SOY-DERIVED MICROBIAL METABOLITE EQUOL IN CANCER PREVENTION

Mureşan Liliana[1,*)], T. Rusu[1)], T. Y. Wang[2)], J. B. Park[2)], Doina Clapa[1)]
*[1]Univeristy of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, Mănăştur St.3-5, 400372, Cluj-Napoca, Romania; *Corresponding author:* muresanliliana@yahoo.com
[2]Diet, Genomics and Immunology Laboratory, U.S. Department of Agriculture−Agricultural Research Service, Beltsville, Maryland 20705, United States

Abstract. The human diet is integral part of overall health, and human response to foods and their components is often dependent on their dynamic interrelationship with microbes residing in the oral and gastrointestinal tract. Part of this dynamic relationship arises from the ability of bacteria to metabolize dietary components. This microbial involvement can alter the nutritional value of food components by generating specific compound(s) that are formed by gut bacteria. Thus, microbial metabolites may have a positive or negative influence on the risk of multiple diseases, including cancer. Soybeans were selected for this review, because there is considerable worldwide consumption and have been linked as cancer risk modifiers. Considerable evidence indicates that these soy foods are involved with cancer prevention, yet there is considerable variability in the response in terms of cancer risk and tumor behavior. Equol, an active metabolite of the soy isoflavone daidzein has been intensely studied in the last 25 years for its potential health benefits, not just for its estrogenic or anti-estrogenic activities but also for its antioxidant, anti-inflammatory or neuroprotective properties. The aim of this review is to assess the strengths of evidence that links microbially generated metabolite equol from soy foods with cancer risk and tumor behavior. Through a systematic analysis of existing evidence, the review focuses on biological consequences of soyderived equol on cancer-related processes as a function of the amount and duration of exposure, and when possible, human genetics and tissue specificity. While this evaluation specifically focuses on soy, the findings may also serve as a proof-of-principle for examination of food/disease/microbiome relationship with other foods.

Keywords: equol, isoflavones, microbial metabolites, food, cancer

INTRODUCTION

Soybean (Glycine max) is one of the top agricultural commodities in the United States and around the world, and one of the most valuable crops with important nutritional and nutraceutical value. Today soybeans are known for being one of the best source of vegetable protein, for being the second largest source of vegetable oil, andfor potentialnutraceutical properties for human health (28). Along with essential amino acids and polyunsaturated fatty acids, soybeans contain various secondary metabolites such as isoflavones, phytic acid, peptides or trypsin inhibitors (101). In fact, soy foods are the greatest source of isoflavones in human diet, which are structurally similar with 17 β-estradiol, and may have important implications in human health. These biologically active phytoestrogens have been associated with potential effects on cardiovascular diseases, obesity, diabetes, osteoporosis, and several types of cancer (104).

THE HUMAN MICROBIOTA

In 1675, Antoine van Leeuwenhoek first observed microorganisms reside in the intestine. However, over two hundred years passed before Louis Pasteur demonstrated their critical role in food digest (90). Today, it is well accepted that tens of trillions of

microorganisms play critical/dyanmic roles in many human body parts such asskin, oral cavity, gastrointestinal tract and vagina. The major site of these microbes is the gastrointestinal tract with the colon alone containing 70 % of all the microbes in the human body (61).

The collections of the immense number of microbes that colonize the human body are collectively referred to as the human microbiota (all of the genes inside the microbial cells are known as human microbiome) (66). These microorganisms perform functions that can influence a number of the body's physiological and pathological processes. As the gastrointestinal microbes are essential for the human body in producing some essential vitamins, metabolizing food into energy, and shaping immune system, they can influence various disorders such as inflammatory bowel diseases, diabetes, inflammation, autoimmunity, allergy, liver diseases, obesity and cancer (109). Interest in understanding the complex interactions between the host, microbiota and nutrition has grown rapidly during the last decade, even though the importance of these interactions was suggested many centuries ago. For examples, in 400 BC Hippocrates said that "all disease begins in the gut", and later, at the beginning at 20 century, Elie Metchnikoff, who is considered the father of the modern immunology, proposed that the interaction of diet and intestinal bacteria contributes not only to many diseases onset and ill-consequences, it may also influence the aging process (36).

The emergence of the genomic era, where new technologies such next generation gene sequencing (NGS) analysis of conserved regions of microbial 16SrRNA, has introduced new thoughts and approaches to help understand better aboutthe interactions between food compounds, andthe microbiome, and their implications in human health and disease prevention (106). Even though only a small percentage of the human intestinal bacteria have been identified so far, it is clear that the colon has many more important roles than previously thought and is being considered more like a metabolic organ similar to the liver, rather than limited functions that relate to trans-epithelial fluid transport, secretion of fluids and discharge of waste products (4).

Consequently, the gut microbiota is believed to have a critical role not only in digestion, but also in human health and disease prevention. Inherent enzymatic activities in microbes provide them a unique ability to produce metabolites, which can not formed by mammalian cells (70). Some of these metabolites have been demonstrated to have enhanced biological activities which parent food components can't deliver. The precise role of these microbial metabolites may arise from several changes in normal cellular/tissue processes including serving as a protector against oxidative stress (94).

In general, the ability of microbes to form specific metabolites may influence human health through modifications in a variety of cellular events including carcinogen bioactivation, DNA-repair, cell cycle regulation, apoptosis, differentiation, hormonal regulation, inflammation, and immunocompetence (24). Those influences can promote both positive and negative effects on human health. Along with assisting in food digestion and nutrientabsorption, microbiota positively influences host health by regulating the metabolism or protecting host from harmful pathogens via its antioxidant and antiinflammatory capabilities (52, 24). At the same time any disruption of commensal microbiota has been associated with various disorders such us inflammatory bowel disease (27), irritable bowel syndrome (85), antibiotic associated diarrhea (47), obesity (45), allergies or even neurophsychiatric illnesses (e.g., schizophrenia, autism, attention-deficit/hyperactivity disorder) (52). Furthermore, the diet can play an essential role in

defining the gut bacterial composition, being able to stimulate either beneficial probiotics or pathogenic bacteria (29).

DIET GUT MICROBIOME AND CANCER PREVENTION

Cancer is a leading cause of death in the world, responsible for 8.8 million deaths in 2015 and projecting an estimated cancer death increase to 13.1 million by 2030 (112). Research results suggest that between 30-40% of these deaths are directly related to diet (25). Food and nutrition can modify the risk and tumor behavior at cancers at a large number of sites. Major progress has been made in understanding the cancer development and accumulated evidence suggest that foods plays an important role in the cancer process (91)(79). Furthermore, data suggest that foods and various dietary patterns can not only prevent the cancer formation but also can protect against cancer after its formation (2)(44)(47).With all the evidence suggesting the important relationship between foods and cancer development or treatment, it stills a lot of inconsistency in literature regarding this phenomenon. This inconsistency may be related with the human microbiota which seems to have more influence in human health and diseases than previously thought (24)(38). The complexity of microbes that reside within the human body participate in all dynamic relationship with our diet and metabolism which is dictated by genetic and epigenetic controls (6)(50).These microbes are essential in helping us to metabolize food into energy, or to produce metabolites from food compounds that can have antimicrobial, antiviral, antitumoral or other biological functions (103, 105, 32).

