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Increased probiotic yogurt or resistant starch intake does not affect isoflavone bioavailability in subjects consuming a high soy diet

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Increased probiotic yogurt or resistant starch intake does not affect isoflavone bioavailability in subjects consuming a high soy diet

Abstract

Objective: Probiotics and prebiotics that affect gut microflora balance and its associated enzyme activity may contribute to interindividual variation in isoflavone absorption after soy intake, possibly enhancing isoflavone bioavailability. This study examined the effects of the consumption of bioactive yogurt (a probiotic) or resistant starch (a known prebiotic) in combination with high soy intake on soy isoflavone bioavailability. **Methods:** Using a crossover design, chronic soy consumption was compared with soy plus probiotic yogurt or resistant starch in older male and postmenopausal females (n 31).

Isoflavone bioavailability was assessed at the beginning and end of each 5-wk dietary period by sampling plasma and urine after a standardized soy meal. **Results:** Chronic soy intake did not significantly affect plasma or urinary isoflavones after the soy meal and there were no significant effects of probiotic or resistant starch treatment. However, there were trends for increased circulating plasma daidzein and genistein after the probiotic treatment and for increased plasma daidzein and genistein 24 h after soy intake with resistant starch treatment. Neither treatment induced or increased equol production, although there was a trend for increased plasma equol in equol-positive subjects (n 12) after probiotic treatment. **Conclusion:** The weak or absence of effects of probiotic yogurt or resistant starch supplement to a chronic soy diet suggests that gut microflora were not modified in a manner that significantly affected isoflavone bioavailability or metabolism.

Keywords

isoflavone, probiotic, prebiotic, bioavailability, daidzein, genistein, CMMB

Disciplines

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Increased probiotic or prebiotic (resistant starch) intake does not significantly affect isoflavone bioavailability in subjects consuming a high soy diet.

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Abstract

Objective: Probiotics and prebiotics that affect gut microflora balance and its associated enzyme activity may contribute to inter-individual variation in isoflavone absorption following soy intake, possibly enhancing isoflavone bioavailability. This study examined the effects of the consumption of a probiotic (bioactive yogurt) or a prebiotic (resistant starch) in combination with high soy intake, on soy isoflavone bioavailability.

Research Methods and Procedures: Using a crossover design, chronic soy consumption was compared with soy plus either probiotic or prebiotic foods in older male and postmenopausal females (n = 31). Isoflavone bioavailability was assessed at the beginning and end of each 5 week dietary period by sampling plasma and urine after a standardized soy meal.

Results: Chronic soy intake did not significantly affect plasma or urinary isoflavones following the soy meal and there were no significant effects of either probiotic or prebiotic treatment. However, there were trends for increased circulating plasma daidzein and genistein after the probiotic treatment and for increased plasma daidzein and genistein 24 h post soy intake with the prebiotic treatment. Neither probiotic nor prebiotic treatment induced or increased equol production, though there was a trend for increased plasma equol in “equol-positive” subjects (n = 12) after probiotic treatment.

Conclusion: The weak or absence of effects of probiotic or prebiotic supplement to a chronic soy diet suggests that gut microflora were not modified in a manner that significantly affected isoflavone bioavailability or metabolism.

Key Words: isoflavone, probiotic, prebiotic, bioavailability, daidzein, genistein

INTRODUCTION

Gut microflora plays an essential role in soy isoflavone absorption, metabolism and bioavailability by virtue of its enzymatic activity (1-3). Differences in gut microfloral profiles may contribute to the large inter-individual variation typical for isoflavone bioavailability following soy intake. The enzymes most pertinent to isoflavone bioavailability are the β -glucosidases, for initial glucoside hydrolysis and subsequent aglycone absorption, and the β -glucuronidases and sulphatases, for reabsorption of the hepatic conjugates and biliary excretion (4). The initial hydrolysis of soy isoflavone glucosides to their respective aglycones is suggested to be the rate-limiting step in isoflavone absorption (5-7); thus, β -glucosidases activity may be particularly important in relation to isoflavone bioavailability. Bacterial species of bacteroides, bifidobacteria and lactobacilli have the highest activity of this enzyme (7-9).

Lactobacilli and bifidobacteria are unique in being exclusively beneficial to the host (10) and thus are termed “probiotic bacteria” (11). Live cultures of these bacteria can be provided in food matrices, such as fermented milk products, to introduce them to the gastrointestinal tract (12) where they subsequently colonize the small intestine and colon (13). Alternatively, the growth and/or activity of specific probiotic bacteria already resident in the gut can be selectively stimulated by intake of prebiotic dietary components (14). Resistant starch is such a prebiotic; it is fermented in the large bowel specifically by bifidobacteria (15) and consequently increases β -glucosidase activity (16). By altering gut microflora balance and increasing β -glucosidase activity, probiotic bacteria may also enhance isoflavone absorption. Furthermore, the metabolism of daidzein to equol relies exclusively on intestinal bacteria (17) and this conversion may be particularly important as equol has significantly enhanced antioxidant activity compared with daidzein (18,19) and may increase potential health benefits (20). Thus, we hypothesized that

intake of either probiotic cultures or a prebiotic dietary component may affect soy isoflavone bioavailability, including equol production. A crossover study was conducted in which plasma and urinary isoflavone levels after 5 weeks of daily intake of soy foods were compared with those after 5 weeks of daily intake of soy in combination with either yogurt containing probiotic bacteria or bread containing the prebiotic, resistant starch.

MATERIALS AND METHODS

Subjects

Male and female subjects, recruited via the local media, were required to be at least 45 years old and mildly hypercholesterolemic (total cholesterol greater than 5.5 mmol/L). A secondary aim of this study was to determine the effects of soy in combination with a probiotic or prebiotic on lipids (Larkin et al, manuscript submitted), hence the latter criterion. Further, women had to be post-menopausal for more than 12 months and not taking hormone replacement therapy (HRT) for the previous 6 months. Further exclusion criteria included the use of cholesterol-lowering medication, antibiotics or probiotic-containing foods or supplements within 2 months prior to the study, an average alcohol intake of greater than 2 standard drinks per day and cigarette smoking.

Study Design

The study consisted of 2 separate cohorts, both of randomized crossover design (Figure 1), with each subject acting as his or her own control. The probiotic cohort was double-blinded; the prebiotic cohort was not blinded. After a two-week wash-in period during which soy foods, yogurt, Hi-MaizeTM bread (high resistant starch content) and probiotic supplements were excluded from the diet, subjects commenced the study, which involved two 5-week dietary periods separated by a 4-week washout, which required the same dietary exclusions as the wash-

in period. Subjects were instructed to otherwise maintain their normal diets for the duration of the study so that other prebiotic dietary components would remain constant. This study design enabled us to examine the effects of increased intake of probiotic yogurt or the prebiotic, resistant starch. Subjects completed a dietary questionnaire during each of the wash-in and washout periods and during both dietary periods.

