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Use of soy isoflavones as alternative to hormone replacement therapy (HRT) for women in menopause

By

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Information

This is the result of a master thesis (30 ects) carried out at the department of horticulture within the horticultural programme at the Swedish University of Agricultural Sciences. The project is a literature study. Marie Olsson and Lars Mogren were the head supervisors for this project, and Hans Lindqvist the examiner.

Abstract

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The loss of estrogen following menopause can have several effects, including reduction of bone mass, menopausal symptoms, such as hot flushes, decreased cognitive function, vaginal atrophy, and hypercholesterolemia. Traditional treatment of menopausal symptoms using hormone replacement therapy is associated with an increased risk of breast- and endometrial cancer, as well as an increased risk of cardiovascular disease. Daidzein, genistein and glycitein are isoflavones found in great amounts in soybeans, and soybean products. Isoflavones are phytoestrogens, or plant estrogens, that may be classed as natural selective estrogen receptor modulators (SERMs). They exert estrogenic effects (e.g. on bone and plasma lipids) and antiestrogenic effects or no effect on tissues where estrogen stimulation may be undesirable (e.g. breast and endometrium).

The current evidence indicates that there are few risks and many potential health benefits for women in menopause to increase their intakes of isoflavones. The effects of isoflavones may be affected by the ability to produce equol. Treating menopausal symptoms via increasing intakes of isoflavones may provide an important alternative to the traditional hormone replacement therapy. Although long-term intervention studies needs to be done before definitive conclusions can be drawn, whether soy isoflavones alone can serve as a safe and effective alternative to hormone replacement therapy.

The main objective of this master thesis was to investigate the effects of isoflavones in women during menopause on the menopausal symptoms, bone mass, breast, endometrium, and cardiovascular disease risk by reviewing current literature in the area.

Keywords: Isoflavone, daidzein, genistein, equol, estrogen receptors, pharmacokinetics, soy foods, supplements, safety, mechanisms

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Contents

1. Objectives

The main objective of this master thesis was to investigate the effects of isoflavones in women during menopause on the menopausal symptoms, bone mass, breast, endometrium, and cardiovascular disease risk by reviewing current literature in the area.

The specific questions addressed were:

- How does isoflavones affect women during menopause?
- How does isoflavones exert its effect?
- How much isoflavones is needed to convey their health benefits, and how can this be obtained?
- How safe is a diet rich in isoflavones?

2. Introduction

Hormone replacement therapy (HRT) has traditionally been used for treatment of menopausal disorders. However, not all women can, or wish to, take HRT (Warren *et al.,* 2002). More and more women rely on phytoestrogens, or plant estrogens, such as isoflavones to tailor their menopausal therapy in a "natural" way. Isoflavones may be classed as natural selective estrogen receptor modulators (SERMs) (Carusi, 2000), that exert selective estrogenic effects (e.g. on bone and plasma lipids) and anti-estrogenic effects or no effect on tissues where estrogen stimulation may be undesirable (e.g. breast and endometrium) (Nussey & Whitehead, 2001). Ideally SERMs would treat such symptoms as hot flushes, vaginal dryness, and mood changes, while protecting women from osteoporosis and cardiovascular disease, without substantially increasing the risk of endometrial cancer (Carusi, 2000).

2.1 Menopause

The menopause is the cessation of menstruation at the end of a woman's reproductive life. Operationally it is defined as the absence of menstruation for a year (Nussey & Whitehead, 2001). The term perimenopause or premenopause is defined by the World Health Organization as the two to eight years prior to menopause and one year following final menses. Natural menopause is a result from the loss of ovarian follicular activity (WHO, 1996). The loss of estrogen following menopause has several effects, not only physically but also psychologically. Some of the effects are limited to the transition period when a woman is adjusting to the loss of her hormones, such as menstrual periods becoming irregular. Other may occur first at the actual menopause, but may have serious consequences in the long term (Nussey & Whitehead, 2001). Some of the

deleterious effects of lowered estrogen levels include reduction of bone mass, menopausal symptoms, and hypercholesterolemia (Somekawa *et al.,* 2001). Osteoporosis is one of the most common endocrine diseases and occurs when the bone resorption exceeds the bone formation. The bone loss is accelerated for several years after the withdrawal of estrogens at the menopause (Nussey & Whitehead, 2001). Primary menopausal symptoms are considered to be the vasomotor symptoms, referring to hot flushes and night sweats. The vasomotor symptoms may also be associated with sleep and mood disturbances, as well as decreased cognitive function. Other menopausal symptoms are urogenital atrophy, urinary tract infections and incontinence, somatic symptoms, sexual dysfunction and decreased libido, and loss of skin elasticity (Utian, 2005; Cheung *et al.,* 2004). Epidemiologically women appear to be protected against cardiovascular disease by their sex hormones (Nussey & Whitehead, 2001), and incidence of cardiovascular disease have been shown to increase substantially after menopause (Wroblewski Lissin & Cooke, 2000). This adverse effect of menopause has been attributed to the metabolic effect of the loss of estrogen increasing the atherogenic potential of circulating lipids leading to coronary artery disease and stroke (Nussey & Whitehead, 2001).

2.2. Hormone replacement therapy

Hormone replacement therapy (HRT) refers to a variety of treatments involving different estrogens and progestins (Cheung *et al.,* 2004). HRT may be used during a short period of time to alleviate menopausal symptoms, such as hot flushes and vaginal atrophy (Nussey & Whitehead, 2001; Cancellieri *et al.,* 2007). HRT may also be used for several years or more to prevent changes in bone density and reduce the risk of cardiovascular disease. However, when HRT is stopped the same physiological changes that accompany the untreated menopause still occur, but later in life (Nussey & Whitehead, 2001). Even if hormone replacement therapy is widely used with evident success in menopausal women, approximately 70% drop out after the first year of treatment. Reasons for dropping out were irregular bleeding, breast discomfort, nausea, migraine, weight gain, water retention, besides the fear of breast cancer (Elkind-Hirsh, 2001). Over the last few years there has been a debate about HRT safety particularly with regard to the risk of developing breast cancer, but also the increased risk of endometrial cancer (Nussey & Whitehead, 2001; Beck *et al.,* 2003). A randomized, double blind placebo-controlled trial from the Women's Health Initiative (WHI) showed an increase in both cardiovascular disease and breast cancer (Writing group for the Women's Health Initiative investigators, 2002). The study involved 16,608 post-menopausal women who were given estrogen plus progestin or a placebo. The study was however terminated early as the risk was considered higher than the benefits. After an average of 5.2 years follow up, there was a 26% increase in breast cancer, 29% increase in coronary heart disease and a 41% in strokes among those who received estrogen plus progestins. However a 34% decrease in hip fractures and a 37% decrease in colorectal cancers were also reported among the hormone users (Writing group for the Women's Health Initiative investigators, 2002). In the U.S. hormone therapy prescriptions have declined as a response to the evidence substantiating cardiovascular disease harms and breast cancer risk (Hersh *et al.,*

2004). Some women may also not be able to treat their menopausal problems with HRT as they have absolute contraindications such as undiagnosed vaginal bleeding, active thromboembolic disease and active breast or endometrial cancer (Nussey and Whitehead, 2001). Concerns about the risks of conventional HRT have led increasingly more women and their practitioners to seek alternative therapies (Wroblewski Lissin & Cooke, 2000).

