Circulating levels of adipose products and differences in fat distribution in the ovulatory and anovulatory phenotypes of polycystic ovary syndrome

Central fat distribution is increased in anovulatory women with polycystic ovary syndrome (PCOS) compared with ovulatory PCOS and matched controls. Among secreted adipocytokines, this is reflected mainly in lower levels of adiponectin. (Fertil Steril® 2009;91:1332–5. ©2009 by American Society for Reproductive Medicine.)

Most women with polycystic ovary syndrome (PCOS) have increased fat quantity and altered fat distribution with increased abdominal adipose tissue (1). In addition, several studies have shown that women with PCOS exhibit an alteration in the secretion of adipose products that may contribute to insulin resistance and cardiovascular risk (2–4). Among these alterations, particularly important is the reduction in circulating adiponectin levels, which may play a significant role in the early endothelial abnormalities of young women with PCOS (4–9).

Some initial reports have found elevated levels of serum visfatin (10–12) and retinol-binding protein 4 (RPB4) (13, 14) compared with control women of similar body weight, although the data have not been entirely consistent. Although the finding of increased RBP4 may be expected because of the known increased insulin resistance of women with PCOS (13, 15), the cause for the elevation of visfatin, if present, remains unclear.

Differences in results may be related to heterogeneity among the patients studied with PCOS, given the expanded diagnostic criteria for the diagnosis of PCOS, which includes ovulatory hyperandrogenic women (14, 15), and anovulatory normoandrogenic women (14). It is known that some of these phenotypes have milder insulin resistance and reduced markers of cardiovascular risk (16–18).

Our study assessed fat distribution and the circulating levels of several well-established (adiponectin and leptin) and newer (visfatin and RPB4) adipocytokines in women with PCOS with different phenotypes as well as in matched controls. Our aim was to determine if fat distribution is altered in women with different phenotypes, and whether there are changes in adipocytokine secretion which in turn may relate to insulin resistance.

Forty-eight women of reproductive age with PCOS, all referred to the Palermo Endocrine Unit because of androgen excess and who were seen consecutively, were included in our study. The diagnosis of PCOS was based on the presence of clinical or biologic hyperandrogenism associated with chronic anovulation and/or the finding of polycystic ovaries on ultrasound scan (15). In all patients, adrenal enzymatic deficiency (by measurement of serum 17-hydroxyprogesterone), Cushing syndrome, or androgen secreting tumors were excluded.

Biochemical hyperandrogenism was defined as serum testosterone (T) >60 ng/dL and/or free T $\geq$3 pg/mL and/or serum dehydroepiandrosterone sulfate (DHEAS) $\geq$3000 μg/L. That these values signify hyperandrogenism has been reported previously in our population using the same assays (19).

Anovulation was defined as serum progestogen (P) <3 ng/mL. In patients with normal menses, at least two consecutive menstrual cycles were studied, and the finding of a low serum P (<3 ng/mL) level in both cycles indicated the presence of anovulation. This study included both anovulatory (n = 34) and ovulatory (n = 14) women with PCOS (16, 17).

The presence of polycystic ovaries was determined by transvaginal sonography. The finding of increased ovarian size (20) and/or of at least 12 follicular cysts measuring 2 to 9 mm were considered indicative of the presence of polycystic ovaries (21).

The study design included a medical examination, biochemical analyses, and dual energy x-ray absorptiometry (DEXA) determinations of fat quantity and distribution. Excluded from the study were women with renal or hepatic diseases, and type II diabetes; no women received any medications for at least 3 months before the study. Fat quantity was determined by total body DEXA using a similar Instrument (QDR-Discovery; Hologic, Bedford, MA). Total fat quantity and quantity of fat in the trunk area were measured. Quantity of fat also was determined as the central

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abdominal area (R1 area), an area of 50 cm^2 around the central point of the midline between the lateral iliac crests and the lowest rib margin at the end of a normal expiration (the same midline used for waist circumference) (1). The central point of R1 area did not always correspond to the umbilicus. To assess fat distribution, percentage of total fat in the trunk and R1 area were calculated.

As controls, we selected a group of 20 healthy women matched for age and body mass index (BMI) using the same exclusion criteria described above. Controls were women with regular menses, no clinical or biologic hyperandrogenism, and normal serum progesterone levels (>7 ng/mL day 22 of the cycle). No patients or controls had a BMI higher than 40.

