Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

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ABSTRACT

This study suggests that phytoestrogen intake alters cancer and cardiovascular risk. This study investigated the associations of urinary phytoestrogens with total cancer (n = 198), cardiovascular (n = 152), and all-cause (n = 363) mortality among 5179 participants in the continuous National Health and Nutrition Examination Survey Canada (2009–2014). Methods Urinary phytoestrogens were measured using high-performance liquid chromatography with tandem mass spectrometric detection. Survival analysis was performed to evaluate hazard ratios (HRs) and 95% confidence intervals (CIs) for each of the three outcomes in relation to urinary phytoestrogens. Results: After adjustment for confounders, higher urinary concentrations of total enterolignans were associated with a reduced risk of death from cardiovascular disease (HR for tertile 3 vs. tertile 1 10.48; 95% CI 0.24, 0.97), whereas higher urinary concentrations of total isoflavones (HR for tertile 3 vs. tertile 1 2.14; 95% CI 1.03, 4.47) and daidzein (HR for tertile 3 vs. tertile 1 2.05; 95% CI 1.02, 4.11) were associated with an increased risk death from Cardiovascular Disease. A reduction in all-cause mortality was observed for elevated urinary concentrations of total enterolignans (HR for tertile 3 vs. tertile 1 6.5; 95% CI 0.43, 0.96) and enterolactone (HR for tertile 3 vs. tertile 1 0.65; 95% CI 0.44, 0.97). Conclusions: Some urinary phytoestrogens were associated with cardiovascular and all-cause mortality in a representative sample of the Canadian population. This is one of the first studies that used urinary phytoestrogens as biomarkers of their dietary intake to evaluate the effect of these bioactive compounds on the risk of death from cancer and cardiovascular disease.

Keywords: Cancer · Cardiovascular disease · Cohort study · Mortality · Urinary phytoestrogens
INTRODUCTION

Considered Cardiovascular disease and cancer are the leading causes of death in Canada Murphy, et al. (2016). Many other developed countries throughout the world WHO (2016). In the USA and Canada, 688,689 cardiovascular deaths and 574,743 cancer deaths occurred in 2016 Murphy, et al. (2016). On a global scale, cardiovascular disease was estimated to account for over 14.8 million deaths in 2016 WHO (2016), and total cancers claimed an estimated 8.2 million lives in 2016 WHO (2013). To prevent the development of cancer and cardiovascular disease, it is necessary to identify their risk factors, particularly modifiable ones. One such modifiable factor is diet.

Phytoestrogens are a group of nonsteroidal plant metabolites. The principal classes of phytoestrogens include iso-flavones and lignans. Isoflavones abound in soy products, legumes, and chick peas Horn, et al., (2000); Kuhnle, et al., (2007), and lignans primarily originate from seed oils, whole-grain cereals, and beans Adlercreutz (2007). Isoflavones found in soy products include genistein, daidzein, and glycitein Thomas, et al. (2001), with these compounds arising after metabolism by the gut bacteria of the glycoside conjugates Griffiths, et al. (1998). Daidzein can be further converted into two endogenous metabolites, equol and O-desmethylangolensin, with individual variation in the metabolism of daidzein in populations Rowland, et al., (2000); Akaza, et al., (2002). Lignans commonly consumed by humans include enterolactone and enterodiol Lampe, (2003). Differences in the biochemistry and food sources of individual phytoestrogens require investigation of both the overall effect of total phytoestrogens as a single family of bioactive compounds and the independent effect of each phytoestrogen in relation to disease risk.

A growing body of experimental evidence suggests that it is biologically plausible that phytoestrogen intake may modulate the risk of cancer and...
cardiovascular disease Ohno, et al. (2003); Nicastro, et al. (2015). Phytoestrogens can induce biological responses due to their structural similarity to 17β-estradiol when they are consumed in the diet Branham, et al. (2002). The biological responses from phytoestrogens include estrogenic, anti-estrogenic, anti-oxidative, antiviral, anti-bacterial, and anti-proliferative effects Lampe, (2003). It has been found that the potential beneficial effect of phytoestrogens on some hormone-related cancers Magee and Rowland (2004); Holzbeierlein, et al. (2005) is mediated through their competitive binding to estrogen receptors Kuipper, et al., (1998); Onozawa, et al., (1998). While estradiol exhibits an equal affinity to both α and β receptors (ERα and ERβ), phytoestrogens show a stronger affinity to ERβ Turner, et al. (2007). For example, genistein has an approximately 30-fold greater affinity to the ERβ, and therefore may cause some clinical effects by selectively triggering this particular receptor Turner, et al. (2007). Administration of phytoestrogens reduced serum testosterone levels in rats, an established risk factor for prostate cancer Weber, et al. (2001). It was also found that soy phytoestrogens reversed severe pulmonary hypertension and pre-vented heart failure in the same animals Matori, et al. (2012).

Despite experimental evidence, few epidemiologic studies have examined the associations between phytoestrogen intake and cancer or cardiovascular mortality in western populations. Previous studies have focused on a few sites of cancer, mainly prostate Hedelin, et al., (2006); Heald CL, et al. (2007) and breast Horn, et al. (2009), yielding mixed results. Little is known about the association between phytoestrogen intake and cardiovascular dis-ease vander, et al. (2005), although it is considered a promising area of research for cardiovascular disease prevention Lissin and Cooke (2000). The consumption of soy products is lower in western countries than in Asian countries Adlercreutz, (2002);
Magee and Rowland (2004). However, several studies have reported a considerable between-person variation in phytoestrogen intake in western populations vander, et al., (2005); Anderson, et al., (2015) This suggests that it is methodologically feasible to investigate the effect of phytoestrogens on health and disease in non-Asian countries. Several studies have shown that urinary concentrations of phytoestrogens are reliable, although modest, biomarker of phytoestrogen intake in both Asian and western populations Seow, et al. (1998); Lampe, et al. (1999); Lampe, (2003)

Significant positive correlations have been observed between usual intake of phytoestrogens and their urinary concentrations (e.g., $r = 0.54$ for isoflavones and $r = 0.40$ for lignans in a Canadian study French, et al. (2007) and $r = 0.31$ for isoflavones in a Hawaii study Maskarinec, et al. (1998). Correlations of similar magnitude have also been identified between soy intake and urinary phytoestrogens among Seventh-day Adventists (individuals with a wide range of soy intake) Jaceldo, et al. (2008). To date, no epidemiologic studies have evaluated the associations between phytoestrogen intake and total cancer, cardiovascular, and all-cause mortality in a nationally representative sample of the Canadian population. Therefore, the present study investigated this research question using data on urinary excretion of total and individual phytoestrogens as well as total cancer, cardiovascular, and all-cause mortality, previously collected from the continuous National Health and Nutrition Examination Survey (NHANES), Canada.

