Miller et al., *Cogent Food & Agriculture* (2016), 2: 1135861
http://dx.doi.org/10.1080/23311932.2015.1135861

cogent food & agriculture

FOOD SCIENCE & TECHNOLOGY | REVIEW ARTICLE

Collected literature on isoflavones and chronic diseases

Katie A. Miller[1], Farrell Frankel[1], Hitomi Takahashr[1], Nicole Vance[1], Celia Stiegerwald[1] and Sari Edelstein[1]*

Received: 26 October 2015
Accepted: 11 December 2015
First Published: 29 January 2016

*Corresponding author: Sari Edelstein, Simmons College, S-334, Boston, MA 02115, USA
E-mail: dre@simmons.edu

Reviewing editor:
Fatih Yildiz, Middle East Technical University, Turkey

Additional information is available at the end of the article

Abstract: Isoflavones are organic compounds, which have been linked to the health benefits and prevention of many diseases. Common isoflavones are genistein, daidzein, and glycitein. Genistein has been researched in regard to its effect on the reduction of menopausal symptoms and reduction of cardiovascular risk factors in osteopenic, post-menopausal women. Research on daidzein focuses on bone mineral density implications in post-menopausal women, therapeutic effects early in prostate cancer, and protection against DMBA-induced mammary carcinogenesis. The most recent research on daidzein has implications for its effect on cardiovascular risk reduction. Research on glycitein focuses on it bioavailability, as well as its role in angiogenesis and invasion of malignant glioma cells. The health benefits of these specific isoflavones are instrumental in the prevention and treatment of many diseases. This review of literature focuses on the effects of genistein, daidzein, and glycitein on health outcomes, such as breast cancer, cardiovascular disease, and prostate cancer.

Subjects: Environment & Agriculture; Food Science & Technology; Health and Social Care

Keywords: isoflavones; genistein; daidzein and glycitein

ABOUT THE AUTHOR

Edelstein's present position is as associate professor in the Nutrition and Dietetics Department at Simmons College. She presently teaches both Food Science and Food service classes. Before coming to Simmons College, Edelstein has previously been in private practice and has served as a hospital food service director and chief dietitian. Additionally, Edelstein is a past president of the Miami Dietetic Association and has served in several other leadership positions in Florida. She is the author of many research articles, inclusive of topics on ethics, yoga, the glycemic index, and athletic performance as well as books, which include:
The Healthy Young Child, Wadsworth Publishing, 1995; Nutrition in Public Health, 2nd ed., Jones & Bartlett, to come out in 2005; Nutrition Pocket Guide for Nurses, Jones & Bartlett, to come out in 2007; Managing Food and Nutrition Services; For Hospitality, Culinary and Dietetics Professionals, Jones & Bartlett, to come out in 2007.
Nutrition in the Lifecycle-An Evidence-Based Approach, Jones & Bartlett, to come out in 2007.
Please note Nicole Vance's credentials: RD, LDN.

PUBLIC INTEREST STATEMENT

It is projected that the US older adult population (age > 65 years) will more than double from 40.5 million in 2010 to 89 million in 2050 (Dall et al., 2013). With the increasing prevalence of weight-related chronic diseases, it is unsurprising that an estimated 78% of the US health care budget was spent on chronic illnesses in 2009 (Bodenheimer et al., 2009). The proportion of older adults reporting at least one chronic disease has increased from 86.9% in 1998 to 92.2% in 2008, while those reporting four or more chronic diseases has increased from 11.7 to 17.4% during the same decade (Hung et al., 2011). Together, the aging US population and increasing prevalence of lifestyle-related chronic diseases is expected to place an even greater burden on the already struggling US health care system in the coming decades. Phytonutrients may become a part of the prevention package in some of these illnesses.

cogent • food & agriculture

1. Introduction

It is projected that the US older adult population (age > 65 years) will more than double from 40.5 million in 2010 to 89 million in 2050 (Dall et al., 2013). With the increasing prevalence of weight-related chronic diseases, it is unsurprising that an estimated 78% of the US health care budget was spent on chronic illnesses in 2009 (Bodenheimer, Chen, & Bennett, 2009). The proportion of older adults reporting at least one chronic disease has increased from 86.9% in 1998 to 92.2% in 2008, while those reporting four or more chronic diseases has increased from 11.7 to 17.4% during the same decade (Hung, Ross, Boockvar, & Siu, 2011). Together, the aging US population and increasing prevalence of lifestyle-related chronic diseases is expected to place an even greater burden on the already struggling US health care system in the coming decades.

Lifestyle factors such as diet and exercise are thought to play a critical role in the primary and secondary prevention of chronic diseases. Recent research has focused on the specific effects of phytoestrogens (bioactive components found in many foods that mimic the effect of estradiol in the human body) on various health outcomes. Isoflavones, a subcategory of the polyphenolic phytonutrient flavonoid class, are largely found in soybeans and soy-based products such as soy milk, tempeh, and tofu (Bhagwat, Haytowitz, & Holden, 2008). In these dietary plant-based sources, isoflavones are typically present in their glycosylated form (e.g. genistin, daidzin, glycitin). When consumed, enterocyte glycosidases hydrolyze the glucose side chain, leaving the isoflavone into its bioactive aglycone form (e.g. genistein, daidzein, glycitein) (Gültekin & Yildiz, 2006). Daidzein can be further transformed by intestinal microflora into equol, a bioactive metabolite that may be responsible for health benefits associated with daidzein intake (Jackson, Greiwe, & Schwen, 2011). Not all humans produce equol after consuming foods containing soy; indeed, only 20–35% of the adult population is an "equol producer" (Setchell et al., 2005).

Though genistein (Figure 1) and daidzein (Figure 2) are thought to be the most potent isoflavones (Singh-Gupta et al., 2010), there is growing evidence suggesting that glycitein (Figure 3) is a bioactive isoflavone that warrants further study (Shinkaruk et al., 2012). This literature review will provide an overview of the current research on the effects of genistein, daidzein, and glycitein on age- and lifestyle-related chronic conditions and illnesses, including osteoporosis, cardiovascular disease (CVD), breast and prostate cancer, age-related cognitive decline, and Alzheimer's disease (AD).

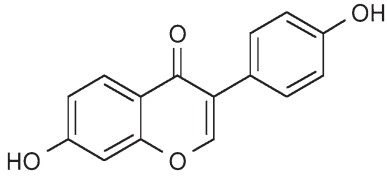

Figure 1. Daidzein is found mostly in soy products, such as tofu, texturized vegetable protein, soymilk, soy flour, miso, and soy-based infant formula.

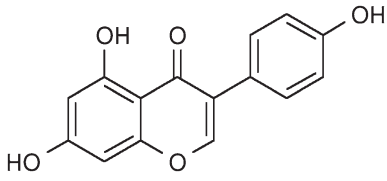

Figure 2. Genistein is naturally found in plants and food, such as lupin, fava beans, soybeans, kuzdu, and psoralea. It is also in miso, tempeh, and tofu.