In all women with PCOS and in normal controls, during the follicular phase (days 5 to 8), a fasting blood sample was obtained between 8:00 and 9:00 AM for measurements of luteinizing hormone (LH), estradiol, testosterone, leptin, adiponectin, visfatin, RBP4, insulin, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides as well as c-reactive protein (CRP) (22–25). Insulin resistance was calculated by the Quantitative Insulin-Sensitivity Check Index (QUICKI) (26). In all hormonal assays, the intra-assay coefficient of variation was <6%, and the interassay coefficient of variation was <15%.

Adiponectin and leptin were measured by enzyme-linked immunosorbent assay (ELISA) systems purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX). Visfatin was measured by a visfatin C-terminal (human) enzyme immunoassay (Phoenix Pharmaceuticals, Belmont, CA) with a sensitivity of 3 ng/mL. Human recombinant full-length visfatin had a 100% cross-reactivity. Retinol-binding protein 4 was measured by ELISA (ALPCO Diagnostics, Windham, NH) with a sensitivity of 0.9 ng/mL.

The protocol and procedures were approved by the local institutional review board, and all patients consented to participate in this study.

Statistical analyses were performed using Statview 4.5 (Abacus Concepts Inc., Berkeley, CA) and SPSS 9.0 for PC (SPSS Inc., Chicago, IL). Univariate analyses were performed using Student's unpaired *t*-test for the numeric variables, and the differences in the prevalence of the nominal variables were analyzed by chi-square test. Analysis of covariance (ANCOVA) was used to assess the differences in fat quantity and distribution and in the biochemical parameters between subgroups of patients and controls. Correlation analyses were performed using the Spearman rank correlation method. All data are expressed as mean ± standard deviation (SD).

Patients with PCOS and matched controls were of similar age (27.3 ± 6.4 years vs. 27.7 ± 4.5 years) and BMI (27.1 ± 5.6 vs. 26.8 ± 3.9), and had a similar total fat quantity (26446 ± 9158 g vs. 26667 ± 9136 g). Trunk fat quantity (10938 ± 5179 g vs. 10890 ± 4058 g), percentage of trunk fat (39.6 ± 5.6% vs. 38.6 ± 4.4%), and R1 fat quantity (728 ± 350 g vs. 654 ± 245 g) were similar in the two groups whereas waist circumference (91.1 ± 13.9 cm vs. 85.5 ± 5.4 cm, $P<.05$) and percentage of R1 fat (2.66 ± 0.54% vs. 2.36 ± 0.24%, $P<.01$) were statistically significantly higher in women with PCOS.

The women with PCOS had increased levels of LH, T, insulin, lower QUICKI, lower HDL cholesterol, and higher triglycerides (all $P<.01$) compared with matched controls.

Leptin was similar in both groups, but circulating adiponectin was lower ($P<.01$), and visfatin ($P<.01$), RBP4 ($P<.05$), and CRP ($P<.01$) were higher in women with PCOS compared with matched controls. Serum leptin correlated positively and adiponectin negatively ($P<.01$) with all fat parameters ($P<.01$). Serum visfatin and RBP4 did not correlate with any fat parameter.

Retinol-binding protein 4 correlated with levels of serum LH ($r = 0.35$, $P<.05$) and estradiol ($r = 0.40$, $P<.05$), and these correlations were independent of BMI or fat quantity. No other correlations between adipose products and gonadotropin or steroid values were found.

