Retinol-Binding Protein 4 in Polycystic Ovary Syndrome—Association with Steroid Hormones and Response to Pioglitazone Treatment

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Context: Polycystic ovary syndrome (PCOS) is frequently associated with insulin resistance.

Objective: The aim of the study was to investigate a putative role of the adipokines retinol-binding protein 4 (RBP4), adiponectin, and visfatin in a cohort of patients with PCOS and their response to treatment with pioglitazone.

Design and Setting: We conducted a randomized, controlled, double-blind study at a tertiary referral center.

Patients and Interventions: Forty premenopausal women with PCOS were allocated to receive treatment with either pioglitazone (30 mg/d) or a placebo for a period of 3 months.

Main Outcome Measures: Serum concentrations of RBP4, adiponectin, and visfatin were determined along with metabolic and hormonal parameters before and after treatment.

Results: Serum adiponectin concentrations were higher after treatment with pioglitazone ($P = 0.003$), whereas RBP4 levels tended to decrease ($P = 0.06$), and visfatin concentrations remained unchanged. We found RBP4 serum concentrations at baseline to be positively correlated with serum levels of testosterone ($R = 0.446$; $P = 0.005$), 17-OH progesterone ($R = 0.345$, $P = 0.037$), and dehydroepiandrosterone sulfate ($R = 0.347$; $P = 0.041$). However, these correlations were abolished after treatment with pioglitazone. Patients with high RBP4 levels had significantly higher hirsutism scores ($P = 0.038$ before and $P = 0.034$ after treatment). In contrast, serum adiponectin concentrations were related to parameters of impaired glucose metabolism, and no significant associations were detected for visfatin.

Conclusions: Our results suggest that RBP4 may contribute to endocrine changes and to the phenotypic manifestation of patients with PCOS because higher RBP4 concentrations are associated with higher androgen levels and higher clinical hirsutism scores independently of pioglitazone treatment. The molecular involvement of RBP4 in human steroid metabolism requires further clarification. **(*J Clin Endocrinol Metab* 94: 1229–1235, 2009)**

It is well acknowledged that both excessive leanness and obesity adversely affect female reproductive function (1). Overweight has become increasingly common and is associated with the polycystic ovary syndrome (PCOS) affecting approximately 6% of women at a reproductive age (2, 3). PCOS is a heterogeneous condition characterized mainly by hyperandrogenism, chronic anovulation, and infertility (4, 5). Although the precise pathogenesis of PCOS remains uncertain, a close link to insulin

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Abbreviations: AUC, Area under the curve; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; HOMA-IR, homeostasis model assessment-insulin resistance; IR, insulin resistance; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; PPAR, peroxisome proliferator-activated receptor; RBP4, retinol-binding protein 4.

resistance (IR) and consecutive hyperinsulinemia, impaired glucose tolerance, type 2 diabetes mellitus, atherogenic dyslipidemia, and visceral obesity has been well established (6). Like PCOS, IR is also associated with both obesity and lipodystrophy (7, 8). PCOS and obesity have a synergistic deleterious effect on glucose tolerance, but IR is found in nonobese PCOS patients as well, thereby contributing to the high cardiovascular risk known to occur in these women (9, 10).

More recently, several studies have demonstrated that effective reduction of IR induces regular menstrual cycles and improves fertility in patients with PCOS (11). This has been shown for administration of diazoxide (12), metformin (13, 14), and more recently also thiazolidinediones (15, 16). Pioglitazone is a thiazolidinedione derivative that has been approved for the treatment of type 2 diabetes and appears to have both antiinflammatory and antiatherosclerotic properties (17).

