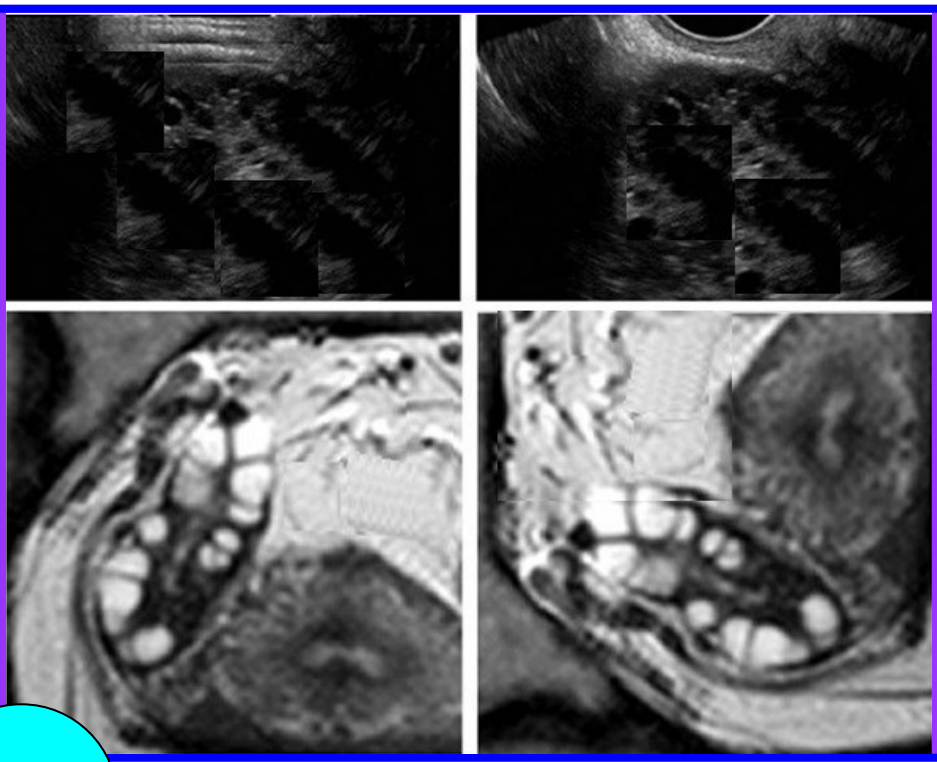

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Effect of glabridinon on insulin resistance, C-reactive protein and endothelial function in young women with polycystic ovary syndrome

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Abstract

In the present study 32 women with polycystic ovary syndrome were treated with glabridin (10 μM) daily for 12 months. The results revealed a significant reduction in serum testosterone from 95.3 ± 23.6 to 49.8 ± 12.7 ng/dL and fasting insulin concentrations from 12.3 ± 5.2 to 8.11 ± 3.42 U/mL after glabridin treatment. It also leads to a marked improvement in insulin resistance indices and reduction in hirsutism score from 12.8 ± 3.2 to 7.0 ± 3.5. More interestingly, all the women reverted to regular menstrual cycles. The sex hormone-binding globulin level increased significantly from 23.1 ± 5.6 to 52.5 ± 15.8 nmol/L after glabridin treatment. The levels of serum hsCRP decreased from 0.32 ± 0.08 to 0.06 ± 0.01 mg/dL and that of endothelium-dependent vascular responses from 17.2 ± 4.3 to 8.8 ± 2.4% after glabridin treatment. Therefore, glabridin acts as a potent candidate for the improvement of insulin sensitivity and androgen production.

