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ORIGINAL ARTICLE

D-lactate levels are elevated in women with polycystic ovary syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a complex syndrome with various metabolic disorders. Previous studies have reported that D-lactate is commonly related to metabolic disturbances. However, the role of D-lactate in PCOS remains unclear.**Methods:** Serum D-lactate levels from control (n = 132) and women with PCOS (n = 132) were measured, and their relationship with several metabolic parameters were analyzed. The correlation of serum D-lactate with PCOS was assessed using logistic regression analysis, and the performance of serum D-lactate as a potential predictor for PCOS was evaluated using receiver operating characteristic curve analysis.**Results:** Serum D-lactate levels were markedly higher in patients with PCOS compared with those of controls (P < 0.001). The proportion of PCOS was substantially higher in increasing quartiles of serum D-lactate levels (P < 0.001). After adjusting for other confounders, there was still a correlation between D-lactate and PCOS (P < 0.001; odds ratio, 5.654; 95% confidence interval, 3.091–10.342). D-lactate levels were positively correlated with fasting serum insulin, homeostasis model assessment of insulin resistance, and triglycerides in patients with PCOS (all P < 0.01), which was not detected in controls (P > 0.05). D-lactate had an area under the curve (AUC) of 79.4% in predicting PCOS, with a similar performance as anti-Müllerian hormone (AMH) and luteinizing hormone (LH), and its combination with AMH and LH yielded a higher AUC of 90.9%.**Conclusions:** Substantially elevated serum D-lactate levels are significantly associated with PCOS, highlighting the importance of further research into the role of D-lactate in the pathogenesis of PCOS.

Keywords: D-lactate, polycystic ovary syndrome, obesity, insulin resistance, dyslipidemia

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder that affects approximately 5%–20% of women of childbearing age worldwide.[1–3] Monosaccharides are the simplest carbohydrates and principal energy sources of biological processes.[4] Using RNA sequencing technology, we have recently found that monosaccharide biosynthesis involved in the pathogenesis of PCOS.[5] Our previous study also showed that the serum levels of fructose, a major monosaccharide, were markedly elevated in women with PCOS.[6]

D-lactate, an optical isomer of lactic acid, is a downstream metabolic product of fructose.[7] Many studies have demonstrated that D-lactate is highly relevant to insulin resistance and obesity, which are common features of PCOS.[8–10] For instance, Erkin-Cakmak *et al.* showed that a decrease in D-lactate levels can improve insulin sensitivity in children with obesity and metabolic syndrome.[10] Elevated serum D-lactate

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levels are positively associated with insulin resistance and low-density lipoprotein cholesterol (LDL-C) levels in obese adolescents.[9] In addition, recent research suggests that urinary and blood levels of D-lactate are increased in patients with diabetes.[11] Considering the known role of insulin resistance and obesity in PCOS,[12–14] we hypothesized that D-lactate may be involved in the pathogenesis and progression of PCOS.

To date, no study has investigated the serum D-lactate levels of women with PCOS or the association between serum D-lactate and PCOS. This study aimed to measure and analyze the serum D-lactate levels in women with PCOS and healthy participants and elucidate the relationship between serum D-lactate and PCOS.

PATIENTS AND METHODS

Patients and samples collection

A total of 132 women with PCOS and 132 healthy women without PCOS (controls) were enrolled at the Shengjing Hospital of China Medical University from July 13 to December 20, 2020. Women with PCOS were diagnosed according to the new guidelines for the diagnosis and treatment of PCOS.[15] For the diagnosis of PCOS in adults, PCOS was present if at least two of the following three features were observed: biochemical or clinical evidence of hyperandrogenism, menstrual disturbance (oligomenorrhea, amenorrhea, or anovulation), and polycystic ovarian morphology on ultrasonography. The exclusion criteria are briefly described in our previous publication, which were the presence of endocrine diseases and recent hormonal medication.[16] The women without PCOS comprised the control group, and had clinical infertility due to fallopian tube or male factor infertility. Women with PCOS and the control group were matched for age and body mass index (BMI). Data on age and BMI were collected from the electronic medical record database of the Shengjing Hospital of China Medical University.

Venous blood samples were collected early in the morning under 12 h of fasting conditions on the third to fifth days of spontaneous menstruation or progestin-induced withdrawal bleeding. The blood was centrifuged at 2,000 × g for 10 minutes at 4°C, and the serum was divided into two parts, one for serum parameter detection in the clinical laboratory at Shengjing Hospital, which included fasting plasma glucose (FPG), fasting serum insulin (FSI), lipids, total testosterone (TT), follicle-stimulating hormone (FSH), estradiol, prolactin, progesterone, anti-Müllerian hormone (AMH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and the other for assessing free testosterone (FT), dehydroepiandrosterone sulfate (DHEAS), sex hormone-binding globulin (SHBG), and D-lactate in our internal laboratory. Repeated freezing and thawing were avoided throughout the experimental procedure.

The study was performed in accordance with the principles of the Declaration of Helsinki. The present study was approved by the Institutional Review Board of China Medical University (approval number 2020PS198K). All participants were exempted from providing informed consent, as the patient specimens used in the study were all discarded specimens after routine clinical diagnosis, and privacy-related information was removed.

