Association of heme oxygenase-1 with the risk of polycystic ovary syndrome in non-obese women

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STUDY QUESTION: Is circulating heme oxygenase-1 (HO-1) associated with the risk of polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: Lower circulating HO-1 is associated with a higher risk of PCOS in non-obese women, in a dose-related manner.

WHAT IS KNOWN ALREADY: PCOS is one of the most common endocrine disorders in women of reproductive age, with increasing worldwide incidence. HO-1 plays a crucial role in many physiological systems, with potent anti-inflammatory, antioxidant and antime-tabolic properties.

STUDY DESIGN, SIZE, DURATION: This hospital-based case–control study included 80 women with PCOS and 80 healthy control women seen at the Reproductive Center of Tongji Hospital (Wuhan, China) from November 2011 to May 2012. Cases and controls were frequency-matched on age and BMI and were enrolled into the study once written informed consent had been obtained.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Serum hormones, glucose, insulin and lipid concentrations were measured using an automated platform. Correlation coefficients and multiple linear regression models were calculated in the combined group (both cases and controls) using serum HO-1 concentration as the independent variable and age and BMI as covariate variables to explore the association between HO-1 and the pathophysiology of PCOS. To examine the independent association of serum HO-1 levels with the likelihood of PCOS, multivariate logistic analysis was used. The strength of the association was tested further by receiver-operating characteristic (ROC) curve models, with or without the addition of HO-1.

MAIN RESULTS AND THE ROLE OF CHANCE: Compared with controls, women with PCOS were found to have significantly increased insulin resistance (IR), oxidative stress (OS) and inflammation levels, creating a vicious circle of effects in the pathophysiology of PCOS. However, serum HO-1 was negatively associated with this vicious circle. Women with the highest tertile of HO-1 (≥5.29 ng/ml) had an odds ratio (OR) of PCOS of 0.02 (95% CI 0.0034–0.07) compared with women with the lowest quartile (<3.14 ng/ml) (P < 0.01). This trend remained after adjustment for potential confounders in the multivariable model (all P < 0.01). ROC analysis based on an existing prognostic model yielded significantly discriminative values for PCOS, with or without the addition of HO-1 (areas under the curves were 0.86 (95% CI 0.81–0.92) versus 0.95 (95% CI 0.92–0.98); P for difference = 0.0005).

LIMITATIONS, REASONS FOR CAUTION: It is difficult to establish a time-integrated measure of circulating HO-1 during the progression of PCOS and these findings should be confirmed in large-scale studies involving different ethnic groups. Moreover, the study lacks measurements of glycated hemoglobin (HbA1c) to provide an index of blood glucose concentrations over time.

† The authors consider that the first two authors should be regarded as joint first authors.
Heme oxygenase-1 and polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is an exceptionally common endocrine disorder in women of reproductive age, with a prevalence of up to 10% (Fauser et al., 2012). Increased insulin resistance (IR) is viewed as a central feature of PCOS irrespective of body mass index (BMI) (Teede et al., 2010; Fauser et al., 2012). IR combined with compensatory hyperinsulinemia, which is frequently encountered in PCOS patients, increases the risk of developing type 2 diabetes mellitus (T2DM), metabolic disorders and cardiovascular disease (Moran and Teede, 2009; Moran et al., 2010). Although the etiology of the syndrome remains enigmatic, enhanced oxidative stress (OS) in the form of lipid peroxidation and DNA oxidative damage has been noted in previous studies of PCOS (Murri et al., 2013). Moreover, evidence exists to suggest that low-grade chronic inflammation also contributes to the development of PCOS and its complications (Escobar-Morreale et al., 2011; Gao et al., 2013).

Heme oxygenase-1 (HO-1), also known as heart shock protein 32 (Hsp32), is a ubiquitously expressed inducible enzyme that catalyzes the decomposition of heme into carbon monoxide (CO), biliverdin and Fe²⁺, which have potent anti-inflammatory, antioxidant, and antimitabolic properties (Kim et al., 2011). Though HO-1 is expressed at low levels in most tissues under normal basal conditions, it is highly inducible in response to various pathophysiological stresses (Grochot-Przeczek et al., 2012). Numerous studies have indicated that HO-1 induction is an adaptive defense mechanism to protect cells and tissues against injury in many disease settings, which makes it a biomarker for early diagnosis of clinical conditions such as T2DM (Bao et al., 2010), Alzheimer’s disease (Butterfield et al., 2012) and Silicosis (Sato et al., 2006). However, much less is known about whether circulating HO-1 concentrations are related to PCOS.