Food derived metabolites created by intestinal microbes may influence human health more than previously considered via a variety of mechanisms including changes in carcinogen bioactivation (48), apoptosis (20), differentiation (3), cell cycle regulation (20) inflammation (118), immunocompetence or hormonal regulation(105). For example, the link between lower rate of breast cancer in Asian women population and the consumption of soy foods may be attribute to the microbially derivated metabolite equol, rather than the soy isoflavone dazdein (59). The protective effects of equol on hormone-related types of cancer have been attributed mainly to its ability to selectively bind estrogen receptors, regulation of the estrogen level or to indirectly influence androgen level (58, 73).Thus, equol-producers may receive protection from soy consumption, which may clarify the unevenness of breast cancer among populations and explain the inconsistencies in the literature. As to soy and soy products as a whole, Huang and colleagues suggested that soy foods have a favorable effect on the microbiota by incresing the bifidobacterial and lactobacilli population and diminishing disease-promoting microorganisms (34). Undeniably, the interrelationship between the gut microbiota, diet and human health is exceedingly complex, due to shifts in these and other cellular processes including energy homeostasis and the formation of essential nutrients (95, 30).

SOY-DERIVED BIOACTIVE MICROBIAL METABOLITE EQUOL

Epidemiological evidence reveals that people of Asian descent are statistically less likely to develop certain types of cancers (i.e breast and colon) than people of European/American ancestry (3). At least part of this relationship may be attributed to the soy content in a typical Asian diet (74) and more specifically to its isoflavone content (100). Isoflavones are flavonoids with a chemical structure similar with the human estrogen and therefore possess pseudo-hormonal properties. Soybeans and soy foods represent the richest

sources of isoflavones in human diet. Typical isoflavonoids in soy are dazdein,genistein and glycitein,with the most notable for health being considered soy genistein and daidzein.

Genistein, which represent about 53% of soy isoflavone, was found to have both estrogenic and anti-estrogenic functions and the action of genistein as a cancer promoter or having anticancer activity is still highly controversial (13). Both genistein and daidzein are found in soy foods as conjugated glucosides and require several conjugation and de-conjugation steps in order to be absorbed (67). Intestinal bacteria are the key players in these steps and in charge for converting the conjugated isoflavones in their aglycon form and then further metabolized to produce para-etilfenol from genistein and equol from daidzein (91).

The isoflavone daidzein is particularly important as studies suggest that its microbially derivate metabolite, equol, may have anticancer effects (96). Equol is exclusively produced by intestinal bacterial biotransformation of daidzein. This compound was discovered in 1932 in the urine of a pregnant mare and thus named after its equine origin (11). It did not receive much attention until it was discovered to be associated with the infertility of Australian sheep eating a particular species of Trifolium in Australia (11). It was presumed this infertility was related to abnormal estrogen homeostasis.

It is now recognized that once daidzein enters the large intestine, it is converted into dihydrodaidzein with the help of beta-glucosydase. It is then further converted to S-equol 7-hydroxy-3-(4'-hydroxyphenyl)-chroman or O-DMA by way of dihydrodaidzein with the help of intestinal microbes (92)(94). Equol's phenolic ring has a similar structure to human estrogen which allows binding to the estrogen receptor (95). Estrogen has been identified as a positive effecter for several hormone sensitive cancers, including breast cancer and prostate cancer (25, 84, 87). Thus, its effecter action as an antagonist or partial antagonist to estrogen receptor in humans may account for part of its anticancer property (95). Furthermore, equol is more bioavailable and has stronger antioxidant activity than daidzein, dihydrodaidzein and O-DMA (109). Even more, equol is up to 100-fold more potent that its isoflavone parent daidzein to down-regulating the estrogen receptors in human breast cancer cells (92, 104).

MULTIPLE MICROORGANISMS PRODUCE EQUOL

Multiple bacteria isolated from humans and animals can contribute to the conversion of daidzein to equol. It is generally accepted that a consortium of bacteria is necessary for this process. *Lactococcus garvieae* is recognized as one of the most efficient bacteria in the synthesis of equol, and has been used in the production of equol supplements (SE5-OH) (115). Some other bacteria that contribute to equol production are: *Aldercreutzia equolifaciens, Streptococcus intermedius, Bacteriodes ovatus, Slackia equolifaciens and Slackia isoflavoniconvertes*, which is one of the few bacterium isolated from humans, that is known to have the ability to produce equol (65). More recently, *Eggerthella sp. Bacterium* was also isolated from human microbiome and found to convert daidzein into S-equol (116). In addition, a gram-negative, rod-shaped bacteria called *Julong 732*, has been reported to have a role in equol production (110).

If one or more of the aforementioned bacteria are missing from a human microbiome, it is impossible to optimally produce equol (7). However, the microorganism alone is insufficient for production since there are several factors that may determine overall equol formation. For instance, along with the presence of specific equol- producing bacteria, an organism needs the optimal conditions that will permit the redox reactions to occur along with the presence of a source of substrate (i.e. daidzein).

While animals are reported to produce equol after soy consumption (95, 12), not all humans are capable to produce equol, and this capability differs greatly among populations (91). Between 50-55% of Asian population are equol producers compare with only 20-35% of Western population capabale of producing equol (7, 20). These statistics may clarify the unevenness of breast cancer among populations, as only equol-producer may be protected by soy consumption. Psychographic factors may also contribute to equol production (57). For example, the frequency of equol-producers is 59% among vegetarians soy consuming, but only 25% in non-vegetarians (30). Furthermore, Hong K.W. and colleagues(40) found that the genetic background of the host is another important factor in determining the microbiota profile, as the specific gene HACE1 (HACE1 is an E3 ubiquitin protein ligase located in 6q21, the genomic region frequently deleted in natural killer cell malignancy), expressed in many human tissues was found to be an important determinant in equol-producing phenotype (40).

EQUOL AND HORMONE-RELATED CANCERS

There is evidence that equol may reduce breast cancer risk. Epidemiological studies provide evidence that breast cancer incident is significantly lower in population consuming an Asian diet compared to those consuming a Western diet, which has been accredited to the consumption of soy foods (114, 85). For example, Japanese women have one of the lowest rates of breast cancer in the world is associated with their soy-rich diets (35). It should be noted the amount of soy consumed differ markedly between populations, with Asian population consuming 100mg/day isoflavones compare with 1mg/day consumed by Western population (73).

A cohort study including 73,223 Chinese women with a soy-rich diet during adolescence and early adulthood, and followed for 7.5 years, observed a reduction of 43% of breast cancer risk in premenopausal women who consumed a diet high in soy as compared to those with a regular diet (59). Furthermore, when Japanese immigrants switch to a Western diet, their cancer rates totally change, which suggests that diet is critically important in protection against breast cancer (54).Nevertheless, which constituent or metabolite from soy accounts for this protection remains controversial (52). It is possible that equol may be one factor contributing to this association, but many other factors in their diet may account for the reduced risk (98, 103).

