Dietary intake of isoflavones and coumestrol and the risk of prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Novelty and Impact: Although phytoestrogen intake influences prostate carcinogenesis in experimental studies, little is known about whether intake of these bioactive compounds is associated with prostate cancer risk in human populations. The authors investigated this association among 27,004 participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Our study is the first to reveal a positive association of dietary intake of total isoflavones, genistein, daidzein, and glycitein with risk of advance, but not total, prostate cancer.

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Abstract

Experimental studies have revealed that phytoestrogens may modulate the risk of certain sites of cancer due to their structural similarity to 17β-estradiol. The present study investigates whether intake of these compounds may influence prostate cancer risk in human populations. During a median follow up of 11.5 years, 2,598 cases of prostate cancer (including 287 advanced cases) have been identified among 27,004 men in the intervention arm of the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Dietary intake of phytoestrogens (excluding lignans) was assessed with a food frequency questionnaire. Cox proportional hazards regression analysis was performed to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for dietary isoflavones and coumestrol in relation to prostate cancer risk. After adjustment for confounders, an increased risk of advanced prostate cancer [HR (95% CI) for quintile (Q) 5 vs. Q1] was found for the dietary intake of total isoflavones [1.91 (1.25-2.92)], genistein [1.51 (1.02-2.22), daidzein [1.80 (1.18-2.75), and glycitein [1.67 (1.15- 2.43)] (p-trend for all associations ≤0.05). For example, HR (95% CI) for comparing the Q2, Q3, Q4, and Q5 with Q1 of daidzein intake was 1.45 (0.93-2.25), 1.65 (1.07-2.54), 1.73 (1.13-2.66), and 1.80 (1.18-2.75), respectively (p-trend: 0.013). No statistically significant associations were observed between the intake of total isoflavones and individual phytoestrogens and non-advanced and total prostate cancer after adjustment for confounders. This study revealed that dietary intake of isoflavones was associated with an elevated risk of advanced prostate cancer.

INTRODUCTION

Prostate cancer is one of the most common cancers in Western countries and its incidence has been rapidly increasing in Asian countries during the last several decades [1, 2]. Age, ethnicity, and family history are the only established, but non-modifiable, risk factors for this malignancy [3]. Although migrant and temporal trend studies suggest that diet may play a role in prostate cancer etiology [2], few specific nutrients that appear to alter its occurrence have been identified.

Phytoestrogens are a family of estrogen-like bioactive compounds found in plants [4]. Individual phytoestrogens derived from dietary sources are classified into three major categories: isoflavones (e.g. genistein, daidzein, glycitein, formononetin, and biochanin A), lignans (e.g. matairesinol and seco-isolariciresinol), and coumestans (e.g. coumestrol) [5]. While the main food sources of isoflavones are soybeans, kudzu root, American groundnuts, those of lignans are flaxseed, green tea, and strawberries. Coumestans are abundant in legumes, clover, and soybean sprouts [6]. Experimental studies have revealed that phytoestrogen intake may modulate the risk of certain types of cancer [7] due to their structural similarity to 17β-estradiol [8] and the resulting competitive binding to estrogen receptors [9]. Of particular relevance to prostate carcinogenesis is that rats fed a phytoestrogen-rich diet experienced a reduction in serum testosterone concentrations [10]. Given that high serum testosterone was observed to be a risk factor for prostate cancer in some, although not all, epidemiologic studies [11, 12], these animal studies suggest that high intake of phytoestrogens may be associated with a reduced risk of this disease. Conversely, another study in mice showed that genistein enhances the proliferation and metastasis of prostate cancer cells derived from human surgical samples, suggesting that phytoestrogens may play a role in the development of aggressive prostate tumor [13].

Despite biological plausibility, it still remains unknown whether phytoestrogen intake may influence prostate cancer risk in human populations. A few studies examining this association produced mixed results [14-16]. Phytoestrogen intake is lower and has a smaller between-person variation in Western countries than in East Asia countries (e.g. China and Japan) [17, 18]. However, reported significant, inverse association of adolescent phytoestrogen intake with postmenopausal breast cancer in Canadian women [19] suggests that variations in phytoestrogen intake among individuals in Western countries are sufficient for investigators to examine the effects of these bioactive compounds on human health and disease. To date, few prospective cohort studies have evaluated whether phytoestrogen intake is associated with the occurrence of total and advanced prostate cancers in a large sample of the U.S. population [16]. Therefore, the present study investigates this hypothesis using data previously collected from participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO).

SUBJECTS AND METHODS

Study Population

Approximately 155,000 men and women were enrolled to the PLCO from 10 screening centers across the U.S. between November 1993 and July 2001. In the present study, only men (n=76,683) were considered because the outcome of interest was prostate cancer. Subjects were excluded if they did not complete the baseline questionnaire (n=892), were not asked to complete the Dietary Questionnaire (DQX) at baseline (n=38,343, all enrolled to the control arm), completed the DQX but determined to be invalid [eight or more missing responses, extreme energy

intakes (top or bottom 1%), or missing the DQX completion date)] (n=6,594), had a history of cancer (n=705), or were not followed up after the enrollment (n=52). Subjects who were outliers for total isoflavone intake (defined as value that falls above the sum of third quartile and twice the interquartile range) were also excluded (n = 2,287). Finally, cases of prostate cancer diagnosed within two years of enrollment into the study were excluded to eliminate preclinical disease at baseline (n = 806). After all above exclusions, a total of 27,004 men in the PLCO met the eligibility criteria of the present study. As control arm participants were not offered a food frequency questionnaire (FFQ) (i.e. the Dietary Questionnaire), all subjects included in the present analysis were in the intervention arm.