Laboratory measurements

Serum levels of FPG, FSI, total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using enzymatic assays on an ARCHITECT ci16200 Automatic Biochemical Analyzer (Abbott Laboratories, USA) with corresponding kits (Architect glucose Reagent Kit, Architect insulin Reagent Kit, Abbott Laboratories, USA; and TC Test Kit, LDL-C Test Kit, HDL-C Test Kit, TG Test Kit, Kyowa Medex, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: HOMA-IR = (FSI, μU/mL × FPG, mmol/L) / 22.5.

The concentrations of several hormones, including TT, FSH, estradiol, prolactin, progesterone, AMH, LH, and TSH, were assessed using chemiluminescence assays on an UniCel DxI 800 Automatic Immunoassay System (Beckman Coulter, USA) with commercial kits (C10160, C10155, C10156, C10161, C10159, C10158, B13127, C10153, Beckman Coulter, USA).

DHEAS (Cusabio Biotech, Wuhan, China; CSB-E05 105 h), FT (Cusabio Biotech; CSB-E05 096 h), and SHBG (Human SHBG ELISA Kit; RayBiotech, Norcross, GA, USA) were measured using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. The assay's sensitivity limits for detecting DHEAS, FT, and SHBG were defined by the manufacturer as 10 μg/L, 3.75 ng/L, and 1.2 pmol/L, respectively. The intra-assay coefficients of variation (CVs) were 6.8% for FT, 5.5% for DHEAS, and 9.2% for SHBG, and the inter-assay CVs were 10.2%, 8.3%, and 6.1%, respectively. Serum concentrations were calculated by comparing the optical density (450 nm) of each sample with that of the standard curve.

D-lactate concentration was measured using a D-lactate fluorometric assay kit (BioVision Inc., Milpitas, CA, USA; K668–100). The assay was performed as per the manufacturer's instructions. By recognizing a fluorescent

signal (Ex/Em = 535/587 nm), the serum concentration of D-lactate can be computed using the formula listed in the instructions. The intra-and inter-assay CVs were 5.8% and 8.7%, respectively.

Participants grouping

To explore whether the difference of D-lactate levels between women with PCOS and control women was attributed to several D-lactate-related metabolic disorders, including obesity, insulin resistance, and dyslipidemia, the participants were further classified into subgroups.

According to the criteria proposed by the World Health Organization, overweight and obesity in Asian women were defined as BMIs between 23 and 25, and over 25, respectively.[17] To explore whether the difference in D-lactate levels between PCOS and control groups was attributed to obesity, PCOS and control groups were subdivided into lean, overweight, and obese subgroups (BMI < 23, 23–25, and > 25; Supplementary Table 1).

Insulin resistance was defined as an HOMA-IR of more than 2.5.[18] To investigate whether the difference in D-lactate levels between PCOS and control groups was due to insulin resistance, PCOS and control groups was subclassified into the non-insulin-resistant and insulin-resistant subgroups (HOMA-IR < 2.5, and $\geqslant$ 2.5; Supplementary Table 2).

Dyslipidemia was defined as TC $\geqslant$ 6.2 mmol/L, TG $\geqslant$ 2.3 mmol/L, LDL-C $\geqslant$ 4.1 mmol/L, HDL-C < 1.0 mmol/L, fulfilling at least one criteria described above.[19] To investigate whether the difference in D-lactate levels between PCOS and control groups was due to the presence of dyslipidemia, these two groups were subdivided into dyslipidemia and normolipidemia groups (Supplementary Table 3).

Statistical analysis

Statistical Package for Social Sciences, version 22.0 (SPSS 22.0) (IBM Corporation, Armonk, NY, USA) was used to analyze the acquired values. The normal distribution of continuous data was examined using the *Kolmogorov–Smirnov* test. Normal distribution variables were presented as mean ± standard deviation and were compared using *Z*-test. Non-normal distribution variables were presented as the median (interquartile range) and were compared using the *Mann–Whitney U*-test. The total participants were grouped based on quartile D-lactate levels, and differences among these four groups were analyzed with one-way analysis of variance or the *Kruskal–Wallis* test for continuous variables. Categorical variables were compared using the *Chi–square* test.

Univariate logistic regression analysis was used to investigate the covariates affecting PCOS, and multivariate logistic regression analysis was carried out on the significant variables defined by univariate analysis. The correlations of D-lactate concentration with all the other clinical indicators were evaluated with Pearson's or Spearman correlation coefficient analyses.

The performance of D-lactate, AMH, LH, and other parameters for predicting PCOS was evaluated by analyzing the receiver operating characteristic (ROC) curves. The area under the curve (AUC) was used to calculate the sensitivity, specificity, and 95% confidence interval (CI) for the prediction of PCOS. The performance of D-lactate, AMH, LH, and the combination of D-lactate with AMH or LH in predicting PCOS was compared according to the method proposed by Hanley and McNeil.[20] All statistical tests were two-sided, and statistical significance was set at $P < 0.05$ between the two groups.

RESULTS

Women with PCOS presented with higher serum D-lactate levels than control participants

Serum D-lactate levels in women with PCOS were markedly higher than those in control participants (3.59 ± 0.77 pmol/μL *vs.* 2.73 ± 0.71 pmol/μL; $P < 0.001$; Table 1; Figure 1A). To explore whether the difference in D-lactate levels between PCOS and control groups was attributed to several D-lactate-related metabolic disorders, serum D-lactate levels between PCOS and control groups were further compared in several metabolic subgroups.

