THE JOURNAL OF
Obstetrics and Gynaecology Research

The official Journal of Asia and Oceania Federation of Obstetrics and Gynaecology
Japan Society of Obstetrics and Gynecology

doi:10.1111/j.1447-0756.2009.001059.x J. Obstet. Gynaecol. Res. Vol. 35, No. 6: 1091–1095, December 2009

Influence of red clover-derived isoflavones on serum lipid profile in postmenopausal women

Milan M. Terzic[1], Jelena Dotlic[1], Sanja Maricic[2], Tihomir Mihailovic[3] and Bratislava Tosic-Race[1]

[1]*Department of Obstetrics and Gynecology, School of Medicine, University of Belgrade,* [2]*General Health Center 'Savski Venac', and* [3]*Ultramedica Clinic, Belgrade, Serbia*

Abstract

Aim: Menopause is associated with adverse metabolic changes, especially in plasma lipoprotein and cholesterol levels. Estrogens have beneficial effects on lipid metabolism. Phytoestrogens are plant substances that are structurally and functionally similar to 17β-estradiol and are capable of producing estrogenic effects. The goal of the present study was to estimate the effects of red clover-derived isoflavones on serum lipid levels in postmenopausal women.

Methods: The study comprised 40 healthy postmenopausal women with an average age of 56 years. The women were divided into two groups: 22 were allocated to a red clover-derived isoflavone medication group, and 18 were allocated to a non-medication group. Total blood cholesterol, cholesterol fractions and triglycerides in the women of both groups were investigated before treatment and at 4-month intervals over the following 12 months.

Results: Both total serum cholesterol and low-density lipoprotein (LDL) cholesterol levels, as well as triglyceride levels, were decreased significantly in the group receiving phytoestrogens. However, high-density lipoprotein (HDL) cholesterol showed a significant increase.

Conclusion: Red clover phytoestrogen supplementation in postmenopausal women had favorable metabolic effects on serum lipids. Furthermore, red clover phytoestrogens have no side-effects and can be considered safe.

Key words: phytoestrogen, plasma lipid, postmenopause, red clover-derived isoflavone.

Introduction

Menopause, with its well-known hormonal profile, is associated with adverse metabolic changes, especially in plasma lipoprotein and cholesterol levels. These unfavorable changes contribute to the development of arterial disease and myocardial infarction in the postmenopausal period. Hormone replacement therapy (HRT) containing synthetic/animal estrogens, has beneficial effects on lipid metabolism.[1,2]

However, HRT has numerous potentially dangerous adverse effects, prompting the search for alternative estrogen-based treatments.[3] One such class of estrogenic molecules is produced by plants: phytoestrogens. Phytoestrogens are plant substances that are structurally and functionally similar to 17β-estradiol. Even though in the circulatory system phytoestrogens bind to the estrogen receptor at low levels compared with endogenous estrogen, they are capable of producing estrogenic effects.[4]

Several researchers have evaluated the effects of isoflavones on plasma lipid levels in postmenopausal women. Although the results of this research have been promising overall, some of the data has been less

Received: May 4 2008.
Accepted: December 28 2008.
Reprint request to: Professor Dr Milan M. Terzic, Department of Obstetrics and Gynecology, School of Medicine, University of Belgrade, Belgrade 11000, Serbia. Email: terzicmilan@yahoo.co.uk

convincing, but it is now accepted that isoflavone products are very useful for improving lipid profiles.[5–7] Considering that red clover has a high isoflavone content, the aim of the present study was to estimate the effects of red clover-derived isoflavones on serum lipid levels in postmenopausal women.

Methods

The study was conducted as a prospective open randomized controlled clinical trial at the University of Belgrade, Institute of Obstetrics and Gynecology from December 2006 to February 2008.

Eligible subjects were healthy postmenopausal women (confirmed anamnestically and by routine health check-up, including examination of respiratory and cardiovascular functions, such as blood pressure, heart rate and electrocardiogram; gynecological findings; mammography in the past 12 months; hematological and biochemical analysis; laboratory analysis of urine sediment and culture) who were not taking anti-osteoporotic, lipid-lowering, anti-hypertensive or hormonal drugs and who had their last menstrual period more than 12 months before the study began. Exclusion criteria were one or more of the following: occurrence of any pathological state, especially one that could affect lipid levels and therefore affect the results of the study, such as disturbances of liver, kidneys, diabetes and endocrine glands; allergy to the components of the investigated medication; appearance of any serious adverse reaction that could affect the health or life of the patient; change in regular dietary habits; study protocol interruption; and the patient's wish to withdraw from the study.

