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CLINICAL ARTICLE

The effects of soygerm extracts on blood lipoproteins, antioxidative capacity and urinary estrogen metabolites in postmenopausal women on hormone therapy

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Abstract

Objective: To evaluate the effects of soygerm isoflavones extracts on blood lipoproteins, antioxidative capacity and urinary estrogen metabolites in postmenopausal women who receive hormone therapy (HT). *Method:* Thirty-nine volunteers receiving HT were recruited, and 33 completed the study. All subjects received 6 g of soygerm extracts per day for 4 weeks. Blood and urine samples were collected for study at the beginning and at the end of study. *Result:* Plasma HDL-C levels increased markedly with significant decreases of plasma LDL-C/HDL-C ratio and LDL-TG levels. The lag time of conjugated dienes formation prolonged for 9.9% and thiobarbituric acid reactive substances production in copper-catalyzed oxidation of LDL decreased. The differences were statistically significant. Urinary ratio of 2-OHE_1 to 16α-OHE_1 increased without statistical significance. *Conclusion:* Soygerm extracts may improve serum lipid profile in postmenopausal Taiwanese women who receive HT, and probably provide a favorable effect on estrogen metabolism.

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1. Introduction

Evidence from randomized clinical trials of the Women's Health Initiative (WHI) suggested that, compared with placebo, combined estrogen/progestin replacement therapy increased the risks of coronary heart disease (CHD), stroke, pulmonary embolism and invasive breast cancer [1]. Diet supplements that may counteract the adverse effects from combined estrogen and progestin may be beneficial to women who receive hormone therapy (HT). For instance, soy or isoflavones consumption has been shown to be inversely associated with risks of cardiovascular disease (CVD) [2] and breast cancer [3]. Thus, these soy-based supplements could potentially mitigate the negative effects from HT.

Previous study has shown HT with combined estrogen and progestin causes a decrease in high-density lipoprotein cholesterol (HDL-C) [4]. Soy isoflavones supplement might improve plasma lipids in normocholesterolemic [5] and hypercholesterolemic [6] postmenopausal women. Nevertheless, some studies did not support the effect of isoflavones on plasma lipids [7,8]. Given isoflavones are phytoestrogens with estrogenic and antiestrogenic effects, it would be important to examine whether the combination of extracts of soygerm isoflavones with HT provides synergistic or antagonistic actions.

Besides reducing the risk of CVD, some studies showed inverse relationships between soy or isoflavones consumption and breast cancer risk [3,9]. Most of the significant results reported were conducted among Asian populations [9] and Australians [3]. Estrogens are involved in the etiology of breast cancer primarily by mitogenic stimulation [10]. The carcinogenic effect of estrogens is also mediated through its various oxidative metabolites which may provide persistent estrogenic activity or trigger DNA damage by forming DNA adducts [11]. Among the estrogen metabolites, 4- and 16α-hydroxyestrone (OHE_1) are thought to be carcinogenic, whereas, 2-OHE_1 is not [12]. 16α-OHE_1 and 2-OHE_1 are two main metabolites in humans [12]. Therefore, the ratio of urinary 2-OHE_1 to 16α-OHE_1 (2/16α ratio) has been hypothesized as a biomarker of breast cancer risk and some evidence supported this hypothesis [13]. Isoflavones have been shown to increase the 2/16α ratio [14]. However, they have not been evaluated for their influence on exogenous estrogens that postmenopausal women receive.

Therefore, the present study was designed to investigate the effect of soygerm isoflavones extract on blood lipoproteins and estrogen metabolites in postmenopausal women with HT in an attempt to decrease the risks of CVD and breast cancer. Soygerm was used because it was the most concentrated source of isoflavones in soy.

2. Materials and methods

2.1. Subjects

Postmenopausal women, who had discontinued menstrual periods for at least 1 year, were recruited from Taiwan Adventist Hospitals during the period between Jan. 2000 and Dec. 2000. The inclusion criteria included menopausal women under 65 years old, a body mass index less than 30, no history of cardiovascular, metabolic, or endocrinologic disease, no intake of isoflavones supplement within recent six months, and already receiving HT with 0.625 mg conjugated equine estrogen and 5 mg medroxyprogesterone acetate daily for at least 3 months. Written informed consent was obtained from each participant before the study. The present study was approved by the Institutional Review Board for Research on Human Subjects, National Taiwan Normal University. Subjects did not get any payment for completing the study and they were free to withdraw from the study whenever they wanted to and for any reason.

