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

Gamil F. Mahmud [1] ; Jones W. Zhang [1] · Kathryn T. Josette [2] · Jones B. Else [3] · Shahat , M. S. [4]

[1] Functional Foods Department, National Institute of Nutrition Canada, Ottawa.
[2] Center for Disease Prevention and Control, Ontario
[3] National Health and Nutrition Examination Survey (NHANES) Canada
[4] Food Science and Technology Department, faculty of Agriculture, Al Azhar university, Cairo, Egypt

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 per-formed to evaluate hazard ratios (HRs) and 95 % confi-dence 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 10.48; 95 % CI 0.24, 0.97), whereas higher urinary concentrations of total isoflavones (HR for tertile 3 vs. tertile 12.14; 95 % CI 1.03, 4.47) and daidzein (HR for tertile 3 vs. tertile 12.05; 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 10.65; 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 associ-ated with cardiovascular and all-cause mortality in a rep-resentative 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 bioac-tive compounds on the risk of death from cancer and car-diovascular 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 cardiovas-cular 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 origi-nate from seed oils, whole-grain cereals, and beans **Adlercreutz (2007)**. Isoflavones found in soy products include genistein, daid-zein, 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 popula-tions **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 phytoestro-gens 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 rela-tion 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, anti-viral, 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 clini-cal effects by selectively triggering this particular recep-tor **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 phytoes-trogens 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 mor-tality, 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 examina-tion. As the objective of the present study was to investi-gate urinary phytoestrogens in relation to cancer, cardio-vascular, 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 par-ticipant burden and facilitate scheduling and completion of examinations. All subjects in the subsample were ran-domly 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)**. Exclud-ing 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 iden-tified during a mean follow-up of approximately 6 years (2009–2014). The de-identified data analyzed in the pre-sent 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 (mar-ried 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/m^2) was calculated from height and weight measured during the medical examination portion of data collection.

Urinary phytoestrogen measurement

Phytoestrogen biomonitoring was accomplished by meas-uring urinary excretion of isoflavones (including daidzein, genistein, equol, and *O*-desmethylangolensin) and enterol-ignans (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 phy-toestrogen 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 preci-sion,

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 isofla-vones and total enterolignans. Demographic, anthropomet-ric, and lifestyle characteristics of subjects (including age, gender, race, BMI, education, smoking status, and alcohol intake) were compared by the tertiles of total urinary phy-toestrogen (ng/ml) (tertile 14–414; tertile 2415–1047; ter-tile 31048–112,457). Chi-square tests and analysis of vari-ance were employed to compare differences in categorical and continuous variables among tertiles, respectively. Uri-nary 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 inter-vals (CIs) for cancer, cardiovascular, and all-cause mortal-ity 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.

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 outcomes of interest in univariate models or did not sub-stantively alter any risk estimates for all outcomes consid-ered (<10 %). No interactions tested were found to be sta-tistically 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 phytoes-trogens in relation to each of the three outcomes included age, gender, 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.

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

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 uri-nary concentrations of total phytoestrogens were lower in individuals who died of each of the three outcomes exam-ined 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 sub-jects 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 phy-toestrogens 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 uri-nary daidzein (HR for tertile 3 vs. tertile 12.05; 95 % CI 1.02, 4.11). Conversely, higher total enterolignan 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 ter-tile 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 posi-tively associated with cardiovascular

mortality. In addition, higher urinary concentrations of enterolactone were signifi-cantly associated with lower all-cause mortality.

Genistein is a main isoflavone present in soy products and has been one of the most widely investigated phy-toestrogen 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 pros-tate 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 metabo-lite 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 pre-sent study showed low all-cause mortality associated with elevated urinary excretion of both total enterolignans and

enterolactone. The consumption of lignanrich 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 pros-tate 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 par-tially contributes to its inverse association with all-cause mortality.

Experimental and epidemiologic data are scarce examin-ing the influence of intake of total and individual phytoes-trogens 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 asso-ciated 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

cell counts), obesity, and metabolic syndrome in human studies **Frankenfeld, (2014); Struja et al (2014)**. Animal and in vitro studies have offered additional mechanistic basis for the reduced cardiovascular mortality associated with elevated levels of urinary enterolactone **Prasad,(2005); Penumathsa , et al. (2007)**. Specifically, lignan complex [including secoisolariciresinol diglucoside (SDG)] isolated from flaxseed reduced the extent of hypercholesterolemic atherosclerosis and promoted its regression in rabbits **Prasad, (2005); Prasad , (2008)**. SDG induced an elevated expression of vascu-lar endothelial growth factor (VEGF) in human coronary arteriolar endothelial cells **Penumathsa, et al. (2007)**, and lack of VEGF led to ischemic cardiomyopathy in mice **Carmeliet, et al. (1999)**.

