# Antioxidant properties of high-density lipoproteins are impaired in women with polycystic ovary syndrome

Jinxia Zhang, M.D.,[a] Yujin Zhang, M.D.,[b] Hongwei Liu, M.D.,[b] Huai Bai, M.D., Ph.D.,[a] Ying Wang, M.D.,[b] Changan Jiang, Ph.D.,[a] and Ping Fan, M.D., Ph.D.[a]

[a] Laboratory of Genetic Disease and Perinatal Medicine, Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, West China Second University Hospital, Sichuan University; and [b] Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China

**Objective:** To determine the relationships among the inflammatory index, intrinsic oxidation levels, lipid and apolipoprotein (apo)A-I concentrations of high-density lipoprotein (HDL), and polycystic ovary syndrome (PCOS).
**Design:** Cross-sectional study.
**Setting:** University hospital.
**Patient(s):** A total of 425 patients with PCOS and 441 control women were included.
**Intervention(s):** None.
**Main Outcome Measure(s):** The HDL inflammatory index (HII) was determined using a cell-free fluorometric assay. Intrinsic HDL oxidation levels, HDL-free cholesterol, HDL-cholesterol ester, HDL-triglyceride, serum apoA-I, and malondialdehyde levels were also measured.
**Result(s):** The mean HII value and the frequency of HII $\geq 1$ were significantly higher in the PCOS group ($0.77 \pm 0.54$, 27.1%) than in the control group ($0.53 \pm 0.37$, 8.4%). These values were also higher in each of the 4 PCOS phenotypes based on the Rotterdam criteria than in the controls, and higher in patients with hyperandrogenism (HA) + oligo- and/or anovulation (OA) phenotype than in those with OA + polycystic ovary (PCO) phenotype. Furthermore, patients with PCOS with OA + PCO had lower malondialdehyde and intrinsic HDL oxidation levels compared with those with HA. Multivariate regression analysis demonstrated that PCOS, HDL-cholesterol ester, and $E_2$ levels were the main predictors of HII value.
**Conclusion(s):** The impairment of HDL antioxidant/anti-inflammatory function in PCOS is related to HA status, increased oxidative stress, and abnormalities in HDL components and thus may contribute to PCOS pathogenesis and increase the risks of future cardiovascular diseases. (Fertil Steril® 2015;103:1346–54. ©2015 by American Society for Reproductive Medicine.)
**Key Words:** Polycystic ovary syndrome, dysfunctional high-density lipoprotein, oxidative stress, inflammation, dyslipidemia

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Polycystic ovary syndrome (PCOS) is a common female endocrine and metabolic disorder that affects 6%–15% of women of reproductive age (1). Polycystic ovary syndrome is often associated with long-term health risks, such as obesity (2), dyslipidemia (3), increased oxidative stress (4, 5), chronic inflammation (6), elevated metabolic syndrome (MS), and type 2 diabetes risks (7). Several studies have also demonstrated an increase in subclinical atherosclerosis in PCOS, as measured by carotid artery intima media thickness, coronary artery calcium scores, and endothelial dysfunction (8). A systematic review and meta-analysis has revealed a significant twofold risk of coronary artery disease and stroke for patients with

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J.Z. and Y.Z. should be considered similar in author order.
Reprint requests: Ping Fan, M.D., Ph.D., Sichuan University, Chengdu, West China Second Hospital, Laboratory of Genetic Disease and Perinatal Medicine, Sichuan 610041, China (E-mail: fanping15@scu.edu.cn).

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PCOS relative to women without PCOS, not fully depending on body mass index (BMI) (9). However, few prospective studies have examined nonfatal and fatal cardiac events in women with well-defined PCOS (8, 9). The pathogenesis of PCOS remains unclear, but studies have suggested that PCOS has a complex, multifactorial etiology resulting from the interactions between genetic, environmental, and intrauterine factors (10).

Studies have indicated that atherosclerosis is a chronic inflammatory disease of the arterial wall that is mediated in part by oxidized low-density lipoproteins (LDLs) (11, 12). High-density lipoproteins (HDLs) possess many important atheroprotective functions. In addition to mediating reverse cholesterol transport, they also have antioxidant, anti-inflammatory, antithrombotic, and antiapoptotic activities (11, 13). In mouse and human, the main component of HDL, apolipoprotein (apo)A-I, is capable of removing "seeding molecules" of lipid peroxidation from LDL, thus preventing further oxidation of LDL-derived phospholipids and dramatically reducing the inflammatory properties of LDL (11). High-density lipoprotein–associated antioxidant enzymes, such as paraoxonases (PON1, PON3) and platelet-activating factor acetylhydrolase (PAF-AH), can prevent or destroy the formation of oxidized lipids (11). High-density lipoprotein also seems to be the major carrier of lipid hydroperoxides in plasma and may transport oxidized cholesteryl esters to the liver for excretion (14, 15). Through these distinct mechanisms, HDLs degrade and remove oxidized lipids and decrease atherosclerosis risk (14). The antioxidant/anti-inflammatory activities of HDLs are dependent on the presence of HDL-binding apo and antioxidant enzymes (11, 16). Modification of these proteins, such as oxidation, may weaken the antioxidant/anti-inflammatory activities of HDLs and even convert them from anti-inflammatory to proinflammatory particles (11).

