Association of galactose and insulin resistance in polycystic ovary syndrome: A case-control study

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Summary

Background Polycystic ovary syndrome (PCOS) is closely linked to metabolic disorders. Recent reports have identified galactose as having strong associations with metabolic disorders, however, the correlation between galactose and PCOS remains largely unknown.

Methods The serum galactose levels of 104 patients with PCOS and 98 controls were measured, and their relationships with several metabolic parameters were analyzed. The study took place at the Center for Reproductive Medicine at Shengjing Hospital of China Medical University, Shenyang, China from July 13 to Oct 20, 2020. The relationships between serum galactose and PCOS as well as PCOS-related insulin resistance were investigated via logistic regression analyses, and the performance of serum galactose as a potential biomarker for PCOS was evaluated using receiver operating characteristic curve analysis.

Findings Higher serum galactose levels were observed in the patients with PCOS than in the controls ($p = 0.001$). There was still a correlation between circulating galactose levels and PCOS after adjusting for covariates ($p = 0.002$; odds ratio (OR), 1.133; 95% confidence interval (CI) 1.047−1.227). Serum galactose levels were shown to be most closely related to the fasting serum insulin level ($r = 0.370$, $p = 0.001$) and were higher in the insulin-resistant subgroup than in the non-insulin-resistant subgroup of patients with PCOS ($p = 0.001$). There was no difference in serum galactose levels between the insulin-resistant and non-insulin-resistant subgroups of women in the control group ($p > 0.05$). Furthermore, higher serum galactose levels were shown to be associated with insulin resistance in PCOS ($p = 0.004$; OR, 26.017; 95% CI, 2.907−232.810). The area under the curve for galactose-mediated prediction of PCOS was 80.0%, with a sensitivity of 71.0% and a specificity of 86.4%.

Interpretation Higher circulating galactose levels correlate with PCOS and PCOS-related insulin resistance; therefore, it may serve as a potential biomarker for patients with PCOS. These findings require further validation in a study with a larger sample size.

Keywords: Galactose; PCOS; Insulin resistance; Monosaccharide

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Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrinopathy of girls and women of reproductive age. PCOS strongly correlates with the presence of several metabolic disorders, including insulin resistance, obesity, and dyslipidemia.[1] Several monosaccharides, including glucose, fructose, mannose, and galactose, act as the principal source of energy for most cellular functions.[2] Our previous study used RNA sequencing to show that changes in the monosaccharide synthesis pathway could be a marker in PCOS.[3] However, except for glucose, little is known about how these monosaccharides may influence the pathogenesis of PCOS.

The monosaccharide galactose is responsible for energy delivery and the galactosylation of complex molecules and is vital for human metabolism.[4,5] The toxic effects of galactose on the ovary are widely recognized. Patients with galactosemia often have low follicle numbers and signs of follicle atresia.[6] The precise mechanism by which galactose exerts these ovotoxic effects is poorly elucidated, yet it is established that galactose itself and its metabolites can inhibit the development of oocytes, attenuate follicle-stimulating hormone bioactivity, and induce ovarian apoptosis.[7,8]

Several recent studies have linked galactose with PCOS-related metabolic disorders such as dyslipidemia and insulin resistance.[9,10] However, to our knowledge, no study has investigated the serum galactose levels of patients with PCOS, or an association between serum galactose and PCOS. The present study evaluated the serum galactose levels of patients with PCOS relative to those of healthy women, and investigated whether serum galactose levels correlated with PCOS and PCOS-related insulin resistance.

Methods

Study design and participants

This study included 104 patients with PCOS and 98 non-PCOS women, randomly selected from the Center for Reproductive Medicine at Shengjing Hospital of China Medical University, Shenyang, China from July 13 to Oct 20, 2020. PCOS was diagnosed strictly in accordance with the Rotterdam criteria, that is, when any two of the following three conditions were present: two or more oligo- or anovulatory cycles; clinical or biochemical signs of hyperandrogenism; and polycystic ovary manifestations after exclusion of other etiologies.[11,12] The women without PCOS comprised the control group, and had clinical infertility due to fallopian tube or male factor insufficiencies.

The exclusion criteria were consistent with our previous publications.[13,14] Specifically, patients characterized by any of the following were excluded: fewer than 3 years post menarche; smoking; pregnant; breastfeeding; on hormone medication; with hyperprolactinemia; or who had any other disease such as thyroid diseases, diabetes, adrenal diseases, tumors, and inflammatory diseases. All participants were exempted from informed consent before participating in the study, as the patient specimens and data had all identifying information removed, which fully protected the privacy of the patients. The specimens used in the study were all discarded specimens after routine clinical diagnosis and treatment, which had no impact on the routine diagnosis and treatment of patients. This study abides by the Declaration of Helsinki and was approved by the Ethical Review Board at China Medical University. According to a previous study that reported prevalence of PCOS as 13% in females of childbearing age,[15] we calculated the sample size using the ClinCalc.com Sample Size Calculator (https://clincalc.com/stats/samplesize.aspx), with an effect size of 0.95, type I error of 0.05, and a power of 95%. A calculated number of subjects of 90 per group was derived, and thus a total of 202 subjects were enrolled to replace drop-out patients. The characteristics of the participants in this study are summarized in Table 1.