In the lean, overweight, and obese subgroups, circulating D-lactate levels were all higher in women with PCOS than those in the control participants ($P < 0.001$, $P = 0.002$, $P < 0.001$; Figure 1B; Supplementary Table 1). Serum D-lactate levels were higher in women with PCOS in both insulin-resistant and non-insulin-resistant subgroups as compared to the corresponding control participants of these subgroups (both $P < 0.001$; Figure 1C; Supplementary Table 2). For the dyslipidemia and normolipidemia subgroups, higher serum D-lactate levels were observed in women with PCOS than in the control group (both $P < 0.001$; Figure 1D; Supplementary Table 3). Collectively, women with PCOS presented with higher serum D-lactate levels, which seemed to be attributed to PCOS and independent of the D-lactate-related metabolic disturbances.

Higher serum D-lactate levels correlated with PCOS

We focused on the correlation between serum D-lactate

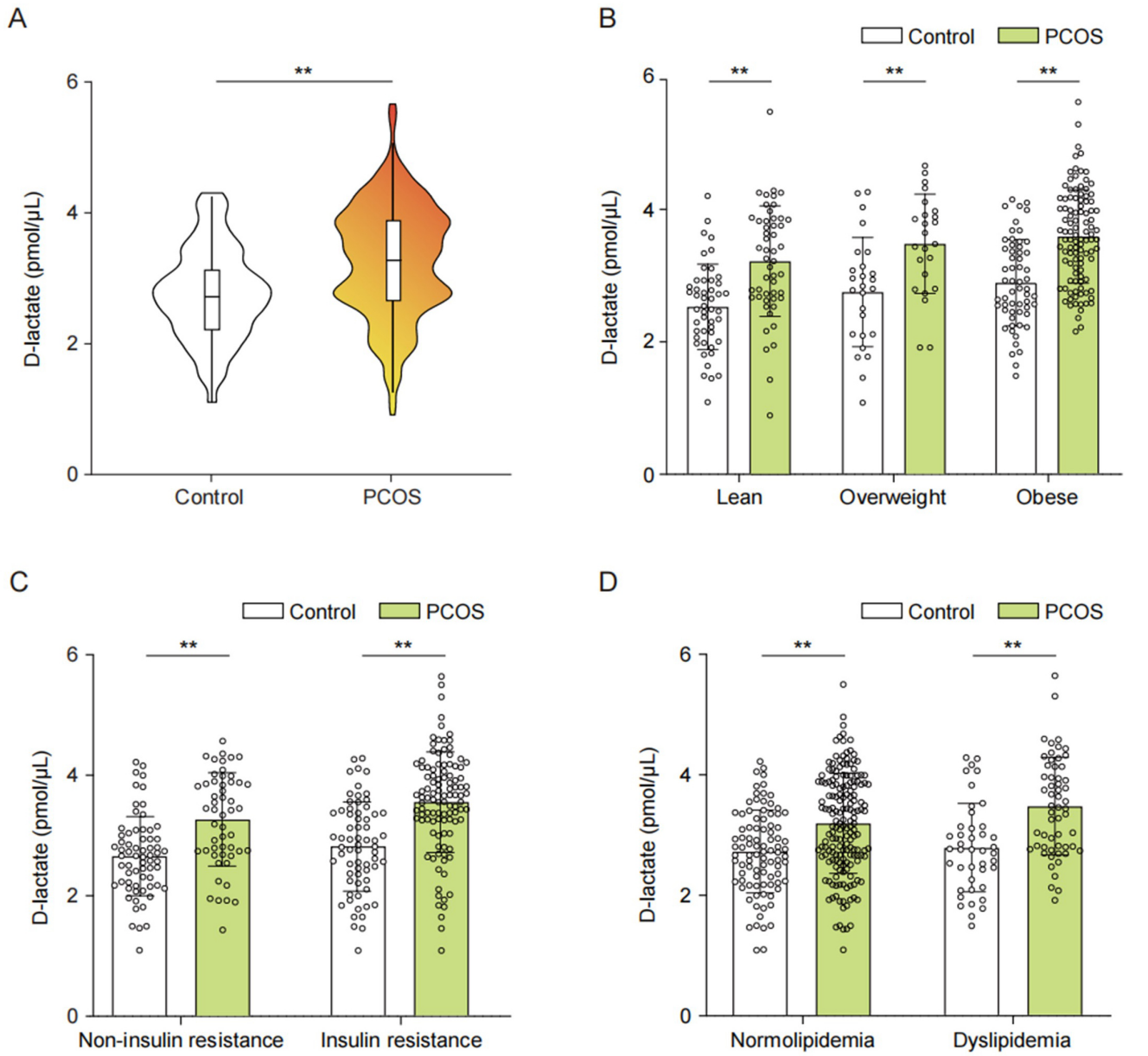


Figure 1. Serum D-lactate levels in women with PCOS and control participants. (A) Differences in serum D-lactate levels between women with PCOS and control participants. (B) Differences in serum D-lactate levels between women with PCOS and the control participants in the lean, overweight and obese subgroups. (C) Differences in serum D-lactate levels between women with PCOS and the control participants in the insulin-resistant and noninsulin-resistant subgroups. (D) Differences in serum D-lactate levels between women with PCOS and the control participants in the dyslipidemia and normolipidemia subgroups. $^{**}P < 0.01$. PCOS, polycystic ovary syndrome.

levels and PCOS. All participants were grouped according to quartiles of serum D-lactate levels (Table 2). The proportion of PCOS was substantially higher combined with increasing quartiles of serum D-lactate levels ($P < 0.001$). After adjusting for age, BMI, and the covariates affecting PCOS identified in the univariate logistic regression analysis, including TT, SHBG, AMH, FSH, LH, FSI, HOMA-IR, and TG, there was still a significant correlation between the circulating D-lactate levels and PCOS ($P < 0.001$; odds ratio (OR) 5.654; 95% CI, 3.091–10.342; Table 3).