Mounting evidence suggests that obesity induces OS in humans and plays a pivotal role in inflammatory processes relevant to cardiovascular risk in women with PCOS (Kelly et al., 2001). However, 20–50% of the women with PCOS are either normal weight or lean (Fauser et al., 2012), and may be subject to a different PCOS pathophysiology than obese women (Nestler and Jakubowicz, 1997). Therefore, this study was designed to delineate and quantify the association between serum HO-1 concentrations and PCOS while taking into account the effect of important confounding factors. Additionally, the discriminative value of models for PCOS without or with serum HO-1 was also evaluated by receiver-operating characteristic (ROC) analysis.

Materials and Methods

Participants

The study was conducted in Wuhan (Hubei, China) between November 2011 and May 2012. Through an advertisement in the hospital newspaper of the Reproductive Center of Tongji Hospital, subjects who received no medical treatment were invited to undergo a screening test after an overnight fast. The screening included medical history, clinical examinations and ultrasound as previously described (Gao et al., 2013). A total of 160 females including 80 patients with PCOS and 80 healthy control women participated in this investigation (age range 21–38 years; BMI range <30 kg/m²). To minimize confounding factors, we focused our study on one center and one ethnic group.

Controls were primarily selected from women who visited the Reproductive Center of Tongji Hospital for routine health checkup. The control women did not have a history of diagnosed diabetes or endocrinological or autoimmune disorders and were age and BMI frequency-matched with the subjects. All controls were monitored by menstrual calendar for 6 months and by urinary luteinizing hormone (LH) testing for 1 month before the study to establish the regularity of their cycles (every 21–35 days) and had proven fertility. None exhibited clinical or diagnostic evidence of PCOS during examinations. No participants were taking any medication known to affect sex hormone metabolism, such as combined oral contraceptives, lipid-lowering agents or insulin sensitizer, within the latest 3 months.

Definition of PCOS

The diagnostic criteria for PCOS were defined according to the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus 2003 (ESHRE/ASRM) (Fauser et al., 2012): oligo- or anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries (by ultrasound measurement of ovarian volume and number of antral follicles). Patients were diagnosed as having PCOS on the basis of their clinical, laboratory and ultrasound outcomes, the results of which satisfied at least two of the three PCOS diagnostic criteria listed above. Given that PCOS is a diagnosis of exclusion, conditions including thyroid dysfunction and hyperprolactinemia were excluded biochemically, and more rare conditions were also excluded clinically (Cushing’s syndrome, virilizing tumors, congenital adrenal hyperplasia, etc.).

Collection of data

A trained examiner who was masked to the status of each participant conducted in-person interviews with all subjects using a self-administrated, structured questionnaire. To avoid delay and to minimize nonparticipation, information about age, body weight and other personal data (such as history of diseases, menstrual cycle) were collected at the time of the checkup.

WIDER IMPLICATIONS OF THE FINDINGS: Circulating HO-1 that provides protection against IR, OS and chronic inflammation is markedly reduced in non-obese women with PCOS. Low serum HO-1 is suggested as an independent risk factor for PCOS; thus, circulating HO-1 levels may be a novel biomarker for PCOS in young, non-obese women.

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Key words: polycystic ovary syndrome / insulin resistance / oxidative stress / inflammation / heme oxygenase-1
Laboratory investigations
Venous blood samples were taken at the early follicular phase, defined as Day 3–5 of a menstrual cycle for the control group or a spontaneous bleeding episode for the PCOS group and collected in vacutainer tubes. All subjects were sampled in the morning between 7:30 and 9:30 a.m. under fasting conditions. Sera were decanted into cryovials and frozen at −80°C until analysis.