Introduction

Polycystic ovary syndrome is characterized by two main features which are anovulation and hyperandrogenism. About 6% of the women of reproductive age suffer from this disease having resistance to insulin as well as hyperinsulinemia (Franks, 1995; Chang et al., 1983; Dunaif et al., 1989). Hyperinsulinemia leads to an increase in ovarian androgen production and decrease in serum sex hormone–binding globulin concentration (Nestler, 1994; Barbieri et al., 1986; Cara and Rosenfield, 1988). Inhibition of insulin secretion by diazoxide (Nestler et al., 1989) or metformin (Velazquez et al., 1994) or by diet (Kiddy et al., 1989; Kiddy et al., 1992) has been shown to decrease serum free testosterone concentrations. In adolescent girls, insulin resistance with hyperandrogenism suggests the role of hyperinsulinemia in polycystic ovary syndrome (Apter et al., 1995). Polycystic ovary syndrome women suffer from adverse coronary heart disease risk profiles at relatively young age, suggesting premature coronary atherosclerosis (Dahlgreen et al., 1992; Conway et al., 1992; Dahlgreen et al., 1992; Talbott et al., 1998). The endothelial cell dysfunction leads to atherosclerotic disease (Ross, 1999; Shimokawa, 1999; Verma and Anderson, 2002). For prediction of coronary heart disease the assessment of endothelial function is measured by flow-mediated dilatation (FMD) of the brachial artery.

Glycyrrhiza, a Chinese herb is used an expectorant for arresting cough, reducing fever and comfort the stomach (Tian et al., 2008; Sabbioni et al., 2005). Licorice prevents carcinogenesis induced by toxicants or hormones and also has a significant hepatoprotective activity (Mori et al., 2001; Yokozawa et al., 2005; Lee et al., 2009). From the extract of *Glycyrrhiza,* flavonoids and triterpenoids were isolated (Lee et al., 2009). Glabridin (Figure 1), a polyphenolic flavonoid exhibits multiple pharmacological activities, such as cytotoxic, antimicrobial, anti-fatigue, estrogenic and anti-proliferative activity against human breast cancer cells (Shang et al., 2009). It also affects melanogenesis, inflammation, low-density lipoprotein oxidation and

protection of mitochondrial functions from oxidative stress (Choi, 2005). Thus, the aim of this study was to investigate the hypoglycemic effects of glabridin from licorice in the patients with DM.

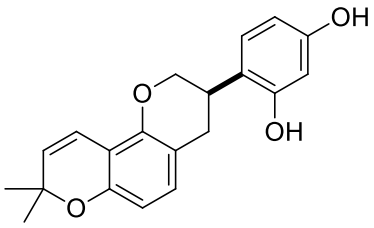

Figure 1: Structure of glabridin

Materials and Methods

Patients

Thirty two women (mean age, 26.5 ± 4.2 years) suffering of polycystic ovary syndrome were enrolled for the present study. The criteria used for the selection of patients include at least two of the following three features: oligo- or anovulation; clinical Ferriman-Gallwey score greater than 8 and/or biochemical signs of hyperandrogenism and polycystic ovaries. The biochemical criteria included abnormal luteinizing (LH):follicle-stimulating hormone (FSH) ratio (>2) and/or enhanced levels of testosterone. The ultrasound with 12 or more follicles per 2-9 mm in diameter ovary, and/or increased ovarian volume (>10 mL) indicated polycystic ovary syndrome. The mean cycle length in the patients was 55.4 ± 9.2 days. The thyroid, renal and hepatic functions in all the patients were normal. Patients with pregnancy, use of oral contraceptives in 6 months period before the study, anti-androgens, anti-diabetics and known cardiovascular disease were excluded from the study. We used dexamethazone suppression test and follicular phase serum 17-OH progesterone to determine Cushing's syndrome and late-onset congenital adrenal hyperplasia.