Men in the intervention arm were offered annual prostate-specific antigen (PSA) blood test and digital rectal exam (DRE) for screening prostate cancer during their first 6 years of participation in the trial and follow-up continued for at least 7 additional years. A positive test is considered to be either a PSA test >4 ng/mL or one of the following signs from a DRE: nodularity, induration, asymmetry, or a loss of anatomic landmarks. Participants with a positive screening result received a subsequent diagnostic evaluation (e.g. prostate biopsy). During a median follow up of 11.5 years, 2,598 cases of prostate cancer (including 287 advanced cases) have been identified from the 27,004 eligible men. Advanced prostate cancer cases were defined as patients who were diagnosed with stage II (Gleason score of ≥8), stage III, or stage IV of the disease, or died from it [20].

Data Collection

PLCO participants in the intervention arm were asked to complete a sex-specific baseline questionnaire and the FFQ mentioned above. The vast majority (96.8%) of the PLCO participants completed the baseline questionnaire that solicits information on age, race, body mass index (BMI, kg/m^2), marital status, education level, physical activity, cigarette smoking, family history of prostate and other cancers, aspirin use, ibuprofen use, and personal history of vasectomy [20].

The FFQ was offered to the participants in the intervention arm at the time of randomization into the PLCO. The FFQ is a 137-item food frequency questionnaire developed to assess usual diet and alcohol consumption during the past year, and this dietary assessment instrument has not been specifically validated for isoflavones. It also contained questions on use of vitamins and other dietary supplements. Dietary intake of energy and nutrients were calculated by multiplying the amount of energy and nutrients in a standard portion size of each food item by the reported frequency of consumption and summing over all food items. Individual phytoestrogens considered for this study included genistein, daidzein, glycitein, formononetin, biochanin A, and coumestrol. These six individual phytoestrogens represent all kinds of the phytoestrogens with data available from the PLCO. Dietary intake of lignans was not estimated from the FFQs completed by PLCO participants because lignan values are not available from the USDA food composition database. Dietary intake of total isoflavones was calculated by summing each of the five individual isoflavones examined. The nutrient contents of food items were based on values from the USDA Database for the Isoflavone Content of Selected Foods (Release 2.0) (that also contains data on coumestrol) [21] and other two national dietary databases, the USDA's 1994-96 Continuing Survey of Food Intakes by Individuals and the University of Minnesota's Nutrition Data Systems for Research [20].

Statistical Analysis

Demographic, anthropometric, and lifestyle characteristics of subjects were compared across the quintiles of total isoflavone intake using chi-square tests for categorical variables and analysis of variance for continuous variables. As intakes of individual phytoestrogens were not normally distributed, the Wilcoxon rank-sum test was employed to compare their differences between subjects who developed any or advanced prostate cancer and those who were free from this malignancy during the specified follow-up period.

Cox proportional hazards regression analysis was conducted to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for prostate cancer risk in relation to dietary intake of isoflavones and coumestrol. The follow-up period for each individual started from the date of cohort entry to date of diagnosis with prostate cancer, death, study dropout, or the censor date (December 31, 2009), whichever came earlier. The date of cohort entry was defined as the date of randomization, baseline questionnaire completion, or dietary survey completion, whichever occurred later. Dietary intake of total isoflavones, each individual isoflavone, and coumestrol were divided into quintiles based on the entire sample, and the HRs for quintiles 2 through 5 were calculated with the lowest quintile as referent category. Covariates included in the final models were age (years), race (non-Hispanic White, non-Hispanic Black, Asian, and other), BMI (kg/m^2) (continuous), smoking status (never smoker, former smoker, and current smoker), alcohol intake (drinkers, non-drinkers), and family history of prostate cancer (yes, no). Other variables evaluated as potential confounders were education, marital status, physical activity, family history of other cancer, aspirin use, ibuprofen use, vasectomy, as well as intake of energy, iron, processed meats, red meat, and caffeine. None of those variables were included in the final models because they were not significantly associated with prostate cancer risk or did not materially alter risk estimates (<10%). Potential interactions of dietary isoflavones and coumestrol with age, race, BMI, education, smoking status, alcohol intake, caffeine intake, red meat intake, and family history of prostate cancer on prostate cancer risk were tested. None of the interaction terms examined were statistically significant and were thus not included in the final models.

All above analyses were conducted separately for advanced, non-advanced, and total prostate cancer because epidemiologic studies suggest that aggressive prostate cancer has a different etiology from indolent disease and is the outcome that is clinically important [22]. Linear trends across quintiles of isoflavone and coumestrol intake were examined using the median in each quintile to create a continuous variable and entering it into regression models. All constructed models were graphically checked for the proportional hazards assumption, and none of the models was found to violate the assumption. SPSS version 23 (Armonk, NY) was used for statistical analysis. A p-value of <0.05 (2-sided) was considered statistically significant.

