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Adiponectin and Adiponectin Receptor-1 in Patients with Polycystic Ovarian Syndrome: Impact of Insulin Sensitization by Metformin

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Published: 01 March 2013

Ibnosina J Med BS 2013,5(2):52-61

Received: 25 July 2012

Accepted: 15 September 2012

This article is available from: <http://www.ijmbs.org>

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Abstract

Background: Abdominal obesity, insulin resistance and hyperinsulinemia play a central role in the pathogenesis of polycystic ovarian syndrome (PCOS). Abdominal adipose tissue is a source of adiponectin. Metformin has been widely used in the treatment of PCOS and has been shown to improve the metabolic and hormonal disturbances of PCOS. **Objective:** The current study aimed to investigate the relationships between serum adiponectin and adiponectin receptor-1 (AdipoR1) with insulin resistance, hormonal variables, and anthropometric measures in patients with polycystic ovarian syndrome (PCOS), and to find the short-term effect of metformin treatment on adiponectin and AdipoR-1 levels in these patients. **Patients and Methods:** 38 PCOS patients and 14 age- and body mass index (BMI)-matched healthy controls were recruited. In all participants, BMI, waist circumference, serum levels of fasting glucose, insulin, adiponectin, AdipoR1, total testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH)

were assessed. PCOS patients received metformin treatment (1500 mg/daily) for two menstrual cycles followed by measurement of all previous parameters. All subjects gave informed consent. **Results:** PCOS patients had higher waist circumference, fasting glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), testosterone, LH and LH/FSH ratio than did controls. In PCOS patients, adiponectin and AdipoR1 were lower than in controls, and both correlated negatively with waist circumference, insulin, and HOMA-IR ($P=0.048$, $P=0.0003$, $P=0.0003$, respectively for adiponectin and $P=0.039$, $P=0.023$, $P=0.025$, respectively for AdipoR-1). HOMA-IR followed by testosterone were independent predictors of adiponectin while HOMA-IR was an independent predictor of AdipoR1. Metformin decreased fasting glucose ($P=0.003$), insulin ($P=0.042$), HOMA-IR ($P=0.006$), testosterone ($P=0.001$), LH ($P=0.0001$) and LH/FSH ratio ($P=0.003$) and increased adiponectin ($P=0.014$) and AdipoR1 ($P=0.001$) levels in PCOS patients. **Conclusion:** Reduced adiponectin and Ad-

ipoR1 in PCOS patients is independently associated with insulin resistance and the improvement of insulin sensitivity by short-term metformin treatment results in increased adiponectin and AdipoR-1.

Key words: Adiponectin, Adiponectin receptor, Metformin, Polycystic ovarian syndrome.

Introduction

Abdominal obesity, insulin resistance and compensatory hyperinsulinaemia play a central role in the pathogenesis of polycystic ovarian syndrome (PCOS), the most common endocrine disorder that cause anovulatory infertility in women (1). Abdominal adiposity is a risk factor for insulin resistance, glucose intolerance and type 2 diabetes mellitus (2-4). Insulin resistance may be a trigger point in the pathophysiology of PCOS (5). Insulin resistance and hyperinsulinemia promote abnormal ovarian androgen secretion and subsequently abnormal follicular development leading to dysfunctional ovarian and menstrual activity (6). Increased ovarian theca cell androgen production occurs by either binding to insulin or IGF-1 receptors, or by stimulating the release of LH. Hyperinsulinemia could also enhance the bioavailability of androgen by decreasing the biosynthesis of sex hormone-binding globulins (7). Insulin sensitizing agents like metformin have been widely used in the treatment of PCOS and also to improve the metabolic and hormonal disturbances of PCOS (8). Moreover, adipose tissue is a source of adipocytokines, the adipocyte-derived biologically active molecules that may influence the function as well as the structural integrity of other tissues (4,9). Adiponectin is adipocytokine abundantly expressed in white adipose tissue, and is the most abundant circulating adipose-specific protein in humans (10). It acts through two main receptors, adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) (11). Adiponectin has anti-atherogenic and anti-inflammatory effects (12,13). Adiponectin may have a role in preventing the development of insulin resistance (5,14) also AdipoR1 is a determinant of first-phase insulin secretion (15). The production of adiponectin decreased in obesity (5), and its serum levels have been shown to correlate negatively with waist-to-hip ratio (16) and body mass index (BMI) (10). Adiponectin receptors are down-regulated in mouse models of obesity and insulin resistance (17). Several studies have shown that women with PCOS have reduced adiponectin levels, which may play a significant role in early endothelial abnormalities of young women with PCOS (18-20). Hypoadiponectinemia in one study was