The correlation of D-lactate with the hormonal and metabolic parameters in PCOS and non-PCOS groups were further detected, respectively (Table 4). Spearman correlation coefficient analyses revealed that D-lactate levels of women with PCOS were positively correlated with BMI, FSI, HOMA-IR, and TG (all $P < 0.01$). Among these indicators, BMI was the most related parameter with D-lactate in women with PCOS ($R = 0.290$, $P < 0.001$). However, there was no correlation between D-lactate and FSI, HOMA-IR, and TG in the non-PCOS group (all $P > 0.05$). Collectively, higher serum D-lactate levels correlated with PCOS, and was

Table 1: Comparison of the basic clinical data for the PCOS and control groups

Clinical data	Control group (*N* = 132)	PCOS group (*N* = 132)	*P*-value
Age (years)	31.00 (29.00-33.75)	31.00 (28.00-33.00)	0.354
BMI	24.60 (22.05-27.48)	24.50 (21.90-26.95)	0.618
D-lactate (pmol/μL)	2.73 ± 0.71	3.59 ± 0.77	< 0.001
TT (μg/L)	0.48 (0.34-0.64)	0.63 (0.48-0.79)	< 0.001
FT (nmol/L)	0.03 (0.02-0.04)	0.04 (0.02-0.05)	< 0.001
SHBG (nmol/L)	31.40 (22.25-51.65)	24.90 (17.35-36.85)	0.001
DHEAS (μmol/L)	3.18 (2.17-4.46)	4.65 (2.87-7.00)	< 0.001
AMH (pmol/L)	27.99 (13.85-39.84)	62.05 (39.70-87.82)	< 0.001
FSH (mIU/mL)	7.24 ± 2.25	6.56 ± 1.95	0.010
LH (mIU/mL)	3.83 (3.01-5.62)	10.22 (5.74-15.49)	< 0.001
Estradiol (ng/L)	43.00 (33.00-56.00)	47.00 (36.00-68.00)	0.086
Prolactin (μg/L)	11.85 (8.60-16.64)	10.02 (7.58-13.77)	0.004
P (μg/L)	0.55 (0.40-0.81)	0.58 (0.40-1.05)	0.281
TSH (μIU/mL)	1.84 (1.29-2.69)	1.89 (1.31-2.62)	0.924
FPG (mmol/L)	5.31 (4.91-5.66)	5.18 (4.92-5.57)	0.241
FSI (mIU/L)	10.55 (8.43-14.50)	12.40 (8.90-18.90)	0.055
HOMA-IR	2.53 (1.93-3.56)	2.94 (1.94-4.37)	0.113
TC (mmol/L)	4.65 (4.05-5.20)	4.63 (4.21-5.34)	0.840
LDL-C (mmol/L)	2.93 ± 0.85	2.89 ± 0.76	0.804
HDL-C (mmol/L)	1.21 (1.05-1.41)	1.19 (1.04-1.35)	0.346
TG (mmol/L)	0.97 (0.69-1.56)	1.27 (0.87-2.10)	0.003

Data were presented as mean ± standard deviation and the median (interquartile range). The *Z*-test was used for data with normal distribution and the *Mann–Whitney U*-test was used for data with non-normal distribution. PCOS, polycystic ovary syndrome; BMI, body mass index; SHBG, sex hormone-binding globulin; TT, total testosterone; FT, free testosterone; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

associated with FSI, HOMA-IR, and TG only in women with PCOS.

The performance of serum D-lactate levels in predicting PCOS

Considering the higher serum D-lactate levels in women with PCOS and the correlation between D-lactate and PCOS, we explored the clinical value of serum D-lactate levels for PCOS. We first compared the performance of all the parameters included in this study for predicting PCOS using the ROC curves (Table 5). AMH, LH, and D-lactate presented the highest area under the curve (AUCs) in predicting PCOS among all parameters, which had significantly higher AUCs than testosterone, the typical markers for PCOS. We then compared the performance of D-lactate with AMH and LH in predicting PCOS, and explored the performance of their combined use (Figure 2).

The AUC of D-lactate for PCOS prediction was 79.4%, with a sensitivity of 73.1%, and a specificity of 75.2%, and the cutoff value was set at 3.14 pmol/μL (Figure 2A, E). As for the typical markers AMH and LH in predicting PCOS, AMH had an AUC of 81.7%, a sensitivity of 72.3%, and a specificity of 78.0% (Figure 2A, E). LH could predict PCOS with an AUC of 80.4%, a sensitivity of 70.0%, and a specificity of 83.5% (Figure 2A, E). When we compared the performance of these parameters with the method proposed by Hanley and McNeil,[20] there was no difference in terms of performance between D-lactate, AMH, and LH predicting PCOS (Supplementary Table 4).

