical Committee for Clinical Trials, and written consent was obtained from each participant.

One group (n = 6) received one capsule of 10 mg equol (41.7 μmole; group Eq10) and another (n = 6) received one capsule 30 mg equol (125 μmole; Eq30) just before breakfast. From each participant, 2 ml of blood were collected at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, and 36 h after the administration. The third group (n = 6) took 10 mg of equol three times (Eq10*3; once before each meal) for 1 day, and 2-ml blood samples were gathered similarly for 72 h. Participants were asked to record the time and volume of urination over the experimental period, and 10-ml samples were collected from each urination event and stored in plastic tubes.

Blood and urine samples were analyzed for equol, daidzein, genistein, and glycitein after enzymatic hydrolysis to aglycone. Plasma isoflavones were measured by time-resolved fluoroimmunoassay (TR-FIA) ,[12] and those in urine samples were measured using high-performance liquid chromatography with multi-channel coulometric electrochemical detection.[13] Sex hormone binding globulin (SHBG), T3, and T4 were also measured by the DELFIA research assay kits (Perkin Elmer Japan, Co.. Ltd.) Using plasma obtained at 0, 24, and 48 h after the supplement intake, the Serum Research Laboratory (Tokyo, Japan) performed serum biochemical analyses for total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, protein, albumin, AST, GOT, γ-GTP, UA, BUN, and blood glucose.

All statistical analyses were performed using SPSS Ver. 14 (SPSS, Inc., Chicago, IL, USA). Statistical significance was examined by the paired t-test. Plasma half-life of equol was calculated from the peak value using the equation $Y = Y1 \times 2^{-\alpha(t-1)}$, where Y is the plasma equol concentration, Y1 is the equol concentration at 1 h after intake, t is the number of hours after the intake, and α is the plasma half-life.

Results

Physical characteristics and dietary nutrient intake of the participants are shown in Table 2 *(Table 2)*. In the month prior to the experiment, the average consumption of isoflavones was about 30 mg/day/capita in both males and females. One of 6 males and 4 of 12 females showed more than 1 μmol/L equol in the urine at the baseline. These individuals were considered to be equol producers, and the remaining participants were nonproducers.

Blood pressure and other health conditions did not change after equol supplement ingestion. Participants did not report any physical and mental complaints throughout the experimental period.

Table 2 Physical characteristics and dietary habit of participants

	Males			Females		
n	6			12		
Age	20.3	±	0.6	21.4	±	0.3
height	168.2	±	5.4	158.8	±	3.7
weight	66.3	±	5.5	50.5	±	4.2
BMI	23.5	±	2.4	19.9	±	1.5
Dietary intake (g)	2944	±	965	2508	±	861
Energy intake (kcal)	2437	±	863	1887	±	708
protein (g)	92.7	±	29.3	64.6	±	74.5
fat (g)	86.6	±	28.8	60.5	±	34.2
carbohydrate (g)	311.8	±	130	260.1	±	64.4
soy bean product (g)	55.5	±	35.1	41.7	±	18.1
daidzein (mg)	12.7	±	7.8	10.6	±	5.4
genistein (mg)	21.3	±	13.3	17.3	±	8.9

Table 3 Changes of plasma concentration of equol by time (nmole/ml plasma)

	Group 1(Eq10)			Group 2(Eq30)			Group 3(Eq10*3)			
	Male(1)	Females(5)		Males(2)	Females(4)		Males(3)		Females(3)	
hr	value	mean	sd	mean	mean	sd	mean	sd	mean	sd
0	1.8	2.1	± 2.5	2.1	3.0	± 2.0	2.5	± 1.9	1.3	± 1.0
0.5	199.7	334.1	± 266.0	313.1	2515.3	± .	129.8	± 54.8	487.6	± 135.1
1	289.8	479.3	± 117.4	1418.2	1293.3	± 424.7	295.3	± 180.1	434.7	± 112.5
2	142.4	219.6	± 63.7	736.6	657.9	± 202.6	217.7	± 95.4	212.1	± 47.5
3	51.1	131.9	± 110.6	278.6	309.9	± 126.9	62.6	± 11.6	108.3	± 6.9
4	35.3	74.3	± 40.9	205.7	188.4	± 53.2	53.5	± 26.3	57.9	± 2.6
5	60.3	106.8	± 79.9	170.4	194.6	± 121.0	296.1	± 23.6	603.2	± 120.8
							140.6	± 21.5	213.7	± 87.3
							99.7	± 14.9	148.5	± 36.4
8	19.2	54.0	± 10.0	122.8	219.1	± 86.1	67.7	± 17.6	129.1	± 3.5
							49.7	± 9.4	106.0	± 17.7
							314.2	± 84.9	598.6	± 34.1
							123.0	± 13.8	255.1	± 65.4
12	8.7	28.7	± 12.1	73.0	126.8	± 111.1	96.8	± 19.2	147.5	± 28.9
							102.0	± 30.7	120.9	± 17.0
							98.1	± 35.0	87.9	± 4.0
24	5.5	11.6	± 4.5	34.3	68.8	± 39.4	46.6	± 6.3	71.3	± 14.7
36	1.5	7.5	± 4.0	6.9	28.0	± 17.9	6.6	± 7.6	41.6	± 31.9
							4.7	± 3.2	29.6	± 20.8