Clinical examinations and laboratory evaluations

For all the patients oral glucose tolerance test and transvaginal ultrasonography were performed 3 days after the initial dosage. The blood samples were collected and serum concentrations of FSH, LH, testosterone, prolactin and sex hormone-binding globulin were measured using chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA). The serum glucose levels were measured using glucokinase technique. The levels of total cholesterol, high- and low-density lipoprotein (HDL and LDL)-cholesterol and triglyceride were measured using commercial enzymatic methods (Aeroset automated analyzer, Abbott Diagnostics, IL, USA). Friedewald's formula was used to calculate LDL-cholesterol. The chemiluminescent enzyme immune-oassay (Immulite 1000 Analyser) and chemiluminescent enzyme immunoassay (Immulite 2000) were used for measuring plasma insulin levels and plasma hsCRP concentrations respectively. To determine insulin resistance various methods like fasting insulin, the homeostasis model assessment (HOMA) and the quantitative insulin sensitivity check index (QUICKI) were used. Insulin resistance was estimated using HOMA score.

Brachial artery responses to endothelium-dependent (FMD) and endothelium-independent stimuli were used to measure arterial endothelium and smooth muscle function with ultrasonography. B-mode ultrasound images for determination of brachial artery diameter were captured by a 7.5 MHz transducer (Toshiba Power Vision 8000). The arterial diameters were measured during rest, during reactive hyperemia (FMD), again at rest and after treatment with 0.4 mg sublingual NTG. The end-diastolic arterial diameter was measured from one to the other media-adventitia interface and the measurements were made in triplicate at baseline, every 20 s after reactive hyperemia and after administration of NTG. All of the women were treated with 10 μM glabridin daily. The patients were examined every month for 12 months and were then admitted to the clinical research center. The clinical examinations and laboratory evaluations were performed, the results obtained were then analysed and compared with baseline values.

Statistical analysis

For statistical analysis statistical package for the social sciences (SPSS version 11.5) was used. The results are expressed as mean ± S.D and the characteristics of distribution were examined using the Kolmogorov–Smirnof test. Student's unpaired *t*-test was used for the analysis of differences and the differences were considered statistically significant at $p<0.05$.

Results

All the patients received 10 μM glabridin daily for 12 months with reporting any adverse effect during the treatment period. None of the enrolled patients stopped taking treatment during study. No change was observed in serum transaminases in all the tested patients on treatment with glabridin. After 12 months of glabridin treatment all the thirty two patients reverted to the regular ovulatory cycles. The length of the cycles at the end of the treatment was 29.5 ± 5.0 days. We recorded the hormonal parameters and clinical characteristics for all the patients under the study (Table I). The representative ultrasound images of the patients prior to treatment were also recorded (Figure 2).

Table I

Patient characteristics and hormone studies prior to and after the glabridin treatment

	Before glabridin (n = 32)	Post-glabridin (n = 32)	P
BMI (kg/m²)	25.4 ± 5.3	26.0 ± 5.0	0.387
Waist circumference (cm)	80.6 ± 8.2	79.6 ± 8.3	0.418
Ferriman–Gallwey score	9.8 ± 1.8	9.2 ± 1.6	0.110
LH (IU/L)	9.3 ± 3.4	9.0 ± 3.6	0.524
FSH (IU/L)	4.7 ± 2.1	5.8 ± 2.5	0.502
Testosterone (ng/dL)	89.7 ± 31.7	49.5 ± 13.3	0.040
Sex hormone-binding globulin (nmol/L)	20.4 ± 7.2	52.4 ± 10.2	0.033
Total cholesterol (mg/dL)	170.2 ± 39.6	165.4 ± 38.4	0.549
Triglyceride (mg/dL)	130.7 ± 68.8	108.5 ± 58.4	0.202
HDL-cholesterol (mg/dL)	41.5 ± 6.2	52.5 ± 6.4	0.213
LDL-cholesterol (mg/dL)	96.3 ± 16.4	98.5 ± 21.7	0.213