All subjects consumed soy milk and soy cereal daily during both dietary periods; this diet was further supplemented with either the probiotic or prebiotic foods for the corresponding intervention. A test soy meal was consumed prior to and at the end of both 5-week dietary periods for determination of subsequent isoflavone bioavailability. Subjects were allocated to one of four groups (Pro-1, Pro-2, Pre-1 or Pre-2), which determined the intervention (probiotic or prebiotic) and the order of crossover (Figure 1). The control and active dietary treatments for the probiotic cohort were soy + control yogurt (S + YC) and soy + probiotic yogurt (S + YP), respectively and for the prebiotic cohort were soy control (SC) and soy + resistant starch bread (S + RS), respectively. To assist with compliance, subjects with a preference for either yogurt or bread were allocated accordingly; otherwise, allocation to the probiotic or prebiotic intervention was random as was the order of the crossover.

For each test meal and daily during both 5-week dietary periods, all subjects consumed 250 mL soy milk (So Natural, UHT Calciforte, Taren Point, NSW, Australia) and 45 g soy cereal (Specialty Cereals, Mt Kuring-gai, NSW, Australia). This provided a total of 38 mg daidzein and 68 mg genistein and 4.5 mg glycitein, with the majority of this provided by the cereal (29 mg daidzein + 55 mg genistein + 4.5 mg glycitein). Sufficient quantities of each food were provided to subjects at the beginning of each dietary period; soy milk was provided in 1 L cartons, from which subjects were asked to measure and consume 250 mL daily and soy cereal was pre-packaged as individual 45 g serves.

For the probiotic intervention, subjects consumed 100 mL yogurt per day (Vaalia, Pauls Dairy, Brisbane, Qld, Australia) either with (probiotic) or without (control) live bacterial cultures. The probiotic yogurt contained 10^8 colony forming units (CFU)/100 g daily serve of each of *Lactobacillus GG*, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*. Both control and probiotic yogurts were vanilla flavored with “low” fat (1.4%) content; there were no flavor or texture differences between the two types and the containers were unmarked except for a numerical code. Individual 100 mL serves of yogurt were provided fresh to subjects every 3 weeks and stored under refrigeration. For the prebiotic intervention, bread was baked using 70 % resistant starch flour (Penford Australia Ltd, Sydney, NSW, Australia) by a local bakery (K & M Bakery, Woonona, NSW, Australia) to provide approximately 4 g resistant starch per slice. Bread was baked every 2 - 3 weeks and either delivered to subjects on the day of baking or frozen in the clinic area for pick-up. Subjects consumed 4 - 5 slices of bread per day (to provide a total of 16 - 20 g RS).

At the start of weeks 0, 5, 9 and 14, subjects attended the clinic at the University of Wollongong on two consecutive days. On the first morning, a fasted blood sample was taken, a food frequency questionnaire was administered, and subjects commenced a 24 h urine collection. Next, a test meal consisting of 250 mL soy milk and 45 g soy cereal was eaten as breakfast in the clinic. Subjects were instructed not to consume any other soy foods, probiotics or prebiotics for 48 h post-meal. Blood was again collected 8 and 24 h after the test meal and a second 24 h urine sample was collected between 24 and 48 h post-meal. Urine was collected in 2.5 L bottles containing 1 g ascorbic acid; total volume of each 24 h collection was recorded and aliquots were stored at -80°C until analysis. Blood was collected by a registered nurse in the clinic area into EDTA tubes (Sarstedt) which were inverted and placed on ice until centrifuged at 4°C for 10 min at 2100 g; plasma was harvested and stored at -80°C until analysis.

Sample Analysis

Extraction of isoflavones from plasma and urine was based on published methods using β -glucuronidase enzymatic extraction (21) with modifications to optimize the procedure for local use (22) and measurement via HPLC with ECD (22). All reagents and standards were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia) and solvents used for extractions, standard preparations and HPLC analysis were HPLC grade (Crown Scientific, Moorebank, NSW, Australia). In these purified, extracted samples, isoflavones were separated on a reverse phase C-18 column (SGE Wakosil II 18RS), identified by HPLC (Shimadzu auto-injector) with an isocratic mobile phase of 50 mM sodium acetate buffer pH 4.8 with acetic acid / methanol (45 / 55) and quantified by electrochemical detection at +750 mV (VT-03, Antec Leyden, Zoeterwoude, The Netherlands). Mixed standards of genistein (Sigma, G6649), daidzein (Sigma, D7802), equol (Sigma, 45405), O-desmethylangolensin (ODMA; Plantech, UK) at 4 different concentrations (between 0.2 μ M and 2 μ M for plasma and between 0.2 μ M and 4 μ M for urine) were included in each HPLC run at least in triplicate. Limits of quantification for plasma and urine, respectively were 5 ng/mL and 1 μ g/mL, for daidzein and genistein, 15 ng/mL and 2 μ g/mL, for equol and 60 ng/mL and 6 μ g/mL, for ODMA (22). Within-run coefficients of variation for peak area of standards (% standard deviation/mean) were less than 5% for daidzein and genistein, less than 10% for equol and less than 20% for ODMA. Recoveries were greater than 70% and calculated values were not adjusted for recovery.

Ethics

Ethical approval for this study was granted by the University of Wollongong Human Research Ethics Committee (Ethics Number 02/248) and all procedures complied with National Health and Medical Research Council standards required in Australia. Written, informed consent was obtained from all subjects prior to commencement of the study.

Statistics

Initially, the normality of data sets was examined and outliers were identified (SPSS 11.0 and 11.5, SPSS Inc. Chicago, Illinois). ANOVA with repeated measures and Bonferroni post-hoc analysis (SPSS 11.5, SPSS Inc. Chicago, Illinois) were used and reported with F values, df (hypothesis df, error df) and p values. Student's t-tests were 2-tailed (Microsoft Excel/SPSS 11.5, SPSS Inc. Chicago, Illinois). All values are reported as mean \pm SEM.

RESULTS

Thirty-six subjects (15 females and 21 males) commenced the study, however 5 people withdrew for personal reasons; consequently, 31 subjects (12 female and 19 male Australians of Caucasian background) completed the study. The median subject age was 57.5 years (range 49 – 72 years) and subject age was not significantly different between the groups ($F = 1.020$, $p = 0.399$, one-way ANOVA with between groups analysis). Mean intake of daidzein and genistein for each test soy meal and for the daily soy intake during dietary periods was 0.48 ± 0.01 and 0.84 ± 0.02 mg/kg body mass, respectively.