RESULTS

Characteristics of study subjects are shown in Table 1. Statistically significant differences existed in age, race, BMI, education, smoking status, alcohol intake, red meat intake, and family history of prostate cancer across total isoflavone quintiles. Subjects in the highest quintile of total isoflavone intake were more likely to be Asian, better educated, never smokers, and have a slightly lower BMI than those in other quintiles.

For the PLCO participants included in the present study, the top three food contributors to intake of total isoflavones were tofu/soybeans (66.9%), peas (10.8%), and tea (10.3%), and the corresponding three food

items/groups contributing most to intake of coumestrol were tea (52.1%), beans (37.7%), and other soups (not vegetable and tomato soups) (8.7%) (data not shown).

The median intake of total and individual isoflavones and coumestrol were compared between total prostate cancer cases, advanced cases, and those who had not developed prostate cancer through the censor date (December 31, 2009) (Table 2). Subjects who were diagnosed with advanced prostate cancer had a significantly higher median intake of total isoflavones, genistein, daidzein, glycitein, formononetin, and biochanin A than those who were not diagnosed with prostate cancer during follow-up (all p ≤0.044). Similar differences between total prostate cancer and non-cases were found for total isoflavones, genistein, daidzein, and glycitein (all p ≤0.043).

HRs (95% CIs) for each of three prostate cancer outcomes considered in relation to dietary intake of total and individual isoflavones and coumestrol are presented in Table 3. After adjustment for confounders, HR (95% CI) of advanced prostate cancer for comparing quintile 5 with quintile 1 of dietary intake of total isoflavones, genistein, daidzein, and glycitein were 1.91 (1.25-2.92) (p-trend: 0.007), 1.51 (1.02-2.22) (p-trend: 0.012), 1.80 (1.18-2.75) (p-trend: 0.013), and 1.67 (1.15-2.43) (p-trend: 0.003), respectively. As indicated by significant p-trend values, the risk of advanced prostate cancer increased monotonically with increasing intake of genistein, daidzein, and glycitein, although the strength of the associations were generally modest over the whole range of their dietary intake. For example, HR (95% CI) for quintiles 2 to 5 compared with quintile 1 of daidzein intake was 1.45 (0.93-2.25), 1.65 (1.07-2.54), 1.73 (1.13-2.66), and 1.80 (1.18-2.75), respectively. No statistically significant associations were observed between the intake of total isoflavones and individual phytoestrogens and non-advanced and total prostate cancer after adjustment for confounders.

A separate Cox proportional hazards regression analysis that included the 2287 outliers of total isoflavones was also carried out. The results obtained were largely similar to those of the analysis that excluded those outliers, whereas the associations of advanced prostate cancer with intake of total and individual isoflavones were overall stronger and more significant for linear trends across quintiles in the latter analysis. A sensitivity analysis that included the 806 cases of prostate cancer diagnosed within the first two years of enrollment showed weaker, less significant but still positive associations between intake of individual isoflavones and the risk of advanced prostate cancer. Similar results were obtained when intakes of total and individual isoflavones were evaluated as continuous variables. For example, HRs (95% CIs) of advanced prostate cancer per an interquartile range increase in intakes of total isoflavone, genistein, daidzein, and glycitein were 1.25 (1.07-1.47), 1.21 (1.03-1.43), 1.22 (1.06-1.39), and 1.18 (1.05-1.32), respectively. In addition, the results of this nutrient-based analysis were largely confirmed by food-based analysis. Specifically, subjects who were in the highest quintile of dietary intake of soy products experienced a significantly increased risk of advanced prostate cancer as compared with those in the lowest quintile of intake [RR (95% CI): 1.64 (1.11, 2.43)].

DISCUSSION

In this prospective cohort study, we found a significant positive association of dietary intake of total isoflavones, genistein, daidzein, and glycitein with the risk of advanced prostate cancer. All these associations were independent of established or suspected risk factors for prostate cancer.

Genistein is one of the most extensively investigated phytoestrogens. The present study showed that dietary intake of genistein was significantly associated with an elevated risk of advanced prostate cancer. Previous studies have reported an inverse association between plasma concentrations of genistein and the risk of prostate cancer [23, 24]. Some experimental studies have also revealed a protective effect of genistein on prostate cancer [25, 26], although total phytoestrogens may be more effective than genistein alone for inhibiting the proliferation of prostate cancer [27]. It is possible that an inverse association between genistein intake and prostate cancer is in part ascribed to its effect of lowering serum testosterone concentrations, which was found in rats fed a phytoestrogen-rich diet [10]. We were not able to examine the potential role of serum testosterone in the association between isoflavone intake and prostate cancer as data on this androgenic hormone are not available from the PLCO.

