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## MENOPAUSE

# Effect of *Trifolium pratense*-derived isoflavones on the lipid profile of postmenopausal women with increased body mass index

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### Abstract

**Background.** Since current clinical evidence indicates that conventional estrogen hormone therapy (HT) increases cardiovascular risk, alternatives to estrogens are growing in popularity, especially among high-risk populations.

**Objective.** To determine the effect of *Trifolium pratense*-derived isoflavone supplementation on the lipid profile of postmenopausal women with increased body mass index (BMI).

**Methods.** Sixty postmenopausal women aged > 40 years, HT non-users, were randomly assigned to one of two groups: either two capsules of *T. pratense* (80 mg red clover isoflavones) daily for a 90-day period or placebo of equal design. After a 7-day washout period, medication was crossed-over for another 90 days. Total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C) and lipoprotein A (LpA) levels were assessed at baseline, 90 and 180 days. Women were divided into two groups: those with increased BMI ( $\geq 25$  kg/m<sup>2</sup>) and those with BMI < 25 kg/m<sup>2</sup>.

**Results.** Fifty-three women (88.3%) completed the trial. *T. pratense* isoflavone supplementation had a positive effect on the lipid profile of women with increased BMI, evidenced by a significant decrease in TC, LDL-C and LpA levels.

**Conclusions.** Isoflavones derived from *T. pratense* are an attractive alternative therapeutic option for high-risk populations such as postmenopausal women with increased BMI and abnormal lipid profile.

**Keywords:** *Isoflavones, Trifolium pratense, red clover, dyslipidemia, cardiovascular risk, obesity*

### Introduction

Worldwide, women have received the benefits of hormone therapy (HT) for the alleviation of the climacteric syndrome and the prevention of osteoporosis and other age-related conditions [1]. Despite this, in developed and non-developed countries, long-term HT compliance is low and related to several factors, among them risk–benefit concerns [2]. In this context, the use of HT decreased further after the unexpected findings of the Women's Health Initiative (WHI) study in which one HT regimen significantly increased the risk for cardiovascular events and breast cancer [3]. As a result of this, physicians and patients have changed their attitude toward the use of HT for management of the menopause [4,5], focusing their interest on alternatives to estrogens [6,7]. Under this category one can mention phytoestrogens, which are plant-derived molecules, mainly represented by

isoflavones, exhibiting estrogenic effects [8,9]. Despite being less potent than conventional estrogenic compounds, their selective  $\beta$ -estrogenic receptor binding properties allow positive effects on various organs (bone, vagina, brain) with no effect on the uterus or breast [10]. Although isoflavones derived from soy have been the most extensively studied, interest in those obtained from extracts of *Trifolium pratense* (red clover), a type of phytoestrogen, are increasing among women and researchers. Such tendency is currently supported by experimental [11,12] and clinical evidence [13–15]. With regard to the latter, studies related to the effect of *T. pratense* isoflavones on the lipid profile of men and climacteric women have rendered conflicting results, some being positive [15–18] and others not [19,20].

Because the prevalence of the metabolic syndrome and thus cardiovascular risk increases after the menopause [21–23], more research is required to

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determine the effect of red clover isoflavones on such high-risk populations (increased body mass index (BMI)); moreover, data in this regard are still scarce. The objective of the present study was therefore to determine the effect of *T. pratense*-derived isoflavones on the lipid profile of postmenopausal women with increased BMI.

## Methods

### Subjects

From July 2003 to August 2004, a prospective randomized, double-blind, placebo-controlled trial was carried out to evaluate the effect of supplementation with *T. pratense* (red clover, MF11RCE) extract on climacteric symptoms, lipids and vagina of postmenopausal women. Data of the global project, including general lipid profile, are presented elsewhere [15]; however, the lipid data have been further exploited and analyzed according to BMI and are presented in the current paper. After research protocol approval by the Bioethics Committee of Medical Faculty, Universidad Católica de Guayaquil, 60 postmenopausal women (amenorrhea > 12 months and basal follicle-stimulating hormone > 30 mIU/ml) aged > 40 years, non-users of HT with a Kupperman index of  $\geq 15$ , were recruited. The study protocol and objectives were explained in detail and sociodemographic data obtained among women consenting to participate. BMI, blood pressure and lipid profile were recorded at baseline, 90 and 180 days. Subjects not consenting to participate, not expressing their willingness to take the prescribed preparations and adhere to the control dates, as well as those on conventional HT, isoflavone-derived supplements, thyroid medication (or history of thyroid disease) or on medication that could interfere with lipid serum levels were excluded. BMI was calculated as weight (kg) divided by the square of height ( $m^2$ ) and was considered to be increased if  $\geq 25 \text{ kg}/m^2$  [15]. Blood pressure determinations were performed after women had been sitting for 15 min, those having values  $\geq 140/90$  mmHg or already on antihypertensive drugs were defined as having hypertension. Women on hypoglycemic drugs indicated by a physician were considered diabetic [15].

