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Serum Isoflavone Concentrations and Equol-Producing Intestinal Flora in Prostate Cancer in Japanese-American in Hawaii

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Abstract

Studies have reported that soy isoflavones and the ability to produce equol may play a role in the prevention of prostate cancer (PCa). To further investigate this relationship, we evaluated Japanese-Americans (JA) and Caucasians (CA) in Hawaii with and without PCa, with regards to dietary soy intake, serum isoflavone levels, and the presence of equol-producing *Slackia* sp. in the intestine. The serum levels of isoflavone were determined in 65 JA (34 PCa, 31 controls) and 68 CA (34 PCa, 34 controls). All completed a lifetime dietary soy consumption survey. The serum levels of genistein and o-DMA were significantly higher in JA ($p = 0.04$). Daidzein and isoflavone levels trended higher in JA, but no differences were noted in dihydrodaidzein (DHD) and equol levels. There were no differences in serum isoflavone levels between PCa patients and controls for both JA and CA. Only 8.6% stool samples were positive for equol-producing *Slackia* sp., a rate much lower than seen in Japan. JA reported a higher intake of soy than CA. The high soy consumption and isoflavone levels in JA may be related to the lower incidence of PCa relative to CA in Hawaii. The near absence of equol producers in Hawaii JA may contribute to their higher incidence of PCa compared to Japan Japanese. These results suggest that both soy isoflavone levels and equol production may be inversely associated with PCa risk.

Keywords: Prostate cancer; Isoflavones; Equol; Intestinal bacteria; Japanese-American men

Abbreviations

PSA: Prostate Specific Antigen

Introduction

Prostate cancer is the most commonly diagnosed cancer in Western countries in men and the second leading cause of cancer death among men in the United States [1]. However, the incidence of prostate cancer is markedly different among various races and countries. Asians, especially Japanese and Chinese have incidence rates lower than Caucasians [2]. The incidence in Japanese-Americans is intermediate between Japanese living in Japan and Caucasians in the US [3]. The higher incidence among Japanese born in the US compared with Japanese born in Japan suggests that Japanese migrants have some lifestyle characteristics that make their risk for prostate cancer higher than that of Japanese in Japan. Diet is one of the important environmental factors that change after migration to the West. Several studies indicate that traditional Japanese diet may decrease the risk of prostate cancer. Recently, several studies have reported that soy isoflavones and the ability to produce equol, an active derivative of the soy isoflavone daidzein, may play a role in the prevention of prostate cancer [4-7]. Isoflavones such as genistein and daidzein exhibit anticarcinogenic properties and weak estrogenic activity. Recent studies indicate that not only genistein and daidzein, but equol, which is metabolized from daidzein by intestinal microbiota, acts as an antiandrogen and inhibits the development of prostate cancer [8]. We identified an equol-producing bacterium, *Slackia* sp. strain NATTS in fecal cultures [9]. The prevalence of the equol-producing *Slackia* sp. including of *Slacika* sp. strain NATTS [9], *Slackia isoflavoniconvertens* JCM 16137^T [10] and *Slackia* sp. TM30 [11] in Japanese adults was examined by the Yakult Intestinal Flora-SCAN (YIF-SCAN®) based on 16S rRNA-targeted RT-quantitative PCR (RT-qPCR) technology, which was found to be 40%. We confirmed that it has the ability to convert daidzein in soy isoflavones into equol with high efficiency. Our study is starting to make clear that equol-producing *Slackia* sp. clarify that NATTS have not been detected in all humans and that there is a

noticeable difference in serum equol concentrations measured in so-called equol-producers, depending on the country or region [12]. To further investigate the relationship between the risk of prostate cancer and dietary isoflavones, we evaluated Japanese-Americans (JA) and Caucasians (CA) in Hawaii, with and without prostate cancer, with regards to dietary soy intake, serum isoflavone levels, and the presence of equol-producing *Slackia* sp. in the stool.