General participant information was collected from the electronic medical record database of the Shengjing

	Control	PCOS	*p*-value
N	98	104	
Age (year)	33.00 (29.00−36.00)	29.00 (26.00−31.00)	0.001
BMI (kg/m^2)	23.28 ± 3.67	26.24 ± 4.18	0.001
Galactose (μM)	**14.81 (9.23−18.33)**	**22.36 (15.40−33.07)**	**0.001**
TT (ng/mL)	0.40 (0.30−0.56)	0.66 (0.51−0.85)	0.001
FT (nM)	0.022 (0.018−0.029)	0.043 (0.031−0.051)	0.001
SHBG (nM)	37.35 (19.33−57.05)	23.10 (17.05−31.45)	0.003
DHEAS (nM)	3076 (2217−4093)	5076 (3685−7164)	0.001
AMH (ng/mL)	2.98 (1.55−5.45)	9.49 (6.36−12.77)	0.001
FSH (mIU/mL)	7.94 (6.85−9.28)	6.68 (5.51−8.04)	0.001
LH (mIU/mL)	4.25 (3.03−5.78)	10.81 (6.93−15.25)	0.001
Estradiol (pg/mL)	40.00 (28.75−55.00)	49.00 (37.00−64.00)	0.010
Prolactin (ng/mL)	11.91 (8.34−16.71)	9.68 (7.41−13.29)	0.013
P (ng/mL)	0.60 (0.38−0.81)	0.64 (0.44−0.88)	0.251
TSH (μIU/mL)	1.73 (1.24−2.51)	1.61 (1.22−2.19)	0.148
FPG (mM)	5.25 (4.89−5.59)	5.20 (4.90−5.61)	0.722
FSI (mIU/L)	9.90 (7.45−14.00)	13.65 (9.08−19.08)	0.001
HOMA-IR	2.30 (1.62−3.34)	3.25 (1.97−4.79)	0.002
TC (mM)	4.56 (4.10−5.15)	4.69 (4.17−5.37)	0.302
LDL-C (mM)	2.90 (2.26−3.29)	2.94 (2.42−3.56)	0.117
HDL-C (mM)	1.29 (1.06−1.59)	1.14 (0.95−1.31)	0.001
TG (mM)	0.93 (0.68−1.54)	1.33 (0.91−1.85)	0.001

Table 1: **Comparison of the basic clinical data for each of the study participants in each group.**
Abbreviations: 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. Mean ± standard deviation or median (interquartile range) are shown. The Student's *t*-test was used for data with normal distribution and the Mann−Whitney *U* test was used for data with non-normal distribution.

Hospital of China Medical University, including the age and body mass index (BMI). Venous blood samples were collected from each participant on day 3 to 5 of their spontaneous menstruation (early follicular phase) after continuous fasting for at least 12 h prior to collection. Blood samples were centrifuged at $3000 \times g$ for 15 min, and the obtained serum was separated into two portions. One portion was used for measuring indicators in the clinical laboratory at Shengjing Hospital. These included hormones, lipids, fasting blood glucose (FPG), and fasting serum insulin (FSI). The other portion was stored at −80 °C in our internal laboratory to assess free testosterone, dehydroepiandrosterone sulfate (DHEAS), sex hormone-binding globulin (SHBG), and galactose. Before measurements, the frozen serum samples were thawed at 4 °C and centrifuged at $1000 \times g$ for 15 min. No samples were exposed to repeated freeze-thaw cycles.

Outcomes

The following hormones in blood samples were measured using a chemiluminescence assay 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) in accordance with the manufacturer's and supplier's instructions: total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, prolactin, anti-Müllerian hormone (AMH), and thyroid-stimulating hormone. The concentrations of FPG, FSI, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were assayed by enzymatic method on an ARCHITECT ci16200 Automatic Biochemical Analyzer (Abbott Laboratories, USA) using the appropriate kits in accordance with the manufacturer's and supplier's instructions: 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 as follows: HOMA-IR=(FSI, μU/mL × FPG, mmol/L) / 22.5.

The concentrations of free testosterone (CSB-E05096h, Cusabio Biotech, Wuhan, China), DHEAS (CSB-E05105h, Cusabio Biotech, Wuhan, China), and