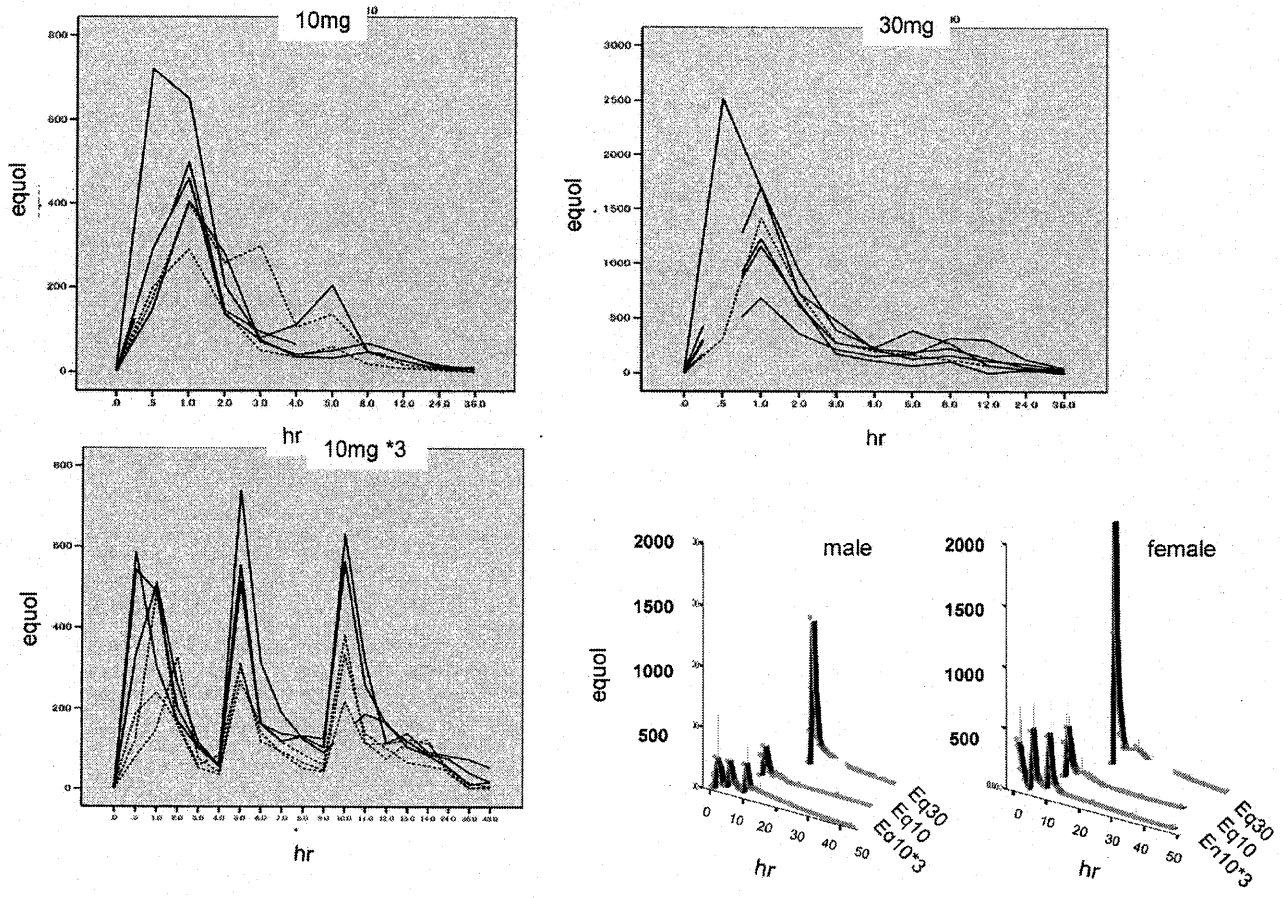

Fig. 1. Changes of individual equol plasma concentrations in groups Eq10 (one 10-mg dose), Eq30 (one 30-mg dose), and Eq10*3 (three 10-mg doses). Dose dependency (Eq10 vs. Eq30) is illustrated in the lower-right figure.