At week 0, females had significantly higher concentrations of plasma daidzein than males, but there was no gender difference in plasma genistein ($F = 8.386$, $p = 0.007$ and $F = 2.859$, $p = 0.102$, respectively; one-way ANOVA with repeated measures and between groups analysis). There were no differences between males and females for week 0 urinary excretion of either daidzein or genistein ($F = 1.555$, $p = 0.223$ and $F < 0.0001$, $p = 0.992$ respectively, one-way ANOVA with repeated measures and between groups analysis). There was no significant order effect for either dietary intervention and therefore, the two groups of each cohort were combined for all analyses. In week 0 of each dietary period, plasma daidzein and genistein were still

significantly elevated 24 h after the test soy meal compared with 0 h, as expected. The 0 h plasma concentrations of daidzein and genistein were significantly increased after all four soy dietary periods, independent of treatment.

In the probiotic cohort, S + YP did not affect plasma daidzein or genistein concentrations significantly differently compared with S + YC ($F_{1,15} = 0.416$, $p = 0.528$ and $F_{1,15} = 0.813$, $p = 0.382$, respectively, three-way ANOVA with repeated measures; Figure 2, Table 1); however, there was a greater increase in 0 h plasma daidzein concentration after probiotic treatment compared with control, though this was not significant ($F_{1,15} = 1.897$, $p = 0.189$, two-way ANOVA with repeated measures). After probiotic treatment, the plasma concentrations of daidzein and genistein at 0 h of week 5 were higher than those at 24 h of week 0; this was significant for genistein and almost so for daidzein ($p = 0.043$ and $p = 0.058$, respectively, Student's paired t-tests). In the prebiotic cohort, there were also no significant differences between S + RS and SC for plasma concentrations of either daidzein or genistein ($F_{1,15} = 0.416$, $p = 0.528$ and $F_{1,15} = 0.813$, $p = 0.382$, respectively, three-way ANOVA with repeated measures; Figure 3, Table 2).

When the active treatments for the probiotic and prebiotic cohorts (S + YP and S + RS, respectively) were compared, there were no significant differences between them for their effects on plasma isoflavones ($F = 0.440$, $p = 0.513$ for daidzein and $F = 0.623$, $p = 0.437$ for genistein, two-way ANOVA with repeated measures and between-groups analysis, Figure 4).

None of the four 5-week dietary treatments significantly affected urinary daidzein or genistein concentrations and there were no significant differences between S + YC and S + YP (daidzein: $F_{1,15} = 0.013$, $p = 0.909$; genistein: $F_{1,15} = 0.499$, $p = 0.491$) nor SC and S + RS (daidzein: $F_{1,13} = 0.537$, $p = 0.477$; genistein: $F_{1,13} = 3.287$, $p = 0.093$, three way ANOVA with repeated measures, Table 3).

There was high inter-individual variability in the occurrence of equol and ODMA in plasma and urine and subjects were classified into one of three subgroups based on distinct differences between the pattern and frequency of occurrence of equol in their plasma or urine. These subgroups were approximate tertiles: equol-positive (eql(+)) included 12 subjects who consistently had equol in their plasma and/or urine, equol-occasional (eql(o)) included 10 subjects who had plasma or urinary equol in a single plasma or urine sample and equol-negative (eql(-)) included 9 subjects who never had equol in their plasma or urine. There were no significant differences between subjects in these subgroups for body mass, BMI or age at baseline, nor in week 0 plasma or urinary concentrations of daidzein or genistein. None of the treatments significantly affected the plasma or urinary concentrations of equol in either the eql(+) or eql(o) subjects, nor did any of the treatments induce equol formation in eql(-) subjects.

Throughout the study, mean plasma daidzein and genistein tended to be lowest in eql(-) subjects and highest in eql(+) subjects; however, variability was large and there were no significant differences between the three subgroups ($F = 1.113$, $p = 0.343$ for daidzein and $F = 2.228$, $p = 0.127$ for genistein, two-way ANOVA with repeated measures and between groups analysis, Figure 5). There were no significant differences or trends between the subgroups for urinary excretion of daidzein or genistein ($F = 0.157$, $p = 0.856$ and $F = 0.315$, $p = 0.732$ respectively, two-way ANOVA with repeated measures and between groups analysis).

DISCUSSION

In the current study, neither the addition of probiotic bacteria nor a prebiotic ingredient to a soy diet significantly affected isoflavone absorption or metabolism. This was in contrast to our hypothesis that intake probiotic and prebiotic intake, by virtue of their individual effects on gut microflora and enzyme activity necessary for isoflavone absorption and metabolism could potentially increase isoflavone bioavailability.

The lack of any significant effects of probiotic intake may indicate either that the levels of probiotic bacteria provided were insufficient to affect gut microfloral balance or that any gut microfloral changes that did occur did not further affect isoflavone absorption. Our probiotic yogurt was purported by the supplier to contain 10^8 colony forming units (CFU)/100 g of each of *Lactobacillus acidophilus*, *Bifidobacterium bifidus* and *Lactobacillus GG*. This level of bacterial activity should be sufficient to affect gut microfloral balance as probiotic effects in the gastrointestinal tract have been reported with minimum daily doses of between 10^8 and 10^9 viable cells (13,23-25). Oral administration of these probiotic bacteria results in increased populations in the human gastrointestinal tract and faeces (25-28, 31-35) and increased fecal β -glucosidase activity (36). Such increases in lactobacilli and bifidobacteria intake and concomitant enhancement in intestinal β -glucosidase activity would be expected to enhance initial hydrolysis and absorption of dietary isoflavone glycosides. However, a recent randomized, crossover trial with 40 postmenopausal women also reported no effect of bacterial supplementation (10^9 CFU *Lactobacillus acidophilus* and *Bifidobacterium longum*) with daily intake of soy protein isolate plus 44 mg isoflavones on plasma isoflavone concentrations after 6 weeks (37). Together with our study, this suggests that soy isoflavone bioavailability is not affected by concurrent probiotic intake as either a supplement (37) or a dietary component (current study).

