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Effect of metformin administration on plasma advanced glycation end product levels in women with polycystic ovary syndrome

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Abstract

Metformin therapy in polycystic ovary syndrome (PCOS) improves metabolic and hormonal profiles. Its therapeutic effect on cardiovascular risk factors is under investigation. Advanced glycation end products (AGEs), well-known atherogenic molecules, were recently found to be elevated in plasma of women with PCOS. The purpose of the study was to investigate the effect of metformin treatment in plasma AGE levels of women with PCOS. This was a descriptive clinical trial. The study involved 22 patients with PCOS (age, 25.09 ± 1.05 years; body mass index [BMI], 28.44 ± 1.51 kg/m^2) and 22 healthy women (age, 26.50 ± 0.85 years; BMI, 25.62 ± 1.30 kg/m^2). Measurements of plasma AGE levels (U/mL) were performed, and the metabolic and hormonal profiles were determined in all subjects. All women with PCOS received a dose of 1700 mg metformin daily for 6 months. AGEs levels were reduced after metformin administration in 22 women with PCOS (9.98 ± 0.13 [before metformin] vs 9.86 ± 0.11 [after metformin], $P = .05$). In a subgroup analysis, of 16 women with PCOS and normal glucose tolerance, the drop of AGE levels was potentiated (9.98 ± 0.19 [before] vs 9.81 ± 0.15 [after], $P = .02$). Body mass index as well as the other parameters studied remained unchanged after metformin therapy apart from a drop of testosterone levels ($P = .01$) and free androgen index ($P = .009$). In conclusion, after metformin therapy, the atherogenic AGE molecules were reduced in the serum of women with PCOS. The clinical relevance of this finding in PCOS, a high-risk group for type 2 diabetes mellitus and cardiovascular disease, remains to be seen. Future studies are required to confirm the need of therapeutic intervention for short-term abnormalities and for prevention of long-term sequelae characterizing this syndrome.

1. Introduction

Polycystic ovary syndrome (PCOS), in addition to hyperandrogenemia and chronic anovulation [1], is associated with a number of cardiovascular risk factors such as insulin resistance, glucose intolerance, dyslipidemia, coagulopathy, endothelial dysfunction, inflammation, and oxidative stress [1-6]. Recently, elevated serum levels of advanced glycation end products (AGEs) and their receptors have been found in young women with PCOS. Furthermore, orlistat, a lipase inhibitor, has been shown in a short-term administration to reduce the serum levels of AGEs in women with this syndrome. [7]. AGEs levels and their receptors have been correlated with molecular damage, oxidative stress, and endothelial cell activation, and they have been implicated in long-term vascular sequelae in diabetic patients [8-11]. These molecules, the most well characterized of which are *N*-ε-(carboxymethyl)lysine, pentosidine, 3-deoxyglucosone, and methylglyoxal [12,13], constitute a complex and heterogeneous group of compounds with diverse molecular structure and biologic function.

Aminoguanidine, a guanidine, represents the most extensively investigated inhibitor of AGEs formation in vitro and in vivo [14,15]. Metformin (dimethylguanidine), the most widely used insulin sensitizer in the management of PCOS [16-18], has been shown to react strongly with methylglyoxal and glyoxal, and to decrease their formation in experimental animals [19].

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The aim of the present study was to investigate the effects of metformin on AGE levels in women with PCOS.

2. Subjects and methods

2.1. Subjects

The study consisted of 44 women: 22 patients with PCOS, all recruited from the Outpatient Section of Endocrinology of the First Department of Internal Medicine in Laiko University Hospital in Athens, Greece, and 22 healthy women (physicians and students) who volunteered to participate in the study. The diagnosis of women with PCOS (age, 25.09 ± 1.05 years; body mass index [BMI], 28.44 ± 1.51 kg/m^2) was based on the Rotterdam criteria [20]. Twenty-two healthy women served as the control group (mean age, 26.50 ± 0.85 years; BMI, 25.62 ± 1.30 kg/m^2). In this group, there was no hyperandrogenemia or evidence of hyperandrogenism (hirsutism, acne, or alopecia) on physical examination. They had regular menstrual cycles (intermenstrual intervals between 28 and 32 [±2] days but with no more than 4 days variation from cycle to cycle), and ovulation was confirmed by estimation of progesterone levels on days 21 to 23 of their cycle. All the participants in the study were in good health. Inclusion criteria for controls and women with PCOS did not allow any medication that could affect carbohydrate or sex hormone metabolism, for at least 3 months before the study. Current smokers were excluded from the study.

