Effects of a supplement containing isoflavones and *Actaea racemosa* L. on asymmetric dimethylarginine, lipids, and C-reactive protein in menopausal women

Marieke O. Verhoeven, M.D.,[a] *Tom Teerlink, Ph.D.,*[b] *Peter Kenemans, Ph.D.,*[a] *Sonja D. Zuijdgeest-van Leeuwen, Ph.D.,*[c] *and Marius J. van der Mooren, Ph.D.*[a,c]

[a] Departments of Obstetrics and Gynecology, Project "Aging Women," and [b] Clinical Chemistry, Institute for Cardiovascular Research-Vrije Universiteit, VU University Medical Center, Amsterdam; and [c] Numico Research, Wageningen, The Netherlands

Objective: To investigate the effects of a supplement containing soy isoflavones and *Actaea racemosa* L. on several coronary heart disease (CHD) risk markers in menopausal women.
Design: Randomized, placebo-controlled, double-blind study.
Setting: Nine hospitals in The Netherlands.
Patient(s): One hundred twenty-four menopausal women.
Intervention(s): Daily placebo (n = 64) or supplement containing soy isoflavones and *Actaea racemosa* L. (n = 60) for 12 weeks.
Main Outcome Measure(s): Fasting blood concentrations of asymmetric dimethylarginine (ADMA), lipids, and C-reactive protein (CRP) at baseline and week 12.
Result(s): In the supplement group, total cholesterol and low-density lipoprotein cholesterol showed a small absolute reduction at week 12 (−0.2, 95% confidence interval [CI] −0.3 to −0.0; and −0.2, 95% CI −0.3 to −0.0; respectively). Concentrations of ADMA, triglycerides, lipoprotein(a), and CRP did not change significantly. Analysis of covariance over the 12-week study period revealed no significant between-group differences for all parameters. No significant correlations were found between the concentrations of isoflavones and the CHD risk markers investigated.
Conclusion(s): Twelve-week administration of a supplement containing soy isoflavones and *Actaea racemosa* L. had little or no influence on the CHD risk markers studied. This supplement probably has neither protective nor adverse effects on the cardiovascular system; however, large long-term studies are needed to test this. (Fertil Steril® 2007;87:849–57. ©2007 by American Society for Reproductive Medicine.)

Key Words: Actaea racemosa L., ADMA, asymmetric dimethylarginine, Cimicifuga racemosa L., C-reactive protein, lipids, lipoproteins, menopause, phytoestrogens, soy isoflavones

Estrogens alone or combined with a progestogen are the first choice of treatment for women with disabling climacteric complaints, such as hot flushes and night sweats (1). However, large randomized controlled trials reported negative effects of long-term use of menopausal hormone therapy (HT) (2–5). As a result of these publications and the subsequent media exposure, women have become hesitant to start or continue HT even though climacteric symptoms may seriously interfere with their quality of life (6).

Therefore, it is necessary to search for an alternative option to obtain climacteric symptom relief, with fewer disadvantages. Isoflavones are nutritional components, present in soy foods that in vitro bind to the estrogen receptor (7). This has led to the hypothesis that isoflavones might reduce climacteric symptoms. However, so far the results of several studies have been inconsistent (8–10). An extract of black cohosh (*Actaea racemosa* Linnaeus [formerly called *Cimicifuga racemosa* L.]) has been suggested to reduce climacteric symptoms as well and is another potential alternative for HT (11–13).

The modulation of cardiovascular risk markers by estrogens alone or combined with a progestogen has been studied widely. Favorable effects of HT on the lipid profile are well documented (14). An emerging cardiovascular risk marker is asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor (15). High levels of ADMA have been associated with increased overall mortality and cardiovascular events in specific patient groups (16–21). Concentrations of ADMA were reduced by HT given orally (~8%) or transdermally (~4%) (22–24).

Another cardiovascular risk factor is C-reactive protein (CRP), a marker for inflammation that is associated with coronary heart disease (CHD) (25). Oral HT has been found

Received March 28, 2006; revised and accepted July 21, 2006.
Supported by Numico Research, Wageningen, The Netherlands, through a grant to the Biocare Foundation (grant no. 02-60). Drs. Zuijdgeest-van Leeuwen and van der Mooren are employed by Numico Research.
Presented as an abstract at the 7th European Congress on Menopause, Istanbul, Turkey, June 3–7, 2006.
Reprint requests: M.J. van der Mooren, M.D., Ph.D., M.Sc., Associate Professor, Department of Obstetrics and Gynecology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands (FAX: +31-20-444 4422; E-mail: mj.vandermooren@vumc.nl).

to increase CRP in postmenopausal women, whereas transdermal HT did not (26, 27).