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 middleaged 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

those studies, indicators of cardiovascular functions or biomarkers of cardiovascular lesions were examined; instead, the present study evaluated urinary excretion of total isofla-vones and daidzein in relation to cardiovascular mortality among adult women and men of all ages. The potential bio-logical 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 meas-uring their urinary concentrations. Urinary excretion of phytoestrogens is free of recall bias inherent in food fre-quency questionnaires and is an integrated reflection of phytoestrogen intakes from all sources, including those that may be inadequately represented in food composi-tion 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 nat-urally present in lower concentrations in other foods such as vegetables **Liggins, et al., (2000)**, fruits, and nuts **Liggins, et al. (2000)**. Another theoreti-cal 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 Agricul-ture 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 phy-toestrogens 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 mor-tality 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 cardiovascu-lar 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 normal-ized to urinary creatinine levels by including urinary cre-atinine in the Cox models, a commonly used method **Seow, et al., (1998)**, **Atkinson; et al., (2002)** because creatinine is excreted by glomerular filtra-tion 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 con-centrations 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 phytoes-trogens were measured only once, and a single measure-ment 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 par-tially ascribed to reverse causality due to the presence of subclinical disease. As NHANES did not exclude individu-als with diseases at baseline, some individuals with clini-cal 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 interpret-ing 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 signifi-cantly 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 concentra-tions of total isoflavones and daidzein were significantly associated with an increased risk of death from cardiovas-cular 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 pre-vention of these major diseases among people across the world.

REFERENCES

Adlercreutz H (2002):
Phyto-oestrogens and cancer. *Lancet Oncol 3:364–373*

Adlercreutz H (2007):
Lignans and human health. *Crit Rev Clin Lab Sci 44:483–525*

Akaza H; Miyanaga N; Takashima N; Naito S; Hirao Y; Tsukamoto T and Mori M (2002):
Is daidzein non-metabolizer a high risk for prostate cancer? A case–controlled study of serum soybean isoflavone concentration. *Jpn J Clin Oncol 32(8):296–300*

Analytic and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES) (2006)
Centers for Disease Control and Prevention, Hyattsville

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):
Overnight urinary isoflavone excretion in a population of women living in the United States, and its relationship to isoflavone intake. *Cancer Epidemiol Biomarkers Prev 11:253–260*

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 meas-urements. *Environ*

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):

Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. *J Nutr 132(4):658–664*

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

Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. *Prostate42:304–314*

Carmeliet P; Ng YS; Nuyens D; Theilmeier 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):

Impaired myocar-dial angiogenesis and ischemic cardiomyopathy in mice lacking the vascular endothelial growth factor isoforms VEGF 164 and VEGF188. Nat Med 5(5):495–502 cer. *Br J Nutr 91:513–531*

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

Enterolactone inhibits insulin-like growth factor-1 receptor signaling in human prostatic carcinoma PC-3 cells. *J Nutr 139(4):653–659*

Eichholzer M; Richard A; Nicastro HL; Platz EA;

Linseisen J and Rohrmann S (2014):
Urinary lignans and inflammatory markers in the US National Health and Nutrition Examination Survey (NHANES) 2009-2014and 2005–2008. Cancer Causes Control *25(3):395–403*

Frankenfeld CL (2014):
Cardiometabolic risk factors are associ-ated 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 con-centrations of isoflavones and lignan metabolites in premenopau-sal 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 Dis10:216–223*

Grace PB; Taylor JI; Low Y-L; Luben RN; Mulligan AA; Bot-ting NP; Dowsett M; Welch AA; Khaw K-T; Wareham NJ; Day NEand Bingham SA (2004):
Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European Prospective Investigation of Cancer and Nutrition-Norfolk. *Cancer Epide-miol Biomarkers Prev 13(5):698–70*

Griffiths K; Denis L; Turkes A and Morton MS (1998):
Possible relationship between dietary factors and pathogenesis of prostate cancer. *Int J Urol 5:195–213*