2.3. Isoflavones

More and more women rely on phytoestrogens, or plant estrogens, such as isoflavones to tailor their menopausal therapy in a "natural" way (Carusi, 2000). Isoflavones may be classed as natural selective estrogen receptor modulators (SERMs) (Carusi, 2000), that exert selective estrogenic effects (e.g. on bone and plasma lipids) and anti-estrogenic effects or no effect on tissues where estrogen stimulation may be undesirable (e.g. breast and endometrium) (Nussey & Whitehead, 2001). Asian people consume 10-100 times more isoflavones than Western people (Barnes *et al.,* 1995; Wu *et al.,* 1998), and it has been shown that Japanese women experience fewer hot flushes (Lock, 1991), fewer hip fractures (Cooper *et al.,* 1992) and a decreased rate of hormone-dependent cancers (Parkin, 1989) in comparison to American women. Phytoestrogens are diphenolic compounds that structurally resemble human estrogens (Hurst, 2002; Setchell & Adlercreutz 1988). As seen in Fig. 1. the isoflavone daidzein and its metabolite (Section 3.4.) equol are almost identical to endogenous 17β-estradiol.

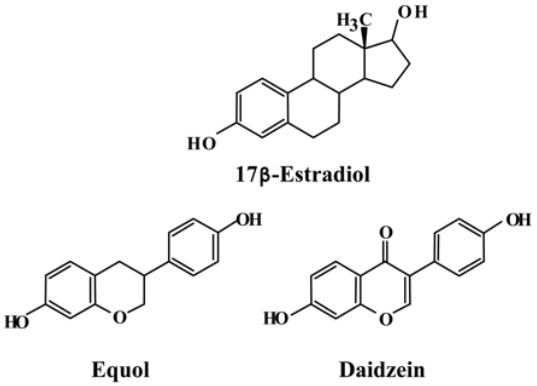

Figure 1. Similarity of the endogenous estrogen (17b-estradiol) to isoflavones (Equol, Daidzein)

The structural similarity of isoflavones to estrogens enables them to bind to estrogen receptors and provides them the ability to mildly mimic and in some cases act as antagonist to estrogen (Hurst, 2002; Jordan, 1990). When the level of estradiol is low, as it is in menopause, isoflavones exert more agonist properties and thus mildly mimic endogenous estrogen actions (Setchell & Cassidy, 1999; Collins *et al.,* 1997). These effects are also tissue specific (Setchell & Cassidy, 1999).

2.4. Isoflavones and estrogen receptors α and β

Hormones and signaling molecules control gene expression by binding to nuclear receptor proteins. Nuclear receptors act as transcription factors. By binding to specific response elements they regulate the expression of target genes (Härd & Gustafsson, 1993; Tsai & O'Malley, 1994).

In the 1960s the existence of a protein responsible for specific binding of 17β-estradiol in the uterus was recognized (Jensen & Jacobson, 1962). In 1996 a novel estrogen receptor was discovered in the rat prostate. It was named estrogen receptor β (ER-β) to distinguish it from the previously cloned estrogen receptor α (ER-α) (Kuiper *et al.,* 1996). These two known estrogen receptors ER-α and ER-β may play different roles in gene regulation (Paech *et al.,* 1997).

The DNA-binding-domains (DBD) of the two ERs share approximately 97% similarity. In the ligand-binding-domain (LDB), the overall amino acid identity is 59 % see Fig. 2. (Gustafsson, 1999). Those parts of the LDB involved in the actual binding of ligand are similar in ER-β and ER-α and show similar binding specificities (Kuiper *et al.,* 1997).

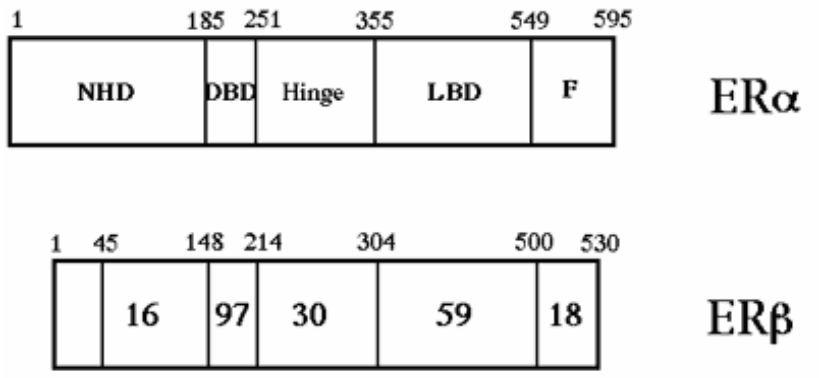

Figure 2. Comparison of the amino acid sequences of the two estrogen receptors. The separate domains are identified in the ER-α diagram. The numbers in the ER-β diagram represent the degree of homology (%) between respective domains in the two receptors (Adapted from Gustafsson, 1999).

The selective action of estrogens in different tissues may be explained by the distribution and ligand binding affinities for ER-α and ER-β (Kuiper *et al.,* 1997; Paech *et al.,* 1997). ER-β is found in the brain, bone and vascular epithelia sites that are specific targets where classical estrogen replacement therapy is beneficial, see Fig. 3. (Setchell & Cassidy, 1999).

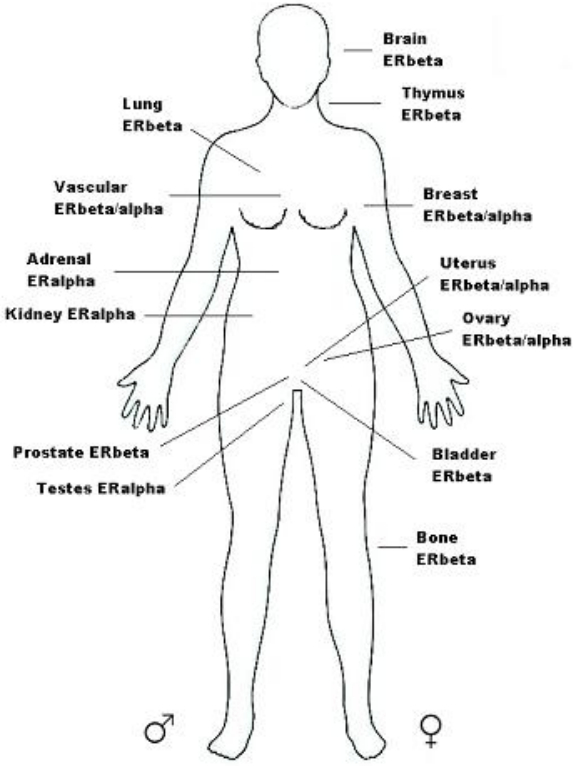

Figure 3. The anatomical distribution of ER-α and ER-β. (Setchell & Cassidy, 1999)

Interaction between hormones and their receptors depends on the number of receptors, the concentrations of circulating hormones and the affinity of the hormone receptors. The affinity of the hormone receptor is the concentration of hormone required to occupy 50% of the receptors. The higher the affinity the lower the concentration of hormone is required. The affinity of hormone receptors does not generally change and as a result the biological response depends on the number of receptors and the concentration of the hormone (Nussey & Whitehead, 2001). Phytoestrogens exhibit weak estrogenic activity on the order of 10^{-2}–10^{-3} that of 17ß-estradiol, but may be present in the body in concentrations 100-fold higher than endogenous estrogens, which may explain their biological response (Tham *et al.,* 1998)

The affinities for various compounds and the transcriptional response a given compound is able to elicit differ between the two receptor types (Pettersson & Gustafsson, 2001). 17β-estradiol binds to ER-α and ER-β with equal affinity, whereas soy isoflavones have a preference for binding to ER-β (An *et al.,* 2001). However, the maximal transcriptional stimulation by phytoestrogens achieved with ER-β is only about half that of ER-α, despite their higher affinity for ER-β (Barkhem *et al.,* 1998). Daidzein has been shown to have lower affinity for ER-α and ER-β compared with genistein (Kuiper *et al.,* 1998).