Goodman and colleagues conducted a multiethnic cohort study that included 36,458 postmenopausal women (32). The results of their study indicated a significant reduction of breast cancer risk in the Japanese-American women group and a less significant risk in white women group with the higher - urinary excretion of daidzein, compare with the lower or not any urinary dazdein excretion, supporting that the equol-producer postmenopausal women had greater protection against breast cancer from equol (32).

Epidemiological evidence support these findings, as an international comparison of prostate cancer cases indicates that the incident rates in Asian men consuming a diet rich in soy (living in the countries such as Japan, China, India and Singapore) are significantly lower when compared to men consuming a Western diet (living in Canada, United States and Europe) (84). Experimental data also suggested that equol-producing men have a lower risk of developing prostate cancer (4). Equol also can act as an anti-androgen. Although, equol does not bind to the androgen receptor, it influences its activity by specifically binding to 5 alpha-dihydrotestosterone and prevents its binding to the androgen receptor. Since prostate cancer cells depend on androgen, the ability of equol to decrease the androgen level can serve as potent protection against prostate cancer (63). Equol may also have protective effects in

ovarian cancer, as i*n vitro* results suggested that equol induces apoptosis in SKOV-3 cells via a TRAIL and caspase 8-dependent pathway (when human SKOV-3 ovarian cancer cells where treated with equol, the increased level of TUNELpositive cells were observed in a time-dependent manner, between 4–48 hours) (118). Similar results were observed also in other studies where equol express estrogenic responses in ovarian cells (9, 27). Also, based case-control study,that included 832 chinese women with endometrial cancer concluded that regular consumption of soya foods was inversely associated with the risk of endometrial cancer (111).

Another influencing factor that may contribute to the effect of soy intake on carcinogenesis may be the time of life when soy rich diet is consume, as a good number of case-control studies suggested that soy intake during early years and adolescence has stronger protection against breast cancer compare with adult soy diet which suggest maturation of the breast tissue likely determines the response (96, 113, 83). Finally, the type of soy foods consumed may also influence the benefits of soy diet, as components from fermented soy are more bioavailable than regular soy such as soy milk or tofu (68). Among the Japanese population, for example, at least 40% of isoflavones comes from fermentated soy foods such miso and natto, where the isoflavones are available in their aglycone form, compared with the Western population who gets most of the isoflavones in their glucoside form from unfermented soy foods such soy flour or soy milk (40, 62). However, one of the most significant factors in influencing soy protection against breast cancer seems to be the ability of producing equol, as there is mounting evidence that equol's anti-estrogenic activity may account for its potential health benefits, but those benefits may be restricted to the subgroup of equol-producers (98). Thus, equol-producers population would be expected to receive greater anticancer benefits from soy consumption compared to those without such microbial capability, which may partially explain the variation in cancer protection from epidemiological and intervention studies using soy foods.

The health benefits of equol on cancer protection maybe indirect. Brown and colleagues reported in a rodent study that R-equol has preventive activity in breast tumor(14). Interestingly, both enantiomers, R-equol and S-equol, significantly reduced body weight of the animals suggesting that a controlling obesity, a risk factor for breast cancer may also contribute to part of the response to equol (103). This relationship with cancer risk may arise because adipocytes may increase the concentration of circulatory endogenous estrogen and therefore contribute to the postmenopausal risk of breast cancer (50). It should be noted that mounting evidence points to the importance of the intestinal flora to influence obesity (91). Therefore, a shift in the human microbiota may assist in reducing obesity and thereby reduce endogenous estrogen generation and consequently reduce breast cancer risk or other estrogen-related cancers.

EQUOL AND PREVENTION OF BREAST CANCER-POSSIBLE MECHANISMS

More than 30 years ago, Setchell and colleagues first provided evidence that soy foods may have protective effects against breast cancer (97). More recent clinical studies suggest that soy foods consumption improves breast cancer survival rate (102) by reducing the recurrence risk in breast cancer survival (33, 102). Reducing the estrogen production is critical in breast cancer, as all data indicate that exposure to estrogen significant influence breast cancer development and about 80 percent of the breast cancer cells are recognized to be estrogen-dependent (53, 20). Today, equol's estrogenic activity is viewed as a possible contributor to prevention of breast cancer risk but its mechanism of actions are not fully

understood. The most important mechanism by which equol may influence breast cancer is by competing with the endogenous estrogens for estrogen receptors, reducing or even suppressing estrogen cellular bioactivity (Tabel 1). Equol was found to have affinity for both estrogen receptors, ER-alpha and ER-beta, acting as an antagonist or partial antagonist to these receptors (81).

Choi and Kim demontrasted that equol has **anti-proliferative** effects on human estrogen-receptor negative breast cancer cells in a dose- and time-dependent manner (57.7% decrease of proliferation at high concentration of 100 μM equol for 72h), However, the protective effects equol has on estrogen receptor-negative cancer may not be achivable by diet as *in vitro* studies detected equol is at less than 10 μM (71). Bosviel and colleagues focused on the capacity of equol to reverse the DNA methylation as a plausible mechanism by which equol inhibits tumor proliferation profile of breast cancer cells (when the cells where treated with 2 μM S-equol for three weeks) (12) They found that the demethylating effects of S-equol on the CpG islands inside the promoters of the two genes leads to higher expression of the oncosuppresors in the breast cancer cells (12).

Another important mechanism widely examined *in vitro* is the **pro-apoptotic** effects of equol in breast cancer cells.Considerable data show that equol has the ability to initiate apoptosis via cell cycle arrest in estrogen receptor-negative breast cancer cells (MDA-MB-453 cells at high concentration 50 -100 μM equol) (20). Therefore, equol can induce cell cycle arrest that would lead to apoptosis, but not through the estrogen-dependent pathway, rather through the estrogen-independent pathway (20). Also, equol was found to be able to induce apoptosis through cytochrome c-mediated caspases cascade in breast cancer cell (21). Furthermore, equol (at high dose of 100 μM) in combination with tamoxifen were able to induce apoptosis in breast cancer cells by inactivation of capsase-9 and -7 along with cytochrome-c (17). Results from *in vivo* model, when equol was administrated, demonstrated markedly promoted apoptosis. Significant decrease in the expression of anti-apoptotic protein Bcl-2 and an increase in the level of pro-apoptotic protein Bax was observed when a high dose of equol of 5 and 25 mg/kg body weight were administrated for eight weeks (19). In both, *in vitro* and *in vivo* studies apoptosis was often initiate only at high concentrations of equol (21, 19, 24). Columba de la Parra and colleagues observed that equol increases breast cancer malignancy by upregulating the eukaryotic protein synthesis initiation factor elF4G (when using different concentrations of equol, 1,5,10,25,50 μM on metastatic cancer cells MDA-MB-435 and MDA-MB-231) (23). However, in a more recent *in vivo* study the same author demonstrated that equol may have breast cancer promoting effects (when two metastatic human breast cancer cell lines, MDA-MB-435 (ER-) and Hs578t(ER-) were treated with 25 μM of (R,S) Equol for 24h) by up-regulating the c-Myc gene transcription (22).

