



High Urinary Isoflavone Excretion Phenotype Decreases Plasma Cholesterol in Golden Syrian Hamsters Fed Soy Protein

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Abstract

Apparent absorption of isoflavones varies greatly among individuals but is relatively stable within an individual. We hypothesized that high urinary isoflavone excretors would show less plasma non-HDL cholesterol (non-HDL-C) than low isoflavone excretors after soy protein feeding. Fifty Golden Syrian hamsters were fed a high-fat/casein diet ($n = 10$) or a high-fat/soy protein diet ($n = 40$) for 4 wk. We identified 2 distinct urinary isoflavone excretion phenotypes based upon HPLC analysis of urinary glycitein using a pairwise correlation plots analysis, or based upon total urinary isoflavone using a hierarchical cluster test. High isoflavone excretors showed greater urinary isoflavones ($P < 0.05$) than did low isoflavone excretors at wk 1 and 4. The low urinary glycitein excretion phenotype was more stable than the high urinary glycitein excretion phenotype by McNemar's test. High urinary isoflavone excretors had significantly less non-HDL-C than did the low isoflavone excretors or casein-fed controls ($P < 0.05$). Plasma total and non-HDL-C were negatively correlated with urinary daidzein, glycitein, and total isoflavone excretion ($r = -0.45$ to -0.58 , $P < 0.05$). Urinary isoflavone excretion phenotypes predicted the cholesterol-lowering efficacy of soy protein. Isoflavone absorbability, probably due to gut microbial ecology, is an important controllable variable in studies of effects of soy protein on blood lipids. *J. Nutr.* 136: 2773–2778, 2006.

Introduction

Increased intake of soy protein lowers blood cholesterol, as confirmed by a recent meta-analysis of 22 randomized human trials (1). The role of isoflavones in this effect of soy protein was determined to be negligible. However, moderately hypercholesterolemic men and women who ate 62 mg total isoflavones/d in soy protein had decreased total and LDL cholesterol (LDL-C)¹ compared with casein-fed controls (2). LDL-C was less in premenopausal women who ate soy protein high in isoflavones compared with controls during the midfollicular and periovulatory phases of the menstrual cycle (3). These same soy foods and doses fed to postmenopausal women in a crossover study showed that only the greatest isoflavone intake caused significantly less LDL-C than controls (4). A minimal dose of 96 mg isoflavone/d was required for cholesterol lowering in a meta-analysis of 8 human studies with no-, low-, and high-isoflavone containing soy protein treatments (5).

The hamster model has also provided contrasting results in studies of the effects of isoflavones. Song et al. (6) fed male and female 6–8-wk-old Golden Syrian hamsters various diets with or without isoflavones. Plasma total cholesterol and non-HDL cholesterol (non-HDL-C) concentrations were significantly less in hamsters fed soy protein with or without isoflavones or fed 1.3

mmol daidzein/kg diet in a casein-based diet (approximately the concentration of total isoflavones in a 25% soy protein diet) compared with concentrations in those fed a high-fat, casein control diet, illustrating similar efficacy for cholesterol lowering by soy protein and daidzein). The cholesterol-lowering effects of isoflavones increased with increasing isoflavone doses in 6-mo-old ovariectomized Golden Syrian hamsters (7). In another study, plasma isoflavones showed a positive correlation with plasma LDL + VLDL-cholesterol concentration in female hamsters. Male hamsters fed isoflavones as part of a casein/lactalbumin diet did not have lower cholesterol concentrations than those fed the control diet, but isoflavones within a soy diet increased the cholesterol-lowering effect (8). The addition of lactalbumin to casein seemed to alter the effects of isoflavones on blood lipids.

Inter-individual variability in apparent absorption of isoflavones may account for the observed negligible effects of isoflavones in meta-analyses of soy protein feeding trials (1). In a randomized crossover design, 7 women fed various doses of soy milk showed distinct patterns of urinary isoflavone excretion; 2 women had greater excretion of isoflavones than did the other 5 women (9). Human fecal isoflavone disappearance phenotypes were observed in vitro (10). The phenotypes were stable in 12 of 15 subjects reexamined after 10 mo, and fecal isoflavone disappearance was strongly inversely correlated with plasma isoflavones in 8 men fed a single isoflavone dose (10). Gut microbes seemed played an important role in the metabolism of isoflavones. Zheng et al. (11) identified fecal isoflavone disappearance

¹ Abbreviations used: HDL-C, HDL-cholesterol; IDL, intermediate density lipoprotein; LDL-C, LDL-cholesterol; non-HDL-C, non-HDL-cholesterol.