The inflammatory/anti-inflammatory properties of HDL could be evaluated by a cell-free assay measuring the HDL inflammatory index (HII) (11, 17–19). The HII represents the net action of all components in HDL, including oxidized phospholipids, lipid hydroperoxides, HDL-associated antioxidases and apolipoproteins, serum amyloid A, and antioxidant vitamins (11). The impaired antioxidant/anti-inflammatory activities of HDL have been associated with acute coronary syndrome (20) and type 2 diabetes (17).

Mounting data have demonstrated that patients with PCOS are associated with abnormal circulating markers of oxidative stress, such as increased malondialdehyde (MDA), homocysteine, asymmetric dimethylarginine, and superoxide dismutase activity, as well as decreased glutathione levels and PON1 activity (4). Our previous studies demonstrated that serum apoA-I levels, HDL-associated PAF-AH (H-PAF-AH), and apoE-containing H-PAF-AH activities were lower, and LDL-associated PAF-AH (L-PAF-AH) activities as well as the ratio of L-PAF-AH to H-PAF-AH activities were higher in patients with PCOS (3, 5, 21). We observed that the PAF-AH G994T gene mutation, which completely abolishes PAF-AH activity, and the PON1 Q192R gene variation are the risk factors for PCOS (22, 23). These results suggest that impaired antioxidant activities of HDL may be associated with increased oxidative stress in women with PCOS. In this study, we investigated the relationships among the HII, intrinsic oxidation levels, and lipid and apoA-I concentrations of HDL and PCOS.

## MATERIALS AND METHODS

### Subjects

Women with or without PCOS, aged 20–40 years, were recruited during 2006–2013 from the Outpatient Clinic of Reproductive Endocrinology, West China Second University Hospital, Sichuan University. All participants gave their informed consent, and the study was approved by the institutional review board of the West China Second University Hospital, Sichuan University.

Polycystic ovary syndrome was defined by the presence of two or more of the following features based on the revised 2003 Rotterdam diagnostic criteria (24): oligo- or anovulation (OA), which was assessed as oligomenorrhea (fewer than eight cycles per year); biochemical and/or clinical hyperandrogenism (HA), which was assessed by total T (TT) levels above the 95th percentile (2.60 nmol/L) of the levels that were detected in a group of normal menstruating women with normal cycles (22); clinical presence of obvious acne, which was defined as the number of comedones (>10) and inflammatory papules/pustules, as well as nodules/cysts (>10) spread on the face, back, and chest (3, 25, 26) and/or hirsutism with a modified Ferriman–Gallwey (F-G) score of more than 6 (3, 27, 28); polycystic ovaries (PCOs) were confirmed if there were 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10 mL) by ultrasonic examination, with exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting carcinomas, and Cushing's syndrome. All of the control women had regular menstrual cycles (between 21 and 35 days), exhibited normal circulating androgen levels, the absence of obvious acne or hirsutism on physical examination, and normal ovarian morphology as determined by ultrasound.

Subjects were excluded if they met one of the following criteria: [1] clinically evident chronic or acute diseases, such as infection, tumors, thyroid dysfunction, cardiovascular disease, endometriosis, hyperprolactinemia, hypogonadotropic hypogonadism, or premature ovarian failure; [2] pregnant or in the luteal phase according to P measurement (>9.54 nmol/L); [3] taking medication known to affect the metabolism of carbohydrates, lipids, or hormones within 3 months before the study; [4] smokers; [5] diabetes patients with fasting glucose $\geq$7.0 mmol/L and/or 2-hour glucose $\geq$11.1 mmol/L.

Clinical and anthropometric variables, including waist circumference, hip circumference, waist-to-hip ratio, BMI ($kg/m^2$), systolic and diastolic blood pressure (SBP and DBP), and the degree of hirsutism and acne, were evaluated in all of the subjects. Ultrasound ovarian volume was also assessed using the formula for the volume of an ellipsoid (29): 0.523 × length × width × thickness.

Blood samples were obtained in the morning after an overnight fast on the 3rd–10th days of the menstrual cycle