Informed consent was obtained from all subjects on the basis that the substance to be administered was safe and that the subjects had freely taken part in the study. The study was approved by the local Research Ethics Committee.

We opted to form a classic clinical sample because there was no information available from previous studies regarding the variability (SD) or the size effect of our population, which made it impossible for us to determine the sample size. However, allocation to each group according to the random order of patients visiting the Institute guaranteed the absence of bias in the analysis.

A total of 96 consecutive postmenopausal women who visited the Institute over a 3-month period complaining of postmenopausal symptoms (hot flushes, sleep disturbances, mood swings and vaginal dryness) were recruited for the investigation. Only 50 of the women fulfilled the inclusion criteria. Therapy was administered to every second patient visiting the Institute (even numbers), while the remaining patients (odd numbers) were allocated to the control group (no medication). In such a way patients were randomly divided in two groups containing an equal number of patients ($n = 25$). However, some patients dropped out of the study. Three women were excluded from the medication group because they stopped taking the medication regularly. Seven women withdrew from the control group because they wished to begin treatment. Therefore, 40 women in total completed the study: 22 women received red clover-derived isoflavones while the 18 women did not receive any treatment. The follow-up period lasted 1 year.

Patients in the medication group ingested one capsule per day containing 40 mg red clover-derived isoflavones, early in the morning, before breakfast. Each patient kept a daily diary of therapy administration, symptoms and adverse reactions. The red clover pill contained the four most important isoflavones: biokain A (23 mg), diadzein (1 mg), formononetin (15 mg) and genistein (1 mg).

Total cholesterol, cholesterol fractions and triglyceride levels of all patients were recorded, together with height and bodyweight, before treatment and at 4-month intervals over the next 12 months. Standard tests for lipid level assessment were performed using an Olympus AU 400 automatic analyzer (Jadran Galenski Laboratorij, Rijeka, Croatia) with normal rates for triglycerides from 0.61 to 2.10 mmol/L, total cholesterol 3.63–6.46 mmol/L, HDL 0.75–1.99 mmol/L, LDL 1.60–4.78 mmol/L and very low density lipoproteins (VLDL) 0.10–1.03 mmol/L.

Data analysis was performed using SPSS version 15 (SPSS, Chicago, IL, USA). Differences between the groups were evaluated by using Student's t test. $P < 0.05$ was considered statistically significant.

Results

There were no significant differences in the average age ($t = -1.302$), body mass index (BMI; $t = -0.144$), and time after menopause ($t = 0.263$), between the isoflavone supplemented and control groups (Table 1).

The results of the serum biochemical parameters, which are presented as average values in Table 2, show that the triglyceride level significantly decreased from the baseline after 12 months of therapy in the isoflavone supplemented group ($t_{12\ \text{months}} = 1.686$). In contrast,

Table 1 Age and body mass index (BMI) of patients in the phytoestrogen and control groups

	Phytoestrogen group mean ± SD‡	Control group	*P*-value†
Patient age	55.0 ± 5.4	57.0 ± 3.7	0.201
Age at menopause	46.7 ± 6.2	47.0 ± 6.6	0.794
BMI	28.0 ± 5.0	27.0 ± 7.0	0.881

†Value of probability of H_0; ‡mean value ± standard deviation (SD).

Table 2 Mean triglyceride and cholesterol values in the phytoestrogen and control groups

	mean ± SD‡											
	Triglycerides (mmol/L)			Total cholesterol (mmol/L)			LDL (mmol/L)			HDL (mmol/L)		
	Phytoestrogen group	Control group	*P*-value†	Phytoestrogen group	Control group	*P*-value†	Phytoestrogen group	Control group	*P*-value†	Phytoestrogen group	Control group	*P*-value†
Start rate	1.75 ± 0.50	1.73 ± 0.37	0.891	5.62 ± 0.45	5.55 ± 0.40	0.005	3.38 ± 0.40	3.11 ± 0.50	0.157	1.47 ± 0.23	1.50 ± 0.40	0.808
4 months	1.69 ± 0.42	1.75 ± 0.50	0.689	4.87 ± 0.40	5.54 ± 0.30	0.003	2.87 ± 0.32	3.14 ± 0.45	0.051	1.78 ± 0.36	1.50 ± 0.36	0.022
8 months	1.60 ± 0.38	1.75 ± 0.35	0.219	4.96 ± 0.45	5.57 ± 0.50	0.045	2.84 ± 0.40	3.12 ± 0.34	0.027	1.76 ± 0.41	1.48 ± 0.30	0.024
12 months	1.55 ± 0.37	1.75 ± 0.36	0.099	4.92 ± 0.30	5.65 ± 0.45	0.028	2.79 ± 0.43	3.10 ± 0.42	0.032	1.75 ± 0.38	1.51 ± 0.20	0.024