evident in both obese and lean PCOS patients with variable degrees of insulin resistance (21). We hypothesize that insulin resistance in patients with PCOS could be involved in the downregulation of adiponectin and its receptors. The improvement of insulin sensitivity that is seen during metformin treatment could result in modifying these levels.

To test our hypothesis, this study was conducted to investigate the relationship between serum levels of adiponectin and AdipoR1 and insulin resistance, hormonal variables, and anthropometric measures in patients with PCOS. Also, we wanted to assess the short-term effect of metformin treatment on adiponectin and AdipoR1 levels in these patients.

Patients and Methods

Protocol

This was a cohort study involving 38 PCOS patients who attended the infertility clinic in the tertiary maternity-childhood hospital in Burraidah, Al-Qassim provenance, Saudi Arabia during May, 2011 to April, 2012. Informed consents were signed by patients.

Patient's characteristics

The patient inclusion criteria included females aged 20-38 years, Arab population, met the criteria for diagnosis of PCOS according to the Rotterdam Consensus Group (22). Patients with a history of failed induction of ovulation by Clomiphene Citrate (CC) for at least three months with doses of 150 mg/ day for five days (CC resistance) were included. The exclusion criteria were patients who received gonadotropins or hormonal contraception in the three months prior to the study, patients with hyperprolactinemia (morning plasma prolactin ≥ 30 ng/ml) or other endocrine, hepatic, or renal disorders. Fourteen healthy, fertile non-pregnant females with cross-matched age and BMI were recruited as a control group. BMI was calculated as weight in kilograms divided by height in meters squared for all eligible subjects. Normal weight was defined as BMI 18.5-24.9 kg/m² (23). Waist circumference was also measured (24). The research was carried out in accordance with the Declaration of Helsinki of the World Medical Association, and was approved by Research and Ethics Committee of Faculty of Medicine, Qassim University, Saudi Arabia.

Laboratory assays

Venous blood samples (10 ml) collected by venipuncture between 8-10 a.m. after overnight fasting were allowed to clot and centrifuged at 3,000 rpm for five minutes. Serum

was stored at -20°C for biochemical assays. Blood samples were taken from patients and controls on days 2-7 of their menstrual cycles (early follicular phase). Glucose level was measured by oxidase method and spectrophotometric quantitation (Diamond diagnostics, Germany) (25). Insulin was detected by Enzyme-linked immunosorbent assay (ELISA) kits (IMMUNOSPEC Corporation Catalog No: E29-072, USA). Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) by the following formula: $\text{HOMA-IR} (\text{mmol/L} \times \text{IU/ml}) = \text{fasting blood glucose} (\text{mmol/L}) \times \text{fasting insulin} (\text{IU/L}) / 22.5$ (26). Patients were considered to have insulin resistance if $\text{HOMA-IR} > 2.77$ (23). Adiponectin, AdipoR1 levels were measured by ELISA kits (Assay Max Human Adiponectin, Catalog No. EA2500-1, USA and Glory Science Co., Ltd, LOT 100102P, USA, respectively). The ADIPOR1 ELISA assay was used for competitive inhibition enzyme linked immunosorbant assay. A standard curve was drawn by reading absorbance values and concentrations of the samples obtained on the standard curve by inverse computation.

The levels of total testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were assessed by using ELISA kits (IBL International, Catalog No. RE52151, Germany; DRG Catalog No. EIA-1289, USA; and DRG Catalog No. EIA-1288 respectively). The PCOS- patients were assigned to a short course of metformin treatment. Beginning on the first day of the first spontaneous or induced menstrual cycle, they received 500-mg tablets of metformin hydrochloride (Julphar, Gulf Pharmaceutical Industries, UAE) three times per day for two menstrual cycles, or 70 days, whichever came first (27). The biochemical and hormonal parameters measured if affected by this drug, the action was expected to be after short treatment duration. This step was followed by a second post-treatment blood sample taken on the 2nd day of the next menstrual period, after completing treatment for measurement of all previous parameters.