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phenotypes among 35 Chinese and 33 Caucasian women. The high urinary isoflavone excretion phenotype affected urinary excretion of genistein when accompanied by relatively rapid gut transit time. Low fecal isoflavone degradation rate in 12 women fed soy for 7 d was related to ~50% greater apparent absorption of isoflavones compared with 13 women with high fecal isoflavone degradation rates (12).

Golden Syrian hamsters may be good models to study the role of isoflavone bioavailability in the cholesterol-lowering efficacy of isoflavones, because greater isoflavone bioavailability was associated with increased cholesterol lowering by these compounds when isoflavones were fed in a casein-based diet (13). Inter-individual variability in isoflavone bioavailability, which we think is an important issue in understanding the health effects of these compounds in humans, seemed to be greater among female than among male hamsters (13), making female hamsters a particularly useful model for humans. The objectives of this study were to identify in a hamster model urinary isoflavone excretion phenotypes using statistical methods and to test the hypothesis that a high excretor isoflavone bioavailability phenotype was associated with cholesterol-lowering efficacy of dietary soy protein.

Materials and Methods

Diets

In the experimental period, hamsters were fed a hyperlipidemic casein-based control diet high in saturated fatty acids (14) or a treatment diet with soy protein (Supro 670, Protein Technologies International) substituted for casein. The isoflavone concentration of soy protein was analyzed by Waters HPLC linear gradient with UV detection (15). The soy protein contained 405 μg of daidzein, 647 μg of genistein, and 72 μg of glycitein/g. The saponin fractions of soy protein were analyzed by HPLC (16); the soy diet contained 1.2 mg saponin/g. We prepared and stored experimental diets at 4°C and measured food intake weekly and over 2–3 consecutive d in metabolic cages.

Animals and sample collection

Fifty 8-wk-old female Golden Syrian hamsters, 118–128 g, were obtained from Charles River Breeding Laboratories. Hamsters were fed AIN 93 mol/L diet for 2 mo (17), then assigned to 2 groups to achieve similar mean body wt/group: 10 hamsters fed casein diet and 40 hamsters fed soy protein diet. The hamsters had free access to food and drinking water in a temperature-controlled room (23°C) with a 12-h light/dark cycle during the 4-wk experimental period. Clinical observation and body wt measurements were conducted 1 \times /wk. Urinary and fecal samples were collected over 24 h at the end of wk 1 and 4 when hamsters were put in metabolic cages individually for 2–3 d. At the end of the feeding period, diets were withdrawn from hamsters 14–16 h before they were killed by exposure to CO₂. We collected blood samples by cardiac puncture in EDTA tubes and centrifuged at 5000 \times g; 10 min, 4°C. Plasma samples were then frozen at –20°C until analysis. The animal experimental protocol was approved by the Iowa State University Animal Use Committee.

Plasma lipid analysis

Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were measured with Infinity Cholesterol and Triglycerides Reagent (Thermo DMA) and HDL Cholesterol Reagent (PTA/MgCl₂) Diagnostics kits (Sigma Diagnostics). Non-HDL-C was calculated by subtracting HDL cholesterol (HDL-C) from total cholesterol and represented LDL + intermediate density lipoprotein (IDL) + VLDL cholesterol.

Chemicals

Daidzein (4',7-dihydroxyisoflavone), genistein (4',5,7-trihydroxyisoflavone), glycitein (4',7-dihydroxy-6-methoxyisoflavone), and THB (2,4,4'-trihydroxybeozoin) were synthesized (18). Milli-Q (Millipore) HPLC grade water was used. We purchased other HPLC solvents from Fisher Scientific.