Excess visceral adipose tissue is thought to play a key role in mediating metabolic disturbances characteristic of IR (18). Several adipose tissue-derived hormones, named adipokines, such as leptin, adiponectin, visfatin, retinol-binding protein 4 (RBP4), or resistin, have been shown to facilitate changes in carbohydrate and lipid metabolism, thereby being involved in the pathogenesis of IR (19, 20). Serum concentrations of leptin, RBP4, and resistin are increased in insulin-resistant states, whereas inadequately low concentrations of adiponectin are characteristic of IR. The relationship of visfatin to IR remains controversial (20). It was therefore hypothesized that RBP4, adiponectin, and visfatin may be involved in the pathogenesis of PCOS. Hence, we aimed to investigate the effect of thiazolidinedione treatment on serum RBP4, adiponectin, and visfatin levels and potential relationships between these adipokines and parameters of glucose metabolism as well as endocrine changes in patients with PCOS. At present, no study dealing with PCOS was performed in which the role of those novel factors has been studied in a prospective randomized setting.

Subjects and Methods

Subjects and study protocol

Forty patients were included in the study, and the diagnosis of PCOS was established as described (15). Statistical power analysis was performed for the initial study assessing the metabolic and hormonal therapeutic properties of pioglitazone in patients with the PCOS, and therefore no additional statistical power calculation was performed. The present study was a follow-up investigation on that study. PCOS was diagnosed by the presence of 1) long-standing ovulatory dysfunction (oligo- or amenorrhea); 2) hirsutism (Ferriman-Gallwey score >7) and/or circulating serum total testosterone greater than 2.5 nmol/liter and SHBG concentrations less than 50 nmol/liter; and 3) exclusion of other endocrine disorders, such as thyroidal dysfunction, adrenal diseases, and hyperprolactinemia. Other exclusion criteria were desire for pregnancy or existing pregnancy, basal FSH concentration greater than 20 IU/liter, diabetes mellitus, past hysterectomy, intake of medication known or suspected to affect reproductive or metabolic function, history of liver disease and/or alcohol abuse, elevated liver enzymes, or severe uncontrolled illness. All subjects showed a polycystic appearance of the ovaries on transvaginal sonography. All potentially fertile patients were asked to use barrier methods of contraception during the entire study period. After having signed written informed consent, patients were requested to adhere to a written list of recommendations concerning a healthy diet and physical activity for weight maintenance during a period of 4 wk while knowingly receiving placebo (run-in phase). Thereafter, randomization was performed, and treatment with either 30 mg pioglitazone or placebo (identical tablets, taken once daily) was begun. Patients and physicians were blinded to the applied treatment. Every other week, each patient had an appointment with the treating physician; vital signs and body measurements [body mass index (BMI)] were determined, and serum was taken for the determination of progesterone concentrations. At each visit, compliance with the medication was checked by pill count. The study protocol was approved by the regional ethics committee.

Hormonal parameters

Venous blood samples were obtained after an overnight fast in cyclic women in the follicular phase (d 3–8) of the cycle, at the end of the run-in phase, and at the end of the treatment phase for measurement of serum concentration of total testosterone, SHBG, dehydroepiandrosterone sulfate (DHEAS), FSH, LH, progesterone, low-density lipoprotein, high-density lipoprotein, cholesterol, triglycerides, and liver enzymes. LHRH test with measurement of concentrations of LH and FSH after iv injection of 100 μg LHRH (Ferring, Wallisellen, Switzerland) was performed to assess pituitary function, and an oral glucose tolerance test (OGTT) was conducted to assess glucose tolerance, insulin sensitivity, and β-cell function [homeostasis model assessment-insulin resistance (HOMA-IR)]. Whole body insulin sensitivity was defined by the Matsuda index calculated as: 10,000/square root of [fasting glucose $\times$ fasting insulin] $\times$ [mean glucose $\times$ mean insulin during OGTT]. In addition, insulin resistance was calculated using HOMA-IR: fasting insulin (μmol/liter)$*$fasting glucose (mmol/dl)/22.5. The area under the curve (AUC) of insulin was calculated by integrating insulin levels obtained during OGGT. Serum samples were immediately stored at -70 C, and all measurements were performed after completion of the study (Laboratories Schönenbuch, Allschwil, Switzerland); serum 17-OH progesterone (reference range, follicular phase, 0.6–4.7 nmol/liter), LH (reference range, follicular phase, 0.4–12.6 IU/liter; Second NIBSC 80/552), FSH [reference range, follicular phase, 3.5–12.5 IU/liter; Second International Reference Preparation (World Health Organization) 78/549], testosterone (reference range, 2.7–2.9 nmol/liter), DHEAS (reference range, 2.7–9.2 μmol/liter), and insulin [reference range, 21–118 pmol/liter; First International Reference Preparation (World Health Organization) 66/304] were measured by electrochemiluminescence immunoassays (Roche, Rotkreuz, Switzerland). Liver enzymes, glucose, and lipids were measured using enzymatic methods (Roche Hitachi). The free androgen index (FAI) was calculated as: testosterone (nmol/liter) $\times$ 100/SHBG (nmol/liter). The occurrence of ovulation was assessed for each patient by serial measurement of serum progesterone in combination with self-reported menstruation. Ovulation was defined as progesterone levels exceeding 9 nmol/liter with consecutive menstruation after 2 wk as an indicator of ovulation. The inaccuracy of the test systems (interassay coefficient of variation) was on average less than 5%.