Assays for serum hormone, insulin and lipid concentrations were performed as previously described (Gao et al., 2013). IR was determined by a number of different methods including fasting insulin (FI), the homeostasis model assessment of β cell function (HOMA-β) and insulin resistance (HOMA-IR) (Matthews et al., 1985). The estimate of IR by HOMA score was calculated using the formula: HOMA-IR = [22.5 (fasting glucose (FG) (mmol/l)) − 3.5], HOMA-IR = FI (mmol/l) × FI (μIU/l)/22.5. Serum HO-1 and HO-2 concentrations were determined from a fasting sample using a specific ELISA technique (Enzo Life Sciences, mIU/l)/22.5. Serum HO-1 and HO-2 concentrations were determined from a fasting sample using a specific ELISA technique (Enzo Life Sciences, Farmingdale, NY, USA) in accordance with the manufacturer’s recommendations. The respective intra- and inter-assay coefficients of variation were 5.5 and 9.7% for total testosterone (TESTO), 5.6 and 8.5% for prolactin (PRL), 1.6 and 3.2% for follicle stimulating hormone (FSH), 2.8 and 4.1% for LH, and 3.4 and 6.2% for estradiol (E2). All samples were measured in duplicate, and results are expressed as mean ± SD from those experiments.

Statistical analysis
Deviation of the data from a normal distribution was examined using the Shapiro–Wilks test of normality. Comparisons between PCOS cases and controls were made using Student’s t-test or Mann–Whitney U-test when appropriate. Spearman or Pearson correlation coefficients were calculated according to the distribution of relevant variables to estimate the interrelationship between HO-1 and the variables of interest. After adjusting for age and BMI, partial correlation coefficients were calculated. To examine the independent association between serum HO-1 levels and the likelihood of PCOS, a multivariate logistic regression model was used. Statistical analyses were performed using SPSS for windows software version 12.0 (SPSS, Inc., Chicago, IL, USA). A two-tailed P-value of <0.05 was considered significant.

Finally, we investigated the addition of serum HO-1 levels to an existing risk prediction model of PCOS (Palmert et al., 2002; Wilgen et al., 2009; Gao et al., 2013) and the subsequent impact on discrimination quantified by the area under the receiver–operating characteristics curve (AUC of ROC). This portion of the statistical analysis was performed using Stata 11.0 (Stata Corp., College Station, TX, USA).

Ethical approval
Study protocols were approved by the review boards of Tongji Medical College Ethics Committee. Not until written informed consent was obtained from each participant could she enter the study. At the end of the current study, each patient was provided with educational materials, advised to implement lifestyle changes as appropriate and underwent routine treatment.

Results
Clinical characteristics and metabolic parameters
The clinical features as well as baseline hormonal and metabolic parameters of the study population, according to status, are summarized in Table I. The levels of serum HO-1 were significantly lower in patients with PCOS when compared with controls (3.20 ± 0.96 ng/ml and 5.31 ± 1.14 ng/ml, respectively, P < 0.01). There were significant differences in TESTO, LH and LH-to-FSH ratio between the PCOS group and the control group, whereas there was no difference in PRL, E2 or FSH levels. In regard to metabolic profile, serum triglyceride (TG) was statistically higher in PCOS patients than controls, whereas no differences in cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were observed between groups. At the same time, the PCOS group had significantly higher HOMA-IR values, but notably lower HOMA-β values.

Because C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) are key predictors of inflammation, we measured their serum levels to determine whether there was chronic low-grade inflammation in PCOS. As shown in Fig. 1, the CRP (Fig. 1A) and TNF-α (Fig. 1B) levels in the PCOS group were markedly elevated relative to the corresponding control group (3.8 ± 0.89 ng/ml versus 3.0 ± 0.22 ng/ml, P < 0.01, and 131 ± 15.6 pg/ml versus 122 ± 8.9 pg/ml, P < 0.01, respectively). It is generally accepted that malondialdehyde (MDA), 8-Hydroxy-desoxyguanosine (8-OHdG) and nitric oxide (NO) are major peroxide products of lipids, arginine and DNA that serve as biomarkers of OS. In order to understand the role of OS in the pathogenesis of PCOS, we also monitored serum levels of MDA (Fig. 1C), 8-OHdG (Fig. 1D) and NO (Fig. 1E), which were all significantly higher in PCOS patients than in healthy controls (5.9 ± 1.70 nmol/l versus 3.2 ± 1.30 nmol/l, P < 0.01, 1.7 ± 0.28 ng/ml versus 1.3 ± 0.26 ng/ml, P < 0.01 and 89 ± 26.5 μmol/l versus 57 ± 23.1 μmol/l, P < 0.01, respectively).