Recently, however, an experimental study suggested that genistein promoted the proliferation and metastasis of patient-derived prostate cancer cells by increasing phosphorylation of epidermal growth factor receptors and its downstream molecules [13]. In that study, a prostatectomy sample was grafted into mice, and it is found that lymph node and secondary organ metastases were significantly increased in genistein-treated mice compared with untreated controls. In addition, genistein-treated mice had more proliferating and fewer apoptotic cancer cells than untreated mice, resulting in an enhanced tumorigenic activity [13]. This animal study offered some biological plausibility for our present observation that dietary intake of genistein was positively associated with the risk of advanced prostate cancer. To our knowledge, this potential adverse effect has not been reported in other epidemiologic studies, which might have arisen from two main reasons. First, most previous studies have investigated Asian populations that ingested higher amounts of phytoestrogens than Western populations. Second, few epidemiologic studies on this research topic have presented separate results for total and advanced prostate cancer [28], a distinction substantially relevant to the etiology, prevention, and treatment of the disease.

Some other biological mechanisms are available for the increased risk of advanced prostate cancer associated with intake of genistein and other individual isoflavones. As stated previously, phytoestrogens may induce estrogenic responses in the body due to their structural similarity to 17β-estradiol [8]. It has been proposed that estrogen is implicated in prostate carcinogenesis through its genotoxic metabolites and regulation of estrogen receptor-β (ERβ) expression during prostate tumor progression [29]. Moreover, the Prostate Cancer Prevention Trial (PCPT) showed that some repeat polymorphisms in genes involved in estrogen synthesis and metabolism were associated with a more than two-fold increased risk of aggressive prostate cancer (Gleason score ≥7). Of note, the most pronounced promoting effect on advanced prostate cancer was observed in PCPT patients who were treated with finasteride, a medication that inhibits the conversion of testosterone to dihydrotestosterone, leading to elevated concentrations of estrogen [30]. Therefore, it is reasonable to infer that an increased risk of advanced prostate cancer related to higher intake of isoflavones was likely mediated through increased estrogenic activity. Nevertheless, precise mechanisms for the effects of isoflavones on prostate cancer and particularly its advanced phenotype merit further investigation as it has been found that isoflavones have both estrogenic and anti-estrogenic functions [31].

Like genistein, daidzein, one of the main isoflavones found in soy products, has also been widely investigated in relation to the risk of cancer and other diseases [32]. The present study did not find a significant association of dietary daidzein intake with both non-advanced and total prostate cancer, an observation in agreement with the

results of several studies in which dietary intake of phytoestrogens was assessed or plasma or urinary concentrations of their biomarkers were measured among European or Jamaican residents [24, 28]. Similarly, two double-blind, placebo-controlled randomized controlled trials showed that daidzein supplementation was not significantly associated with a decreased risk of prostate cancer [33, 34]. Our results of an increased risk of advanced prostate cancer in relation to dietary intake of daidzein have not been reported previously. As both daidzein and genistein are the isoflavones that are abundant in soy products and some other commonly consumed food items, it is biologically plausible that daidzein may also confer an elevated risk of advanced prostate cancer due to its estrogenic effect on the prostate gland.

Prostate cancer can be broadly classified into slow-growing indolent and fast-growing aggressive tumors, and most advanced or metastatic prostate cancers are derived from the latter category. A major strength of the present study is that its large sample size permitted the performance of separate analyses for these two distinct types of tumor in relation to intake of isoflavones and coumestrol. Our study revealed that the effect of isoflavones differs by the biological behavior of prostate tumor. However, this differential effect has seldom been investigated in epidemiologic studies due to small sample sizes (n<250 prostate cancer cases in most studies) [28, 35]. Although it remains to be further investigated whether phytoestrogens exert a differential effect on indolent and aggressive prostate cancers, our results provide additional evidence supporting that these two types of the disease have different etiologies. It is possible that this differential effect of isoflavones is in part ascribed to differences of indolent and aggressive prostate tumors in their expression levels of estrogen receptors. It has been found that the expression of ERα was increased but that of ERβ was reduced in aggressive, androgen-refractory prostate cancer compared with its indolent phenotype [36]. Ample experimental evidence supports that ERβ possesses antiproliferative and even tumor-suppressive properties [36]. Therefore, the competitive binding of increased intake of isoflavones that have a weak estrogenic effect with endogenous estrogen to ERβ in the context of its reduced expression in early aggressive prostate tumor may promote the progression of this type of tumor to screening-detectable or clinically-diagnosable disease. However, lack of data on the expression of estrogen receptors from the PLCO precludes us from examining this possibility.

Another major advantage of the present study is that the prospective nature of the PLCO largely excluded the possibility of reverse causality between intake of total and individual isoflavones and the risk of prostate cancer, a bias that is not uncommon in case-control studies, particularly those measuring exposure biomarkers in biological samples. This reverse causality might still exist to some extent in prospective cohort studies due to the presence of preclinical prostate cancer at baseline. However, the results presented in this paper were obtained after excluding cases of prostate cancer diagnosed with two years of enrollment.

