THE DIFFERENTIAL EFFECT OF THE PHYTOESTROGEN GENISTEIN ON CARDIOVASCULAR RISK FACTORS IN POSTMENOPAUSAL WOMEN: RELATIONSHIP WITH THE METABOLIC STATUS.

Short title: Genistein effects in postmenopausal women

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ABSTRACT

Context: The wide family of the phytoestrogens has become an alternative to the classical hormonal therapy in menopause, nevertheless some findings are still conflicting.

Objective: To examine the effect of genistein administration on metabolic parameters and vascular reactivity considering the basal endocrine status of the patients.

Design: Randomized placebo controlled study.

Setting: University Hospital.

Participants: 50 postmenopausal women.

Intervention(s): Thirty subjects (group A) were randomized to receive 54 mg/day of genistein while twenty subjects (group B) were treated with the placebo for 24 weeks. In the group A we distinguish two subgroups: 14 normoinsulinemic and 12 hyperinsulinemic patients.

Main Outcome Measure(s): Anthropometric measures, hormonal and lipid assays, OGTT with glycemic, insulin and C-peptide evaluation, indexes of insulin sensitivity and endothelial function, euglycemic hyperinsulinemic clamps were performed.

Results: The insulin basal values significantly decreased in group A, whereas the homeostasis model index of insulin sensitivity (HOMA-IR) and the fasting glucose levels significantly improved compared to placebo group. The genistein administration decreased fasting glucose and AUC-glucose levels in the normoinsulinemic patients after treatment. In the hyperinsulinemic patients a significant reduction in fasting insulin, fasting C-peptide and AUC-insulin levels as well as an increase in FHIE was shown. In these patients HDL cholesterol levels were significantly improved. The endothelium-dependent and independent dilatation improved in the treated group. Normoinsulinemic patients showed both a significantly enhanced flow mediated and nitrate mediated dilatation, while no significant changes were found in the hyperinsulinemic group.

Conclusions: The glycoinsulinemic metabolism and the endothelial function were significantly influenced by genistein. In particular normoinsulinemic patients showed an improvement in glycemic
and vascular reactivity indexes. Conversely, an improvement in the insulin sensitivity indexes was noted in hyperinsulinemic patients.
INTRODUCTION

Decreased ovarian function during menopause is associated with the widely described increase in cardiovascular diseases occurring in this period of a woman’s life (1). The cardiovascular risk is in part attributable to the altered lipid and glycemic metabolism as well as to the development of the chronic vascular dysfunction (2,3). Postmenopausal hormone therapy (HT) exerts some beneficial effects on the glycemic and lipid metabolism but many postmenopausal women either cannot or will not comply with a HT regimen (4,5).

In this regard phytoestrogens (PHs), a wide family of plant derived compounds, have become an alternative treatment. In fact the growing body of literature indicates that PH may exert both estrogenic and antiestrogenic effects on the metabolism, depending on their concentration and molecular characteristics (6). Genistein is one of the most studied PHs, arousing great interest at present. The relative potencies of the different PHs compared with E2 have been assessed using human cell culture bioassays and the relative potency of genistein in comparison with E2 is well established (7).

Some clinical trials have shown that soy supplementation is associated with a reduction in lipids and lipoproteins in hyper and non-hypercholesterolemic subjects (8) with an improvement in lipid peroxidation biomarkers (9) as well as in vascular reactivity (10). However, other recent works show soy supplementation to have little effect on the lipid and glycemic metabolism (11). Thus, despite these conflicting findings, the effects of genistein on vascular reactivity seem to be relevant (12) while the relationship of this PH to the glycemic metabolism might be examined.

Many studies suggest that soy has beneficial effects on diabetes mellitus and obesity. In fact, the supplementation of soy protein supplements seems to determine a reduced insulin response to the oral glucose test challenge in hyperlipidemic subjects (13), while soy consumption increases the SHBG levels (14) and is inversely associated with impaired glucose tolerance. Jayagopal et al. reported that PH supplementation significantly lowers the mean values for fasting insulin and insulin resistance in postmenopausal type 2 diabetic patients (15). Recently therefore, more it has been demonstrated that
genistein supplementation improves insulin and glucose metabolism in healthy postmenopausal subjects (16).