Patients and Methods

A total of 65 Japanese-American men (34 prostate cancer patients and 31 cancer-free controls) and 68 Caucasian men (34 prostate cancer patients and 34 cancer-free controls) were enrolled. All cancer patients had histologically confirmed prostate cancer; the control group consisted of age-matched (± 5 years) and geographically-matched cancer-free male subjects. All prostate cancer patients and controls were residents of Hawaii. We obtained the patients' race, age and cancer status (PSA, stage, Gleason score) from hospital records (Table 1). This study was approved by the institutional review board of the Queen's Medical Center in Honolulu, Hawaii, the study design was fully explained to all subjects, and written informed consent was obtained.

Cases	Caucasian (CA) n = 68		Japanese (JA) n = 65		p value
	Cases (Pca)	Controls	Cases (Pca)	Controls	
n	34	34	34	31	
Age					
Mean ± SD	73. 5 ± 10.1	70.2 ± 7.8	71.8 ± 8.8	69.8 ± 8.9	CA vs. JA 0.53*
Median (Range)	72 (56 ~ 93)	70 (57 ~ 87)	71.5 (55 ~ 91)	69 (56 ~ 92)	Pca vs. Cont 0.09*
PSA					
Mean ± SD	7.5 ± 5.1	-	77.8 ± 409.3	-	0.32*
Median (Range)	6.3 (0.45 ~ 25)	-	7 (1.9 ~ 2394)	-	
Stage					
T1c	25 (74%)	-	19 (56%)	-	0.33**
≥ T2a	9 (26%)	-	15 (44%)	-	
Gleason score					
Median (Range)	7 (6 ~ 9)		7 (4 ~ 8)		0.87**
≤ 6	16 (47%)	-	15 (44%)	-	
7	12 (35%)	-	14 (41%)	-	
≥ 8	6 (18%)	-	5 (15%)	-	

Table 1: Demography of cases and controls, *Wilcoxon's test, **Chi-square test.

Isoflavone level analysis

Fasting blood samples were drawn from all patients and controls. The serum was separated and stored at -70°C. Serum levels of daidzein, genistein, glycitein, dihydrodaidzein (DHD), equol, o-desmethylangolensin (o-DMA) and total isoflavone were measured by reverse-phase high performance liquid chromatography-multiple reaction monitoring-mass spectrometry (HPLC-MS) [13]. We show the limit of detection (LOD) and quantification (LOQ) of our method in Table 2.

Isoflavone	LOD (ng/ml)	LOQ (ng/ml)
Daidzein	0.1	1
Dihydrodaidzein	0.1	1
Equol	2.5	25
O-desmethylangolensin	1	10
Genistein	0.1	1
Glycitein	0.1	1

Table 2: LOD and LOQ of isoflavones, LOD: Limit of Detection; LOQ: Limit of Quantification.

Equol-producing ability

Small amounts (100 mg) of fresh stool sample from subjects were placed in containers with 2 ml of RNA stabilization regent, RNAlater™ (Ambion Inc., Austin, TX USA). These samples were stored at 4°C until use. We extracted total RNA fractions from stools using a method that has also been described previously and measured the count of equol-producing *Slackia* sp. using the YIF-SCAN® [9].

Soy intake analysis

To evaluate the intake of isoflavones in JA and CA in Hawaii, the subjects completed a brief questionnaire about dietary habits, current medications, and general health status. The questions addressed total consumption of soy food and individual consumption of soybeans, miso, tofu, soybean milk, natto, and other soy products (textured vegetable protein, etc.). Soy intake was divided into three categories: low (< 0.1 servings/day), medium (0.1-0.43 servings/day), and high (> 0.43 servings/day).

Statistical methods

The Wilcoxon's test (nonparametric) was used to compare the mean values of isoflavones and patients' background (age, PSA, Gleason score) between two groups (JA and CA in Hawaii, with and without prostate cancer). The chi-square test was used to compare the numbers of patients with and without equol-producing *Slackia* sp., and to assess soy intake. Multivariate logistic regression analysis was used to evaluate the association between total isoflavone levels, age, race and prostate cancer risk. All analyses were performed using JMP version 12 (SAS Institute, Inc) and a $p < 0.05$ was considered statistically significant.