Table 1 Major components of soygerm extract consumed by study subjects

Components	Content (mg/g)	Daily intake (mg/day) [a]
Total isoflavones	19.9	119.4
Genistein [b]	1.2	7.2
Daidzein [b]	10.9	65.4
Glycitein [b]	7.8	46.8
Protein [c]	384	2304

[a] Calculated from 6 g of daily soygerm extract supplementation.
[b] Measured in duplicate.
[c] Manufacturer's data.

2.2. Supplements

Supplements (Phytogen 25; SoyLife Nederland B.V., the Netherlands) were extracts from soygerm and contained 4.9% moisture, 38.4% crude protein, 9.2% crude fat, 4.2% ash and 43.3% carbohydrate. The contents of isoflavones were measured via a reverse phase high performance liquid chromatography after extraction and acid hydrolysis of conjugates [15]. The analysis showed that it contained 19.9 mg isoflavones per gram, lower than the value (25 mg/g) claimed by the manufacturer. The major components of soygerm extracts consumed by the subjects were listed in Table 1.

2.3. Study design

This present study was a pilot clinical trial. All subjects consumed soygerm extract powder (119 mg isoflavones/day) twice daily for 4 weeks. Fasting blood and first morning urine samples were collected at the beginning and the end of the experimental periods. All participating subjects were advised to keep their usual lifestyles, diet habits, and continue to use HT. Since the intestinal microflora were needed for the metabolism of non-estrogenic daidzein to estrogenic equol [16], all participating subjects were advised to avoid antibiotics whenever possible during the study period.

2.4. Collection and analysis of blood and urine samples

After a 12-hour fast, blood sample was obtained and collected into tubes containing EDTA (2.8 mg/ml of blood). Plasma was separated from whole blood by centrifugation at 2500 rpm for 15 min. Early morning spot urine samples were collected into tubes containing vitamin C (1 mg/ml of urine) and kept at 4 °C immediately. After centrifugation at 3000 rpm for 15 min, clear supernatants were stored at −70 °C until the end of the study when aliquots from each subject were analyzed together.

Plasma isoflavones were analyzed by HPLC method after enzymatic hydrolysis and extraction. According to the method of Franke et al. [17], flavone was added as internal standard for recovery calculation. With this method, the recovery was 94 ± 9%.

Plasma lipoproteins were isolated by sequential flotation ultracentrifugation at densities of 1.006–1.019, 1.019–1.063, 1.063–1.125, 1.125–1.20 kg/l for very low density lipoprotein (VLDL), LDL, HDL_2 and HDL_3 in NaBr density solution containing 10 μmol EDTA/l. Plasma cholesterol, triglycerides and lipoproteins were measured using enzymatic kits (Randox Lab., Antrim, UK). Lag time of copper-catalyzed oxidation of LDL and thiobarbituric acid reactive substances (TBARS) formation was measured as previously described [18].

Urinary creatinine was determined using a commercial kit (Randox Lab. Antrim, UK) after the samples were heated at 100 °C for 5 min to destroy vitamin C. Urinary 2-OHE_1 and 16α-OHE_1 were measured in triplicate using a competitive solid-phase enzyme immunoassay kit (Immuna Care Corporation, Bethlehem, PA, USA). Samples from the same subject were analyzed in the same run. The kinetics of the immunoreaction was monitored at 2-min intervals for 20 min, using a MR5000 plate reader (Dynatech Lab. VI, USA). The within-assay coefficients of variation were 5.33% and 5.35%, and between-assay coefficients of variation were 6.64% and 9.24% for 2-OHE_1 and 16α-OHE_1, respectively. The results were expressed as ng/mg creatinine to adjust for the differences arising from variations of urine concentration.