In order to analyse thoroughly how genistein administration affects cardiovascular risk markers we investigated the metabolism of glucose and lipids as well as vascular reactivity in postmenopausal subjects with differing metabolic status.

MATERIALS AND METHODS

Fifty postmenopausal women (aged 53.91 ± 3.94 yr) who had attended the Gynecologic Department of our University for the treatment of menopausal symptoms, were consecutively enrolled in the study protocol. No subjects had taken drugs which might have affected the lipid or glucose metabolism for at least three months before the enrolment or had had diseases known to affect the lipoprotein and the glucose metabolism.

They were 3.9 ± 0.8 yr postmenopausal. Before the beginning of the study an assessment of the plasma follicular stimulating hormone (FSH) and 17-β estradiol ($E_2$) concentration, a mammography, a cervical cytology and a transvaginal ultrasound examination of the ovaries as well as of the endometrial thickness were performed. These parameters were found to be normal and compatible with the menopausal status. Diabetes, breast cancer, liver or kidney parameter alterations, history of major thromboembolism, thyroid diseases, and uncontrolled or treated hypertension (systolic blood pressure >160 mmHg or diastolic >90 mm Hg) represented exclusion criteria.

Informed consent was obtained from each patient, and the study protocol was approved by our Institutional Review Board.

The body mass index (BMI) was evaluated according to the ratio of weight (kilograms) to height (square meters). To determine of the waist to hip ratio (WHR), the waist circumference was used as the minimum value between the iliac crest and the lateral costal margin, whereas the hip circumference was determined as the maximum value over the buttocks.

The randomization of the subjects was carried out by computer set-up system. The ratio of 2/3 was chosen in order to have as few subjects as possible experiencing menopausal symptoms while excluded
from therapy for the 6 months study duration. Patients were therefore assigned to one of two treatment
groups: group A (thirty subjects) underwent a therapy with the oral dose of 54 mg/day (three
tablets/day) of genistein, while group B (twenty subjects) was treated with a placebo. The active drug
and the placebo were similar in appearance and both were administered for at least 24 weeks.
At baseline patients underwent initial hospitalization. Following a standard carbohydrate diet (300 g/d)
for 3 days and an overnight fast for 10–12 h, all patients had blood samples for the hormone assessment
(testosterone, dehydroepiandrosterone sulfate, androstenedione, 17-hydroxyprogesterone, progesterone,
FSH, LH, SHBG, E2) the serum lipid assay [triglycerides, total cholesterol, high and low density
lipoproteins (HDL and LDL), very low density lipoprotein (VLDL)], the complete blood count and
hepatic and renal markers. On the following day, the patients had an oral glucose tolerance test (OGTT)
while the day after the insulin sensitivity was tested with an euglycemic-hyperinsulinemic clamp.
Oral glucose tolerance test data were analyzed as AUC after glucose ingestion, calculated by the
trapezoidal rule, and expressed as µIU/mL × 240 minutes for insulin, as ng/mL × 240 minutes for C-
peptide, and as mg/dL × 240 minutes for glucose. The fractional hepatic insulin extraction (FHIE) was
calculated by the difference between the incremental AUC-C-peptide (AUC-Cpep) and AUC-insulin
(AUC-I) divided by the incremental AUC-Cpep. A normal glycemic response to OGTT was defined
according to the criteria of the American Diabetes Association (17).
According to AUC-I values patients were classified as normoinsulinemic and hyperinsulinemic
patients, assuming an AUC-I cut-off value of 10,000 µIU/mL × 240 minutes chosen from arbitrary
initial cut-off values examined by using ROC graphs and tested for sensibility and specificity. These
values were obtained from about 100 OGTTs that were performed in our postmenopausal population and
the 10,000 µIU/mL × 240 minutes threshold showed an optimal predictive performance compared with
several other indexes of insulin resistance (18).
The hyperinsulinemic-euglycemic clamp was performed after a 10-hour overnight fast to estimate
peripheral insulin sensitivity. At 8:00 am, an IV catheter was placed in the antecubital vein for the
infusion of glucose and insulin. Another catheter was placed in the dorsal vein of the contralateral hand
for blood withdrawal which was warmed to 65°C with a warming box. A primed constant infusion of insulin was given (Actrapid HM, 40 mIU/m² per min; Novo Nordisk, Copenhagen, Denmark) (19). After achieving steady-state insulin levels of 100 µIU/mL within 10 minutes during the clamp (range, 80–125 µIU/mL), a variable infusion of 20% glucose was begun via a separate infusion pump, and the rate was adjusted, on the basis of plasma glucose samples drawn every 5 minutes, to maintain plasma glucose between 79.3 mg/dL and 89.9 mg/dL. The plasma glucose level was determined by using the glucose oxidase technique with a glucose analyzer (Beckman Instruments, Palo Alto, CA). The glucose infusion rate during the last 60 minutes of a 2-hour infusion then was taken as the estimate of peripheral insulin sensitivity and was measured as M (mg/kg per min) (20). The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: fasting glucose (mg/dl)x fasting insulin (µIU/ml) / 22.5, while the prediction of the pancreatic β-cells function was measured by the HOMA-B index : fasting insulin (µIU/ml) x 20/ [fasting glucose (mg/dl)-3.5 ] (21,22).