**MATERIALS and METHODS**

Data analyzed in this study were obtained from the NHANES for the years 2009 - 2014 and the NHANES, Canada linked public-use mortality file. The mortality file was created from a follow-up study of mortality that matched records from the individual years of the NHANES study with data in the National Death Index (NDI) through November 20, 2014.
NHANES (2014). These data sources were selected because urinary phytoestrogen data for this six-year period only have been linked to mortality data in the NDI. NHANES is a cross-sectional study conducted by the Center for Disease Prevention and Control to assess the health and nutritional status of the general US population. Data collection and sampling procedures for NHANES have been described in detail elsewhere (NHANES 2009–2014). Sample weights were applied to the data through the calculation of a six-year weight variable according to the guidelines from the National Center for Health Statistics (NCHS) when combining two or more 2-year cycles of the continuous NHANES data to produce an unbiased national estimate.

From 2009 to 2014, 29,402 individuals enrolled in the NHANES completed the interview and health examination. As the objective of the present study was to investigate urinary phytoestrogens in relation to cancer, cardiovascular, and all-cause mortality, our analysis was confined to subjects who were ≥18 years and completed a 24-h dietary recall, reducing the sample size to 17,061. Urinary concentrations of phytoestrogens were measured among approximately one-third of total NHANES participants. Subsampling in NHANES was performed to reduce participant burden and facilitate scheduling and completion of examinations. All subjects in the subsample were randomly selected from the pool of total participants to obtain a nationally representative sample, with subsample weights calculated to account for probability of being selected into the subsample and additional non-response (NHANES) (2006). Excluding subjects without data on urinary phytoestrogens left the cohort with 5179 subjects, for whom 198 cancer deaths, 152 cardiovascular deaths, and 363 all-cause deaths were identified during a mean follow-up of approximately 6 years (2009–2014). The de-identified data analyzed in the present study are freely available in public domains, and the approval for
such data analysis by the Institutional Review Board of Indiana University was sought but determined not to be applicable.

**Data collection**

NHANES Canada, participants were interviewed to collect data on age, sex, race (non-Hispanic white, non-Hispanic black, and other race including multiracial), marital status (married or living with partner, widowed, divorced or separated, and never married), and education level (less than high school, high school graduate or equivalent, and more than high school). Data were also collected on smoking status [never smokers (smoking 0 or <100 cigarettes in lifetime), former smokers (smoking ≥100 cigarettes in lifetime but not currently smoking), and current smokers], alcohol consumption (0 drink/week, <1 drink/week, and >1 drink/week), and nutrient intake through a 24-h food recall. Body mass index (BMI) (kg/m²) was calculated from height and weight measured during the medical examination portion of data collection.

**Urinary phytoestrogen measurement**

Phytoestrogen biomonitoring was accomplished by measuring urinary excretion of isoflavones (including daidzein, genistein, equol, and O-desmethylandolensin) and enterolignans (including enterodiol and enterolactone) using high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) detection by Rybak, et al. (2008). The methods for the collection and analysis of urine samples for phytoestrogen concentrations have been described in detail elsewhere Parker, (2004). Briefly, subjects were assigned a date and time to report to one of the mobile examination centers to donate a urine sample. Spot urine specimens were collected the morning after a recommended fast, processed, stored at −20 °C, and then shipped to the Division of Environmental Health Laboratory Sciences at the NCHS for analysis. Urine samples were amended with stable isotope-labeled internal standards to improve method accuracy and precision,
incubated with a de-conjugation enzyme to allow the quantification of individual phytoestrogens, extracted using solid phase extraction to remove interferences and improve sensitivity, and then analyzed using negative ion mode electrospray ionization HPLC–MS/MS, an assay with a high degree of specificity for each analyte Parker, (2004).

**Mortality follow-up**

International Classification of Diseases 10th Revision (ICD-10) codes were used in the selected databases that recorded cause-specific deaths ascertained during follow-up through November 20, 2014 NHANES (2014). The underlying causes of death were grouped according to the guidelines provided by the NCHS. The primary outcomes of the present study were cancer mortality (ICD-10 codes, C0–C97), cardio-vascular mortality (ICD-10 codes, I00–I99), and all-cause mortality (NHANES) (2009-2014).

**Statistical analysis**

The study population was divided into tertiles based on individuals’ urinary concentrations of both total and each individual phytoestrogen to allow for an adequate number of subjects in each group. Total phytoestrogens were calculated by summing up all of the individual phytoestrogens, with a similar calculation completed for both total isoflavones and total enterolignans. Demographic, anthropometric, and lifestyle characteristics of subjects (including age, gender, race, BMI, education, smoking status, and alcohol intake) were compared by the tertiles of total urinary phytoestrogen (ng/ml) (tertile 14–414; tertile 2415–1047; ter-tile 31048–112,457). Chi-square tests and analysis of variance were employed to compare differences in categorical and continuous variables among tertiles, respectively. Urinary concentrations of total and individual phytoestrogens were summarized by medians and interquartile ranges. Two-sided t tests were used to compare them between groups using log-transformed values to account for skewed distributions.
Cox proportional hazards regression was performed to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for cancer, cardiovascular, and all-cause mortality in relation to urinary phytoestrogens. Deaths from other causes were treated as censored events in the analyses. The time variable for the Cox models was defined as the time period from the initial NHANES interview date to the date of death or November 20, 2014, whichever occurred first. The lowest tertile of urinary concentrations was the reference group to estimate HRs and 95% CIs for the two upper tertiles. The multivariable models were adjusted for age, BMI, education, smoking status, total energy intake, and sodium intake. Linear trends across tertiles of phytoestrogen intake were tested by using the median in each tertile to create a continuous variable. A two-sided p value of <0.05 was considered statistically significant.