After 3 days of avoiding soy products, the plasma concentration of equol decreased to 1–3 nmol/L. In the Eq10 group, plasma equol started to increase 30 min after the intake and reached a peak value of 289 nmol/L in male and 479 nmol/L in females at 1 h *(Table 3, Fig. 1)*. In the Eq30 group, the peak concentration reached 1,418 nmol/L in male and 2,515 nmol/L in female at 1 h. Plasma equol concentration decreased at 3 to 4 h, and a second peak appeared 5 h after intake in some participants. By comparing the Eq10 and Eq30 groups, a significant dose-dependent increase in plasma concentration of equol was evident *(Fig. 1)*. In both groups, the average plasma half-life of equol was about 83.2 min. The model satisfied to show the goodness of fit (data not shown). After 24 h, plasma concentration decreased to 2–3% of the peak concentration in the Eq10 and Eq30 groups.

In the Eq10*3 group, three distinct equol peaks appeared after each intake, and only a small percentage seemed to remain in the plasma. In both males and females, it was about 15% at 24 h; the value decreased to 1.5% in males and 5.0% in females at 48 h.

Equol appeared in the urine 1 h after intake, reached a peak at around 5–6 h, and had nearly disappeared at 36 h. Cumulative mean equol excretion (± SD) was 23.3 ± 22.8 μmol/L (median 11.8 μmol/L) in the Eq10 group, 46.9 ± 23.0 μmol/L (median 53.1 μmol/L) in the Eq30 group, and 49.7 ± 37.0 μmol/L (median 38.1 μmol/L) in the Eq10*3 group *(Fig. 2)*. These values correspond to 55.9%, 37.5%, and 39.2% of the intake amount in each group, respectively. The maximum equol excretion was 66.7 μmol/L in the Eq10 group, 68.4 μmol/L in the Eq30 group, and 119.9 μmol/L in the Eq10*3 group. The equol recovery rate in urine was higher in males (37.6 ± 43.3 μmol/L, median 45.8 μmol/L) compared to that in females (37.6 ± 22.7 μmol/L, median 30.4 μmol/L). Producers excreted more equol in the urine (66.1 ± 41.4 μmol/L, median 62.5 μmol/L) compared to nonproducers (31.4 ± 20.8 μmol/L, median 25.8 μmol/L).

Absorption and excretion of daidzein and genistein reached a peak more slowly than equol did. The peak plasma concentration of daidzein was about 30 nmol/L in males and about 50 nmol/L in females at 5 h, and that of genistein was about 40 nmol/L in both sexes at 5 h.

Serum biochemical analyses revealed no significant change *(Table 4)*. Serum T3 and T4 appeared to increase after intake of equol, but a paired t-test did not show a statistically significant difference.

In the Eq10 group SHBG ranged from 9 to 15 nmol/L in males and was about 60 nmol/L in females; in the Eq30 group SHBG was 48 nmol/L in males and around 60 nmol/L in females. In the Eq10*3 group, the multiple intake in one day appeared to decrease the level of SHBG in both sexes *(Table 4)*.