	Control			PCOS		
	Non-IR	**IR**	*p*-value	**Non-IR**	**IR**	*p*-value
N	55	43		37	67	
Age (year)	33.00 (29.00−37.00)	32.00 (29.00−35.00)	0.440	30.00 (26.50−32.00)	28.00 (26.00−30.00)	0.080
BMI (kg/m^2)	22.07 ± 3.61	24.77 ± 3.20	0.001	23.39 ± 2.96	27.82 ± 3.92	0.001
Galactose (μM)	14.78 (7.87−17.54)	14.82 (10.00−28.38)	0.067	17.93 (8.82−24.96)	24.45 (19.10−38.43)	0.001
TT (ng/mL)	0.38 (0.29−0.52)	0.42 (0.31−0.64)	0.103	0.62 (0.51−0.77)	0.68 (0.51−0.90)	0.299
FT (nM)	0.021 (0.016−0.028)	0.023 (0.019−0.034)	0.137	0.039 (0.031−0.048)	0.044 (0.030−0.055)	0.325
SHBG (nM)	52.95 (37.83−67.95)	20.55 (16.38−31.30)	0.001	31.40 (22.90−71.08)	20.80 (13.90−25.80)	0.001
DHEAS (nM)	2909 (2038−3386)	3750 (2642−4661)	0.012	4561 (2972−6209)	5590 (3847−8315)	0.030
AMH (ng/mL)	2.71 (1.26−5.03)	3.78 (2.05−5.85)	0.054	9.14 (7.60−12.37)	9.59 (6.14−13.47)	0.773
FSH (mIU/mL)	8.37 (7.31−10.31)	7.52 (6.10−8.38)	0.003	6.97 (5.69−7.49)	6.57 (5.36−8.31)	0.893
LH (mIU/mL)	4.54 (3.07−5.88)	4.15 (2.98−5.74)	0.467	11.95 (6.07−15.88)	10.75 (7.80−14.39)	0.498
Estradiol (pg/mL)	45.00 (33.00−57.00)	35.00 (26.00−55.00)	0.119	48.00 (37.50−73.00)	50.00 (31.50−64.00)	0.786
Prolactin (ng/mL)	9.96 (7.71−17.39)	13.80 (9.75−16.67)	0.113	10.51 (7.85−15.72)	9.57 (7.36−12.81)	0.263
P (ng/mL)	0.60 (0.41−0.76)	0.50 (0.35−0.97)	0.802	0.68 (0.41−1.02)	0.62 (0.45−0.83)	0.431
TSH (μIU/mL)	1.64 (1.25−2.39)	2.00 (1.20−2.65)	0.392	1.61 (1.35−2.07)	1.61 (1.15−2.23)	0.911
FPG (mM)	5.09 (4.71−5.34)	5.45 (5.20−5.92)	0.001	4.97 (4.77−5.21)	5.38 (5.01−5.72)	0.001
FSI (mIU/L)	8.00 (6.00−9.10)	14.50 (11.80−20.20)	0.001	7.70 (6.35−9.45)	17.30 (14.00−23.00)	0.001
HOMA-IR	1.78 (1.34−2.15)	3.67 (2.87−4.69)	0.001	1.76 (1.38−2.06)	3.98 (3.25−5.57)	0.001
TC (mM)	4.38 (3.86−5.05)	4.69 (4.36−5.40)	0.031	4.38 (3.92−5.06)	4.90 (4.40−5.43)	0.003
LDL-C (mM)	2.73 (2.13−3.16)	2.95 (2.56−3.54)	0.037	2.62 (2.10−3.35)	3.17 (2.59−3.57)	0.007
HDL-C (mM)	1.38 (1.12−1.65)	1.19 (0.96−1.49)	0.009	1.25 (1.11−1.37)	1.07 (0.90−1.25)	0.009
TG (mM)	0.81 (0.65−1.14)	1.20 (0.76−2.44)	0.003	1.01 (0.72−1.25)	1.62 (1.15−2.56)	0.001

Table 2: Comparison of the basic clinical data for each group of study participants categorized based on their HOMA-IR.
Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; PCOS, polycystic ovary syndrome; IR, insulin resistance; 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. Mean ± standard deviation or median (interquartile range) are shown. The Student's *t*-test was used for data with normal distribution and the Mann−Whitney *U* test was used for data with non-normal distribution.

SHBG (Human SHBG ELISA Kit; RayBiotech, Norcross, GA, USA) were determined using Enzyme-Linked Immunosorbent Assay (ELISA). The limits for the assay sensitivity for the free testosterone, DHEAS, and SHBG were, respectively, 3.75 pg/mL, 10 ng/mL, and 1.2 pmol/L. Inter-assay coefficients of variation (CVs) were 10.2, 8.3, and 9.2%, respectively, whereas intra-assay CVs were 6.8, 5.5, and 6.1%. The final concentration was obtained by comparing the optical density of each sample to the standard curves at 450 nm using a microplate reader.

Galactose concentrations were assessed using a galactose fluorometric assay kit (K668−100; BioVision, Milpitas, CA, USA) as described by the manufacturer. Briefly, each serum sample was diluted in the assay buffer prior to measurement and the metabolite enzymatically generated from the free galactose in the samples reacted with the probe, producing a fluorescent signal under the excitation wavelength (535 nm) and emission wavelength (587 nm). The inter-assay CV was 7.2%, and the intra-assay CV was 8.9%. Galactose interference was eliminated using sample cleanup

mix reagents, and the assay range was 5 to 500 pmol/well.

Grouping of participants

With consideration of the effects of galactose in metabolism, and the metabolic phenotypes of PCOS, the participants were grouped variously by the presence or absence of several metabolic disorders and the serum galactose levels were compared.

Overweight and obesity were defined, respectively, according to the definitions of the World Health Organization for Asians as BMIs between 23 and 25 kg/m^2, and ≥ 25 kg/m^2.[16] To explore associations between circulating galactose levels and PCOS in the context of BMI, the PCOS and control groups were stratified into lean, overweight, and obese subgroups (BMI < 23, 23−25, and ≥ 25 kg/m^2; Supplementary Table 1).

Insulin resistance was diagnosed according to the HOMA-IR formula and the cut-off point was set to more than 2.5, which has been widely used.[17,18] Thus, to investigate the relationship of serum galactose with

PCOS in the context of HOMA-IR as well as the association between serum galactose level and PCOS-related insulin resistance, each subject within the PCOS and control groups was classified as either non-insulin-resistant or insulin-resistant (i.e., HOMA-IR < 2.5 or ≥ 2.5, respectively; Table 2, Supplementary Table 2.)

Dyslipidemia was diagnosed if any one of the following conditions was met, indicated by higher-than-normal TC, TG, and LDL-C, and lower-than-normal HDL-C, respectively:[19] TC ≥ 6.2 mmol/L; TG ≥ 2.3 mmol/L; LDL-C ≥ 4.1 mmol/L; and HDL-C < 1.0 mmol/L. The patients with PCOS and control women were each subdivided as either with dyslipidemia or normolipidemia (Supplementary Table 3).

Metabolic syndrome was defined according to the criteria of the American Association of Clinical Endocrinologists/American College of Endocrinology,[20] in which any 3 of the following 5 conditions must be met: BMI ≥ 25 kg/m²; TG ≥ 1.70 mmol/L; HDL-C < 1.29 mmol/L; blood pressure ≥ 130/85 mmHg; 2 h postprandial plasma glucose > 7.8 mmol/L, 6.1 mmol/L ≤ FPG ≤ 7.0 mmol/L; other risk factors including type 2 diabetes, family history of hypertension or cardiovascular diseases, PCOS, sedentary lifestyle, older age, ethnicity with high risk of type 2 diabetes, or cardiovascular disease. The PCOS and control groups were then stratified as either with or without metabolic syndrome (Supplementary Table 4).