In addition to the aforementioned study, equol's ability to inhibit the growth of breast cancer cells may relate to its **antioxidant properties**. Equol can donate electrons through its hydroxyl groups. Since it can scavenge free radicals this may also contribute to soy's health benefits (79). Furthermore, equol's binding to estrogen receptor beta will also confer antioxidant properties through increase in endothelial nitric oxide synthase (eNOS), which in turn will regulate the accumulation of the Nrf2 nuclear transcription factor that will induce the expression of genes that encode for antioxidant enzymes (44). Equol antioxidant capacity was also observed in animal studies as evident by a significantly increase the activities of glutathione reductase and peroxidase (which reduced O^2 and H_2O_2 intracellular content) (19). It has also been suggested that equol has the greatest antioxidant activity, when compared to the other isoflavones examined (99). Even though it is well known that equol has the greater antioxidant activity among isoflavones, there are no human studies to confirm the possibility

that equol may act as an antioxidant agent (51). Equol antioxidant properties were well documented by Choi E (21).

Overall, the most evidence which links equol to cancer risk/behavior comes from in vitro experiments which are not supported by *in vivo* studies. For example, the inhibitor effects of equol in brest cancer cells were only noticed at high concentrations, ranging from 50 to100 M (20). Results from the in vitro experiments may not be able to have the same effects on in vivo subjects , as studies have shown that the serum equol concentration is much lower at 40 nmol/L in non-producer and over 83nmol/L in equol producers (98). In a recent nested case-control study (2016) conducted among Chinese women by Atkinson C. and colleagues no association was found between plasma equol concentration and breast cancer and fibrocystic breast condition(18). As studies continue to show that equol may have either protective effects or promote breast cancer in humans, it is accepted that the concentration and the estrogen receptor status play an important role in its properties; more recent results accepted that this fidings may not have the same effects when it comes to clinical studies which observed that even a high amount of daily isoflavone intake (50mg- equivalent with about 5 servings of soyfoods) has no the same effects (15, 71).Also, studies show that rodents metabolize isoflavones differently than humans, resulting that animal findings may not be very relevant when it comes to human health (96).

Moreover, the length of exposure over a lifetime to equol may also have a major impact on the equol protection status agains breast cancer, as in vivo studies have shown that equol may have no benefits in postmenopausal women even if they are equol producers(64). Considerable evidence suggests that consumption of soy in childhood and adolescence may reduce the risk of breast cancer by up to 60% (55, 59). Recently, Baglia and colleagues observed in a large human study (over 70,000 participants) that soy consumption early in life, before puberty, may have protective effects agains breast cancer even if the consumption was not continued during and after puberty (9). Similar protective results were observed if isoflavones were available during early preganancy (71). Even more, Trichopoulous observed that the fetal environment, more specifically the hormone concentrations do influence the development of the breast, so in utero soy exposure may play a role in offspring protection (105). In Japan, where the incidents of breast cancer are historically low, not only is soy exposure available in utero, but infants are weaned on soy-containing foods as early as 6 month (1, 59). Overall, the protective effects of equol on breast cancer have been attributed mainly to its ability to selectively bind to estrogen receptors and regulation of the estrogen level. However, their non-hormonal properties that may also contribute to their biological activity.

Epidemiological studies, while intriguing, are not convincing, as other compounds, which are common in the Asian diet may account for the reduced risk of breast cancer in Asian populations. Hence, more in vivo studies will help to translate this in vitro founding that will allow new approaches in understanding the effects of equol in preventing breast cancer.

CONCLUSION

Overall, the health benefits of soy foods on human cancer prevention may come from the microbial derivate equol, as he maybe more potent that its isoflavone parental daidzein. These findings hold promise for new frontiers for fighting diseases such as cancer and related diseases. A better understanding of potential interactions between human host, nutrition and miocrobiota may provide powerful preventative and therapeutic potentials to reduce cancer or related disorders.

Unfortunately, the clinical evidence about the impact of soy on estrogen-related types of cancer is not particularly compelling possibly because microbiome was not taken into consideration. Therefore, determination of this relationship may be a key step in formulating estrogen-containing hormone prevention strategies which may benefit both men and women. Even though equol's correlation with cancer is still debatable, it is accepted that he is a distinguished non-steroidal estrogen with two diasteroisomers which have biological properties to deliver benefits on the prevention or treatment of various estrogen- and androgen-mediated diseases. However, in the future, additional clinical studies should be conducted to understand better the importance of equol in various types of cancers.

Table 1.

Summary of studies of equol and breast cancer

Findings	Amount and time	Mechanisms of action	Experiment	Reference
Equol down-regulates estrogen receptor	Equol has high affinity for the estrogen receptor in a concentration-dependent manner: 10`8-10`5M	-by directly biding to estrogen receptor and inhibit its activity	Human breast cancer MCF-7 cells	Sathyamoorthy & Wang, 1997
Equol inhibits cell proliferation	At concentrations of 1-100μM equol inhibits cell proliferation in a time-dependent manner at any 24,48,or 72 h exposure	-by inducing cell cycle arrest by decreasing the expression of CDKs	Human breast cancer MDA-MB-453 cells	Choi and Kim 2008
Equol initiates apoptosis	At high concentration of 50-100μM and a72 h exposure, equol induces apoptosis gradually in a dose-dependent manner.	-by increasing the cytochrome as a result of increasing expression of pro-apoptotic protein Bax - through cytochrome c-mediated caspases cascade	Human breast cancer cells Human breast cancer cells	Choi and Kim 2008 Choi et al.2008
Equol stimulates apoptosis Equol is a strong antioxidant	When administrating 5, 25 mg/kg BW equol	-by decreasing the expression of Bcl-2 and increasing the level of Bax - by increasing the activities of glutathione reductase and peroxidase	Rats breast cancer cells	Choi and Kim 2011
Equol inhibits tumor growth	At 2μM equol for three weeks	-by demethylation of BRCA1 and BRCA2 genes which will lead to the expression of oncosupressors	Human breast cancer cells	Bosviel et al. 2012

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of Ph.D, Harold Seiffried for its thorough and helpful comments.

REFERENCES

1. Adlercreutz H, Yamada T, Wähälä K, Watanabe S. (1999). Maternal and neonatal phytoestrogens in Japanese women during birth. Am J Obstet Gynecol. 1999 Mar;180 (3 Pt 1):737-43.

