Association of abdominal obesity, insulin resistance, and oxidative stress in adipose tissue in women with polycystic ovary syndrome

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Objective: To study the expression of insulin signaling–related genes and oxidative stress markers in the visceral adipose tissue obtained from polycystic ovary syndrome (PCOS) patients and healthy control subjects and to investigate the relationships among abdominal obesity, insulin resistance, and oxidative stress at the tissue level.
Design: Case-control study.
Setting: University teaching hospital.
Patient(s): In total, 30 PCOS patients and 30 healthy control subjects, who underwent laparoscopic surgery, were included in the study.
Intervention(s): Abdominal obesity was defined based on waist circumference (WC). The homeostasis model index was used to assess insulin resistance (HOMA-IR).
Main Outcome Measure(s): Gene expression of glucose transporter 4 (GLUT4) and insulin receptor substrate 1 (IRS1) in visceral adipose tissue (VAT) and the parameters of oxidative stress, such as superoxide dismutase, enzyme glutathione reductase, and dimethylarginine, were measured, and the expression of protein oxidative damage product 3-nitro-tyrosine residues (nitrotyrosine) in VAT was identified with the use of immunohistochemistry.
Result(s): PCOS was associated with lower expression of GLUT4 and IRS1 and a higher level of oxidative stress in VAT, which was strongly correlated with WC and HOMA-IR. Presence of abdominal obesity further intensified the correlations observed in our measurements. The nitrotyrosine expression in VAT was stronger in PCOS patients.
Conclusion(s): The strong correlation of insulin resistance with oxidative stress at the VAT level suggests that local oxidative stress and abnormalities of insulin signaling in adipose tissue play critical roles in the pathogenesis of PCOS. (Fertil Steril® 2014;102:1167–74. ©2014 by American Society for Reproductive Medicine.)
Key Words: Polycystic ovary syndrome (PCOS), insulin resistance, oxidative stress, abdominal obesity, waist circumference (WC), visceral adipose tissue (VAT)

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Polycystic ovary syndrome (PCOS) is the most common complex endocrine disorder, affecting 4%–12% women of fertile age around the world (1). Recent studies have reported its prevalence to be as high as 12%–21% (2, 3). In addition, PCOS has major reproductive (leading cause of anovulatory infertility), psychologic (anxiety and depression) (4), and metabolic (increased type 2 diabetes mellitus and cardiovascular risk factors) (5) impact. Thus, it represents a substantial health burden. Insulin resistance is a central characteristic of PCOS in the majority of affected women (6), driving both hyperandrogenism and clinical features. Although the exact cause of

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PCOS remains to be established, most studies have demonstrated that abdominal obesity, insulin resistance (IR), and oxidative stress are of vital importance for its pathogenesis (7, 8).

Obesity plays a very important role in the development of PCOS in women. As many as 42% of women with PCOS were reported to be overweight or obese in population-based studies in the United States (9). The dysfunction of adipose tissue can lead to diabetes and metabolic syndrome. It is well known that adipose tissue is not only an energy storage organ but also an important endocrine organ (10). The cytokines secreted by fat cells are associated with chronic inflammation during obesity (11). Recently, a significant finding reported that compared with subcutaneous adipose tissue (SAT), the visceral adipose tissue (VAT) had a more direct connection with metabolic diseases (12–14).

Insulin resistance is another key biochemical feature of PCOS. Based on noneuglycemic-hyperinsulinemic clamping data, the prevalence of IR has been reported to range from 50% to 70% in women with PCOS (15, 16). Using the glucose infusion rate (GIR) on euglycemic-hyperinsulinemic clamping as a marker of insulin sensitivity, Stepto reported that IR was present in 75% of lean PCOS case subjects, 62% of overweight control subjects, and 95% of overweight PCOS case subjects (17). Studies have found that the numbers of insulin receptor in fat cells and activity are normal in PCOS patients but that the level of autophosphorylation of insulin receptors is reduced (18). In normal physiologic conditions, insulin-stimulated glucose uptake mainly occurs through the insulin-sensitive glucose transporter 4 (GLUT4) in muscle and fat tissue. Chen et al. found that insulin sensitivity decreases in PCOS patients owing to decreased expression of GLUT4 as caused by overexpression of miR-93 (19). Insulin receptor substrate 1 (IRS1) is another protein critical for transmission of insulin signaling, via phosphorylation of insulin receptor, and it plays important roles in metabolic regulation. Early studies have demonstrated that low GLUT4 and IRS1 protein expression in fat cells is an early sign of IR in fat tissue (19–22). Carvalho et al. demonstrated that low IRS1 expression in fat cells is associated with low GLUT4 protein expression and impaired insulin-stimulated glucose transport in IR patients, indicating that low expression of GLUT4 and IRS1 may be causatively related to IR (21).