Several limitations of the present study should be considered when interpreting the results obtained. Although dietary intake of total isoflavones, individual isoflavones, and coumestrol was estimated using a food frequency questionnaire modified from the validated Willet and Block FFQs [37], it is well known that recall errors often occur in questionnaire-based dietary assessment. Such dietary measurement errors, if non-differential, could result in an attenuation of the strength of the associations of interest. Thus, the associations found in this study may be conservative. In addition, dietary intake of total isoflavones, individual isoflavones, and/or coumestrol may be

underestimated due to inadequate coverage of all food items containing phytoestrogens in most available food composition databases. For example, soy additives are found in some processed foods [38], and certain isoflavones are naturally present in lower concentrations in commonly consumed foods such as vegetables [39], fruits, and nuts [40]. Therefore, it is possible that the isoflavones from these food products are underrepresented in the food composition databases and the actual impact of isoflavones on prostate cancer risk was thus not observed. The findings of the present study were based on a single dietary assessment. Thereby, it is also possible that some study subjects had changed their dietary habits during follow-up, which might have somewhat distorted our reported effects of isoflavone intake on prostate cancer risk. Another limitation of using a dietary questionnaire to assess phytoestrogen nutritional status is that this method is unable to capture phytoestrogen metabolites (e.g. equol, O-desmethylangolensin) produced by intestinal bacteria [41]. Circulating or urinary biomarkers of phytoestrogens are free from recall bias, are an integrated reflection of phytoestrogens from both food sources and bacteria synthesis, and account for individual differences in the absorption and metabolism of these chemical compounds. Although some studies have shown that phytoestrogen biomarkers are only modestly correlated with their dietary intake [42, 43], it would be preferable to measure dietary exposure to phytoestrogen using both dietary questionnaire and biomarkers in the same populations to better evaluate its effect on prostate cancer risk. However, data on phytoestrogen biomarkers are not available from the PLCO at this time. The FFQ used in the PLCO has not been specifically validated for isoflavones. It is thus possible that some subjects were misclassified with regard to their dietary intake of isoflavones, which could have led to a further attenuation of the associations examined that is ascribed to recall bias in dietary assessment. Taken together, the positive results obtained in the present study based on dietary intake data warrant replication from epidemiological studies using a FFQ specifically validated for this class of nutrients and/or analyzing their circulating biomarkers. One more limitation of our study is lack of data on dietary intake of lignans for the reasons mentioned above. As lignans substantially contribute to total phytoestrogen intake in Western diets, inability to investigate the effect of lignan intake on prostate cancer risk has somewhat weakened the impact of the present study. Finally, the exclusion of men in the control arm who were not asked to complete a food frequency questionnaire should have not substantially reduced the generalizability of our obtained results as men in the intervention arm and the control arm were overall comparable with regard to demographic, anthropometric, socioeconomic, and lifestyle factors due to the successful implementation of randomization in the PLCO [44].

A question may arise as to whether the observed dietary intake of isoflavones and coumestrol in the present study is biologically meaningful. Intake of total isoflavones among our study subjects (median: 0.42 mg/day) is largely comparable to that of studies conducted in other Western populations, e.g. 0.36 mg/day (median) for Dutch women [45] but much lower than that of studies carried out among Asian populations, e.g. 36 mg/day (mean) for Shanghai men [46]. Intake of phytoestrogens or their biomarkers have been associated with an altered risk of colorectal cancer [47] and cardiovascular disease [48] and reduced concentrations of C-reactive protein [49] among residents of North America. It is unknown what blood or urinary concentrations of phytoestrogens for PLCO subjects were in response to the dietary intake of these nutrients due to lack of biomarker data. However, the median urinary levels of isoflavones were reported to be 160 ng/mL among U.S. men and women in the continuous National

Health and Nutrition Examination Survey, 1999–2004 (about the same time period as for the PLCO) [48]. It is apparent that phytoestrogen concentrations in blood and urine among individuals with a typical Western diet are generally far lower than those achieved in most intervention studies with a common isoflavone supplementation dose of 30 mg/day or greater [48, 50].

The present study shows that higher intake of total isoflavones, genistein, daidzein, and glycitein were associated with an increased risk of advanced prostate cancer. The findings of the present study need to be confirmed in more prospective cohort or nested case-control studies to be conducted by measuring both dietary intake of phytoestrogens and their biomarkers among populations with diverse dietary habits. A thorough understanding of the role of phytoestrogens in prostate cancer etiology is expected to offer innovative practical avenues for the primary prevention of this disease.

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COMPLIANCE WITH EITHICAL STANDARDS

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ETHICAL APPROVAL: The PLCO was approved by the institutional review boards of the U.S. National Cancer Institute as well as the 10 screening centers, and written informed consent was obtained from all participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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INFORMED CONSENT: Informed consent was obtained from all individual participants included in the study.