Results

Table 1 summarizes the demography prostate cancer patients and controls. No significant difference in age was detected between CA and JA men. There was no significant difference in PSA, clinical stage and Gleason score between prostate cancer patients and controls.

The serum isoflavone level

Caucasian (CA) vs. Japanese-American (JA): Serum level of genistein and o-DMA were significantly higher in JA (Genistein: $p = 0.04$; o-DMA: $p = 0.04$) and there was a trend for higher daidzein levels in JA ($p = 0.09$), whereas glycitein levels were significantly higher in CA ($p = 0.03$). No differences were noted in DHD and equol levels between CA and JA. Total isoflavone levels trended higher in JA ($p = 0.07$) (Table 3).

Serum Isoflavone level (ng/ml)	Mean / Median	CA (n = 68)	JA (n = 65)	p value*
Total				
isoflavone	Mean ± SD	76.340 ± 104.855	135.093 ± 245.597	0.07
	Median	41.03 (14.62-717.3)	56.895 (10.0-1658.6)	
Daidzein	Mean ± SD	13.949 ± 34.744	33.297 ± 84.031	0.08
	Median	0 (0-179.99)	4.19 (0-600.19)	
Genistein	Mean ± SD	21.416 ± 54.021	61.770 ± 150.878	0.042**
	Median	4.9 (0-339.42)	13.29 (0-931.69)	
Glycitein	Mean ± SD	35.736 ± 10.410	30.674 ± 15.502	0.029**
	Median	34.69 (14.62-63.23)	29.85 (0-82.74)	
DHD	Mean ± SD	1.747 ± 5.935	1.431 ± 6.151	0.76
	Median	0 (0-34.15)	0 (0-43.93)	
Equol	Mean ± SD	3.493 ± 19.240	5.906 ± 26.813	0.55
	Median	0 (0-145)	0 (0-180)	
o-DMA	Mean ± SD	0	2.013 ± 7.949	0.040**
	Median	0	0 (0-37.58)	

Table 3: Serum Isoflavone level, Caucasian vs. Japanese-American, *Wilcoxon's test, **Statistically significant ($p < 0.05$).

Cancer cases vs. controls: There were no differences in serum isoflavone levels between prostate cancer cases and controls for both JA and CA (Table 4). Also, multivariate logistic regression analysis didn't show any significant association between total isoflavone levels ($p = 0.99$), age ($p = 0.75$), race ($p = 0.12$) and prostate cancer risk.

Serum Isoflavone level (ng/ml)	Mean / Median	Cases (n = 68)	Control (n = 65)	p value*
Total				
isoflavone	Mean ± SD	107.910 ± 225.455	102.133 ± 144.376	0.86

	Median	47.52 (10.6-1658.6)	44.26 (13.52-717.34)	
	Mean ± SD	24.408 ± 78.325	22.379 ± 46.415	0.85
Daidzein	Median	0 (0-600.19)	0 (0-208.08)	
	Mean ± SD	44.565 ± 138.419	37.645 ± 82.263	0.72
Genistein	Median	6.9 (0-931.69)	7.07 (0-512.68)	
	Mean ± SD	32.698 ± 214.934	33.837 ± 11.935	0.62
Glycitein	Median	31.71(0-82.79)	34.64 (4.92-65.81)	
	Mean ± SD	1.959 ± 7.483	1.221 ± 4.059	0.48
DHD	Median	0 (0-43.93)	0 (0-26.89)	
	Mean ± SD	3.273 ± 15.744	6.092 ± 3.273	0.48
Equol	Median	0 (0-101)	0 (0-180)	
	Mean ± SD	1.008 ± 5.748	0.959 ± 5.542	0.96
o-DMA	Median	0 (0-34.36)	0 (0-37.58)	

Table 4: Serum Isoflavone level, Cases vs. Control, *Wilcoxon's test.

Equol producer: Only 7 subjects (5.3%) were positive for equol in all subjects. No significant difference was noted between CA and JA (Table 5).