### Assignment

Subjects consenting to participate were randomly assigned to initiate daily either two capsules of *T. pratense*-derived isoflavones or placebo for a 90-day period. After a 7-day washout period, participants switched to receive the opposite treatment for 90 days more (net trial duration 180 days) [15]. Placebo and *T. pratense* isoflavone capsules, identi-

cally manufactured, were placed in opaque containers (60 capsules each) labeled as A or B and provided by Melbrosin International. Investigators and participants were blinded until the end of the study, after which the code was broken. Each capsule of *T. pratense* isoflavone supplement provided approximately 40 mg isoflavones (Menoflavon<sup>®</sup>; Melbrosin International, Vienna, Austria).

### Lipid profile

A 15–20 ml fasting blood sample was withdrawn from each participant at the planned intervals. After centrifugation at 5°C for 10 min at 3000 rev/min, serum was decanted into 1.5 and 2.0 ml aliquots and stored at  $-70^\circ\text{C}$  until further analysis for total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and lipoprotein A (LpA), which were assayed with a Hitachi 717 automatic photometric analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

### Statistical analysis

Data analysis was performed using EPI-INFO 2000 statistical software (Centers for Disease Control, Atlanta, GA, USA; World Health Organization, Geneva, Switzerland); data are presented as means and standard deviations, or frequencies and percentages. Comparison of continuous and categorical data was performed with the non-paired Student *t* test and the  $\chi^2$  test, respectively. A *p* value of < 0.05 was considered as significant. Women who abandoned the trial were not included in the final statistical analysis.

## Results

A total of 60 postmenopausal women were enrolled in the present clinical trial. Seven subjects (11.7%) did not complete the trial therefore were excluded from the analysis: five abandoned treatment for no specified reasons and two due to side-effects (headache: one woman during the placebo phase and the other during the *T. pratense* supplementation phase). Overall baseline characteristics, as well as according to the initially assigned group, are depicted in Table I. No significant differences were observed among groups.

The lipid profile after each phase of the trial according to BMI is shown in Table II. *T. pratense* isoflavone supplementation had a positive effect on the lipid profile of women with increased BMI, as evidenced by a significant decrease in TC, LDL-C and LpA. Women with increased BMI displayed significantly higher baseline TG levels compared with their non-overweight counterparts. After

isoflavone supplementation this level decreased, but this did not reach statistical significance. After the placebo phase, the TG levels of women with increased BMI were significantly increased compared with baseline and the post-isoflavone level. The same trend was observed in LpA levels among women with increased BMI, i.e. a significant decrease after isoflavone supplementation and a concomitant significant rise after the placebo phase.

## Discussion

Epidemiological data indicate that cardiovascular risk increases after the onset of the menopause [23], a situation directly correlated to the rise in the prevalence of the metabolic syndrome [21,22]. We have previously reported that obesity plays a central role as a risk factor for the metabolic syndrome in postmenopausal women, in whom quality of life has been found to be impaired [24] and related to increased menopausal symptoms [25]. Within this scenario of high-risk populations in need of treatment in the post-WHI era in which risk-benefit concerns have increased, interest in alternatives to estrogens is currently growing among clinicians and patients [4–7].

Table I. Demographic and general data of the postmenopausal women.

	Group A (n = 31)	Group B (n = 22)	Overall (n = 53)
Age (years)	51.3 ± 3.3	51.2 ± 3.9	51.3 ± 3.5
BMI (kg/m <sup>2</sup> )	27.2 ± 4.3	25.8 ± 3.2	26.6 ± 3.9
Hysterectomy	2 (6.5)	1 (4.5)	3 (5.7)
Diabetes	2 (6.5)	1 (4.5)	3 (5.7)
Hypertension	2 (6.5)	1 (4.5)	3 (5.7)
Married	12 (38.7)	11 (50.0)	23 (43.4)
Schooling < 12 years	17 (54.8)	12 (54.5)	29 (54.7)
Non-professionals	23 (74.2)	16 (72.7)	39 (73.6)

BMI, body mass index; data are presented as mean ± standard deviation or n (%); no significant differences between Group A and Group B using Student's *t* test,  $\chi^2$  or Yates' corrected  $\chi^2$  calculation.