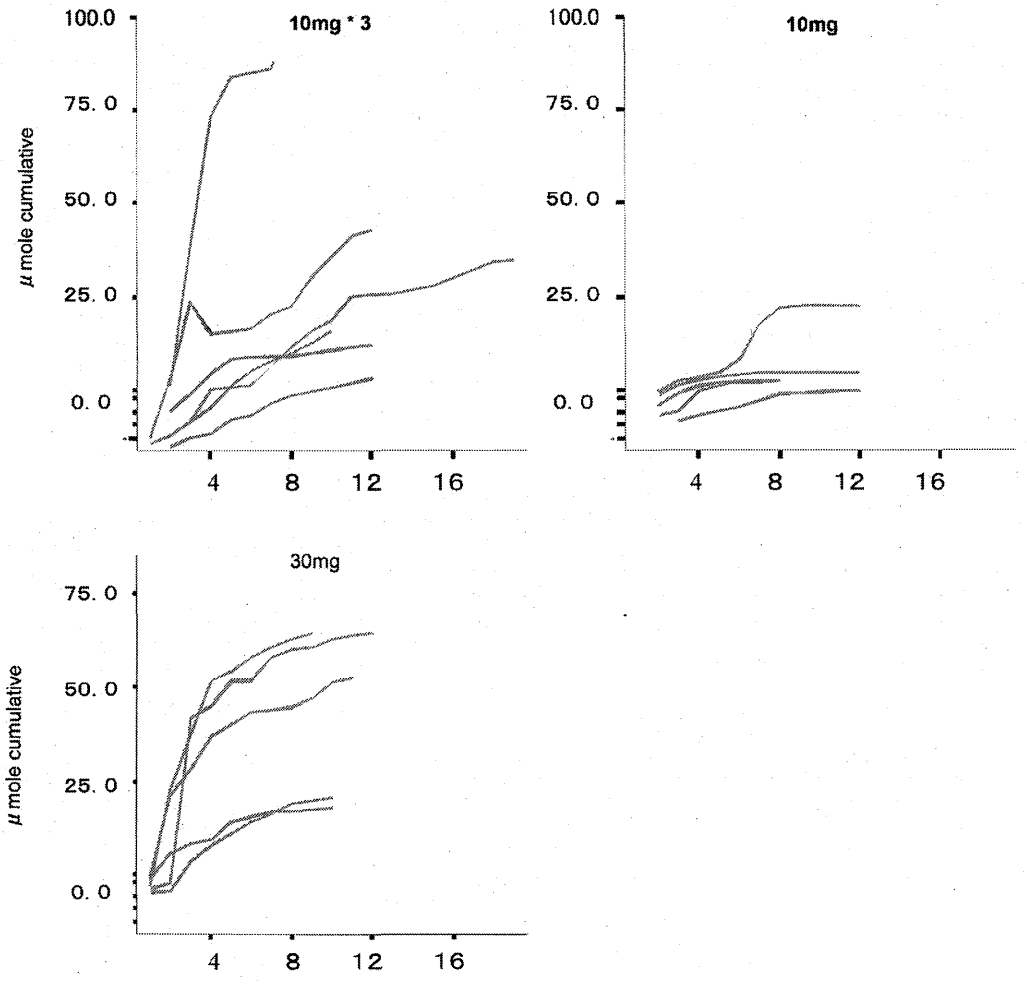

Fig. 2. Cumulative urinary excretion of equol in groups Eq10 (one 10-mg dose), Eq30 (one 30-mg dose), and Eq10*3 (three 10-mg doses).

Table 4 Biochemical changes after taking equol supplement (Group 3: Eq10*3)

		Males						Females					
day		0		24hr		48hr		0		24hr		48hr	
		mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
protein	g/dl	7.6 ±	0.3	7.3 ±	0.1	7.5 ±	0.3	7.4 ±	0.1	7.1 ±	0.1	7.4 ±	0.1
albumin	g/dl	4.8 ±	0.2	4.6 ±	0.2	4.8 ±	0.2	4.7 ±	0.0	4.3 ±	0.1	4.7 ±	0.1
AST	unit/dl	19.7 ±	2.3	15.0 ±	3.5	17.7 ±	4.0	20.0 ±	1.0	16.0 ±	1.0	18.3 ±	1.2
ALT	unit/dl	21.3 ±	5.1	19.0 ±	4.4	19.7 ±	5.5	12.7 ±	2.1	10.7 ±	1.2	11.7 ±	0.6
g-GTP	unit/dl	20.0 ±	1.7	19.7 ±	2.5	19.7 ±	2.5	13.3 ±	3.1	13.7 ±	3.2	13.7 ±	3.2
TG	mg/dl	55.7 ±	17.6	57.3 ±	14.2	59.7 ±	22.3	42.3 ±	25.7	48.3 ±	11.0	49.3 ±	18.6
phospholipid	mg/dl	198.7 ±	29.6	187.0 ±	20.7	193.0 ±	19.1	199.7 ±	13.6	193.7 ±	22.7	203.7 ±	22.1
cholesterol	mg/dl	187.0 ±	41.1	176.0 ±	34.6	176.7 ±	30.4	171.3 ±	15.5	166.0 ±	24.8	170.0 ±	26.2
HDL_chol	mg/dl	61.0 ±	10.4	56.0 ±	8.9	59.0 ±	7.2	70.0 ±	11.1	68.0 ±	7.0	72.3 ±	10.0
LDL_chol	mg/dl	112.3 ±	25.0	102.3 ±	19.1	103.0 ±	18.2	88.0 ±	11.3	83.0 ±	19.3	83.7 ±	21.1
BUN	mg/dl	17.7 ±	1.6	13.1 ±	1.1	14.3 ±	4.2	12.0 ±	1.8	11.1 ±	0.9	12.5 ±	0.4
UA	mg/dl	5.8 ±	0.8	5.2 ±	0.6	5.8 ±	0.9	4.6 ±	0.5	4.0 ±	0.3	4.6 ±	0.4
glucose	mg/dl	88.7 ±	3.2	92.0 ±	2.6	89.3 ±	3.2	87.0 ±	9.2	93.7 ±	4.2	91.0 ±	3.6
SHBG	nM/L	42.8 ±	19.5	31.5 ±	8.1	27.6 ±	6.9	126.8 ±	21	108 ±	27	98.8 ±	25.8
T3	nM/L	3.3 ±	2.1	7.2 ±	7.9	7.3 ±	4.8	2.1 ±	0.4	2.0 ±	0.7	3.2 ±	0.7
T4	nM/L	154.0 ±	47.0	181.0 ±	94.0	182.0 ±	54.0	161.0 ±	19.0	138.0 ±	33.0	165.0 ±	23.0