Adipokine measurement

Sera of women with PCOS treated according to the study protocol were analyzed. Serum RBP4 was measured by an ELISA (Immundiagnostic, Bensheim, Germany) that was used in groups 1 and 3 of the original studies showing an association of RBP4 with IR (21). The concentrations of adiponectin and visfatin in the serum were measured using the Human Adiponectin/Acrp30 immunoassay (R&D Systems GmbH, Wiesbaden- Nordenstadt, Germany) and Human Visfatin Elisa Kit (AdipoGen, Brisbane, Australia), respectively. Intra- and interassay coefficients of variation of immunoassays were less than 8%. All measurements were performed before and after treatment with pioglitazone.

Statistical methods

The Gaussian distribution of all parameters was confirmed by Kolmogorov-Smirnov tests. The efficacy of treatment (placebo *vs.* pio-

glitazone; within-subject effects before *vs.* after treatment) was compared between the two study groups by ANOVA with repeated measurements; ovulation rates were compared by χ^2 tests. Multivariate regression analysis was performed to calculate independent associations between variables. Paired *t* test was performed to compare adiponectin, visfatin, and RBP4 concentrations before and after pioglitazone treatment. Data are the mean ± SEM. Data analysis was performed using the statistical software package SPSS for Windows (SPSS, Inc., Chicago, IL).

Results

Clinical, metabolic, and hormonal characteristics of the study cohort

The characteristics of the study cohort and the effect of pioglitazone treatment on hormonal and metabolic parameters have been published previously (15), and these results are summarized in Table 1.

Thirty-five (87.5%) of the initially enrolled 40 patients completed the trial, and data were available for analyses. Treatment with pioglitazone resulted in a decrease in both fasting serum insulin concentrations and AUCs of serum insulin during the OGTT ($P < 0.02$). Consistent with these results, we observed an increase in calculated insulin sensitivity ($P < 0.05$) and a decrease in estimated insulin secretion ($P < 0.02$) compared with placebo. Thus, the expected metabolic effects of pioglitazone treatment were confirmed. Waist/hip ratio, hirsutism, and BMI remained unchanged during the course of the study in both groups. In the pioglitazone treatment group, 41.2% of the patients presented with the laboratory and clinical signs of ovulatory menstrual cycles (*i.e.* three ovulations during the study period) as compared with 5.6% of subjects treated with placebo ($P < 0.02$).

Adiponectin

We found that serum adiponectin concentrations increased in response to pioglitazone treatment ($P = 0.003$; Fig. 1A), whereas they remained unchanged in the placebo group ($P = 0.985$; Fig. 1B). We found correlations between baseline adiponectin concentrations and serum triglycerides ($R = -0.338$; $P = 0.05$), high-density lipoprotein cholesterol ($R = 0.571$; $P < 0.001$), response to oral glucose tolerance testing (AUC; negative correlation; $P < 0.05$ at 30, 60, 90, and 120 min), and insulin sensitivity index ($R = 0.445$; $P = 0.009$). Serum insulin concentrations (AUC) during OGTT at the end of the treatment period correlated negatively with serum adiponectin levels at 60, 90, and 120 min ($P < 0.05$). Serum baseline and end of treatment glucose and insulin concentrations were found to be inversely linked to baseline adiponectin levels ($P < 0.05$). IR, as calculated by HOMA-IR, was not statistically correlated with adiponectin concentrations at baseline, but was after treatment ($R = -0.362$; $P = 0.038$).