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Table 1. Baseline characteristics of study subjects by quintiles (Q) of dietary intake of total isoflavone intake in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), 1993-2009

Characteristics	Total Isoflavones (mg/day)					p-value
	Q1 (0.0-0.17) n = 5,405	Q2 (0.18-0.31) n = 5,415	Q3 (0.32-0.48) n = 5,384	Q4 (0.49-0.74) n = 5,410	Q5 (0.75-2.03) n = 5,390	
Age [Mean (SD)]	61.6 (5.3)	61.9 (5.2)	63.7 (5.1)	63.6 (5.3)	62.5 (5.1)	<0.001*
Race/Ethnicity (%)						
Non-Hispanic White	19.8	20.2	20.2	20.1	19.7	<0.001*
Non-Hispanic Black	26.7	19.0	21.1	18.9	14.4	
Asian	3.0	5.9	5.9	19.0	66.2	
Other	23.8	21.3	14.3	17.8	22.8	
BMI [Mean (SD)]	27.8 (4.2)	27.9 (4.2)	27.7 (4.1)	27.5 (4.1)	27.6 (4.1)	<0.001*
Education (%)						
Less than High School	23.0	21.6	23.1	18.1	14.3	<0.001*
High School Graduate or Equivalent	24.4	23.3	21.0	17.3	14.0	
Post High School Education	20.7	20.6	20.1	19.8	18.6	
College Graduate or Higher	16.8	17.8	18.6	21.9	24..9	
Smoking Status (%)						
Never Smoker	17.7	18.9	20.1	21.0	22.3	<0.001*
Former Smoker	26.6	23.3	19.2	16.0	14.9	
Current Smoker	20.3	20.1	19.9	20.2	19.4	
Alcohol Intake (%)						
Do Not Drink Alcohol	25.2	24.1	16.1	14.7	19.9	<0.001*
Drinks Alcohol	18.9	19.2	20.8	21.2	20.0	
Total Energy Intake (kcal/day) [Mean (SD)]	1927 (696)	2338 (776)	2328 (849)	2388 (835)	2630 (875)	<0.001*
Caffeine Intake (mg/day) [Mean (SD)]	558 (664)	585 (665)	544 (648)	543 (630)	570 (611)	0.002*
Red Meat Intake (g/day) [Mean (SD)]	39.6 (34.4)	47.1 (38.7)	43.8 (38.8)	40.1 (36.7)	44.7 (41.4)	<0.001*
Family History of Prostate Cancer						
Yes	17.7	19.6	21.4	21.6	19.6	0.026*
No	20.1	20.2	19.7	20.0	20.1	

*Significant value

Table 2. Differences in dietary intake of total and individual isoflavones and coumestrol (mg/day) between subjects who did and did not develop prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO)[a] 1993-2009

Phytoestrogens	Total Prostate Cancer (1) (n = 2,598)	Advanced Prostate Cancer (2) (n = 287)	Non-Prostate Cancer (3) (n = 24,406)	p-value: (1) vs. (3)	p-value: (2) vs. (3)
Total Isoflavones	0.420 (0.211 - 0.699)	0.476 (0.257 - 0.733)	0.392 (0.194 - 0.682)	0.002*	<0.001*
Genistein	0.169 (0.050 - 0.318)	0.191 (0.058 - 0.340)	0.152 (0.046 - 0.315)	0.043*	0.001*
Daidzein	0.245 (0.143 - 0.372)	0.272 (0.178 - 0.399)	0.231 (0.131 - 0.361)	0.001*	<0.001*
Glycitein	0.005 (0.002 - 0.017)	0.014 (0.002 - 0.026)	0.004 (0.002 - 0.016)	0.001*	<0.001*
Formononetin	0.011 (0.007 - 0.018)	0.012 (0.007 - 0.019)	0.011 (0.006 - 0.018)	0.515	0.044*
Biochanin A	0.050 (0.032 - 0.077)	0.053 (0.036 - 0.080)	0.049 (0.031 - 0.075)	0.138	0.008*
Coumestrol	0.077 (0.038 - 0.150)	0.089 (0.041 - 0.160)	0.079 (0.039 - 0.153)	0.555	0.325

[a] Values are medians (the interquartile range)

*Significant value

Table 3 HRs (95% CIs) for advanced, non-advanced, and total prostate cancer according to quintiles (Q) of intakes of total and individual isoflavones and coumestrol in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), 1993-2009