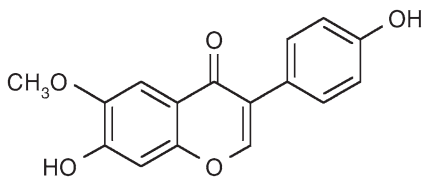

Figure 3. Glycitein is mostly found in soy products, such as soy flour, soybeans, and soy isolate.

2. Osteoporosis

Osteoporosis is a degenerative skeletal disease characterized by deteriorating bone microarchitecture, low bone mineral density (BMD), and greater bone resorption than bone formation (bone involution). Using data gathered by National Health and Nutrition Examination Survey, a recent data brief found that among non-institutionalized US adults older than 50 years, 9% reported osteoporosis at either the femur neck (3%), lumbar spine (4%), or both sites (2%), while 49% report osteopenia at either site (22% femur neck, 10% lumbar spine) or both sites (17%). Prevalence of both osteoporosis and osteopenia significantly increases with age among all adults (p-for-trend < 0.05), but a greater percentage of females between 70 and 79 years reported osteoporosis and osteopenia (27 and 68%, respectively) than men of the same age category (4 and 45%) (Looker, Borrud, Dawson-Hughes, Shepherd, & Wright, 2012).

Decreasing estrogen levels post-menopause are a critical risk factor for osteoporosis development among older female adults. However, it is well established in the literature that hormone replacement therapy (HRT) significantly increases risk of both fatal and non-fatal CVD as well as breast cancer (Rossouw et al., 2002). As phytoestrogens, isoflavones having a binding affinity with estrogen receptors (ER), particularly ER-β, and therefore can have estrogenic effects related to bone health (Gültekin & Yildiz, 2006). It has also been hypothesized that the high soy content of the Asian diet contributes to the lower prevalence of osteoporosis observed among Asian women compared to Western women (Ricci, Cipriani, Chiaffarino, Malvezzi, & Parazzini, 2010). Thus, isoflavones have been examined for their ability to reduce risk of osteoporosis in post-menopausal women through decreased bone resorption and increased BMD.

A randomized, double-blind placebo-controlled trial (Marini et al., 2007) investigated the effects of daily genistein supplementation (54 mg/d) on BMD at anteroposterior lumbar spine and femoral neck in osteopenic, post-menopausal Italian women (n = 389) after 24 months. Both treatment and placebo tablets contained 500 mg of calcium carbonate and 400 IU of vitamin D. All participants were required to maintain an isocaloric, fat-restricted diet (compliance assessed by nutritionist). At 24 months, BMD at the anteroposterior lumbar spine and femoral neck had increased among genistein treatment participants, and these values were significantly different than placebo group participants (0.10 and 0.062 g/cm^2, respectively; $p < 0.001$ for both). In addition, genistein treatment participants exhibited higher levels of bone growth markers (i.e. bone-specific alkaline phosphatase) and lower levels of bone resorption markers (i.e. pyridinoline and deoxypyridinoline), reinforcing the hypothesis that genistein acts through mechanisms that increase osteoblast activity and reduce osteoclastogenesis. Although this dosage had no significant adverse effects on the uterus (as indicated by non-significant changes in endometrial thickness), the authors did report that significantly more genistein treatment participants experienced adverse gastrointestinal (GI) side effects and discontinued the study (19% vs. 8%; $p = 0.002$).

Despite these promising preliminary results, recent meta-analyses suggest that the relationship between isoflavones and BMD is more complex. A 2010 meta-analysis analyzed 12 randomized controlled trials (RCTs) that included a total of 1,433 Western female subjects. The mean pooled difference between isoflavone treatment and placebo groups on mean BMD change from baseline at the lumbar spine was non-significant (0.73, 95% CI −2.79, 4.25), and no time- or dose-dependent effects emerged (Ricci et al., 2010). However, the authors did note that the two studies which employed isolated genistein supplementation (one of which was Marini et al., 2007) reported the greatest difference between treatment and placebo, suggesting that interventions with isolated genistein supplementation requires further study.

The varying results observed in mixed isoflavone supplementation may be related to an individual's equol-producing status. A 12-month double-blind RCT by Wu et al. (2007) assessed the effect of 75 mg/d isoflavone conjugates on BMD among post-menopausal Japanese women. In the isoflavone treatment group (n = 25), BMD at the hip and intertrochanteric regions were significantly higher ($p < 0.05$) among equol producers (−0.46 and −0.04%, compared to non-equol producers (2.28 and

−2.61%, respectively). Further research, particularly those involving Western women, is required to ascertain the efficacy of pure genistein supplementation and the role of equol status in mediating the association between isoflavone intake and bone metabolism (Uehara, 2013).

3. CVD

Between 2013 and 2025, the prevalence of CVD and diabetes are projected to increase by 27 and 21%, respectively (Dall et al., 2013). The pathophysiology of CVD and type 2 diabetes mellitus (T2DM) appears to be inextricably linked through inflammatory and vascular pathways, and presence of one disease is a risk factor for the other (Dokken, 2008). Due to the strongly adverse effects of HRT (Rossouw et al., 2002), much research has explored the effects of isoflavone supplementation on CVD and T2DM risk factors such as hypertension, dyslipidemia, fasting serum glucose, and fasting insulin. As Zhuo, Melby, and Watanabe (2006) discuss, the body of literature regarding the effect of human isoflavone consumption and serum lipids is controversial. An early meta-analysis of controlled clinical trials by Anderson, Johnstone, and Cook-Newell (1995) found that soy protein consumption was significantly associated with improved serum lipids, but these strong effects have not been replicated in more recent studies. Contradicting results may be due to differences in study design, mediating variables such as sex and equol status, or differences in study population.

Using the same data-set as the aforementioned 2007 study by Marini and colleagues, Atteritano et al. (2007) examined the effects of 54 mg/d genistein supplementation on various cardiometabolic markers among healthy post-menopausal Italian women. At both 12-month and 24-month assessments, the authors observed significantly reduced fasting glucose and fasting insulin in the treatment group ($n = 198$) compared to the placebo group ($n = 191$; $p < 0.05$). Specifically, average fasting insulin in the treatment group decreased from 7.24 μIU/ml (SD 0.17) to 6.66 μIU/ml (SD 0.16) after 24 months of treatment, whereas the average fasting insulin in the placebo group increased from 7.24 μIU/ml (SD 0.18) to 7.95 μIU/mL (SD 0.19) over the same time period. Women in the treatment group also exhibited other serum markers suggesting lower CVD risk, including decreased fibrinogen and cell adhesion molecules (i.e. soluble intercellular adhesion molecules [sICAM-1] and soluble vascular cellular adhesion molecule [sVCAM-1]). Elevated levels of serum fibrinogen and cell adhesion molecules are risk factors for CVD due to their role in clotting and atherosclerosis. Overall, the study results suggest that genistein supplementation, in combination with calcium, vitamin D and a healthy diet, may reduce certain CVD risk factors as fasting glucose, fasting insulin, fibrinogen, and cell adhesion molecules in osteopenic, post-menopausal women.