Each subject was asked to avoid making any changes in her lifestyle or dietary habits during the study. After three months, patients underwent a medical examination and a transvaginal ultrasonography only while a second hospitalization was performed six months later and the basal study was repeated.

OGTT blood samples were obtained basally and 30, 60, 90, 120, 180, and 240 minutes after the ingestion of 75 g of glucose. In all samples the glucose, insulin and C-peptide (C-pep) plasma levels were determined. For each interpretation, all samples from the same patient were assayed simultaneously. Samples for the measurement of basal and stimulated insulin and C-peptide, together with other hormones, were centrifuged promptly and the plasma stored at – 20°C until required for assay.

All hormones were measured by commercial RIA kits (Radim, Rome, Italy). The intra- and interassay coefficients of variation were less than 8% and 15% for all hormones, respectively. Total cholesterol and triglyceride concentrations were determined by an enzymatic assay (Bristol, Paris, France). HDL concentrations were determined after precipitation of chylomicrons, VLDL, and LDL (Roche, Mannheim, Germany), and VLDL were separated (as the supernatant) from LDL and HDL by
lipoprotein ultracentrifugation. A magnesium chloride/phosphotungstic acid technique was used to precipitate LDL from the bottom fraction after ultracentrifugation. All lipid assays were performed according to our standard laboratory procedures.

Ultrasound assessment of endothelial-dependent and independent vasodilation of the brachial artery

The method of evaluating the endothelial function consists of measuring the variation of vascular flow in response to local or systemic stimuli. The evaluation time was between 8.30 and 10.00 h, after overnight fasting. The subjects rested supine in a quiet, air conditioned room for 30 minutes before the endothelial function was assessed. The endothelial function was evaluated by the flow mediated dilatation (FMD) of the brachial artery in response to hyperemia of the arm, a nitric oxide (NO) mediated process. The FMD was measured in accordance with the Coretti’s guidelines (23). The Eco-Doppler ultrasound imaging of the right brachial artery was carried out with a 7.5 MHz linear-arrayed transducer and a standard Esaote AU5 Harmonic system (Ansaldo, Milan, Italy).