The absence of any significant changes in isoflavone bioavailability with resistant starch intake again suggests either a lack of effect on the gut microflora or that such effects had no impact on isoflavone absorption and metabolism. Although we did not determine gastrointestinal microbial activity in the current study, prebiotic effects have been reported after 2 – 4 weeks of daily RS intake of between 15 and 55 g (38-42), thus it is likely the RS intake in the current study had a similar effect. Resistant starch specifically stimulates the growth of bifidobacteria in the

colon (15,43) and increases caecal lactobacilli and bifidobacteria levels in human flora-associated rats (16). Bifidobacteria can hydrolyze daidzin and genistin from soy milk to their respective aglycones (44) and metabolize daidzein in human faecal samples (45,46) and these effects have been attributed to its β -glucosidase activity (36,44). Both bifidobacteria and lactobacilli possess high β -glucosidase activity (16,36) and are also the dominant native microflora in the duodenum and jejunum where most of the initial isoflavone hydrolysis is likely to occur (9). Interestingly, *Lactobacillus acidophilus* and *Bifidobacterium bifidus* do not increase β -glucuronidase activity in the human gastrointestinal tract (29,36,47,48), thus the lack of prebiotic or probiotic observed here suggest that β -glucuronidase activity may be more important than β -glucosidase activity in isoflavone bioavailability.

Although subjects were instructed to maintain their normal diets, many foods contain prebiotics and contribute to the microbial activity of the gastrointestinal tract. With no measure of this in the current study, we are unable to attribute the results of resistant starch intake to a specific prebiotic effect. Fructooligosaccharide (FOS), a prebiotic similar to RS, also increases fecal bifidobacteria (49) and affects gut microflora balance (50) and was reported to prolong the clearance of daidzein and genistein and increase their bioavailability in rats (51). We previously found altered isoflavone metabolism with resistant starch intake, including enhanced metabolism of daidzein to equol (Larkin et al, manuscript submitted). In the current study, the mean plasma isoflavone concentrations 24 h post-meal were highest after 5 weeks of soy and resistant starch intake and although not significant, this finding is interesting to note as it also suggests prolonged plasma isoflavone clearance following a single soy meal. Overall, there may be a potential prebiotic effect on isoflavone bioavailability; however, the relevance of dietary-induced changes

in gastrointestinal microfloral activity to isoflavone bioavailability is rather poorly understood and requires further investigation.

In the current study, 12 subjects (39%) consistently metabolized daidzein to equol, a similar proportion to that reported in other soy studies (52-54). All four soy dietary periods increased plasma equol levels in the initial (0 h) measurement, implying that 5 weeks of daily soy intake (with or without a probiotic or prebiotic) was sufficient to increase plasma equol in those who are predisposed to this conversion. However, the concurrent consumption of either a probiotic or prebiotic with soy did not affect equol concentration or the number of people who produced equol, nor did it induce equol production. Lactobacilli and bifidobacteria have been identified as playing a role in the metabolism of daidzein to equol (17,44,55) (56) and the short chain fatty acid products of resistant starch intestinal fermentation (42,57,58) increase daidzein conversion to equol in faeces (17); however, increased intake of either these probiotic bacteria or resistant starch did not affect equol production in the current study. Previous studies also report no effect of either probiotic (37) or dietary fibre (52) supplementation with soy consumption on equol-producing capacity, suggesting genetics may contribute to this ability (59). In the current study, equol occurrence varied greatly between individuals and within subjects on different occasions, as has been previously reported (60-62). This large variability, combined with a small numbers of subjects, was a clear limitation. There was a pattern however, although not significant, for subjects who produced equol to have higher plasma concentrations of daidzein and genistein, suggesting that a high level of initial isoflavone absorption may be a determining factor for equol production, as previously proposed (44).

CONCLUSIONS

Concurrent consumption of soy with either a probiotic or prebiotic dietary component did not significantly alter plasma or urinary daidzein or genistein concentrations or equol-producing

ability of the subjects in this study. However, our results suggest minor effects on soy isoflavone bioavailability with probiotic or prebiotic intake. Further investigation, including exploration into the roles of specific gut microflora, will assist in the establishment of dietary conditions to enhance isoflavone bioavailability and equol production, particularly if the latter is conclusively associated with increased health benefits.

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FIGURE LEGENDS

Figure 1. Study Design. There were 2 groups for each cohort: probiotic (Pro-1 and Pro-2) and prebiotic (Pre-1 and Pre-2); these followed complementary orders of dietary treatments. S+YC = soy plus control yogurt; S+YP = soy plus probiotic yogurt; SC = soy control, S+RS = soy plus resistant starch. Soy test meals were given at the beginning and end of each 5 week dietary period.

Figure 2. Probiotic cohort - Effect of 5 weeks soy + control yogurt (S + YC) and 5 weeks soy + probiotic yogurt (S + YP) on mean plasma daidzein and genistein concentrations. Mean \pm SEM, n = 17. *Significant $p < 0.017$, post-hoc Student's paired t-tests with Bonferroni adjustment.

Figure 3. Prebiotic cohort - Effect of 5 weeks soy control (SC) and 5 weeks soy + resistant starch (S + RS) on mean plasma daidzein and genistein concentrations. Mean \pm SEM, n = 14.

*Significant $p < 0.017$, post-hoc Student's paired t-tests with Bonferroni adjustment.

Figure 4. Change in plasma daidzein and genistein after 5 weeks of soy + probiotic yogurt (S + YP) and 5 weeks of soy + resistant starch (S + RS).

Figure 5. Mean plasma daidzein and genistein concentrations throughout the study based on equol-producing ability of subjects as determined by HPLC quantification of samples: eql (+) subjects consistently had plasma and/or urinary equol, n = 12; eql (o) subjects had equol in a

single plasma or urine sample, $n = 10$; eql (-) subjects had no observable plasma or urinary equol,
 $n = 9$.