Heald CL; Ritchie MR; Bolton-Smith C; Morton MS and Alexander FE (2007):
Phyto-oestrogens and risk of prostate cancer in Scot-tish men. *Br J Nutr 98:388–396*

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):
Dietary phytoestrogen, serum entero-lactone and risk of prostate cancer: the cancer prostate Sweden study. Cancer Causes Control 17:169–180

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):
Isoflavone soy protein supplementation and athero-sclerosis progression in healthy postmenopausal women: a rand-omized controlled trial. *Stroke 42:3168–3175*

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):
Assessing phytoestrogen exposure epidemiologic studies: development of a database (United in States). *Cancer Causes Control 11:299–302*

Horn-Ross PL; John EM; Lee M; Stewart SL; Koo J; Sakoda LC; Shiau AC; Goldstein J; Davis P and Perez-Stable EJ (2009):
Phytoes-trogen consumption and breast cancer risk in a multiethnic popu-lation. Am J Epidemiol 154(5):434–441 intakes. *Am J Clin Nutr 87(5):1422–1427*

Jaceldo-Siegl 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):
Urinary phytoestrogens and risk of prostate cancer in Jamaican men. *Cancer Causes Control 21(12):2249–2257*

Kelly LA; O'Leary JJ; Seidlova-Wuttke D; Wuttke W and Norris LA (2010):
Genistein alters coagulation gene expression in ova-riectomised 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 estrogenic chemicals and phytoestrogens with estrogen recep-tor beta. *Endocrinology 139(10):4252–4263*

Kurahashi N; Iwasaki M; Inoue M; Sasazuki S and Tsugane S (2008):
Plasma isoflavones and subsequent risk of prostate cancer in a nested case–control study: the Japan Public Health Center. *J Clin Oncol 26:5923–5929*

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*

Latest world cancer statistics—Press release number 223 (2013):
World Health Organization, Lyon

Li S-H; Liu X-X; Bai Y-Y; Wang X-J; Sun K; Chen J-Z and Hui R-T (2013):
Effect of oral supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of rand-omized placebo-controlled trials. *Am J Clin Nutr 91(2):480–486*

Liggins J; Bluck LJ; Runswick S; Atkinson C; Coward WA and Bingham SA (2000):
Daidzein and genistein contents of vegeta-bles. *Br J Nutr 84:717–725*

Liggins J; Bluck LJ; Runswick S; Atkinson C; Coward WA and Bingham SA (2000):
Daidzein and genistein content of fruits and nuts. *J Nutr Biochem 11(6):326–331*

Liggins J; Mulligan A; Runswick S and Bingham SA (2002):
Daid-zein and genistein content of cereals. *Eur J Clin Nutr 56:961–966*

Lissin LW and Cooke JP (2000):
Phytoestrogens and cardiovascular health. *J Am Coll Cardiol 35(6):1403–1410*

Magee PJ and Rowland IR (2004):

Phytoestrogens, their mechanism of action: current evidence for a role in breast and prostate can-

Maskarinec G; Singh S; Meng L and Franke AA (1998):
Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. *Cancer Epidemiol Biomarkers Prev7(7):613–619*

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*

Murphy SL; Xu J and Kochanek KD (2016):
Deaths: final data for2015. Centers for Disease Control and Prevention, Atlanta

National Health and Nutrition Examination Survey (2009-2014):
Linked Mortality Files (2009) Office of Analysis and Epidemiology, Hyatsville

NHANES (2014):
Public-use Linked Mortality Files (2006) (2013) National Health and Nutrition Examination Survey

NHANES 2009–2014 Public Data Release File Documentation (2015):
Canada Department of Health and Human Services, Centers for Disease Control and Prevention, Hyattsville

Nicastro HL; Mondul AM; Rohrmann S and Platz EA (2015):
Associations between urinary soy isoflavonoids and two inflammatory markers in the United States in 2005-2008. *Cancer Causes*

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 Waka-bayashi K (1998):
Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell link 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):
Serum phytoestrogens and prostate cancer risk in a nested case–control study among Japanese men. *Cancer Sci95(1):65–71*

Park SY; Wilkens LR; Franke AA; Le Marchand L; Kakazu KK; Goodman MT; Murphy SP; Henderson BE and Kolonel LN (2009):
Urinary phytoestrogen excretion and prostate cancer risk: a nested case–control study in the Multiethnic Cohort. *Br J Cancer 101(1):185–191*