The vessel diameter was measured at the end of diastole, corresponding with the R wave on the ECG, at a fixed distance from an anatomical marker. In order to measure the FMD, the images were obtained at baseline and after 90 seconds from the deflation of a wrist cuff inflated to suprasystolic pressure (to at least 50 mmHg over the systolic blood pressure) for 5 minutes. The images to measure the nitrate mediated (endothelium-independent) dilatation (NMD) were acquired 4 minutes after the administration of sublingual glycerine trinitrate (0.3 mg). The FMD and NMD were calculated as the percentage increase in the arterial diameter during hyperemia compared with the diameter at rest.

Statistical Analysis

Data were stored and analyzed using SPSS software (statistical package for social science, release 6.0, SPSS, Inc., Chicago, IL) on an IBM-compatible computer. Distribution of the data was tested by the Kolmogorov-Smirnov test to verify whether the samples followed a normal distribution. The absolute differences between the two groups were compared using the Student’s t test while the non parametric tests were applied to biochemical data that violated the assumption of normality. The estimated sample size for a two sided t-test with a 5% alpha error and with an 80% of power was equal to 12 subjects per
group. A two-tailed $P < 0.05$ was considered statistically significant. All data are presented as mean ± SD.

RESULTS

Three patients from the placebo group A, dropped out because of the menopausal neurovegetative symptoms and two patients decided to withdraw. In the genistein treated group B, one patient withdrew due to another health problem and one patient was lost for unknown reason. Two more patients who completed the follow up, refused to undergo the hyperinsulinemic euglycemic clamps in the second hospitalization; therefore the data of the patients were removed from the table 2.

Table 1 shows the clinical and hormonal characteristics of the group A and B before and after the 24-week treatment. The studied groups did not differ statistically in age, BMI, or menopausal status and hormonal assays at baseline. No significant difference between the two groups in terms of BMI and body fat distribution was observed after treatment. No significant changes in FSH, LH, E2, T and A levels after 6 months of therapy were found in either group. Comparing the two groups in terms of the variation after the treatment ($\Delta$), the SHBG values significantly increased in group A after treatment ($P<0.05$). The insulin basal levels significantly decreased ($P<0.02$) in group A and the delta values were significantly different from group B ($P<0.05$). A trend towards a decrease in basal glucose levels was noted in group A while there was a significant difference in delta values between the two groups ($P<0.02$).

The HOMA-IR index of insulin sensitivity significantly improved in group A ($P<0.02$) and the difference in delta values between the two groups was confirmed ($P<0.01$). On the contrary the $M$ values, arising from a normal range in all the groups, tended to improve in group A without reaching a significant statistical level.

To better evaluate the effect of genistein administration on the glyco-insulinemic metabolism, we categorized the treated population into a hyperinsulinemic group (H), 14 patients, and a normoinsulinemic group (N), 12 patients, revealing some differences between the two groups as shown in table 2.
Table 2 illustrates the glycoinsulinemic metabolism and the lipid profile in these two subgroups. At baseline, BMI (P<0.05), fasting insulin and the AUC-I levels were significantly different between the two groups (both with P<0.005), as expected. Hyperinsulinemic group showed also basal C-Pep and stimulated AUC-pep values higher than normoinsulinemic patients as well as a higher HOMA-IR and HOMA-B index (P<0.005). The two groups didn’t differ in basal total cholesterol, triglycerides and LDL-chol levels while HDL-chol levels were lower in hyperinsulinemic patients (P<0.005).

In the normoinsulinemic patients, a significant reduction in both fasting glucose and AUC-glucose plasma levels (P<0.05 and P<0.01 respectively) was observed after genistein treatment even if the delta values didn’t differ significantly in comparison whit the hyperinsulinemic patients. No changes in the fasting insulin and C-pep levels were noticed and no relevant differences were found both in the insulin response to OGTT and in the lipid parameters. Conversely, in the H group a 6-month course of genistein treatment could reduce both the fasting insulin and the AUC-I levels (P<0.05) even in terms of delta values (P<0.01). In these patients the C-pep values significantly decreased and the FHIE values increased after therapy. The AUC-Cpep and the AUC-glucose didn’t show any consistent change. HOMA-IR significantly improved in hyperinsulinemic patients (P<0.05) as well as the HOMA-B indicated a reduced pancreatic activity. However the peripheral insulin glucose utilization index (M) showed a trend towards improvement in normoinsulinemic patients only, although without showing any statistical significance.

