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Effects of the phytoestrogen genistein on cardiovascular risk factors in postmenopausal women

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Abstract

Objective: The phytoestrogen genistein has been shown to be the most efficacious in clinical and experimental studies. We studied whether genistein treatment affects some cardiovascular risk markers in postmenopausal women.

Design: Sixty healthy postmenopausal women, who were 52 to 60 years of age, were enrolled in a 6-month double-blind, placebo-controlled, randomized study. After a 4-week stabilization on a standard fat-reduced diet, participants were randomly assigned to receive either genistein (n = 30; 54 mg/d) or placebo (n = 30). At baseline and after a 6-month treatment, we measured fasting glucose, insulin, insulin resistance (HOMA-IR), osteoprotegerin (OPG), fibrinogen, and sex hormone-binding globulin (SHBG).

Results: By comparison with placebo, genistein treatment decreased significantly fasting glucose (genistein = $-8.7 \pm 2.3\%$; placebo = $3.2 \pm 2.3\%$; $P < 0.001$), fasting insulin (genistein = $-12 \pm 3.33\%$; placebo = $36 \pm 3.29\%$; $P < 0.001$), and HOMA-IR (genistein = $-14 \pm 5.8\%$; placebo = $42 \pm 0.6\%$; $P < 0.001$). After genistein-treatment, fibrinogen decreased (genistein = 3.18 ± 0.12 g/L; placebo = 3.83 ± 0.04 g/L; $P < 0.001$) with respect to placebo. In the genistein group, serum OPG was lower ($-2 \pm 0.3\%$) than in placebo ($9 \pm 1.5\%$; $P < 0.001$), and serum SHBG was higher (63 ± 3.8 nmol/L) compared with placebo (53 ± 2.9 nmol/L; $P < 0.05$).

Conclusion: Our study suggests that genistein may have a favorable effect on some cardiovascular markers.

Key Words: Cardiovascular risk markers – Phytoestrogen – Genistein – Menopause – Osteoprotegerin – Insulin resistance.

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Coronary artery disease (CAD) is the leading cause of death among women in developed nations. It is well recognized that its incidence increases substantially after menopause, purportedly due to the loss of estrogen protection.[1,2] In fact, loss of ovarian function leads to a range of potentially unfavorable and interrelated metabolic alterations in women,[3] including changes in lipid and lipoprotein,[4] body fat distribution,[5] glucose and insulin metabolism,[6,7] coagulation and fibrinolysis,[8] and increased arterial resistance to flow.[9]

Hormone therapy (HT), in population-based observational studies,[10-12] has cut in half the risk of

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cardiovascular events, but a recent meta-analysis showed that the incidence of coronary heart disease was not reduced significantly when only studies that adjusted for socioeconomic status, alcohol use, exercise, and major cardiovascular risk factors were included in the statistical evaluation.[13] Furthermore, the Women's Health Initiative study has shown a lack of coronary heart disease protection of hormone therapy for primary prevention in postmenopausal women[14] and an increase of the coronary heart disease and ischemic stroke risk.[15,16]

Finally, the Heart and Estrogen/Progestin Replacement Study, a randomized, placebo-controlled, secondary prevention trial of estrogen/progestin, found no overall reduction in coronary events among women assigned to active hormone treatment and no benefit has been observed in a recent angiographic trial, the Estrogen Replacement and Atherosclerosis trial.[17,18]

The adverse effects of hormone therapy, including increased incidence of breast cancer,[19] endometrial cancer,[20] and thromboembolic events[21] in combination with low compliance to HT, and the above mentioned negative effects on cardiovascular risk prevention, have stimulated in recent years research on alternatives to classical HT.