Analytical methods

Fecal and urinary sample preparation and HPLC analysis. Isoflavone extraction from urine and fecal samples was performed as described previously (19). Fecal saponin metabolite analysis in wk 4 samples was as described (16,20).

Statistical analysis. We conducted pairwise correlation plots analysis and hierarchical clustering test to classify isoflavone excretion phenotypes. One-way ANOVA followed by *t* tests determined the differences in urinary and fecal isoflavones between 2 isoflavone excretion phenotypes at wk 1 and 4, differences in excretion of daidzein, genistein, and glycitein within excretion phenotype, differences in the plasma lipid levels between the soy protein-fed and control groups, between isoflavone excretion phenotypes, and between isoflavone excretion phenotypes and the controls. We used McNemar's test (21) to verify high and low isoflavone phenotype stability. Intraclass Correlation Analysis (22) was done to verify individual phenotype stability. Pearson correlation analysis determined the correlations between urinary and fecal isoflavone excretion and between isoflavone excretion and plasma lipids. All results were reported as mean \pm SEM. All differences were considered significant at *P* < 0.05. Statistical analysis was performed with R software (23) and SAS (SAS Institute, version 8.1; 1998).

Results

Identification of urinary isoflavone excretion phenotypes.

Food intakes and body wt did not differ between the groups at wk 1 and 4. For casein-fed hamsters, food intake was 7.7 \pm 0.5 g/d (wk 1) and 7.1 \pm 0.6 g/d (wk 4), whereas soy protein-fed hamsters had food intakes of 7.1 \pm 0.6 and 7.0 \pm 0.5 g/d at wk 1 and 4, respectively. Body wt for both groups were 123 \pm 3 g initially; casein-fed hamsters had body wt of 130 \pm 5 g (wk 1) and 134 \pm 7 g (wk 4), whereas soy protein-fed hamsters had body wt of 128 \pm 6 g (wk 1) and 131 \pm 6 g (wk 4).

Urinary isoflavone excretion phenotypes were classified using pairwise correlation plots. The plot of the ranked values showed a gap in urinary glycitein excretion between individuals at wk 1 (Fig. 1). From these rankings, hamster 2 had the maximum excretion of all isoflavones. Hamster 8 remained among the least

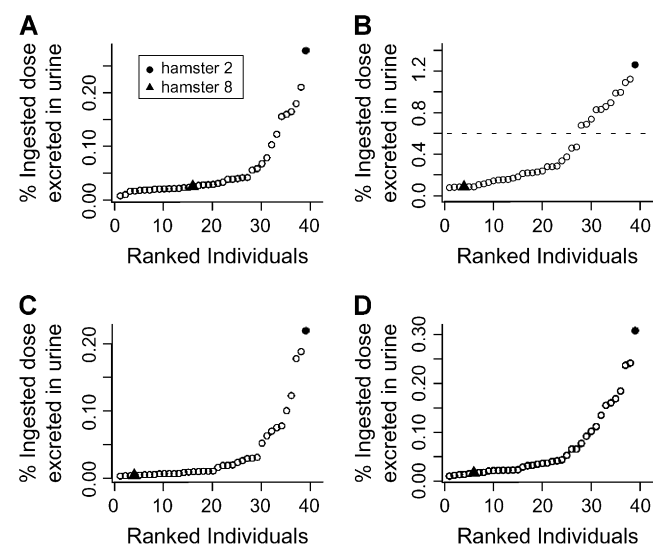


Figure 1 Display of the ranked values of the percentage of ingested isoflavone dose excreted in hamster urine at wk 1 for (A) daidzein, (B) glycitein, (C) genistein, and (D) total isoflavone. Rankings on the x-axis were different for each isoflavone. According to the percentage of ingested dose of glycitein excreted in urine, high and low urinary excretion phenotypes were identified by a natural break point in (B) glycitein.

in urinary excretion of all isoflavones. The highlighted data for these 2 individuals indicates the consistency of apparent absorption for all isoflavones and the extreme difference among individuals. The number of observations in the 2 categories were great enough for inference; thus, the statistical analysis showed a natural cut-off for high and low urinary glycitein excreters (Fig. 1).

