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Effects of a High Dose, Aglycone-Rich Soy Extract on Prostate-Specific Antigen and Serum Isoflavone Concentrations in Men With Localized Prostate Cancer

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Abstract

The efficacy and safety of consuming high-dose isoflavone supplements for prostate cancer is not clear. A double-blind, placebo controlled, randomized trial was conducted in 53 men with prostate cancer enrolled in an active surveillance program. The treatment group consumed a supplement containing 450 mg genistein, 300 mg daidzein, and other isoflavones daily for 6 mo. Prostate-specific antigen (PSA) was measured in both groups at baseline, 3 mo, and 6 mo, and serum concentrations of genistein, daidzein, and equol were assessed at baseline and 6 mo in the treatment group. Following the completion of the 6-mo double-blind study, men were enrolled in a 6-mo open label trial with the same isoflavone-rich supplement, and PSA was measured at 3 and 6 mo. PSA concentrations did not change in either group after 6 mo or after 12 mo when the open-label study was included. The 6 mo serum concentrations of genistein and daidzein (39.85 and 45.59 $\mu\text{mol/l}$, respectively) were significantly greater than baseline values and substantially higher than levels previously reported in other studies. Equol levels did not change. Although high amounts of aglycone isoflavones may result in significantly elevated serum concentrations of genistein and daidzein, these dietary supplements alone did not lower PSA levels in men with low-volume prostate cancer.

INTRODUCTION

Widespread screening for prostate cancer, combined with an aging population, has substantially increased detection of this cancer in the United States and worldwide.

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Annually, more than 200,000 men are newly diagnosed with prostate cancer in the United States, and more than 30,000 die from the disease (1). Measurement of prostate-specific antigen (PSA) as a screening index is commonly used as a biomarker for prostate cancer; but after diagnosis is confirmed, many cases are deemed insignificant due to their low volume and slow progression. Low-volume prostate cancer and slowly rising PSA do not necessitate aggressive intervention and are best monitored through active surveillance; but in practice, a majority of men seek a more proactive approach (2). Therefore, secondary chemoprevention is an attractive option for men seeking a way to slow disease progress, which at the very least should safely delay the need for surgery, radiation, or hormonal therapy. If secondary chemoprevention is successful and safe, a realistic expectation is that prostate cancer will remain insignificant for the remainder of life, and more aggressive and costly treatment will not be required (3).

A lower incidence of prostate cancer occurs in men from certain Asian countries, such as China and Japan, compared to the incidence in the United States, and the lower incidence may be due in part to a higher intake of isoflavone-rich soyfoods in Asian cultures (4,5). The Japan Collaborative Cohort Study reported serum concentrations of genistein, daidzein, and equol to be dose-dependently related to a reduced prostate cancer risk among 14,105 men followed for 9 to 11 yr (6).

Genistein is a competitive inhibitor of a number of tyrosine kinases, including epidermal growth factor receptor and Src, and can induce apoptosis of PC-3 cells by inhibition of Akt/PKB serine/threonine kinase activity and downstream NF- κ B signaling (7) and through regulation of gene expression associated with cell cycle regulation and angiogenesis (8). Genistein has been shown to substantially inhibit the growth and angiogenesis of LNCaP prostate cancer xenografts (9); delay the progression from benign to malignant tumors by inhibiting osteopontin, an extracellular matrix protein secreted by macrophages infiltrating prostate tumors (10); and downregulate androgen receptor (AR) mRNA expression and growth factor signaling pathways (11) in the TRAMP model. Prostate biopsy tissue from men at high risk of prostate cancer who consumed a soy protein isolate daily for 6 mo also displayed significant suppression of AR expression with no alteration in ER- β expression or circulating hormones (12).