Genistein, a phytoestrogen with selective estrogen receptor modulator properties, has received a great deal of attention over the last few years because of its potentially preventive roles against cardiovascular diseases.[22] Genistein may interact with nuclear estrogen receptors by either activating or inhibiting transcription of cell-specific genes.[23] In experimental models, genistein has been shown to enhance the dilator response to acetylcholine of atherosclerotic arteries, and to relax rat arteries by a nitric oxide-dependent mechanism.[24,25] Furthermore, genistein improves endothelial dysfunction induced by oophorectomy in rats and reduces infarct size in an experimental model of myocardial ischemia/reperfusion injury.[26,27] In a randomized clinical trial, genistein therapy increased the ratio of nitric oxide to endothelin and improved flow-mediated endothelium dependent vasodilation in healthy postmenopausal women.[28]

The possible effects of genistein on other risk factors for the development of cardiovascular disease have not been widely studied. Therefore, we investigated the effects of genistein and placebo on predictors of cardiovascular disease in selected healthy postmenopausal women.

METHODS

Participants

We analyzed serum stored from our previous randomized, double-blind, placebo-controlled trial assessing the genistein role on endothelial function in postmenopausal women.[28] The study subjects and procedures are described in detail there[28] and are summarized briefly here. All 60 women were healthy, 52 to 60 years of age, and with at least 12 months of menopause at baseline, were in good general health without any clinical or laboratory evidence of confounding systemic diseases. Other exclusion criteria were smoking habit of more than 10 cigarettes per day, previous treatment with oral or transdermal estrogen, progestin, androgen or other steroids, and cholesterol-lowering or cardiovascular medications.

Treatments

After a 4-week stabilization on the standard fat-reduced diet, participants to the study were randomly assigned to receive the phytoestrogen genistein (n = 30; 54 mg/d, Lab Plants Messina, Italy) or placebo (n = 30) for 6 months.

Assays

At baseline and after 6-month treatment, we measured fasting glucose, insulin, osteoprotegerin (OPG), follicle-stimulating hormone (FSH),[28] luteinizing (LH), and sex hormone-binding globulin (SHBG) on serum samples. Fibrinogen, platelet (PLT) count, and fasting glucose were evaluated immediately after the venous blood drawing.

Fibrinogen and PLT were determined by automated routine procedures. LH was measured by a chemiluminescent immunoassay (Medical System SpA, Genova, Italy).

SHBG was measured by an immunoradiometric assay (RADIM SPA, Rome, Italy) (intra-assay CV 4%; interassay CV 5%; lower detection limit, 2.5 nmol/L).

OPG was measured by a commercially available ELISA kit according to the protocol of the manufacturer (Immunodiagnostik Bensheim Germany). This assay detects monomeric, dimeric, and ligand-bound forms of OPG (intra-assay CV 5%; interassay CV 6%; lower detection limit, 0.14 pmol/L).

Insulin was measured by a commercially available ELISA kit according to the protocol of the manufacturer (DRG Diagnostik, Frauenberg Germany) (intra-assay CV 4%; interassay CV 6%; lower detection limit, <1.5 μIU/mL).

Serum glucose was measured by an enzymatic kit (BioSystemSA, Barcelona, Spain), (intra-assay CV 1%; interassay CV 1.8%; lower detection limit, <0.0126 mmol/L). Glucose in the sample produces, by means of the coupled reactions, a colored complex that can be spectrophotometrically measured.

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The insulin resistance was calculated using the Homeostasis Model Assessment method (HOMA-IR = (insulin × glucose)/22.5).[29]

To evaluate genistein plasma levels, blood samples (0.5 mL) were collected in polypropylene tubes containing 50 μL of heparin (50,000 IU) and after centrifugation at 3,000*g* at 4°C for 10 minutes, each sample was stored at −70°C until analysis. The assay was performed by using an HPLC method with UV detection with some modifications.[28] The concentration of plasma genistein was expressed in μmol/L.

Statistics

Data are given as mean ± SEM. The significance of difference was assessed by analysis of variance. A value of *P* less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristics

Our published results[28] indicated that no statistically significant difference was observed in the baseline clinical characteristics of the two groups. All the groups had a similar age and body mass index (placebo BMI = 24 ± 0.43; genistein BMI = 23 ± 0.51).