Another method to classify urinary isoflavone excretion phenotypes was based on hierarchical clustering using a complete linkage method (Fig. 2), which ensured that all items in a cluster were within some maximum distance of each other. Only hamsters in 2 of the 4 groups were used in further studies (Group 3, high isoflavone excreters; Group 2, low isoflavone excreters) because they displayed more consistent characteristics within a cluster than did the 2 other groups. Hamster 2 was removed from this analysis, because it consistently formed its own cluster. Hamster 1 had missing values for the clustering variables (no HPLC data). The full clustering tree for 38 hamsters indicated 4 excretion phenotypic clusters (Fig. 3). Bivariate plots showed each observation's categorization with the respective phenotypic cluster number. We chose to compare Clusters 2 and 3 for the effect of isoflavone excretion on plasma lipids, because Clusters 1 and 4 had no universal ordering (i.e., individuals in Clusters 2 and 3 remained grouped for their excretion of each of the isoflavones and for total isoflavone excretion). We classified urinary isoflavone excretion phenotypes for wk 4 by the same methods.

Influence of urinary isoflavone excretion phenotypes on isoflavone and saponin excretion. Glycitein excretion (as percentage of ingested dose) was greater than daidzein and genistein excretion in urine within urinary excretion phenotype ($P < 0.05$; Tables 1 and 2).

According to high and low urinary glycitein excretion phenotypes identified by a natural break point, high excreters excreted more urinary daidzein, genistein, glycitein, and total isoflavone ($P < 0.05$) than did individuals of the low excretion phenotype at wk 1 and 4 (Table 1). Fecal daidzein, genistein, and total isoflavone excretions did not differ between excretion phenotypes at wk 1 or 4.

According to total urinary isoflavone excretion at wk 1 (Figs. 2 and 3), high and low urinary excretion phenotypes were identified by a hierarchical cluster test. Individuals of the high excretion

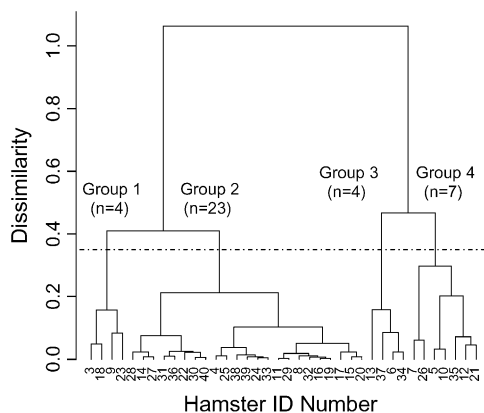


Figure 2 Hierarchical clustering based on hamster urinary excretion of total isoflavone in wk 1. A dendrogram of the urinary excretion of total isoflavone in wk 1 was computed by complete-link clustering that divided all hamsters (except hamsters 1 and 2) into 4 clusters (as indicated by the dotted line). Dissimilarity between the clusters was defined as the maximum dissimilarities between members. The height at which 2 cases joined a single group indicated their dissimilarity.

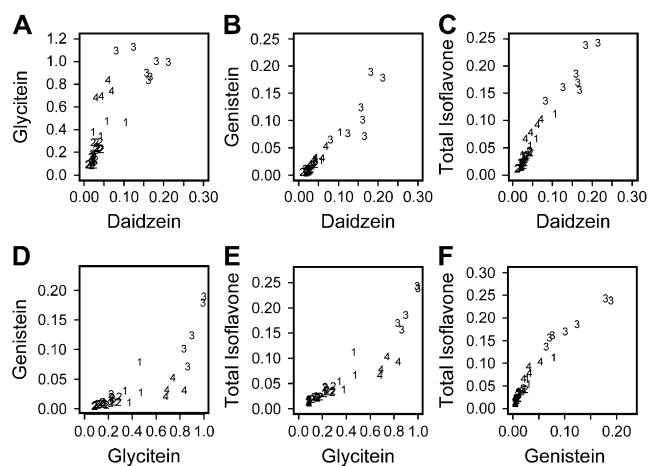


Figure 3 Scatterplots of the fractions of the ingested dose of isoflavones excreted in hamster urine during wk 1. Pairwise relations were compared among urinary excretion of glycitein, daidzein, genistein, and total isoflavones in bivariate scatterplots (A–F). The numbers indicate groupings of individuals as defined in Fig. 2 (hierarchical clustering). Group 2 constituted low urinary excreters of all isoflavones, whereas Group 3 comprised high urinary excreters of all isoflavones compared with the other groups. Groups 1 and 4 fell between groups 2 and 3 and had no universal ordering.

phenotype (cluster 3) excreted more urinary daidzein, genistein, glycitein, and total isoflavone ($P < 0.05$) than did those of the low urinary excretion phenotype (cluster 2) at wk 1 and 4 (Table 3).