Isoflavones in soyfoods are typically present in their glucoside form, but aglycone isoflavones are absorbed faster and in higher amounts (13). Based on this difference in bioavailability, a novel aglycone isoflavone-rich extract [genistein combined polysaccharide (GCP); Amino Up Chemical Company, Sapporo, Japan] was produced by culturing soybean extract with mycelia from the mushroom *Ganoderma lucidum*, which produces beta-glucosidase and cleaves glucoside forms of isoflavones into the more biologically active aglycone forms (14). The resulting concentration of aglycone isoflavones, genistein (93 mg/g) and daidzein (57 mg/g), is substantially higher than concentrations found in a typical serving of soy milk (0.06 mg/g genistein; 0.04 mg/g daidzein) or tofu (soybean curd; 0.19 mg/g genistein; 0.09 mg/g daidzein) (15). GCP has antiangiogenesis properties (14) and can induce apoptosis in human breast cancer cell xenografts (16). GCP has been shown to reduce the growth of PC-3 prostate cancer xenografts by modulation of molecular markers of p21, a major cell cycle inhibitory protein; p27, a cell cycle inhibitor protein; and p53, a transcription factor that regulates the cell cycle and functions as a tumor suppressor (17). GCP has also been shown to reduce concentrations of cyclin B, a mitotic molecule involved in the regulation of the cell cycle, and to activate PARP, a protein involved in the repair of single-strand DNA nicks and in apoptosis (17). GCP mediates prostate cancer growth inhibition and apoptosis through multiple mechanisms, including molecular mimicry of androgen ablation through downregulation of AR concentrations, as well as by providing a proapoptotic signal through mTOR inhibition, an AR-independent process (18).

Combination treatment of GCP with perifosine, a strong Akt inhibitor, produced a greater degree of inhibition of pAKT activation, increased apoptosis, and decreased clonegenicity than perifosine alone in various prostate cancer cell lines, both with and without androgen receptors (19). GCP has also been shown to enhance the effects of the antimicrotubule taxane docetaxal (20).

A case study reported that 1.5 g/day of GCP taken for 6 wk prior to surgery reduced PSA concentrations from 19.7 to 4.2 ng/ml in a man with histologically confirmed prostate cancer and that no cancer was identified in the tissue sample following radical prostatectomy (21). We previously reported results from an open-label clinical trial utilizing 5 g of GCP (providing 450 mg genistein and 300 mg daidzein) daily in patients with prostate cancer and elevated PSA (22). After 6 mo of GCP intake, all participants who had undergone previous treatment (radical prostatectomy, radiation therapy, or a combination of both) showed increases in their PSA. In contrast, 8 of 13 men following active surveillance showed stable or lowered PSA concentrations. This randomized, double-blind, placebo controlled study was designed to more fully evaluate the effect of GCP as a sole therapy on PSA concentrations in patients engaged in active surveillance as well as to monitor serum isoflavone concentrations.

SUBJECTS AND METHODS

A 2-part study was conducted over a 12-mo period: a double-blind study from 0 to 6 mo and an open-label study from 6 to 12 mo. In the first 6 mo, a placebo-controlled, randomized study was conducted in men who were following an active surveillance protocol for prostate cancer. Sixty-six men with histologically confirmed prostate cancer and a PSA concentration that had been rising for two consecutive readings were enrolled. None had been previously treated for prostate cancer with radiation, surgery, or hormones. All had a PSA concentration between 0.7 and 22.6 ng/ml and a Gleason score of 10 or less; 5 of the men were admitted with PSA concentrations > 10 ng/ml and had been on an active surveillance protocol for at least 12 mo prior to admission to the study. The study was approved by the University of California, Davis, Institutional Review Board; and all participants provided written informed consent before enrollment.

Based on power calculations using data from our previous study (22), 66 men enrolled and were randomly assigned. The study was designed to be able to detect the difference of 60% vs. 25% in the probability of reduction or stabilization of PSA between the treatment and the placebo groups evaluated at 6 mo. The analysis was based on the comparison of proportions at the significant level of 0.05. The power of at least 80% was achieved with 60 patients. The observed effects were smaller than anticipated at the time of the planning. Men in the treatment group consumed 5 g/day of GCP, which contained 450 mg genistein and 300 mg daidzein and other isoflavones; whereas those in the placebo group consumed 5 g/day of inert cellulose. The supplements and placebos were packed into solid white, 500-mg capsules that looked identical and were consumed in divided doses 3 times daily. Compliance was monitored at each clinic visit.

Exclusion criteria included use of isoflavone-rich dietary supplements or intake of 3 or more servings of soy-based foods per day. Other dietary supplements, most commonly multi-vitamin/mineral or antioxidant supplements, were permitted. Men were instructed to maintain their normal diet, exercise, and lifestyle patterns throughout the study. Use of urologic agents, such as finasteride, was not permitted. Compliance to the exclusion criteria, and to the intake of capsules, was assessed at 3 and 6 mo. Common Toxicity Criteria (CTC) Version 2.0 (National Cancer Institute, NIH, 1999) was utilized for assessment of toxicities.