As concerns the lipid profile, the normoinsulinemic group didn’t reveal any significant variation in the post-treatment assessment, while the hyperinsulinemic women showed a statistically significant increase in HDL cholesterol (P<0.05).

In the placebo group nine patients were normoinsulinemic and six patients were hyperinsulinemic, any statistical difference was observed after treatment (data not shown).

**Endothelial function**

In group A we observed a significant increase both in the FMD values (12.9±9 before therapy vs 22.8±18 after therapy, P<0.05) and in the NMD values (42.4±22 before therapy vs 143.5±90 after
therapy, P<0.001) while we failed to find any significant variation both in FMD and in NMD in the placebo group. On the other hand substantial differences were found when comparing the normo and the hyperinsulinemic subgroups. After the 24-week therapy, the normoinsulinemic women showed an evident enhanced endothelial response to stimuli both in FMD and in NMD (P<0.02 and P<0.001 respectively) (Figure 1). In the hyperinsulinemic patients no significant differences in endothelial function were found after genistein treatment.

DISCUSSION

Recent studies have pointed out some beneficial effects of soy protein supplementation on several cardiovascular risk factors (24,25). Nevertheless during the last few years there have been many conflicting reports. Our data partially confirm the recent results on the glycemic metabolism and on vascular reactivity in postmenopausal women and show that the individual metabolic status influences the kind of response that genistein administration achieves.

In the present study, we found that the 6-month genistein treatment significantly improves the glycemic metabolism and endothelial function in postmenopausal women compared to the placebo group. Two previous studies showed either only modest estrogen-like effects of soy supplementation on insulin levels (26) or no effects on lipids or on insulin sensitivity in non-diabetic postmenopausal women who had taken 114mg/d of isolated isoflavones, respectively (27). On the other hand, more recently, Crisafulli et al. have reported that genistein administration for 6 months significantly lowers fasting insulin and glucose levels as well as improves HOMA-IR in healthy postmenopausal women (12). Furthermore, a following study showed the significantly positive effect of 54mg/d of genistein for 24 months on the glycemic and insulin metabolism when compared with the placebo group (16). Our results on treated patients are consistent with these findings and further support the beneficial effects of genistein on glycemic metabolism, although our population had a higher BMI.

In our study, we found different responses to the therapy in according to the patients’ normoinsulinemic and hyperinsulinemic status. The glycoinsulinemic metabolism of our patients was studied in detail by analysing of the response to glucose load, and assessing the AUC-I as an index of hyperinsulinemia as
well as by the evaluation of pancreatic insulin secretion (C-pep levels) and of hepatic insulin clearance (FHIE values). Therefore the indexes of insulin resistance were measured not only by the homeostasis model but also by the euglycemic hyperinsulinemic clamp technique, which is still considered as the gold standard for the determination of total body insulin action.

The subgroup of normoinsulinemic patients showed significant decrease in basal glucose levels as well as a reduction in AUC-glucose after genistein treatment. No change in the basal or stimulated insulin levels has been found in these patients, while the direct utilization of glucose by peripheral tissue (indicated by M index) tended to increase. The basal glucose levels reduction may be partially explained by an increased peripheral utilization but other mechanisms might also be involved. In fact, some studies have shown that isoflavones can inhibit the intestinal brush border uptake of glucose (28) by blocking intestinal glucose absorption. Thus isoflavones may directly interfere with the activity of some enzymes involved in glycemic homeostasis such as alpha-glucosidase and tyrosine-kinase (29,30).