Figure 1

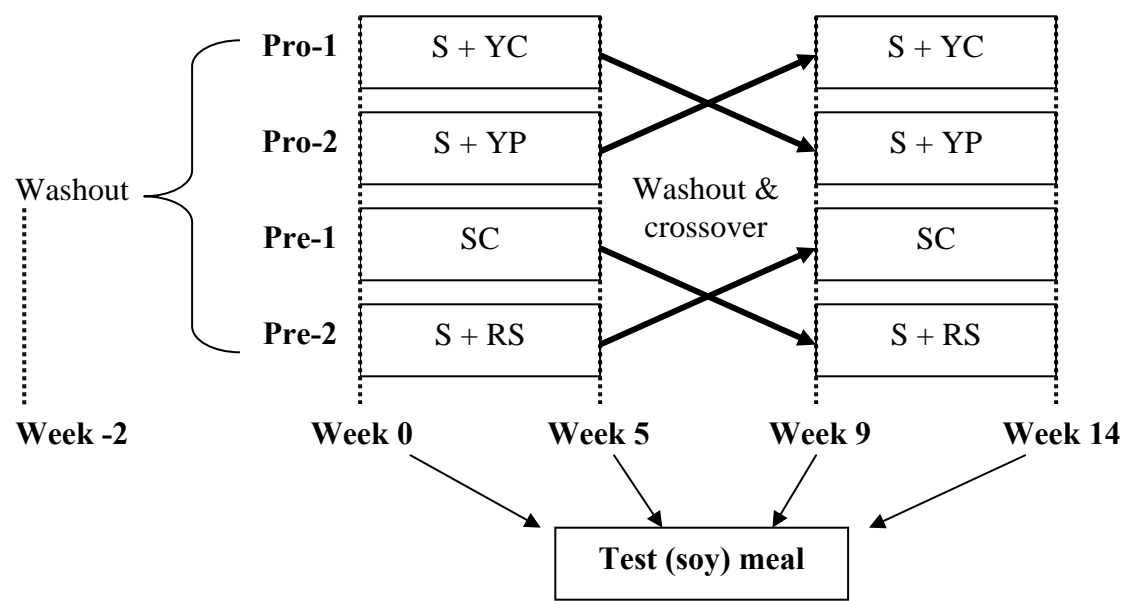


Figure 2.

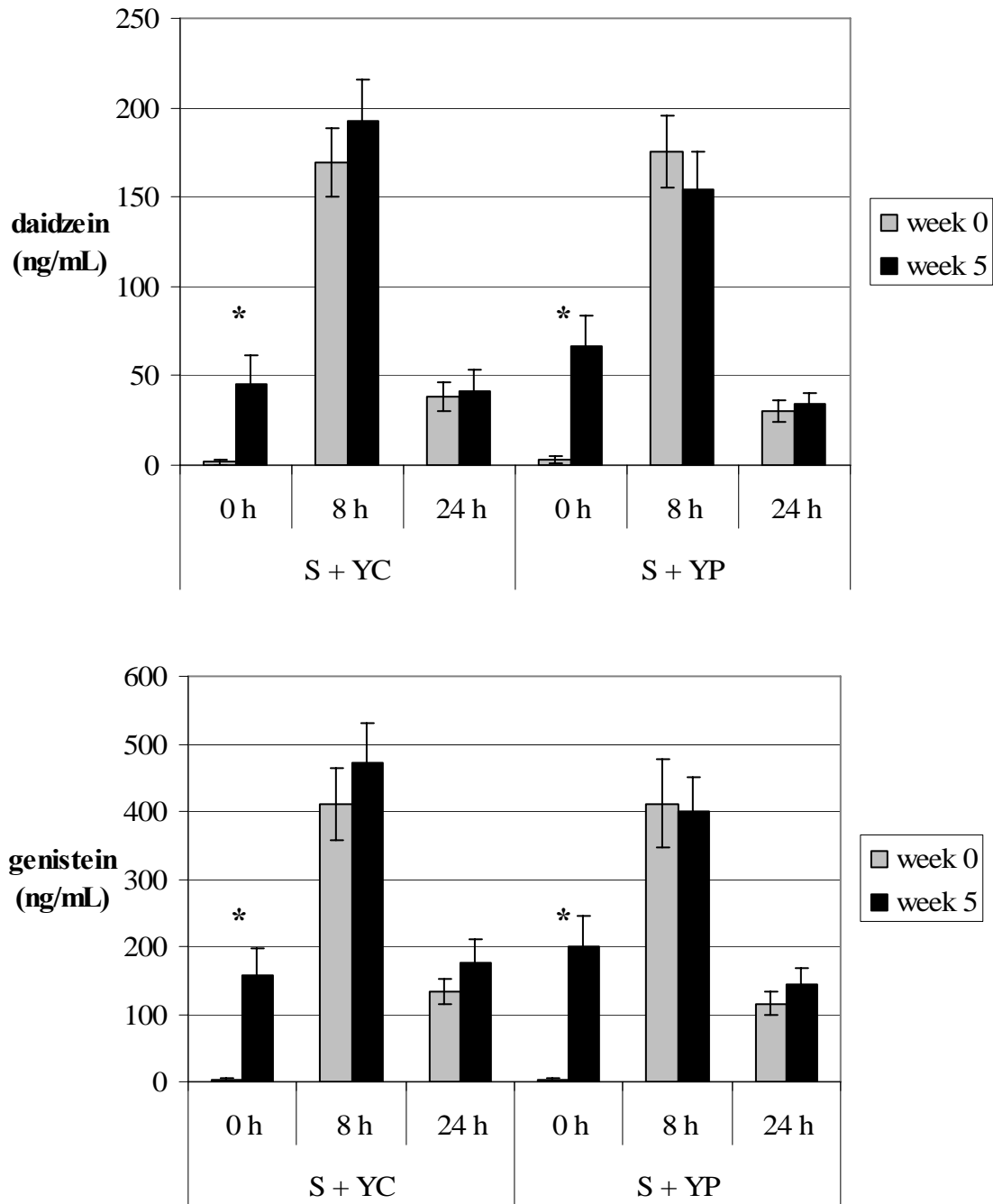


Figure 3.