Blood was collected by venipuncture at baseline and after 3 and 6 mo. The serum PSA concentrations were measured according to standard methodology in the Department of Pathology, University of California (UC) Davis Medical Center. Blood for the isoflavone measurements was centrifuged for 15 min at 2,000 rpm and serum separated and stored at -70°C by the UC Davis Cancer Center Specimen Repository. Serum concentrations of genistein, daidzein, and equol were measured at baseline and 6 mo at the University of Alabama at Birmingham using reversed-phase HPLC with an electrospray ionization interface and mass spectrometry by methods described elsewhere (23,24). Testosterone and estradiol measurements were not obtained, since we previously noted no significant changes in these parameters after 6 mo of GCP intake (22). No men were removed from the study for a rising PSA level.

Following the initial 6-mo double-blind study, an open-label study was conducted (Months 6–12). A total of 18 eligible participants in the original placebo group were given 5 gm/day GCP for 6 mo. A total of 17 eligible participants in the initial GCP group also continued taking 5 g/day of GCP from Months 6 to 12. Blood samples were collected at Months 9 and 12, processed as described previously, and measured for PSA concentrations (Fig. 1).

The observed proportion of men with a clinical response was calculated from all participants for which full data sets were available (intent-to-treat analysis was not used), and an exact 5%, 2-sided confidence interval was obtained. In secondary analyses, exact confidence intervals were also obtained for observed proportions for other characterizations of outcome. Finally, the longitudinal patterns of the change in PSA for the duration of the study were characterized using random-effects regression models for repeated measures (25). This approach allowed the use of all available data for each patient, including the pretreatment PSA measurements. The models allowed for both differing pretreatment concentrations and different overall rates of change in PSA by including person-specific random effects that were assumed to follow bivariate normal distribution. Because the PSA measurements had a skewed distribution, they were log-transformed before analysis and the results interpreted as the percentage of change. A term for a participant examined the difference between the prestudy and poststudy concentrations, and interaction terms examined the impact on treatment effect of the baseline Gleason score and treatment subgroup.

RESULTS

Double-Blind Study (0–6 Mo)

Of the 66 men randomized to the study, 30 were enrolled in the placebo group and 36 in the GCP group. Based on CTC version 2.0 criteria, 13 participants discontinued the study prior to 6 mo: 7 had diarrhea (5 in the GCP group, 2 in the control group), 2 were moved to other therapies, 2 had unrelated medical issues, 1 had a skin rash (GCP group) that disappeared on discontinuation of the study, and 1 complained of too many capsules. Thus, 53 participants completed the first 6 mo of the study, with 25 in the placebo and 28 in the GCP groups. The 2 groups were similar in age and Gleason score on admission (Table 1).

Fifty percent of the participants in the GCP group (14/28) and 32% of the placebo group (8/25) showed stabilization or reduction in PSA concentrations after 6 mo (Table 2). The differences were not statistically significant between groups ($P = 0.29$).

Of the 28 participants in the GCP group who completed the first 6-mo intervention, serum samples from 24 were analyzable for concentrations of genistein, daidzein, and equol at baseline and at 6 mo. Of these 24, 21 were self-identified as Caucasians and 3 were self-identified African Americans. Twenty-one men (20 Caucasians and 1 African American) showed substantial increases in serum genistein (at least 100% above baseline) and in serum

daidzein (at least 40% above baseline). Three men (2 African Americans and 1 Caucasian) had low concentrations of genistein at baseline (0.0–0.015 $\mu\text{mol/l}$) and showed minimal or no increase after 6 mo, with final values ranging from 0.014 to 0.426 $\mu\text{mol/l}$. The same 3 men had low concentrations of daidzein at baseline (0.006–0.155 $\mu\text{mol/l}$) and showed minimal or no change after 6 mo, with final values ranging from 0.0 to 0.175 $\mu\text{mol/l}$. Equol, a metabolite of daidzein, was low (0.011–0.019 $\mu\text{mol/l}$) and did not change in 8 participants (6 Caucasians and 2 African Americans), only 1 of whom also showed no change in serum daidzein. Of the 25 participants in the placebo group, serum samples from 22 were analyzable for isoflavone concentrations at baseline and 6 mo. The median values with interquartile (25%–75%) range of isoflavones are shown in Table 3.