Statistical analysis
Data analyses were performed using SPSS version 23.0, and the normality of the continuous variables was assessed by Kolmogorov-Smirnov test. Normally distributed continuous variables were reported as mean ± standard deviation and compared with Student's t-test. Non-normally distributed continuous variables were reported as the median (interquartile range), and variables were compared using the Mann-Whitney U test. Univariate logistic regression analysis was utilized to select the covariates affecting PCOS, and variables found significant by the univariate analysis were included in the multivariate logistic regression analysis.

Non-normally distributed variables were logarithmically transformed (log10), and the Kolmogorov-Smirnov test as well as Q-Q plots were used to confirm whether the raw data were transformed from non-normal distribution to normal distribution successfully. The correlation between galactose concentration and metabolism parameter was evaluated with Pearson's correlation coefficient. The association between galactose levels and FSI was further assessed using univariate and multivariate linear regressions. Univariate logistic regression analysis was utilized to investigate the covariates that correlated with insulin resistance in PCOS, and patients with PCOS were grouped based on quartile galactose

levels. After adjusting for the significant covariates, multivariate logistic regression analysis was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the presence of insulin resistance in PCOS, according to quartiles of serum galactose concentrations, with the lowest quartile as the reference.

Receiver operating characteristic (ROC) curves were used to examine the diagnostic value of galactose for PCOS and included an evaluation of the area under the curve (AUC), sensitivity, specificity, positive predictive value, and negative predictive value. The performance of galactose, androgens and the combination of galactose and androgens in predicting PCOS was compared according to the method proposed by Hanley and McNeil.[21] All the statistical tests were 2-sided, and $p < 0.05$ was considered significant.

Role of the funding source
The funding bodies had no role in study design, data collection, analysis, interpretation, manuscript writing, or submission decision. All authors had full access to the dataset, and the decision to submit for publication was jointly taken by all authors.

Results

Patients with PCOS presented with higher serum galactose levels
As shown in Table 1, serum galactose levels were higher in the patients with PCOS than in the control women. Considering the function of galactose in metabolism, and the metabolic phenotypes of PCOS, we went on to investigate whether its higher levels in PCOS were dependent on several metabolic disorders, and we compared the serum galactose levels between the PCOS and control groups among participants with different metabolic statuses.

When the participants were split into lean, overweight, and obese subgroups, the serum galactose levels were higher in the patients with PCOS for both the lean and obese subgroups as compared to the corresponding control of these subgroups (Figure 1a, Supplementary Table 1). Additionally, in both the insulin-resistant and non-insulin-resistant population, the serum galactose levels of patients with PCOS were higher than those of the control women (Figure 1b, Supplementary Table 2). When the relationship between the serum galactose levels and PCOS in the context of lipid metabolism was evaluated, higher serum galactose levels were observed in the patients with PCOS for both the dyslipidemia and normolipidemia subgroups compared to the corresponding control of these subgroups (Figure 1c, Supplementary Table 3). Considering that obesity and dyslipidemia are both components of the diagnostic criteria of metabolic syndrome, we further grouped the
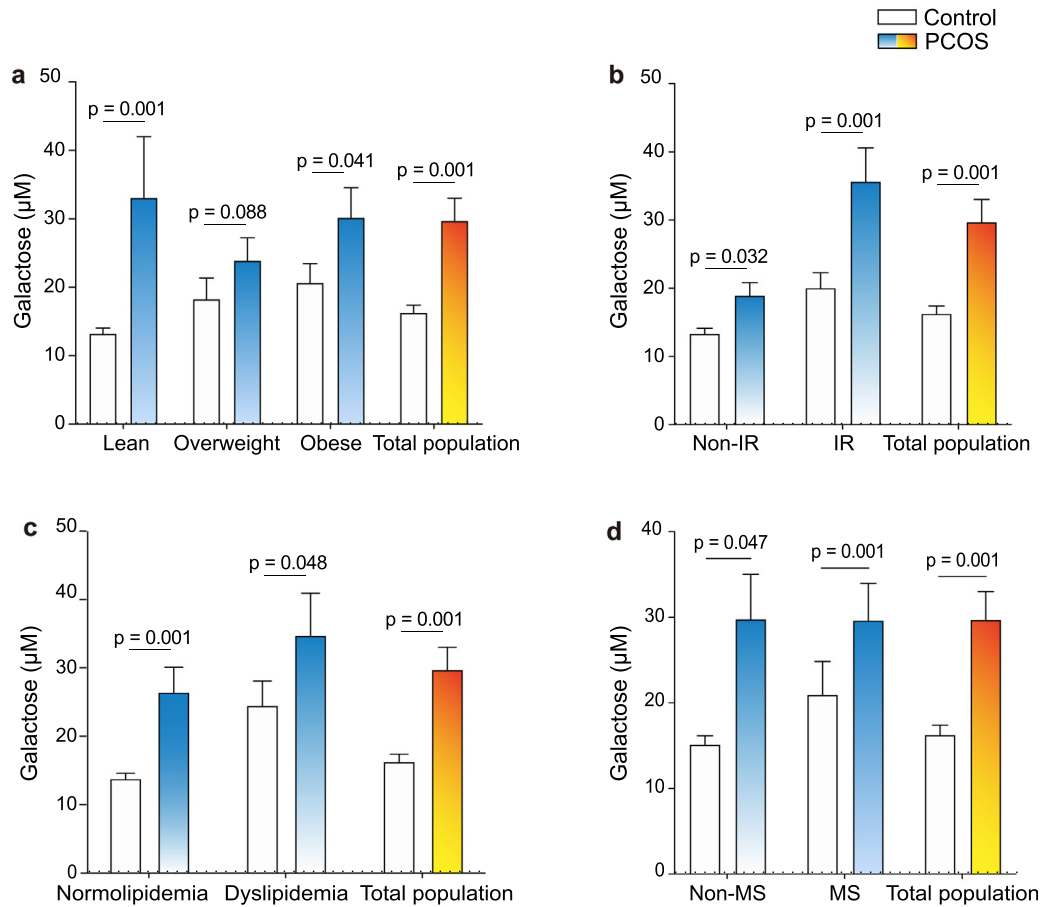
Figure 1. *Serum galactose levels in the control group versus patients with PCOS*. a, Differences in serum galactose levels between control women and patients with PCOS in the lean, overweight and obese groups. b, Differences in serum galactose levels between control women and patients with PCOS in the insulin-resistant and noninsulin-resistant groups. c, Differences in serum galactose levels between control women and patients with PCOS in the dyslipidemia and normolipidemia groups. d, Differences in serum galactose levels between control women and patients with PCOS in the metabolic syndrome and nonmetabolic syndrome groups. Bars represents the mean ± standard error of the mean. PCOS, polycystic ovary syndrome; IR, insulin resistance; MS, metabolic syndrome.