†Values of the probability of H_0; ‡mean values ± standard deviation (SD). HDL, high-density lipoprotein; LDL, low-density lipoprotein.

there was no significant change over time in the control group. The mean total cholesterol level in the investigated group significantly decreased after 4 months and remained significantly lower after 8 and 12 months of therapy in comparison to the control group ($t_{4\ months} = -3.190$, $t_{8\ months} = -2.068$, $t_{12\ months} = -2.288$). Significant falls in the mean LDL cholesterol values were registered after 4, 8 and 12 months of therapy ($t_{4\ months} = -2.015$, $t_{8\ months} = -2.295$, $t_{12\ months} = -2.230$, respectively). The mean HDL cholesterol levels in the phytoestrogen and control groups at the beginning of the study were not significantly different ($t = -0.244$). However, after 4, 8 and 12 months of therapy, the mean HDL levels cholesterol increased significantly ($P < 0.05$) in the group receiving phytoestrogens compared to the group not receiving medication ($t_{4\ months} = 2.393$, $t_{8\ months} = 2.353$, $t_{12\ months} = 2.353$). No side-effects were detected.

Discussion

In postmenopausal women, total cholesterol, LDL cholesterol and triglyceride levels are increased and HDL cholesterol is decreased compared with premenopausal women of the same age and BMI.[8] High LDL cholesterol levels are associated with atherosclerosis.[9] The majority of conventional HRT-regimens lower total cholesterol and LDL cholesterol and slightly increase HDL cholesterol. Therefore, the achieved favorable lipoprotein levels are associated with a lowered risk of cardiovascular disease in postmenopausal period. However, this treatment can cause numerous adverse effects and consequences, most importantly, an association with the risk of breast cancer, as well as endometrial malignancy if these estrogens are prescribed unopposed in patients without hysterectomy. Contrary to conventional HRT, there is an association between a high intake of phytoestrogens and a low risk of both breast and endometrial cancer.[10–12] This effect could be explained by specific phytoestrogens binding to the estrogen receptor. There are two types of intranuclear estrogen receptors (ER): ERα and ERβ.[1] Phytoestrogens bind weakly to ERα receptors and more strongly to ERβ receptors, and possess organ-specific estrogenic and antiestrogenic effects, working as partial agonists in some tissues and antagonists in others. ERβ receptors are located in vascular walls and bone cells, while ERα receptors are found in the endometrium and breast tissue. Therefore, women receiving phytoestrogen medication experience two benefits: (1) an increase of HDL cholesterol and (ii) downregulation of ERα receptor caused by phytoestrogens binding to ERβ receptors.[13–15] It has been confirmed that phytoestrogens have favorable effects on serum lipids.[6]

Biological effects of isoflavones are different depending on their origin. Isoflavones derived from black cohosh show little potential for relief of menopausal symptoms and provide little benefit to blood lipids.[16,17] Contrary to these findings, recent investigations have shown beneficial biological effects of red clover-

derived isoflavones.[8] The present study shows that red clover exerts a positive effect on lipid levels in postmenopausal women. However, it must be stressed that although body mass index (BMI) does not significantly affect age at menopause,[2] our patients were of a similar age and BMI, and women with a lower BMI have more menopausal complaints.[17]

The observed benefits appeared to be attributable to isoflavones; mainly to daidzin and glycitin. Red clover has some advantages in comparison with other plants containing phytoestrogens.[18] Red clover is the only plant from which all four of the most important isoflavones can be derived. Soy, for example, contains only two isoflavones: diadzein and genistein. Furthermore, the amount of isoflavones that can be derived from red clover is greater than from other plants (almost 20 times greater than from soy). Red clover is a safe and nontoxic plant that cannot be genetically modified in production. Moreover, red clover contains other beneficial substances, such as polifenoles, which are potent antioxidants. Standard daily dietary supplements made from soy or red clover contain at least 40 mg of isoflavones.[3,7] Our patients took the advised amount of phytoestrogens.[7,19] Isoflavone supplementation over a period of 4 weeks to 2 months, has been proven safe in numerous studies, as judged by no side-effects in clinical laboratory blood tests, physical examinations and on the basis of subjective symptoms. Therefore, investigations based on longer isoflavone administration have been undertaken.[20] Because no side-effects were detected in our study, which lasted for a period of 12 months, it can be concluded that isoflavone supplementation may be safe for even longer usage than previously advised.