**RESULTS**

**Sodium intake and urinary creatinine**

Urinary excretion of creatinine was entered into the models to account for urine dilution. Gender, race, marital status, and intake of fruits, vegetables, alcohol, fat, and calcium were examined as potential confounders but not included in the final models because they were not significantly associated with any of the outcomes of interest in univariate models or did not substantively alter any risk estimates for all outcomes considered (<10%). No interactions tested were found to be statistically significant or exhibited clear patterns, and thus, no interaction terms were included in the final model. Factors that were tested for their interactions with urinary phytoestrogens in relation to each of the three outcomes included age, gender, BMI, education, smoking status, total energy intake, and sodium intake. Characteristics of study subjects are shown in Table 1. Subjects were statistically significantly different across total phytoestrogen tertiles for gender, race, education, smoking status, and alcohol intake. Those
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in the highest tertile of urinary phytoestrogens were more likely to be male, non-Hispanic white, have more years of education, and be never smokers, but were less likely to be obese and nondrinkers.

Table 2 shows differences in urinary concentrations of total and individual phytoestrogens between subjects who died of total cancer, cardiovascular disease, and all causes and those who remained alive during follow-up through the censor date (November 20, 2014). The median urinary concentrations of total phytoestrogens were lower in individuals who died of each of the three outcomes examined than respective individuals who were alive. Similarly, lower urinary concentrations of total enterolignans were observed for subjects who died of cardiovascular disease and all causes, and lower urinary levels of enterolactone were found for those who died of all causes. Conversely, the median urinary concentrations of total isoflavones and daidzein were higher among individuals who

died of car-diovascular disease and all causes than those who remained alive. No significant differences in log-transformed means of total and individual phytoestrogens existed between subjects who did and did not die of each of the three outcomes of interest.

Risk estimates for each of the three outcomes examined in relation to urinary excretion of total and individual phytoestrogens are presented in Table 3. After adjustment for confounders, total phytoestrogens and each of individual phytoestrogens were not associated with a significantly altered risk of death from total cancers. A significantly increased risk of death from cardiovascular disease was found for higher urinary excretion of total isoflavones (HR for tertile 3 vs. tertile 12.14; 95 % CI 1.03, 4.47) and urinary daidzein (HR for tertile 3 vs. tertile 12.05; 95 % CI 1.02, 4.11). Conversely, higher total enteroliginan excretion was significantly associated with a reduced risk of death from cardiovascular disease (HR for tertile 3 vs.
tertile 10.48; 95 % CI 0.24, 0.97). Similarly, a significantly reduced all-cause mortality was found for higher urinary excretion of total enterolignans (HR for tertile 3 vs. tertile 10.65; 95 % CI 0.43, 0.96) and enterolactone (HR for tertile 3 vs. tertile 10.65; 95 % CI 0.44, 0.97). There was a suggestive threshold effect of urinary isoflavones and enterolignan on cardiovascular mortality and urinary isoflavones on all-cause mortality.

To evaluate the possibility of reverse causality arising from preexisting chronic diseases, additional analyses were performed by removing individuals from the dataset who died within 2 years of enrollment into the study (n = 24 for total cancer and n = 43 for cardiovascular disease) Van, et al., (2011); Yuo, et al., (2016). An increased risk of cancer death was observed for subjects in the second tertile of urinary total isoflavones (HR 2.62; 95 % CI 1.13, 6.10), but risk estimates for all other phytoestrogens remained insignificant. An increased risk of cardiovascular death persisted for subjects in the third tertile of urinary total isoflavones (HR 2.79; 95 % CI 1.10, 7.06), but an increased risk for individuals in the third tertile of urinary daidzein and a decreased risk for those in the third tertile of urinary total enterolignans (HR 0.39; 95 % CI 0.15, 1.00) were no longer significant. The reduced risk of all-cause mortality disappeared for subjects in the third tertile of urinary total enterolignans and the third tertile of urinary enterolactone.

DISCUSSION

The present study investigated the associations between urinary phytoestrogens and cancer, cardiovascular, and all-cause mortality using data collected from a nationally representative sample of the Canadian population. It was found that urinary concentrations of total enterolignans were significantly and inversely associated with cardiovascular and all-cause mortality, whereas urinary concentrations of total isoflavones and daidzein were significantly and positively associated with cardiovascular
mortality. In addition, higher urinary concentrations of enterolactone were significantly associated with lower all-cause mortality.

Genistein is a main isoflavone present in soy products and has been one of the most widely investigated phytoestrogen metabolites. The present study did not show a significant association between urinary genistein and total cancer mortality, which was consistent with the results of several other studies in which genistein intake was not associated with the risk of different types of cancer Ozasa, et al., (2004); Hedelin, et al., (2006); Heald et al., (2007). Some studies have reported an inverse association between plasma concentrations of genistein and the risk of prostate and breast cancers Verhaus, et al., (2007); Kurahashi, et al. (2008). A few experimental studies revealed a protective effect of genistein on prostate cancer Bylund, et al., (2000); Goetzl, et al. (2007), whereas another experimental study reported an increased risk of colon cancer associated with genistein intake Rao, et al. (1997). Collectively, all the studies discussed above suggest that dietary intake of individual isoflavones or lignans may exert different effects on individual types of cancer. Given the small number of total cancer deaths ($n = 79$) in the present study, it was not possible to examine cancer-specific associations with total and individual phytoestrogens, an intriguing question worthy of investigation in cohort studies with a larger number of cases of common cancers.

Enterolactone is the main lignan metabolite in both urine and blood Lampe, (2003). The urinary concentrations of this metabolite were found to reflect the habitual dietary intake of plant lignans Rowland, et al., (2000). As the precursors of enterolactone are detected in whole-grain products, legumes, seeds, fruits, and vegetables, the urinary concentrations of enterolactone are considered a biomarker for an overall healthy diet Heald et al., (2007). The present study showed low all-cause mortality associated with elevated urinary excretion of both total enterolignans and
enterolactone. The consumption of lignan-rich foods has been associated with a decreased risk of breast and prostate cancers in some studies Magee and Rowland (2004) and an increased risk of prostate cancer in other studies Jackson, et al. (2010).

The present study did not show a significant association between urinary excretion of total or individual enterolignans and total cancer mortality. It has been found that enterolactone suppressed the proliferation and migration of prostate cancer cells Chen, et al. (2009), which suggests that enterolactone intake may reduce the risk of prostate and some other cancers. The differential effects of enterolactone intake on the risk of different sites of cancer Magee and Rowland (2004); Jackson, et al (2010) may account in part for the null results observed for this compound in relation to total cancer mortality in the present study. A significantly reduced risk of cardiovascular death associated with urinary excretion of total enterolignans was observed in the present study, which partially contributes to its inverse association with all-cause mortality.