Phytoestrogens (mg/day)	No. of Cases	Person-Years	Crude HR (95% CI)	Adjusted HR (95% CI)[a]	No. of Cases	Person-Years	Crude HR (95% CI)	Adjusted HR (95% CI)[a]	No. of Cases	Person-Years	Crude HR (95% CI)	Adjusted HR (95% CI)[a]
		Advanced	Prostate Cancer			Non-advanced	Prostate Cancer			All	Prostate Cancer	
Total Isoflavones												
Q1 (0.0 - 0.17)	33	2,094	Reference	Reference	438	27,088	Reference	Reference	471	29,182	Reference	Reference
Q2 (0.18 - 0.31)	54	3,366	1.60 (1.04 - 2.47)*	1.56 (1.00 - 2.42)*	440	27,289	0.99 (0.87 - 1.13)	0.97 (0.85 - 1.11)	494	30,655	1.03 (0.91 - 1.17)	1.02 (0.89 - 1.16)
Q3 (0.32 - 0.48)	58	3,777	1.68 (1.10 - 2.58)*	1.49 (0.96 - 2.30)	475	30,397	1.07 (0.94 - 1.21)	1.01 (0.88 - 1.15)	533	34,174	1.11 (0.98 - 1.26)	1.04 (0.91 - 1.18)
Q4 (0.49 - 0.74)	72	4,682	2.06 (1.37 - 3.11)*	1.70 (1.11 - 2.61)*	485	30,807	1.08 (0.95 - 1.23)	1.01 (0.88 - 1.15)	557	35,489	1.15 (1.01 - 1.30)*	1.06 (0.93 - 1.20)
Q5 (0.75 - 2.03)	70	4,535	2.07 (1.37 - 3.13)*	1.91 (1.25 - 2.92)*	473	29,798	1.07 (0.94 - 1.22)	1.02 (0.90 - 1.17)	543	34,333	1.14 (1.01 - 1.29)*	1.09 (0.96 - 1.23)
p-trend			0.001*	0.007*			0.183	0.560			0.018*	0.151
Genistein												
Q1 (0.0 - 0.04)	45	2,915	Reference	Reference	461	28,699	Reference	Reference	506	31,614	Reference	Reference
Q2 (0.05 - 0.09)	47	2,900	1.07 (0.71 - 1.60)	1.03 (0.68 - 1.58)	433	26,762	0.96 (0.84 - 1.09)	0.96 (0.84 - 1.09)	480	29,662	0.97 (0.86 - 1.10)	0.96 (0.85 - 1.09)
Q3 (0.10 - 0.19)	56	3,608	1.22 (0.82 - 1.80)	1.13 (0.75 - 1.69)	470	29,921	1.02 (0.90 - 1.16)	0.96 (0.84 - 1.09)	526	33,529	1.04 (0.92 - 1.17)	0.97 (0.86 - 1.10)
Q4 (0.20 - 0.34)	70	4,492	1.51 (1.04 - 2.19)*	1.31 (0.89 - 1.94)	504	32,336	1.09 (0.96 - 1.24)	1.02 (0.90 - 1.17)	574	36,828	1.13 (1.00 - 1.27)	1.05 (0.92 - 1.19)
Q5 (0.35 - 1.12)	69	4,539	1.53 (1.05 - 2.23)*	1.51 (1.02 - 2.22)*	443	27,661	0.97 (0.85 - 1.11)	0.94 (0.82 - 1.08)	512	32,200	1.02 (0.90 - 1.16)	0.99 (0.87 - 1.13)
p-trend			0.006*	0.012*			0.829	0.716			0.264	0.64
Daidzein												
Q1 (0.0 - 0.11)	45	2,087	Reference	Reference	461	26,675	Reference	Reference	464	28,762	Reference	Reference
Q2 (0.12 - 0.19)	47	3,365	1.54 (1.00 - 2.38)	1.45 (0.93 - 2.25)	433	28,870	1.04 (0.91 - 1.19)	1.00 (0.87 - 1.14)	514	32,235	1.08 (0.95 - 1.22)	1.03 (0.91 - 1.17)
Q3 (0.20 - 0.28)	56	4,117	1.87 (1.23 - 2.85)*	1.65 (1.07 - 2.54)*	470	29,756	1.08 (0.94 - 1.23)	1.02 (0.89 - 1.17)	533	33,873	1.13 (1.00 - 1.28)	1.07 (0.94 - 1.21)
Q4 (0.29 - 0.40)	70	4,272	1.91 (1.26 - 2.91)*	1.73 (1.13 - 2.66)*	504	29,948	1.08 (0.94 - 1.23)	1.01 (0.88 - 1.15)	540	34,220	1.14 (1.00 - 1.29)*	1.06 (0.93 - 1.20)
Q5 (0.41 - 1.64)	69	4,613	2.08 (1.37 - 3.14)*	1.80 (1.18 - 2.75)*	443	30,130	1.09 (0.95 - 1.24)	1.03 (0.90 - 1.18)	547	34,743	1.16 (1.02 - 1.31)*	1.09 (0.96 - 1.23)
p-trend			0.001*	0.013*			0.222	0.637			0.025*	0.208
Glycitein												
Q1 (0.0 - 0.001)	50	3,165	Reference	Reference	505	31,418	Reference	Reference	555	34,583	Reference	Reference
Q2 (0.002 - 0.003)	54	3,398	1.02 (0.70 - 1.50)	1.07 (0.72 - 1.58)	516	31,886	0.98 (0.86 - 1.10)	1.00 (0.88 - 1.14)	570	35,284	0.98 (0.87 - 1.10)	1.01 (0.90 - 1.14)
Q3 (0.004 - 0.014)	61	3,936	1.32 (0.91 - 1.92)	1.23 (0.83 - 1.80)	462	29,526	1.02 (0.90 - 1.16)	0.98 (0.86 - 1.12)	523	33,462	1.05 (0.93 - 1.18)	1.00 (0.88 - 1.13)
Q4 (0.015 - 0.026)	53	3,490	1.53 (1.04 - 2.25)*	1.25 (0.83 - 1.88)	396	25,783	1.19 (1.04 - 1.36)*	1.10 (0.96 - 1.27)	449	29,273	1.22 (1.08 - 1.38)*	1.11 (0.98 - 1.27)
Q5 (0.027 - 0.090)	69	4,465	1.64 (1.14 - 2.35)*	1.67 (1.15 - 2.43)*	432	26,766	1.03 (0.90 - 1.17)	1.02 (0.89 - 1.16)	501	31,231	1.08 (0.96 - 1.22)	1.08 (0.95 - 1.22)
p-trend			0.001*	0.003*			0.184	0.449			0.020*	0.095
Formononetin												
Q1 (0.0 - 0.005)	42	2,655	Reference	Reference	472	29,566	Reference	Reference	514	32,221	Reference	Reference
Q2 (0.006 - 0.009)	59	3,806	1.34 (0.90 - 1.99)	1.30 (0.87 - 1.96)	499	31,228	1.01 (0.89 - 1.15)	1.01 (0.89 - 1.15)	558	35,034	1.04 (0.92 - 1.17)	1.03 (0.91 - 1.17)
Q3 (0.010 - 0.013)	62	3,955	1.45 (0.98 - 2.15)	1.47 (0.99 - 2.19)	440	27,544	0.92 (0.81 - 1.05)	0.92 (0.80 - 1.05)	502	31,499	0.97 (0.85 - 1.09)	0.96 (0.85 - 1.09)
Q4 (0.014 - 0.021)	67	4,327	1.54 (1.05 - 2.27)*	1.50 (1.01 - 2.22)*	460	29,139	0.95 (0.83 - 1.08)	0.91 (0.80 - 1.04)	527	33,466	1.00 (0.88 - 1.13)	0.96 (0.85 - 1.09)
Q5 (0.022 - 0.170)	57	3,711	1.46 (0.98 - 2.17)	1.42 (0.95 - 2.13)	440	27,902	1.00 (0.88 - 1.14)	0.98 (0.85 - 1.11)	497	31,613	1.04 (0.92 - 1.18)	1.01 (0.89 - 1.15)
p-trend			0.139	0.192			0.990	0.593			0.615	0.944
Biochanin A												
Q1 (0.0 - 0.028)	45	2,916	Reference	Reference	461	28,783	Reference	Reference	506	31,699	Reference	Reference