Linear mixed model for the $\log(\text{PSA} + 1)$ -transformed response was fitted to the data to assess the association between measured concentrations of genistein, daidzein, and equol with PSA. Group assignment was not included as an independent variable into the model under the assumption that its effect is fully explained by the measurement of the marker panel. A random normally distributed intercept term was used to adjust for repeated measurements on the same subject. None of the changes in the 3 isoflavone concentrations showed significant association with PSA ($P > 0.25$; Table 4). The only significant variable was time, reflecting the pattern of generally increasing PSA concentrations. Backward variable selection procedure removed all variables except Time that remained highly significant ($P < 0.0001$) in the model with $\log(\text{PSA} + 1)$ average within-subject increase per unit time of 0.0096 (SE = 0.0024).

Extended Open-Label Study (Months 6–12)

Following the 6-mo double-blind study, a 6-mo open-label study was conducted. PSA concentrations from men in the original placebo group were assessed at 3 and 6 mo after GCP intake, and PSA concentrations in men from the original GCP group were assessed at 9 and 12 mo following GCP intake. No metastasis of prostate cancer was observed in any participant. Results from the 6-mo open label study are shown in Table 5. No significance differences between groups were noted ($P = 0.915$), although a trend toward increasing PSA in the placebo group was noted (Fig. 2).

Analysis of the trajectory of PSA change from baseline to 12 mo for the placebo (6 mo)-to-GCP (6 mo) and GCP (12 mo) groups distinguished the effect of cumulative dose of GCP. The time from start of GCP was taken as a surrogate of cumulative dose. Following log transformation of PSA values to normalize data and analyze trajectories (26), a linear mixed model with random intercept was used to adjust for repeated measurements on the same subject. A plot of residuals showed no systematic pattern, which indicates the model provides an adequate description of the data.

DISCUSSION

Despite the high serum concentrations of genistein and daidzein among men in the treatment group, no association with changes in PSA concentrations were observed after either 6 or 12 mo, both in terms of absolute changes and when calculating PSA doubling times (27). Our earlier study reported 8 of 13 men with low volume prostate cancer who showed stabilized or reduced PSA concentrations following the same intervention used in this study (22). Although the dosage of isoflavones was identical in both studies, the differences in outcome between our earlier study and this one may be due to the larger sample size or the potentially problematic accuracy of the endpoint measure (PSA) as an indicator of prostate cancer in the study population.

Isoflavone supplementation at lower doses than used in this study have generally shown little or no effect on lowering PSA levels in men with prostate cancer (28). Recently, the intake of 141 mg/day of isoflavones for 1 yr appeared to decrease, but not reverse, the rate of increase in PSA among 20 men with rising PSA levels who had previously been treated for prostate cancer with radiation therapy and/or prostatectomy (29). Consumption of 107 mg/day isoflavones for 6 mo among men at high risk of prostate cancer or with low grade prostate cancer also showed no effect on PSA levels (30). Intake of 80 mg/day of soy isoflavones among men with rising serum PSA who had completed primary therapy for localized disease showed 43% (9/21), with lower PSA after 4 wk (31), but treatment efficacy was not the primary outcome measure of this study, and no longer term data was recorded. A significant reduction of PSA has been reported in one study using an isoflavone-rich supplement as part of an intensive diet and lifestyle intervention (32), and trends toward a reduction in PSA have been noted from some other small trials (33,34).

The relationship between PSA concentration or doubling time and detection of prostate cancer continues to be debated (35,36). PSA screening can lead to overdiagnosis but has also been shown to reduce the rate of death from prostate cancer by 20% (37). PSA concentration may be most useful to monitor the effectiveness of hormonal therapies or as a tumor marker for disease progression after treatment with surgery or radiation. Our study monitored PSA in men who did not receive surgery, radiation, or hormonal therapy, and their disease state and glandular production of PSA may be different than previously treated men whose PSA is also rising. Rather than measurement of PSA concentrations, tissue histopathology and assessment of molecular markers and cell signaling pathways may be more appropriate outcome measures in men with prostate cancer following an active surveillance protocol. However, collection of prostate cancer tissue at the end of our study for molecular studies was not practical given the biopsy costs and the small volume of disease in the prostate gland. This further complicates efforts to define effectiveness of chemopreventive agents in a reasonable time period for a relatively small number of patients as opposed to large cohort studies.