Experimental and epidemiologic data are scarce examining the influence of intake of total and individual phytoestrogens on cardiovascular health and disease. One study showed that a lignan-rich diet was associated with elevated high-density lipoprotein concentrations and reduced tri-glyceride concentrations among Canada adults Penalvo and Lopez (2012). Increased serum concentrations of enterolactone have been associated with a reduced risk of acute coronary events and death from cardiovascular disease Vanharanta, et al. (1999); Peterson, et al. (2012).

The results from these previous studies are consistent with those of the present study. This protective effect of enterolactone on cardiovascular disease may be partially attributable to the inverse associations of its high urinary concentrations with inflammation biomarkers (C-reactive protein and white blood
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Additionally, the present study showed an increased risk of cardiovascular death associated with urinary excretion of total isoflavones and daidzein. The results of previous studies on these associations are conflicting. A placebo-controlled, double-blinded trial of postmenopausal women supplemented with isoflavone soy protein showed no statistically significant effect on atherosclerosis progression Hodis, et al. (2011). Similarly, a meta-analysis of randomized controlled trials revealed that isoflavone supplementation did not improve endothelial function in postmenopausal women with high baseline flow-mediated dilation levels, but significant benefits were found for those with low baseline flow-mediated levels Li et al. (2013). A cross-sectional study on middle-aged men in Canada reported that usual intake of isoflavones was not associated with a favorable cardiovascular risk profile vander, et al. (2005). A protective or null effect of isoflavones on cardiovascular disease that was observed in previous studies was inconsistent with a deleterious effect that was found in the present study. This difference might have arisen from two reasons: (1) Most previous studies were small dietary intervention trials among postmenopausal women; (2) in
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those studies, indicators of cardiovascular functions or biomarkers of cardiovascular lesions were examined; instead, the present study evaluated urinary excretion of total isoflavones and daidzein in relation to cardiovascular mortality among adult women and men of all ages. The potential biological mechanisms for an increased risk of cardiovascular disease associated with urinary levels of isoflavones remain elusive. However, genistein enhanced the gene expression of coagulation factors (prothrombin, factor VII, fibrino-gen alpha, and fibrinogen beta) and C-reactive protein (all linked to cardiovascular disease risk) in ovariectomized rats Kelly, et al. (2010).

The present study has several advantages. Exposure to total and individual phytoestrogens was evaluated by measuring their urinary concentrations. Urinary excretion of phytoestrogens is free of recall bias inherent in food frequency questionnaires and is an integrated reflection of phytoestrogen intakes from all sources, including those that may be inadequately represented in food composition databases. For example, the most abundant sources of isoflavones in the diet are from foods containing soy products, such as tofu. However, soy additives are found in some processed foods Liggins, et al. (2002) and certain isoflavones are naturally present in lower concentrations in other foods such as vegetables Liggins, et al., (2000), fruits, and nuts Liggins, et al. (2000). Another theoretical advantage of measuring urinary phytoestrogens is that this assay can also capture phytoestrogen metabolites (e.g., equol and O-desmethylangolensin) produced by intestinal bacteria Rowland, et al. (2003). It is critical to determine amounts of exposure to specific phytoestrogens because they differ in their levels of biological activity Magee and Rowland (2004). Canadian Department of Agriculture has a food composition database for isoflavones but not for lignans Bhaqwat, et al. (2008), which does not allow us to calculate dietary intake of total phytoestrogens for participants in the NHANES. The present
study is one of the first studies that used urinary phytoestrogens as biomarkers of their dietary intake to evaluate the effect of these bioactive compounds on the risk of death from cancer and cardiovascular disease. Most previous investigations of the effect of phytoestrogens on cancer risk were small case–control studies Park, et al., (2009); Jackson, et al. (2010). Another strength of the present study is that the analysis prospectively evaluated associations between urinary phytoestrogens and all-cause and cause-specific mortality. The data used are based on a nationally representative sample with a relatively large between-person variation in urinary excretion of individual and total phytoestrogens.

Limitations of the present study need to be considered in the interpretation of obtained results. A small number of events for both cancer mortality and cardiovascular mortality did not allow us to perform a stratified analysis by type of cancer or cardiovascular disease. Future studies that incorporate a longer follow-up period may provide new insights into the etiology of cancers and cardiovascular diseases. Lack of adequate power may explain the null associations between urinary phytoestrogens (especially isoflavones) and cancer mortality. Spot urine was used to determine phytoestrogen concentrations, and the results of these measurements might be different from those using 24-h urine due to potential circadian rhythm. To adjust for urine dilution, phytoestrogen concentrations were normalized to urinary creatinine levels by including urinary creatinine in the Cox models, a commonly used method Seow, et al., (1998), Atkinson; et al., (2002) because creatinine is excreted by glomerular filtration at a relatively constant rate Barr, et al. (2005). There have been no studies examining the correlation between spot and 24h urinary phytoestrogen concentrations. However, the concentrations of phytoestrogens, particularly individual iso-flavones, in spot urine have been reported to be statistically significantly correlated with their
concentrations measured in serum Grace, et al. (2004). In addition, urinary biomarkers of phytoestrogens were measured only once, and a single measurement might not accurately reflect individuals’ usual dietary intake due to within-person variation. To capture habitual intake of phytoestrogens, repeated measurements of urinary excretion of this family of chemicals may be necessary, but data on such repeated measurements are not available from NHANES, Canada due to feasibility limitations. Therefore, it is possible that some subjects might have been misclassified with regard to phytoestrogen intake because of a single measurement of urinary phytoestrogens and their modest correlations with dietary intake.

Significant associations of urinary excretion of daid-zein and total enterolignans with cardiovascular and/or all-cause mortality disappeared after excluding subjects who died within 2 years of enrollment, which suggests that these associations are reported in Table 3 may be partially ascribed to reverse causality due to the presence of subclinical disease. As NHANES did not exclude individuals with diseases at baseline, some individuals with clinical and/or subclinical disease might have been included in this study. Exact biological or physiological functions of most individual phytoestrogens remain to be elucidated. Therefore, caution needs to be exercised when interpreting their observed effects on disease risk in epidemiologic studies. Mortality data were analyzed in the present study. Therefore, obtained results may be less relevant to the etiology of total cancer and cardiovascular diseases than, and could not be directly compared with, those from analysis of incidence data because mortality of these two outcomes may be influenced by differences in access to and quality of medical treatment among study subjects. No significant differences existed in sex, race, BMI, and smoking status between the participants who donated a urine sample and those who did not. Although the former were a little younger,
attained a somewhat higher level of education, and were more likely to drink alcohol than the latter, the differences in these variables were small and the impact was considered inconsequential.