However, findings from a murine study (Al-Nakkash et al., 2012) suggest that genistein may have sex-dependent effects on CVD risk factors. A 4-week treatment of 600 mg genistein per kilogram of food significantly enhanced basal vascular reactivity compared to no treatment in male and female mice ($p < 0.01$ for both). However, other significant observations differed by sex; female mice that received genistein exhibited significant reduction in systolic blood pressure compared to their female control counterparts, while male mice in treatment group gained significantly less weight and exhibited higher serum insulin and lower serum glucose than the male control group ($p < 0.05$ for all).

Similarly, a recent nested case-control study (Zhang et al., 2012) observed a sex-dependent association between urinary equol excretion and incidence of coronary heart disease (CHD) among Chinese adults in Shanghai. Conditional logistic regression was used to compare baseline urinary isoflavonoids of cases ($n = 377$; developed incident CHD during follow-up) age- and sex-matched controls ($n = 753$). Although no association was found between total urinary isoflavonoids and CHD in either sex, there was a significant inverse association between higher urinary equol excretion and lower incidence of CHD among the female participants (p for trend = 0.02). Compared to women with the lowest equol levels, the adjusted ORs for women in the third quartile of equol levels was 0.51 (95% CI 0.26, 0.98) and the fourth quartile 0.46 (95% CI 0.24, 0.89).

These results observed by Zhang et al. (2012) may be due to sex-dependent differences in equol producers, as studies have noted a higher prevalence of female equol producers (Qin et al., 2013). A recent cross-sectional study (Liu, Ho, Chen, Liu, & Woo, 2014) observed that among prehypertensive post-menopausal Chinese women, equol producers exhibited significantly lower systolic and diastolic blood pressure ($p = 0.01$), serum triglycerides (TGs) ($p = 0.023$) and C-reactive protein ($p = 0.015$) compared to non-equol producers. However, a double-blind placebo-controlled RCT by Qin et al. (2013) conversely observed that independent of equol status, 6-month supplementation of daidzein significantly reduced serum TGs among hypercholesterolemic (fasting TC > 200 mg/dL) Chinese men and women compared to placebo ($p < 0.05$). Daidzein supplementation did not significantly affect other cardiometabolic markers, including TC, LDL cholesterol, HDL cholesterol, fasting glucose, and fasting insulin. Of note, these results were not dose-dependent, as no significant differences were observed between treatments groups receiving 40 and 80 mg/d daidzein; the authors also reported no adverse effects occurred during the trial. In general, the efficacy of isoflavone intake and supplementation on CVD and related cardiometabolic risk factors is uncertain, and further human observational studies are required to ascertain the mediating role of sex and equol status.

4. Breast cancer

Breast cancer is the leading type of cancer affecting US women (Howlader et al., 2015), and its prevalence is significantly higher among Western populations compared to Asian populations (Gültekin & Yildiz, 2006). Isoflavones are known to be potent radiosensitizers, enhancing the effectiveness of radiotherapy on malignant tumors (Singh-Gupta et al., 2010). Liu et al. (2013) found that varying concentrations of genistein enhanced the radiosensitivity of both MCF-7 (ER-positive, hormone-dependent) and MDA-MB-231 (ER-negative, hormone-independent) breast cancer cell lines. Pretreatment with 5, 10, and 20 μM genistein for 24 h led to concentration-dependent effects on cell cycle arrest at the G_2/M phase, during which cells are most sensitive to radiation. This may be the mechanism by which genistein increases radiosensitivity. In addition, MCF-7 and MDA-MB-231 cells pretreated with 20 μM genistein for 24 h exhibited higher apoptotic rates (22.7 ± 1.4 and 20.7 ± 2.3%, respectively) compared to the control groups (8.3 ± 1.6 and 10.5 ± 2.0%, respectively) at 24 h post-irradiation. The authors noted that, contrary to previous study results, this study found non-significant differences between the ER-positive and ER-negative breast cancer cell lines, indicating that the effects of genistein are not mediated by ER status. It is thought that the radiosensitizing mechanism of genistein is related to its ability to arrest cell cycle at vulnerable G2/M stage and induce apoptosis through a mitochondrial-mediated pathway.

Results from other *in vitro* studies examining the effects of isoflavone treatment on breast cancer cell proliferation support this mechanistic pathway. Choi and Kim (2008) exposed MCF-7 and MDA-MB-453 cells to increasing concentrations of daidzein (range 1–100 μm) over 24-, 48-, and 72-h exposure periods. Significant dose- and time-dependent effects were observed, as well as significant cell cycle arrest in the G1 and G2/M phases after 72-h treatment at concentrations exceeding 5 μM in MCF-7 cells and 10 μM in MDA-MB-453 cells ($p < 0.05$ for all). Further analysis demonstrated that daidzein treatment significantly increased caspase-9 activity in both cell lines (indicating increased apoptotic activity), and decreased levels of cyclin-dependent kinase-1 (CDK1), a protein that regulates G2/M phase activity. Jin, Zhang, Kang, Wang, and Zhao (2010) similarly found that daidzein (concentrations ranging from 25 to 100 μM, exposure 24-, 48-, and 72-h periods) significantly inhibited MCF-7 cell proliferation in dose- and time-dependent manner ($p < 0.01$ for all). The MCF-7 cells produced high levels of reactive oxygen species (ROS) that acted as upstream signals to promote apoptosis; thus, the authors postulated an ROS-induced caspase-dependent apoptotic mechanism. In addition, it was recently demonstrated that daidzein also induces ROS-induced mitochondrial-mediated apoptosis in BEL-7402 cancer cell lines (Han et al., 2015).

Researchers have also investigated the effects of glycitein on mammary carcinogenesis. Zhang, Su, Bai, and Liu (2015) recently reported that glycitein may have a biphasic effect on cell proliferation, an effect that has also been observed for genistein (Karahalil, 2006). Exposing breast cancer SKBR-3 cells to lower concentrations of glycitein (<10 mg/mL) led to increased cell growth and *de*

novo DNA synthesis, while higher concentrations (>30 mg/mL) significantly inhibited cell growth and DNA synthesis. Specifically, approximately 50% cell proliferation was inhibited at 40 mg/mL and 75% at 100 mg/mL; cells treated with the latter concentration of glycitein had severely damaged cell membranes, which may be related to the inhibited proliferation observed.

Isoflavone efficacy in chemoprevention of breast cancer has also been explored through murine studies. Sahin et al. (2011) examined the association between genistein and lycopene dietary supplementation and incidence of 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer using five experimental groups consisting of 10 female Wister rats each: (1) normal control, (2) DMBA control, (3) DMBA + lycopene, (4) DMBA + genistein, and (5) DMBA + lycopene + genistein. Supplementation was fed to the rats by oral gavage three times per week during the two weeks preceding the DMBA injection; Groups 3 and 5 received 20 mg lycopene per kg body weight for, and Groups 4 and 5 received 2 mg genistein kg body weight. Mammary tumor incidence was 100% in the Group 1 (DMBA control), 70% in Group 3 (lycopene only), 60% in Group 4 (genistein only) and 40% in Group 5 (lycopene/genistein combination). The combined supplement group also presented with the lowest mean tumor weight and volume of the four groups, though tumors in Groups 3 and 4 were smaller compared to the DMBA-control group. These results indicate that while lycopene and genistein may each inhibit DMBA-induced breast cancer alone, supplementation with both may be more effective.