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

Penalvo JL and Lopez-Romero P (2012):
Urinary enterolignan con-centrations are positively associated with serum HDL cholesterol and negatively associated with serum triglycerides in U.S. adults. *J Nutr 142(4):751–756*

Penumathsa SV; Koneru S; Thirunavukkarasu M; Zhan L; Prasad K and Maulik N (2007):
Secoisolariciresinol diglucoside: relevance to angiogenesis and cardioprotection against ischemia-reperfu-sion injury. *J Pharmacol Exp Ther 320(2):951–959*

Peterson J; Dwyer J; Adlercreutz H; Scalbert A; Jacques P and McCullough ML (2012):
Dietary lignans: physiology and potential for cardiovascular disease risk reduction. Nutr Rev 68(10):571–603

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

Prasad K (2008):
Regression of hypercholesterolemic atheroscle-rosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. *Atherosclerosis 197(1):34–42*

Rao CV; Wang C-X; Simi B; Lubet R; Kelloff G; Steele V and Reddy BS (1997):
Enhancement of experimental colon cancer by genistein. *Cancer Res 57:3717–3722*

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

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*

Rybak ME; Parker DL and Pfeiffer CM (2008):

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):

Quantitative analysis of the principle soy isoflavones genistein, daidzein and glycitein, and their primary conjugated metabolites in human plasma and urine using reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl 760(2):191–205 three repeated measurements in the Swedish AMORIS study.

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 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*

Vander Schouw YT; Kreijkamp-Kaspers S; Peeters PHM; Keinan-Boker L; Rimm EB and Grobbee DE (2005):
Cardiovascular disease in women: prospective study on usual dietary phytoes-trogen intake and cardiovascular disease risk in western women. *Circulation 111(4):465–471*

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*

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):

Plasma phytoestrogens and subsequent breast cancer risk. *J Clin Oncol 25(6):648–655*

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*

World Health Organization (2016):

The top 10 causes of death: Fact Sheet Number 310

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

Use of statins and the risk of death in patients with prostate cancer. *J Clin Oncol 32(1):5–11*

Ziegler RG (2004):

Phytoestrogens and breast cancer. *Am J Clin Nutr 79(2):183–184*

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

Characteristics	Tertile 1	Tertile 2	Tertile 3	*p* value
	(4–414)	(415–1047)	(1048–112,457)	
	n = 1726	*n* = 1727	*n* = 1726	
Age [Mean (SD)]	44.7 (16.8)	45.5 (17.9)	44.8 (17.4)	0.28
Gender (%)				
Male	45.9	47.2	51.0	0.006
Female	54.1	52.8	49.0	
Race/ethnicity (%)				
Non-Hispanic white	70.7	71.2	72.5	0.029
Non-Hispanic black	9.9	11.8	11.7	
Other	19.4	17.0	15.8	
BMI [Mean (SD)]	28.3 (6.4)	28.2 (5.9)	27.7 (6.4)	0.004
Education (%)				
Less than high school	23.1	20.7	19.5	<0.001
High school graduate or equivalent	27.3	27.5	22.9	
More than high school	49.6	51.8	57.6	
Smoking status (%)				
Never smoker	48.2	51.4	53.6	<0.001
Former smoker	22.9	24.1	25.3	
Current smoker	28.9	24.5	21.1	
Alcohol intake (%)				
0 drinks/week	20.2	21.0	16.6	0.025
<1 drinks/week	41.9	42.8	46.1	
>1 drinks/week	37.9	36.2	37.3	

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

Phytoestrogens	Total cancer				Cardiovascular diseases				All causes			
	Death		Survival		Death		Survival		Death		Survival	
	(*n* = 198)		(*n* = 5802)		(*n* = 152)		(*n* = 5848)		(*n* = 363)		(*n* = 5637)	
Total phytoestrogen	607	(416, 1311)	679	(306, 1440)	437	(268, 1083)	682	(308, 1442)	531	(294, 1117)	687	(308, 1453)
Isoflavone	160	(67, 294)	114	(44, 345)	163	(62, 260)	114	(44, 346)	139	(54, 286)	113	(44, 346)
Genistein	32	(13, 88)	26	(9, 89)	28	(13, 79)	26	(9, 90)	31	(12, 79)	26	(9, 90)
Daidzein	78	(28, 170)	56	(18, 191)	84	(32, 143)	56	(18, 191)	68	(21, 167)	56	(18, 191)
Equol	8	(3, 19)	8	(2, 17)	6	(3, 14)	8	(2, 17)	7	(3, 18)	8	(2, 17)
O-desmethylangolensin	3	(0, 16)	4	(1, 19)	5	(1, 21)	4	(1, 19)	3	(1, 16)	4	(1, 19)
Enterolignan	437	(213, 809)	415	(148, 928)	299	(124, 706)	416	(149, 931)	347	(152, 750)	417	(148, 940)
Enterodiol	53	(18, 112)	39	(14, 92)	32	(16, 66)	40	(14, 93)	33	(15, 86)	40	(14, 93)
Enterolactone	371	(171, 743)	347	(104, 821)	240	(75, 622)	349	(105, 824)	289	(124, 628)	351	(104, 825)