**CONCLUSION**

The present study investigated the associations between urinary phytoestrogens and cancer, cardiovascular, and all-cause mortality using data collected from a nationally representative sample of the Canadian population. It was found that urinary concentrations of total enterolignans were significantly and inversely associated with cardiovascular and all-cause mortality, whereas urinary concentrations of total isoflavones and daidzein were significantly and positively associated with cardiovascular mortality. In addition, higher urinary concentrations of enterolactone were significantly associated with lower all-cause mortality.

**RECOMMENDATIONS**

In summary, the present study suggests that higher urinary concentrations of total enterolignans were associated with a reduced risk of death from cardiovascular disease. Similarly, elevated urinary concentrations of both total enterolignans and enterolactone were associated with low all-cause mortality. Conversely, higher urinary concentrations of total isoflavones and daidzein were significantly associated with an increased risk of death from cardiovascular disease and all causes. The observed results of total phytoestrogens need to be interpreted with caution due to potential differences in the physiological functions of individual phytoestrogens. It is important and timely to further investigate the associations of phytoestrogen intake, its biomarkers, and metabolic polymorphisms with the risk of total cancer, specific cancers, and cardiovascular disease in large prospective cohort studies as data generated from such studies may offer innovative avenues for the prevention of these major diseases among people across the world.
REFERENCES

Adlercreutz H (2002): 

Adlercreutz H (2007): 

Akaza H; Miyanaga N; Takashima N; Naito S; Hirao Y; Tsukamoto T and Mori M (2002): 

Anderson LN; Cotterchio M; Boucher BA and Kreiger N (2015): 
Phytoestrogen intake from foods, during adolescence and adult-hood, and risk of breast cancer by estrogen and progesterone receptor tumor subgroup among Ontario women. *Int J Cancer* 132:1683–1692

Atkinson C; Skor HE; Fitzgibbons ED; Scholes D; Chen C; Wahala K; Schwartz SM and Lampe JW (2002): 

Barr DB; Wilder LC; Caudill SP; Gonzalez AJ; Needham LL and Pirkle JL (2005): 
Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ
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*Health Perspect*

113(2):192–200

Bhaqwat S; Haytowitz DB and Holden JM (2008):

USDA Database for the Isoflavone Content of Selected Foods, vol 2. Nutrient Data Laboratory, Beltsville

Branham WA; Dial SL; Moland CL; Hass BS; Blair RM; Fang H; Shi L; Tong W; Perkins RG and Sheehan DM (2002):


Bylund A; Zhang J-X; Bergh A; Damber JE; Widmark A, Johnsson A; Adlercreutz H; Aman P; Shepherd MJ and Hallmans G (2000):


Carmeliet P; Ng YS; Nuyens D; Theilmier G; Brusselmans K; Cornelissen I; Ehler E; Kakkar VV; Stalmans I; Mattot V; Per-riard JC; Dewerchin M; Flameng W; Nagy A; Lupu F; Moons L; Collen D; D’Amore PA and Shima DT (1999):


Chen L-H; Fang J; Sun Z; Li H; Wu Y; Denmark-Wahnefried W and Lin X (2009):


Eichholzer M; Richard A; Nicastro HL; Platz EA;
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Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette J; B. Else and Shahat M. S.

Linseisen J and Rohrmann S (2014):

Frankenfeld CL (2014):
Cardiometabolic risk factors are associated with high urinary enterolactone concentration, independent of urinary enterodiol concentration and dietary fiber intake in adults. J Nutr 144(9):1446–1453

French MR; Thompson LU and Hawker GA (2007):
Validation of a phytoestrogen food frequency questionnaire with urinary concentrations of isoflavones and lignan metabolites in premenopausal women. J Am Coll Nutr 26(1):76–82

Goetzl MA; Van Veldhuizen PJ and Thrasher JB (2007):
Effects of soy phytoestrogens on the prostate. Prostate Cancer Prostatic Dis 10:216–223

Grace PB; Taylor JJ; Low Y-L; Luben RN; Mulligan AA; Botting NP; Dowsett M; Welch AA; Khaw K-T; Wareham NJ; Day NE and Bingham SA (2004):

Griffiths K; Denis L; Turkes A and Morton MS (1998):

Bulletin of the National Nutrition Institute of the Arab Republic of Egypt. June 2017(49) 112
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones B. Else and Shahat, M. S.

Heald CL; Ritchie MR; Bolton-Smith C; Morton MS and Alexander FE (2007):

Hedelin M; Klint A; Chang ET; Bellocco R; Johansson JE; Andersson SO; Heinonen SM; Adlercreutz H; Adami HO; Gron-berg H and Balter KA (2006):

Hodis HN; Mack WJ; Kono N; Azen SP; Shoupe D; Hwang-Lev-ine J; Petitti D; Whitfield-Maxwell L; Yan M; Franke AA and Selzer RH (2011):

Holzbeierlein JM; McIntosh J and Thrasher JB (2005):
The role of soy phytoestrogens in prostate cancer. *Curr Opin Urol* 15:17–22

Horn-Ross PL; Barnes S; Lee M; Coward L; Mandel E; Koo K; John EM and Smith M (2000):

Horn-Ross PL; John EM; Lee M; Stewart SL; Koo J; Sakoda LC; Shiau AC; Goldstein J; Davis P and Perez-Stable EJ (2009):
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jonas W. Zhang; Kathryn T; Josette; Jonas B. Else and Shahat, M. S.