Although mammary tumors in rats are morphologically comparable to those in humans, it should be noted that applying the dosage utilized in this study (2 mg/kg body weight) to a human adult would result in 100 mg for a 50-kg (110 lb) woman; this is much higher than the dosages utilized (30–54 mg/d) during human studies discussed in previous sections (Atteritano et al., 2007). Accounting for biological differences between species to determine effective dosage is one of the factors currently complicating the benefit/risk analysis of phytoestrogen and isoflavone consumption (Karahalil, 2006), underscoring the need for more human studies.

Within the extant body of human studies, there is a lack of consensus regarding the association between isoflavone consumption and breast cancer. A 2012 meta-analysis (Nechuta et al., 2012) of three longitudinal studies found that among 9,514 breast cancer survivors (two US cohorts and one Chinese cohort), high isoflavone consumption (≥10 mg/d) was significantly associated with a reduced risk of breast cancer reoccurrence (hazard ratio [HR] 0.75, 95% CI 0.61, 0.92) and a borderline significant reduced risk of breast cancer mortality (HR 0.83, 95% CI 0.64, 1.07).

Wu et al. (2015) recently published the first long-term double-blind RCT on soy supplementation and mammographic density (an acknowledged biomarker of breast cancer) in US breast cancer patients. After 12 months of isoflavone supplementation (50 mg/d), the treatment group (n = 46) did not exhibit statistically significant differences in mammographic density (p = 0.38) or fibroglandular tissue volume (p = 0.48) compared to the placebo group (n = 49). Although this study duration was longer than previous RCTs studying this relationship, the sample was relatively small and included both pre- and post-menopausal women. The authors suggest that the contradicting results may be due to differences between dietary sources of soy and processed supplements, supplementation at the midlife stage may be too late, or that the protective effects of isoflavone supplementation may not been reflected in breast density changes. In addition, findings from a meta-analysis of 8 short-term (6 months to 3 years) RCTs suggests that isoflavone consumption may have different effects on mammographic density in pre- and post-menopausal women. Hooper, Madhavan, Tice, Leinster, and Cassidy (2010) observed that dietary isoflavone intake was statistically associated with breast density among pre-menopausal women (mean difference [MD] 1.83%, 95% CI 0.25, 3.40) but not post-menopausal women (MD −1.10%, 95% CI −3.22, 1.03).

Different results have also been noted based on the geographic location of the study. A meta-analysis of 35 studies conducted by Chen et al. (2014) concluded that while subgroup analysis of studies based in Asian countries shows a preventive effect for isoflavone consumption in both

pre- and post-menopausal women, no significant effect is seen for studies based in Western countries. This is in concurrence with findings from earlier data (Karahalil, 2006).

Overall, results from recent *in vitro* studies suggest that all three major isoflavones (genistein, daidzein, and glycitein) may act through ROS- and mitochondrial-mediated mechanistic pathways to arrest cell cycle growth at G_2/M phase and induce apoptosis. These findings support the biological plausibility of isoflavone consumption enhancing the chemoprevention of breast cancer. Although the body of *in vivo* research on the association between isoflavones and breast cancer continues to grow, there is not enough consistent evidence to conclude that isoflavones exert a protective effect against breast cancer in humans. Further longitudinal studies and controlled trials are needed to clarify this relationship.

5. Prostate cancer

Among US men, prostate cancer currently has the highest incidence rate and second-highest mortality rate of all cancer types (Howlader et al., 2015). The hormone insulin-like growth factor-1 (IGF-1) is thought to play a critical role in prostate cancer development. Binding of IGF-1 to insulin-like growth factor-1 receptor (IGF-1R) on malignant cells initiates a phosphorylation cascade that ultimately blocks apoptosis, thereby increasing cancer cell proliferation. Lee, Ju, Park, Hong, and Yoon (2012) recently demonstrated that genistein effectively inhibits IGF-1-stimulated cell growth in PC-3 prostate cancer cells by blocking phosphorylation of IGF-1R and its downstream targets.

In addition, past murine studies have indicated that pure genistein supplementation inhibits prostate tumor growth but also increases metastasis, while mixed isoflavone pills (containing genistein, daidzein, and glycitein) do not have further metastatic effects. Singh-Gupta et al. (2010) examined the daidzein efficacy in PC-3 prostate tumor growth inhibition within nude mice and in cell growth inhibition within PC-3 and C4-2B prostate cancer cell lines. Mice with established PC-3 prostate tumors were divided into five experimental groups and received either 1 mg/d isoflavone mixture (43% genistein, 21% daidzein, 2% glycitein), 0.64 mg/d genistein/daidzein mixture, 0.43 mg/d pure genistein, 0.21 mg/d pure daidzein, or no treatment. Compared to the control group, all treatment groups significantly inhibited tumor growth by 30–50% ($p \leq 0.05$) but no statistical significance was observed between groups. However, mice that received pure genistein exhibited a significant two-fold increase in lymph node weight and size compared to mice in the control group ($p = 0.03$), while mice that received pure daidzein, genistein/daidzein, or isoflavone mixture had lymph node sizes comparable to the control group. Post-irradiation, mice in the pure daidzein and genistein/daidzein groups showed a significant 90% tumor growth inhibition ($p < 0.0001$) compared to the control group, suggesting daidzein is an efficacious radiosensitizer. Though *in vitro* studies indicate that genistein is more potent than daidzein, these results suggest the latter may play a critical role in preventing adverse metastatic effects.

Despite generally promising results from *in vitro* and *in vivo* murine studies, the results from human studies have been conflicting. Findings from early case-control studies based in China and Japan suggest that high isoflavone intake is associated with decreased risk of prostate cancer (Zhuo et al., 2006). Also, a 2009 meta-analysis of observational studies found that high soy intake significantly reduced risk of prostate cancer by 26% (combined RR/OR 0.74; 95% CI 0.63, 0.89) compared to low intake (Yan & Spitznagel, 2009). However, it should be noted that in a subgroup analysis based on geographic study location, the Asian-based studies still exhibited a significantly reduced risk of 48% (combined RR/OR 0.52; 95% CI 0.34, 0.81) while the analysis of Western-based studies was no longer significant (combined RR/OR 0.99; 95% CI 0.85, 1.16). In addition, the separate analysis of the studies focusing on isoflavone (not soy) consumption and prostate cancer risk was not significant either (combined RR/OR 0.88; 95% CI 0.76, 1.02).

Some interventional studies have found tenuous evidence of an association between isoflavone supplementation and prostate cancer. For example, a small ($n = 20$) open-labeled non-randomized Phase II trial (Pendleton et al., 2008) found that 12-month supplementation of 141 mg/d

isoflavonoids (administered as 47 mg powdered beverage supplement, 3 servings/d) had no effect on serum prostate specific antigen (PSA) levels, an established biomarker for prostate cancer. However, PSA levels rose at a significantly slower rate (20%) during the intervention period compared to before the intervention (56% per year; $p = 0.05$).