participants according to the existence of metabolic syndrome. Regardless of the presence of metabolic syndrome, the galactose levels of the patients with PCOS were higher than those of the control women (Figure 1d, Supplementary Table 4). Collectively, the patients with PCOS presented with higher serum galactose levels, which seemed to be due to PCOS itself and independent of the metabolic disorder status in the population.

Next, we focused on the correlation between the serum galactose levels and PCOS itself. After adjusting for the covariates affecting PCOS identified in the univariate logistic regression analysis, including age, BMI, free testosterone, DHEAS, AMH, LH, FSH, prolactin, FSI and HOMA-IR, there was still a significant correlation between the circulating galactose levels and PCOS ($p = 0.002$; OR, 1.133; 95% CI, 1.047−1.227; Table 3).

Serum galactose levels correlated with insulin resistance in patients with PCOS

We then set out to explore the deeper relationship between the serum galactose levels and PCOS. Pearson correlation analyses revealed that there were correlations between the serum galactose levels and several metabolic indicators of patients with PCOS (Table 4). The galactose levels of patients with PCOS were positively associated with the FSI level, HOMA-IR, and TG concentration, with the FSI correlation being the most significant ($r = 0.370$, $p = 0.001$; Figure 2a).

Given this finding, the relationship between the serum galactose levels and insulin resistance in PCOS based on HOMA-IR was further evaluated. We segregated the PCOS and control groups into insulin-resistant and non-insulin-resistant subgroups and found that the circulating galactose concentrations of the

	Univariate regression			Multivariate regression		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
Age (year)	0.767	0.702−0.838	0.001	0.808	0.679−0.962	0.017
BMI (kg/m^2)	1.217	1.121−1.321	0.001	1.380	1.087−1.751	0.008
FT (pM)	1.107	1.071−1.145	0.001	1.024	0.961−1.091	0.462
DHEAS (nM)	1.000	1.000−1.001	0.001	1.000	1.000−1.000	0.577
AMH (ng/mL)	1.558	1.375−1.765	0.001	1.223	0.992−1.507	0.059
LH (mIU/mL)	1.361	1.243−1.491	0.001	1.375	1.130−1.672	0.001
FSH (mIU/mL)	0.719	0.613−0.844	0.001	0.725	0.494−1.066	0.102
Prolactin (ng/mL)	0.939	0.891−0.990	0.019	1.053	0.933−1.188	0.404
FSI (mIU/L)	1.073	1.028−1.120	0.001	1.522	0.842−2.753	0.165
HOMA-IR	1.198	1.028−1.396	0.020	0.111	0.009−1.398	0.089
Galactose (μM)	**1.060**	**1.032−1.089**	**0.001**	**1.133**	**1.047−1.227**	**0.002**

Table 3: Univariate and multivariate logistic regression analyses evaluating the factors affecting PCOS.
Abbreviations: PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval; BMI, body mass index; FT, free testosterone; DHEAS, dehydroepian-drosterone sulfate; AMH, anti-Müllerian hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; FSI, fasting serum insulin; HOMA-IR, homeostasis model assessment of insulin resistance.

insulin-resistant subgroup were higher than those of the non-insulin-resistant subgroup in patients with PCOS (*p* = 0.001; Figure 2b, Table 2). Additionally, there was no difference in serum galactose levels between the insulin-resistant and non-insulin-resistant subgroups of control women (*p* > 0.05). Univariate and multivariate linear regression analyses were then used to assess the effects of galactose on FSI, and we found that galactose levels were correlated with FSI after adjusting for several co-variables, including age, BMI, SHBG, DHEAS, FPG, TC, and LDL-C levels (*β* coefficient, 0.247; 95% CI, 0.009−0.075; *p* = 0.015; Table 5). We also grouped the patients with PCOS based on the galactose level quartile and evaluated the relationship between each galactose level quartile and the incidence of insulin resistance in patients with PCOS using

	r	*p*-value
Age (year)	−0.009	0.927
BMI (kg/m2)	0.096	0.331
FPG (mM)	0.125	0.206
FSI (mIU/L)	0.370	0.001*
HOMA-IR	0.365	0.001*
TC (mM)	0.059	0.550
LDL-C (mM)	0.063	0.523
HDL-C (mM)	−0.016	0.871
TG (mM)	0.247	0.011*

Table 4: Correlation between serum galactose concentration and the metabolic parameters of patients with PCOS.
Abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; 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. *p*-value were tested with Pearson analysis. **p*-value < 0.05.

logistic regression analysis (Table 6). This evaluation revealed that the incidence of insulin resistance in the group of patients with galactose levels more than 33.07 μM was significantly higher (*p* = 0.004; OR, 26.017; 95% CI, 2.907−232.810) as compared to the group of patients with galactose levels less than 15.40 μM. Taken together, higher circulating galactose concentrations were associated with insulin resistance in patients with PCOS.