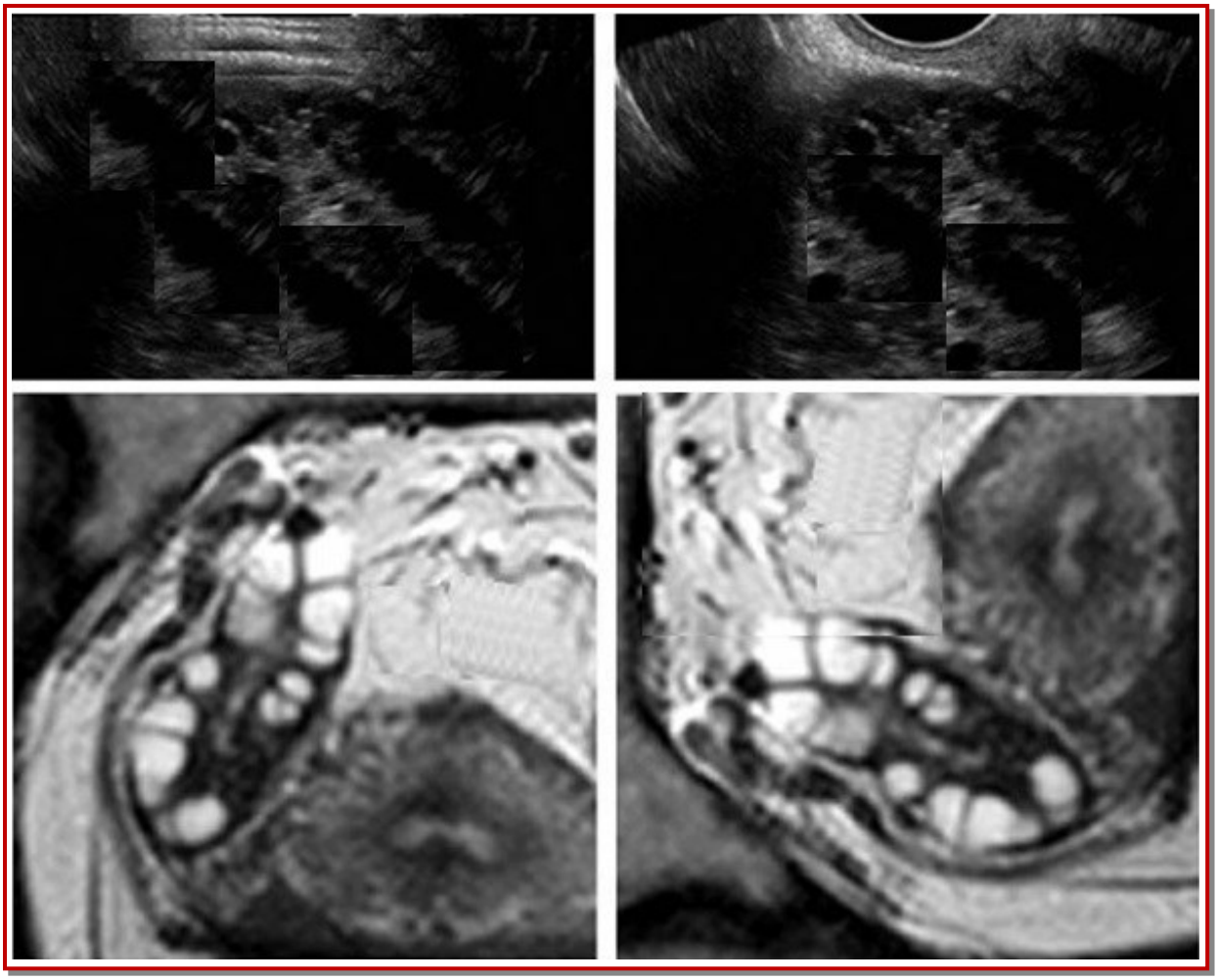

Figure 2: Ultrasonography of patients with polycystic ovary syndrome

Glabridin treatment for 12 months did not produce any significant change in body weight and the waist circumferences. Also the levels of total cholesterol, LDL, LH and FSH levels showed no significant change prior to and after the glabridin treatment. However, glabridin treatment for 12 months resulted in significant decrease in the hirsutism score. Hirsutism score decreased from a baseline value of 13.7 ± 3.1 to 5.6 ± 1.4 after 12 months (Figure 3). The levels of serum HDL were increased where as that of serum triglyceride decreased after 12 months but the changes were statistically insignificant ($p<0.05$). Glabridin treatment markedly decreased the levels of serum testosterone from 89.7 ± 31.7 to 49.5 ± 13.3 ng/dL (Figure 3). On the other hand, the levels of sex hormone-binding globulin were increased from 24.8 ± 9.5 to 49.1 ± 13.5 nmol/L. Glabridin treatment also caused a significant decrease in serum hsCRP levels after 12 months (Figure 3).