High and low fecal saponin metabolite (20) did not differ between excretion phenotypes in wk 4 according to glycitein and total isoflavone phenotypes (data not shown).

Stability of urinary excretion phenotypes over 4 wk.

McNemar's test showed the stability of the urinary excretion phenotypes between wk 1 and 4 (Tables 2 and 3). Only 4 of 39 individuals switched their urinary glycitein excretion phenotypes between wk 1 and 4. Twenty-six hamsters remained low excreters and 1 hamster became a high excretor. The low urinary glycitein excretion phenotype was more stable than the high glycitein urinary excretion phenotype. Three of 11 hamsters of the high glycitein excretor phenotype became low excreters. By the other classification method, the low excretion phenotype (cluster 2) was more stable (2 individuals switched) than the high excretion phenotype (cluster 3) in which 3 individuals switched.

TABLE 1 Urinary isoflavone recoveries in female Golden Syrian hamsters according to urinary glycitein excretion phenotypes (high and low) in wk 1 and 4¹

Urinary excretion phenotype	n	Daidzein	Genistein	Glycitein	Total isoflavone
Wk 1					
<i>% Ingested dose recovered in urine over 24 h</i>					
High	11	27.7 ± 5.9	25.1 ± 4.2	88.2 ± 8.2 [#]	45.8 ± 5.9
Low	27	8.8 ± 3.8*	7.9 ± 3.6*	27.2 ± 11.2 ^{**}	11.9 ± 4.8*
All	38	14.3 ± 4.8	12.9 ± 3.2	44.8 ± 9.3 [#]	21.7 ± 6.1
Wk 4					
High	9	25.9 ± 6.8	23.8 ± 3.2	82.8 ± 9.7 [#]	43.8 ± 6.2
Low	29	9.1 ± 4.1*	8.2 ± 4.1*	29.9 ± 10.7 ^{**}	13.8 ± 4.9*
All	38	13.1 ± 5.2	11.9 ± 3.9	42.4 ± 11.3 [#]	20.9 ± 5.2

¹ Values represent means ± SEM. *Different from high excretor, [#]different from daidzein and genistein, $P < 0.05$.

TABLE 2 Urinary isoflavone recoveries in female Golden Syrian hamsters according to urinary total isoflavone excretion phenotypes in wk 1 and 4¹

Urinary excretion phenotype	n	Daidzein	Genistein	Glycitein	Total isoflavone
<i>% Ingested dose recovered in urine over 24 h</i>					
Wk 1					
High	7	30.8 ± 5.2	26.2 ± 3.8	86.9 ± 7.3 [#]	46.0 ± 5.8
Low	23	8.1 ± 3.9*	7.5 ± 3.9*	25.9 ± 9.9* [#]	11.1 ± 5.1*
All	30	13.4 ± 4.8	11.8 ± 4.1	40.1 ± 10.5 [#]	19.2 ± 6.2
Wk 4					
High	9	28.9 ± 5.7	25.9 ± 4.1	87.9 ± 8.1 [#]	44.9 ± 6.1
Low	21	8.3 ± 4.2*	7.6 ± 3.9*	26.5 ± 8.9* [#]	12.3 ± 9.3*
All	30	14.5 ± 4.8	13.1 ± 4.2	44.9 ± 9.9 [#]	22.1 ± 7.0

¹ Values represent means ± SEM. *Different from high excretor, [#]different from daidzein and genistein, *P* < 0.05.