On the other hand, the antidiabetic properties of PHs have also been revealed “in vivo” both in diabetic and obese patients even if the precise molecular mechanisms by which PHs exert their beneficial effect on diabetes and obesity are still unclear. Goodman-Gruen et al. observed a significant reduction in fasting insulin in women consuming high isoflavones in their diet (31). Furthermore, most recently, Jayagopal et al demonstrated that dietary supplementation with high PHs doses reduced fasting insulin and HOMA-IR in post menopausal women with diet-controlled type-2 diabetes (15).

Our data on hyperinsulinemic patients, while showing a significant reduction of basal insulin levels and of AUC-I, did not show any increase of glucose levels. The insulin resistance measured by HOMA-IR significantly improved even though the M index didn’t change significantly. In these patients the positive effect on insulin metabolism may be explained by the influence of genistein on the hepatic metabolism as indicated by the FHIE. Moreover genistein influences the pancreatic insulin secretion as suggested by the significant decrease of the C-peptide and AUC-Cpep levels as well as by the HOMA-B index. Thus, in a patient with a persistent hyperinsulinemia and a higher pancreatic activation compared with a normoinsulinemic one, the genistein may improve the insulin levels by acting on the hepatic
insulin clearance and by reducing the β-cells activation and consequently affecting the circulating insulin levels.

The discrepancies regarding M and HOMA-IR values have been reported in other studies (32) and could be due to different sensitivity of the two parameters or to the limited number of our cases. Nevertheless, in our population, the M index is the truest expression of the peripheral glucose utilization and tends towards an improvement arising from normal values, while HOMA-IR is directly drawn from the insulin and glucose circulating levels and provides a reasonable approximation of insulin sensitivity (33). Our data also show a significant increase in SHBG levels after treatment in the whole population, particularly evident in hyperinsulinemic patients. These findings agree with an in vitro study showing a stimulating genistein effect on SHBG production in human hepatocarcinoma cells (34,35) as well as with Aubertin-Leheudre’s and Pino’s in vivo studies (36, 14). Higher levels of SHBG may contribute to insulin metabolism restoration as well as being a marker of an improved metabolism or consequence of a direct estrogenic-like effect on the hepatocytes.

The benefits of PHs on the lipid metabolism in healthy postmenopausal women are still a controversial matter. Several studies suggested different effects on the lipid panel probably in relation to different doses and types of isoflavone supplementation. More recently a European meta-analysis (37) and the statements from the American Heart Association (38) have asserted the lack of efficacy of PHs on lipid levels.

According to previous observations (11) our work, while not observing any change in lipid markers in the whole population, indicated a significant increase of HDL in the hyperinsulinemic group and a trend toward a reduction in LDL-col levels.

Some recent studies have demonstrated that PHs may improve endothelial function (10, 25, 39, 40).

Genistein acts through a receptor pathway having a high affinity with the beta estrogen receptor in the vessel wall, as well as a relative affinity to the alpha-estrogen receptor (41). It has also been demonstrated that genistein shows antioxidant properties thorough the direct stimulation of endothelial
nitric oxide synthesis and the inhibition of the tyrosine kinase protein (41) while genistein induces an endothelium-independent relaxation without involving the endothelium or the eNOS pathway (42).

As reported in previous randomized clinical trial, although genistein therapy improved the endothelium-dependent vasodilatation, it did not change the endothelial independent vasodilatation because of the limited number of patients tested (10). The positive influence of isoflavones on endothelium-dependent vasodilatation was also demonstrated in healthy postmenopausal women by Colacurci et al. but no effect on the endothelium-independent mechanisms was observed even if a significant increase of basal brachial artery diameter after therapy was shown (24).

In the present study, we have shown a significant improvement both in dependent and independent endothelium vasodilatation in normoinsulinemic patients. These findings agree both with the data demonstrating the improvement of FMD as well as with Hallund’s study concerning the endothelium independent vasodilatation (43). Conversely patients with insulinemic imbalance lost the beneficial effects on the endothelium function. This may depend on the pre-existing endothelial dysfunction as it appears even in the early phases of type-2 diabetes (44).