The study protocol was approved by the local ethics board, and informed consent was obtained from all participants.

2.2. Protocol

The baseline study and the follow-up visits were performed in the Outpatient Department of Endocrinology, First Department of Internal Medicine in Laiko University Hospital, Athens. After a 10-hour overnight fasting period, blood samples were collected (time 0). Subsequently, an oral glucose tolerance test (OGTT) with 75-g glucose load was performed at 30-minute intervals (times 30, 60, 90, and 120 minutes).

Weight, height, and waist and hip circumferences were measured. Waist circumference was obtained as the smallest circumference at the level of the umbilicus. Hip circumference was obtained as the widest circumference at the level of the buttocks. BMI (kg/m^2) and waist-to-hip ratio (WHR) were calculated.

Blood pressure was measured by a mercury sphygmomanometer with the subject in a sitting position, after a rest of at least 5 minutes. At the end of this resting period, the average of 3 measurements was obtained.

The evaluations were conducted within the follicular phase of the menstrual cycle in control women and at any time in the women with PCOS, who were chronically anovulatory. In the amenorrheic women, recent ovulation was excluded by progesterone measurement (<5 nmol/L).

After baseline studies, metformin was administrated in women with PCOS for 6 months. At the end of this period they underwent evaluation of hormonal and metabolic parameters under the same conditions. The women with PCOS were closely followed up for the entire period of the study.

2.3. Assay methods

Blood samples were collected between 8:00 and 10:00 AM after an overnight fast. Blood samples were centrifuged immediately, and serum was stored at −20°C until assayed. The samples were assayed within 8 to 9 months of their collection.

All measurements were performed with the Chemwell analyzer (Palm City, FL), unless otherwise stated.

Plasma GLU was determined by the glucose (GLU) oxidase–color method (Glucose LR, GOD-PAP; Linear Chemicals, Barcelona, Spain). High-density lipoprotein cholesterol (HDL) was assessed enzymatically using a direct method (HDL-Cholesterol, DIRECT, Linear Chemicals). Total cholesterol (TC) was determined by the Enzymatic Cobas Mira method (Cholesterol LR, CHOD-PAP; Linear Chemicals). Insulin (INS) was measured by a solid-phase enzyme-amplified sensitivity immunoassay (INS-EASIA; Biosource, Nivelles, Belgium). Total testosterone (TT) was measured by enzyme-linked immunosorbent assay (Testosterone Enzyme Immunoassay Test Kit, LI7603; Linear Chemicals). Sex hormone–binding globulin (SHBG) serum levels were measured by ELISA (SHBG ELISA, MX 520 11; IBL, Hamburg, Germany). The expected values of SHBG for women are 15 to 120 nmol/L. The intra- and interassay coefficients of variation for low and high levels, respectively, were as follows: for INS, 5.3% and 3.0% and 9.5% and 4.5% respectively; for TT, 5.0% and 6.4% and 4.4% and 8.4%; for SHBG, 3.0% and 5.3% and 7.2% and 8.4%, respectively.

2.4. Hormonal and biochemical parameters

Serum levels of TT (ng/dL), SHBG (nmol/L), INS (μU/mL), GLU (mg/dL), HDL (mg/dL), and TC (mg/dL) were measured at baseline (time 0), before metformin, as well as after 6 months metformin administration. GLU and INS concentrations were determined additionally during the OGTT (times 30, 60, 90, 120 minutes) at baseline as well as 6 months after metformin administration.

2.5. Chemicals and reagents

Bovine serum albumin, D-GLU, alkaline phosphatase–conjugated goat antimouse immunoglobulin G, and anti-goat immunoglobulin G–fluorescent isothiocyanate as well as *p*-nitrophenyl phosphate tablets were purchased from Sigma Chemical (St Louis, MO). Superblock blocking buffer was from Pierce (Rockford, IL) and normal goat serum from GIBCO-BRL (Gaithesburg, MD). Mouse antihuman AGE monoclonal antibody (6D12) was obtained from Research Diagnostics.

2.6. Specificity of anti-AGE antibody (6D12)

6D12 is a mouse antihuman monoclonal antibody with immunospecificity to a common structure among AGE proteins.