Correlations between serum HO-1 and relevant variables of PCOS
To explore the correlation between HO-1 and other variables of PCOS, Spearman or Pearson correlation coefficients were calculated on the distribution of relevant variables, and all the data were included in correlation models (Table II).

Significant negative correlations were identified between HO-1 and other variables of PCOS, Spearman or Pearson correlation coefficients were calculated on the distribution of relevant variables, and all the data were included in correlation models (Table II).

Overall, serum HO-1 exhibited an inverse correlation with the index of HOMA-IR (Fig. 2A), but a positive correlation with HOMA-β (Fig. 2B), with linearly dependent coefficients (r) of −0.13 (P = 0.02) and 0.45 (P < 0.01), respectively. In addition, HO-1 was found to have a negative dose–response relationship with CRP (Fig. 2C) and TNF-α (Fig. 2D), with r of −0.31 (P < 0.01) and −0.26 (P < 0.01), respectively. Similar negative associations, but of even greater magnitude, were observed for MDA (Fig. 2E), 8-OHdG (Fig. 2F) and NO (Fig. 2G), with r of −0.39 (P < 0.01), −0.64 (P < 0.01) and −0.42 (P < 0.01), respectively.

Association between serum HO-1 and PCOS
Considering our finding that HO-1 was linked to other variables, we performed a subsequent multivariate analysis to gain further insight. All subjects were categorized according to the tertiles of HO-1 concentration in the study population, and the odds ratios (ORs) for PCOS analyzed (Table III). Compared with women in the lowest HO-1 tertile
Table I Clinical characteristics, hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS) and normal cycling women (controls).

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Controls</th>
<th>P*</th>
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<tbody>
<tr>
<td>N</td>
<td>80</td>
<td>80</td>
<td>/</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>27.0 ± 3.5</td>
<td>27.4 ± 3.4</td>
<td>0.52</td>
</tr>
<tr>
<td>BMI (kg/m²), mean ± SD</td>
<td>22.4 ± 3.33</td>
<td>21.7 ± 3.51</td>
<td>0.25</td>
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<tr>
<td>Hormonal profile</td>
<td></td>
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<tr>
<td>TESTO (ng/dl), geometric mean (95% CI)</td>
<td>48.3 (44.14–52.41)</td>
<td>28.3 (25.52–31.15)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PRL (ng/dl), geometric mean (95% CI)</td>
<td>13.5 (11.62–15.42)</td>
<td>13.7 (12.08–15.24)</td>
<td>0.79</td>
</tr>
<tr>
<td>E₂ (pg/ml), median (quartiles)</td>
<td>63.7 (53.60, 77.23)</td>
<td>61.2 (50.78, 76.99)</td>
<td>0.77</td>
</tr>
<tr>
<td>FSH (mIU/ml), mean ± SD</td>
<td>6.8 ± 1.59</td>
<td>7.1 ± 2.10</td>
<td>0.25</td>
</tr>
<tr>
<td>LH (mIU/ml), geometric mean (95% CI)</td>
<td>7.9 (6.72–8.98)</td>
<td>3.4 (2.88–3.82)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LH/FSH, median (quartiles)</td>
<td>1.0 (0.70, 2.10)</td>
<td>0.5 (0.38, 0.66)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
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<tr>
<td>CHO (mg/dl), geometric mean (95% CI)</td>
<td>209 (195.3–223.7)</td>
<td>212 (199.4–225.5)</td>
<td>0.64</td>
</tr>
<tr>
<td>TG (mg/dl), geometric mean (95% CI)</td>
<td>99 (81.9–116.0)</td>
<td>90 (74.1–106.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-C (mmol/l), mean ± SD</td>
<td>0.92 ± 0.40</td>
<td>0.90 ± 0.34</td>
<td>0.70</td>
</tr>
<tr>
<td>LDL-C (mmol/l), mean ± SD</td>
<td>4.5 ± 2.12</td>
<td>4.1 ± 1.96</td>
<td>0.22</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FG (mmol/l), geometric mean (95% CI)</td>
<td>6.7 (6.20–7.18)</td>
<td>6.4 (6.01–6.76)</td>
<td>0.65</td>
</tr>
<tr>
<td>FI (μU/ml), median (quartiles)</td>
<td>10.2 (9.01, 12.35)</td>
<td>8.8 (8.10, 9.72)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HOMA-β, geometric mean (95% CI)</td>
<td>628 (50.0–74.5)</td>
<td>79 (66.3–92.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HOMA-IR, geometric mean (95% CI)</td>
<td>3.2 (2.85–3.55)</td>
<td>2.5 (2.25–2.72)</td>
<td>&lt;0.01</td>
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<tr>
<td>Enzyme levels</td>
<td></td>
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<tr>
<td>HO-1 (ng/ml), mean ± SD</td>
<td>3.2 ± 0.96</td>
<td>5.3 ± 1.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HO-2 (ng/ml), geometric mean (95% CI)</td>
<td>1.3 (1.11–1.44)</td>
<td>1.3 (1.11–1.50)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Data collected from November 2011 to May 2012.
CHO, cholesterol; E₂, estradiol; FG, fasting glucose; FI, fasting insulin; HDL-C, high-density lipoprotein cholesterol; HO-1, heme oxygenase-1; HO-2, heme oxygenase-2; HOMA-β, homeostasis model assessment of β cell function; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; PRL, prolactin; TESTO, total testosterone; TG, triglycerides.