Q2 (0.029 - 0.041)	51	3,285	1.10 (0.74 - 1.64)	1.03 (0.69 - 1.54)	465	29,155	0.98 (0.86 - 1.12)	0.97 (0.85 - 1.11)	516	32,440	0.99 (0.88 - 1.12)	0.98 (0.86 - 1.11)
Q3 (0.042 - 0.057)	61	3,895	1.29 (0.88 - 1.90)	1.17 (0.79 - 1.73)	462	29,024	0.96 (0.85 - 1.10)	0.95 (0.83 - 1.08)	523	32,919	0.99 (0.88 - 1..12)	0.97 (0.85 - 1.10)
Q4 (0.058 - 0.086)	66	4,266	1.47 (1.01 - 2.15)*	1.39 (0.95 - 2.03)	451	28,470	0.99 (0.87 - 1.13)	0.95 (0.84 - 1.09)	517	32,736	1.03 (0.92 - 1.17)	0.99 (0.88 - 1.13)
Q5 (0.087 - 0.360)	64	4,092	1.45 (0.99 - 2.13)	1.26 (0.85 - 1.85)	472	29,947	1.05 (0.92 - 1.19)	1.01 (0.88 - 1.15)	536	34,039	1.09 (0.96 - 1.23)	1.03 (0.91 - 1.17)
p-trend			0.030*	0.146			0.328	0.773			0.100	0.451
Coumestrol												
Q1 (0.0 - 0.03)	50	3,252	Reference	Reference	488	30,672	Reference	Reference	538	33,924	Reference	Reference
Q2 (0.04 - 0.06)	55	3,484	1.13 (0.77 - 1.65)	1.16 (0.79 - 1.72)	453	28,418	0.95 (0.84 - 1.09)	0.94 (0.82 - 1.07)	508	31,902	0.97 (0.86 - 1.10)	0.96 (0.85 - 1.09)
Q3 (0.07 - 0.10)	65	4,216	1.32 (0.92 - 1.91)	1.30 (0.89 - 1.90)	478	29,812	1.00 (0.88 - 1.13)	0.98 (0.86 - 1.11)	543	34,028	1.03 (0.91 - 1.16)	1.01 (0.89 - 1.14)
Q4 (0.11 - 0.19)	58	3,671	1.19 (0.82 - 1.74)	1.22 (0.83 - 1.79)	450	28,591	0.95 (0.83 - 1.08)	0.93 (0.81 - 1.06)	508	32,262	0.97 (0.86 - 1.10)	0.95 (0.84 - 1.08)
Q5 (0.20 - 1.27)	59	3,831	1.24 (0.85 - 1.81)	1.25 (0.85 - 1.84)	442	27,886	0.95 (0.83 - 1.08)	0.94 (0.83 - 1.08)	501	31,717	0.97 (0.86 - 1.10)	0.97 (0.86 - 1.10)
p-trend			0.441	0.438			0.435	0.502			0.631	0.706

[a] Adjusted for age, race/ethnicity, BMI, smoking status, alcohol intake, and family history of prostate cancer.
*Significant value