Serum galactose levels showed similar diagnostic performance to androgens for PCOS

Finally, we aimed to explore the clinical value of serum galactose levels for patients with PCOS. As previously described, we found that the patients with PCOS presented with higher serum galactose concentrations as compared to those in control women. Therefore, we assessed the diagnostic performance of serum galactose for PCOS using ROC curves and compared the performance of these evaluations to those of the typical markers for PCOS, including total testosterone, free testosterone, and DHEAS levels (Figure 3).

The AUC of galactose for PCOS diagnosis was 80.0%, and this indicator could diagnose PCOS with a sensitivity of 71.0% and a specificity of 86.4% when the cutoff was set at 17.77 μM (Figure 3a,e). As for the typical androgen markers in predicting PCOS, total testosterone had an AUC of 75.9%, with a sensitivity of 85.0% and a specificity of 56.1% (Figure 3a,e). Free testosterone could diagnose PCOS with an AUC of 83.9%, producing a sensitivity of 72.0% and a specificity of 87.9% (Figure 3a,e). The AUC of DHEAS was 77.0%, with a sensitivity of 77% and a specificity of 69.7% for predicting PCOS (Figure 3a,e). When we compared the performance of these parameters as proposed by Hanley and McNeil,[21] there were no differences between
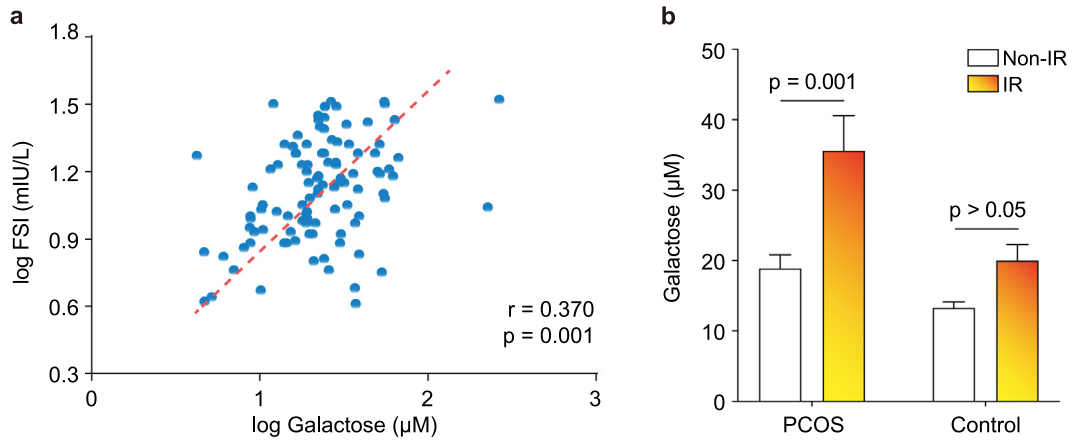
Figure 2. *Association between serum galactose and insulin resistance in patients with PCOS.* a, Association between serum FSI and galactose levels. Both parameters were log-transformed for the plot. b, Serum galactose levels of the insulin-resistant and non-insulin-resistant subgroups of the PCOS and control groups. Bars represents the mean ± standard error of the mean. FSI, fasting serum insulin; PCOS, polycystic ovary syndrome; IR, insulin resistance.

galactose and androgens in the performance of predicting PCOS (Supplementary Table 5).

The performance of combining serum galactose and androgens to predict PCOS was further explored. The combination of galactose and total testosterone produced an AUC of 80.5%, with a reduced sensitivity (68.0%) and a high specificity (90.9%) for PCOS diagnosis (Figure 3b,e). The specificity value of the combination of galactose and total testosterone was higher than that of total testosterone alone. The combination of galactose and free testosterone yielded the highest AUC of 87.2%, with moderate sensitivity (84.0%) and
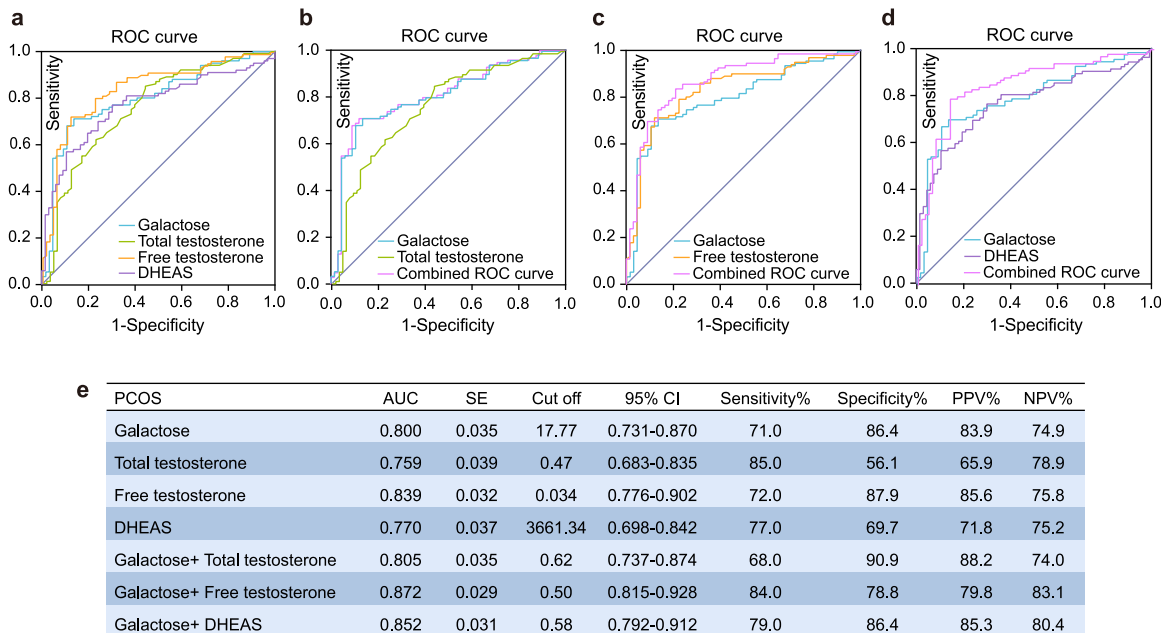