The epitope of 6D12 is *N*-ε-(carboxymethyl)lysine–protein adduct. This antibody also recognizes carboxyethyllysine and does not recognize the early products, Schiff bases, and Amadori products. However, it shows positive reaction to AGE samples obtained from proteins, lysine derivatives, or monoamino-carboxylic acids. Recent immunologic studies using 6D12 demonstrated the presence of AGE-modified proteins in several human tissues, indicating its usefulness in the biochemical quantification of AGE-modified proteins [21].

2.7. Preparation of AGE-modified proteins

Advanced glycation end product–modified bovine serum albumin was prepared as previously described by Diamanti-Kandarakis et al [22]. The degree of AGE modification of the protein was further determined by competitive AGE-ELISA as described below.

2.8. Competitive AGE-ELISA

The competitive AGE-ELISA procedure was performed as previously described by Diamanti-Kandarakis [22].

2.9. Hyperandrogenemia estimation

The hyperandrogenemia index (free androgen index [FAI], %) was estimated by the formula:

$$FAI = [\ totalT\ (\mathrm{ng/dL})/SHBG\ (\mathrm{nmol/L})] \times 100$$

2.10. Insulin resistance estimation

Insulin resistance was estimated by the GLU/INS ratio, the quantitative insulin sensitivity check index (QUICKI), and the Matsuda index.

QUICKI is defined as:

$$QUICKI = 1/[\log(Fasting\ INS) + \log(Fasting\ GLU)]$$

[23].

The Matsuda index is obtained using the following formula:

$$\begin{aligned}\mathrm{MATSUDA}(M) &= 10.000/\text{square root of} \\ &\times [(fasting\ GLU \times fasting\ INS) \\ &\times (mean\ GLU \times mean\ INS\ during\ OGTT)]\end{aligned}$$

[24].

2.11. Metformin protocol

Women with PCOS received a dose of 1700 mg metformin daily for 6 months (Lipha Sante; Aron Medicia Division, Lyon, France). Initially, metformin was administered in incremental doses (ie, 425 mg, 850 mg, 1275 mg, 1700 mg) every 7 days until the final dose of 1700 mg daily. All women were urged to maintain the same diet followed before treatment and were checked monthly. No severe side effects were reported during the study. After 6 months of treatment with metformin, hormonal and metabolic studies were repeated.

2.12. Statistical analysis

Results are reported as mean values ±SE. Statistical analysis was accepted at $P < .05$. Normal distribution of continuous variables was assessed by applying the nonparametric Kolmogorov-Smirnov test. An independent-sample *t* test was used for comparisons between women with PCOS and the control group, and a paired *t* test was applied to evaluate changes between measurements at baseline and after the 6-month treatment period. Mann-Whitney *U* and Wilcoxon tests were performed for variables that were not normally distributed. Correlations between variables were evaluated by Pearson coefficient except for variables not normally distributed, which were evaluated by Spearman

Table 1
Anthropometric characteristics and hormonal and metabolic profiles of patients with PCOS before metformin, after metformin, and of normal control women

Variables studied	Studied groups			*P*		
	P pre-M (n = 22)	P post-M (n = 22)	C (n = 22)	P pre-M vs C	P pre-M vs P post-M	P post-M vs C
Age (y)	25.09 ± 1.05	25.09 ± 1.05	26.50 ± 0.85	.30		
BMI (kg/m^2)	28.44 ± 1.51	28.98 ± 1.51	25.62 ± 1.30	.16	.15	.11
Body weight (kg)	76.58 ± 4.44	78.12 ± 4.79	70.79 ± 3.5244	.31	.13	.22
WHR	0.79 ± 0.02	0.80 ± 0.01	0.74 ± 0.01	.08	.14	.06
TT (ng/dL)	90.13 ± 6.02	63.97 ± 7.20	40.39 ± 2.74	< .0001	.01	.003
SHBG (nmol/L)	29.49 ± 3.07	33.89 ± 3.64	43.49 ± 4.03	.009	.36	.08
FAI index (%)	405.118 ± 58.72	261.37 ± 58.42	110.95 ± 13.28	.0001	.009	.002
GLU (mg/dL)	84.09 ± 2.38	86.73 ± 2.28	80.32 ± 2.23	.25	.43	.09
INS (μU/mL)	16.46 ± 2.97	15.06 ± 2.52	9.03 ± 1.23	.01	.60	.03
TC (mg/dL)	180.38 ± 12.11	153.25 ± 9.31	167.43 ± 7.65	.37	.09	.41
HDL (mg/dL)	50.76 ± 2.78	46.55 ± 2.37	51.40 ± 2.37	.86	.14	.29
QUICKI	0.333 ± 0.007	0.334 ± 0.007	0.361 ± 0.006	.007	.97	.04
Matsuda	3.45 ± 0.42	2.92 ± 0.33	7.03 ± 1.01	.003	.20	.001
SBP (mm Hg)	116.00 ± 2.82	110.71 ± 3.45	110.90 ± 2.56	.18	.22	.97
DBP (mm Hg)	77.50 ± 2.64	72.62 ± 2.73	72.05 ± 1.99	.10	.14	.62