Sample Size Calculation

Sample size was calculated according to differences in adiponectin levels in normal women compared to PCOS patients with suspected changes in patient groups after metformin therapy. In previous studies (21, 28), the plasma adiponectin levels in normal women had been reported in the range of $18\text{--}33 \mu\text{g/ml}$, with an average level of $22 \mu\text{g/ml}$. On the other hand, the studies reported an average of $10 \mu\text{g/ml}$ in PCOS patients. On the assumption that a 25%

difference in adiponectin level before and after metformin treatment in PCOS patients was clinically significant, we needed 38 patients in the PCOS group to demonstrate this difference in statistical significance with a type I error probability (α) of 0.05, type II error (β) of 0.2 with a power of 80%.

Statistical analyses

Data were analyzed using Statistical Package for the Social Science, version 16 (SPSS, Chicago, IL, USA) and was expressed as mean \pm SD and/or percentages. Comparison between patients and controls was performed with unpaired student “*t* test”. Paired student “*t* test” was used for comparison between means of different parameters before/after treatment. The degree of correlation between adiponectin, AdipoR1, and the variables of interest was assessed using Pearson’s correlation coefficient. In addition, multivariate stepwise regression analysis was performed to identify important predictors of adiponectin and AdipoR1. For all tests, a probability (*P*) < 0.05 was considered statistically significant.

Results

PCOS patients and healthy controls had no significant differences in age, BMI or FSH serum levels. Waist circumference, fasting glucose, insulin, as well as HOMA-IR, were significantly higher in PCOS patients than in healthy controls (Table 1). From the total 38 PCOS patients, seven had normal weight (BMI $24.7 \pm 0.6 \text{ kg/m}^2$) and 31 were either overweight or obese (BMI $31.4 \pm 2.0 \text{ kg/m}^2$). HOMA-IR in normal weight PCOS patients was 2.38 ± 0.54 . HOMA-IR increased non-significantly to 3.27 ± 0.24 in patients with higher BMI ($p = 0.127$). In addition, 43% of normal weight patients were insulin resistant, while 58% of overweight and obese patients were insulin resistant. The mean serum levels of adiponectin and AdipoR1 were significantly lower in patients than in controls (Table 1). No differences were observed in adiponectin levels between the normal weight PCOS patients and those with increased BMI (14.5 ± 2.3 vs 13.7 ± 2.7) respectively, ($p = 0.50$). The measured hormonal parameters, total testosterone, LH, and LH/FSH ratio in PCOS patients were significantly higher than in controls (Table 1). In PCOS patients, both adiponectin and AdipoR1 serum levels correlated both negatively and significantly with waist circumference, fasting insulin, and HOMA-IR. Both had no significant correlation with BMI, fasting glucose, LH, FSH, or LH/FSH ratio. Adiponectin had a significant positive correlation with AdipoR1, and correlated both

Table 1. Age, anthropometric and biochemical parameters investigated in polycystic ovarian syndrome (PCOS) patients and in age-matched healthy control subjects.

Variables	PCOS patients (n=38)	Controls (n=14)	P value
Age (years)	30.3 ± 4.6	31.9 ± 4.5	0.269
BMI (kg/m ²)	30.2 ± 3.1	28.4 ± 3.3	0.066
Waist circumference (cm)	98.1 ± 7.0	85.4 ± 8.3	0.0001
Glucose (mmol/l)	6.1 ± 0.6	5.1 ± 0.6	0.0001
Insulin (μIU/ml)	11 ± 5	5 ± 1	0.0001
HOMA-IR (mmol/L-μIU/ml))	3.1 ± 1.4	1.0 ± 0.3	0.0001
Adiponectin (ng/ml)	13.9 ± 2.6	15.6 ± 1.0	0.017
AdipoR1(ng/ml)	16.5 ± 4.3	19.7 ± 1.9	0.011
Total testosterone (ng/dl)	93 ± 40	47 ± 20	0.0001
LH (mIU/ml)	13.9 ± 3.9	6.6 ± 1.5	0.0001
FSH (mIU/ml)	7.2 ± 1.7	7.4 ± 1.4	0.693
LH/FSH ratio	2.0 ± 0.8	0.9 ± 0.3	0.0001