3. Isoflavones

3.1. Dietary sources

In most populations soy is the major source of phytoestrogens such as isoflavones (Tham *et al.,* 1998). Traditional soy food products have been consumed for more than 1000 years by people throughout Asia. However, it is only for the past 25 years the Western cultures have begun to add soy food to their diets. Today soy foods are available in many types throughout the world. Soy foods can be divided into two categories: nonfermented and fermented. Traditional nonfermented soyfoods are fresh green soybeans, whole dry soybeans, soy nuts, soy sprouts, whole-fat soy flour, soymilk and soymilk products, tofu, okara and yuba. Fermented soyfoods are tempeh, miso, soy sauces, natto and fermented tofu and soymilk products (Golbitz, 1995).

Some of these foods have wholeheartedly been adopted by Westerners, whereas others have not yet been accepted. Tofu, soymilk, soy sauce, miso and tempeh belong to the most popular soy food products in the U.S. The Americans have also created a whole new class of "second generation" soyfoods such as tofu hot dogs, tofu ice cream, veggie burgers, tempeh burgers, soymilk yoghurt, soymilk cheeses, soy flour pancake mix and several other prepared Americanized soyfoods (Golbitz, 1995).

3.2. Function in Plants

Isoflavones are substances that the plant produces to defend itself, also known as phytoalexins (Messina *et al.,* 1994). There are also other classes of substances apart from the phenolic compounds that work as phytoalexins, such as terpenoids and alkaloids (de Bruxelles and Roberts, 2001). Phytoalexins are produced in greater amounts when the plants are stressed. Dehydration, mechanical injury, microbial infections and ultraviolet irradiation are some of the known plant stressors (Messina *et al.,* 1994). Not only do the isoflavones play important roles as defensive compound against microorganisms, but they enjoy a very intricate relationship with certain nitrogen-fixing bacteria and actually help the soybean to grow (Subramanian *et al.,* 2004; Aoki *et al.,* 2000; Zhang *et al.,* 2000; Messina *et al.,* 1994).

3.3. Content in soybeans and soy food

Soybeans contain three types of isoflavones (daidzein, genistein and glycitein) found in four chemical forms (aglycons, glucosides, acetylglucosides and malonylglucosides) (Kudou *et al.,* 1991):

Aglycons	*Glucosides*	*Acetylglucosides*	*Malonylglucosides*
Daidzein	Daidzin	6''-O-acetyldaidzin	6''-O-malonyldaidzin
Genistein	Genistin	6''-O-acetylgenistin	6''-O-malonylgenistin
Glycitein	Glycitin	6''-O-acetylglycitin	6''-O-malonylglycitin

Isoflavones accumulate in soybeans during seed development. They are synthesized within seed tissues from simple precursors, but transport from maternal tissues may also contribute to the accumulation of these natural products in seeds (Dhaubhadel *et al.*, 2003). There is considerable variation in the phytoestrogen concentrations of different plants. The concentrations of these compounds can be influenced by a number of factors including plant species, strain, crop year and geographical location (Elridge & Kwolek, 1983; Wang & Murphy, 1994a). The mean content of isoflavones in raw soybeans from different locations is presented in Table 1.

Soybeans from different locations	**mg isoflavones/100g edible portion**
Brazil	87.63
Japan	118.51
Korea	144.99
Taiwan	59.75

Table 1. The mean content of isoflavones in raw soybeans from different locations (U.S. Department of Agriculture, Agricultural Research Service, 2007).

Nearly all isoflavones are found in the soybean hypocotyl with low to moderate amounts found in the cotyledon (Fig. 4.).

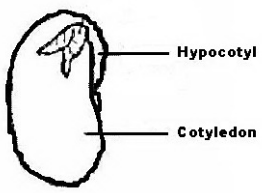

Figure 4. Anatomy of a bean seed.

Traditional processing of soybeans into food does not separate these seed parts (Wang & Murphy, 1994b). The glucoside forms genistin and daidzin account for 95-99% of the total isoflavone content of whole soybeans (Naim *et al.*, 1974). Nonfermented soy foods have greater levels of glucosides, whereas fermented soy foods contain greater levels of aglycons. The retention and distribution of isoflavone isomers in soy foods are affected by the variety of soybean, method of processing and addition of other components (Wang & Murphy, 1994b). For example soy concentrate can be produced by a water or alcohol wash of soy flakes to remove soluble carbohydrates and improve functionality. However alcohol washing removes most of the isoflavones (Wang & Murphy, 1994b).

3.4. Metabolism of isoflavones

The effects of isoflavones in humans are to a great extent mediated by intestinal microflora (Adlercreutz, 2002), and the variation in the effects among individuals has been attributed to differences in their metabolism (Rafii *et al.,* 2003) Once they have been ingested isoflavones are metabolized into diverse compounds in the intestine that might have increased or decreased estrogenic activity (Hwang *et al.,* 2006; Rafii *et al.,* 2003). Genistin and daidzin, the two most prevalent forms in soy foods, are biologically inactive. Once they have been ingested they are cleaved by glucosidases to the corresponding aglycones genistein and daidzein (Setchell *et al.,* 2002). Genistein is further metabolized by intestinal microflora to dihydrogenistein, while daidzein is converted either to equol or O-desmethylangolensin (O-DMA) (Heinonen *et al.,* 1999; Kelly *et al.,* 1993; Setchell *et al.,* 2002), equol being more estrogenic than either daidzein or O-DMA (Setchell *et al.,* 2002). Steps involved in the absorption and metabolism of isoflavones are illustrated in Fig. 5.

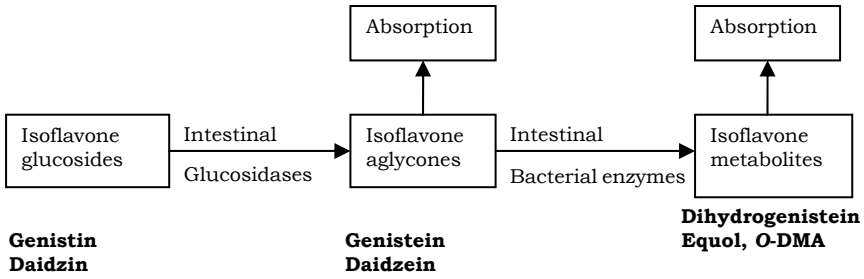

Figure 5. Summary of the absorption and metabolism of isoflavones.

Following absorption, isoflavones undergo hepatic conjugation to glucoronic acid or sulphate. They, as well as endogenous steroids, undergo enterohepatic circulation whereby they are deconjugated in the intestine and re-absorbed or excreted in the faeces (Setchell, 1998; Setchell & Cassidy, 1999). The absorption of isoflavones is dose-dependent, meaning that serum and urinary concentrations of isoflavones increase in accordance with the amount consumed (Karr *et al.,* 1997).