Table 2 Plasma levels of isoflavones and lipids, plasma antioxidant status and urine excretions of estrogen metabolites at baseline and after ingestion of soygerm extract supplementation (n = 33)

	Baseline	After soygerm	p-value
Plasma isoflavones levels			
Daidzein (nmol/l)	127 ± 93.1	316.6 ± 482.2	0.002
Genistein (nmol/l)	596.9 ± 340.7	607.2 ± 281.5	0.80
Plasma lipid profile			
Total-TG (mg/dl)	90.3 ± 45.2	85.9 ± 36.3	0.647
VLDL-TG (mg/dl)	55.8 ± 38.1	57.6 ± 33.7	0.506
LDL-TG (mg/dl)	21.3 ± 8.8	17.7 ± 4.4 *	0.002
Total-C (mg/dl)	177.9 ± 26.3	181.7 ± 27.8	0.200
VLDL-C (mg/dl)	9.7 ± 5.0	10.8 ± 7.0	0.442
LDL-C (mg/dl)	105.2 ± 24.7	104.4 ± 21.3	0.996
HDL-C (mg/dl)	63.0 ± 18.2	67.7 ± 19.7 *	0.012
HDL_2-C (mg/dl)	40.9 ± 16.6	44.0 ± 18.5 *	0.050
HDL_3-C (mg/dl)	22.0 ± 3.8	23.2 ± 2.7 *	0.039
LDL-C/HDL-C	1.79 ± 0.61	1.65 ± 0.49 *	0.006
Plasma oxidative status			
Lag time (min)	33.4 ± 8.3	36.7 ± 7.6 *	0.004
TBARS (nmol/mg protein) (n = 17)	72.5 ± 13.0	67.8 ± 10.6 *	0.048
Urinary estrogen metabolites			
2-OHE_1 (ng/mg Creatinine)	10.84 ± 5.60	10.48 ± 4.58	0.711
16α-OHE_1 (ng/mg Creatinine) (n = 31)	5.96 ± 2.67	4.96 ± 2.49	0.090
2/16α ratio (n = 31)	1.84 ± 0.80	2.26 ± 1.63	0.150

Values are means ± SD. Lag time, lag time of conjugated dienes formation in LDL oxidized by copper; TBARS, thiobarbituric reactive substances produced in LDL oxidized by copper; E_1, estrone; 2/16α ratio: ratio of 2-OHE_1 to 16α-OHE_1.
* Values are significantly different with a $p < 0.05$ by a two-tailed paired.

2.5. Statistical analysis

Results were expressed in terms of means and standard deviations. Comparison between the values was made by paired, two-tailed t-test and performed with SPSS 11.5. The difference was statistically significant when a p-value was less than 0.05.

3. Results

The ages of the 39 subjects ranged from 49 to 64 years and BMI from 17 to 29.8 kg/m^2. Six subjects did not complete the study for the following reasons: two immigrated to other countries, one was not supported by her family to continue, one was averse to soy smell, one stopped receiving HT, and one felt bloating. Finally, data collected from 33 subjects were used for the statistical analysis. Mean age and mean menopausal age of the subjects were 54.8 ± 5.2 and 48.6 ± 4.2 years, respectively. Compliance of subjects was monitored by self-reported daily consumption sheets and confirmed by the changes in plasma isoflavone levels following supplementation. Two subjects had one undetectable level of 16α-OHE_1, so the number of 16α-OHE_1 and 2/16α ratio data became 31. Due to instrumental error, half of the copper-oxidized LDL samples were not heated to 100 °C as required to react with TBA reagent, so the number of TBARS data was reduced to 17.

The results were summarized in Table 2. Ingestion of soygerm extracts resulted in a 2.5-fold increase of plasma levels of daidzein. The change of serum levels for genistein was not significant.

Soygerm extract supplementation significantly increased the levels of plasma HDL-C (7.4%), subfraction HDL_2-C (7.5%), and HDL_3-C (5.3%) and decreased LDL-C/HDL-C ratio (Table 2). It also decreased plasma LDL-TG levels by 16.7%. No significant differences were found in plasma levels of total cholesterol, LDL-C and triglycerides after soygerm extract supplementation. The ex vivo LDL oxidation decreased by prolonging the lag time of conjugated dienes formation by 9.9% and decreased TBARS production by 6.5% after soygerm extract supplementation. Urinary 16α-OHE_1 decreased by 16.8%, and 2/16α ratio increased by 22.8%, but neither reached statistical significance.