No single subject was less than 1.3 years postmenopausal when treatment was started. Similar trends were seen also when groups were subdivided according to smoking status and history of coronary artery disease.

Serum hormone concentration and sex hormone-binding protein

Table 1 shows that at baseline there was no statistically significant difference in the levels of estradiol, genistein, FSH, LH, and SHBG. Genistein levels increased only in the women treated with genistein, whereas estradiol concentrations did not change in the two groups (Fig. 1). By comparison with placebo, serum LH and FSH were lower (genistein LH = 28 ± 2 IU/L, $P < 0.05$ vs placebo LH 33 ± 1.09 IU/L; genistein FSH = 81 ± 1.94 IU/L, $P < 0.001$ vs placebo LH = 98 ± 1.29 IU/L) after 6 months of genistein treatment (Table 2). When compared with placebo, serum SHBG was higher in the genistein group (genistein 63 ± 3.8 nmol/L, $P < 0.05$ vs placebo, 53 ± 2.9 nmol/L) (Table 2).

TABLE 1. *Basal biochemical parameters in the two groups of postmenopausal women*

	Placebo (n = 30)	Genistein (n = 30)
Age (y)	57 ± 1.09	54 ± 1.28
BMI	24 ± 0.43	23 ± 0.51
Fasting glucose (mmol/L)	5 ± 0.12	4.74 ± 0.11
Fasting insulin (μIU/mL)	6.6 ± 0.85	7 ± 0.55
HOMA-IR	1.45 ± 0.20	1.47 ± 0.12
Fibrinogen (g/L)	3.7 ± 0.05	3.6 ± 0.12
PLT (mmc)	232,333 ± 15,954	228,286 ± 9,844
LH (IU/L)	28 ± 1.19	31 ± 4.02
FSH (IU/L)	94 ± 2.37	88 ± 2.01
Estradiol (pmol/L)	71 ± 2.37	73 ± 2.19
Genistein (μmol/L)	0.06 ± 0.002	0.07 ± 0.004
SHBG (nmol/L)	75 ± 2.92	71 ± 4.2
OPG (pmol/L)	4.98 ± 0.16	4.67 ± 0.13

Values are mean ±SEM.

Body mass index, blood glucose, insulin, and insulin resistance

Table 1 shows that at baseline there was no statistically significant difference in the levels of serum fasting glucose, insulin, and insulin resistance (HOMA-IR). BMI did not change after a 6-month treatment in the two groups.

Genistein treatment decreased the levels of fasting glucose (−8.7 ± 2.3%, $P < 0.001$ vs placebo, 3.2 ± 2.3%), fasting insulin (−12 ± 3.33%, $P < 0.001$ vs placebo, 36 ± 3.29%), and HOMA-IR (−14 ± 5.8%, $P < 0.001$ vs placebo, 42 ± 0.6%) (Fig. 2).

Fibrinogen and platelet count

No significant difference was observed between placebo and genistein groups for fibrinogen and platelet count at baseline (Table 1). In the genistein group, fibrinogen concentration was decreased (3.18 ± 0.12 g/L, $P < 0.001$ vs placebo, 3.83 ± 0.04 g/L) with respect to placebo after 6-month treatment (Table 2). Platelet count did not modify after genistein and placebo administration.

Serum osteoprotegerin levels

Table 1 shows basal serum OPG levels. No statistically significant difference was observed between the groups. By comparison with placebo, serum OPG was lower in the genistein group (−2 ± 0.3%, $P < 0.001$ vs placebo, 9 ± 1.5%) (Figure 2).

DISCUSSION

Epidemiological observations have pointed out lower insulin levels in Japanese people compared with Japanese-Americans,[30-33] and an improvement in insulin concentration and resistance in healthy and diabetic patients taking soy supplement.[34-36]

Jayacopal et al[34] showed an 8% decrease in fasting insulin and a 6.47% reduction in insulin resistance in postmenopausal women with type 2 diabetes after administration of 300 mg of isolated soy protein with 132 mg of isoflavones for 12 weeks.