3.4.1. Individual differences

The effects of dietary isoflavonoids vary among individuals. This has been attributed to differences in their metabolism by intestinal bacteria (Rafii *et al.,* 2003). Equol, metabolite of daidzein, is more estrogenic than both daidzein and O-DMA (Setchell *et al.,* 2002), and it binds to both human ER forms (ERα- and β), has high antioxidant activity, and is relatively stable (Dixon, 2004). Equol is found in the urine of 30-40% of individuals who consume isoflavones (Setchell *et al.,* 2002). The efficacy of soy foods differs depending on the ability of an individual to produce equol, and the frequency varies both among individuals and

populations. It was found that the frequency of eqoul producers in vegetarians was 59%, similar to the reported frequency in Japanese adults consuming soy, which is much higher than for non-vegetarian adults (25%), suggesting that dietary components other than soy influence equol synthesis by intestinal bacteria (Setchell *et al.,* 2006), such as carbohydrate and dietary fiber levels (Slavin *et al.,* 1998; Lampe *et al.,* 1998). This fact can complicate epidemiological and dietary intervention studies (Dixon, 2004). Compared with Western populations, Asian populations have a higher eqoul-producer frequency (Song *et al.,* 2006). There are also significant interspecies differences in isoflavone metabolism. In one study female pigs had an overall metabolic profile closer to woman than to either female cynomolgus monkeys or rats, making the pig perhaps a better animal model for studying the health effects of isoflavones in nonequol producers (Gu *et al.,* 2006). The inability to produce equol in some humans may be due to the fact that they are host to a different population of intestinal microorganisms (Setchell *et al.,* 2002). There might be ways to convert a nonequol-producer into a producer through administering a mixed microbial culture which have shown to efficiently transform daidzein to equol. In one study it was found that administration of eqoul-producing bacteria to a dynamic *in vitro* model of the gastrointestinal tract, inoculated with a nonequol-producing fecal sample, resulted in the formation of equol in the distal colon parts. The effect also maintained beyond the period of inoculum administration. However further studies and experiments with *in vivo* models are necessary to explore the efficacy and safety of supplementation with equol-producing bacteria (Decroos *et al.,* 2006).

One study found that isoflavone metabolism and disposition were affected by the duration of soy ingestion and by sex (Lu & Anderson, 1998). They found that some women who initially were unable to metabolize daidzein to equol, developed this ability during chronic soy ingestion, but this was however not noticed in men. Thus, in women, ability to metabolize daidzein to equol can be increased by prolonged ingestion (Lu & Anderson, 1998).

There are conflicting results in the literature on the bioavailability of isoflavones when consumed in the aglycone form compared with when consumed in the glucoside form. Izumi *et al.,* 2000, reported that the bioavailability of both genistein and daidzein from the aglycone form was significantly higher and faster than from the glucoside form when consumed by 8 Japanese volunteers. However, a study with only Americans with typical American dietary habits showed no significant difference when isoflavones were consumed as either the aglycone or glucoside form (Zubik & Meydani, 2003). Yet another study demonstrated that the overall bioavailability may increase if the compounds are ingested as their glucosides rather than as aglycones (Setchell *et al.,* 2001). The differences in reported bioavailability of isoflavones may be accounted for by differing intestinal microfloral populations between Japanese and American women, if the isoflavones were ingested together with food and a potentially different food matrix (Zubik & Meydani, 2003).

When equal amounts of genistein and daidzein are consumed, genistein attains higher plasma concentrations than daidzein. Daidzein is to a larger extent distributed in the whole body compared with genistein. Genistein have greater

systemic bioavailability than daidzein. These differences in pharmacokinetics and metabolism may have implications for clinical studies, while assumptions cannot be made that all isoflavones are comparable in their pharmacokinetics and bioavailability (Setchell *et al.,* 2001).

Bioavailability of daidzein can be affected by the ability to produce equol (Vergne *et al.,* 2007). Vergne *et al.,* 2007 showed that equol producers excreted significantly less daidzein than equol non-producers. The difference disappeared when equol excretion was added to daidzein excretion in equol producers.

4. Potential clinical effects of isoflavones in menopause

4.1. Cardiovascular disease

Coronary artery disease (CAD) is the most common cause of mortality for women and increases dramatically after menopause (Wroblewski Lissin & Cooke, 2000). A high blood concentration of low-density lipoprotein (LDL) is an established risk factor for cardiovascular disease (Ballantyne, 1998). Women have more favorable lipoprotein profiles than men starting in adolescence. At the time of menopause, however, adverse changes in lipoprotein profiles occur, including increases in total and LDL cholesterol, decrease in high-density lipoprotein (HDL), and shift in LDL particle size toward smaller, denser particles. These adverse changes have been attributed to the changes in estrogen levels that occur at the time of menopause (Bittner, 2002). This theory is further supported by the increased incidence of cardiovascular events in younger, surgically postmenopausal women (Wroblewski Lissin & Cooke, 2000).

Epidemiologic data have shown that women ingesting high amounts of phytoestrogens from soy products have less cardiovascular disease, than those eating Western diets (Wroblewski Lissin & Cooke, 2000). Soy, and especially the isoflavones contained in soy, has been suggested to improve lipoprotein levels, thus reducing the risk of CAD. However, this hypothesis is not uniformly accepted (Dewell *et al.,* 2006). A pivotal paper in this field is an often cited meta-analysis of 38 controlled clinical trials of soy consumption in humans. Improvements in total cholesterol by 9.3% and LDL by 12.9%, as well as a decrease in triglyceride levels of 10.5% were revealed. The average intake of soy was 47g/day in these trials. The extent of reduction was dependent upon the initial serum cholesterol concentrations. In subjects with moderate hypercholesterolemia, a decrease in total cholesterol of 7.4% was observed, whereas subjects with severe hypercholesterolemia achieved a decline of 19.6% (Anderson *et al.,* 1995). However, according to recent reviewers (Sacks *et al.,* 2006; Dewell *et al.,* 2006) this meta-analysis was limited by the quality of the studies. Dewell *et al.,* 2006 and Lichenstein *et al.,* 2002, noted that 77% of the studies in this meta-analysis had 95% confidence intervals that encompassed zero and hence the findings should be

viewed with caution. In the majority of 22 randomized trials, isolated soy protein with isoflavones decreased LDL cholesterol concentrations. The average effect was approximately 3%, as compared with milk or other proteins. The reduction is considered small relative to the large amount of soy protein tested in these studies, averaging 50g, which is about half the usual total daily protein intake in the US. Most recent studies favor soy protein rather than soy isoflavones as the responsible nutrient although it cannot be ruled out that another component in soybeans could be the active factor (Sacks *et al.,* 2006). Dewell *et al.,* 2006, noted that "the changes reported in studies using purified isoflavones supplements, although not statistically significant, are quantitatively similar to those observed in the soy protein studies". Many studies during the past 10 years have not confirmed earlier research indicating that soy protein has clinically important favorable effects on LDL cholesterol and other cardiovascular disease risk factors, as compared with other proteins. However, because of soy products high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat it still should be beneficial to cardiovascular and overall health (Sacks *et al.,* 2006).

Dietary isoflavones have also been attributed cardioprotective benefits beside the reduction in LDL cholesterol, including an inhibition of pro-inflammatory cytokines, cell adhesion proteins and inducible nitric oxide production, potential reduction in the susceptibility of the LDL particle to oxidation, inhibition of platelet aggregation and an improvement in vascular reactivity. Increasing number of studies reveal a significant impact of genetic variation on changes in cardiovascular risk factors in response to dietary intervention. This might explain some of the differences in the current literature concerning isoflavones and cardiovascular health (Rimbach *et al.,* 2007).