Equol-producing *Slackia* sp., detected from patients fecal samples

Of 133 enrolled patients, 120 cases were able to be measured for equol-producing *Slackia* sp., detected from patients' fecal samples. Only 10 of the 120 stool samples were positive for equol-producing *Slackia* sp. (8.3%). No significant difference was noted between JA and CA (Chi-square test: p = 0.11) (Table 6).

Soy questionnaire

Japanese cases reported higher soy intake than Caucasian cases (P < 0.0001). Fifty eight Japanese patients compared with 26 Caucasian patients consumed at least an intermediate amount of soy (> 0.1 servings/day) (Figure 1). JA showed a higher intake of soy than CA in Hawaii. There was no difference in soy intake between prostate cancer cases and controls.

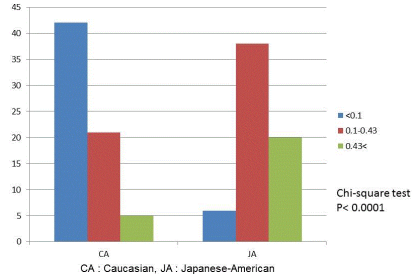

Figure 1: Life time soy intake per ethnicity (Servings/day). Caucasian vs. Japanese-American.

Discussion

As a result of our study, we know that JA higher serum isoflavone concentration than CA. Especially; genistein was significantly higher in JA. Several studies suggest that genistein is a promising agent for cancer chemoprevention and / or treatment [14]. It may be related to higher intake of soy in JA as our diet survey noted. We previously investigated the isoflavone concentration in Japanese in Japan. JA showed lower level isoflavone concentrations than Japanese in Japan as previously reported [15,16]. These results indicate that moderate intake of isoflavones in JA may be related to the intermediate incidence of prostate cancer in JA between Japanese living in Japan and CA in the US. Interestingly, our study showed that only glycitein levels were significantly higher in CA. Different soy product intake may be related to the higher glycitein level in CA. The influence of higher glycitein levels in CA and its relationship to the incidence of prostate cancer is not well understood.

In addition, surprisingly only 7 patients (5.3%) in Hawaii showed equol positivity. Our LOD for equol was higher than other studies, thus we underestimated the percentage of equol producers compared to other studies [16,17]. But our lower equol positive rate was consistent with the low positive rate (8.3%) of equol-producing *Slackia* sp. in patients in Hawaii. Our results showed that only one patient was both equol positive and equol-producing *Slackia* sp. positive. Equol is converted from daidzein, but serum daidzein levels were not detected or were markedly low in equol-producing *Slackia* sp. positive cases as shown in Table 6, whereas daidzein levels were higher among equol positive cases as shown in Table 5. This may explain why equol was negative in the cases that were equol-producing *Slackia* sp. positive.

Race	Ca / Con	age	*Slackia* sp. count*	total isoflavone	Daidzein	Genistein	Glycitein	DHD	Equol	o-DMA	Soy intake
CA	Con	81	6.9	83.7	0	34.6	16.1	0	33	0	Medium
CA	Con	71	0	717.3	180	339.4	44.8	8.2	145	0	Low
CA	Pca	80	0	181.6	48	36.9	30.1	10.6	56	0	High
JA	Con	76	0	250.5	17.8	155.1	39.6	0	38	0	Medium
JA	Con	82	0	553.7	208.1	99.9	65.8	0	180	0	High
JA	Pca	61	0	71.6	1.7	10.9	0	0	59	0	Medium
JA	Pca	80	0	146.5	24	21.5	0	0	101	0	High

Table 5: Characteristics of equol positive subjects (n = CA: 68cases, JA: 65 cases), *Equol-producing *Slackia* sp. count. (log10 cells/g feces) CA: Caucasian, JA: Japanese-American, Con: Control, Pca: Prostate Cancer.