The performance of combining serum D-lactate and AMH or LH in predicting PCOS was further explored. The combination of D-lactate and AMH produced an AUC of 88.4%, a sensitivity of 80.0%, and a specificity of 78.7% in PCOS prediction (Figure 2B, E). The AUC of combining D-lactate and LH was 88.3%, with a sensitivity of 79.2% and a specificity of 83.5% in predicting PCOS (Figure 2C, E). The combination of D-lactate, AMH, and LH yielded the highest AUC of 90.9%, with a sensitivity of 74.6% and a specificity of 91.3% for predicting PCOS (Figure 2D, E). The combination of D-lactate with AMH, LH, or both AMH and LH had a better predictive performance than AMH

Table 2: Clinical and biochemical characteristics by quartile of Fasting serum D-lactate levels in all participants

Characteristics	Quartile 1 (*N* = 66)	Quartile 2 (*N* = 68)	Quartile 3 (*N* = 67)	Quartile 4 (*N* = 63)	*P*-value
D-lactate (pmol/μL)	⩽ 2.57	> 2.57 and ⩽ 3.13	> 3.13 and ⩽ 3.84	> 3.84	
PCOS	10 (15.2%)	25 (36.8%)	43 (64.2%)	54 (85.7%)	< 0.001
Age (years)	31.00 (29.00-33.00)	31.00 (28.00-33.75)	30.00 (28.00-33.00)	32.00 (29.00-34.00)	0.213
BMI	22.95 (21.68-25.68)	23.35 (21.46-26.08)	25.40 (22.40-28.00)	25.80 (23.50-28.00)	< 0.001
TT (μg/L)	0.49 (0.35-0.66)	0.50 (0.38-0.68)	0.59 (0.46-0.84)	0.63 (0.47-0.76)	0.005
FT (nmol/L)	0.03 (0.02-0.04)	0.03 (0.02-0.04)	0.03 (0.02-0.05)	0.03 (0.02-0.05)	0.032
SHBG (nmol/L)	35.05 (23.33-57.05)	31.50 (23.10-50.90)	24.75 (18.75-32.35)	24.10 (16.33-40.68)	0.001
DHEAS (μmol/L)	3.31 (2.20-4.80)	3.38 (2.26-5.71)	4.24 (2.85-6.02)	4.18 (2.83-6.50)	0.015
AMH (pmol/L)	29.77 (17.49-43.77)	39.56 (27.77-68.47)	39.84 (20.71-69.04)	56.76 (32.06-87.11)	< 0.001
FSH (mIU/mL)	7.43 ± 2.25	6.91 ± 2.14	6.83 ± 2.08	6.39 ± 1.95	0.049
LH (mIU/mL)	4.26 (3.01-7.32)	4.79 (3.46-12.14)	7.36 (3.61-12.62)	8.99 (4.65-12.17)	0.002
Estradiol (ng/L)	43.00 (34.00-59.00)	45.00 (34.00-67.00)	43.00 (32.00-59.75)	46.00 (34.00-57.00)	0.987
Prolactin (μg/L)	12.66 (9.04-17.33)	10.61 (7.66-14.10)	9.74 (7.72-14.88)	10.21 (7.48-14.55)	0.014
P (μg/L)	0.54 (0.39-0.97)	0.59 (0.45-0.82)	0.57 (0.40-1.03)	0.54 (0.37-1.07)	0.892
TSH (μIU/mL)	2.08 (1.35-2.79)	1.86 (1.20-2.75)	1.53 (1.07-2.66)	1.93 (1.41-2.56)	0.189
FPG (mmol/L)	5.27 (4.91-5.67)	5.16 (4.84-5.59)	5.21 (4.96-5.63)	5.20 (4.93-5.52)	0.734
FSI (mIU/L)	9.70 (8.23-12.43)	10.80 (8.13-14.30)	13.60 (9.30-19.60)	14.00 (10.10-18.90)	0.001
HOMA-IR	2.31 (1.85-2.96)	2.38 (1.81-3.42)	3.16 (2.08-4.63)	3.05 (2.35-4.27)	0.002
TC (mmol/L)	4.54 (4.03-5.04)	4.53 (3.98-5.24)	4.61 (4.23-5.25)	5.00 (4.40-5.50)	0.056
LDL-C (mmol/L)	2.82 ± 0.81	2.86 ± 0.92	2.90 ± 0.68	3.06 ± 0.78	0.327
HDL-C (mmol/L)	1.22 (1.05-1.44)	1.21 (1.00-1.39)	1.21 (1.07-1.36)	1.16 (1.04-1.32)	0.743
TG (mmol/L)	0.96 (0.65-1.48)	1.21 (0.77-1.74)	1.12 (0.72-1.81)	1.48 (0.98-2.51)	0.001

Data are mean ± SD, or median (interquartile range) unless otherwise indicated. Differences among groups were analysed with one-way analysis of variance (ANOVA) or the *Kruskal-Wallis* test for continuous variables. Categorical variables were compared using the *chi-square* test. PCOS, polycystic ovary syndrome; BMI, body mass index; TT, total testosterone; FT, free testosterone; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Table 3: Univariate and multivariate logistic regression analyses evaluating the factors affecting PCOS