4.2. Breast cancer

At the beginning of the 1980s it was proposed that isoflavonoids may prevent breast cancer. This was the starting point of numerous epidemiological, experimental, case-control and prospective studies investigating the hypothesis (Adlercreutz, 2002). Epidemiological observations show that Asian women have significantly lower risk of breast cancer compared with Western women (Parkin, 1989). Studies assessing cancer risk and diet have provided support for the theory that high intake of isoflavone-rich soy contributes to this risk difference. The findings of these studies showed significant inverse associations between breast cancer and soy isoflavones, both in relation to their consumption and their urinary excretion (Duncan *et al.,* 2003). Observations that Asian women who emigrate to the U.S. and adopt Western diet lose their lower risk of breast cancer further support the hypothesis that isoflavones may prevent breast cancer (Ziegler *et al.,* 1993). In the Asian countries the diets are also low in fat and red meat and are often rich in fish, elements that have independently been linked to a decreased cancer risk (Adlercreutz, 2002).

4.2.1. Mechanisms

Some epidemiological evidence suggests that the chemoprotectant effects of isoflavones are dependent on lifelong exposure from childhood (Limer and Speirs, 2004). Studies in rodents have shown that the favourable effect of an isoflavone-rich diet on breast-cancer risk may only be significant if consumption takes place before puberty or during adolescence (Lamartiniere, 2000). Lamartiniere, 2000, concludes that "genistein at physiological concentrations enhances cell differentiation, resulting in programming of the mammary gland, with no observed toxicity to the reproductive tract". He also concluded that "reduced EGFR (epidermal growth factor receptor) expression at the time of carcinogen exposure may account for genistein programming against mammary cancer".

Soy isoflavones may reduce the risk of breast-cancer by affecting endogenous sex-hormone concentrations and the menstrual cycle (Adlercreutz, 2002). Studies have shown that phytoestrogens stimulate the production of SHBG (sex-hormone binding globulin) in liver cells (Adlercreutz *et al.,* 1987). A low lifetime exposure to estrogen is associated with a reduced breast-cancer risk (Limer and Speirs, 2004) thus an increase in SHBG levels, leading to lower free-sex-hormone concentrations and longer menstrual cycles would lower the risk of breast-cancer (Adlercreutz, 2002).

Equol production in the gut has been associated with a lower risk of breast-cancer (Duncan *et al.,* 2000). People that produce high concentrations of equol have an increased ratio of 2-hydroxyestrone to 16α-hydroxyestrone in their urine. High urinary excretion of 2-hydroxyestrone relative to 16α-hydroxyestrone is thought to decrease breast-cancer risk (Atkinson *et al.,* 2003). Equol producers also show higher concentrations of SHBG than non-equol producers (Duncan *et al.,* 2000).

Genistein have been shown by both *in vitro* and *in vivo* studies to be a promising agent for cancer chemoprevention and/or treatment. Genistein has been shown to inhibit the growth of cancer cells through the modulation of genes that are related to the homeostatic control of cell cycle and apoptosis (programmed cell death). Genistein inhibits the activation of the nuclear transcription factor NF-κB and Akt signaling pathway, both of which are known to uphold a balance between cell survival and apoptosis (Sarkar and Li, 2002). NF-κB is needed for tumor cell proliferation, invasion, and angiogenisis, and tumor cells have been found to constitutively express the activated form of NF-κB. Inhibition of NF-κB is therefore likely to prevent tumor metastasis (Bharti and Aggarwal, 2002). Akt, which is also known as protein kinase B, represents a subfamily of the serine/threonine kinase. The activation of the Akt pathway plays a pivotal role in malignant transformation and chemoresistance by inducing cell survival, growth, migration, and angiogenesis. Inhibition of Akt kinase results in suppression of cell growth and induction of apoptosis in human cancer cells that have constitutively activated Akt (Yang *et al.,* 2004). Genistein is also known for its anti-oxidant property, which targets estrogen and androgen-mediated signaling pathway in the process of carcinogenesis. Genistein have also been found to be a potent inhibitor of angiogenesis and metastasis (Sarkar and Li, 2002).

4.2.2. Soy for breast cancer patients, is it safe?

Tamoxifen is a selective estrogen receptor modulator (SERM) used clinically in the adjuvant treatment of estrogen-dependent breast cancer. Individuals at high risk of developing breast cancer are also administered tamoxifen as a prophylactic. Therapies using tamoxifen is associated with menopausal symptoms such as hot flushes, joint pain, sleep disorders and depression, which may be reduced by the use of HRT. However long-term use of HRT for breast cancer patients is discouraged as HRT is associated with an increased risk for mammary carcinogenesis (Limer and Speirs, 2004). Soy isoflavone supplements may be used as a natural alternative to alleviate the tamoxifen-induced menopausal symptoms (Wuttke *et al.,* 2002). The ingestion of dietary phytoestrogens by breast cancers patients and survivors is, however, controversial (Messina and Loprinzi, 2001). Concerns over possible detrimental effects of soy in breast cancer patients have arisen because of the estrogen-like effects of isoflavones. Genistein have been shown to have a biphasic effect on the growth of MCF-7 cells (an estrogen receptor-positive cell line), stimulating proliferation at low concentrations but inhibiting it at high concentrations. Both genistein and soy protein stimulate tumor growth in a dose-dependent manner in ovariectomized athymic mice implanted with MCF-7 cells. In intact mice fed estrogen, however, genistein inhibits tumor growth. These studies might suggest that in a low-estrogen environment, perhaps as exist in a postmenopausal women (and possibly breast cancer patients as a result of chemotherapy-induced menopause), genistein is estrogenic and has a proliferative effect on breast tissue. In a high estrogen environment such as exists in a premenopausal woman genistein has an antiproliferative and possibly antiestrogenic effect. Two studies in premenopausal women suggest that soy exert estrogenic-like effects on breast tissue. However, two other studies, both 1 year long, indicated that isoflavone supplements do not affect breast tissue density in premenopausal women and may decrease density in postmenopausal women (Messina and Loprinzi, 2001). Messina and Loprinzi, 2001, conclude in their review that “the data are not impressive that the adult consumption of soy affects the risk of developing breast cancer or that soy consumption affects the survival of breast cancer patients. Consequently, if breast cancer patients enjoy soy products, it seems reasonable for them to continue to use them”.

4.3. Endometrial cancer

Over the last few years there has been a debate about HRT safety particularly with regard to the risk of developing breast cancer, but also the increased risk of endometrial cancer (Nussey & Whitehead, 2001; Beck, 2003).

Some studies have investigated the effect of isoflavones on the endometrium. Isoflavones have had no effect on the endometrium thickness when given alone (Upmalis *et al.,* 2000; Penotti *et al.,* 2003), or in combination with soy protein (Han *et al.,* 2002). Neither have isoflavones had an estrogenic effect on endometrial histology (Duncan *et al.,* 1999; Balk *et al.,* 2002).

One randomized, double-blind, placebo-controlled study determining the effects of 5 years treatment with soy isoflavones on histological characteristics of endometrium in postmenopausal women suggested that long-term treatment with soy isoflavones was associated with an increased occurrence of endometrial hyperplasia (Unfer *et al.,* 2004). However, most isoflavone supplements are given at a dosage of no more than 80mg/day, and in this study the isoflavones was administered at a higher dosage of 150mg/day (Unfer *et al.,* 2004).

4.4. Osteoporosis

The continual loss of bone mass when you get old is a natural process of aging. Osteoporotic fractures are more common among women than men, due to their lower peak bone mass. But also, the abrupt decrease in estrogen secretion in postmenopausal women accelerates bone loss (Tham *et al.,* 1998).