Recent evidence indicates that oxidative stress also contributes to metabolic diseases, such as IR (23), and may be a leading aspect of the pathogenesis of PCOS (7, 24). Cell metabolism products, such as reactive oxygen species (ROS) derived from molecular oxygen, including oxygen ions, free radicals, and peroxides, contribute to macromolecular oxidation, including protein, lipid, and DNA oxidation. Dimethylarginine (MDA) is a by-product of lipid peroxidation and therefore regarded to be a good marker of oxidant-mediated damage (25). On the other hand, ROS production is a two-way balanced process in which oxidant formation and antioxidant defense molecules are involved, including total superoxide dismutase (T-SOD), the only natural antioxidant enzyme, and glutathione peroxide (GPx), which catalyzes H_2O_2 to water and oxidized glutathione, which, indirectly, can reflect the body's antioxidant activity. T-SOD, GPx, and MDA have been used for evaluating oxidative stress (7). However, studies addressing the circulating markers of oxidative stress in women with PCOS have yielded controversial results (7, 8), and few studies have measured these parameters in fat tissue. Considering the central role of fat cells in the development of oxidative stress, it would be meaningful to determine whether oxidative stress markers in fat tissue might be more sensitive markers compared with circulating markers.

Given the central role of visceral adipose deposits, IR, and oxidative stress in the development of PCOS in obese women, we undertook the present study to measure GLUT4 and IRS1 mRNA expression and oxidative stress indexes of VAT obtained from women with or without PCOS at the time of laparoscopic surgery. We further analyzed the relationships among abdominal obesity, IR, and oxidative stress in patients with PCOS.

SUBJECTS AND METHODS

Subjects

PCOS patients who failed induction of ovulation and did not become pregnant after clomiphene treatment for three cycles (age 24.93 ± 2.43 y) and 30 race- and body mass index (BMI)–matched endocrine infertility control subjects undergoing laparoscopic surgery because of hydrosalpinx, salpingemphraxis, or fallopian tube adhesion (age 28.30 ± 4.19 y) were recruited to our hospital. The control groups were also excluded of acute salpingitis and endometriosis. The diagnosis of PCOS was based on the 2003 American Society for Reproductive Medicine Rotterdam diagnostic criteria (1), excluding hyperprolactinemia and other endocrine diseases, such as thyroid disease, Cushing syndrome, congenital adrenal hyperplasia, and ovarian or adrenal tumors that generate hyperandrogenism. Patients were excluded if they had a family history of diabetes or had not used hormone drugs for 3 months.

Demographic features of patients, such as weight, height, and waist circumference (WC) were recorded before the experiment. BMI was calculated with the use of the following formula: weight (kg)/[height (m) squared]. Omental adipose tissue (one part of VAT) was collected at the beginning of the operation (within 30 minutes) from all subjects. The following tests were performed for all participants, who were in the early follicular phase of the menstrual cycle (the first 3–5 days of menstruation without dominant follicles according to B-ultrasound for irregular menstruation) and were instructed to follow a fast overnight (8–12 h) and rest for 30 minutes before their blood draw: 1) biochemical measurements, including serum fasting glucose levels and fasting insulin levels with the use of the hexokinase method (ADVI12400; Siemens) and a chemiluminescence immunoassay method (Advia Centaur; Siemens) separately; and 2) hormonal measurements, including total testosterone with the use of an RIA method. Although the criterion standard for measurement of IR is the euglycemic clamping test, owing to technical difficulties we preferred to use the homeostasis-model assessment of insulin resistance (HOMA-IR) score [fasting insulin (FINS; mIU/L) × fasting plasma glucose (FPG)/22.5] instead (26, 27). For FPG, Bio-Rad Liquid Unassayed Multiqual was used for internal control; for FINS, Bio-Rad Lyphochek Immunoassay plus control was used for internal control; the variability of FPG was <3% and the