Characteristics	Univariate regression			Multivariate regression		
	OR	95%CI	*P*-value	OR	95%CI	*P*-value
Age (years)	0.980	0.922-1.042	0.514	1.056	0.953-1.170	0.298
BMI	0.978	0.914-1.047	0.523	0.833	0.735-0.944	0.004
TT (μg/L)	12.721	4.049-39.964	< 0.001	2.494	0.467-13.306	0.285
AMH (pmol/L)	1.391	1.272-1.522	< 0.001	1.259	1.119-1.416	< 0.001
FSH (mIU/mL)	0.855	0.757-0.965	0.011	0.813	0.662-0.998	0.047
LH (mIU/mL)	1.277	1.191-1.371	< 0.001	1.192	1.096-1.297	< 0.001
Prolactin (μg/L)	0.934	0.891-0.978	0.004	1.000	0.934-1.070	0.995
FSI (mIU/L)	1.038	1.001-1.076	0.041	1.044	0.983-1.109	0.161
TG (mmol/L)	1.262	1.038-1.533	0.019	1.245	0.952-1.629	0.110
D-lactate (pmol/μL)	**4.744**	**3.127-7.197**	**< 0.001**	**5.654**	**3.091-10.342**	**< 0.001**

PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval; BMI, body mass index; TT, total testosterone; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; FSI, fasting serum insulin; TG, triglycerides.

or LH alone (Supplementary Table 4).

As described previously, women with PCOS all presented with higher serum D-lactate levels regardless of their metabolic status. We were interested in exploring whether D-lactate and the combination model of D-lactate, AMH, and LH had certain suitability for people with different metabolic statuses. Therefore, we compared their performance in predicting PCOS in

Table 4: Correlation coefficients of serum D-lactate levels with other clinical parameters in patients with PCOS and control participants

Characteristics	PCOS (*N* = 132)		Control (*N* = 132)	
	R	*P*-value	R	*P*-value
Age (years)	0.095	0.119	0.012	0.845
BMI	0.290	< 0.001	0.170	0.004
TT (μg/L)	0.022	0.717	0.070	0.237
FT (nmol/L)	0.039	0.556	0.057	0.390
SHBG (nmol/L)	-0.105	0.106	-0.089	0.143
DHEAS (μmol/L)	-0.031	0.606	0.028	0.642
AMH (pmol/L)	0.048	0.419	-0.061	0.303
FSH (mIU/mL)	-0.064	0.283	-0.034	0.565
LH (mIU/mL)	-0.072	0.226	0.009	0.880
Estradiol (ng/L)	-0.048	0.418	-0.008	0.889
Prolactin (μg/L)	-0.037	0.528	-0.114	0.057
P (μg/L)	-0.049	0.410	-0.111	0.065
TSH (μIU/mL)	0.045	0.446	-0.033	0.571
FPG (mmol/L)	0.098	0.101	-0.028	0.631
FSI (mIU/L)	0.210	< 0.001	0.081	0.171
HOMA-IR	0.211	< 0.001	0.068	0.252
TC (mmol/L)	0.111	0.061	0.120	0.043
LDL-C (mmol/L)	0.110	0.064	0.110	0.063
HDL-C (mmol/L)	-0.084	0.161	0.012	0.846
TG (mmol/L)	0.155	0.009	0.075	0.204

Data are mean ± SD, or median (interquartile range) unless otherwise indicated. The *P*-values were tested with Pearson's or Spearman analyses. PCOS, polycystic ovary syndrome; BMI, body mass index; TT, total testosterone; FT, free testosterone; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

several metabolic subgroups (Table 6). AUC values varied depending on metabolic phenotypes but remained in the moderately to highly accurate range for all populations. Especially in obese, insulin-resistant and dyslipidemia population, D-lactate produced an AUC of 86.2%, 83.4%, and 80.2% in predicting PCOS, respectively. Moreover, the combination of D-lactate, AMH, and LH yielded an AUC of over 90.0% in the obese, lean, insulin-resistant, non-insulin-resistant and normolipidemia subgroups. Taken together, these data suggest that the serum D-lactate levels had a similar predictive performance to AMH and LH in PCOS identification, and its combined use with AMH and LH improved the performance of AMH and LH. Moreover, D-lactate and its combination model with AMH and LH were likely applicable across populations with different metabolic statuses.

DISCUSSION

In the current study, we reported for the first time that serum D-lactate levels in women with PCOS were distinctly higher than those in the control group and were associated with PCOS even after adjusting for covariates affecting PCOS. This prompted us to further investigate the potential role of serum D-lactate levels in women with PCOS.

Our previous study found that women with PCOS had abnormal monosaccharide metabolism, and their serum fructose levels were significantly higher than those of healthy participants.[6] Fructose upregulates aldolase B to increase methylglyoxal production in the liver, and excess methylglyoxal can be converted by glyoxalase to D-lactate.[21] Consistent with this finding, short-term restriction of dietary fructose intake in obese children can result in reduced levels of D-lactate.[10] Therefore, we speculate that active monosaccharide metabolism in PCOS may promote more conversion of fructose to D-lactate, resulting in high D-lactate levels in women with PCOS.