Individual glycitein excretion phenotype stability from wk 1 to 4 (Fig. 4) was investigated using Intraclass Correlation Analysis, defined as $\delta_B^2 / (\delta_B^2 + \delta^2)$, which compared the variability between hamsters to the total variability in the data. The 95% CI estimate for this ratio was [0, 0.89]. This interval was wide due to high variability, or low precision, in the estimate of this ratio. However, the variability of the values within a hamster can be seen as the length of the lines connecting the 2 measurements. In general, there was less change from wk 1 to 4 when a hamster had a relatively low original glycitein excretion phenotype.

Plasma lipids and urinary isoflavone excretion phenotypes. Compared with the control diet, the soy protein diet lowered plasma total cholesterol by 18% (*P* < 0.05), non-HDL-C by 30% (*P* < 0.05), and non-HDL-C/HDL-C (*P* < 0.05; Table 3). Plasma HDL-C and triglyceride concentrations did not differ between groups. Plasma total cholesterol (by 26% vs. 14%; *P* <

0.05), non-HDL-C (by 51% vs. 36%, respectively; *P* < 0.05), and non-HDL-C/HDL-C (*P* < 0.05) were less in individuals of the high urinary glycitein excretion phenotype (identified at wk 1 or 4) compared with individuals of the low glycitein excretion phenotype (Table 3). Individuals of the low glycitein excretion phenotype did not differ from hamsters fed the casein diet in plasma lipid status. Individuals of the high isoflavone excretion phenotype at either wk 1 or 4 had less plasma total cholesterol (by 18% and 28%, respectively; *P* < 0.05), non-HDL-C (by 49% and 53%, respectively; *P* < 0.05), and the ratio of non-HDL-C to HDL-C (*P* < 0.05) was less compared with individuals of the low isoflavone excretion phenotype (Table 3). Individuals of the low excretion phenotype were similar to casein controls in plasma lipid status. Isoflavone excretion phenotypes did not significantly affect plasma HDL-C and triglycerides.

Relation between plasma lipids and isoflavone or saponin metabolite excretion. Strong relations existed between urinary and fecal isoflavone excretion within individuals at wk 1 and 4 according to correlation analysis (*r* = 0.79–0.93, *P* < 0.05). The plasma total cholesterol level was positively correlated with non-HDL-C and the ratios of non-HDL-C to HDL-C (*r* = 0.87 and 0.79, *P* < 0.05). Non-HDL-C was positively correlated with non-HDL-C/HDL-C (*r* = 0.97, *P* < 0.05). There was no correlation of HDL-C or triglyceride with total or non-HDL-C.

Total and non-HDL-C were negatively correlated with urinary daidzein, glycitein, and total isoflavone excretion (*r* = –0.45 to –0.58, *P* < 0.05). HDL-C and triglycerides were not significantly correlated with urinary or fecal isoflavone excretion. Total and non-HDL-C were not significantly correlated with fecal saponin metabolite excretion.

Discussion

We identified 2 distinct urinary isoflavone excretion phenotypes based on urinary glycitein or total isoflavone excretion in hamsters using 2 statistical methods. Zheng et al. (12) showed

TABLE 3 Plasma cholesterol level in female Golden Syrian hamsters fed casein or soy protein with different urinary isoflavone excretion phenotypes^{1,2}

Group	n	Total cholesterol	HDL-C	Non-HDL-C ³	Triglyceride	Non-HDL-/HDL-C
<i>mmol/L</i>						
Casein	10	5.71 ± 0.88	3.02 ± 0.42	2.41 ± 0.91	1.19 ± 0.32	0.79 ± 0.28
Soy protein	38	4.68 ± 0.72*	2.79 ± 0.57	1.67 ± 0.69*	1.15 ± 0.57	0.59 ± 0.19*
Wk 1						
High glycitein	11	4.21 ± 0.71*	2.81 ± 0.63	1.18 ± 0.75*	1.12 ± 0.42	0.41 ± 0.21*
Low glycitein	27	4.88 ± 0.81	2.78 ± 0.37	1.87 ± 0.88	1.16 ± 0.31	0.67 ± 0.33
Wk 4						
High glycitein	9	4.01 ± 0.81*	2.61 ± 0.69	1.09 ± 0.65*	1.09 ± 0.38	0.39 ± 0.27*
Low glycitein	29	4.93 ± 0.79	2.92 ± 0.48	1.92 ± 0.83	1.13 ± 0.36	0.69 ± 0.38
Wk 1						
High total isoflavone	7	4.10 ± 0.56*	2.75 ± 0.51	1.12 ± 0.62*	1.13 ± 0.59	0.41 ± 0.39*
Low total isoflavone	23	5.01 ± 0.83	2.51 ± 0.48	2.23 ± 0.87	1.17 ± 0.31	0.88 ± 0.37
Wk 4						
High total isoflavone	9	3.98 ± 0.57*	2.65 ± 0.39	1.11 ± 0.52*	1.03 ± 0.61	0.40 ± 0.41*
Low total isoflavone	21	5.21 ± 0.73	2.62 ± 0.50	2.33 ± 0.77	1.12 ± 0.35	0.87 ± 0.39