Data are expressed as means ± SE ($P < .05$, statistically significant; $P < .001$, after Bonferroni correction). P indicates PCOS; M, metformin; C, control women.

Table 2
Serum levels of AGEs (U/mL) in patients with PCOS before metformin, after metformin, and in control women

	Studied groups			*P*		
Total PCOS population						
	P pre-M (n = 22)	P post- M (n = 22)	C (n = 22)	P pre-M vs C	P pre-M vs P post-M	Post-M vs C
AGEs (U/mL)	9.98 ± 0.13	9.86 ± 0.11	5.26±0.20	<.001	.05	<.001
PCOS population with normal glucose tolerance*						
	P pre-M (n = 16)	P post-M (n = 16)	C (n = 22)	P pre-M vs C	P pre-M vs P post-M	Post-M vs C
AGEs (U/mL)	9.98 ± 0.19	9.81 ± 0.15	5.26 ± 0.20	<.001	.02	<.001

Data are expressed as means ± SE. Results are from a subpopulation of women with PCOS and NGT.
*$P < .05$, statistically significant.

coefficient. Multiple regression analysis was performed in the total population to assess if the presence of PCOS (control group: 0, PCOS group: 1), BMI, Matsuda index, and TT predicted AGE values. In addition, multiple regression analysis was performed in PCOS population to assess which from TT, BMI, and Matsuda index as independent variables predict AGE values as dependent variables. A χ^2 test was applied to compare the family history of diabetes for the 2 groups.

Analysis was performed using SPSS (Statistical Package for the Social Sciences, version 11.01; SPSS, Chicago, IL) for Windows XP (Microsoft, Corp, USA).

3. Results

The women with PCOS did not differ in age, BMI, or WHR with the control group before metformin administration as well as after 6 months of metformin treatment (Table 1). In addition, when women with PCOS posttreatment were compared with the control group, no statistically significant difference was observed in BMI ($P = .11$) and WHR ($P = .06$). Furthermore, women with PCOS and controls did not differ in family history of type 2 diabetes mellitus ($P = .54$).

Six women with PCOS were found to exhibit impaired glucose tolerance (IGT). They were not excluded from the study because IGT is considered to be a metabolic feature of PCOS. The analyses were also repeated in the population with normal glucose tolerance (NGT) to have more information on the effect of metformin on metabolic parameters of the study in a subpopulation of these young women with PCOS without IGT.

After 6 months of metformin administration, serum levels of AGEs were significantly decreased in the PCOS population ($P = .05$, Table 2). In the subpopulation of 16 women with PCOS and NGT, AGEs values were further decreased ($P = .02$, Table 2). All anthropometric characteristics, and hormonal and metabolic profiles showed no difference between the PCOS population with NGT and the PCOS population with IGT. Their anthropometric characteristics (age [$P = .89$], BMI [$P = .67$], WHR [$P = .89$]), as well as their hormonal (TT [$P = .50$], SHBG [$P = .77$], FAI index [$P = .79$]) and metabolic (GLU [$P = .08$], INS [$P = .31$], TC [$P = .83$], HDL [$P = .33$], QUICKI [$P = .93$], Matsuda index [$P = .99$]) profiles were comparable.

Women with PCOS showed increased serum levels of AGEs compared with controls ($P < .001$). The same profile was observed in the subpopulation of women with PCOS and NGT.

Hormonal and metabolic profiles at baseline in women with PCOS and the control group as well as the comparison of their values after 6 months of metformin administration are described in Table 1.