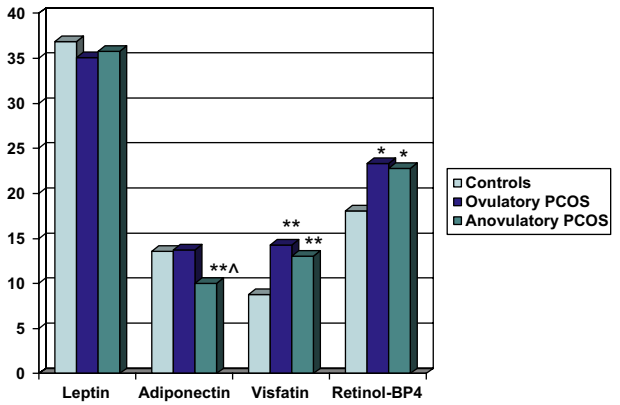
In classic (anovulatory) PCOS, waist circumference was statistically significantly larger (93.2 ± 14.9 vs. 86 ± 8.5 cm, $P<.05$) and trunk fat, R1 fat, percentage of trunk fat, and percentage of R1 fat were statistically significantly greater ($P<.05$) than in ovulatory women with PCOS. Compared with controls, ovulatory PCOS patients had similar fat parameters, including waist circumference, percentage of trunk fat, and percentage of R1 fat, whereas patients with classic PCOS, despite being of similar BMI and having similar total fat, had larger waist circumferences ($P<.05$) and higher trunk R1 fat and percentage of trunk and percentage of R1 fat. Patients with classic PCOS had higher insulin ($P<.01$), lower QUICKI ($P<.01$) values, and higher LH levels than the ovulatory group, and their levels were higher compared with matched controls.

C-reactive protein was higher in classic PCOS versus ovulatory PCOS. Leptin, visfatin, and RBP4 were similar in the two subgroups of women with PCOS, but adiponectin was statistically significantly lower in the anovulatory group (Fig. 1). Comparing the serum levels of the adipocytokines in both phenotypes with those of the matched controls, it was observed that classic (anovulatory) PCOS patients had increased serum visfatin and RBP4 and lower levels of adiponectin while ovulatory PCOS patients had higher serum visfatin and RBP4 but similar levels of adiponectin compared with matched controls (see Fig. 1).

Our study assessed for the first time whether there are differences in body fat distribution as well as in certain adipocytokines between women with classic and ovulatory phenotypes, and whether these parameters may relate to

FIGURE 1

Comparison of serum levels of the adipocytokines in ovulatory and anovulatory polycystic ovary syndrome with controls. *$P<.05$ versus controls. **$P<.01$ versus controls. **^$P<.005$ versus ovulatory PCOS.



* p<.05 versus controls ** p<.01 versus controls **^ p<.005 versus ovulatory PCOS

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differences in insulin sensitivity. We were limited in our ability to assess insulin sensitivity other than by measures of QUICKI, which in our hands correlates well with other methods (27).

Our data suggest that fat distribution is normal in women with ovulatory PCOS and is similar to that found in controls when matched for total fat and BMI. Anovulatory women with classic PCOS have greater abdominal adiposity in spite of similar BMI. Our patients with ovulatory PCOS who had normal fat distribution had insulin levels and insulin sensitivity that were similar to those of matched controls. Previous data have found women with ovulatory PCOS to have insulin levels that were slightly but significantly higher compared with controls, but lower than in patients with classic PCOS (17). Other studies, however, have reported normal insulin levels in this subgroup of patients with PCOS (18, 28). It is clear that there is heterogeneity in the degree of insulin sensitivity in women with PCOS who have ovulatory function, and this may relate to the specifics of the group studied, including variations such as age, BMI, ethnicity, and the method for determining insulin sensitivity (as already noted). However, in spite of normal fat distribution and insulin parameters, women with ovulatory PCOS were found to have increased levels of visfatin and RBP4, which were similar to those found in classic PCOS.

The findings of elevated visfatin in PCOS, in spite of a lack of correlation with fat mass or insulin, is intriguing and needs to be studied further. This relationship also existed for RBP4, which showed an interesting and novel small but statistically significant correlation between RBP4 and LH as well as with estradiol. It is anticipated that more work in this area will be forthcoming in that these newer assays are currently available commercially.

However, adiponectin was statistically significantly decreased in women with the classic syndrome but not in women with ovulatory PCOS (see Fig. 1). Serum adiponectin correlated with fat distribution, confirming that abdominal fat is perhaps the principal factor influencing adiponectin secretion (4).

We have shown that patients with ovulatory PCOS have a similar fat distribution to matched controls but altered levels of visfatin and RBP4. The relationship of the latter more novel observations to insulin dynamics in PCOS remains to be determined. Our data further underscore the significant metabolic and hormonal differences in these two phenotypes of PCOS, warranting caution in the interpretation of studies that do not differentiate between various types of patients.

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