Both bone resorption and bone formation increase during menopause (Heikkinen *et al.,* 1997). When bone resorption exceeds its formation osteoporosis takes place (Nussey and Whitehead, 2001). Two different cell lines are involved in bone remodeling. Osteoblasts are responsible for bone formation, and it responds to changes in the activity of osteoclasts, the bone resorbing cells. Maintaining bone homeostasis involves many hormones, growth factors, and cytokines that play regulatory roles. Estrogen in particular suppresses osteoclast activity and thereby prevents bone resorption (Setchell and Lydeking-Olsen, 2003).

The incidence of hip fractures is significantly lower among Asian women relative to Caucasian women (Lauderdale *et al.,* 1997). Differences in bone mineral density (BMD) and fracture rates among Asian and Western people are probably due to multiple factors. Studies on cultured bone cells and rat models of postmenopausal osteoporosis support a significant bone-sparing effect of the soy isoflavones genistein and daidzein. However, reported findings on phytoestrogens and bone in human studies have given variable results. This might be explained by differences in the ability to produce equol (Setchell and Lydeking-Olsen, 2003). In a study by Lydeking-Olsen *et al.,* 2004, the group of women able to produce equol showed an increase of 2.4% in lumber spine BMD ($p<1$ compared with control group), while there were no significant change in BMD in the "non-equol" producer group.

4.4.1. Mechanisms

The mechanism of action for isoflavones is still elusive. However, from the many lines of evidence it is evident that there are probably multiple pathways, genomic and nongenomic, that help conserve the integrity and activity of the two cell lines (osteoblasts, and osteoclasts) to maintain stable bone mass in adults (Setchell and Lydeking-Olsen, 2003).

Estrogen receptors ER-β (Onoe *et al.,* 1997) and ER-α (Bodine *et al.,* 1998) are found in human osteoblasts. The expression of ER-β is greatly increased during bone mineralization (Arts *et al.,* 1997), which is particularly important to the potential hormonal effects of phytoestrogen, considering that compounds such as genistein show much higher affinity for ER-β than ER-α. (An *et al.,* 2001).

Stimulatory effects on protein synthesis and on alkaline phosphatase release by various types of osteoblasts cells *in vitro* have been reported for both daidzein and genistein. It has been suggested that daidzein and genistein influence transcriptional or translational events to convey these effects (Setchell and Lydeking-Olsen, 2003). Genistein have been found to stimulate the production of osteoprotegerin, a factor that prevents bone resorption, providing a further mechanism for the bone-sparing effects of soy isoflavones (Viereck *et al.,* 2002).

Estrogen receptors seem to not be present in the nucleus of osteoclasts cell. Osteoblast and osteoclast activities are coupled, but the action of isoflavones on the osteoclasts could also be independent of their effects on osteoblasts. There are a number of possible mechanisms by which genistein and daidzein can suppress osteoclast activity by means of apoptosis, activation of protein tyrosine phosphatase, inhibition of cytokines, changes in intracellular Ca^{2+}, and membrane depolarization (Setchell and Lydeking-Olsen, 2003).

4.5. Cognitive function

At menopause the depletion of estrogen may be associated with increased risk of neurodegenerative diseases (Dixon, 2004). Estrogen is thought to be neuroprotective (Warren *et al.,* 2002) and estrogen treatment has been found to improve memory in menopausal women (File *et al.,* 2001). Researchers have begun to investigate the effects of isoflavones on the brain (warren *et al.,* 2002). In a study examining the effects of supervised high versus low soy diets on attention, memory and frontal lobe function in young healthy adults of both sexes, both sexes improved memory within 10 weeks (File *et al.,* 2001). 12 weeks of consumption of a supplement containing soy isoflavones showed significant cognitive improvements in postmenopausal women (Duffy *et al.,* 2003). Studies in rodents on brains structure, learning, memory and anxiety has led to the conclusion that high consumption of dietary isoflavones over a relatively short time period can significantly alter the volume of sexually dimorphic brain regions, increase anxiety, and improve learning and visual-spatial memory in females but not in males (Lephart *et al.,* 2002). Isoflavones are suggested to prevent degeneration of the central nervous system and the development of Alzheimer's disease in menopausal women (Kim *et al.,* 2000). Linford and Dorsa, 2002, demonstrated that genistein and 17β-estradiol have comparable anti-apoptotic properties in primary cortical neurons and that these properties are mediated through estrogen receptors.

4.6. Vasomotor symptoms

Vasomotor symptoms such as hot flushes, night sweats, palpitations and insomnia are often associated with menopause (Soliman, 2005). The potential for phytoestrogens to alleviate menopausal hot flushes is an area of active research (Duncan *et al.,* 2003). Asian women have considerably fewer menopausal symptoms than Western women, and it has been hypothesized that this difference is due to their high intake of isoflavones (Adlercreutz *et al.,* 1992). A recent meta-analysis of 17 randomized, controlled, parallel group studies that had compared isoflavones therapy (using either soy products or red clover products) to a non-

isoflavone, non estrogenic comparator, suggest that isoflavone supplementation may produce a slight to modest reduction in the number of daily flushes in menopausal women and that the benefit may be more apparent in women experiencing a high number of flushes (Howes *et al.,* 2006). Numerous intervention studies using isoflavone extract have showed inconsistent results, with some reporting significantly fewer hot flushes, whereas others report no significant effects secondary to an apparent placebo effect (Duncan *et al.,* 2003). A large number of clinical trials have reported a decrease in hot flushes in women treated with soy protein, however many of these trials fail to prove that soy is superior to placebo (Kang *et al.,* 2002). In a community-based study the incidence of hot flushes was inversely related both to the amount of soy foods consumed and the daily intake of isoflavones; thus, there is indirect evidence for a role for isoflavones (Nagata *et al.,* 2001). In a study of 180 Japanese women given a standardized questionnaire to evaluate the severity of menopausal symptoms only 5% reported symptoms of hot flushes (Uchiyama *et al.,* 2001). The daily isoflavone intake from soy foods was calculated to be 22±14mg (Uchiyama *et al.,* 2001), which is much lower dose than doses used in clinical studies of isoflavones (Setchell *et al.,* 2002). In the study of Uchiyama *et al.,* 2001, 53.5% of the group was found to be equol-producers on the basis of their urinary equol excretion. All of the equol-producers recorded the least severe symptoms assessed by a simplified menopausal index score (Uchiyama *et al.,* 2001). Equol-producers may therefore gain the most benefit from soy isoflavones for relief of hot flushes. Furthermore, not all clinical trials on soy isoflavones stratify women according to eqoul status. This might explain why some studies fail to show isoflavones effectiveness on hot flushes (Setchell *et al.,* 2002).

The mechanism by which isoflavones may ease vasomotor symptoms, like hot flushes, is by acting as weak estrogen agonists. In a low-estrogen environment, as in a postmenopausal woman, isoflavones will exhibit their most potent estrogenic effects (Carusi, 2000).