PCOS	AUC	SE	Cut off	95% CI	Sensitivity%	Specificity%	PPV%	NPV%
Galactose	0.800	0.035	17.77	0.731-0.870	71.0	86.4	83.9	74.9
Total testosterone	0.759	0.039	0.47	0.683-0.835	85.0	56.1	65.9	78.9
Free testosterone	0.839	0.032	0.034	0.776-0.902	72.0	87.9	85.6	75.8
DHEAS	0.770	0.037	3661.34	0.698-0.842	77.0	69.7	71.8	75.2
Galactose+ Total testosterone	0.805	0.035	0.62	0.737-0.874	68.0	90.9	88.2	74.0
Galactose+ Free testosterone	0.872	0.029	0.50	0.815-0.928	84.0	78.8	79.8	83.1
Galactose+ DHEAS	0.852	0.031	0.58	0.792-0.912	79.0	86.4	85.3	80.4

Figure 3. *Diagnostic performance of serum galactose levels in patients with PCOS.* a, ROC curves illustrate the value of serum galactose, total testosterone, free testosterone, and DHEAS levels in predicting PCOS. b, ROC curves for galactose, total testosterone, and a combination of total testosterone and galactose levels for this same application. c, ROC curves for galactose, free testosterone, and a combination of free testosterone and galactose levels. d, ROC curves for galactose, DHEAS, and a combination of DHEAS and galactose levels. 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; DHEAS, dehydroepiandrosterone sulfate; AUC, the area under the curve; SE, standard error; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

	Univariate regression			Multivariate regression		
	β coefficient	95%CI	*p*-value	β coefficient	95%CI	*p*-value
Age (year)	−0.115	−0.662 to 0.171	0.245	−0.031	−0.440 to 0.322	0.756
BMI (kg/m2)	0.254	0.015 to 0.101	0.009	0.500	0.514 to 1.392	0.001
SHBG (nM)	−0.373	−0.175 to −0.034	0.004	−0.151	−0.096 to 0.015	0.148
DHEAS (nM)	0.264	0.000 to 0.001	0.008	0.094	0.000 to 0.001	0.465
FPG (mM)	0.341	2.188 to 7.342	0.001	0.104	−1.895 to 4.806	0.386
TC (mM)	0.303	0.764 to 3.226	0.002	0.746	−0.338 to 12.690	0.063
LDL-C (mM)	0.240	0.434 to 3.758	0.014	−0.618	−12.665 to 1.403	0.114
Galactose (µM)	**0.254**	**0.015 to 0.101**	**0.009**	**0.247**	**0.009 to 0.075**	**0.015**

Table 5: Linear regression describing the relationship between galactose and FSI levels in patients with PCOS.

Abbreviations: FSI, fasting serum insulin; PCOS, polycystic ovary syndrome; CI, confidence interval; BMI, body mass index; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; FPG, fasting plasma glucose; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol.

Galactose (µM)	OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
< 15.40	1.000	—	—	1.000	—	—
15.40−22.36	3.022	0.976−9.356	0.055	4.251	0.522−34.579	0.176
22.36−33.07	10.389	2.728−39.560	0.001	18.302	2.284−146.647	0.006
> 33.07	6.296	1.861−21.298	0.003	26.017	2.907−232.810	0.004

Table 6: Logistic analysis of the correlation between galactose concentration and risk of insulin resistance in women with PCOS.

Abbreviations: PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval. *OR was adjusted for age, BMI, FPG, TC, LDL-C, and TG.

specificity (78.8%) for predicting PCOS (Figure 3c,e). The sensitivity value of the combination of galactose and free testosterone was higher than that of free testosterone alone. The AUC of combining galactose and DHEAS was 85.2%, with moderate sensitivity (79.0%) and specificity (86.4%) for PCOS prediction (Figure 3d, e). The specificity and sensitivity values of the combination of galactose and DHEAS were both higher than those of DHEAS alone, and the combination of galactose and DHEAS had a better diagnostic performance than DHEAS alone (Supplementary Table 5). Taken together, these data suggest that the serum galactose levels demonstrate a similar diagnostic performance to the androgen levels in PCOS identification and display a better diagnostic performance when combined with DHEAS than DHEAS alone.

Discussion

This study demonstrated that circulating galactose levels were significantly higher in women with PCOS, and were associated with PCOS even after adjustment for covariates affecting PCOS. Another interesting finding was that the galactose levels were positively associated with insulin resistance in PCOS.