* Differences between means were tested with Student’s t-test. Differences between medians were evaluated using Mann–Whitney U-test.

(3.41 ng/dl), which served as a reference, the OR (95% confidence interval (CI)) for PCOS in the highest tertile (≥5.29 ng/dl) was 0.02 (0.0034–0.07) (P for trend <0.01). To examine whether the negative association between increasing HO-1 levels and the prevalence of PCOS in a longitudinal analysis was independent of other known risk factors (age, BMI, FG, Fl, HOMA-β cell, HOMA-IR, CHO, TG, LDL-C and HDL-C), we also controlled for levels of these relevant variables as continuous variables in different multivariable models progressively. These results remained relatively robust after multivariable adjustment of confounders (all P for trend <0.01). As shown in Table III, the overall results were similar from Model 1 to Model 5, and decreasing ORs for PCOS were associated with increasing quartiles of serum HO-1 concentrations in a dose-related manner in each analysis model.

Discriminative performance of HO-1 for PCOS

Differences between the AUCs for two independent ROC curves were tested following the recommendation given by Cleves and Rock (2002). As shown in Fig. 3, the AUC for a model with known risk factors (Model 1), comprising age, BMI, TESTO, HOMA-IR, CHO and TG, was 0.86 (95% CI 0.81–0.92) for PCOS. However, when serum HO-1 concentration was added to the model (Model 2, including Model 1 plus HO-1), the AUC significantly increased to 0.95 (95% CI 0.92–0.98; P = 0.0005 for the difference of the AUCs).

Association between serum HO-2 and PCOS

Similar to our approach to HO-1, we examined the baseline characteristics of the participants by increasing quartiles of HO-2. However, the results for HO-2 were substantially different from those for HO-1. The circulating levels of serum HO-2 did not show any significant difference between the two groups. Moreover, there was no correlation between HO-2 and any relevant variable for PCOS. Even in evaluation of the diagnosis effect, the areas under the curves of the two established models were not significantly different (data not presented).

Discussion

Despite its increasing worldwide prevalence, the etiology of PCOS remains largely unclear. Emerging evidence suggests that IR, low-grade chronic inflammation and OS states are associated with the development...
of PCOS (Escobar-Morreale et al., 2011; Fauser et al., 2012; Murri et al., 2013). A unifying view of the pathophysiology proposes that PCOS and its metabolic comorbidities may be explained by the existence of a vicious circle among these risk factors (Fig. 4). PCOS represents a chronic inflammatory process (Duleba and Dokras, 2012) and the associated inflammation causes IR (Nicolai et al., 2009), whilst IR might promote inflammation by impairing the anti-inflammatory effect of insulin (Dandona et al., 2004). In addition, mononuclear cells in the polycystic ovary activated by glucose can generate OS that could stimulate a local inflammatory response (Gonzalez et al., 2006), which creates a vicious cycle of inflammation and formation of OS. Moreover, IR encourages OS as hyperglycemia and higher levels of free fatty acids lead to reactive oxygen species (ROS) production (Inoguchi et al., 2000). Importantly, OS impairs glucose uptake in tissues, and results in IR and impaired insulin secretion, both in vitro (Nguyen et al., 2005; Gao et al., 2010) and in vivo (Furukawa et al., 2004; Masharani et al., 2011). Taken together, this vicious circle plays a key role in mediating the effects of many known risk factors for PCOS.