To our knowledge, the mean concentrations of serum genistein and daidzein (42.96 and 51.24 $\mu\text{mol/l}$, respectively) after 6 mo of supplementation are significantly higher than levels achieved in other studies that have used dietary supplements of isoflavones and are in the range of concentrations shown effective in in vitro studies of prostate cancer (38).

High concentrations of isoflavones in the serum or plasma may be clinically important, as isoflavone levels in prostate tissue are about half the plasma concentrations (38). Men with benign prostatic hyperplasia consuming a supplement with 112.5 mg isoflavone aglycone eq/day for 3 days prior to surgery had plasma concentrations ranging from 4 to 27 $\mu\text{mol/l}$ (38). Men with prostate cancer consuming a soy extract rich in genistein (300 mg/day for 28 days, then 600 mg/day for 56 days) had mean peak plasma genistein concentrations of 8.1 $\mu\text{mol/l}$ (39). Lower concentrations have been reported from a 12-wk intake of 80 mg/day isoflavones by 50 men with localized prostate cancer (mean plasma levels of 0.84–1.46 $\mu\text{mol/l}$ for genistein and daidzein, respectively) (40) and from a 1-yr trial among men who had undergone previous treatment for prostate cancer and consumed 141 mg/day isoflavones from soy milk (mean serum levels of 1.94 and 1.84 $\mu\text{mol/l}$ for genistein and daidzein, respectively). If high isoflavone concentrations in prostate tissue influence PSA levels or other molecular determinants of prostate cancer, then achieving high isoflavone concentrations, as we report here, may be clinically useful.

Consistent with other reports (29,38), high interindividual variability of serum isoflavones levels was noted in our study. Despite the high intake of aglycone isoflavones, 3 men showed no change in their initially low concentrations of genistein and daidzein after 6 mo,

suggesting that some men are unresponsive to this supplement; 8 men showed no increase in serum equol, although 7 of them had substantial increases in serum daidzein. Serum equol was in the nanomolar range, similar to levels reported in other studies, and no differences were noted between the GCP and placebo groups.

The high intake of aglycone isoflavones in our study was generally well tolerated, with loose stools the most common complaint from a small number of men in the GCP group. Other human studies that have used lower doses of isoflavones have not reported serious adverse events (31,40,41). Nonetheless, the safety of high intakes of isoflavones in men with prostate cancer is important to consider. A small number of men (2/17) showed elevated liver transaminase levels after consuming 60 mg of a red clover isoflavone extract for 1 yr (34). In animal models, nude mice orthotopically implanted with RM-9 murine prostate cancer cells and then given pure genistein showed increased metastasis to regional lymph nodes compared to a control group (42), but these effects may be due to exposure from genistein alone rather than a mixture of isoflavones used in clinical tests. As might be expected, no metastasis was noted in participants following 6 or 12 mo of supplementation in our study. Future studies should consider longer term follow-up.

A rationale for secondary chemoprevention was an attempt to maintain the benefits of screening, primarily for the early detection of high risk prostate cancer that if left untreated, constitutes a risk to the patient. While seeking to avoid overtreatment of indolent prostate cancer, our study was designed to assess whether GCP would retard the growth of this indolent prostate cancer without significantly impacting quality of life or causing side effects. GCP could not be proven effective as a sole treatment for low volume prostate cancer with this study design, using the prostate-cancer related endpoint PSA as the primary outcome measure. However, a combination approach to secondary chemoprevention merits further exploration. The intake of highly bioavailable isoflavone supplements, consumed in high amounts to raise serum levels to concentrations shown effective in in vitro studies, may still hold promise, particularly when combined with other cancer therapies.

CONCLUSION

Serum concentrations of genistein and daidzein can increase to micromolar concentrations after high intake of an aglycone isoflavone supplement for 6 mo in men with low volume prostate cancer, although some men showed no elevation of genistein, daidzein, or equol. The micromolar concentrations of plasma isoflavones did not correlate with changes in PSA concentrations after 6 or 12 mo in the population group studied.