We did not observe associations between adiponectin and steroid hormone parameters at baseline. However, although FSH serum concentrations in response to LHRH stimulation were not related to adiponectin levels at baseline, the response after treatment was significantly negatively associated with adiponectin levels ($R > -0.45$; $P < 0.02$ at 0, 20, 30, and 60 min, respectively), reflecting the simultaneous increase of serum adiponectin concentration and the improvement in LHRH reactivity in the treated group. Moreover, the increase of adiponectin serum concentrations in response to treatment correlated with ovulation rates ($R = 0.468$; $P = 0.011$).

Visfatin

We found that in both treatment groups the serum visfatin concentrations underwent no significant changes (Fig. 1, C and D). Although visfatin is considered to be involved in the pathophysiology of IR, we did not observe any statistically significant association between serum visfatin concentration and metabolic parameters. Furthermore, we did not detect correlations be-

TABLE 1. Clinical, metabolic, and hormonal parameters of the study cohort before and after pioglitazone treatment

Parameter	Pioglitazone (n = 17)		Placebo (n = 18)	
	Before	After	Before	After
Age (yr)	30.2 ± 1.4		30.6 ± 1.1	
BMI (kg/m^2)	29.4 ± 1.7	30.1 ± 1.7	27.5 ± 1.2	27.7 ± 1.2
Waist/hip ratio	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0
Hirsutism (Ferriman-Gallwey score)	15.5 ± 1.2	15.6 ± 1.3	15.6 ± 2.0	15.8 ± 2.8
Fasting plasma glucose (mmol/liter)	4.8 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	5.0 ± 0.1
Glucose AUC (mmol/liter · min)	789.9 ± 53.3	694.2 ± 42.9[a]	804.3 ± 52.6	788.8 ± 40.6
Fasting serum insulin (pmol/liter)	68.3 ± 9.7	53.2 ± 5.3[b]	47.8 ± 4.6	62.0 ± 8.4[a]
Insulin AUC (pmol/liter · min)	51,615.3 ± 6,910.5	33,506 ± 3,732[a,b]	37,731 ± 4,380	40,050 ± 4,680
HOMA index (mIU/liter/mg/dl)	16.1 ± 2.1	12.6 ± 1.1[a,b]	11.6 ± 1.1	14.1 ± 1.7
Insulin sensitivity index (mmol^{-1}/pmol^{-1}/liter)	16.3 ± 3.5	19.6 ± 2.7[c]	16.9 ± 2.0	15.2 ± 1.8
Serum triglycerides (mmol/liter)	1.2 ± 0.1	1.08 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
Serum cholesterol (mmol/liter)	4.8 ± 0.2	4.6 ± 0.1	4.8 ± 0.2	4.7 ± 0.2
DHEAS (μmol/liter)	5.4 ± 0.6	5.8 ± 0.7	6.3 ± 0.6	6.8 ± 0.5
Testosterone (nmol/liter)	2.4 ± 0.3	2.1 ± 0.2	2.8 ± 0.2	2.5 ± 0.2[a]
SHBG (nmol/liter)	36.8 ± 4.3	40.8 ± 3.3[b]	40.9 ± 3.5	35.8 ± 4.0[a]
FAI (U)	9.3 ± 2.2	6.4 ± 1.2[b]	8.5 ± 1.6	9.8 ± 2.0

[a] $P < 0.05$ *vs.* baseline (by ANOVA).

[b] $P < 0.02$ pioglitazone *vs.* placebo (by ANOVA).

[c] $P < 0.05$ pioglitazone *vs.* placebo (by ANOVA).