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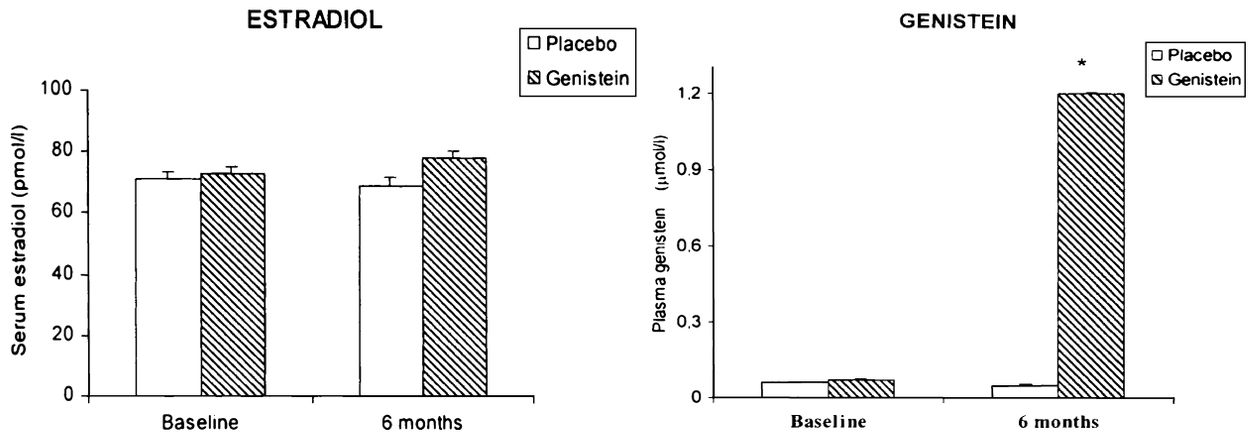

FIG. 1. Mean (± SEM) genistein and estradiol concentrations at baseline and after 6 months of placebo and genistein treatment (*, $P < 0.05$ vs placebo).

TABLE 2. *Biochemical parameters in the two groups of postmenopausal women after 6 months of treatment*

	Placebo (n = 30)	Genistein (n = 30)
Fasting glucose (mmol/L)	5.3 ± 0.19	4.3 ± 0.10[a]
Fasting insulin (µIU/mL)	8.23 ± 0.71	6.24 ± 0.45[a]
HOMA-IR	2 ± 0.21	1.18 ± 0.08[a]
Fibrinogen (g/L)	3.83 ± 0.04	3.18 ± 0.12[a]
PLT (mmc)	235,100 ± 7,981	244,428 ± 13,304
LH (IU/L)	33 ± 1.09	28 ± 2.0[a]
FSH (IU/L)	98 ± 1.29	81 ± 1.94[a]
SHBG (nmol/L)	53 ± 2.92	63 ± 3.83[a]
OPG (pmol/L)	5.5 ± 0.13	4.4 ± 0.11[a]

Values are mean ± SEM; [a]$P < 0.05$ vs placebo.

Furthermore, soy protein significantly decreased serum insulin in healthy postmenopausal women,[35] but no effect was observed in healthy premenopausal women,[37] thus suggesting that some soy-induced metabolic effects might be influenced by the menopausal status.

However, in the above-mentioned studies, it is hard to dissect out which one of the two major soy components (protein or isoflavones) is primarily responsible for the benefits. In addition it is difficult to identify the role of the specific isoflavone molecule eventually involved in the therapeutic effect.