Data are presented as the mean ± SD, P versus controls. BMI: body mass index, HOMA- IR: homeostasis model assessment of insulin resistance, AdipoR1: adiponectin receptor 1, LH: luteinizing hormone, FSH: follicle-stimulating hormone.

negatively and significantly with total testosterone, while ApioR1 did not (Table 2). Multivariate stepwise regression analysis was performed for PCOS patients to identify the best predictors of adiponectin and AdipoR1 levels. Adiponectin was introduced as a dependent variable. Waist circumference, fasting insulin, HOMA-IR and total testosterone were introduced as independent variables (variables that significantly correlated with adiponectin with $P < 0.05$). After adjusting the effects of other variables, HOMA-IR, followed by total testosterone, were found to be independent predictors of adiponectin levels ($\beta = -0.491$, $P = 0.001$ and $\beta = -0.294$, $P = 0.037$ respectively) whereas other values were not. This model was used because it had the best Adjusted R Square = 0.354. AdipoR1 was also introduced as a dependent variable; waist circumference, fasting insulin and HOMA-IR were introduced as independent variables. After adjusting the effects of other variables, HOMA-IR was found to be an independent predictor of AdipoR1 levels, whereas other values were not ($\beta = -0.365$, $P = 0.024$ and Adjusted R Square = 0.109). The mean duration of metformin treatment for PCOS patients

was 64 ± 4 days (range 58-70 days). All patients received metformin in the second cycle as no women became pregnant in the first cycle and no one dropped from the study. After metformin treatment, there were significant reductions in waist circumference, fasting glucose and insulin. Insulin resistance significantly declined after metformin therapy as indicated by significant decreases in HOMA-IR. Moreover, metformin treatment significantly increased adiponectin and AdipoR1. Total testosterone, LH, and LH/ FSH ratio were significantly reduced, but there were no significant differences observed in BMI or FSH levels in those patients after treatment (Table 3).

Discussion

The present results are in line with those of previous studies on PCOS, where lower adiponectin levels were found compared with the BMI-matched controls (23, 29). The decreased serum adiponectin levels in cases with PCOS were independent from BMI as that was found in both normal weight as well as obese PCOS patients, consistent with previous studies (21, 24).

Table 2. Baseline Pearson correlations coefficients (r) of adiponectin and adiponectin receptor-1 with anthropometric and biochemical parameters in polycystic ovarian syndrome patients.

Variables	Adiponectin (ng/mL)		AdipoR1(ng/mL)	
	R	P value	R	P value
BMI (kg/m ²)	- 0.25	0.126	- 0.18	0.272
Waist circumference (cm)	- 0.32	0.048	- 0.34	0.039
Glucose (mmol/l)	- 0.21	0.196	- 0.13	0.442
Insulin (uIU/ml)	- 0.54	0.0003	- 0.37	0.023
HOMA-IR (mmol/L -μIU/ml))	- 0.55	0.0003	- 0.36	0.025
Total testosterone (ng/dl)	- 0.39	0.013	- 0.18	0.286
LH (mIU/ml)	- 0.27	0.104	0.06	0.717
FSH (mIU/ml)	0.13	0.436	0.17	0.315
LH/FSH ratio	- 0.28	0.092	- 0.04	0.813
AdipoR1(ng/ml)	0.45	0.005		

BMI body mass index, HOMA- IR homeostasis model assessment of insulin resistance, LH luteinizing hormone, FSH follicle-stimulating hormone, AdipoR1 adiponectin receptor 1, r Pearson correlation coefficient.

Table 3. Clinical and biochemical variables in 38 patients with polycystic ovarian syndrome (PCOS) before and after metformin treatment.