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FIG. 1.
Design for double blind (0–6 mo) and open label (6–12 mo) studies. GCP, genistein combined polysaccharide; PSA, prostate-specific antigen.

FIG. 2.
Changes in log (PSA+1) over 12 months (placebo group switched to GCP after 6 months).
PSA, prostate-specific antigen; GCP, genistein combined polysaccharide.

TABLE 1

Participant demographics for double-blind study^a

Group	N	Mean Age, yr (SE)	Age Range (SD)	Gleason Score				Baseline PSA Range (SD)
				2-4	5-6	7	8-10	
GCP	28	70.5 (1.8)	49.8-85.4 (9.3)	0	25	2	1	0.7-16.2 (3.7)
Placebo	25	68.6 (1.5)	51.5-79.3 (7.3)	2	21	2	0	1.1-22.6 (4.7)

^aAbbreviations are as follows: PSA, prostate-specific antigen; GCP, genistein combined polysaccharide.

TABLE 2Change in PSA response by treatment group in double-blind study^a

Response	GCP^b	Placebo^c
Stable/reduced (%)	14 (50)	8 (32)
>20% reduction	3	1
Increased (%)	14 (50)	17 (68)
>20% increase	6	7

^a Abbreviations are as follows: PSA, prostate-specific antigen; GCP, genistein combined polysaccharide.^b *N* = 28.^c *N* = 25.

TABLE 3Mean/median serum isoflavone concentrations in double-blind study^a

Group	Placebo		GCP	
Isoflavone/Time	Baseline	6 Months	Baseline	6 Months
Genistein (μmol/l)	3.05/0.17	2.57/0.48	1.23/0.046	42.96/38.94
Range	0–33.18	0.057–23.70	0–7.05	0.014–99.25
Quartiles	0.064–0.92	0.15–2.81	0.0099–1.23	27.07–60.06
Interquartile Range	0.86	2.66	1.23	32.99
95% confidence interval	(0–6.26)/(0.064–0.96)	(0.41–4.77)/(0.15–2.90)	(0.34–2.12)/(0.011–1.12)	(31.90–54.02)/(27.33–59.75)
Daidzein (μmol/l)	3.49/0.19	2.70/0.71	2.06/0.14	51.24/51.71
Range	0.012–23.85	0.027–13.23	0–9.18	0–123
Quartiles	0.07–1.75	0.091–3.42	0.011–2.69	30.55–61.88
Interquartile Range	1.68	3.33	2.68	31.33
95% confidence interval	(0.52,6.45)/(0.071,2.14)	(1.15,4.25)/(0.090,3.47)	(0.75,3.36)/(0.011,2.32)	(38.35,64.13)/(30.98,59.75)
Equol (μmol/l)	0.070/0.020	0.048/0.017	0.030/0.018	0.049/0.020
Range	0.011–0.73	0.010–0.54	0.011–0.17	0.011–3.00
Quartiles	0.018–0.030	0.014–0.022	0.015–0.021	0.015–0.20
Interquartile Range	0.013	0.0077	0.0062	0.19
95% confidence interval	(0.0034–0.14)/(0.018–0.031)	(0.0011–0.096)/(0.014–0.022)	(0.013–0.048)/(0.015–0.021)	(0.11–0.86)/(0.015–0.089)

^a Abbreviation is as follows: GCP, genistein combined polysaccharide.

TABLE 4Relationship of isoflavones to PSA levels^a

	Value	SE	df	P Value
(Intercept)	1.8834	0.0815	135	0.0000
Genistein	0.0021	0.0171	135	0.9035
Daidzein	−0.0020	0.0017	135	0.2521
Equol	0.01388	0.0435	135	0.7500
Time	0.0107	0.0031	135	0.0008

^a Abbreviation is as follows: PSA, prostate-specific antigen.

TABLE 5Change in PSA response by original treatment group in extended open-label trial^a

	Continuing GCP ^b	New GCP(Originally Placebo) ^c
Stable/reduced (%)	5 (29)	5 (28)
>20% reduction	0	2
Increased (%)	12 (71)	13 (72)
>20% increase	8	7

^a Abbreviation is as follows: GCP, genistein combined polysaccha-ride.^b *N* = 17.^c *N* = 18.