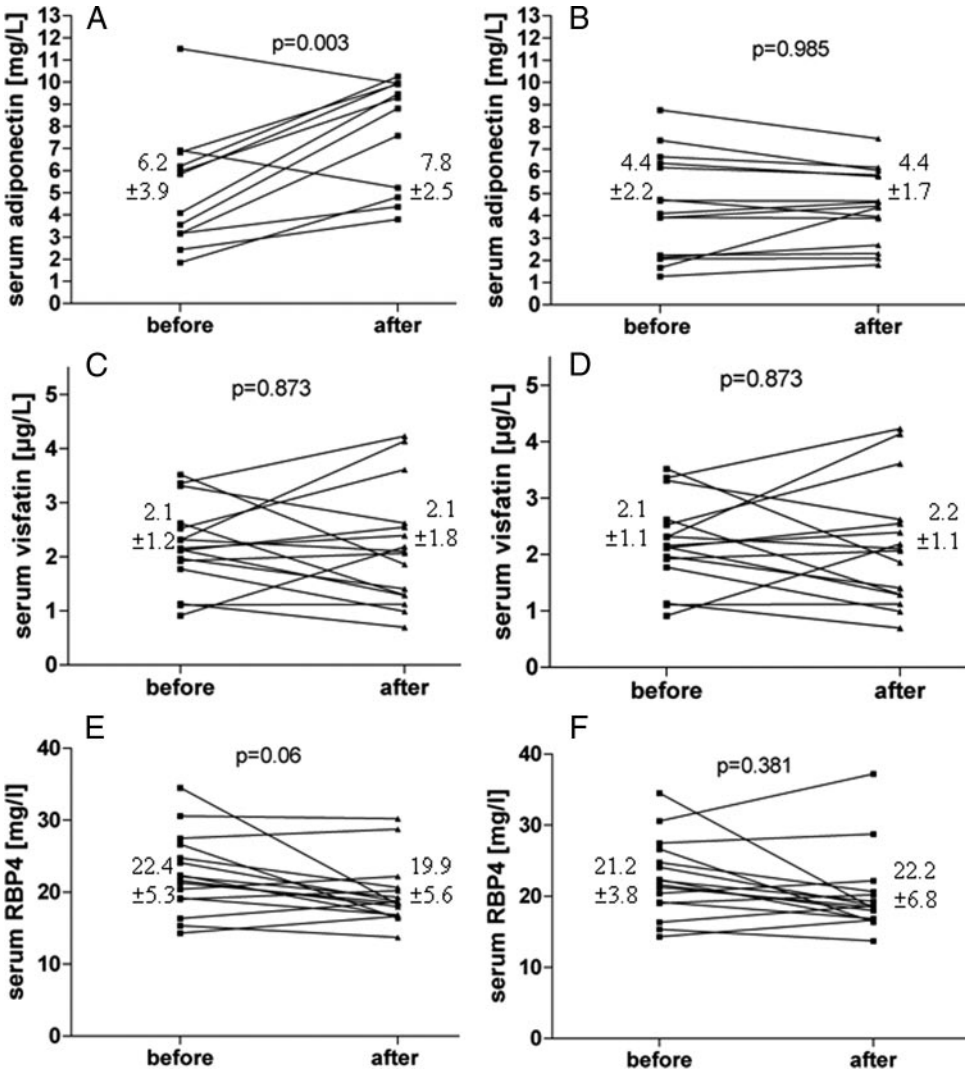

FIG. 1. Adipokine serum concentrations before and after pioglitazone treatment. Diagrams depict the serum concentrations of the adipokines adiponectin (A and B), visfatin (C and D), and RBP4 (E and F) in the treatment group and in patients receiving placebo. Paired *t* test was performed to compare adiponectin, visfatin, and RBP4 concentrations before and after pioglitazone treatment.

tween serum visfatin concentration and any of the hormonal parameters associated with PCOS.