Discussion

Equol, a metabolite of daidzein, is considered to be the most effective estrogen modifiers in the human body and has been shown to improve osteoporosis and climacteric syndrome.[3,4,9,14] Because most soy isoflavones are conjugated to sugars of the β-glycoside type, their absorption from the intestine requires hydrolysis by bacterial β-glucosidases.[13,15] Equol is converted from daidzein by particular intestinal bacteria,[6,7] such that only those individuals with these bacteria produce equol. The rate of equol producers is reported to be about 20–30% among Caucasians and 30–50% among Japanese.[16,17] High soy food intake may be relevant to equol production, and fewer equol producers are being found in the younger generation in Japan, probably due to their increasingly Westernized dietary habits.

Setchell et al.[3,8] proposed that, after consuming soy food, people who have plasma equol concentrations of < 40 nmol/L (10 μg/L) can be classified as nonproducers and concentrations > 85 nmol/L (20 μg/L) define equol producers. This distinction can also be applied to urine, with an equol producer defined as someone excreting > 1000 nmol/L. In the present study, 1 of 6 males and 4 of 12 females (i.e., 5 of 18, 28%) were equol producers.

In our previous study, after consumption of soy bean powder, the peak of plasma daidzein appeared at 3 h followed by an equol peak at 6 h.[19] The plasma half-life of daidzein was 6–7 h [19,20]. In the present study, remnant daidzein and genistein in the supplement showed similar movement through the body, but equol aglycone showed much faster kinetics. Peak plasma equol concentration was found at 30 min or 1 h, and the average plasma half-life was 83 min; the recovery rate in urine was also high (40–60%). Setchell et al.[3] reported the bioavailablity and metabolism of equol in one healthy adult. When given as a single-bolus oral 25-mg dose, equol required 4–6 h to attain the maximal plasma concentration, and the plasma half-life was 8.8 h. They used a mixed-type equol, so that extra time was required for hydrolysis in the intestine.

Quick excretion in the urine suggested that most equol was glucuronized. A second plasma peak at 5 h suggested the recirculation of equol through the bile and reabsorption in the small intestine. Equol is considered to be metabolically inert once it is formed, and the predominant phase II reactions are glucuronidation and to a minor extent sulfation.[21] Negel et al. [22] reported that 49.7% of equol circulates in the free or unbound form. This value is considerably greater than the percentage of free daidzein (18.7%) or estradiol (4.6%), the unbound fraction that is available for receptor occupancy. This greater availability of free equol may enhance the overall potency of this metabolite.

In our experiment, only a small proportion of equol remained in the plasma after 1 day, and this would represent the bioactive fraction of equol. A different pattern in the cumulative urinary excretion after repeated intake of equol was noteworthy. In females 5% of the peak plasma equol concentration remained at 48 h compared to 1.5% in males, and the recovery rate in the urine was higher in males compared to that in females. In addition to showing a higher absorption rate, these findings suggest that the bioavailability of equol was higher in females. Equol producers, however, excreted more equol in the urine compared to nonproducers, suggesting that the efficacy of glucuronization by the phase II enzyme is high in producers.

Equol binds to SHBG and competitively inhibits estradiol and testosterone binding in a dose-dependent manner.[23] Because of the small number of participants, the effect of equol on SHBG production was not confirmed in this study, although the administration of multiple doses seemed to decrease the SHBG level. In this study we measured SHBG levels using immunological methods, but measurement of mRNA is necessary to determine the real effect of equol. Further clinical study is required to clarify the effect of equol on SHBG in humans.

This newly developed equol supplement should prove clinically useful as a selective estrogen modifier for climacteric women, especially for equol nonproducers. In addition, the antioxidant activity of equol may inhibit lipid peroxidation and reduce the risk of cardiovascular disease.[24] Thus, for equol nonproducers, the further development of equol supplements could yield some beneficial effects.

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