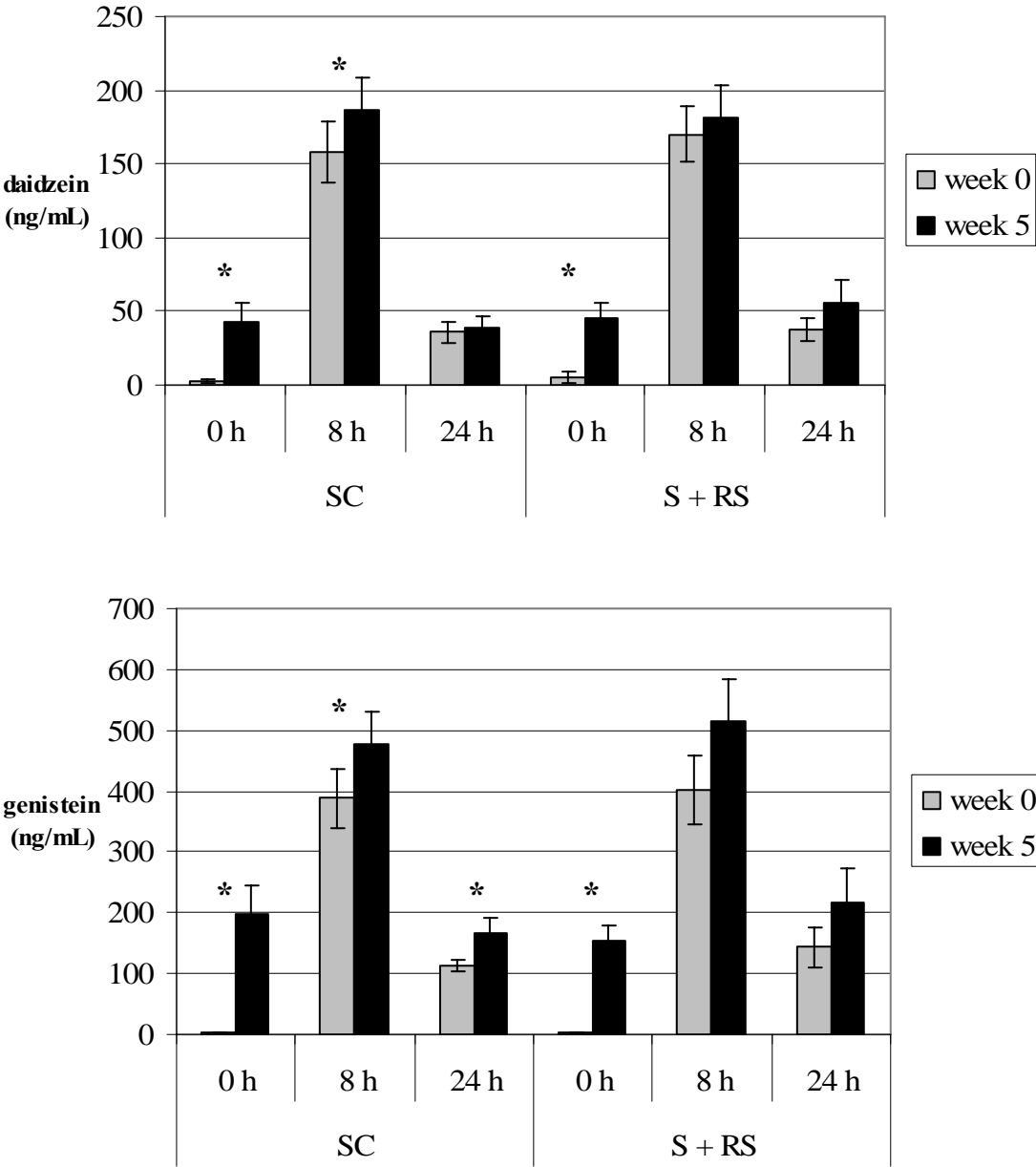


Figure 4.

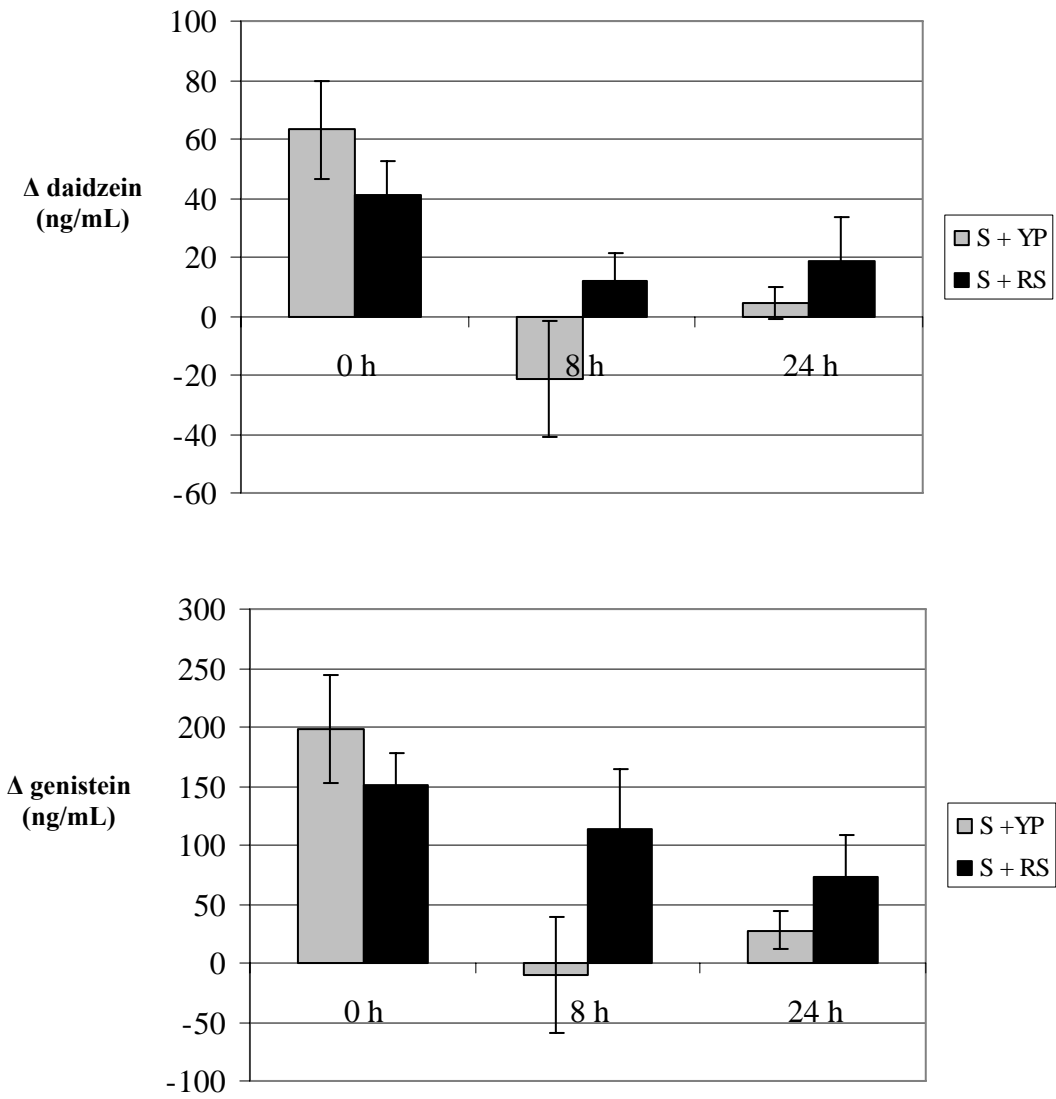


Figure 5.