In this population-based study of a genetically homogeneous group, we found that serum HO-1 was negatively associated with this vicious circle. The results indicate that serum HO-1 had a negative correlation to FI and HOMA-IR, while exerted a positive effect on b cell function. Thus, one may speculate an association of HO-1 with insulin action in PCOS. Interestingly, the interaction between HO-1 and IR in PCOS appears to be bidirectional. Up-regulation of HO-1 might enhance insulin sensitivity, improve glucose tolerance and decrease insulin levels (Nicolai et al., 2009). Nonetheless, i.m. HO-1 mRNA levels were reduced in patients with T2DM compared with age-matched controls (Bruce et al., 2003), which indicates that IR might down-regulate the activity of HO-1 (Seow et al., 2011). This is consistent with our result of lower levels of HO-1 in women with PCOS compared with the corresponding controls.

Apart from IR, systemic inflammation seems to be responsible, at least in part, for the decreased circulating HO-1 levels in PCOS. Evidence suggests that activation of HO-1 attenuates inflammation and modulates immune response, while HO-1 inhibition increases the secretion of...
TNF-α in obese mice (Kapturczak et al., 2004; Li et al., 2008). Additionally, CRP treatment is also capable of inhibiting HO-1 in human monocyte-derived macrophages (Singh et al., 2006). In support of this notion, the serum HO-1 expression was decreased in PCOS in the present study, together with its negative correlation with overexpression of CRP and TNF-α, unequivocally suggesting an essential role of HO-1 in protecting against inflammation in PCOS (Tzima et al., 2009).

Although HO-1 as a powerful antioxidant can be rapidly induced in response to OS; however, the current authors found a decreased HO-1 expression in women with PCOS and observed a negative correlation between HO-1 and OS. One possible explanation is that the HO-1 gene promoter polymorphisms may reduce the inducibility of HO-1 by ROS (Yamada et al., 2000), thereby resulting in different susceptibility to damage in PCOS. Another possibility is that HO-1 might be increased in the early stage of disease (active defense capacity against OS), but decreased in later stage (impaired defense capacity against OS), as evidenced in T2DM (Song et al., 2007; Bao et al., 2010) and chronic obstructive pulmonary disease (Maestrelli et al., 2003).

In light of these findings, it seems clear that the serum HO-1 expression/activity is inhibited as an adaptive response to the extent of IR, inflammation and OS. This may suggest increased vulnerability of the patients to IR, inflammation and OS, which in turn promotes further development of PCOS (Paine et al., 2010; Kim et al., 2011; Grochot-Przeczek et al., 2012). We then ask whether women with low HO-1 activity are associated with high risk of PCOS. The answer is clearly yes. We noted a negative correlation between ORs for incident PCOS and serum HO-1 that seems to be dose-dependent. Moreover, the predictive value of HO-1 revealed by ROC curve analysis suggests it is a crucial protective factor and may prove to be of some clinical