In the PCOS population, serum levels of AGE were positively related to TT plasma levels ($r = 0.434$, $P = .044$), FAI index ($r = 0.438$, $P = .041$), and TC ($r = 0.45$, $P = .041$).

In multiple regression analysis, in the total population, it was revealed that the presence of PCOS predict AGEs values with inclusion ($b = 4.11$, $P < .001$, $R^2=0.90$, F = 82.09) or with exclusion ($b = 4.68$, $P < .001$, $R^2=0.89$, F = 100.79) of TT from the model. Multiple regression analysis in the PCOS population also revealed that TT plasma levels predicted AGE values ($b = 0.012$, $P = .031$).

4. Discussion

In the present study, plasma AGEs levels were reduced after 6 months of metformin administration in women with PCOS.

The mechanisms by which metformin lowers plasma levels of AGEs in the studied group of women with PCOS are not clear; however, there is evidence that its effect could interfere with production as well as with clearance of these products. It has been suggested that metformin, a well-known insulin sensitizer, is a guanidine, which has an inhibitory effect in glycation process [14,15,19] by interfering with the synthetic pathway of AGEs through trapping of methyl-glyoxal and of other dicarbonyl compounds responsible for glycation process in vivo [25].

Therefore, metformin's action may lead to reduced synthesis and/or increased detoxification of AGE metabolites, as it has been shown by increased excretion in urine in vitro [26,27]. Regarding the clearance of AGEs, intact phosphor-inositol 3 kinase (PI3K) activity is required for the activation of macrophage scavenger receptor, which is one of the major mechanisms for their clearance [28]. PI3K, a key intracellular signaling step of insulin action, has been shown to be defective in insulin-resistant state and in PCOS [29]. Interestingly, hyperglycemia was not present in the

studied group of patients with PCOS, and the pathogenetic mechanism for elevated serum levels of AGEs that has been suggested in the present study is the hypothesis of a PI3K pathway disruption. The decreased PI3K activity present in insulin-resistant PCOS [29] may link the pathogenic mechanisms of PCOS insulin resistance with decreased AGEs clearance. On the other hand, it has been suggested that the presence of a guanidine group in the metformin structure confers a potential use of this drug for the inhibition of the Maillard reaction by reacting with carbonyl groups of reducing sugars and dicarbonyl compounds. Dicarbonyl compounds seem to be more reactive in the glycation reaction than reducing sugars, and they consist an important step for cross-linking proteins in the Maillard reaction. It has been suggested that increased chemical modification of proteins by carbohydrates and lipids in diabetes is the result of overload on metabolic pathways involved in detoxification of reactive carbonyl species leading to an increased carbonyl stress and, consequently, metformin reacting with a-dicarbonyl compounds could prevent subsequent AGE formation on susceptible proteins acting as a carbonyl scavenger [25]. Nevertheless, a mechanism independent from the antihyperglycemic effect has been shown for metformin in preventing microvascular alterations [30].

On the other hand, metformin has been shown to have a beneficial effect in metabolic and reproductive aspects of PCOS [16-18].

No statistical difference was observed in insulin sensitivity indices before and after metformin administration. This finding cannot however exclude a nonmeasurable by these mathematical indices, change of insulin resistance, because it has been shown that these formulas are less efficient in subjects with mild insulin resistance and do not correlate well with glucose utilization values of the euglycemic clamp in lean and overweight women with PCOS [31,32].

Metformin improved the hyperandrogemic profile as it has been shown in several previous studies [16-18]. Furthermore, the positive correlation of AGEs levels with testosterone levels has been demonstrated in this as well as in a previous study [22], implying an interaction between plasma AGEs and hyperandrogenemia. This finding is in accordance with previous studies in women with PCOS, which have also shown a positive correlation of testosterone with other atherogenic factors such as endothelin 1 [2], adhesion molecules [6], and PAI-1 [3], but the potential mechanism underlying this correlation remains unknown.

Because the reduction of AGEs levels was even higher when the subgroup of 6 women with PCOS and IGT was excluded, it is likely that longer duration treatment might be required for this subgroup.

The clinical relevance of elevated AGEs levels in PCOS, a high-risk group for type 2 diabetes mellitus and cardiovascular disease, remains to be seen. Considering that clinical implications of elevated AGE levels on normoglycemic individuals are not yet known, their presence in women with PCOS remains to be defined, and future studies are required to confirm the need of a therapeutic agent, when prevention of long-term complications may be feasible.

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