Eight weeks' consumption of fruit cereal bars enriched with soy isoflavones did not affect ADMA concentrations in postmenopausal women (28). The reported effects of isoflavones on the lipid profile vary greatly (29–31), and supplements containing isoflavones have been found not to influence CRP concentrations (32–35). No data have been available about effects of *Actaea racemosa* L. on ADMA and CRP until now, and studies investigating effects on lipids are rare (36).

Previously, we reported on a 12-week randomized, placebo-controlled, and double-blind study of a combination of soy isoflavones and *Actaea racemosa* L., with climacteric symptoms as primary end point (37). As secondary end points we also studied the effects on plasma concentrations of ADMA, arginine, symmetric dimethylarginine (SDMA), and the calculated arginine/ADMA ratio as a marker for NOS activity (38). Furthermore, we studied the effects on serum concentrations of lipids (total cholesterol, low-density lipoprotein [LDL]–cholesterol, high-density lipoprotein [HDL]–cholesterol, triglycerides, lipoprotein(a) [Lp(a)], and CRP. Correlations between these CHD risk markers and the previously reported plasma concentrations of isoflavones (37) after 12 weeks of supplementation were investigated as well.

MATERIALS AND METHODS

Subjects

Healthy menopausal women aged 45 to 65 years with at least five hot flushes per day were recruited from the outpatient clinics of eleven centers in The Netherlands and through regional newspaper advertisements. Participants had to be amenorrhoic for at least 6 months, and their serum FSH concentration had to be higher than 25 IU/L. Women were not allowed to use medication, supplements or diets interfering with the supplement ingredients or the CHD risk markers investigated. Other exclusion criteria were published earlier (37). Dietary and physical habits were not registered during the study period; however, women were asked to maintain their usual dietary and physical habits during study participation.

The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the central Institutional Review Board and the local Institutional Review Board of each participating center. All participants gave written informed consent before study entry.

Study Design

Details of this randomized, placebo-controlled, and double-blind study were published previously (37). In short, eligible women were randomly assigned to either the placebo (n = 64) or the supplement (n = 60) group, using a computer-generated randomization schedule in blocks of six. For a period of 12 weeks, women took orally two soft gel capsules twice per day during or just after breakfast and the evening meal to enhance absorption by bowel activity.

Women in the supplement group thus received daily among others 125 mg soy extract (providing 50 mg isoflavones, including 24 mg genistein and 21.5 mg daidzein) combined with 100 mg *Actaea racemosa* L. extract (providing 8 mg deoxyacetein), whereas the women in the placebo group received daily 2,000 mg olive oil. The supplement and placebo capsules were provided by Numico (Wageningen, The Netherlands), and were identical in appearance, smell, and taste. Women were considered noncompliant if, after one of the supplementation periods of 6 weeks, they returned more than 20% of the number of capsules handed out during the previous visit.

Measurements

Venous blood samples for the determination of the concentrations of ADMA, arginine, SDMA, total cholesterol, LDL- and HDL-cholesterol, triglycerides, Lp(a), and CRP were collected between 8:00 and 10:00 a.m. at the screening visit and during the last visit at week 12. Subjects had fasted and refrained from smoking for at least 12 hours and from consuming alcohol for more than 24 hours before blood sampling. After 20 minutes of rest, blood was collected with a Vacutainer system (Becton Dickinson, Meylan, France) in tubes containing tripotassium ethylenediaminetetraacetic acid (K_3EDTA) (Becton Dickinson) for ADMA, arginine, and SDMA measurements in plasma and in plain tubes (Becton Dickinson) for lipids, including Lp(a), and CRP measurements in serum. Plasma and serum were separated by centrifugation at 2,000*g*, plasma at 4°C serum at 20°C and both for 30 minutes within 1 hour of collection, and divided into aliquots, snap-frozen, and stored at −80°C until analysis. All samples from individual patients were analyzed in the same analytical series for each parameter.

The ADMA, arginine, and SDMA concentrations were measured by high-performance liquid chromatography with fluorescence detection (39). The interassay coefficients of variation (CVs) were less than 3% for ADMA and arginine and less than 4% for SDMA. For each participant, the plasma arginine/ADMA ratio was calculated.

Serum lipid levels were measured with a Modular P system (Roche, Mannheim, Germany). For total cholesterol, HDL-cholesterol, and triglycerides, the following reagents were used: CHOD-PAP, HDL-C plus, and GPO-PAP, respectively (all by Roche). The interassay CVs were less than 3.7%. The LDL-cholesterol was calculated using the Friedewald formula (40). Serum Lp(a) concentrations were measured with a standard commercially available one-step sandwich ELISA using Immunozym Lp(a) (Progen Biotechnik, Heidelberg, Germany). The intra-assay CV for this ELISA was 3.3%, and the interassay CV was 4.5%.

The CRP was assayed using an in-house highly sensitive ELISA with a lower limit of detection of 0.01 mg/L. The