2. AICR. (2007). Food, Nutrition, Physical activity and the Prevention of Cancer: A Global perspective.
3. Abeer M. Mahmouda, Wancai Yanga, B, Maarten C. Boslanda. (2013). Soy isoflavones and prostate cancer: A review of molecular mechanisms. https://doi.org/10.1016/j.jsbmb.2013.12.010
4. Akaza, H. (2004). Comparisons of Percent Equol Producers between Prostate Cancer Patients and Controls: Case-controlled Studies of Isoflavones in Japanese, Korean and American Residents. Japanese Journal of Clinical Oncology, 34(2), 86-89. doi: 10.1093/jjco/hyh015
5. Andres S, Abraham K, Appel KE, Lampen A (2011). Risks and benefits of dietary isoflavones for cancer. Critical Reviews in Toxicology, 463-506.
6. Ann M O'Hara and Fergus Shanahan. (2006). The gut flora as a forgotten organ. EMBO Rep. 2006 Jul; 7(7): 688–693. doi: 10.1038/sj.embor.7400731
7. Atkinson C, Frankenfeld CL, Lampe JW. (2005). Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. PMID:15734719 Exp Biol Med (Maywood). 2005 Mar;230(3):155-70.
8. Arora A, Nair MG, Strasburg GM. (1998). Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. Arch Biochem Biophys. 1998; 356: 133–41.
9. Baglia ML, Zheng W, Li H, Yang G, Gao J, Gao YT, Shu Xo. (2016). The association of soy food consumption with the risk of subtype of breast cancers defined by hormone receptor and HER2 status. Int J Cancer. 2016 Aug 15;139(4):742-8. doi: 10.1002/ijc.30117. Epub 2016 May 5.
10. Barnes S, et al. (1990). Soybeans inhibit mammary tumors in models of breast cancer. Prog. Clin. Biol. Res. 1990; 347:239–253. PubMed.
11. Bennetts, H. W., Uuderwood, E., & Shier, F. L. (1946). A Specific Breeding Problem Of Sheep On Subterranean Clover Pastures In Western Australia. Australian Veterinary Journal, 22(1), 2-12. doi: 10.1111/j.1751-0813.1946.tb15473.x
12. Bosviel, R., Durif, J., Dechelotte, P., Bignon, Y., & Bernard-Gallon, D. (2012). Epigenetic modulation of BRCA1 and BRCA2 gene expression by equol in breast cancer cell lines. The British Journal of Nutrition, 1-7.
13. Bouker K. B. and Hilakivi-Clarke L. (2000). Genistein: does it prevent or promote breast cancer? Environ Health Perspect. 2000 Aug; 108(8): 701–708. PMCID: PMC1638285
14. Brown, N. M., Belles, C. A., Lindley, S. L., Zimmer-Nechemias, L. D., Zhao, X., Witte, D. P., Setchell, K. R. (2010). The chemopreventive action of equol enantiomers in a chemically induced animal model of breast cancer. Carcinogenesis, 31(5), 886-893. doi: 10.1093/carcin/bgq025
15. Cheng G., Wilczek B., Warner M., Gustafsson Jan-Ake, Landgren Britth-Marie. (2007). Isoflavone treatment for acute menopausal symptoms Menopause: May/June 2007 - Volume 14 - Issue 3 - pp 468-473doi: 10.1097/GME.0b013e31802cc7d0
16. Cahill W. M., Schroeder L. J., Smith A. H. (1994). Digestibility and biological value of soybean protein in whole soybeans, soybean flour, and soybean milk. J. Nutr. 1944;28:209–218.
17. Charalambous, C.; Pitta, C. A.; Constantinou, A. I. (2013). Equol enhances tamoxifen's anti-tumor activity by induction of caspase-mediated apoptosis in MCF-7 breast cancer cells. BMC Cancer 2013.
18. Charlotte Atkinson, Roberta M. Ray, Wenjin Li, Ming-Gang Lin, Dao Li Gao, Jackilen Shannon, Helge Stalsberg, Peggy L. Porter, Cara L. Frankenfeld, Kristiina Wähälä, David B. Thomas, Johanna W. Lampe. (2016). Plasma equol concentration is not associated with breast cancer and fibrocystic breast conditions among women in Shanghai, China. doi: http://dx.doi.org/10.1016/j.nutres.2016.03.008
19. Choi, E. & Kim, G. (2011). Anticancer mechanism of equol in 7,12-dimethylbenz(a)anthracene-treated animals. International Journal of Oncology. doi: 10.3892/ijo.2011.1067
20. Choi, E. & Kim, T. (2008). Equol induced apoptosis via cell cycle arrest in human breast cancer MDA-MB-453 but not MCF-7 cells. Molecular Medicine Reports, 44.

21. Choi, E. (2009). Evaluation of Equol Function on Anti- or Prooxidant Status. Journal of Food Science, 74(2), H65-H71. doi: 10.1111/j.1750-3841.2008.01039.
22. Columba de la Parra, Luis D. Borrero-Garcia, Ailed Cruz-Collazo, Robert J. Schneider, and Suranganie Dharmawardhane. (2015). Equol, an Isoflavone Metabolite, Regulates Cancer Cell Viability and Protein Synthesis Initiation via c-Myc and eIF4G. J Biol Chem. 2015. 290(10): 6047–6057. doi:10.1074/jbc.M114.617415
23. Columba de la Parra, Elisa Otero-Franqui, Michelle Martinez-Montemayor and Suranganie Dharmawardhane. (2012). The Soy Isoflavone Equol May Increase Cancer Malignancy via Up-regulation of Eukaryotic Protein Synthesis Initiation Factor eIF4G. October 24, 2012.
doi: 10.1074/jbc.M112.393470The Journal of Biological Chemistry287, 41640-41650.
24. David A. Relman. (2012). The human microbiome: ecosystem resilience and health. Nutr Rev. 2012 Aug; 70(Suppl 1): S2–S9. doi: 10.1111/j.1753-4887.2012.00489.
25. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst. 1981;66(6):1191–1308.
26. E Schmitt. W Dekant.H Stopper. Assaying the estrogenicity of phytoestrogens in cells of different estrogen sensitive tissues. https://doi.org/10.1016/S0887-2333(01)00048-0
27. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA. (2011). NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011 May 27;145(5):745-57. doi: 10.1016/j.cell.2011.04.022. Epub 2011 May 12.
28. Frank D. Gunstone and Tong Wang. Vegetable Oils in Food Technology: Composition, Properties and Uses, Second Edition DOI: 10.1002/9781444339925.ch3 Published Online: 17 MAR 2011.
29. Fredrik Bäckhed, Claire M. Fraser, Yehuda Ringel, Mary Ellen Sanders, R. Balfour Sartor, Philip M. Sherman, James Versalovic, Vincent Young, B. Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications
doi: http://dx.doi.org/10.1016/j.chom.2012.10.012
30. Fujimoto, K., Tanaka, M., Hirao, Y., Nagata, Y., Mori, M., Miyanaga, N., Kim, W. (2008). Age-stratified serum levels of isoflavones and proportion of equol producers in Japanese and Korean healthy men. Prostate Cancer and Prostatic Diseases, 11(3), 252-257.
doi: 10.1038/sj.pcan.4501030
31. Garrett W.S. (2015). Cancer and the microbiota. Science. 2015 Apr 3;348(6230):80-6. doi: 10.1126/science.aaa4972.
32. Goodman MT, Shvetsov YB, Wilkens LR, Franke AA, Le Marchand L, Kakazu KK, Nomura AM, Henderson BE, Kolonel LN. (2009). Urinary phytoestrogen excretion and postmenopausal breast cancer risk: the multiethnic cohort study.Cancer Prev Res (Phila). 2009 Oct; 2(10):887-94. doi: 10.1158/1940-6207.CAPR-09-0039. Epub 2009 Sep 29. PMID: 19789300
33. Guha N, Kwan ML, Quesenberry CP Jr, Weltzien EK, Castillo AL, Caan BJ. (2009). Soy isoflavones and risk of cancer recurrence in a cohort of breast cancer survivors: the Life After Cancer Epidemiology study. Breast Cancer Res Treat. 2009 Nov;118(2):395-405. doi: 10.1007/s10549-009-0321-5. Epub 2009 Feb 17.
34. Haiqiu Huang, Hari B. Krishnan, Quynhchi Pham, Liangli Lucy Yu, and Thomas T. Y. Wang. (2016). Soy and Gut Microbiota: Interaction and Implication for Human Health DOI: 10.1021/acs.jafc.6b03725 J. Agric. Food Chem. 2016, 64, 8695−8709.
35. Hirose Kaoru, Toshiro Takezak, Nobuyuki Hamajima, Shigeto Miura and Kazuo Tajima. (2003). Dietary factors protective against breast cancer in japanese premenopausal and postmenopausal women. J. Cancer: 107, 276 –282 (2003) © 2003 Wiley-Liss, Inc.
36. Hawrelak JA1, Myers SP. The causes of intestinal dysbiosis: a review. PMID:15253677, Altern Med Rev. 2004 Jun;9(2):180-97.
37. Helena Tlaskalová-Hogenová, Renata Štěpánková, Hana Kozáková, Tomáš Hudcovic, Luca Vannucci, Ludmila Tučková, Pavel Rossmann, Tomáš Hrnčíř, Miloslav Kverka, Zuzana Zákostelská, Klára Klimešová, Jaroslava Přibylová, Jiřina Bártová, Daniel Sanchez, Petra Fundová, Dana Borovská, Dagmar Šrůtková, Zdeněk Zídek, Martin Schwarzer, Pavel Drastich