We investigated only one group of the equol-producing bacterium. There have been several studies investigating the isolation of bacteria with the ability to convert equol in human feces. Setechell and Clerici described more than 10 intestinal bacteria that can convert daidzein into equol in a review of equol. We suspect that the patients with undetectable counts of equol-producing *Slackia* sp. had other types of intestinal bacteria that can convert daidzein into equol. Akaza et al. reported that the percentage of equol producers among patients and controls was 29% and 46% in Japanese in Japan [12]. Equol-producing *Slackia* sp. was detected from feces from Japanese adults in Japan and the prevalence of equol-producing *Slackia* sp.in Japanese in Japan was found to be 40% [9]. These studies indicated that equol-producing *Slackia* sp. must be the major bacteria species that are equol producers in Japanese in Japan. Considering these results, the proportion of JA who had equol-producing *Slackia* sp. and equol decreased and the makeup of the intestinal microbiota may have changed due to the different environment found in Hawaii and be responsible for the increase in prostate cancer incidence among Japanese Americans in Hawaii. We need to elucidate the reason why Japanese Americans lost their equol producing ability, including equol-producing *Slackia* sp., in Hawaii.

Race	Ca/Con	Age	*Slackia* sp. count*	total isoflavone	Daidzein	Genistein	Glycitein	DHD	Equol	o-DMA	Soy intake
CA	Con	65	6.2	36.9	0	0	36.9	0	0	0	Low
CA	Con	58	6.9	41	0	6.1	34.9	0	0	0	Medium
CA	Con	81	6.9	83.7	0	34.6	16.1	0	33	0	Medium
CA	Con	69	7.2	33.6	0	0	33.6	0	0	0	Low
CA	Con	70	7.6	46.2	0	5.7	40.5	0	0	0	Low
CA	Pca	78	7.4	40	0	0	40	0	0	0	Low
CA	Pca	86	7.6	39	7.3	3.3	28.4	0	0	0	Low
CA	Pca	60	8.1	36.7	0	2.8	33.9	0	0	0	Low
JA	Con	76	7.8	24.9	0	0	24.9	0	0	0	Low
JA	Pca	66	4	38.1	3.8	11.6	22.7	0	0	0	Low

Table 6: Characteristics of equol-producing *Slackia* sp. positive subjects (n = CA: 60 cases, JA: 60 cases), *Equol-producing *Slackia* sp. count. (log10 cells/g feces) CA: Caucasian, JA: Japanese-American, Con: Control, Pca: Prostate Cancer.

Several studies reported an association between serum isoflavone levels and prostate cancer risk. But the results are still controversial. For a Japanese case-control study, Ozasa et al. reported that high serum levels of isoflavones were associated with a reduced risk of prostate cancer [18]. Also, Kurahashi et al. found that the highest levels of genistein and equol were significantly associated with a decreased risk of localized prostate cancer in a case-control study [7]. However, Sugiyama et al. reported that counts of equol-producing *Slackia* sp. correlated with serum concentrations of equol both in prostate cancer cases and controls, but serum isoflavone concentrations were not associated with prostate cancer risk in the Japanese population [19].

Although a few previous studies from US and other Asian countries showed that lower serum isoflavone concentrations were associated with an increased risk of prostate cancer [20,21], several other studies including European countries did not show any correlation between serum isoflavone levels and an increased prostate cancer risk [22,23].

Similarly, serum isoflavone concentrations did not correlate with the risk of prostate cancer in our study. But our study had several limitations. The small sample size of this study reduced the statistical power necessary to identify risk factors for prostate cancer. Also, the level of serum isoflavones measured was a reflection of recent dietary intake. Soy intake may vary over a lifetime. Isoflavone levels, as a reflection of recent dietary intake, which may vary, may not accurately reflect lifetime soy intake and its effect on prostate cancer development.

In conclusion, Japanese-Americans showed higher serum isoflavone levels and significantly higher soy food intake than Caucasians in Hawaii. The incidence of equol-producers and counts of equol-producing *Slackia* sp., in Japanese Americans in Hawaii, were much lower than that seen in previous studies of Japanese in Japan. Serum isoflavone concentrations were not associated with an increased risk of prostate cancer in our study. Nevertheless, high soy consumption and serum isoflavone levels in Japanese Americans may be related to the lower incidence of prostate cancer relative to Caucasians in Hawaii. The near absence of equol producers in Hawaii's Japanese Americans may contribute to their higher incidence of prostate cancer relative to Japanese in Japan.

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Disclosure Statement

The authors have no conflict of interest.

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