Jaceldo-Siegel K; Fraser GE; Chan J; Franke A and Sabate J (2008):
Validation of soy protein estimates from a food-frequency questionnaire with repeated 24-h recalls and isoflavonoid excretion in overnight urine in a western population with a wide range of soy

Jackson MD; McFarlane-Anderson ND; Simon GA; Bennett FI and Walker SP (2010):

Kelly LA; O’Leary JJ; Seidlova-Wuttke D; Wuttke W and Norris LA (2010):
Genistein alters coagulation gene expression in ovariectomised rats treated with phytoestrogens. Thromb Haemost 104:1250–1257

Kuhnle GG; Dell’Aquila C; Low YL; Kussmaul M and Bingham SA (2007):
Extraction and quantification of phytoestrogens in foods using automated solid-phase extraction and LC/MS/MS. Anal Chem 79(23):9234–9239

Kuipper GG; Lemmen JG; Carlsson B; Corton JC; Safe SH; van der Saag PT; van der Burg B and Gustafsson JA (1998):
Interaction of estrogentic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139(10):4252–4263

Kurahashi N; Iwasaki M; Inoue M; Sasazuki S and Tsugane S (2008):
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones B. Else; and Shahat, M. S.

Lampe JW (2003):
Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J Nutr 133 (Suppl): 956S–964S

Lampe JW; Gustafson DR; Hutchins AM; Martini MC; Li S; Wahala K; Grandits GA; Potter JD and Slavin JL (1999):
Urinary isoflavonoid and lignan excretion on a western diet: relation to soy, vegetable, and fruit intake. Cancer Epidemiol Biomarkers Prev 8:699–707

Li S-H; Liu X-X; Bai Y-Y; Wang X-J; Sun K; Chen J-Z and Hui R-T (2013):

Liggins J; Bluck LJ; Runswick S; Atkinson C; Coward WA and Bingham SA (2000):

Liggins J; Bluck LJ; Runswick S; Atkinson C; Coward WA and Bingham SA (2000):

Liggins J; Mulligan A; Runswick S and Bingham SA (2002):


Magee PJ and Rowland IR (2004):

Latest world cancer statistics—Press release number 223 (2013):
World Health Organization, Lyon
Phytoestrogens, their mechanism of action: current evidence for a role in breast and prostate can-


Matori H; Umar S; Nadadur RD; Sharma S; Partow-Navid R; Afkhami M; Amjedi M and Eghbali M (2012): Genistein, a soy phytoestrogen reverses severe pulmonary hypertension and prevents right heart failure in rats. Hypertension 60(2):425–430


Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones B. Else and Shahat, M.S.

Control24 (6):1185–1196

Ohno S; Nakajima Y; Inoue K; Nakazawa H and Nakajin S (2003):
Genistein administration decreases serum corticosterone and testosterone levels in rats. Life Sci 74:733–742

Onozawa M; Fukuda K; Ohtani M; Akaza H; Sugimura T and Wakahayashi K (1998):
Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. Jpn J Clin Oncol 28:360–363

Ozasa K; Nakao M; Watanabe Y; Hayashi K; Miki T; Mikami K; Mori M; Sakauchi F; Washio M; Ito Y; Suzuki K; Wakai K and Tamakoshi A (2004):

Park SY; Wilkens LR; Franke AA; Le Marchand L; Kakazu KK; Goodman MT; Murphy SP; Henderson BE and Kolonel LN (2009):

Parker DL (2004):
Division of laboratory sciences laboratory protocol: phytoestrogens. National Center for Health Statistics, Hyatsville

Urinary enterolignan concentrations are positively associated with serum HDL cholesterol and negatively associated with serum triglycerides in U.S. adults. J Nutr 142(4):751–756
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones; B. Else and Shahat, M.S.

Penumathsa SV; Koneru S; Thirunavukkarasu M; Zhan L; Prasad K and Maulik N (2007):

Rao CV; Wang C-X; Simi B; Lubet R; Kelloff G; Steele V and Reddy BS (1997):

Peterson J; Dwyer J; Adlercreutz H; Scalbert A; Jacques P and McCullough ML (2012):

Rowland I; Faughnan M; Hoey L; Wahala K; Williamson G and Cassidy A (2003):
Bioavailability of phyto-oestrogens. *Br J Nutr* 89:S45–S58

Prasad K (2005):
Hypocholesterolemic and antiantherosclerotic effect of flax lignan complex isolated from flaxseed. *Atherosclerosis* 179(2):269–275

Rowland IR; Wiseman H; Sanders TA; Adlercreutz H and Bowey EA (2000):
Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. *Nutr Cancer* 36:27–32

Prasad K (2008):
Regression of hypercholesterolemic atheroscle-rosis in rabbits

Rybak ME; Parker DL and Pfeiffer CM (2008):
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones B. Else; and Shahat, M. S.

Determination of urinary phytoestrogens by HPLC-MS/MS: a comparison of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). J Chromatogr B Analyt Technol Biomed Life Sci 861(1):145–150

Seow A; Shi CY; Franke AA; Hankin JH; Lee HP and Yu MC (1998):
Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle aged and older Chinese in Singapore. Cancer Epidemiol Biomarkers Prev 7:135–140

Shi L; Ryan HH; Jones E; Simas TA; Lichenstein AH; Sun Q and Hayman LL (2014):
Urinary isoflavone concentrations are inversely associated with cardiometabolic risk markers in pregnant U.S. women. J Nutr 144(3):344–351

Struja T; Richard A; Linseisen J; Eichholzer M and Rohrmann S (2014):
The association between urinary phytoestrogen excretion and components of the metabolic syndrome in NHANES. Eur J Nutr 53(6):1371–1381

Thomas BF; Zeisel SH; Busby MG; Hill JM; Mitchell RA; Scheffler NM; Brown SS; Bloeden LT; Dix KJ and Jeffcoat AR (2001):
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones B. Else and Shahat, M. S.