RBP4

We found that serum RBP4 levels tended to decrease in response to treatment with pioglitazone ($P = 0.06$), whereas no change was found in the placebo group ($P = 0.381$; Fig. 1, E and F). The change of RBP4 serum concentration did not reach statistical significance, perhaps due to the limited number of patients in our analysis. At baseline (all patients combined), RBP4 serum concentrations were positively associated with serum concentrations of 17-OH progesterone (R = 0.354; $P = 0.037$), DHEAS (R = 0.347; $P = 0.041$), and testosterone (R = 0.466; $P = 0.005$). Because insulin is related to both RBP4 and steroid hormones, potentially confounding effects of insulin were addressed by multivariate regression analysis. However, significant associations of RBP4 with testosterone ($P < 0.001$), DHEAS ($P = 0.008$), and 17-OH progesterone ($P = 0.042$) were maintained after adjustment for insulin. None of these parameters correlated with the concentration of RBP4 after pioglitazone treatment. The FAI correlated with serum RBP4 concentrations (R = 0.406; $P = 0.016$), and this correlation remained present after pioglitazone treatment (R = 0.438; $P = 0.009$). As demonstrated previously, no changes in serum DHEAS and testosterone concentrations were found in response to pioglitazone treatment in this cohort; however, treatment was associated with an increase in SHBG, resulting in a decrease in the FAI ($P < 0.05$). We therefore examined whether patients with low and high serum RBP4 concentrations differed with regard to the phenotypical expression of PCOS. The study population was divided into two groups using the median level of 22 mg/dl as cutoff value. We found that 17 patients with serum RBP4 concentrations ranging from 15.2 – 21.6 mg/dl had lower hirsutism scores than 18 patients with serum RBP4 concentrations ranging from 22.1–31.6 mg/dl ($P = 0.038$ before treatment; $P = 0.034$ after treatment; Fig. 2, A and B). Moreover, the patients with higher initial hirsutism scores had a more pronounced reduction in serum RBP4 concentrations in response to pioglitazone treatment (R = 0.629; $P = 0.009$). Baseline serum FSH concentrations were negatively correlated with RBP4 (R = −0.334; $P = 0.050$). However, the

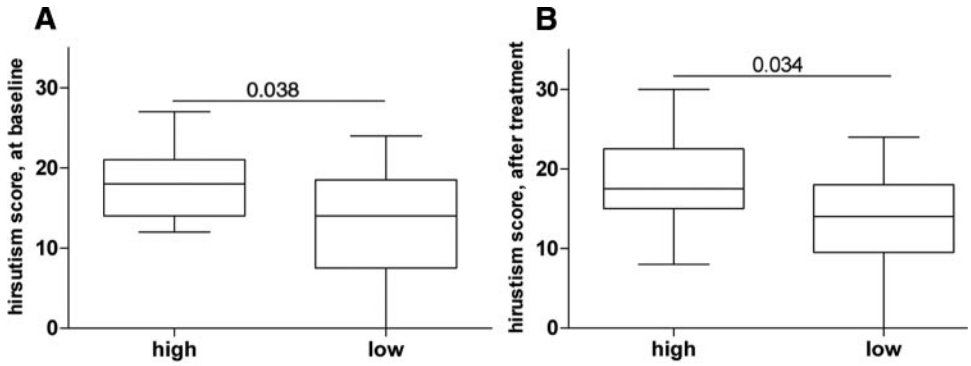

FIG. 2. Hirsutism scores at baseline (A) and after treatment (B) in patients with high *vs.* low RBP4 serum concentrations. Low, Patients with low RBP4 serum concentrations (n = 17); high, patients with high serum RBP4 concentrations (n = 18). Values are depicted as means (*horizontal lines*), 25th and 75th percentiles (*boxes*), and minimum/maximum ranges. Calculations for statistically significant differences between the groups were performed by ANOVA.

response to the LHRH stimulation test was not related to serum RBP4 concentrations at 20, 40, or 60 min. No correlation was found between serum RBP4 and SHBG concentrations. Although serum RBP4 levels tended to decrease in response to pioglitazone treatment, we did not observe significant associations with parameters of impaired glucose metabolism such as fasting glucose, response to oral glucose tolerance testing, and baseline and end-of-treatment insulin. After adjustment for BMI and high-density lipoprotein cholesterol, HOMA-IR as an indicator of IR was weakly correlated to RBP4 ($R = 0.342$; $P = 0.049$). End-of-treatment RBP4 was weakly linked to ovulation rates ($R = -0.331$; $P = 0.063$).