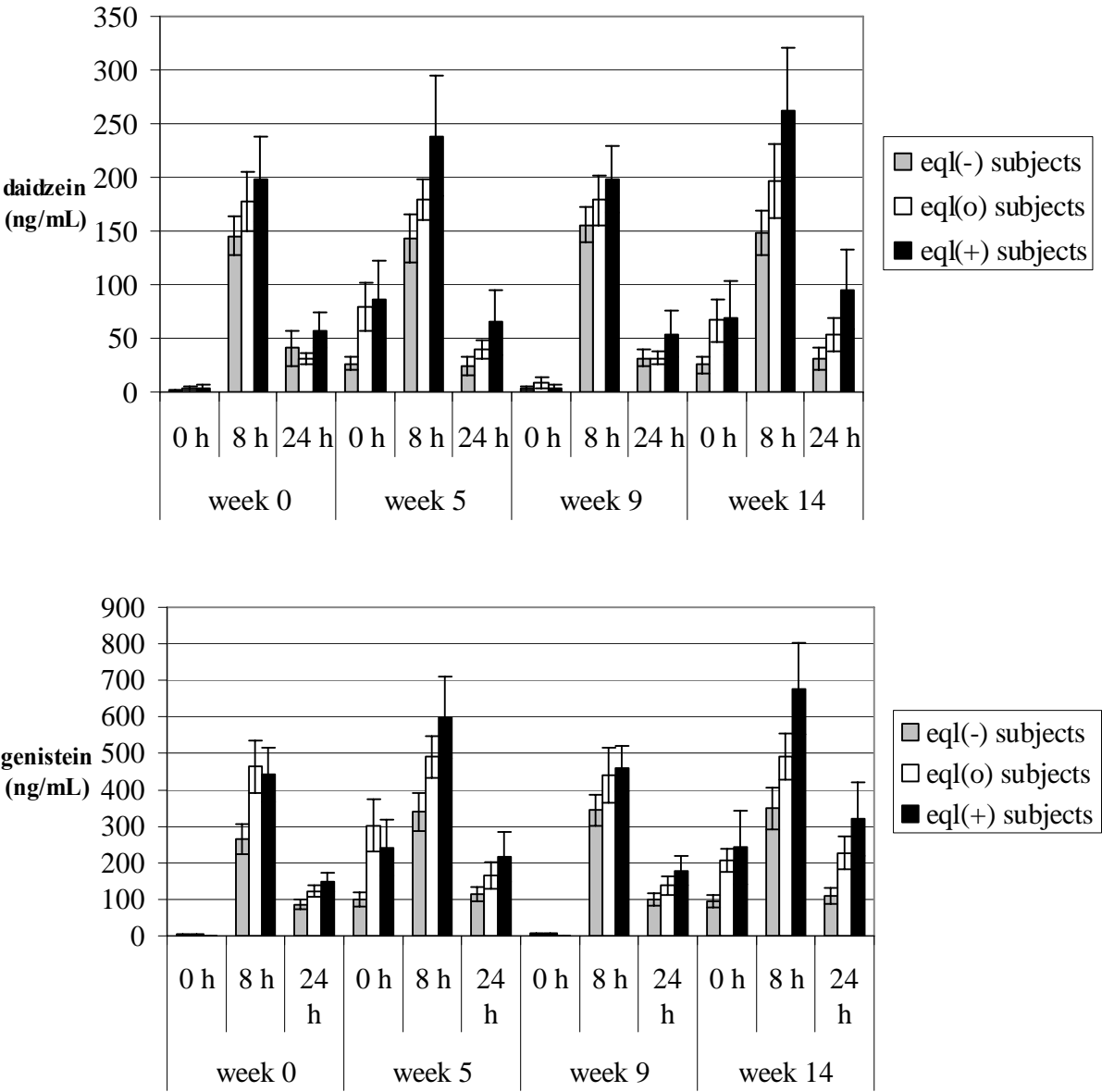


Table 1. Mean plasma daidzein and genistein concentrations after each test soy meal for probiotic cohort

			Plasma sample *			time [†]		week [‡]		week x time [‡]	
		Week	0 h	8 h	24 h	F _{2,14}	p	F _{1,15}	p	F _{2,14}	p
daidzein (ng/mL)	S + YC	0	1.9 ± 1.5 ^a (0 – 23.6)	169 ± 19.3 ^b (65.3 – 333)	38.3 ± 8.5 ^c (0 – 156)	36.615	<0.0001	9.905	0.007	5.393	0.018
		5	45.0 ± 16.8 ^a (0 – 260)	193 ± 22.7 ^b (50.4 – 350)	41.2 ± 12.0 ^a (4.1 – 190)	35.195	<0.0001				
	S + YP	0	3.0 ± 2.2 ^a (0 – 34.2)	176 ± 20.4 ^b (33.0 – 374)	30.3 ± 5.7 ^c (0 – 78.5)	33.805	<0.0001	5.636	0.031	5.646	0.016
		5	66.2 ± 17.8 ^d (4.1 – 237)	154 ± 20.8 ^e (12.2 – 364)	34.5 ± 5.9 ^d (0 – 97.0)	17.035	<0.0001				
genistein (ng/mL)	S + YC	0	3.5 ± 2.4 ^a (0 – 31.9)	412 ± 53.5 ^b (129 – 1009)	134 ± 18.0 ^c (23.0 – 281)	34.022	<0.0001	8.205	0.012	5.641	0.061
		5	158 ± 38.8 ^a (0 – 514)	472 ± 57.8 ^b (149 – 1019)	175 ± 34.4 ^a (32.5 – 487)	23.725	<0.0001				
	S + YP	0	2.8 ± 1.7 ^a (0 – 20.3)	412 ± 64.7 ^b (67.2 – 972)	116 ± 17.8 ^c (14.8 – 295)	21.271	<0.0001	13.810	0.002	8.199	0.004
		5	201 ± 45.5 ^d (46.1 – 682)	401 ± 49.4 ^e (38.8 – 701)	144 ± 24.9 ^d (42.4 – 454)	16.387	<0.0001				

* Values in a row with different superscript letters are significantly different. a, b, c - $p < 0.002$, d – $p < 0.05$, Bonferroni post-hoc analysis.

† One-way ANOVA with repeated measures.

‡ Two-way ANOVA with repeated measures.