4. Discussion

The present study has shown that soygerm isoflavones have favorable effects on plasma lipids in postmenopausal women already receiving HT. Most previous studies focused on postmenopausal women without HT. To our best knowledge, this is the first clinical trial to investigate the effect of soy isoflavones on postmenopausal women who receive HT.

The predominant component in this soygerm extract supplement was daidzein, followed by glycitein and a trace

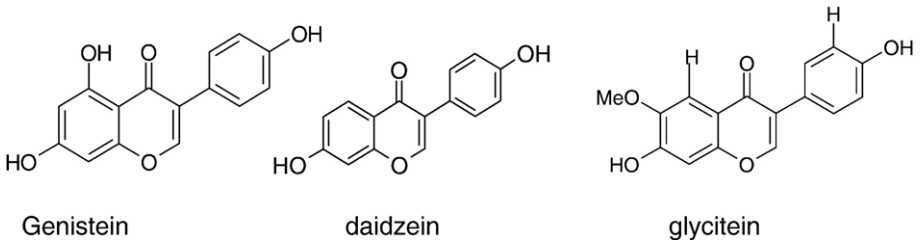

Figure 1 Structures of genistein, daidzein and glycitein.

amount of genistein (Table 1). The level of daidzein in the soygerm extract was 8-fold higher than that of genistein; whereas usual soy foods contain about equal amounts of genistein and daidzein, and a low amount of glycitein (5-10%). Daidzein and glycitein do not possess a hydroxyl group at the 5-position of the A ring while genistein does, and glycitein has an additional $-OCH_3$ at the 6-position of the A ring while daidzein does not (Fig. 1). Daidzein has less estrogenic effect than genistein [19]. When daidzein was metabolized by intestinal microflora to equol, it became more estrogenic and the capacity to produce equol from daidzein determined the effectiveness of soy isoflavones [20]. Therefore, the variable biological function of isoflavones might partly result from variability of intestinal microflora, which could be affected by habitual diets [16]. A recent study found that in apparently healthy men, subjects with ability to produce equol was higher among Japanese (46%) and Korean (59%) than that of Americans (14%) [21].

Combined hormone therapy with estrogens plus progestin was associated with a hazard ratio for coronary heart disease, stroke and thromboembolic events [1]. Although the dose of medroxyprogesterone in the present study was different with that of Women's Health Initiative's study, the effect of 5 mg progestins on CHD might not be less than that of 2.5 mg [4]. Our results indicate that the addition of isoflavones to HT users may reduce the risk of CVD that is associated with HT. The favorable change in plasma lipoproteins included an increase in HDL-C and its subfractions HDL_2-C and HDL_3-C, as well as a decrease in LDL-TG. LDL-TG was recently found to be an independent predictor of coronary artery disease (CAD) and the association of LDL-TG with CAD was stronger than that of LDL-C [22].

The present study also showed resistance of LDL to oxidation increased after soygerm extract supplementation, similar to other studies with high genistein isoflavones [23]. The reduced LDL oxidation might result from the antioxidative property of isoflavones and a decrease in LDL-TG which indicated less oxidizable substrate in LDL and less dense LDL [22].

An insignificant increase in 2/16α ratio in postmenopausal women using HT after soygerm extract supplementation was noted. However, the clinical significance for this finding remained to be determined. A possible adverse effect of simultaneous administration of soy and HT on endometrium and mammary gland was not evaluated in this study. A prior study in ovariectomized nonhuman primates showed that addition of soybean phytoestrogens to estrogen replacement therapy did not provide excessive estrogenic effects, but it protected breast and endometrial tissues from the proliferative stimulation of exogenous mammalian estrogen [24,25].

Limitations of the present study included small study population, which may have caused selection bias, and lack of control group. However, this pilot clinical trial first pointed out the possible effects of soygerm extract on postmenopausal women receiving HT.

In conclusion, the supplementation of 119 mg isoflavones with high daidzein and glycitein contents per day for 4 weeks in Taiwanese postmenopausal women who received HT has favorable effects on plasma lipids in increasing HDL-C and decreasing LDL oxidizability. Long-term, placebo-controlled clinical trials are needed to confirm these results.

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