¹ Values represent means ± SEM. *Different from casein, *P* < 0.05.

² Urinary isoflavone excretion clusters were determined by either glycitein (Fig. 1) or total isoflavone excretion (Fig. 2).

³ Represents the VLDL + IDL + LDL fractions (by difference: total – HDL).

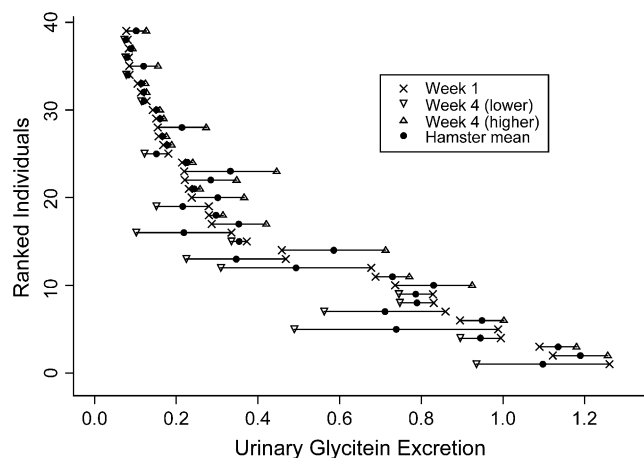


Figure 4 Stability of urinary glycitein excretion within hamsters between wk 1 and 4. The vertical axis is the hamster number (1–39) ranked in order by urinary glycitein excretion at wk 1. The fraction of ingested dose of glycitein excreted in urine in each hamster is represented on the horizontal axis and the horizontal line connects wk 1 and wk 4 measurements for each hamster.

that 13 women of a low *in vitro* fecal isoflavone disappearance phenotype and relatively rapid gut transit time (GTT) showed ~50% greater urinary isoflavone excretion (apparent absorption) than did 12 women of a high fecal isoflavone disappearance phenotype and slow GTT after consuming soy isoflavones for 7 d. Hamsters showed *in vitro* cecal isoflavone degradation rate phenotypic clusters (24) that could account for the urinary isoflavone excretion phenotypes observed in the present study, suggesting that the isoflavone bioavailability phenotypes observed in both humans (11,12) and hamsters (13) are due to gut microbial degradation of isoflavones that differs greatly and relatively stably among individuals.

In hamsters, urinary isoflavone excretion was greater than fecal isoflavone excretion, presumably due to gut microbial degradation of these compounds, as observed in humans (9,25; Tables 2 and 3). Glycitein showed significantly greater excretion than daidzein or genistein in this study (Tables 2 and 3), indicating its greater bioavailability than the other isoflavones. Feeding 1.18 or 1.77 mmol total isoflavones/kg diet to 1-y-old male ($n = 10$) and female ($n = 9$) hamsters for 10 d produced similar 24-h urinary isoflavone excretion differences among the isoflavones (13). Daidzein was a more bioavailable isoflavone than genistein in women fed single doses of soymilk, as reflected in urinary excretion (9,25). Urinary glycitein excretion was similar to that of daidzein and greater than genistein in 7 women and 7 men fed single doses of soymilk (19). Not all human studies have shown greater bioavailability for daidzein than for genistein. Women who were high excretors of isoflavones did not differ in daidzein and genistein excretion, whereas low excretors showed greater daidzein than genistein excretion (9). We also found that the low glycitein urinary excretion phenotype was more stable and the high glycitein urinary excretion phenotype was less stable in hamsters (Fig. 4). In women, a high fecal daidzein isoflavone disappearance phenotype was not as stable as a low daidzein disappearance phenotype, but both high and low genistein disappearance phenotypes were relatively stable (11). Thus, in a broad sense, hamsters may usefully model human isoflavone bioavailability and its stable phenotypic variations to better study the health effects of these compounds.