and David P Funda. (2011). The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cellular & Molecular Immunology (2011) 8, 110–120.
doi:10.1038/cmi.2010.67

38. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. (2011). Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol. 19(7):349-59. doi: 10.1016/j.tim.2011.05.006. Epub 2011 Jun 22.
39. Holmes MD, Liu S, Hankinson SE, Colditz GA, Hunter DJ, Willett WC. (2004). Dietary carbohydrates, fiber, and breast cancer risk. Am J Epidemiol. 2004, 159: 732-739.
40. Hong, K., Ko, K., Ahn, Y., Kim, C., Park, S., Park, J., Kim, Y. (2012). Epidemiological profiles between equol producers and nonproducers: A genomewide association study of the equol-producing phenotype. Genes and Nutrition, 363-951.
41. Horn-Ross PL, Lee M, John EM, Koo J. (2000). Sources of phytoestrogen exposure among non-Asian women in California, USA. Cancer Causes Control. 2000 Apr; 11(4):299-302.
42. Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC. (2003). Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Annu Rev Med. 2003, 54: 131-152. Epub 2001 Dec 2003.PubMed.
43. Hsieh, C. Y., Santel, R. C., Haslam, S. Z. & Helferich, W. G. (1998). Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Res 58:3833-3838.
44. Hwang, J. (2003). The phytoestrogen equol increases nitric oxide availability by inhibiting superoxide production: An antioxidant mechanism for cell-mediated LDL modification. *Free Radical Biology and Medicine*, *34*(10), 1271-1282. doi: 10.1016/S0891-5849(03)00104-7
45. Ian Rowland, Marian Faughna, Leane Hoey (a1), Kristiina Wähälä . Bioavailability of phyto-oestrogens.DOI: https://doi.org/10.1079/BJN2002796Published online: 26 October 2011
46. Janelle C. Arthur, Ernesto Perez-Chanona, Marcus Mühlbauer, Sarah Tomkovich1, Joshua M. Uronis, Ting-Jia Fan, Barry J. Campbell, Turki Abujamel, Belgin Dogan, Arlin B. Rogers6, Jonathan M. Rhodes, Alain Stintzi, Kenneth W. Simpson, Jonathan J. Hansen1, Temitope O. Keku, Anthony A. Fodor, Christian Jobin. (2012). Intestinal Inflammation Targets Cancer-Inducing Activity of the Microbiota Vol. 338, Issue 6103, pp. 120-123.DOI: 10.1126/science.1224820.
47. Jernberg C, Löfmark S, Edlund C, Jansson JK. (2010). Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology. 2010 Nov;156(Pt 11):3216-23. doi: 10.1099/mic.0.040618-0. Epub 2010 Aug 12.
48. Jose C. Clemente, Luke K. Ursell, Laura Wegener Parfrey, Rob. The Impact of the Gut Microbiota on Human Health: An Integrative View. doi: 10.1016/j.cell.2012.01.035.
49. Ju YH1, Fultz J, Allred KF, Doerge DR, Helferich WG. (2006). Effects of dietary daidzein and its metabolite, equol, at physiological concentrations on the growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in ovariectomized athymic mice. Carcinogenesis. 2006 Apr;27(4):856-63. Epub 2006 Jan 6.
50. Kendal A, Folkerd EJ, Dowsett M. (2007). Influences on circulating oestrogens in postmenopausal women: relationship with breast cancer. J Steroid Biochem Mol Biol 2007, 103:99-109.
51. Kenneth D. R. Setchell and Carlo Clerici. (2010). Equol: Pharmacokinetics and Biological Actions1,2 J Nutr. 2010 Jul; 140(7): 1363S–1368S. doi: 10.3945/jn.109.119784.
52. Khanna S, Tosh PK . A clinician's primer on the role of the microbiome in human health and disease. Mayo Clin Proc. 2014 Jan;89(1):107-14. doi: 10.1016/j.mayocp.2013.10.011.
53. Kohler BA, Sherman RL, Howlader N, et al. (2015). Annual Report to the Nation on the Status of Cancer, 1975-2011, featuring incidence of breast cancer subtypes by race/ethnicity, poverty, and state. Journal of the National Cancer Institute 2015; 107(6):djv048. doi:10.1093/jnci/djv048