Table 2. Mean plasma daidzein and genistein concentrations after each test soy meal for prebiotic cohort

			Plasma sample *			time [†]		week [‡]		week x time [‡]	
		Week	0 h	8 h	24 h	F _{2,11}	p	F _{1,12}	p	F _{2,11}	p
daidzein (ng/mL)	SC	0	2.2 ± 1.2 ^a (0 – 13.1)	158 ± 20.4 ^b (72.9 – 303)	36.0 ± 7.6 ^c (9.1 – 104)	25.893	<0.0001	18.710	0.001	5.401	0.023
		5	43.1 ± 12.9 ^a (0 – 162)	187 ± 21.7 ^b (57.1 – 346)	38.7 ± 7.9 ^a (6.1 – 104)	47.316	<0.0001				
	S + RS	0	4.9 ± 3.9 ^a (0 – 54.7)	170 ± 19.0 ^b (68.3 – 324)	37.5 ± 8.1 ^c (8.4 – 93.4)	39.946	<0.0001	5.355	0.039	4.569	0.036
		5	45.7 ± 10.4 ^a (0 – 133)	182 ± 21.1 ^b (66.0 – 357)	56.3 ± 15.1 ^a (12.3 – 198)	44.655	<0.0001				
genistein (ng/mL)	SC	0	1.7 ± 1.2 ^a (0 – 14.7)	388 ± 49.2 ^b (167 – 744)	113 ± 10.6 ^c (61.8 – 184)	49.971	<0.0001	45.825	0.000	4.900	0.030
		5	197 ± 47.4 ^a (58.3 – 598)	479 ± 51.6 ^b (139 – 800)	167 ± 24.0 ^a (66.6 – 365)	51.883	<0.0001				
	S + RS	0	2.2 ± 1.7 ^a (0 – 24.1)	403 ± 56.3 ^b (129 – 938)	143 ± 33.7 ^c (44.6 – 486)	52.852	<0.0001	14.044	0.003	4.484	0.038
		5	153 ± 27.7 ^a (0 – 355)	516 ± 69.1 ^b (140 – 1010)	215 ± 60.2 ^a (67.5 – 876)	30.018	<0.0001				

* Values in a row with different superscript letters are significantly different. p < 0.02, Bonferroni post-hoc analysis.

[†]One-way ANOVA with repeated measures.

[‡]Two-way ANOVA with repeated measures.

Table 3. Urinary daidzein and genistein concentrations – A. probiotic cohort; B. prebiotic cohort.

			Wk	0 – 24 h*	24 - 48 h	0 – 48 h	F _{1,15} [†]	p	p [‡]	
A.	daidzein (mg)	S + YC	0	12.0 ± 1.0 (5.8 – 21.7)	5.0 ± 1.4 (0.79 – 21.6)	17.0 ± 1.9 (10.1 – 37.6)	0.128	0.726	0.001	
			5	13.2 ± 1.3 (3.2 – 20.4)	4.6 ± 1.4 (0.47 – 23.1)	17.8 ± 2.1 (4.0 – 38.1)			<0.0001	
		S + YP	0	12.0 ± 1.3 (3.8 – 20.1)	4.1 ± 1.1 (0.68 – 16.8)	16.1 ± 2.0 (5.8 – 30.4)	1.576	0.229	<0.0001	
			5	14.2 ± 1.6 (4.0 – 27.8)	4.8 ± 0.91 (1.6 – 15.5)	19.0 ± 2.1 (5.6 – 34.5)			<0.0001	
		genistein (mg)	S + YC	0	6.1 ± 0.68 (2.1 – 11.8)	2.4 ± 0.80 (0 – 11.8)	8.5 ± 1.1 (2.5 – 16.0)	1.242	0.283	0.003
				5	7.6 ± 1.0 (1.5 – 17.1)	3.1 ± 1.5 (0 – 25.3)	10.8 ± 2.2 (2.7 – 38.3)			0.006
S + YP	0		5.8 ± 0.79 (1.3 – 10.9)	2.0 ± 0.91 (0 – 15.1)	7.7 ± 1.3 (1.6 – 20.6)	2.424	0.140	0.005		
	5		7.4 ± 0.97 (0.81 – 16.9)	2.5 ± 0.62 (0 – 7.4)	9.9 ± 1.5 (0.81 – 23.9)			<0.0001		
B.	daidzein (mg)	SC	0	10.4 ± 2.4 (2.8 – 18.3)	2.4 ± 0.45 (0.57 – 18.3)	12.7 ± 1.2 (3.3 – 21.4)	2.650	0.128	<0.0001	
			5	13.5 ± 2.3 (0 – 34.8)	4.9 ± 1.6 (0.69 – 23.0)	18.1 ± 3.2 (2.0 – 57.9)			0.001	
		S + RS	0	10.3 ± 1.4 (3.3 – 21.3)	4.2 ± 1.1 (0.80 – 13.9)	14.2 ± 2.2 (4.3 – 28.9)	0.689	0.421	<0.0001	
			5	12.4 ± 2.1 (4.2 – 39.0)	6.5 ± 2.1 (1.2 – 25.7)	18.5 ± 3.6 (5.9 – 61.3)			0.013	
		genistein (mg)	SC	0	5.7 ± 0.87 (1.4 – 13.6)	0.81 ± 0.14 (0.20 – 2.2)	6.4 ± 0.97 (1.6 – 15.8)	3.781	0.074	<0.0001
				5	7.2 ± 1.3 (0 – 16.7)	1.8 ± 0.52 (0 – 6.2)	8.8 ± 1.6 (0.60 – 22.9)			0.001
			S + RS	0	6.7 ± 1.4 (1.5 – 17.4)	2.6 ± 0.82 (0 – 9.1)	9.1 ± 2.0 (1.5 – 25.2)	0.036	0.853	0.001
				5	7.0 ± 0.84 (2.3 – 13.4)	2.8 ± 0.86 (0.30 – 10.5)	9.7 ± 1.5 (2.6 – 21.9)			<0.0001

*Mean ± SEM (range in parentheses)

[†]Two way ANOVA with repeated measures for effect of dietary period on 0 – 24 h and 24 – 48 h urinary isoflavone excretion.

Table 4. Plasma and urinary equol concentrations for equol-positive subjects (n = 12).

Cohort	Treatment	Wk	Plasma equol (ng/mL)			Urinary equol (mg)	
			0 h	8 h	24 h	0 – 24 h	24 – 48 h
Probiotic	S + YC	0	0	37.5 ± 25.5	46.1 ± 26.9	2.4 ± 2.4	1.1 ± 1.0
		5	49.7 ± 17.7	53.8 ± 32.6	31.7 ± 18.8	1.2 ± 1.1	1.3 ± 1.0
	S + YP	0	26.4 ± 19.6	49.3 ± 30.4	39.7 ± 13.5	1.1 ± 0.47	0.51 ± 0.40
		5	69.9 ± 45.0	60.1 ± 34.8	64.0 ± 31.2	1.9 ± 1.5	2.0 ± 1.2
	SC	0	11.2 ± 11.2	43.6 ± 20.8	31.1 ± 13.3	0.83 ± 0.71	0.28 ± 0.15
		5	37.2 ± 10.5	30.4 ± 16.8	46.9 ± 11.8	1.2 ± 0.68	0.35 ± 0.14
Prebiotic	S + RS	0	6.7 ± 4.5	29.5 ± 17.0	29.8 ± 21.5	0.44 ± 0.43	0.80 ± 0.65
		5	28.2 ± 16.2	44.0 ± 18.6	29.6 ± 10.6	0.85 ± 0.85	0.95 ± 0.54