As far as isoflavone molecules are concerned, several mechanisms may be claimed to explain the beneficial effects on glucose metabolism: isoflavones can inhibit alphaglucosidase,[38] intestinal glucose uptake, and tyrosine kinase[39,40]: all these events may contribute to the positive metabolic effect. In vitro study has shown that a soybean phytochemical extract, containing the phytoestrogen isoflavones, genistein and daidzein, inhibits glucose uptake into rabbit intestinal brush border membrane vesicles in a dose-dependent manner.[39]

Several in vitro studies using the phytoestrogen genistein as a protein tyrosine kinase inhibitor have shown that this compound exerts multiple actions on insulin release from pancreatic islet cells.[40-45] For example, in cultured Langerhans islets, genistein has been shown to increase basal insulin secretion.[40] Furthermore, genistein inhibits islet tyrosine kinase activity and glucose- and sulfonylurea-stimulated insulin release without affecting glucose metabolism.[45] However, another study has reported that genistein inhibits glucose-stimulated insulin secretion.[43]

Alternatively, phytoestrogenic molecules may interact with estrogen receptors. Indeed, to confirm a crucial role of the estrogen receptor in the modulation of glucose metabolism, it has been demonstrated that a man with estrogen resistance, caused by a disruptive mutation in ERα gene, had impaired glucose tolerance and hyperinsulinemia.[46]

The data presented in our study indicate that daily administration of 54 mg of genistein appears effective to improve some cardiovascular risk factors. These results extend and clarify previous published data: the effect of a single phytoestrogen (genistein) has not been investigated so far. We observed lower fasting serum glucose, insulin, and HOMA-IR in patients taking genistein compared with placebo. This finding cannot be attributed to change in BMI because this parameter remained constant during the whole treatment period.

Human studies have also shown that lower SHBG levels are associated with higher insulin resistance[47] and coronary heart disease in women.[48] In our study, genistein-treated women had higher SHBG levels in comparison with placebo.

This finding may be due to an increase in SHBG production according to an in vitro study showing a

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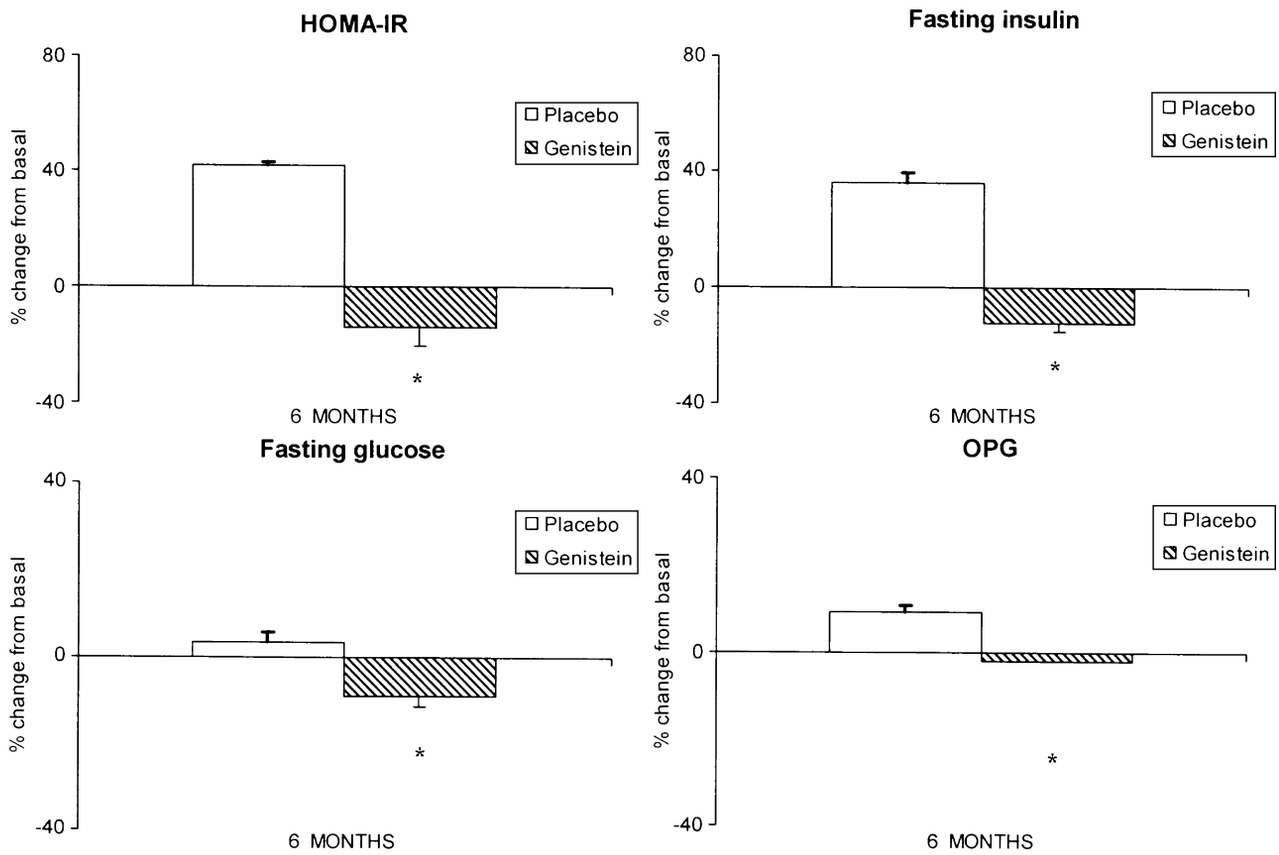