54. Kolonel, L. N. (1988). Variability in diet and its relation to risk in ethnic and migrant groups. Basic Life Sciences, 43.
55. Korde LA, Wu AH, Fears T, Nomura AM, West DW, Kolonel LN, Pike MC, Hoover RN, Ziegler RG. Childhood soy intake and breast cancer risk in Asian American women. Cancer Epidemiol Biomarkers Prev. 2009 Apr;18(4):1050-9. doi: 10.1158/1055-9965.EPI-08-0405. Epub 2009 Mar 24.
56. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998 ; 139 : 4252 –63.
57. Lampe, J. W. (2009). Is equol the key to the efficacy of soy foods? American Journal of Clinical Nutrition, 89(5), 1664S-1667S. doi: 10.3945/ajcn.2009.26736T.
58. Le Jian. (2008). Soy, isoflavones, and prostate cancer.DOI: 10.1002/mnfr.200800167
59. Lee Sa, Shu Xo, Li H, Yang G, Cai H, Wen W, Ji BT, Gao J, Gao YT, Zheng W. Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. Am J Clin Nutr. 2009 Jun;89(6):1920-6. doi: 10.3945/ajcn.2008.27361. Epub 2009 Apr 29.
60. Ley Ruth. E. Obesity and the Human Microbiome. Curr Opin Gastroenterol. 2010;26(1):5-11. © 2010 Lippincott Williams & Wilkins.
61. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol. 2008 Oct;6(10):776-88. doi: 10.1038/nrmicro1978.
62. Lori. Coward, Neil C. Barnes, Kenneth D. R. Setchell, Stephen. Barnes. Genistein, daidzein, and their .beta.-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. J. Agric. Food Chem., 1993, 41 (11), pp 1961–1967. DOI: 10.1021/jf00035a027.
63. Lund, T. D., Blake, C., Bu, L., Hamaker, A. N., & Lephart, E. D. (2011). Equol an isoflavonoid: Potential for improved prostate health, in vitro and in vivo evidence. Reproductive Biology and Endocrinology.
64. Mandeep K. Virk-Baker, Stephen Barnes, Helen Krontiras, and Tim R. Nagy. S-(−)equol producing status not associated with breast cancer risk among low isoflavone consuming US postmenopausal women undergoing a physician recommended breast biopsy. Nutr Res. 2014 Feb; 34(2): 116–125. doi: 10.1016/j.nutres.2013.12.002.
65. Matthies, A., Blaut, M., & Braune, A. (2009). Isolation of a Human Intestinal Bacterium Capable of Daidzein and Genistein Conversion. Applied and Environmental Microbiology, 75(6), 1740-1744. doi: 10.1128/AEM.01795-08.
66. Markus MacGill. (2016). What is the gut microbiota? What is the human microbiome? MedicalNewsToday. Published: March, 24. 2016.
67. Matsuura M. and Obata A. Beta-glucosidases from soybean hydrolyze daidzin and genistin.J.Food Sci.1993,58-144-147.
68. Maskarinec G, Y Morimoto, S Heak, M Isaki, A Steinbrecher, L Custer and A A Franke Urinary estrogen metabolites in two soy trials with premenopausal women. European Journal of Clinical Nutrition (2012) 66, 1044–1049; doi:10.1038/ejcn.2012.71.
69. Maxwell Parkin, Paola Pisani,Jacques Ferlay. Estimates of the worldwide incidence of 25 major cancers in 1990.DOI: 10.1002/(SICI)1097-0215(19990315)80:6<827::AID-IJC6>3.0.CO
70. Mazmanian SK1, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005 Jul 15;122(1):107-18.

71. Messina M1. Soy and Health Update: Evaluation of the Clinical and Epidemiologic Literature. Nutrients. 2016 Nov 24;8(12). pii: E754. 9 DOI: 10.3390/nu8120754.
72. Messina M1, Messina V. Increasing use of soyfoods and their potential role in cancer prevention. J Am Diet Assoc. 1991 Jul; 91(7):836-40.
73. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. Nutr Cancer 2006; 55:1-12.
74. Messina, M. J., Persky, V., & Barnes, S. (1994). Soy intake and cancer risk: A review of the in vitro and in vivo data. Nutrition and Cancer, 21, 31.
75. Messina M1, Wu AH. Perspectives on the soy-breast cancer relation. Am J Clin Nutr. 2009 May;89(5):1673S-1679S. doi: 10.3945/ajcn.2009.26736V. Epub 2009 Apr 1
76. Michael S Donaldson. Nutrition and cancer: A review of the evidence for an anti-cancer diet
77. Minatoya M, Kutomi G, Asakura S, Otokozawa S, Sugiyama Y, Nagata Y, Mori M, Hirata K. Equol, adiponectin, insulin levels and risk of breast cancer. Asian Pac J Cancer Prev. 2013;14(4):2191-9.
78. Nutrition Journal 2004, 3:19. DOI: 10.1186/1475-2891-3-19©
79. Mitchell, J. (1998). Antioxidant Efficacy of Phytoestrogens in Chemical and Biological Model Systems. Archives of Biochemistry and Biophysics, 360(1), 142-148. doi: 10.1006/abbi.1998.0951
80. Morito, K., Hirose, T., Hirakawa, T., Okawa, M., Nohara, T., Ogawa, S., ... Masamune, Y. (2001). Interaction of phytoestrogens with estrogen receptors alpha and beta. Biological and Pharmaceutical Bulletin, 24, 6.
81. Muthyala RS, Ju YH, Sheng S, Williams LD, Doerge DR, et al. Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. Bioorg Med Chem 2004;12:1559–67
82. Nagata C. Factors to consider in the association between soy isoflavone intake and breast cancer risk. J Epidemiol. 2010;20(2):83-9. Epub 2010 Feb 20.
83. Nagel, S. C., Com Saal, F. S., & Welshons, W. V. (march 1998). The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: Physiology of delivery modifies estrogenic activity. Proceedings of the Society for Experimental Biology and Medicine. Society of Experimental Biology and Medicine, 217.
84. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr;55(2):74-108. DOI: 10.3322/canjclin.55.2.74.
85. Paul W. Lepp, Mary M. Brinig, Cleber C. Ouverney, Katherine Palm, Gary C. Armitage and David A. Relman. Methanogenic Archaea and human periodontal disease. Faculty Publications (2004): 6176-6181. DOI: 10.1073/pnas.0308766101.
86. Pilar Gaya, Ángela Peirotén , Margarita Medina, José Maria Landete. Isoflavone Metabolism by a Collection of Lactic Acid Bacteria and Bifidobacteria With Biotechnological Interest. Int J Food Sci Nutr 67 (2), 117-124. 2016 Feb 16.
87. Preetha Anand, Ajaikumar B. Kunnumakara, Chitra Sundaram, Kuzhuvelil B. Harikumar, Sheeja T. Tharakan, Oiki S. Lai, Bokyung Sung, and Bharat B. Cancer is a Preventable Disease that Requires Major Lifestyle Changes. doi: 10.1007/s11095-008-9661-9 Pharm Res. 2008 Sep; 25(9): 2097–2116.
88. Potischman N, Linet MS. Invited commentary: are dietary intakes and other exposures in childhood and adolescence important for adult cancers? Am J Epidemiol. 2013 Jul 15;178(2):184-9. doi: 10.1093/aje/kwt101. Epub 2013 Jun 21.

