Materials and Methods

In the current study, we enrolled 18 female patients with postadolescent severe acne and 18 healthy female controls matched for age and body mass index.

Diagnosis of severe acne was based on the presence of 40--400 papules/pustules and 5 or more larger, deeper, nodular inflamed lesions, mainly present on the face, chest, and back [18, 19]. Postadolescent acne was defined as severe acne in women whose onset of acne was more than 5 years after the onset of menarche. The acne patients were unresponsive to previous topical therapies and systemic antibiotics. Isotretinoin was given at 20 mg/day and the dosage was gradually increased to a maximum of 50 mg/day for each patient.

Acne can be a clinical sign of hyperandrogenism or a manifestation of systemic disorder, which has an unwanted metabolic profile. In order to eliminate the interfering conditions, polycystic ovary syndrome, adrenal enzymatic deficiency, hyperprolactinemia, thyroid dysfunction, Cushing syndrome, and androgen-secreting tumors were excluded.

Polycystic ovary syndrome was diagnosed on the basis of the presence of at least 2 of the following according to the Rotterdam criteria [20]: oligovulation or anovulation, biochemical or clinical hyperandrogenism (hirsutism), and polycystic ovaries defined by ultrasonography [21]. Hirsutism was defined using the modified Ferriman-Gallwey score, and patients with a score $\geq$6 were considered hirsute [22]. Oligomenorrhea was described as fewer than 9 bleeding episodes per year or menstrual cycles <26 days or >35 days in length. In patients with normal menses, at least 2 consecutive menstrual cycles were studied, and the finding of serum P (<4 ng/mL) on days 22--24 of the cycle in both cycles was considered as presence of anovulation. All participants had a pelvic ultrasonography. In the presence of polycystic ovaries in at least 1 ovary, other components of polycystic ovary syndrome were investigated in order to exclude it from the study.

Adiponectin levels were measured at baseline and at 2 h during a 75-g standard oral glucose tolerance test (OGTT) in patients and controls. After 6 months of treatment with

isotretinoin, anthropometric measurements and all biochemical evaluations, including adiponectin levels during OGTT, were repeated in patients with acne.

Exclusion criteria also included previous treatment with oral retinoids, type 2 diabetes, presence of renal, hepatic, and cardiovascular disease, medications known to affect insulin metabolism, and oral contraceptive therapy for at least 3 months before enrollment to the study. Routine precautions, including exclusion criteria for isotretinoin therapy (e.g., pregnancy) were taken. Patients were informed about teratogenic risks of isotretinoin, and use of an effective barrier method of contraception was ensured for patients at risk of pregnancy. The study protocol was approved by the university local ethics committee and was conducted in accordance with the World Medical Association Declaration of Helsinki. All participants provided written informed consent.

On the basis of these sample data with an alpha level of 0.05 and a sample size of 18 patients in each group, the power of the study was 82.6% to find a difference in adiponectin levels between the groups.

Assays

The blood samples were centrifuged at 4,000 rpm for 15 min at 4ºC, and serum samples were stored at --80ºC until assayed. Plasma glucose, insulin, total cholesterol, HDL-C, and triglycerides were measured as previously described [23]. LDL-C levels were calculated using the Friedewald formula [24]. Serum adiponectin levels were measured using an enzyme-linked immunosorbent assay (ELISA), and the intra- and interassay coefficients of variation were 4.2% and 3.1%, respectively (eBioscience, Vienna, Austria).

Statistical Analyses

Statistical analyses were carried out using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Normality of distribution was evaluated by the Shapiro-Wilkins test. Comparison of

variables between the acne patients and the control group was performed by using the Mann-Whitney U test and/or the Student *t* test according to normality of distribution. Pre- and posttreatment data were compared by the Wilcoxon rank test and/or the paired-sample *t* test. Correlation analyses were performed using Spearman/Pearson correlation tests. The results are given as mean ± standard deviation and/or median (range) when appropriate. $p < 0.05$ was accepted as statistically significant.