Similarly, a double-blind, placebo-controlled Phase 2 clinical trial in Norway (Lazarevic et al., 2011) randomized 40 male subjects with localized prostate cancer to receive either 30 mg/d synthetic genistein ($n = 23$ in per-protocol-analysis) or placebo ($n = 17$ in per-protocol-analysis) during the 3–6 weeks before undergoing radical prostatectomy. The mean percent change in PSA levels among men in the genistein treatment arm was −7.8 vs. 4.4% in the placebo group, a difference that was borderline statistically significant ($p = 0.051$). In addition, Pavese and colleagues at Northwestern University have found evidence that genistein significantly reduces expression of matrix metalloproteinase-2 (MMP-2), an enzyme linked to cell degradation associated with cancer metastasis. The full results of this Phase II clinical trial have not yet been published (Pavese, Krishna, & Bergan, 2014).

Meanwhile, other studies have observed no effect in both healthy men (Urban et al., 2001) and men with localized prostatic cancer (deVere White et al., 2004). Most recently, Bosland et al. (2013) published findings from a US-based multi-center, double-blind RCT that randomized patients who had recently undergone radical prostatectomy to receive either 47 g soy protein beverage powder (containing ~173 mg/d isoflavones) or placebo (19.2 g calcium casinate). The primary endpoint was biochemical recurrence, operationally defined by repeated increase in serum PSA levels, and measured by PSA measurements at pre-established intervals. Although the trial was intended to continue until all enrolled completed 2-year supplementation cycle, it was stopped early because 28.3% evaluable participants ($n = 45$; 22 intervention, 23 placebo) reached primary endpoint, nearly equal to the predicted recurrence rate of 30%.

In general, interventional studies supplying isoflavone supplementation, typically in the form of soy protein beverage powder, have reported high compliance by healthy and prostate cancer survivors and non-significant adverse events (i.e. constipation). Though this supplement form is convenient for both investigators and participants (particularly those unaccustomed to consuming soy-based foods), the lack of treatment effect may be related to unrecognized differences between processed isoflavone supplements and functional foods containing isoflavones, such as soybeans and soy-based foods. It has also been suggested that past trials have not provided adequate isoflavone dosage or supplement duration to exert chemopreventive influence in high-risk, post-surgical patients (Bosland et al., 2013). Additionally, the epidemiological data supporting a protective effect for isoflavones and prostate cancer is largely from Asian-based studies, where the population consistently consumes a soy-rich diet over the entire lifetime. Indeed, it is estimated that the Asian diet contains approximately 20–100 mg/d isoflavones; meanwhile, the typical Western diet contains only 1–3 mg/d (Gültekin & Yildiz, 2006; Karahalil, 2006). Thus, soy intake may be more impactful during an earlier life stage (Bosland et al., 2013). Taken together, the body of *in vitro* and murine studies suggests potential mechanistic pathways by which isoflavones may provide chemopreventive benefits, but the conflicting results from human studies raise questions about the efficacy and timing of such supplementation.

6. Age-related cognitive decline and AD

Age-related cognitive decline can be seen in many domains, including memory, visual and auditory learning, and executive higher order functioning (i.e. verbal fluency). It is thought that estrogen may play a role in repairing age-related brain tissue degradation, particularly in structures related to memory and executive function such as the neocortex and hippocampus (White et al., 2000). However, results from the Women's Health Initiative suggested that HRT may be associated with cognitive damage as well as increased cardiovascular risk (Gleason et al., 2009). As a result, much research has explored the effects of isoflavone consumption and supplementation in older adults, particularly post-menopausal women, over the last 10 years.

Some studies have reported no association between isoflavone intake and cognitive function. A *post hoc* analysis of data from the Women's Isoflavone Soy Health randomized clinical trial indicate that 2.5 years of isoflavone supplementation (91 mg aglycone weight) in post-menopausal women did not affect global cognition compared to placebo (St. John et al., 2014). Also, a cross-sectional study in the Netherlands examined association between soy intake among 301 post-menopausal women ages 60–75 and cognitive function in three domains: memory, processing capacity/speed, and executive function. Soy intake during the year prior, assessed using a food frequency questionnaire, was ranged from 0.18 mg/d (median lowest quartile) to 14.6 mg/d (median highest quartile). However, no significant differences in cognitive function between lowest and highest quartile of soy consumption were observed (Kreijkamp-Kaspers et al., 2007). Conversely, an early prospective study found that high consumption of tofu, a soy-rich food, during midlife (age 46–67) was associated with significantly poorer performance on the Cognitive Abilities Screening Instrument (CASI) at age 71–93 (White et al., 2000). Specifically, participants that self-reported consuming tofu twice per week during one of the two midlife dietary interviews were at a statistically significant 74% (95% CI 1.28, 2.37) increased risk of cognitive impairment (CASI score < 74) during the later cognitive test.

However, more recent interventional studies have observed beneficial effect in specific cognitive domains and no effect in other domains. One small, double-blind RCT in London (Duffy, Wiseman, & File, 2003) administered 60 mg isoflavone equivalents ($n = 18$) or placebo ($n = 15$) to post-menopausal women aged 50–65 years for 12 weeks. A battery of cognitive tests was used to assess cognitive function at baseline and post-treatment. Compared to the placebo group, the isoflavone treatment group significantly improved performance ($p < 0.05$) on picture recall task (long-term episodic memory), Paced Auditory Serial Addition Test (sustained attention) and Stockings of Cambridge Test (frontal lobe function). In this study, isoflavone treatment was not associated with participants' self-reported mood. Similarly, a Brazilian RCT found no significant difference in Geriatric Depression Scale scores between post-menopausal women administered 80 mg/d isoflavone supplementation for 4 months and those administered placebo (Santos-Galduróz, Galduróz, Facco, Hachul, & Tufik, 2010). An Italian double-blind crossover RCT of longer duration (6-month treatment or placebo with 1-month washout between) conversely reported 60 mg/d isoflavone treatment was associated with significantly lower scores on Beck Depression Inventory and Profile of Mood States ($p = 0.01$ for both; Casini et al., 2006). These mixed results suggest that more research on is needed before long-term isoflavone supplementation can be recommended to the general public.

Researchers has also examined the relationship between isoflavone intake and AD, an age-related neurodegenerative disease characterized by neuronal loss and accumulation amyloid β-peptide (Aβ) in the brain and the most common form of dementia present in the older adult population (Dyall, 2010). An early study by Gutierrez-Zepeda et al. (2005) explored the separate effects of genistein, daidzein and glycitein supplementation (each 100 µg/mL) in transgenic *Caenorhabditis elegans* expressing the human amyloid-beta (Aβ) peptide. Elevated levels of Aβ is thought to be directly related to the neuronal dysfunction (e.g. memory loss, disorientation) associated with AD. Only the glycitein-supplemented group exhibited significantly delayed Aβ expression-induced paralysis ($p = 0.036$), which correlated with the reduced level of ROS compared to the control group ($p < 0.05$); no significant changes were observed in the genistein- and daidzein-supplemented groups.