Patients from the treatment group were highly motivated to take the medication and, therefore, the dropout rate from this group was low, with no patient wishing to cease medication. There was a higher dropout rate in the control group due to patients wanting to begin treatment. One of the reasons for such motivation could be that a high level of serum lipids was found in the majority of patients at the beginning of the study.

Our study did have its limitations, namely, there was a relatively small sample. Nevertheless, the study groups are comparable to other studies in the field of postmenopausal therapy. Moreover, the sample is representative of the patient population at the University of Belgrade, Institute of Obstetrics and Gynecology clinic, where the study took place; a diverse population in terms of social and financial backgrounds, and from all over the country, both urban and rural areas. Another problem with our study was that it was not designed as a blind or double-blind investigation, but we leave that for future studies. Also, we wanted to avoid a placebo effect in the control group and, therefore, patients from the control group were not given treatment. However, a placebo-controlled study should be performed in further investigations.

Conclusion

Red clover phytoestrogen supplementation in postmenopausal women has favorable metabolic effects on serum lipids. Furthermore, red clover phytoestrogens have no side-effects and can be considered safe. Finally, all of our patients were satisfied with the administered medications.

References

1. Petronijevic A, Terzic M, Argirovic R. *The Menopause*. Belgrade: Medical Book, 1992.
2. Crook D. Interpreting the plasma lipoprotein profile of the postmenopausal women. In: Erkkola R (ed.). *The Menopause*. Toronto: Elsevier, 2006; 33–40.
3. Brockie J. An alternative approach to the menopause. *Obstet Gynaecol (Lond)* 2006; **8**: 96–102.
4. Reinli K, Block G. Phytoestrogen content of foods – a compendium of literature values. *Nutr Cancer* 1996; **26**: 123–148.
5. Geller SE, Studee L. Soy and red clover for midlife and aging. *Climacteric* 2006; **9**: 245–263.
6. Booth NL, Piersen CE, Banuvar S, Geller SE, Shulman LP, Farnsworth NR. Clinical studies of red clover (Trifolium pratense) dietary supplements in menopause: A literature review. *Menopause* 2006; **13**: 251–264.
7. Birkhauser MH, Panay N, Archer DF *et al.* Update practical recommendations for hormone replacement therapy in the peri- and postmenopause. *Climacteric* 2008; **11**: 108–123.
8. De Kleijn MJ, Van der Schouw YT, Banga JD, Van der Graaf Y. The effect of menopause on risk factors for ischemic diseases. *Ned Tijdschr Geneeskd* 1996; **140**: 478–482.
9. Steinberg D. Antioxidants and atherosclerosis: A current assessment. *Circulation* 1991; **84**: 1421–1425.
10. Glazier MG, Bowman MA. A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. *Arch Intern Med* 2001; **161**: 1161–1172.
11. Goodman MT, Wilkens LR, Hankin JH, Lyu LC, Wu AH, Kolonel LN. Association of soy and fiber consumption with the risk of endometrial cancer. *Am J Epidemiol* 1997; **146**: 294–306.
12. Powles TJ, Howell A, Evans DG *et al.* Red clover isoflavones are safe and well tolerated in women with a family history of breast cancer. *Menopause Int* 2008; **14**: 6–12.
13. Rohan TE, Negassa A, Chlebowski RT. Conjugated Equine Estrogen and Risk of Benign Proliferative Breast Disease: A Randomized Controlled. *J Natl Cancer Inst* 2008; **100**: 563–571.

14. Jefferson A. Dietary phytoestrogens – a role in women's health. *Nutr Food Sci* 2003; **33**: 16–22.
15. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999; **340**: 1801–1811.
16. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW Jr. Soy protein and isoflavones: Their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* 1998; **68**: 1375–1379.
17. Reed S, Newton K, Lacroix A, Grothaus L. Assessing the impact of black cohosh on menopausal symptoms among perimenopausal and postmenopausal women. *Menopause* 2008; **15**: 51–58.
18. Beck V, Rohr U, Jungbauer A. Phytoestrogens derived from red clover: An alternative to estrogen replacement therapy? *J Steroid Biochem Mol Biol* 2005; **94**: 499–518.
19. Clarke DB, Bailey V, Lloyd AS. Determination of phytoestrogens in dietary supplements by LC-MS/MS. *Food Addit Contam* 2008; **25**: 534–547.
20. Powles TJ, Howell A, Evans DG *et al.* Red clover isoflavones are safe and well tolerated in women with a family history of breast cancer. *Menopause Int* 2008; **14**: 6–12.