Discussion

As conditions linked to IR like PCOS become increasingly common, it is an important agenda of research to elucidate the complex pathophysiological interplay between excess adipose tissue, IR, and endocrine changes. Furthermore, overweight and hirsutism along with impaired reproductive function may substantially affect the quality of life (22). Over the past few years, adipokines have emerged as an intensively studied group of serum proteins and have been identified as key players in mediating changes between adipocytes and organs affected in IR (20, 23). A close relationship between IR and the PCOS has been firmly established (24, 25). Moreover, distinct changes in steroid hormone metabolism such as increased androgen and decreased SHBG concentrations are typical of PCOS. It is therefore relevant to investigate the contribution of various adipokines in facilitating such diverse pathophysiological alterations.

In our double-blind, randomized, placebo-controlled study, we aimed at elucidating the potential roles of the recently identified adipokine RBP4 together with adiponectin and visfatin in PCOS. Various studies have been performed investigating the potential contribution of adipokines to the pathogenesis of PCOS. Particularly the key adipokine leptin has been shown to be involved in the pathogenesis of PCOS (26). Adiponectin has been extensively investigated recently, and a role in mediating insulin sensitivity in PCOS patients has been well defined as is the case for other conditions associated with IR or obesity (27–32). Adiponectin affects glucose and lipid metabolism and displays antiinflammatory properties (33). In line with previous investigations (34), we found that the serum concentrations of adiponectin rose in response to treatment with the peroxisome proliferator-activated receptor (PPAR)-γ agonist pioglitazone. Similarly, serum adiponectin concentrations correlated with several markers of glucose metabolism in our patients, and an amelioration of insulin sensitivity was observed along with higher adiponectin concentrations after treatment with pioglitazone. In our patients, HOMA-IR correlated with adiponectin only after treatment with pioglitazone, but not at baseline. Our study cohort consisted of young PCOS patients with a relatively homogenous metabolic profile, including a low level of IR. We suggest that small differences within the cohort at baseline account for the fact that no association was detected between adiponectin and HOMA-IR. After pioglitazone treatment, however, adiponectin increased in the treatment group and remained unchanged in the placebo group, which might explain why high adiponectin was then linked to lower IR in the correlation analysis. A close relationship of adiponectin with markers of IR has been conclusively demonstrated in several previous reports. Thus, our results are in line with observations in similar patient groups. Earlier studies identified adiponectin as a potential contributor to IR in PCOS patients but did not find associations with endocrine characteristics of PCOS. Likewise, we did not observe associations between adiponectin and parameters of endocrine abnormalities in our PCOS patients, except for a striking correlation with the response to LHRH after pioglitazone treatment. Romualdi *et al.* (35) treated PCOS women with pioglitazone 45 mg/d and obtained similar results. It has been demonstrated that treatment of obese women with insulin-sensitizing agents results in a decrease of serum LH concentrations (36–38). Lowering of insulin levels reduces pituitary LH secretion as evidenced by *in vitro* studies in cultured rat pituitary cells (39, 40). Interestingly, we observed a decrease of LH levels in the placebo group along with a slight reduction of serum insulin levels, further supporting a link between insulin and pituitary LH biosynthesis. Hence, our results confirm that adiponectin is involved in IR in PCOS patients.

Previous studies demonstrated the presence of higher visfatin serum levels in PCOS compared with healthy controls (41, 42). In our design, we investigated changes in visfatin levels in the serum of women with PCOS treated with pioglitazone. However, we did not detect any change of visfatin serum levels after

treatment. Therefore, the role of visfatin in PCOS remains unclear.