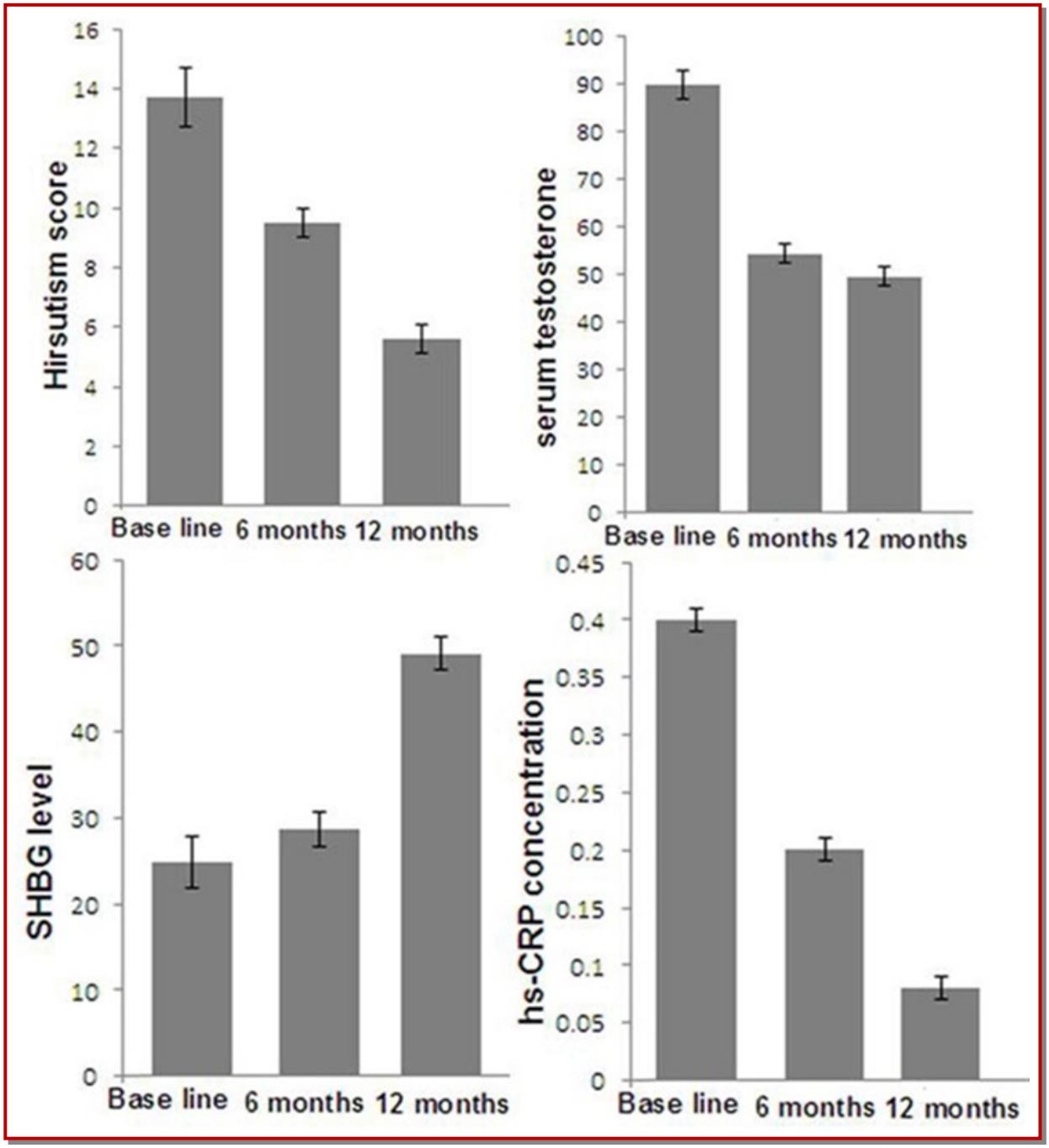

Figure 3: Analysis of hirsutism score, serum testosterone, sex hormone-binding globulin level (SHBG) and hs-CRP level in women with polycystic ovary syndrome before and after glabridin treatment

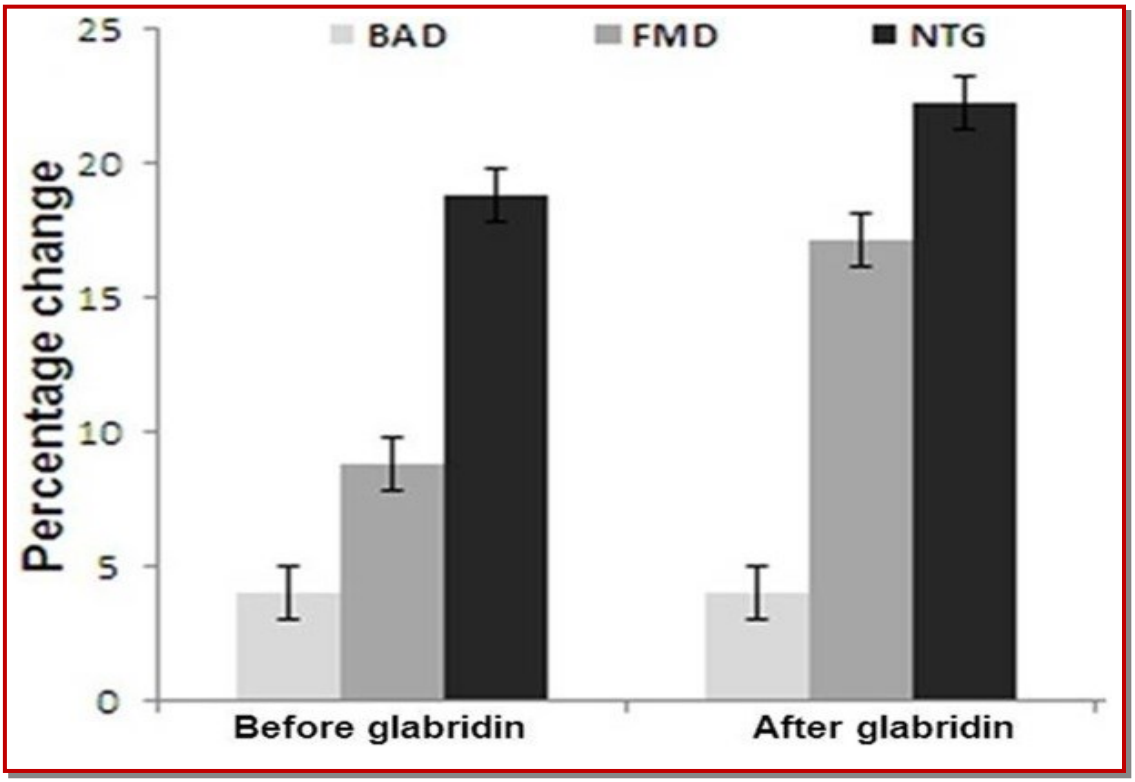

Figure 4: Brachial artery responses in patients with polycystic ovary syndrome after glabridin treatment

Table II

Glucose metabolism parameters of the patients before and after glabridin therapy

	Before glabridin (n =32)	Post-glabridin (n =32)	P
Fasting glucose (mg/dL)	92.5 ± 10.4	84.2 ± 10.4	0.247
Fasting insulin (mU/mL)	13.7 ± 4.5	8.5 ± 3.6	0.034
HOMA	4.6 ± 1.0	2.1 ± 0.4	0.030
QUICKI	0.3 ± 0.04	0.5 ± 0.002	0.030

Values expressed as means ± S.E.