Variables	Before metformin treatment	After metformin treatment	P value
BMI (kg/m ²)	30.2 ± 3.1	29.3 ± 2.7	0.145
Waist circumference (cm)	98.1 ± 7.0	96.29 ± 6.3	0.008
Glucose (mmol/l)	6.1 ± 0.6	5.7 ± 0.8	0.003
Insulin (μIU/ml)	11.4 ± 4.8	9.3 ± 3.6	0.042
HOMA-IR (mmol/L -μIU/ml))	3.1 ± 1.4	2.3 ± 0.9	0.006
Adiponectin (ng/ml)	13.9 ± 2.6	15.2 ± 1.7	0.014
AdipoR1(ng/ml)	16.5 ± 4.3	19.1 ± 1.5	0.001
Total testosterone (ng/dl)	93.1 ± 40.0	69.5 ± 26.9	0.001
LH (mIU/ml)	13.9 ± 3.9	10.2 ± 3.0	0.0001
FSH (mIU/ml)	7.2 ± 1.7	7.1 ± 1.4	0.606
LH/FSH ratio	2.0 ± 0.8	1.5 ± 0.7	0.003

Data are presented as the mean ± SD, P value for values after versus before treatment. BMI: body mass index, HOMA- IR: homeostasis model assessment of insulin resistance, AdipoR1 adiponectin receptor 1, LH: luteinizing hormone, FSH: follicle-stimulating hormone.

Fasting insulin and HOMA-IR, as indicators of insulin resistance, were found significantly higher in PCOS patients than in healthy controls. The percentage of patients with insulin resistance was comparable among normal weight and obese PCOS, which agreed with previous results (21, 29).

Our findings suggest that lower adiponectin levels in PCOS patients could be due to insulin resistance, as significant negative correlations between adiponectin with fasting insulin and HOMA-IR were observed. HOMA-IR was found to be an independent predictor of adiponectin after adjusting for effects of other variables. Our results are in line with the systematic meta-analysis of Toulis et al. (29), who reported that the lower levels of adiponectin in PCOS were related to insulin resistance.

We present a novel data showing decreases in serum levels of AdipoR1 in PCOS patients. This was also related to insulin resistance, since significant inverse correlations of AdipoR1 with insulin levels and HOMA-IR were observed in these patients. It was reported in mouse models that expression of AdipoR1 is downregulated by insulin via the insulin/Foxo1 pathway (30).

Hyperandrogenemia is considered to be part of the metabolic abnormalities of PCOS (31). This was evident in our PCOS patients where the total testosterone concentration was significantly higher than in controls. In PCOS patients, adiponectin correlated negatively and significantly with total testosterone. After adjusting for effects of other variables, total testosterone was found to be an independent predictor of adiponectin levels.

Consistent with others (21), this study indicated that the observed hypoadiponectinemia in women with PCOS was also related to increased testosterone. It was demonstrated that androgen receptors were present in preadipocytes and adipocytes, particularly in visceral adipose tissue, possibly influencing the distribution of body fat. Therefore androgen excess increased abdominal deposition of fat in affected women (32), and administration of testosterone decreased secretion of adiponectin both in vitro (33) and in vivo (34). PCOS patients often showed a waist circumference higher than non-PCOS patients; also ultrasonographic studies demonstrated that the amount of visceral fat was significantly higher in PCOS patients than in weight-matched controls (35, 36). It is expected that adiponectin

levels must be higher with increasing adiposity because it is produced in adipocytes, predominantly in the visceral adipose tissue depot (37). Contradictory to this, in our PCOS subjects, although the measured waist circumference which was used as an indicator of abdominal adiposity was significantly higher than in controls, it correlated negatively with adiponectin levels. Panidis and colleagues (38) found lower adiponectin levels in obese PCOS women compared with lean PCOS women or lean healthy controls. These authors suggested that BMI, not insulin resistance, was the main determinant of adiponectin levels. Nevertheless, Orio's study (39) found comparable concentrations of adiponectin in obese PCOS as well as obese control subjects. It is possible that increased abdominal adiposity decreases adiponectin levels by increasing levels of tumor necrosis factor alpha that interferes with insulin receptor signaling, thus, suppressing adiponectin expression in adipose tissue (40). It is also possible that adiponectin concentrations are decreased in obesity because of insulin resistance in the adipocyte itself (41). Also a high molecular weight component of the serum would act as a downregulator of adiponectin concentration with a major effect on obese subjects compared with leaner subjects (42).