In the current study, either glycitein or total isoflavone bioavailability as reflected in urinary excretion clusters deter-

mined at wk 1 or 4 predicted cholesterol-lowering efficacy of soy protein (Table 3). Hamsters fed 1.3 mmol daidzein/kg diet for 10 wk had less plasma cholesterol compared with casein-fed controls (20). In part because of the apparent similarity in daidzein and glycitein bioavailability in humans (19), the present results are likely to be relevant to humans for cholesterol-lowering efficacy of soy proteins containing isoflavones. To our knowledge, no studies of isoflavone effect on blood lipids have compared isoflavone bioavailability indices with cholesterol status; however, Urban et al. (25) showed that serum isoflavones increased as serum cholesterol significantly decreased after 34 elderly men consumed isolated soy protein high in isoflavone concentration for 6 wk in a randomized crossover design that compared the high isoflavone soy protein to a soy protein that had most of the isoflavones removed. These subjects showed serum isoflavone concentrations after eating the soy protein high in isoflavones that varied among subjects >10-fold for genistein and more than 30-fold for daidzein. This study, along with several others (2–5), supported the role of isoflavones in cholesterol-lowering but did not attempt to relate individual isoflavone status to blood lipid status. Based on our hamster model and the wide range of human variability in isoflavone status after soy intake (25), controlling for the isoflavone bioavailability phenotypes of human subjects may optimize conditions to observe isoflavone effects.

Several studies have found that isoflavones did not improve the blood lipid profile, including a recent meta-analysis (1). For example, dietary intake of isoflavone-rich (80.4 mg isoflavone aglucones/d; $n = 24$) or isoflavone-poor soy protein (4.4 mg isoflavones/d; $n = 24$) for 6 mo did not improve circulating lipid and lipoprotein concentrations in perimenopausal women or in subjects who were mildly hypercholesterolemic ($n = 30$) (26). Hamsters fed isoflavones (0.02 g/100 g diet) for 5 wk did not differ from the casein control in total cholesterol, triglycerides, or HDL-C (27). These studies did not attempt to control for inter-individual variability in isoflavone bioavailability.

Soyasaponins, associated with soy protein in addition to isoflavones, were also assessed for cholesterol-lowering effect. A diet containing 2.4 g group B soyasaponins/kg (saponin concentration similar to that in 25% soy protein diet) caused less plasma total cholesterol and non-HDL-C (by 20 and 33%) in 10 11–12-wk-old female Golden Syrian hamsters compared with those fed casein (20). High producers of a putative fecal saponin metabolite had less total cholesterol/HDL-C compared with low producers. In the present study, high and low saponin metabolite producers did not differ in plasma lipid status (data not shown), and the dietary saponin concentration (1.1 g/kg) was about one-half that shown to be effective in lessening cholesterol in a previous study (20). Thus, isoflavones and not saponins were the main modifiers of plasma lipids, but both components vary among soy protein sources and may contribute to lessening of plasma cholesterol by soy protein.

The cholesterol-lessening effect of soy protein in hamsters largely depended on the extent of apparent absorption of isoflavones, which varies according to distinct phenotypes. These urinary isoflavone excretion phenotypes may be defined according to glycitein or total isoflavone excretion over a given period. These isoflavone absorption phenotypes are probably determined by gut microbial ecology, but isoflavone degrading microbial species and other factors determining this relatively stable variability in isoflavone absorption remain to be identified. Studies examining the effects of isoflavones in humans and animal models might be improved by considering inter-individual variability in isoflavone absorption, as it seems to be a controllable factor.

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