For all the patients under study glucose metabolism parameters prior to and after the 12 month treatment were recorded and compared (Table II). No change was observed in fasting serum glucose concentrations but fasting insulin concentrations and insulin sensitivity indices (HOMA and QUICKI) showed significant changes after 12 months of glabridin treatment.

The endothelium-dependent (FMD) vascular responses for the patients prior to and after the glabridin treatment were recorded. The results revealed a significant increase in level from 7.4 ± 2.2 to 20.0 ± 5.3% after 12 months (Figure 4). The baseline artery diameter and endothelium in-dependent vascular responses remained unchanged after the 12 months of glabridin treatment.

Discussion

It is reported that the administration of metformin reduced the serum insulin concentration during fasting and the insulin response to oral glucose administration in women with polycystic ovary syndrome . It leads to a substantial decrease in the response of serum 17*a*-hydroxyprogesterone to the administration of leuprolide which results in reduced ovarian cytochrome P450c17*a* activity. The reduction in P450c17*a* activity is associated with decrease in the serum free testosterone level. Thus the increased ovarian cytochrome P450c17*a* activity in women with the polycystic ovary syndrome is due to stimulation by insulin and can be reversed by reducing the secretion of insulin. It is reported that both lean and

obese women with polycystic ovary syndrome have peripheral insulin resistance and hyperinsulinemia that appear to play a pathogenic role in the disease (Tarkun et al., 2004). Earlier it was shown that women with polycystic ovary syndrome have endothelial dysfunction (Tarkun et al., 2004). Atherosclerosis represents a chronic inflammatory process and inflammatory markers like CRP provide an adjunctive method for global assessment of cardiovascular risk. It is also reported that CRP may directly promote endothelial dysfunction by increasing the synthesis of soluble adhesion molecules, increasing monocyte chemoattractant protein secretion and facilitating macrophage LDL uptake. In women with polycystic ovary syndrome a high level of hsCRP is present and there is a correlation between insulin resistance and elevated CRP levels (Tarkun et al., 2004).

The use of insulin-sensitizing agents in the ovarian abnormalities of polycystic ovary syndrome patients resulted in beneficial effects on the levels of markers for cardiovascular disease. The endothelial dysfunction and low-grade chronic inflammation being initial stages of atherosclerosis. In the present study the effect of glabridinon hsCRP levels and endothelial dysfunction in women with polycystic ovary syndrome was studied. The results from our study clearly demonstrated a significant decrease in insulin resistance and androgen levels along with improvement of ovulatory cycles in women with polycystic ovary syndrome after glabridin treatment for 12 months. Earlier it was reported that rosiglitazone improves endothelial dysfunction and decrease hsCRP levels in non-diabetic patients with coronary artery disease and also in non-diabetic patients with metabolic syndrome. The results from our study reveal that glabridin treatment improves endothelium-dependent vasodilatation and decreases serum levels of pro-inflammatory marker hsCRP levels in polycystic ovary syndrome patients. Glabridin treatment also improved endothelial dysfunction and hsCRP levels in non-obese young women with polycystic ovary syndrome.

Conclusion

Glabridin treatment improves insulin sensitivity, helps to restore ovulation and decrease androgen production in women with polycystic ovary syndrome.

Financial Support

Self-funded

Ethical Issue

The Ethics Committee for Human Studies of Kocaeli University Hospital approved this study. All the patients were asked to sign a consent explanation of the nature, purpose and potential risks of the study.

Conflict of Interest

Authors declare no conflict of interest

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