**Figure 2** Relation between serum heme oxygenase-1 (HO-1) concentration and insulin sensitivity, β cell function, oxidative stress (OS), and inflammation. (A) Homeostasis model assessment of insulin resistance (HOMA-IR; $r = -0.13$, $P = 0.02$), (B) HOMA of β cell function (HOMA-β; $r = 0.45$, $P < 0.01$), (C) C-reactive protein (CRP; $r = -0.31$, $P < 0.01$), (D) tumor necrosis factor-α (TNF-α; $r = -0.26$, $P < 0.01$), (E) malondialdehyde (MDA; $r = -0.39$, $P < 0.01$), (F) 8-Hydroxy-desoxyguanosine (8-OHdG; $r = -0.64$, $P < 0.01$) and (G) NO ($r = -0.42$, $P < 0.01$). The solid line represents the linear regression line based on all participants and corrected for age and BMI; the dotted lines show 95% confidence limits of the estimate. The variables HOMA-IR and HOMA-β were skewed and were logarithmically transformed to normality before linear regression analysis and the y-axis for these variables is plotted on a logarithmic scale.
However, it is also noteworthy that our study failed to show any link between serum HO-2 and PCOS. This may be because those two HO isoforms are encoded by two distinct genes, and HO-2 is the constitutive non-inducible one that is expressed primarily in brain and testis (Trakshel et al., 1986; Kim et al., 2011).

It should be acknowledged that our study still has several limitations. First, our data do not reflect any causal relationship between HO and PCOS progression. Second, the direct implications of the HO system for testosterone and dihydrotestosterone levels remain unexplained. In addition, the lack of difference in FG in our study may be due to the fact that women with PCOS commonly develop postprandial hyperglycemia prior to the development of impaired fasting glucose (Kim et al., 2011).

### Table III  Odds ratios (95% CI) of PCOS prevalence, by tertiles of serum HO-1 levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tertiles of serum HO-1 levels</th>
<th>P-value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (lowest)*</td>
<td>2</td>
</tr>
<tr>
<td>Serum HO-1 levels (ng/ml)</td>
<td>&lt;3.41</td>
<td>3.41–5.29</td>
</tr>
<tr>
<td>PCOS cases/controls, n/n</td>
<td>47/6</td>
<td>26/27</td>
</tr>
<tr>
<td>Crude OR (95% CI)</td>
<td>1.00</td>
<td>0.43 (0.21–0.86)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI), Model 1</td>
<td>1.00</td>
<td>0.53 (0.29–0.98)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI), Model 2</td>
<td>1.00</td>
<td>0.46 (0.21–1.01)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI), Model 3a</td>
<td>1.00</td>
<td>0.54 (0.28–1.04)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI), Model 3b</td>
<td>1.00</td>
<td>0.41 (0.23–0.73)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI), Model 4</td>
<td>1.00</td>
<td>0.39 (0.17–0.86)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI), Model 5</td>
<td>1.00</td>
<td>0.45 (0.23–0.89)</td>
</tr>
</tbody>
</table>

Results from multivariate logistic regression analysis are presented using the combined data from both PCOS cases and controls.

Model 1, adjusted for age; Model 2, adjusted for Model 1, BMI; Model 3a, adjusted for Model 2, FG and FI; Model 3b, adjusted for Model 2, HOMA-Beta and HOMA-IR; Model 4, adjusted for Model 2, FG, FI, HOMA-Beta and HOMA-IR; Model 5, adjusted for Model 4, CHO, TG, LDL-C and HDL-C. CHO, cholesterol; CI, confidence interval; FG, fasting glucose; FI, fasting insulin; HDL-C, high-density lipoprotein cholesterol; HO-1, heme oxygenase-1; HOMA-β, homeostasis model assessment of β cell function; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; PCOS, polycystic ovary syndrome; TG, triglycerides.

*Women in this category served as the reference group.

PCOS progression. Second, the direct implications of the HO system for testosterone and dihydrotestosterone levels remain unexplained. In addition, the lack of difference in FG in our study may be due to the fact that women with PCOS commonly develop postprandial hyperglycemia prior to the development of impaired fasting glucose (Kim et al., 2011).
2012). Consequently, the combination of FG and HbA1c can be a more sensitive and specific screening tool for depicting glucose concentrations in individuals with PCOS since the glycated hemoglobin (HbA1c) levels represent a long period of blood glucose concentrations (Bennett et al., 2007).

In conclusion, we have provided evidence that circulating HO-1 that initially protects against IR, OS and chronic inflammation is markedly reduced in non-obese women with PCOS. Our results highlight the medically relevant potential of determining HO-1 levels in serum, which may hold promise as a new and useful clinical biomarker for PCOS.

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Authors’ roles


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Conflict of interest

The authors can identify no potential conflicts of interest, neither financial nor any other, involved in the writing or publication of this manuscript.

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