Recent studies have confirmed that the imbalance of the gut microbiota correlate closely with the initiation of PCOS and its metabolic disorders.[22–24] *Bacteroides* and *Lactobacillus* are both common bacterial alterations in women with PCOS and were proved to dominate the

Table 5: The AUC value, sensitivity and specificity of all parameters in women with PCOS

Characteristics	AUC	SE	Cut off	95% CI	Sensitivity (%)	Specificity (%)	*P*-value
Age (years)	0.467	0.036	25.50	0.397-0.537	94.7	10.6	0.356
BMI	0.482	0.036	23.05	0.412-0.552	65.2	38.6	0.618
D-lactate (pmol/μL)	0.794*	0.028	3.14	0.739-0.848	73.1	75.6	< 0.001
TT (μg/L)	0.672	0.033	0.48	0.607-0.737	77.9	49.2	< 0.001
FT (nmol/L)	0.635	0.035	0.04	0.567-0.704	52.8	72.2	< 0.001
SHBG (nmol/L)	0.379	0.037	95.40	0.307-0.452	6.4	94.4	0.001
DHEAS (μmol/L)	0.684	0.034	10.15	0.617-0.750	64.8	68.0	< 0.001
AMH (pmol/L)	0.817*	0.026	5.95	0.766-0.868	72.3	78.0	< 0.001
FSH (mIU/mL)	0.418	0.035	1.90	0.349-0.488	99.2	0.8	0.023
LH (mIU/mL)	0.804*	0.029	7.25	0.748-0.860	70.0	83.5	< 0.001
Estradiol (ng/L)	0.562	0.036	53.5	0.492-0.632	38.9	74.2	0.086
Prolactin (μg/L)	0.397	0.035	0.37	0.328-0.465	100.0	0	0.004
P (μg/L)	0.539	0.036	0.98	0.468-0.609	29.0	83.6	0.281
TSH (μIU/mL)	0.497	0.036	1.43	0.427-0.567	70.5	36.4	0.924
FPG (mmol/L)	0.458	0.036	6.32	0.388-0.528	6.9	96.2	0.241
FSI (mIU/L)	0.568	0.035	14.75	0.499-0.638	40.2	77.3	0.055
HOMA-IR	0.557	0.036	2.89	0.487-0.627	53.1	60.6	0.113
TC (mmol/L)	0.507	0.036	4.07	0.437-0.577	80.8	25.8	0.840
LDL-C (mmol/L)	0.498	0.036	2.39	0.428-0.569	77.7	29.5	0.963
HDL-C (mmol/L)	0.466	0.036	1.11	0.396-0.536	66.2	36.4	0.346
TG (mmol/L)	0.607	0.035	0.98	0.539-0.675	68.5	50.8	0.003

ROC curves illustrate the value of all parameter levels in predicting PCOS. * AUC > 0.7 indicates high and moderate diagnostic performance. AUC, area under the curve; PCOS, polycystic ovary syndrome; SE, standard error; CI, confidence interval; BMI, body mass index; TT, total testosterone; FT, free testosterone; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; TSH, thyroid-stimulating hormone; FPG, fasting plasma glucose; FSI, fasting serum insulin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

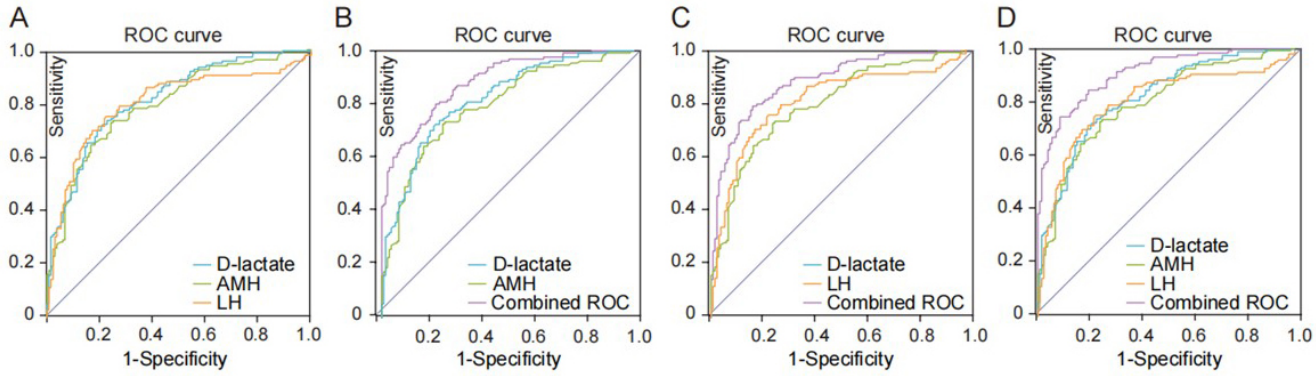
Table 6: AUC (95% CI) values for the detection of PCOS by D-lactate, AMH, and LH among the participants of different metabolic statuses

Metabolic statuses	D-lactate	AMH	LH	D-lactate+AMH	D-lactate+LH	D-lactate+AMH+LH
Obese	0.862 (0.786-0.919)	0.788 (0.702-0.858)	0.779 (0.693-0.851)	0.903 (0.835-0.950)	0.901 (0.832-0.948)	0.919 (0.854-0.961)
Overweight	0.738 (0.592-0.853)	0.846 (0.714-0.933)	0.766 (0.623-0.875)	0.867 (0.739-0.947)	0.845 (0.713-0.932)	0.893 (0.772-0.963)
Lean	0.767 (0.666-0.849)	0.850 (0.760-0.916)	0.851 (0.761-0.917)	0.870 (0.783-0.931)	0.888 (0.805-0.944)	0.910 (0.832-0.960)
IR	0.834 (0.762-0.891)	0.812 (0.737-0.872)	0.787 (0.711-0.852)	0.896 (0.834-0.941)	0.886 (0.822-0.933)	0.910 (0.851-0.952)
Non-IR	0.720 (0.628-0.801)	0.853 (0.774-0.912)	0.842 (0.762-0.904)	0.864 (0.787-0.921)	0.873 (0.798-0.928)	0.903 (0.833-0.951)
Dyslipidemia	0.802 (0.705-0.879)	0.702 (0.596-0.794)	0.775 (0.674-0.856)	0.812 (0.715-0.887)	0.870 (0.782-0.932)	0.851 (0.759-0.917)
Normolipidemia	0.784 (0.714-0.844)	0.872 (0.811-0.918)	0.828 (0.762-0.882)	0.918 (0.865-0.955)	0.890 (0.832-0.933)	0.935 (0.886-0.967)