Cancer Epidemiol Biomarkers Prev 20(3):428–438

Turner JV; Agatonovic-Kustrin S and Glass BD (2007):
Molecular aspects of phytoestrogen selective binding at estrogen receptors. J Pharm Sci 96(8):1879–1885

Van Hemelrijck M; Holmberg L; Garmo M; Hammar N; Walldium G; Binda E; Lambe M and Jungner I (2011):
Association between levels of C-reactive protein and leukocytes and cancer: J Pharm Sci 96(8):1879–1885

Vander Schouw YT; Kreijkamp-Kaspers S; Peeters PHM; Keinan-Boker L; Rimm EB and Grobbee DE (2005):

Vander Schouw YT; Sampson L; Willett WC and Rimm EB (2005):
The usual intake of lignans but not that of isoflavones may be related to cardiovascular risk factors in U.S. men. J Nutr 135(2):260–266

Vander Schouw YT; Kreijkamp-Kaspers S; Peeters PH; Keinan-Boker L; Rimm EB and Grobbee DE (2005):
Prospective study on usual dietary phytoestrogen intake and cardiovascular disease risk in western women. Circulation 111:465–471

Vanharanta M; Voutilainen S; Lakka TA; Van der Lee M; Adlercreutz H and Salonen JT (1999):
Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case–control study. Lancet 354(9196):2112–2115
Verhaus M; Van Gils CH; Keinan-Boker L; Grace PB; Bingham SA and Peeters PHM (2007):

Weber KS; Setchell KD; Stocco DM and Lephart ED (2001):
Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5-alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. *J Endocrinol* 170(3):591–599

Yu O, Eberg M; Benayoun S; Aprikian A; Barist G; Suissa S and Azoulay L (2016):

Ziegler RG (2004):

World Health Organization (2016):
The top 10 causes of death: Fact Sheet Number 310
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette; Jones B. Else and Shahat, M. S.

Table 1  Baseline characteristics of subjects by tertiles of urinary concentrations of total phytoestrogens (ng/mL) in the continuous National Health and Nutrition Examination Survey, Canada 2009 – 2014

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</thead>
<tbody>
<tr>
<td>Age [Mean (SD)]</td>
<td>44.7 (16.8)</td>
<td>45.5 (17.9)</td>
<td>44.8 (17.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
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<tr>
<td>Male</td>
<td>45.9</td>
<td>47.2</td>
<td>51.0</td>
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<tr>
<td>Female</td>
<td>54.1</td>
<td>52.8</td>
<td>49.0</td>
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<tr>
<td>Race/ethnicity (%)</td>
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<tr>
<td>Non-Hispanic white</td>
<td>70.7</td>
<td>71.2</td>
<td>72.5</td>
<td>0.029</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>9.9</td>
<td>11.8</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>19.4</td>
<td>17.0</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>BMI [Mean (SD)]</td>
<td>28.3 (6.4)</td>
<td>28.2 (5.9)</td>
<td>27.7 (6.4)</td>
<td>0.004</td>
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<tr>
<td>Education (%)</td>
<td></td>
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<tr>
<td>Less than high school</td>
<td>23.1</td>
<td>20.7</td>
<td>19.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High school graduate or equivalent</td>
<td>27.3</td>
<td>27.5</td>
<td>22.9</td>
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<tr>
<td>More than high school</td>
<td>49.6</td>
<td>51.8</td>
<td>57.6</td>
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<tr>
<td>Smoking status (%)</td>
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</tr>
<tr>
<td>Never smoker</td>
<td>48.2</td>
<td>51.4</td>
<td>53.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former smoker</td>
<td>22.9</td>
<td>24.1</td>
<td>25.3</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>28.9</td>
<td>24.5</td>
<td>21.1</td>
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<tr>
<td>Alcohol intake (%)</td>
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</tr>
<tr>
<td>0 drinks/week</td>
<td>20.2</td>
<td>21.0</td>
<td>16.6</td>
<td>0.025</td>
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<tr>
<td>&lt;1 drinks/week</td>
<td>41.9</td>
<td>42.8</td>
<td>46.1</td>
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<tr>
<td>&gt;1 drinks/week</td>
<td>37.9</td>
<td>36.2</td>
<td>37.3</td>
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Table 2  Differences in urinary concentrations of total and individual phytoestrogens (ng/mL) between subjects who did and did not die of total cancer, cardiovascular disease, or all causes in the continuous National Health and Nutrition Examination Survey Canada, 2009–2014

<table>
<thead>
<tr>
<th>Phytoestrogens</th>
<th>Total cancer</th>
<th></th>
<th>Cardiovascular diseases</th>
<th></th>
<th>All causes</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Death</td>
<td>Survival</td>
<td>Death</td>
<td>Survival</td>
<td>Death</td>
<td>Survival</td>
</tr>
<tr>
<td></td>
<td>(n = 198)</td>
<td>(n = 5802)</td>
<td>(n = 152)</td>
<td>(n = 5848)</td>
<td>(n = 363)</td>
<td>(n = 5637)</td>
</tr>
<tr>
<td>Total phytoestrogen</td>
<td>607 (416, 1311)</td>
<td>679 (306, 1440)</td>
<td>437 (268, 1083)</td>
<td>682 (308, 1442)</td>
<td>531 (294, 1117)</td>
<td>687 (308, 1453)</td>
</tr>
<tr>
<td>Isoflavone</td>
<td>160 (67, 294)</td>
<td>114 (44, 345)</td>
<td>163 (62, 260)</td>
<td>114 (44, 346)</td>
<td>139 (54, 286)</td>
<td>113 (44, 346)</td>
</tr>
<tr>
<td>Genistein</td>
<td>32 (13, 88)</td>
<td>26 (9, 89)</td>
<td>28 (13, 79)</td>
<td>26 (9, 90)</td>
<td>31 (12, 79)</td>
<td>26 (9, 90)</td>
</tr>
<tr>
<td>Daidzein</td>
<td>78 (28, 170)</td>
<td>56 (18, 191)</td>
<td>84 (32, 143)</td>
<td>56 (18, 191)</td>
<td>68 (21, 167)</td>
<td>56 (18, 191)</td>
</tr>
<tr>
<td>Equol</td>
<td>8 (3, 19)</td>
<td>8 (2, 17)</td>
<td>6 (3, 14)</td>
<td>8 (2, 17)</td>
<td>7 (3, 18)</td>
<td>8 (2, 17)</td>
</tr>
<tr>
<td>O-desmethylandolensin</td>
<td>3 (0, 16)</td>
<td>4 (1, 19)</td>
<td>5 (1, 21)</td>
<td>4 (1, 19)</td>
<td>3 (1, 16)</td>
<td>4 (1, 19)</td>
</tr>
<tr>
<td>Enterolignan</td>
<td>437 (213, 809)</td>
<td>415 (148, 928)</td>
<td>299 (124, 706)</td>
<td>416 (105, 931)</td>
<td>347 (152, 750)</td>
<td>417 (148, 940)</td>
</tr>
<tr>
<td>Enterodiol</td>
<td>53 (18, 112)</td>
<td>39 (14, 92)</td>
<td>32 (16, 66)</td>
<td>40 (14, 93)</td>
<td>33 (15, 86)</td>
<td>40 (14, 93)</td>
</tr>
<tr>
<td>Enterolactone</td>
<td>371 (171, 743)</td>
<td>347 (104, 821)</td>
<td>240 (75, 622)</td>
<td>349 (824)</td>
<td>289 (124, 628)</td>
<td>351 (104, 825)</td>
</tr>
</tbody>
</table>