The present series found that isoflavones derived from *T. pratense* had a positive impact on the lipid profile of postmenopausal women with increased BMI, evidenced by a significant decrease in TC, LDL-C and LpA levels. It has been reported that supplementation with phytoestrogens decreases LDL-C, upgrading liver LDL receptors and decreasing endogenous cholesterol production through inhibition of 7 $\alpha$ -hydroxylase [26]. Despite this, no up-to-date conclusive evidence regarding the effect of red clover (*T. pratense*) isoflavones on lipid profile has been published. Moreover, studies related to the effect of *T. pratense* isoflavones on the lipid profile of men and climacteric women have rendered conflicting results, some being positive [15–18,27] and others not [19,20]. Heterogeneity of the studied populations (including dietary habits) and the use of non-standardized products could possibly explain some of these conflicting results [19,20]. In one study, 46 men and 34 postmenopausal women were randomized to receive supplementation with red clover isoflavone 40 mg/day (40 assigned to biochanin- and 40 to formononetin-enriched capsules) for 6 weeks, after which they crossed over for placebo for another 6 weeks. A positive decrease in LDL-C levels was observed only among men taking the biochanin-enriched presentation [18], with no effect observed among postmenopausal women, a situation that is in contrast to the findings of the present series. In another study (randomized, double-blind, placebo-controlled) in which 177 climacteric women aged 49 to 65 years were assigned to receive red clover isoflavone 43.5 mg/day, a positive effect on TG and plasminogen activator inhibitor type-1 levels was found in the perimenopausal group [17]. A significant trend toward a carryover effect for the concentration of HDL-C has been observed in 14 healthy premenopausal women receiving red clover isoflavones 86 mg/day for 8 weeks, despite no effect on TC, TG levels or LDL oxidizability [16]. Contrary to this, among postmenopausal women relevant observations include an increase in HDL-C and a decrease in apolipoprotein B and TG levels [27,28]. Some of

Table II. The effect of *Trifolium pratense* isoflavone supplementation on lipid profile according to BMI.

Parameter	BMI < 25 kg/m <sup>2</sup> (n = 18)			BMI ≥ 25 kg/m <sup>2</sup> (n = 35)		
	Baseline	After <i>T. pratense</i>	After placebo	Baseline	After <i>T. pratense</i>	After placebo
TC (mg/dl)	215.6 ± 35.6	207.5 ± 36.3	210.6 ± 34.2	228.1 ± 38.4	217.5 ± 30 <sup>a</sup>	225.4 ± 33.5
TG (mg/dl)	164.7 ± 53.6	141.7 ± 45.4	172.8 ± 137.1	217.6 ± 82.7 <sup>c</sup>	201.5 ± 75.7	278.6 ± 171.1 <sup>a,b</sup>
HDL-C (mg/dl)	44.3 ± 8.3	43.5 ± 7.1	44.3 ± 8.4	37.3 ± 12.3 <sup>c</sup>	38.2 ± 10.3	39.5 ± 10.5
LDL-C (mg/dl)	137.9 ± 30.3	133.9 ± 35.3	138.2 ± 31	151.3 ± 29.0	127.6 ± 41.7 <sup>a</sup>	140.9 ± 37.3
LpA (mg/dl)	35.6 ± 35.4	30.3 ± 31.9	31.3 ± 30.9	52.2 ± 38.6	18.9 ± 23.5 <sup>a</sup>	30.1 ± 23.2 <sup>a,b</sup>

TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LpA, lipoprotein A; data are presented as mean ± standard deviation; significant difference ( $p < 0.05$ ) compared with <sup>a</sup>baseline, <sup>b</sup>after *T. pratense* and <sup>c</sup>baseline value of the group with BMI < 25 kg/m<sup>2</sup>.

these findings correlate with those of the present research; however, to date the effect of *T. pratense* focused on postmenopausal women with increased BMI has not been reported. In contrast, data regarding the effect of soy isoflavones on the lipid profile and cardiovascular risk of postmenopausal women can be found in the literature, but with equally conflicting results. While some have reported a positive decrease in TG, LDL-C and TC levels [29,30], others have reported no effect at all [31,32]. In one study, after 6 months of soy isoflavone supplementation and exercise, postmenopausal obese women improved their body composition parameters known to influence cardiovascular risk (i.e. BMI, total and abdominal fat mass, appendicular fat-free mass) [32].

*T. pratense* supplementation in our postmenopausal women with increased BMI significantly decreased TC, LDL-C and LpA levels from baseline, by 4.6%, 15.6% and 63.8% respectively. These changes in general correlate with the findings of others and support the fact that minimal changes in the lipid profile could potentially be associated with an important reduction in cardiovascular risk [27], all the more in postmenopausal women with high lipid values. Schult and colleagues [27] found that the effect of red clover supplementation was most significant in women with high baseline lipid values, a fact that supports our findings; however, we propose that BMI also be taken into account for further research purposes. It is important also to bear in mind the fact that reduction of LpA levels (63.8% in our series), as determined in a large prospective study and in women complicated with type 2 diabetes [33,34], substantially reduces coronary disease.

In conclusion, *T. pratense*-derived isoflavones had a positive effect on the lipid profile of postmenopausal women with increased BMI. Although it presents as an attractive and promising alternative therapeutic option for high-risk populations (increased BMI and abnormal lipid profile), more research is required.

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