In contrast, our results suggest that the adipokine RBP4 may be involved in endocrine perturbations characteristic of PCOS, whereas few correlations of RBP4 were found with the indices of IR. The few relationships between RBP4 and IR have been assessed differently in PCOS patients. Although some investigators found an association of RBP4 with IR or obesity (43), this was not confirmed in another well-designed study (44). In addition, in a study by Hahn *et al.* (45), RBP4 serum concentrations were related to BMI or fat mass in PCOS patients and controls but were not significantly elevated in PCOS *per se*. In contrast to our investigation, study subjects were recruited according to less stringent criteria (45), which would likely result in a broader spectrum of PCOS and would therefore also include subjects who could be regarded "closer to normal." Moreover, the fact that only a minority of our patients was diagnosed as insulin resistant may explain the lack of an association between RBP4 and parameters of IR.

Tan *et al.* (46) showed that steroid hormones, particularly 17-OH-estradiol, were capable of inducing adipose tissue RBP4 biosynthesis, thereby demonstrating that altered expression of steroid hormones may contribute to metabolic perturbations in PCOS patients. In that study, RBP4 and 17-OH-estradiol serum concentrations were compared between controls and PCOS patients. Although the number of patients in our cohort was small, we similarly found a link between steroid hormone levels and RBP4 among the group of patients with PCOS that remained significant even after adjustment for insulin. This finding highlights that the relationship between RBP4 and steroid hormones is not only different between controls and PCOS subjects, but can also be found across the spectrum of PCOS manifestations. This makes RBP4 an attractive target for further research with regard to the pathophysiological mechanisms linking PCOS steroid perturbations and obesity/IR. It is noteworthy that treatment with the PPAR-γ agonist pioglitazone appeared to lower RBP4 serum concentrations ($P = 0.06$) and to improve IR but left testosterone and DHEAS levels unchanged.

Pioglitazone treatment abolished this relationship between RBP4 and steroid hormones both in the treatment group and in all patients combined. Thus, we demonstrate that high RBP4 levels are present in patients with high androgen serum concentrations. Pioglitazone treatment leaves androgen concentrations unchanged but decreases RBP4. Several indirect lines of evidence may explain why the close relationship between RBP4 and steroid hormones was no longer observed after pioglitazone treatment. In cultured ovarian cells, pioglitazone influences ovarian steroid hormone metabolism by up-regulation of progesterone biosynthesis and inhibition of testosterone and estradiol production via insulin-dependent and -independent pathways (47). Glitazones contribute to ovarian insulin sensitization by up-regulation of insulin receptor and insulin receptor substrate-1. Insulin-independent effects of PPAR-γ agonists may be mediated by influencing steroidogenic acute regulatory protein expression (48). Additionally, pioglitazone is capable of inhibiting aromatase-mediated estrogen biosynthesis from testosterone, which would increase serum testosterone concentrations (49). Thus, the multifaceted and partially opposing actions of glitazones on steroid metabolism may explain why no change in androgen concentrations was observed after the treatment period.

Moreover, we found that RBP4 levels were linked to hirsutism scores in our patient cohort and that patients with low RBP4 levels also had lower hirsutism scores, but that hirsutism scores did not change after treatment with pioglitazone. Thus, our findings reflect the potential of glitazones to reduce IR in PCOS patients but questions the ability of glitazones to target some clinical manifestations of steroid abnormalities in PCOS. Pioglitazone treatment enhanced ovulatory rates and resulted in an increase of SHBG levels along with a decrease of the FAI, whereas no effect on androgens and Ferriman-Gallwey hirsutism scores was noted in our patients. It is well established that current treatment modalities are unlikely to show beneficial effects on androgen levels and hirsutism within 3 months of treatment. Thus, the observation time of our study group comprising patients with pronounced endocrine abnormalities may have been too short for assessing the effects of glitazones on all endocrine abnormalities.

In summary, we confirm the potential benefit of pioglitazone treatment in improving serum concentrations of adiponectin and RBP4 leading to a more insulin-sensitive profile in young women with PCOS. We describe an intriguing relationship of the recently identified adipokine RBP4 with several androgens and progesterone. The response to treatment with pioglitazone suggests that steroid hormones have the potential to influence IR through a modification of RBP4 expression. Molecular mechanisms and clinical relevance of this relationship still need to be elucidated.

Acknowledgments

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