AUC, area under the curve; CI, confidence interval; PCOS, polycystic ovary syndrome; AMH, anti-Müllerian hormone; LH, luteinizing hormone; IR, insulin resistance.

production of D-lactate in the gut.[25–27] Thus, another contributor to the higher D-lactate levels in women with PCOS may be the increased production of D-lactate metabolites in the gastrointestinal tract due to the imbalance in the gut microbiota. Moreover, as the product of gut bacterial, D-lactate could induce the inflammatory process and facilitate the systemic inflammatory.[27–29] Interestingly, chronic inflammation is considered a key contributor to the pathogenesis of PCOS.[22,30] Although the precise mechanisms of the relationship of D-lactate with gut microbiota disturbances and chronic inflammation in PCOS remain unclear, serum D-lactate levels might reflect gut microbiota disturbances as well as the chronic inflammation status in women with PCOS to some extent.

An interesting finding of this study is that the circulating D-lactate levels were positively correlated with FSI,



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PCOS	AUC	SE	Cut off	95% CI	Sensitivity%	Specificity%	PPV%	NPV%
D-lactate	0.794	0.028	3.14	0.739-0.848	73.1	75.2	75.0	73.8
AMH	0.817	0.026	5.95	0.766-0.868	72.3	78.0	76.7	73.8
LH	0.804	0.029	7.25	0.748-0.860	70.0	83.5	80.9	73.6
D-lactate+AMH	0.884	0.02	0.46	0.845-0.923	80.0	78.7	79.0	79.7
D-lactate+LH	0.883	0.021	0.56	0.842-0.923	79.2	83.5	82.8	80.1
D-lactate+AMH+LH	0.909	0.017	0.62	0.875-0.943	74.6	91.3	89.6	78.2

Figure 2. Predictive performance of serum D-lactate levels in women with PCOS. (A) ROC curves illustrate the value of serum D-lactate, AMH, LH levels in predicting PCOS. (B) ROC curves for D-lactate, AMH, and a combination of AMH and D-lactate levels in predicting PCOS. (C) ROC curves for D-lactate, LH, and a combination of LH and D-lactate levels in predicting PCOS. (D) ROC curves for D-lactate, AMH, LH, and a combination of AMH, LH and D-lactate levels in predicting PCOS. (E) AUC, cutoff value, SE, 95% CI, sensitivity, specificity, PPV, and NPV for each ROC curve in this evaluation. PCOS, polycystic ovary syndrome; ROC, receiver operating characteristic; AMH, anti-Müllerian hormone; LH, luteinizing hormone; AUC, area under the curve; SE, standard error; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

HOMA-IR, and TG in PCOS, and these interactions seemed unique to women with PCOS. Previous studies provided evidence to support our results.[9,31] In obese adolescents, higher serum D-lactate levels are strongly associated with serum triglyceride and low-density lipoprotein cholesterol levels.[9] D-lactate can promote adipocyte differentiation and contribute to obesity.[31] Obesity can aggravate PCOS symptoms, especially exacerbating insulin resistance.[32–34] Collectively, the potential role of D-lactate in PCOS may be its effects on the metabolic disorders of PCOS, which need to be clarified in follow-up research.

Although studies have reported that the serum levels of lactate, an optical isomer of D-lactate, were elevated in women with PCOS,[35,36] so far no study has investigated the circulating D-lactate levels in women with PCOS. The major strength of this study is that this is the first study to assess the serum D-lactate levels in women with PCOS and evaluate the relationship between serum D-lactate and PCOS. PCOS is a heterogeneous disorder with diverse clinical metabolic phenotypes.[12,13] Another strength of this study is that this study systematically analyzed the D-lactate levels among people with different metabolic statuses. We further confirmed that serum D-lactate levels were likely applicable for predicting PCOS across populations with different metabolic statuses. Additionally, our study has limitations that should be considered. The sample size and non-longitudinal design of the present study were limited; therefore, large-scale, longitudinal, and multicentral trials are needed to validate these findings.

CONCLUSION

In conclusion, substantially elevated serum D-lactate levels are significantly associated with PCOS, which provide new insights for further research on the biological role of D-lactate in PCOS.

DECLARATION

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Author contributions

Yang HY, Song JH, Feng D and Shi B conceived and designed the study. Yang HY and Shi B contributed to the sample handling, data management, and analysis. Yang HY, Song JH, and Feng D wrote the paper. Yang

HY, Song JH, Feng D and Shi B have accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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None.

Ethical approval
The study was performed in accordance with the principles of the Declaration of Helsinki. The present study was approved by the Institutional Review Board of China Medical University (approval number 2020PS198K).

Informed consent
All participants were exempted from providing informed consent, as the patient specimens used in the study were all discarded specimens after routine clinical diagnosis, and privacy-related information was removed.

Conflict of interest
The authors declare no competing interest.

Use of large language models, AI and machine learning tools
AI tools were not used in this paper.

Data availability statement
The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

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