Values are medians (interquartile ranges)

Table 3 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

Phytoestrogens (ng/mL)	Cancer mortality				Cardiovascular mortality				All-cause mortality			
	No. of cases	Person-years	Creatinine-adjusted HR (95 % CI)[a]	Multivariable-adjusted HR (95 % CI)[b]	No. of cases	Person-years	Creatinine-adjusted HR (95 % CI)[a]	Multivariable-adjusted HR (95 % CI)[b]	No. of cases	Person-years	Creatinine-adjusted HR (95 % CI)[a]	Multivariable-adjusted HR (95 % CI)[b]
Total phytoestrogen												
T1 (4–14)	25	1820	Reference	Reference	42	3035	Reference	Reference	102	7250	Reference	Reference
T2 (415–1047)	27	1906	1.76 (0.93, 3.35)	1.41 (0.72, 2.75)	37	2819	0.83 (0.47, 1.49)	0.58 (0.31, 1.09)	100	7269	1.06 (0.76, 1.47)	0.78 (0.55, 1.12)
T3 (1048–112,457)	27	1823	1.36 (0.68, 2.71)	1.18 (0.57, 2.46)	29	2231	0.80 (0.42, 1.53)	0.63 (0.31, 1.28)	88	6263	0.87 (0.60, 1.25)	0.69 (0.46, 1.02)
p-trend			0.73	0.90			0.55	0.36			0.36	0.09
Isoflavone												
T1 (1–58)	20	1451	Reference	Reference	31	2346	Reference	Reference	87	6332	Reference	Reference
T2 (59–219)	30	2081	1.96 (1.00, 3.87)	1.94 (0.96, 3.95)	37	2698	2.07 (1.09, 3.92)	1.97 (0.98, 3.97)	100	7119	1.46 (1.03, 2.08)	1.34 (0.93, 0.95)
T3 (220–55,729)	29	2017	1.62 (0.80, 3.30)	1.67 (0.79, 3.52)	40	3041	1.96 (1.01, 3.82)	2.14 (1.03, 4.47)	103	7331	1.26 (0.87, 1.83)	1.22 (0.82, 1.82)
p-trend			0.61	0.56			0.21	0.15			0.69	0.71
Genistein												
T1 (0–13)	22	1606	Reference	Reference	33	2455	Reference	Reference	87	6325	Reference	Reference
T2 (14–54)	25	1765	1.57 (0.81, 3.06)	1.44 (0.73, 2.87)	38	2828	1.76 (0.95, 3.24)	1.59 (0.83, 3.06)	97	6968	1.60 (1.13, 2.28)	1.44 (1.00, 2.08)
T3 (55–25,700)	32	2178	1.70 (0.88, 3.31)	1.46 (0.73, 2.93)	37	2802	1.70 (0.89, 3.22)	1.39 (0.69, 2.80)	106	7489	1.44 (1.00, 2.08)	1.17 (0.79, 1.74)
p-trend			0.23	0.51			0.28	0.68			0.31	0.97

Con.