4.7. Vaginal atrophy

The lower levels of estradiol during menopause can cause vaginal atrophy; the vaginal mucosa becomes thinner and dry. The symptoms of vaginal atrophy include dryness, burning, itching and painful sexual intercourses. The vaginal epithelium may become inflamed, which contribute to urinary symptoms such as frequency, urgency, painful urination, incontinence, and/or recurrent infections (Castelo-Branco *et al.,* 2005). In a randomized clinical trial analyzing the effects of a 6-month soy-rich diet on the vaginal cell maturation on 187 postmenopausal asymptomatic women, the maturation value score increased significantly in the diet and HRT group but not in the control group (Chiechi *et al.,* 2003). Chiechi *et al.,* 2003, conclude in this study that a diet rich in soy effectively increase the maturation indices of vaginal cells, and that phytoestrogen rich foods should be considered during preventive interventions against vaginal atrophy. However, another randomized, cross-over trial with two 12-week diet periods could not confirm that a soy-rich diet relieves the urogenital symptoms or restore the vaginal epithelium or improve the vaginal health in perimenopausal or postmenopausal Thai women (Manonai *et al.,* 2006).

5. Amounts of isoflavones needed

The amount of isoflavones needed to exert beneficial effects may vary according to the disease in question. Also, the amount of isoflavones needed for health benefits when consumed over a life-time might possibly be lower than that needed to produce benefits in short-term clinical trials. Most clinical trials use ≥40-90mg isoflavones/day. Amounts within this range have produced several beneficial effects (Messina, 2004). Intake recommendation of soy protein is 15g (with a range of 10-25g) and 50mg (aglycone weight) isoflavones (range of 30-100mg)/day. Intakes of 15g soy proteins result in consuming approximately 50mg isoflavones. These amounts of soy protein and soy isoflavones approximately correspond to two servings of traditional soy food. These amounts are also likely to be efficacious for those diseases for which soy is proven to be beneficial, and there is little reason to think that these intake levels will be associated with any adverse effects (Messina, 2002).

5.1. Soy foods

Asian populations widely consume isoflavones predominantly in the form of soy. Soy foods typically contain 1 to 2 mg genistein/g protein, and Asians consume 20 to 80 mg of genistein per day in the usual diet. The average American consumes only 1 to 3 mg of genistein per day (Barnes *et al.,* 1995). The concentration of isoflavones in soy foods and soybeans can vary and are dependent on the soybean and processing conditions used to produce a particular food product (Song *et al.,* 1998). There are also a lot of processed and fast foods with soy additives (e.g. isolated soy protein, soy protein concentrate, soy flour, and hydrolyzed soy protein, which contain significant levels of isoflavones. These products can provide an important source of isoflavones in the diet if consumed regularly (Umphress *et al.,* 2005). The mean content of isoflavones in different soy foods are presented in Table 2.

5.2. Supplements

There is a wide array of isoflavone formulations derived from soy or clover on the market today. These commercial isoflavone supplements may make a significant contribution to total isoflavone intakes in the West. These supplements usually vary in their total isoflavone content, the ratio of individual isoflavones and the chemical forms (glycosides vs. aglycone) in which they are present. Furthermore the isoflavone content can differ significantly from what manufacturers claim and the analysed composition (Rimbach *et al.,* 2007). Setchell and coworkers analyzed the composition of 33 commercially-available isoflavone supplements, and found that there were in some cases considerable discrepancies between the stated and analysed isoflavone concentrations (Setchell *et al.,* 2001).

Soy foods	mg isoflavones/100g edible portion
Instant beverage, soy, powder, not reconstituted	109.51
Miso	42.55
Miso soup mix, dry	60.39
Natto (soybeans, boiled and fermented)	58.93
Soy cheese, unspecified	31.32
Soy cheese, cheddar	7.15
Soy cheese, mozzarella	7.70
Soy cheese, parmesan	6.40
Soy drink	7.01
Soy fiber	44.43
Soy flour (textured)	148.61
Soy flour, defatted	131.19
Soy flour, full-fat, raw	177.89
Soy flour, full-fat, roasted	208.95
Soy hot dog, frozen, unprepared	15.00
Soy meal, defatted, raw	125.82
Soymilk, fluid	9.65
Soymilk, iced	4.71
Soymilk skin or film, cooked	50.70
Soymilk skin or film, raw	193.88
Soy noodles, flat	8.50
Soy paste	31.52
Soy protein concentrate, aqueous washed	102.07
Soy protein concentrate, produced by alcohol extraction	12.47
Soy protein isolate	97.43
Soy sauce made from hydrolyzed vegetable protein	0.10
Soy sauce made from soy and wheat (shoyu)	1.64
Soybean chips	54.16
Soybean curd cheese	28.20
Soybean, curd, fermented	39.00
Soybeans, flakes, defatted	125.82
Soybeans, flakes, full-fat	128.99
Soybeans, green, mature seeds, raw	151.17
Soybeans, mature cooked, boiled, without salt	54.66
Soybeans, mature seeds, dry roasted (includes soy nuts)	176.94
Tempeh	43.52
Tempeh burger	29.00
Tempeh, cooked	53.00
Tofu	13.51-67.49
Tofu, yogurt	16.30

Table 2. The mean content of isoflavones in different soy foods (U.S. Department of Agriculture, Agricultural Research Service, 2007).

5.3. Soy food or isoflavone supplements? Is it safe?

The absorption of isoflavones from food may be saturable, and it may be more difficult to attain supraphysiological levels of isoflavones from food than from supplements (Setchell, 2000). With supplementation, there are dangers of overdosing (Setchell *et al.,* 2001). Setchell and coworkers found in their composition analysis of 33 commercially-available isoflavone supplements numerous compounds that they could not identify, and nothing is known about these components of these mixtures (Setchell *et al.,* 2001).

What is critical to the biological efficacy and safety of isoflavones is a thorough understanding of their bioavailability from different soy foods. Still to be elucidated is whether the pharmacokinetic profile following ingestion of a defined dose is influenced by the food matrix in which the isoflavone is given or by the processing method used (de Pascual-Teresa *et al.,* 2006), if it varies among individuals or whether it is influenced by age (Setchell *et al.,* 2003). The information on the pharmacokinetic behavior of isoflavones when ingested at levels that far exceed normal dietary intakes is insufficient and remains to be elucidated (Setchell *et al.,* 2003). The dose promoted for supplementation, and in some cases, fortified functional foods are higher and far in excess of usual dietary intakes of Asians (Setchell *et al.,* 2001; Setchell *et al.,* 2003), and there is a potential risk for long-term negative effects (Setchell *et al.,* 2003). High levels of isoflavones fed to animals have resulted in deleterious effects, and it is not impossible that adverse effects also could occur in humans as a result of excessive intakes (Setchell *et al.,* 2001).

The data from the study of Setchell *et al.,* 2003, support the concept that the bioavailability of isoflavones from a soy food matrix is nonlinear, with increasing doses of intake in the range typically consumed by people living in Asia. The serum half life (t1/2) of daidzein and genistein are on the order of 8-10h (Setchell *et al.,* 2003). Multiple intakes of soy foods throughout the day are more likely to achieve steady-state serum levels, and whether this would give better results in dietary interventions studies is not known (Setchell el al., 2003). However Setchell *et al.,* 2003, contend on the basis of analogy to many drugs with similar pharmacokinetics, "that a portfolio of soy foods that can be eaten throughout the day is likely to be more effective than once a day intake". Setchell *et al.,* 2003, also conclude from their data that there is no advantage of ingesting large doses of soy isoflavones in soy foods, given the curvilinear relationship between bioavailability and intake.