In conclusion many discrepancies among studies on PHs may be due to differing effective doses and different types of isoflavones, as well as the variations in experimental conditions and in the model used. Moreover the bulk of recent works as well as our data seem to point out some beneficial effects on glycemic metabolism and on the vascular function. Our study has added the evidence that the pre-existent metabolic status of any single patient may determine different effects in response to isoflavone therapy.
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Figure Legend:

Figure 1: Brachial artery flow evaluation: percent increase after flow mediated dilatation (FMD) and non-mediated flow dilatation (NMD), at baseline and after the 24-week therapy.

Dark columns: hyperinsulinemic patients; empty columns: normoinsulinemic patients.

In the frame: significance pre-treatment vs post-treatment values of NMD and FMD in normoinsulinemic women (P<0.02 and P<0.001 respectively).

Significance: * P<0.001 normoinsulinemic vs hyperinsulinemic; ** P<0.01 normoinsulinemic vs hyperinsulinemic.
**TABLE 1**: Clinical and endocrine features of studied population before and after soy isoflavones (A) and placebo (B) treatment.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
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<tbody>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>Post-treatment</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>54.29 ± 4.18</td>
<td>53.54 ± 3.70</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>27.53 ± 4.28</td>
<td>27.22 ± 3.33</td>
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<tr>
<td><strong>WHR</strong></td>
<td>0.81 ± 0.06</td>
<td>0.82 ± 0.05</td>
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<tr>
<td><strong>Estradiol (pg/ml)</strong></td>
<td>15.59 ± 6.4</td>
<td>14.6 ± 15.7</td>
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<tr>
<td><strong>Testosterone (ng/ml)</strong></td>
<td>0.27 ± 0.17</td>
<td>0.22 ± 0.14</td>
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<tr>
<td><strong>FSH (IU/l)</strong></td>
<td>75.54 ± 27.41</td>
<td>78.42 ± 31.71</td>
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<tr>
<td><strong>LH (IU/l)</strong></td>
<td>38.55 ± 15.90</td>
<td>38.12 ± 14.48</td>
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<tr>
<td><strong>SHBG (nmol/l)</strong></td>
<td>42.62 ± 10.18</td>
<td>47.75 ± 10.57</td>
</tr>
<tr>
<td><strong>Fasting insulin (µUI/ml)</strong></td>
<td>10.17 ± 5.78</td>
<td>7.24 ± 2.93 ^*</td>
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<tr>
<td><strong>Fasting glucose (mg/dl)</strong></td>
<td>90.47 ± 10.34</td>
<td>88.71 ± 11.60</td>
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<tr>
<td><strong>HOMA-IR</strong></td>
<td>2.22 ± 1.06</td>
<td>1.6 ± 0.72 ^a</td>
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<tr>
<td><strong>M</strong></td>
<td>6.1 ± 3.8</td>
<td>6.2 ± 2.9</td>
</tr>
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</table>

Data are expressed as mean ± SEM; Δ: absolute difference between post-treatment vs pre-treatment. Conversion factor to SI Units: Insulin: 7.175 to pmol/l; Glucose 0.05551 to mmol/l; FSH and LH: 1 to IU/l; Testosteron: 3.467 to nmol/l; Estradiol: 3.671 to pmol/l.

a: P<0.02 in Group A post-treatment vs pre-treatment; b: P<0.02 Δ values group A vs group B; c: P<0.01 Δ values group A vs group B

d: P<0.05 Δ values group A vs group B.
**TABLE 2:** Lipidic and glicoinsulinemic profile of normoinsulinemic and iperinsulinemic subjects before and after genistein and placebo treatment.