A more recent study by Unno et al. (2015) observed that soybean extract reduced Aβ-40 and Aβ-42 accumulation in aged mice. Conversely, Chatterjee, Roy, Khemka, Chattopadhyay, and Chakrabarti (2015) found that genistein led to enhanced accumulation of Aβ-42 in the SHSY5Y human neuroblastoma cell line. These contradicting results indicate the need for further well-designed *in vitro* and *in vivo* murine studies to discern the potential mechanisms linking isoflavones and AD precursors such as Aβ-42.

Of note, Gleason and colleagues recently published findings from the first isoflavone supplementation RCT conducted among patients diagnosed with AD (Gleason et al., 2015). Among 59 men and women, age > 60 years, 100 mg/d isoflavone supplementation for 6 months did not significantly

affect global cognition, though changes in equol levels were significantly associated with improved verbal fluency ($p = 0.05$). As the authors note, interpretation of these results is limited by the small sample size; in addition, only 26% of the treatment group were equol producers, suggesting that equol status may affect the efficacy of isoflavone supplementation on cognitive function. Overall, this area of research on isoflavones and AD is still growing, and further *in vitro* and *in vivo* murine studies would help elucidate potential mechanisms by which isoflavones are protective or harmful.

7. Conclusion

Genistein, daidzein, and glycitein are the three most bioavailable isoflavones present in soybeans and other soy-based products, and there is a growing body evidence suggests their bioactivity has implications for chronic diseases such as CVD, osteoporosis, cancer, and age-related cognitive decline. Recent *in vitro* studies have demonstrated that isoflavones inhibit breast and prostate cancer cell proliferation, though the mechanistic pathways are still being explored. However, the efficacy and safety of isoflavone supplementation to prevent and treat chronic disease is still highly controversial, due to widely varied findings from *in vivo* murine and human studies. In general, interventional studies have reported high adherence to treatment by healthy and diseased populations, and non-significant adverse events (i.e. constipation). However, the effects of isoflavone supplementation in the human body, particularly within Western populations, are still not understood. More well-designed placebo-controlled studies are needed to ascertain the role played by mediators such as age, sex, and equol status and the long-term implications of isoflavone treatment, as it is not yet possible to determine safe but efficacious dosage levels for general recommendations. Furthermore, ongoing and future prospective cohort studies, which are positioned to study the US population as it continues to age and chronic disease prevalence continues to grow, should consider the importance of assessing isoflavone dietary and supplementary intake for future analysis.

Funding
The authors received no direct funding for this research.

Competing interests
The author declares no competing interests.

Author details
Katie A. Miller[1]
E-mail: katie.miller148@gmail.com
ORCID ID: http://orcid.org/0000-0003-4927-1322
Farrell Frankel[1]
E-mail: farrell.frankel@simmons.edu
Hitomi Takahashr[1]
E-mail: Hitomi.Takahashr@simmons.edu
Nicole Vance[1]
E-mail: Nicole.Vance@simmons.edu
Celia Stiegerwald[1]
E-mail: Celia.Stiegerwald@simmons.edu
Sari Edelstein[1]
E-mail: dre@simmons.edu
[1] Simmons College, S-334, Boston, MA 02115, USA.

Citation information
Cite this article as: Collected literature on isoflavones and chronic diseases, Katie A. Miller, Farrell Frankel, Hitomi Takahashr, Nicole Vance, Celia Stiegerwald & Sari Edelstein, *Cogent Food & Agriculture* (2016), 2: 1135861.

References

Al-Nakkash, L., Martin, J. B., Petty, D., Lynch, S. M., Hamrick, C., & Lucy, D., ... Broderick, T. L. (2012). Dietary genistein induces sex-dependent effects on murine body weight, serum profiles, and vascular function of thoracic aortae. *Gender Medicine, 9*, 295–308. doi:10.1016/j.genm.2012.07.001

Anderson, J. W., Johnstone, B. M., & Cook-Newell, M. E. (1995). Meta-analysis of the effects of soy protein intake on serum lipids. *New England Journal of Medicine, 333*, 276–282. doi:10.1056/NEJM199508033330502

Atteritano, M., Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., ... Squadrito, F. (2007). Effects of the phytoestrogen genistein on some predictors of cardiovascular risk in osteopenic, postmenopausal women: A two-year randomized, double-blind, placebo-controlled study. *The Journal of Clinical Endocrinology & Metabolism, 92*, 3068–3075. doi:10.1210/jc.2006-2295

Bhagwat, S., Haytowitz, D. B., & Holden, J. M. (2008, September). *USDA database for the isoflavone content of selected foods: Release 2.0*. Retrieved from http://www.ars.usda.gov/nutrientdata

Bodenheimer, T., Chen, E., & Bennett, H. D. (2009). Confronting the growing burden of chronic disease: Can the U.S. health care workforce do the job? *Health Affairs, 28*, 64–74. doi:10.1377/hlthaff.28.1.64

Bosland, M. C., Kato, I., Zeleniuch-Jacquotte, A., Schmoll, J., Rueter, E. E., Melamed, J., ... Davies, J. A. (2013). Effect of soy protein isolate supplementation on biochemical recurrence of prostate cancer after radical prostatectomy: A randomized trial. *JAMA, 310*, 170–178. doi:10.1001/jama.2013.7842

Casini, M. L., Marelli, G., Papaleo, E., Ferrari, A., D'Ambrosio, F., & Unfer, V. (2006). Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: A randomized, double-blind, crossover, placebo-controlled study. *Fertility and Sterility, 85*, 972–978. doi:10.1016/j.fertnstert.2005.09.048

Chatterjee, G., Roy, D., Khemka, V. K., Chattopadhyay, M., & Chakrabarti, S. (2015). Genistein, the isoflavone in soybean, causes amyloid beta peptide accumulation in human neuroblastoma cell line: Implications in Alzheimer's disease. *Aging and Disease, 6*, 456–465. Retrieved from http://www.aginganddisease.org/EN/10.14336/AD.2015.0327

Chen, M., Rao, Y., Zheng, Y., Wei, S., Li, Y., Guo, T., & Yin, P. (2014). Association between soy isoflavone intake and breast

cancer risk for pre- and post-menopausal women: A meta-analysis of epidemiological studies. *PLoS One, 9*, e89288. doi:10.1371/journal.pone.0089288

Choi, E. J., & Kim, G.-H. (2008). Daidzein causes cell cycle arrest at the G1 and G2/M phases in human breast cancer MCF-7 and MDA-MB-453 cells. *Phytomedicine, 15*, 683–690. doi:10.1016/j.phymed.2008.04.006