Daidzein												
T1 (0–25)	20	1441	Reference	Reference	30	2265	Reference	Reference	82	5948	Reference	Reference
T2 (26–115)	29	2098	1.41 (0.72, 2.78)	1.29 (0.64, 2.63)	38	2819	1.66 (0.88, 3.15)	1.48 (0.74, 2.97)	101	7384	1.23 (0.86, 1.77)	1.09 (0.74, 1.60)
T3 (116–29,200)	30	2010	1.68 (0.86, 3.29)	1.77 (0.90, 3.49)	40	3001	1.96 (1.02, 3.74)	2.05 (1.02, 4.11)	107	7450	1.44 (1.01, 2.07)	1.43 (0.98, 2.08)
p-trend			0.18	0.11			0.10	0.06			0.07	0.047
Equol												
T1 (0–3)	22	1532	Reference	Reference	36	2629	Reference	Reference	93	6666	Reference	Reference
T2 (4–11)	24	1722	0.96 (0.48, 1.92)	0.94 (0.46, 1.91)	35	2598	1.24 (0.67, 2.27)	1.40 (0.72, 2.74)	86	6222	1.01 (0.70, 1.46)	1.06 (0.72, 1.56)
T3 (12–17,200)	27	1927	1.12 (0.58, 2.19)	1.12 (0.55, 2.27)	29	2220	0.95 (0.48, 1.86)	1.22 (0.57, 2.60)	86	6067	1.07 (0.74, 1.55)	1.18 (0.79, 1.76)
p-trend			0.67	0.69			0.71	0.78			0.71	0.42
O-desmethylangolensin												
T1 (0–1)	29	2124	Reference	Reference	32	2374	Reference	Reference	91	6630	Reference	Reference
T2 (2–9)	25	1730	0.91 (0.49, 1.71)	0.78 (0.41, 1.48)	28	2066	1.15 (0.60, 2.19)	1.07 (0.53, 2.15)	91	6375	1.24 (0.87, 1.77)	1.12 (0.77, 1.62)
T3 (10–9890)	23	1569	0.83 (0.44, 1.56)	0.75 (0.38, 1.48)	40	3025	1.50 (0.81, 2.77)	1.71 (0.87, 3.35)	93	6725	1.13 (0.79, 1.63)	1.12 (0.76, 1.65)
p-trend			0.59	0.58			0.19	0.07			0.90	0.72

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

Phytoestrogens (ng/mL)	Cancer mortality				Cardiovascular mortality				All-cause mortality			
	No. of cases	Person-years	Creatinine-adjusted HR (95 % CI)[a]	Multivariable-adjusted HR (95 % CI)[b]	No. of cases	Person-years	Creatinine-adjusted HR (95 % CI)[a]	Multivariable-adjusted HR (95 % CI)[b]	No. of cases	Person-years	Creatinine-adjusted HR (95 % CI)[a]	Multivariable-adjusted HR (95 % CI)[b]
Enterolignan												
T1 (0–225)	27	1999	Reference	Reference	40	2858	Reference	Reference	101	7106	Reference	Reference
T2 (226–691)	30	2116	1.68 (0.90, 3.13)	1.43 (0.75, 2.73)	39	3009	0.83 (0.47, 1.46)	0.55 (0.30, 1.02)	112	8281	1.26 (0.91, 1.74)	0.99 (0.70, 1.40)
T3 (692–85,847)	22	1434	1.22 (0.62, 2.39)	1.05 (0.52. 2.14)	29	2218	0.73 (0.39, 1.38)	0.48 (0.24, 0.97)	77	5395	0.86 (0.60, 1.25)	0.65 (0.43, 0.96)
p-trend			0.087	0.86			0.36	0.07			0.26	0.019
Enterodiol												
T1 (0–20)	27	1991	Reference	Reference	38	2805	Reference	Reference	105	7566	Reference	Reference
T2 (21–63)	22	1586	0.94 (0.47, 1.88)	1.09 (0.54, 2.22)	38	2961	1.15 (0.65, 2.04)	1.36 (0.74, 2.48)	93	6877	0.92 (0.65, 1.29)	1.05 (0.73, 1.50)
T3 (64–18,000)	28	1835	1.60 (0.85, 3.01)	1.66 (0.85, 3.34)	30	2177	0.92 (0.48, 1.77)	0.71 (0.87, 1.78)	88	6060	0.97 (0.68, 1.37)	0.98 (0.67, 1.43)
p-trend			0.08	0.10			0.69	0.52			0.95	0.85
Enterolactone												
T1 (0–173)	25	1790	Reference	Reference	40	2880	Reference	Reference	102	7177	Reference	Reference
T2 (174–595)	32	2330	1.77 (0.96, 3.29)	1.52 (0.80, 2.90)	37	2837	0.98 (0.56, 1.72)	0.68 (0.37, 1.26)	110	8141	1.34 (0.97, 1.85)	1.09 (0.77, 1.54)
T3 (596–85,300)	22	1429	1.19 (0.60, 2.32)	1.01 (0.50, 2.05)	31	2368	0.78 (0.41, 1.48)	0.54 (0.27, 1.07)	78	5464	0.86 (0.59, 1.25)	0.65 (0.44, 0.97)
p-trend			0.99	0.72			0.43	0.10			0.22	0.014