Oxidative stress found with increased adipose tissue has also been suggested to inhibit the expression of adiponectin (43). AdipoR-1 levels are also negatively correlated with waist circumference in our PCOS patients. This confirmed other data which found that omental and subcutaneous adipose tissue, AdipoR1 mRNA, was lower in obese subjects than in controls, and weight loss caused an up-regulation of the gene expression of the adiponectin receptors in human adipose tissue (44). In contrast to our findings, it was reported in one study that in women with PCOS there was an up-regulation of adiponectin receptors. The authors could not provide a mechanistic explanation for the up-regulation of adiponectin receptors seen in PCOS women but they concluded that it might resemble a pro-diabetic state (45).

Escobar-Morreale and San Millán (46) have proposed that PCOS patients suffer from a vicious circle consisting of androgen excess favoring abdominal deposition of fat, and the dysfunction of visceral adipose tissue results in hypoadiponectinemia, insulin resistance, and compensatory hyperinsulinism, which further facilitates androgen secretion by ovaries and adrenal glands. Moreover, we suggest from our data that lower serum levels of AdipoR 1 related to insulin resistance might result

in decreased adiponectin-binding capacity, and thus will further participate in this proposed vicious circle. Reduced adiponectin and/or AdipoR1 in PCOS patients could provide a link to an elevated risk for type 2 diabetes mellitus and cardiovascular disease in those patients (13,14,47).

Metformin is in widespread clinical use for treatment of PCOS-related symptoms. It improves insulin sensitivity and reduces circulating insulin concentrations in PCOS patients. The major effect of metformin is to reduce glucose production by the liver and improve hepatic insulin resistance (48). This drug may also attenuate the ovarian androgen response to gonadotropin stimulation, and decrease LH presumably by reducing hyperinsulinemia and restore normal menstrual cycles (49).

In our patients, metformin treatment resulted in a significant decrease in waist circumference, caused improvement in insulin sensitivity, and reduced circulating insulin levels which may affect ovarian androgen biosynthesis, thus significantly decreasing total testosterone levels. This agrees with previous studies (50,51). Metformin therapy also leads to significant increases in adiponectin levels confirming findings of a previous study that showed plasma adiponectin increased significantly in PCOS after metformin treatment (52). Contradictory to our results, Trolle et al (53) reported that metformin had no effect on adiponectin despite significant decreases in weight, fasting glucose, and insulin resistance. These different results could be due to different population characteristics including different race, higher BMI in our PCOS patients, and differences in dosage of metformin.

We also observed that AdipoR1 increased significantly after metformin treatment in PCOS women. It was previously reported that metformin clearly modifies adiponectin receptor expression in mice. This action takes place mainly in glucose utilizing tissues inducing a large increase in AdipoR1 and R2 expression in muscle more so for AdipoR1. Also, it stimulates AdipoR1 expression in white adipose tissue (30). Metformin could therefore exert its known beneficial metabolic effects and cardiovascular protective effects (54) by increasing adiponectin and AdipoR1 levels.

Limitations of the study include a small number of recruited subjects. We also were unable to use a double-blind placebo-controlled study design, as the recruited PCOS patients sought fertility evaluation and metformin was an indication, thus using a placebo would have raised

ethical issues.

In conclusion, our data supported that adiponectin and AdipoR1 are reduced in PCOS patients. Adiponectin and AdipoR1 are independently and negatively associated with insulin resistance. Short-term metformin treatment improved insulin sensitivity, and decreased testosterone levels. It resulted in increased adiponectin and AdipoR1 levels, which could be beneficial in increasing insulin sensitivity and decreasing cardiovascular risks in those patients. Controlled clinical trials measuring cardiovascular risks are needed to evaluate the benefits of metformin in PCOS patients and its relevance to adiponectin and adiponectin receptors.

Acknowledgements

This research was supported by Grant number 595 from the Deanship of Scientific Research College of Medicine, Qassim University, KSA.

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