FIG. 2. Mean (± SEM) percent changes from baseline in fasting glucose, insulin, HOMA-IR, and osteoprotegerin (*, $P < 0.05$ vs placebo).

stimulating genistein effect on SHBG production in human hepatocarcinoma cells.[49,50] This latter result might be the consequence of direct estrogenic effects: in fact, estradiol increases liver SHBG in hepatocarcinoma cells.[50]

Alternatively, this effect might also be due to an indirect genistein action: the reduction in insulin concentration and resistance induced by genistein might account for the lack of a negative mechanism on SHBG production. In fact, insulin has been shown to have an inhibitory effect on SHBG secretion by HEP G2 cells.[51,52] Therefore, SHBG levels could be a marker of insulin resistance and/or hyperinsulism and consequently a cardiovascular risk marker.

The recent data on an association between osteoprotegerin, a member of the tumor necrosis factor receptor super families that inhibits osteoclastogenesis,[53] vascular diseases, and cardiovascular risk factor,[54,59] is very interesting. Two studies[54,55] showed the association between serum OPG levels with diabetes, presence of CAD, and cardiovascular mortality. Therefore, we measured serum OPG levels in our samples. In our study, genistein-treated women had lower OPG levels in comparison with placebo: this finding emphasizes that genistein might be cardioprotective. In fact, it has been observed that OPG increases in patients with greater severity of CAD,[55] perhaps as a compensative mechanism. In fact, osteoprotegerin inhibits vessel calcification and endothelial apoptosis.[56-59] In our active-treated patients, this compensative mechanism might be reduced as a consequence of an improvement in some cardiovascular risk factors. In addition, our published results[28] showed that genistein improves endothelial-dependent vasodilation: this might occur to keep from increasing the osteoprotegerin system. In contrast, this compensative mechanism is operative and active in the placebo group, in an effort to reduce the proatherosclerosis tendency of postmenopausal women.

Fibrinogen is an important risk factor for CVD[60-63] and increases in women after menopause.[64]

In our study, we observed a decrease in fibrinogen after a 6-month therapy with genistein: this suggests that this treatment may have a high degree of cardioprotection; in fact, a reduction in 20 mg/dL of fibrinogen is associated with a 50% decrease in the incidence of myocardial infarction in young women in

the Framingham study.[65] Similar decreases in fibrinogen were found in healthy postmenopausal women taking both estrogen only and estrogen/progestogen forms of HT.[66-69]

CONCLUSION

In conclusion, the present data clearly show for the first time that the 54 mg/d of genistein possesses a therapeutic effect on both glycemic control and cardiovascular risk markers in postmenopausal women.

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