HR hazard ratio, *CI* confidence interval [a] Adjusted for urinary creatinine

[b] Adjusted for age, education, smoking status, body mass index, total energy intake, sodium intake, and urinary creatinine

المساهمة بين الفيتواستروجين الموجود بنقط البول كدليل على المتناول الغذائي على العوامل المسببة للوفاة من أمراض السرطان وأمراض القلب الوعائية

جميل فكرى محمود 1؛ جونز زانغ 1 • كاثرين جوزيت 2 • جونز إلس 3 • محمد شحات سالم 4 •

1 قسم الأغذية الوظيفية، المعهد الوطني للتغذية كندا، أوتاوا.
2 مركز الوقاية من الأمراض ومكافحتها، أونتاريو
3 المركز الوطني لدراسات استقصائية للصحة والتغذية (NHANES) - كندا.
4 قسم علوم وتكنولوجيا الأغذية - كلية الزراعة بالقاهرة – جامعة الأزهر - مصر

الملخص العربي

*تشير هذه الدراسة إلى أن تناول الأغذية التي تحتوى على فيتواستروجينات Phytoestrogens يقلل من مخاطر الإصابة بالسرطان ومخاطر أمراض القلب الوعائية. وقد حققت هذه الدراسة في ارتباطات الفيتواستروجينات البولية مع حالات الوفاة بالأنواع المختلفة للسرطان (ن = 198)، إمراض القلب الوعائية (ن = 152)، وجميع الحالات الوفيات (ن = 363) بين 5179 مشارك في المسح المركز الوطني لدراسات استقصائية للصحة والتغذية (NHANES) - كندا (2009- 2014). **الطرق :** وتم قياس الفيتواستروجينات بعينات البول باستخدام كروماتوجراف السائل عالي الأداء المزود بكاشف الطيفي الكتلي. و تم التحليل بعينات البحث لتقييم نسب الخطر (HRs) و 95٪ وعند مستوى ثقة (CIs). لكل من المستويات الثلاث فيما يتعلق بمستوى الفيتواستروجينات البولية. **النتائج** : وكانت النتائج بعد تعديل عند مستويات الثقة وجد أن هناك علاقة بين ارتفاع مستوى Enterolignans الكلي بالبول وانخفاض من نسب الوفيات بإمراض القلب الوعائي بينما ارتفع مستوى التركيز منTotal isoflavones وكذلك Daidzein وزيادة نسب حدوث المخاطر للوفاة من أمراض القلب الوعائي . (HR for tertile 3 vs. tertile 1) 0.48؛ (95 % CIs). 0.24، 0.97)، في حين أن تركيزات أعلى من مجموع isoflavones البولية (HR for tertile 3 vs. tertile 1) 2.14؛ (95 % CIs) 1.03، 4.47) و Daidzein (HR for tertile 3 vs. tertile 1) 2.05؛ (95 % CIs). 1.02، 4.11) ارتبطت بزيادة المخاطر. وقد لوحظ انخفاض في معدل الوفيات لجميع الحالات ، لارتفاع تركيزات البولية من Enterolignans الكلي (HR for tertile 3 vs. tertile 1) 0.65؛ (95 % CIs) 0.43، 0.96) و Enterolactone (HR for tertile 3 vs. tertile 1) 0.65؛ (95 % CIs) 0.44، 0.97). **الاستنتاج :** وكانت الاستنتاجات أن بعض الفيتواستروجينات البولية مرتبطة ارتباط هام بمخاطر الإصابة بإمراض القلب الوعائية والوفاة في جميع الحالات والتي تم دراستها في عينات تمثل المجتمع الكندي ، من خلال المركز الوطني لدراسات استقصائية للصحة والتغذية (NHANES) - كندا . وتعتبر تلك الدراسة واحدة من الدراسات الأولى التي تستخدم الفيتواستروجينات البولية كمؤشرات الحيوية من تناولهم الغذائي لتقييم تأثير هذه المركبات الحيوية على خطر الموت من الإصابة بإمراض السرطان وأمراض القلب والأوعية الدموية.*

الكلمات المفتاحية: *السرطان- امراض القلب والاوعية الدموية – نسبة الوفاه- الفيتواستروجين في البول*