<table>
<thead>
<tr>
<th></th>
<th>NORMOINSULINEMIC</th>
<th></th>
<th>HYPERINSULINEMIC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>Post-treatment</td>
<td>Δ</td>
<td>Pre-treatment</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>27.07 ± 4.22</td>
<td>25.94 ± 3.18</td>
<td>-1.13 ± 1.82</td>
<td>30.6 ± 3.6²</td>
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<td><strong>Fasting Glucose (mg/dl)</strong></td>
<td>89.5 ± 7.5</td>
<td>84.7 ± 9.7³</td>
<td>-4.8 ± 7</td>
<td>95.1 ± 11</td>
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<tr>
<td><strong>Fasting Insulin (μUI/ml)</strong></td>
<td>6.28 ± 3</td>
<td>6.58 ± 2.4</td>
<td>0.3 ± 3</td>
<td>12.8 ± 6³</td>
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<tr>
<td><strong>Fasting C-pep (ng/ml)</strong></td>
<td>1.7 ± 0.7</td>
<td>1.6 ± 0.5</td>
<td>-0.1 ± 0.4</td>
<td>3 ± 1.1³</td>
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<tr>
<td><strong>AUC-Glucose (mg/dlx240’x10⁻³)</strong></td>
<td>25370 ± 3704</td>
<td>23281 ± 3995⁶</td>
<td>-1879 ±7815</td>
<td>29500 ± 6513</td>
</tr>
<tr>
<td><strong>AUC-Insulin (μUI/mlx240’x10⁻³)</strong></td>
<td>6804 ± 1915</td>
<td>7694 ± 2605</td>
<td>889 ± 3170</td>
<td>15893 ± 6314⁶</td>
</tr>
<tr>
<td><strong>AUC-C-pep (ng/mlx240’ x 10⁻³)</strong></td>
<td>1115 ± 209</td>
<td>1241 ± 425</td>
<td>125.9 ± 316</td>
<td>2016 ±71³</td>
</tr>
<tr>
<td><strong>FHIE (%)</strong></td>
<td>86.3 ± 4.6</td>
<td>85.9 ± 3.9</td>
<td>-9 ± 28</td>
<td>82.1 ± 5.2</td>
</tr>
<tr>
<td><strong>M (mgxKg1xmin⁻¹)</strong></td>
<td>6 ± 2.8</td>
<td>7.2 ± 2.2</td>
<td>1.71 ± 3.5</td>
<td>5.3 ± 1.5</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>1.05±0.6</td>
<td>1.68±0.64</td>
<td>0.56±1</td>
<td>3.02±1.8³</td>
</tr>
<tr>
<td><strong>HOMA-B</strong></td>
<td>74±28</td>
<td>126±74</td>
<td>46±78</td>
<td>139±47³</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td>210.4 ± 35.1</td>
<td>217.1 ± 46</td>
<td>6 ± 99</td>
<td>190 ± 43</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mg/dl)</strong></td>
<td>128.2 ± 23.8</td>
<td>137.4 ± 43</td>
<td>21.1 ± 89</td>
<td>125 ± 42</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mg/dl)</strong></td>
<td>56.5 ± 7.9</td>
<td>62 ± 13</td>
<td>4.5 ± 9</td>
<td>45.3 ± 6³</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>95.5 ± 33</td>
<td>88.1 ± 38</td>
<td>-4.4 ± 35</td>
<td>101 ± 32</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM; Δ: absolute difference between post-treatment vs pre-treatment. Conversion factor to SI Units: Insulin: 7.175 to pmol/l; Glucose 0.05551 to mmol/l; C-peptide: 331to pmol/l; Cholesterol.: 0.02586 to mmol/l; Triglycerides 0.01129 to mmol/l.
a: P<0.05 post-treatment vs pre-treatment; b: P<0.01 post-treatment vs pre-treatment; c: P<0.005 basal values hyperinsulinemic vs normoinsulinemic
d: P<0.05 delta values hyperinsulinemic vs normoinsulinemic; e: P<0.01 delta values hyperinsulinemic vs normoinsulinemic
**FIGURE 1**

*:* P<0.001 normoinsulinemic vs hyperinsulinemic; **P<0.01 normoinsulinemic vs hyperinsulinemic.