6. Discussion and Conclusions

The current evidence indicates that there are few risks and many potential health benefits for women in menopause to increase their intakes of soy foods, which are good sources of isoflavones. Treating menopausal symptoms via increasing intakes of isoflavones may provide an important alternative to the traditional hormone replacement therapy. Although long-term intervention studies needs to be done before definitive conclusions can be drawn, whether soy alone can serve as a safe and effective alternative to hormone replacement therapy. Traditional hormone

replacement therapy may involve increased risks of developing breast or endometrial cancer. The current data on the adult consumption of soy concerning development of breast cancer is not impressive enough to discourage women from increasing their intake of isoflavones during menopause. Although some studies have shown genistein to have proliferative effects on breast tissue when present in a low estrogen environment, such as may exist in a menopausal woman, other studies indicate that isoflavones do not affect breast tissue density in premenopausal women and may decrease breast tissue density in postmenopausal women (Messina and Loprinzi, 2001). However more research on the effects of isoflavones on the breast is needed. There seem to be no effect of isoflavones on the endometrial thickness, although excessive intakes may be associated with an increased risk of endometrial hyperplasia. In the randomized, double blind placebo-controlled trial done by the Women's Health Initiative, there was an increase in cardiovascular disease in menopausal women given hormone replacement therapy (Writing group for the Women's Health Initiative investigators, 2002). Isoflavones and soy protein may, however, have beneficial effects on the cardiovascular health by improving lipoprotein profiles, and thus reducing the risk of coronary artery disease. The high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat in soy products may also play a crucial role in the beneficial effects on the cardiovascular system. Soy isoflavones have been shown to significantly improve cognitive functions in postmenopausal women, and it has been suggested to prevent degeneration of the central nervous system and the development of Alzheimer's disease. The effect of isoflavones on the vasomotor symptoms are in some cases modest and sometimes no better than placebo, however the benefit of isoflavones may be more apparent in women experiencing a high number of flushes. Furthermore, isoflavones may be preventive against vaginal atrophy.

The clinical effectiveness of soy protein in cardiovascular, bone and menopausal health may be a function of the ability to produce equol. Equol, with its higher affinity to estrogen receptors, might be more efficient in preventing diseases and treat menopausal symptoms. The variance in results from clinical trials might be explained by the fact that not all nutritionists/scientists distinguish between subjects that are equol-producers from those who are not. It has been shown that equol concentrations are higher in people who have diets containing a lot of plant proteins, carbohydrates, and fibre, compared with to those who have a high-fat diet (Adlercreutz, 2002). Therefore, one may conclude that soy isoflavones would be more efficacious when ingested in the form of soy food, compared to supplementing isoflavones to a high-fat diet. Also, when supplementing with isoflavones tablets, there is always the risk of overdosing. Setchell *et al.,* 2001, found numerous compounds in commercially available isoflavones supplements that they could not identify, which might further support the ingestion of isoflavones in the form of soy food rather than as supplements. An intake of 15g soy protein, resulting in approximately 50mg isoflavones, may be enough to convey the health benefits. This corresponds to approximately two servings of traditional soy food.

Isoflavones exert some of their effects by binding to estrogen receptors and by acting as antioxidants. The exact mechanisms by which isoflavones exerts its benefits are still a major challenge for nutritionists and scientists. How the bioavailability varies among individuals, whether it is influenced by age, gender, or what the effects are of ingesting levels that far exceed normal dietary intakes remains to be elucidated.

7. Glossary

Apoptosis: Cell death as a result of activation of an intracellular ”suicide” programme.
Angiogenesis: Development of new blood vessels by sprouting from pre-existing vessels.
Asymptomatic: Having no symptoms, whether disease is present or not.
Atherogenic: Initiating, increasing, or accelerating atherogenesis.
Atherogenisis: Formation of atheromatous deposits, especially on the innermost layer of arterial walls.
Athymic mice: A laboratory mouse lacking a thymus gland. Athymic mice have no T cells and useful in research because they do not reject tumor or other cells transplanted from mice, humans or other species.
Atrophy: Is a general physiological process of reabsorption and breakdown of tissues, involving apoptosis on a cellular level.
Colorectal cancer: Also called colon cancer or bowel cancer, includes cancerous growths in the colon, rectum and appendix.
Contraindication: A condition or factor that increases the risks involved in using a particular drug, carrying out a medical procedure or engaging in a particular activity.
Cytokine: Any protein or polypeptide produced by a cell and which affects the growth or differentiation of the same or another cell. The term usually excludes endocrine hormones.
Endometrial hyperplasia: Endometrial hyperplasia is a condition that occurs when the lining of the uterus (endometrium) grows too much. It is a benign (not cancer) condition.
Endometrium: The inner membrane of the mammalian uterus.
Enterohepatic circulation: Refers to the circulation of bile from the liver, where it is produced, to the small intestine, where it aids in digestion of fats and other substances, back to the liver.
Epithelium: Sheet of cells tightly bound together, lining all external surfaces and internal surfaces continuous with the external environment in multicellular animals.
Fermentation: Anaerobic breakdown of carbohydrates by living cells, esp. by microorgansims, often with the production of heat and waste gases.
Half life: Time required for the disappearance of half the original quantity of a given substance, e.g. from the circulation, assuming that the substance disappears at a regular rate.
Homeostasis: (1) Maintenance of the constancy of internal environment of the body or part of body; (2) maintenance of equilibrium between organism and environment; the balance of nature.
Homology: Resemblance by virtue of common descent. It refers to structures, DNA sequences and behaviours.

Hypercholesterolemia: (literally: high blood cholesterol) is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease.

Hyperplasia: (1) Excessive development due to an increase in the number of cells; (2) an abnormal increase in cell proliferation.

Metastasis: (1) A change in state, position, form or function; (2) the migration of cancer cells to colonize tissues and organs other than those in which they originated; (3) a secondary tumour caused by migration of cancer cells to another tissue.

Mucosa: The wall of tubular structures such as gut, respiratory tract, urinary and genital tracts, which consists of several distinct layers of tissue.

Nitric oxide: Local signalling molecule produced by nerve cells and endothelial cells lining blood vessels. It causes smooth muscles to relax, causing blood vessel dilation, and regulates nerve cell activity. It is also produced by activated macrophages, neutrophils, and other cells. Nitric oxide produced by endothelial nitric oxide synthase (eNOS) has a vasodilator function and has atherogenic and vascular protective effects. However, nitric oxides produced by inducible nitric oxide synthase (iNOS) in macrophages appear to induce atherosclerosis.

Ovariectomy: ovary removal: the surgical removal of one or both ovaries.

Palpitations: Heartbeat sensations. Unpleasent awareness of your own heartbeat. You may feel that your heart is pounding or racing or you may fell skipped or stopped beats.

Pharmacokinetics: (In Greek: "pharmacon" meaning drug and "kinetikos" meaning putting in motion, the study of time dependency) is a branch of pharmacology dedicated to the determination of the fate of substances administered externally to a living organism.

Prophylactic: A preventive measure. The word comes from the Greek for "an advance guard," an apt term for a measure taken to fend off a disease or another unwanted consequence.

Response element: Is a short sequence of DNA within the promoter of a gene that is able to bind a specific hormone receptor complex and therefore regulate transcription.

Selective Estrogen Receptor Modulators (SERMs): Are a class of medication that acts on the estrogen receptor. A characteristic that distinguishes these substances from pure receptor agonists and antagonists is that their action is different in various tissues, thereby granting the possibility to selectively inhibit or stimulate estrogen-like action in various tissues.

Thrombemolism: Formation in a blood vessel of a clot (thrombus) that breaks loose and is carried by the blood stream to plug another vessel.

Transcription factor: A protein that binds to specific parts of DNA using DNA binding domains and is part of the system that controls the transfer (or transcription) of genetic information from DNA to RNA.

Urogenital: The urogenital system includes the sex organs and the urinary system of vertebrates.

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