Dall, T. M., Gallo, P. D., Chakrabarti, R., West, T., Semilla, A. P., & Storm, M. V. (2013). An aging population and growing disease burden will require a large and specialized health care workforce by 2025. *Health Affairs, 32*, 2013–2020. doi:10.1377/hlthaff.2013.0714

deVere White, R. W., Hackman, R. M., Soares, S. E., Beckett, L. A., Li, Y., & Sun, B. (2004). Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. *Adult Urology, 63*, 259–263. doi:10.1016/j.urology.2003.09.061

Dokken, B. B. (2008). The pathophysiology of cardiovascular disease and diabetes: Beyond blood pressure and lipids. *Diabetes Spectrum, 21*, 160–165. doi:10.2337/diaspect.21.3.160

Duffy, R., Wiseman, H., & File, S. E. (2003). Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacology, Biochemistry and Behavior, 75*, 721–729. doi:10.1016/S0091-3057(03)00116-3

Dyall, S. C. (2010). Amyloid-beta peptide, oxidative stress and inflammation in alzheimer's disease: Potential neuroprotective effects of omega-3 polyunsaturated fatty acids. *International Journal of Alzheimer's Disease, 2010*, 1–10. doi:10.4061/2010/274128

Gleason, C. E., Carlsson, C. M., Barnet, J. H., Meade, S. A., Setchell, K. D. R., Atwood, C. S., … Asthana, S. (2009). A preliminary study of the safety, feasibility and cognitive efficacy of soy isoflavone supplements in older men and women. *Age and Ageing, 38*, 86–93. doi:10.1093/ageing/afn227

Gleason, C. E., Fischer, B. L., Dowling, N. M., Setchell, K. D. R., Atwood, C. S., Carlsson, C. M., & Asthana, S. (2015). Cognitive effects of soy isoflavones in patients with alzheimer's disease. *Journal of Alzheimer's Disease, 47*, 1009–1019. doi:10.3233/JAD-142958

Gültekin, E., & Yildiz, F. (2006). Introduction to phytoestrogens. In F. Yildiz (Ed.), *Phytoestrogens in functional foods* (pp. 3–18). Boca Raton, FL: Taylor & Francis.

Gutierrez-Zepeda, A., Santell, R., Wu, Z., Brown, M., Wu, Y., Khan, I., … Luo, Y. (2005). Soy isoflavone glycitein protects against beta amyloid-induced toxicity and oxidative stress in transgenic *Caenorhabditis elegans*. *BMC Neuroscience, 6*, 54. doi:10.1186/1471-2202-6-54

Han, B.-J., Li, W., Jiang, G.-B., Lai, S.-H., Zhang, C., Zeng, C.-C., & Liu, Y.-J. (2015). Effects of daidzein in regards to cytotoxicity *in vitro*, apoptosis, reactive oxygen species level, cell cycle arrest and the expression of caspase and Bcl-2 family proteins. *Oncology Reports, 34*, 1115–1120. doi:10.3892/or.2015.4133

Hooper, L., Madhavan, G., Tice, J. A., Leinster, S. J., & Cassidy, A. (2010). Effects of isoflavones on breast density in pre- and post-menopausal women: A systematic review and meta-analysis of randomized controlled trials. *Human Reproduction Update, 16*, 745–760. doi:10.1093/humupd/dmq011

Howlader, N., Noone, A. M., Krapcho, M., Garshell, J., Miller, D., Altekruse, S. F., … Cronin, K. A. (Eds). (2015, April). *SEER Cancer Statistics Review, 1975–2012*. Bethesda, MD: National Cancer Institute. Retrieved from http://seer.cancer.gov/csr/1975_2012/, based on November 2014 SEER data submission. Posted to the SEER web site.

Hung, W. W., Ross, J. S., Boockvar, K.S., & Siu, A. L. (2011). Recent trends in chronic disease, impairment and disability among older adults in the United States. *BMC Geriatrics, 11*, 47. Retrieved from http://www.biomedcentral.com/1471-2318/11/4

Jackson, R. L., Greiwe, J. S., & Schwen, R. J. (2011). Emerging evidence of the health benefits of S-equol, an estrogen receptor β agonist. *Nutrition Reviews, 69*, 432–448. doi:10.1111/j.1753-4887.2011.00400.x

Jin, S., Zhang, Q. Y., Kang, X. M., Wang, J. X., & Zhao, W. H. (2010). Daidzein induces MCF-7 breast cancer cell apoptosis via the mitochondrial pathway. *Annals of Oncology, 21*, 263–268. doi:10.1093/annonc/mdp499

Karahalil, B. (2006). Benefits and risks of phytoestrogens. In F. Yildiz (Ed.), *Phytoestrogens in functional foods* (pp. 209–239). Boca Raton, FL: Taylor & Francis.

Kreijkamp-Kaspers, S., Kok, L., Grobbee, D. E., de Haan, E. H. F., Aleman, A., & van der Schouw, Y. T. (2007). Dietary phytoestrogen intake and cognitive function in older women. *Journal of Gerontology: Medical Sciences, 62A*, 556–562.

Lazarevic, B., Boezelijn, G., Diep, L. M., Kvernrod, K., Ogren, O., Ramberg, H., … Karlsen, S. J. (2011). Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: A randomized, placebo-controlled, double-blind phase 2 clinical trial. *Nutrition and Cancer, 63*, 889–898. doi:10.1080/01635581.2011.582221

Lee, J., Ju, J., Park, S., Hong, S. J., & Yoon, S. (2012). Inhibition of IGF-1 signaling by genistein: Modulation of E-cadherin expression and downregulation of β-catenin signaling in hormone refractory PC-3 prostate cancer cells. *Nutrition and Cancer, 64*, 153–162. doi:10.1080/01635581.2012.630161

Liu, X., Sun, C., Jin, X., Li, P., Ye, F., Zhao, T., … Li, Q. (2013). Genistein enhances the radiosensitivity of breast cancer cells via G2/M cell cycle arrest and apoptosis. *Molecules, 18*, 13200–13217. doi:10.3390/molecules181113200

Liu, Z.-M., Ho, S. C., Chen, Y.-M., Liu, J., & Woo, J. (2014). Cardiovascular risks in relation to daidzein metabolizing phenotypes among Chinese postmenopausal women. *PLoS One, 9*, e87861. doi:10.1371/journal.pone.0087861

Looker, A. C., Borrud, L. G., Dawson-Hughes, B., Shepherd, J. A., & Wright, N. C. (2012). *Osteoporosis or low bone mass at the femur neck or lumbar spine in older adults: United States, 2005–2008* (NCHS Data Brief, No. 93). Hyattsville, MD: National Center for Health Statistics.

Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., & Atteritano, M., … Squadrito, F. (2007). Effects of the phytoestrogen genistein on bone metabolism in osteopenic postmenopausal women: A randomized trial. *Annals of Internal Medicine, 146*, 839–847. doi:10.7326/0003-4819-146-12-200706190-00005

Nechuta, S. J., Caan, B. J., Chen, W. Y., Lu, W., Chen, Z., & Kwan, M. L., … Shu, X. O. (2012). Soy food intake after diagnosis of breast cancer and survival: an in-depth analysis of combined evidence from cohort studies of US and Chinese women. *American Journal of Clinical Nutrition, 96*, 123–132. doi:10.3945/ajcn.112.035972

Pavese, J. M., Krishna, S. N., & Bergan, R. C. (2014). Genistein inhibits human prostate cancer cell detachment, invasion, and metastasis. *American Journal of Clinical Nutrition, 100*(Suppl), 431S–436S. doi:10.3945/ajcn.113.071290

Pendleton, J. M., Tan, W. W., Anai, S., Chang, M., Hou, W., Shiverick, K. T., & Rosser, C. J. (2008). Phase II trial of isoflavone in prostate-specific antigen recurrent prostate cancer after previous local therapy. *BMC Cancer, 8*, 132. doi:10.1186/1471-2407-8-132

Qin, Y., Shu, F., Zeng, Y., Meng, X., Wang, B., & Diao, L., … Mi, M. (2013). Daidzein supplementation decreases serum triglyceride and uric acid concentrations in hypercholesterolemic adults with the effect on triglycerides being greater in those with the GA compared with the GG genotype of *ESR-β RsaI*. *The Journal of Nutrition, 144*, 49–54. doi:10.3945/jn.113.182725

Ricci, E., Cipriani, S., Chiaffarino, F., Malvezzi, M., & Parazzini, F. (2010). Soy isoflavones and bone mineral density in perimenopausal and postmenopausal western women: A systematic review and meta-analysis of randomized controlled trials. *Journal of Women's Health, 19*, 1609–1617. doi:10.1089/jwh.2010.2021

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., ... Ockene, J. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the Women's Health Initiative randomized controlled trial. *Journal of American Medical Association, 288*, 321–333. doi:10.1001/jama.288.3.321

Sahin, K., Tuzcu, M., Sahin, N., Akdemir, F., Ozercan, I., Bayraktar, S., & Kucuk, O. (2011). Inhibitory effects of combination of lycopene and genistein on 7,12-dimethyl benz(a)anthracene-induced breast cancer in rats. *Nutrition and Cancer, 63*, 1279–1286. doi:10.1080/01635581.2011.606955

Santos-Galduróz, R. F., Galduróz, J. C. F., Facco, R. L., Hachul, H., & Tufik, S. (2010). Effects of isoflavone on the learning and memory of women in menopause: A double-blind placebo-controlled study. *Brazilian Journal of Medical and Biological Research, 43*, 1123–1126. doi:10.1590/S0100-879X2010007500104

Setchell, K. D. R., Clerici, C., Lephart, E. D., Cole, S. J., Heenan, C., Castellani, D., ... Heubi, J. E. (2005). S-equol, a potent ligand for estrogen receptor β, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *American Journal of Clinical Nutrition, 81*, 1072–1079. Retrieved from http://ajcn.nutrition.org/content/81/5/1072.long

Shinkaruk, S., Durand, M., Lamothe, V., Carpaye, A., Martinet, A., & Chantre, P., ... Bennetau-Pelissero, C. (2012). Bioavailability of glycitein relatively to other soy isoflavones in healthy young Caucasian men. *Food Chemistry, 135*, 1104–1111. doi:10.1016/j.foodchem.2012.03.135

Singh-Gupta, V., Zhang, H., Yunker, C. K., Ahmad, Z., Zwier, D., Sarkar, F. H., & Hillman, G. G. (2010). Daidzein effect on hormone refractory prostate cancer in vitro and in vivo compared to genistein and soy extract: Potentiation of radiotherapy. *Pharmaceutical Research, 27*, 1115–1127. doi:10.1007/s11095-010-0107-9

St. John, J. A., Henderson, V. W., Hodis, H. N., Kono, N., McCleary, C. A., Franke, A. A., & Mack, W. J. (2014). Associations between urine excretion of isoflavonoids and cognition in postmenopausal women in the women's isoflavone soy health clinical trial. *Journal of the American Geriatrics Society, 62*, 629–635. doi:10.1111/jgs.12752

Uehara, M. (2013). Isoflavone metabolism and bone-sparing effects of daidzein-metabolites. *Journal of Clinical Biochemistry and Nutrition, 52*, 193–201. doi:10.3164/jcbn.13-2

Unno, K., Konishi, T., Nakagawa, A., Narita, Y., Takabayashi, F., Okamura, H., ... Imai, S. (2015). Cognitive dysfunction and amyloid β accumulation are ameliorated by the ingestion of green soybean extract in aged mice. *Journal of Functional Foods, 14*, 345–353. doi:10.1016/j.jff.2015.02.011

Urban, D., Irwin, W., Kirk, M., Markiewicz, M. A., Myers, R., Smith, M., ... Barnes, S., (2001). The effect of isolated soy protein on plasma biomarkers in elderly men with elevated serum prostate specific antigen. *The Journal of Urology, 165*, 294–300. http://dx.doi.org/10.1097/00005392-200101000-00082

White, L. R., Petrovitch, H., Ross, W., Masaki, K., Hardman, J., Nelson, J., ... Markesbery, W. (2000). Brain aging and midlife tofu consumption. *Journal of the American College of Nutrition, 19*, 242–255. http://dx.doi.org/10.1080/07315724.2000.10718923

Wu, A. H., Spicer, D., Garcia, A., Tseng, C.-C., Hovanessian-Larsen, L., Sheth, P., ... Pike, M. C. (2015). Double-blind randomized 12-month soy intervention had no effects on breast MRI fibroglandular tissue density or mammographic density. *Cancer Prevention Research, 8*, 942–951. doi:10.1158/1940-6207.CAPR-15-0125

Wu, J., Oka, J., Ezaki, J., Ohtomo, T., Ueno, T., Uchiyama, S., ... Ishimi, Y. (2007). Possible role of equol status in the effects of isoflavone on bone and fat mass in postmenopausal Japanese women: A double-blind, randomized, controlled trial. *Menopause, 14*, 866–874. doi:10.1097/gme.0b013e3180305299

Yan, L., & Spitznagel, E. L. (2009). Soy consumption and prostate cancer risk in men: A revisit of a meta-analysis. *American Journal of Clinical Nutrition, 89*, 1155–1163. doi:10.3945/ajcn.2008.27029

Zhang, B., Su, J.-P., Bai, Y., & Liu, Y.-H. (2015). Inhibitory effects of O-methylated isoflavone glycitein on human breast cancer SKBR-3 cells. *International Journal of Experimental Pathology, 8*, 7809–7817. Retrieved from http://www.ijcep.com/files/ijcep0007779.pdf

Zhang, X., Gao, Y.-T., Yang, G., Li, H., Cai, Q., & Ziang, Y.-B., ... Shu, X.-O. (2012). Urinary isoflavonoids and risk of coronary heart disease. *International Journal of Epidemiology, 41*, 1367–1375. doi:10.1093/ije/dys130

Zhuo, X.-G., Melby, M. K., & Watanabe, S. (2006). Soy isoflavones and health. In F. Yildiz (Ed.), *Phytoestrogens in functional foods* (pp. 243–262). Boca Raton, FL: Taylor & Francis.