89. Raschke M, Rowland IR, Magee PJ, Pool-Zobel BL. Genistein protects prostate cells against hydrogen peroxide-induced DNA damage and induces expression of genes involved in the defence against oxidative stress. Carcinogenesis. 2006 Nov; 27(11):2322-30. Epub 2006 Jun 13.
90. René Vallery-Radot, Louis Pasteur - his life and labours. Translated by Lady Claud Hamilton from the original French titled, M. Pasteur: Histoire d'un Savant par un Ignorant. New York, D Appleton and Company 1,8 and 5 Bond Street. 1885.
91. Ruth E. Ley, Peter J. Turnbaugh, Samuel Klein & Jeffrey I. Gordon. Microbial ecology: Human gut microbes associated with obesity. Nature 444, 1022-1023 (21 December 2006) | doi:10.1038/4441022a.
92. Sathyamoorthy, N., & Wang, T. (1997). Differential effects of dietary phyto-oestrogens daidzein and equol on human breast cancer MCF-7 cells. European Journal of Cancer, 33(14), 2384-2389. doi: 10.1016/S0959-8049(97)00303-1.
93. Sánchez-Calvo J. M., Rodríguez-Iglesias M. A., Molinillo J. M. G., Macías F. A. (2013). Soy isoflavones and their relationship with microflora: beneficial effects on human health in equol producers. Phytochem. Rev. 12 979–1000. 10.1007/s11101-013-9329-x.
94. Scalbert, A., Morand, C., Manach, C., & Rémésy, C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. Biomedicine & Pharmacotherapy, 56(6), 276-282. doi: 10.1016/S0753-3322(02)00205-6.
95. Setchell, K., & Adlercreutz, H. (1988). 1988 Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism, and biological role in health and disease. Role of the Gut Flora in Toxicity and Cancer. London: Academic Press, 315-345.
96. Setchell, K. D. and Clerici, C. (2010). Equol: Pharmacokinetics and Biological Actions. J. Nutr. 140, 1363S-1368S.
97. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M. Nonsteroidal estrogen of dietary origin: possible roles in hormone-dependent disease. Am J Clin Nutr. 1984; 40:569-78.
98. Setchell, K., Brown, N. M., & Olsen, E. L. (2002). The Clinical Importance of the Metabolite Equol—A Clue to the Effectiveness of Soy and Its Isoflavones. *The American Society for Nutritional Sciences*, *132*(12), 3577-3584.
99. Setchell, K., Clerici, C., Lephart, E. D., Cole, S. J., Castellani, D., Wolf, B. E., & Brown, N. (2005). S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. The American Journal of Clinical Nutrition, 81.
100. Setchell, K. D. (1998). Phytoestrogens: The biochemistry, physiology, and implications for human health of soy isoflavones. The American Journal of Clinical Nutrition. 1998 Dec;68(6 Suppl):1333S-1346S.
101. Shashank A. Tidke, D. Ramakrishna, S. Kiran, G. Kosturkova and G.A. Ravishankar, 2015. Nutraceutical Potential of Soybean: Review. Asian Journal of Clinical Nutrition, 7: 22-32.DOI: 10.3923/ajcn.2015.22.32
102. Shu XO1, Zheng Y, Cai H, Gu K, Chen Z, Zheng W, Lu W. Soy food intake and breast cancer survival. JAMA. 2009 Dec 9;302(22):2437-43. doi: 10.1001/jama.2009.1783.
103. Steven R. Gill, Mihai Pop, Robert T. DeBoy, Paul B. Eckburg, Peter J. Turnbaugh, Buck S. Samuel, Jeffrey I. Gordon, David A. Relman, Claire M. Fraser-Liggett, and Karen E. Nelson. Metagenomic Analysis of the Human Distal Gut Microbiome. 2006 Jun 2; 312(5778): 1355–1359.doi: 10.1126/science.1124234.
104. Subedi, Lalita, Eunhee Ji, Dongyun Shin, Jongsik Jin, Joo Hong Yeo and Sun Yeou Kim. Equol, a Dietary Daidzein Gut Metabolite Attenuates Microglial Activation and Potentiates Neuroprotection In Vitro Nutrients 2017, 9, 207; doi:10.3390/nu9030207.
105. Trichopoulos D. Hypothesis: does breast cancer originate in utero? Department of Epidemiology, School of Public Health, Harvard University, 677 Huntington Avenue, Boston, Massachusetts 02115, U.S.A .doi.org/10.1016/0140-6736(90)91000-Z.

106. Turnbaugh PJ, Gordon JI. An invitation to the marriage of metagenomics and metabolomics. Cell. 2008 Sep 5;134(5):708-13. doi: 10.1016/j.cell.2008.08.025.
107. Zhou, J., Li, L., & Pan, W. (2007). Dietary soy and tea combinations for prevention of breast and prostate cancers by targeting metabolic syndrome elements in mice. American Journal of Clinical Nutrition, 86, 882-888.
108. Velentzis LS, Woodside JV, Cantwell MM, Leathem AJ, Keshtgar MR. Do phytoestrogens reduce the risk of breast cancer and breast cancer recurrence? What clinicians need to know. Eur J Cancer. 2008 Sep;44(13):1799-806. doi: 10.1016/j.ejca.2008.05.019. Epub 2008 Jul 7.
109. Vipperla Kishore and Stephen J. O'Keefe. The Microbiota and Its Metabolites in Colonic Mucosal Health and Cancer Risk. Nutrition in Clinical Practice. Doi:10.1177/088453361245, 2012.
110. Wang, X., Hur, H., Lee, J. H., Kim, K. T., & Kim, S. (2005). Enantioselective Synthesis of S-Equol from Dihydrodaidzein by a Newly Isolated Anaerobic Human Intestinal Bacterium. Applied and Environmental Microbiology, 71(1), 214-219. doi: 10.1128/AEM.71.1.214-219.2005.
111. Wang Hong Xu, Wei Zheng, Yong Bing Xiang, Zhi Xian Ruan, Jia Rong Cheng, Qi Dai, Yu Tang Gao, Xiao Ou Shu. Soya food intake and risk of endometrial cancer among Chinese women in Shanghai: population based case-control study.BMJ 2004; 328
doi: https://doi.org/10.1136/bmj.38093.646215.AE .Published 27 May 2004.
112. WHO,Fact sheets. http://www.who.int/mediacentre/factsheets/fs297/en/ , Reviewed 5/24. 2017.
113. Warri A, Saarinen NM, Makela S, Hilakivi-Clarke L. The role of early life genistein exposures in modifying breast cancer risk. Br J Cancer. 2008 May 6;98(9):1485-93. doi: 10.1038/sj.bjc.6604321. Epub 2008.
114. Wu, A. H., Ziegler, R. G., Nomura, A. M., West, D. W., Kolonel, L. N., Horn-Ross, P. L., Pike, M. C. (1998). Soy intake and risk of breast cancer in Asians and Asian Americans. The American Journal of Clinical Nutrition, 68(6), 1443-1437.
115. Yee, S., Burdock, G., Kurata, Y., Enomoto, Y., Narumi, K., Hamada, S., ... Ueno, T. (2008). Acute and subchronic toxicity and genotoxicity of SE5-OH, an equol-rich product produced by Lactococcus garvieae. Food and Chemical Toxicology, 46(8), 2713-2720. doi: 10.1016/j.fct.2008.04.026.
116. Yokoyama, S., Oshima, K., Nomura, I., Hattori, M., & Suzuki, T. (2011). Complete Genomic Sequence of the Equol-Producing Bacterium Eggerthella sp. Strain YY7918, Isolated from Adult Human Intestine. Journal of Bacteriology, 193(19), 5570-5571. doi: 10.1128/JB.05626-11.
117. Yuan J.-P., Wang J.-H., Liu X. (2007). Metabolism of dietary soy isoflavones to equol by human intestinal microbiota. Implications for health. Mol. Nutr. Food Res. 51 765–781. 10.1002/mnfr.200600262 (PubMed).
118. Xue-Mei Gong, Cheng-Jiu Hu, Quan-Jing Zhao and Dong-Mei Shi. Equol induces apoptosis in chemoresistant ovarian cancer cells via external pathway.
doi: 10.3329/bjp.v11i1.238352015