Endocannabinoid system activation may be associated with insulin resistance in women with polycystic ovary syndrome

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Objective: To assess the levels of endocannabinoids and cannabinoid receptors (CB) 1 and 2 in women with polycystic ovary syndrome (PCOS).
Design: Case-control study.
Setting: University teaching hospital.
Patient(s): In total, 20 women with PCOS and 20 healthy women in a control group, who were matched for body mass index and age, were enrolled in this study.
Intervention(s): The homeostasis model index was used to assess insulin resistance.
Main Outcome Measure(s): Omental adipose tissue and human peripheral blood mononuclear cells (PBMCs) from PCOS and the controls were analyzed using real-time polymerase chain reactions for the expressions of CB1 and CB2. The levels of endocannabinoids were analyzed using high-performance liquid chromatography.
Result(s): The levels of anandamide and 2-arachidonoylglycerol, and the expression of CB1 and CB2 mRNA (messenger ribonucleic acid) in the PBMCs were significantly higher in the women with PCOS than in the women serving as controls. We found that expression of CB1, but not CB2, in adipose tissue was significantly higher in the women with, vs. without, PCOS. The expressions of CB1 mRNA and endocannabinoids showed a significant positive correlation with 2-hour glucose and insulin levels 2 hours after glucose loading in the PBMCs and adipose tissue.
Conclusion(s): Activation of endocannabinoids and overexpression of cannabinoid receptors, especially CB1, may be associated with insulin resistance in women with PCOS. (Fertil Steril® 2015;104:200–6. ©2015 by American Society for Reproductive Medicine.)
Key Words: Endocannabinoids, cannabinoid receptor, adipose tissue, human peripheral blood mononuclear cells

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries on ultrasonography (2004). This condition affects 5%–10% of women of reproductive age (1), and several studies have shown that 50% of women with PCOS meet the criteria for metabolic syndrome (2, 3), and as

many as 60% of affected women are obese. The condition is frequently associated with insulin resistance, accompanied by compensatory hyperinsulinemia, which has been reported to increase the risk of developing type 2 diabetes mellitus in these patients by five- to eightfold, compared with weight-matched women serving as controls (3). Several studies have shown that even lean women with PCOS have elevated insulin resistance, compared with women serving as controls, matched on body mass index (4, 5).

Endocannabinoids are defined as endogenous agonists of cannabinoid receptors, which are derived from phospholipids (anandamide [AEA], 2-arachidonoylglycerol [2-AG]) and have complex effects on body weight and metabolic regulation (6, 7). The endocannabinoid system is comprised of 2 G-protein–coupled cannabinoid receptors: CB1 and CB2 (6). Expression of CB1 occurs in the brain, gastrointestinal organs (8), skeletal muscles (9), and adipose tissue (10); whereas CB2 is predominantly expressed in peripheral immune cells (11).

Endocannabinoids increase food intake and promote weight gain by activating central endocannabinoid receptors (12–15). Mice with CB1 knockout are lean, and resistant to diet-induced obesity (16). The CB1 receptor antagonist SR141716A (rimonabant) has been reported to induce a transient reduction of food intake in mice (17, 18). A recent report indicates that SR141716A may restore the secretion of adiponectin to normal levels in lipopolysaccharide-treated mature adipocytes (19).

In obese or hyperglycemic type 2 diabetic patients, circulating levels of AEA and 2-AG have been reported to be higher, compared with those in lean or healthy-weight women serving as controls (20, 21). Plasma level of AEA was found to be significantly enhanced in both women who have anorexia and those who have a binge-eating disorder (22). Furthermore, adipose tissue messenger ribonucleic acid (mRNA) levels of CB1 have been reported to be lower in obese subjects (20).

In addition, the mRNA expressions of both CB1 and fatty acid amide hydrolase have been reported to be higher in visceral than in subcutaneous fat depots (21). However, no studies have been conducted to assess circulating levels of endocannabinoids in women with PCOS, compared with those in healthy women. In this study, we investigated the peripheral endocannabinoid system, namely CB1 and CB2 expression, in visceral adipose tissues, from 20 women with PCOS and 20 healthy women.

MATERIALS AND METHODS

Subjects

Twenty women who met the inclusion criteria for PCOS (detailed later) were enrolled in this study. All were in good health and had not taken oral contraceptives within the preceding 3 months. The protocol was reviewed and approved by the Institutional Review Board (IRB) of Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, under IRB number 20120818R. All of the patients gave written informed consent before enrolling in this study.

The consensus criteria reported by the Rotterdam Group (23) were used to define the clinical, laboratory, and ultrasound criteria for PCOS. The clinical criteria included chronic anovulation or oligomenorrhea (menstrual interval >6 weeks) or amenorrhea (no menstrual loss for >3 months) from menarche. The biochemical criterion was an elevated total serum testosterone (T) level ($\geq$0.8 ng/mL). The ultrasound criteria were enlargement of the ovaries, with an increase in stroma and the presence of $\geq$12 follicles in each ovary measuring 2–9 mm in diameter, and/or an increased ovarian volume (>10 ml) on transvaginal ultrasonography (24). Serum prolactin and thyroid hormone levels were checked in all patients and found to be within healthy limits. Cushing syndrome, congenital adrenal hyperplasia, and androgenic tumors were excluded, using appropriate testing.

Twenty healthy women, matched on body mass index and age, served as controls. None was hirsute, and all had a healthy, regular menstrual period cycle. None was taking oral contraceptives; all had ovaries that had a healthy, nonpolycystic appearance on ultrasound, and did not have elevated luteinizing hormone (LH) or follicle-stimulating hormone (FSH) levels (healthy range for LH and FSH: 5–8 mIU/mL). None of the women had an elevated androgen level.

Oral Glucose Tolerance Test, Fasting Glucose to Insulin Ratio, and Homeostasis Model Insulin Resistance Index

A 2-hour oral glucose tolerance test, with a 75-g glucose load, was performed during the early follicular phase (days 3–7) for all of the women after they had fasted overnight. In the amenorrheic women, progesterone (P) was given, to induce menstrual bleeding. Four blood samples were collected from the antecubital vein, at 0, 60, and 120 minutes, and the serum was stored at −20°C until it was assayed for glucose and insulin. The homeostasis model insulin resistance index (HOMA-IR) was calculated, using the following formula (25): [fasting glucose (mg/dl) × fasting insulin (μIU/ml)]/405. A HOMA-IR value of $\geq$3.8, or a fasting glucose to insulin ratio of $\leq$4.5 has been reported to indicate insulin resistance in patients with PCOS (26).

Hormone Profile

Blood was drawn from the antecubital vein of all participants during the early follicular phase, to measure serum levels of estradiol (E_2), FSH, LH, and T. Women who had PCOS with amenorrhea were given a 75-mg intramuscular dose of P, to induce menstrual bleeding, and the blood samples were collected on cycle day 3 or 4. Progestins are commonly given to induce menstrual bleeding, because their effect has been reported to mimic the status of the early follicular phase after menstrual bleeding.

Serum levels of FSH, E_2, T, and LH were measured by immunoassay, using Immulite kits (Beckman Coulter Inc.). The FSH sensitivity was 0.2 mIU/mL; the intra- and interassay coefficients of variance were 7.7% and 7.9%, respectively. The corresponding values were, respectively, 0.2 mIU/mL, 6.5%, and 7.1% for LH; 15 pg/mL (55 pmol/L), 6.3%, and 6.4%, for E_2; and 0.1 ng/mL, 4.0%, and 5.6% for T. The E_2 and T assays have been validated against liquid chromatography tandem mass spectrometry determinations.