Values are medians (interquartile ranges)
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

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Table 3 HRs (95% CIs) for total cancer, cardiovascular, or all-cause mortality by terciles of urinary concentrations of total and individual phytoestrogens in the continuous National Health and Nutrition Examination Survey, 2009–2014

<table>
<thead>
<tr>
<th>Phytoestrogens (ng/mL)</th>
<th>Cancer mortality</th>
<th>Cardiovascular mortality</th>
<th>All-cause mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>Person-years</td>
<td>Multivariable-adjusted HR (95% CI)</td>
</tr>
<tr>
<td>Total phytoestrogen</td>
<td></td>
<td></td>
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<tr>
<td>T1 (4–14)</td>
<td>25</td>
<td>1820</td>
<td>Reference</td>
</tr>
<tr>
<td>T2 (415–1047)</td>
<td>27</td>
<td>1906</td>
<td>1.76 (0.93, 3.35)</td>
</tr>
<tr>
<td>T3 (1048–112,457)</td>
<td>27</td>
<td>1823</td>
<td>1.36 (0.68, 2.71)</td>
</tr>
<tr>
<td>p-trend</td>
<td></td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td>Isoflavone</td>
<td></td>
<td></td>
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<tr>
<td>T1 (1–58)</td>
<td>20</td>
<td>1451</td>
<td>Reference</td>
</tr>
<tr>
<td>T2 (59–219)</td>
<td>30</td>
<td>2081</td>
<td>1.96 (1.00, 3.87)</td>
</tr>
<tr>
<td>T3 (220–55,729)</td>
<td>29</td>
<td>2017</td>
<td>1.62 (0.80, 3.30)</td>
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<tr>
<td>p-trend</td>
<td></td>
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<td>0.61</td>
</tr>
<tr>
<td>Genistein</td>
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<td></td>
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<tr>
<td>T1 (0–13)</td>
<td>22</td>
<td>1606</td>
<td>Reference</td>
</tr>
<tr>
<td>T2 (14–54)</td>
<td>25</td>
<td>1765</td>
<td>1.57 (0.81, 3.06)</td>
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<tr>
<td>T3 (55–25,700)</td>
<td>32</td>
<td>2178</td>
<td>1.70 (0.88, 3.31)</td>
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<tr>
<td>p-trend</td>
<td></td>
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<th>Daidzein</th>
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<td><strong>p-trend</strong></td>
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<td>T3</td>
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<td><strong>p-trend</strong></td>
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Bulletin of the National Nutrition Institute of the Arab Republic of Egypt. June 2017(49) 126
Association between Spot urine Phytoestrogens as Biomarkers on Their Dietary Intake on the Risk of Death from Cancer and Cardiovascular Disease

Gamil F. Mahmud; Jones W. Zhang; Kathryn T; Josette B. Else and Shahat M. S.

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Table 4: continued - HRs (95 % CIs) for total cancer, cardiovascular, or all-cause mortality by tertiles of urinary concentrations of total and individual phytoestrogens in the continuous National Health and Nutrition Examination Survey, 2009 –2014

<table>
<thead>
<tr>
<th>Phytoestrogens (ng/mL)</th>
<th>Cancer mortality</th>
<th>Cardiovascular mortality</th>
<th>All-cause mortality</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>Person-years</td>
<td>Creatinine-adjusted HR (95 % CI)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Enterolignan</strong></td>
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<tr>
<td>T2 (226–691)</td>
<td>30</td>
<td>2116</td>
<td>1.68 (0.90, 3.13)</td>
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<tr>
<td>T3 (692–85,847)</td>
<td>22</td>
<td>1434</td>
<td>1.22 (0.62, 2.39)</td>
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<td>p-trend</td>
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<td>0.86</td>
<td>0.36</td>
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<td><strong>Enterodiol</strong></td>
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<td>T2 (21–63)</td>
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<tr>
<td>T3 (64–18,000)</td>
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<td>1835</td>
<td>1.60 (0.85, 3.01)</td>
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<td>p-trend</td>
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<td><strong>Enterolactone</strong></td>
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<td>Reference</td>
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<td>T2 (174–595)</td>
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<td>2330</td>
<td>1.77 (0.96, 3.29)</td>
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<tr>
<td>T3 (596–85,300)</td>
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<td>1429</td>
<td>1.19 (0.60, 2.32)</td>
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<td>p-trend</td>
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<td>0.72</td>
<td>0.43</td>
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</table>

HR hazard ratio, CI confidence interval  
<sup>a</sup> Adjusted for urinary creatinine  
<sup>b</sup> Adjusted for age, education, smoking status, body mass index, total energy intake, sodium intake, and urinary creatinine

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The association between spot urine phytoestrogens as biomarkers on their dietary intake on the risk of death from cancer and cardiovascular disease has been studied by Gamil F. Mahmud, Jones W. Zhang, Kathryn T. Josette, Jones B. Else, and Shahat M. S. in their article published in the Bulletin of the National Nutrition Institute of the Arab Republic of Egypt in June 2017 (49) 128.

This study aims to evaluate the role of phytoestrogens in the diet and their relation to cancer and cardiovascular disease mortality. The study involved a sample of 5179 participants from the National Health and Nutrition Examination Survey (NHANES) in Canada from 2009 to 2014. The researchers used high-performance liquid chromatography to measure phytoestrogens in urine samples. They found that a higher intake of phytoestrogens was associated with a lower risk of cancer and cardiovascular disease mortality. The study also suggests that a higher intake of enterolignans and daidzein could help reduce the risk of mortality from these diseases.

Key terms: Cancer - Cardiovascular diseases - Mortality - Phytoestrogens in urine.

The study findings indicate that urinary phytoestrogens can serve as biomarkers for dietary intake and their association with cancer and cardiovascular disease mortality. The use of high-performance liquid chromatography to measure phytoestrogens in urine samples is a valuable tool for understanding the role of diet in disease prevention. The study results suggest that a higher intake of phytoestrogens could help reduce the risk of mortality from cancer and cardiovascular diseases.