Mounting evidence supports the critical role of galactose in the female reproductive system.[7,8,22] As for the relationship between galactose and PCOS, a previous study has revealed that repeated exposure to galactose established a PCOS-like model in mice, presenting with irregular estrous cycles and ovarian cysts.[23] Another bioinformatics analysis has suggested that galactose metabolism is a significantly enriched pathway in PCOS.[24] To date, no study has investigated the circulating galactose levels in patients with PCOS. Our results clearly indicate higher baseline galactose levels in women with PCOS, which are correlated with PCOS itself, and independent of the metabolic status as well as other relevant covariates. Collectively, galactose may be involved in the occurrence and development of PCOS; however, further studies are needed to elucidate the underlying mechanism.

Metabolic dysfunction characterized by insulin resistance is a key element contributing to PCOS progression.[25] Before our research, the serum galactose levels had not been assessed in populations with insulin resistance, but several studies have reported conflicting results on the association between galactose intake and insulin resistance. Galactose intake can induce insulin resistance and inflammation in rats, and the utilization of insulin-sensitizing drugs such as metformin been shown to successfully reverse these harmful effects.[10,26] Conversely, Stahel et al. have suggested that galactose intake can reduce plasma insulin responses compared to both glucose and fructose as well as improve the insulin sensitivity of rat liver tissues.[27] Our results clearly indicate that the circulating galactose levels of insulin-resistant women with PCOS were higher than those of non-insulin-resistant women with PCOS, and this difference in serum galactose levels seemed unique to

women with PCOS. Additionally, the circulating galactose concentrations were positively correlated with both the FSI level and HOMA-IR in PCOS. These findings indicate that insulin resistance may be a key link between galactose and PCOS. Although the exact correlation between galactose and insulin resistance as well as the underlying mechanisms of these interactions remain elusive, these initial results shed light on the potential roles of galactose in PCOS-related insulin metabolism disorders.

The major strength of this study is that this is the first study to assess the serum galactose levels in women with PCOS, and to evaluate the relationships between serum galactose and PCOS as well as PCOS-related insulin resistance. Moreover, our findings also have a clinical implication that the serum galactose may be used as a biomarker for PCOS. Although the diagnostic performance of galactose for PCOS still needs to be further validated, our results highlight the potential clinical role of measuring serum galactose levels in daily practice.

Our study has several limitations. First, this study was a single-center study, which should be further validated by multicenter studies in the future. Second, our study was small and not longitudinal. Thus, we must interpret our data with caution and in the context of our design. Third, the phenotypes of PCOS are heterogeneous, and we were unable to evaluate separately the impact of each phenotype of PCOS on serum galactose, which may limit the generalizability of our results.

Overall, the current study emphasizes that higher circulating galactose concentrations are positively correlated with PCOS and PCOS-related insulin resistance and can be considered a potential diagnostic biomarker for PCOS. Further prospective studies as well as experimental studies are needed to confirm these results and to unveil the role of circulating galactose in the occurrence and development of PCOS.

Data sharing
The datasets generated for this study are available on request to the corresponding author.

Contributors
DL, BS and ZN conceived and designed the study. DL, BS, ZN, HJ, YM, JS, DF, and YF performed data acquisition and interpretation. DL, BS, ZN, HJ, and YM wrote the paper. DL, BS, and ZN have accessed and verified the data. All authors confirmed that they had full access to all the data in the study and accepted responsibility to submit for publication.

Declaration of interests
We declare no competing interests.

References
1. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat Rev Endocrinol.* 2018;14(5):270–284.
2. Sosicka P, Ng BG, Freeze HH. Therapeutic monosaccharides: looking back, moving forward. *Biochemistry.* 2020;59(34):3064–3077.
3. Jiao J, Shi B, Wang T, et al. Characterization of long non-coding RNA and messenger RNA profiles in follicular fluid from mature and immature ovarian follicles of healthy women and women with polycystic ovary syndrome. *Hum Reprod.* 2018;33(9):1735–1748.
4. Coelho AI, Berry GT, Rubio-Gozalbo ME. Galactose metabolism and health. *Curr Opin Clin Nutr Metab Care.* 2015;18(4):422–427.
5. Conte F, van Buuringen N, Voermans NC, Lefeber DJ. Galactose in human metabolism, glycosylation and congenital metabolic diseases: time for a closer look. *Biochim Biophys Acta Gen Subj.* 2021;1865(8):129898.
6. Rubio-Gozalbo ME, Gubbels CS, Bakker JA, Menheere PP, Wodzig WK, Land JA. Gonadal function in male and female patients with classic galactosemia. *Hum Reprod Update.* 2010;16(2):177–188.
7. Thakur M, Shaeib F, Khan SN, et al. Galactose and its metabolites deteriorate metaphase II mouse oocyte quality and subsequent embryo development by disrupting the spindle structure. *Sci Rep.* 2017;7(1):231.
8. Banerjee S, Chakraborty P, Saha P, Bandyopadhyay SA, Banerjee S, Kabir SN. Ovotoxic effects of galactose involve attenuation of follicle-stimulating hormone bioactivity and up-regulation of granulosa cell p53 expression. *PLoS One.* 2012;7(2):e30709.
9. Watkins J, Simpson A, Betts JA, et al. Galactose ingested with a high-fat beverage increases postprandial lipemia compared with glucose but not fructose ingestion in healthy men. *J Nutr.* 2020;150(7):1765–1772.
10. Bo-Htay C, Shwe T, Higgins L, et al. Aging induced by d-galactose aggravates cardiac dysfunction via exacerbating mitochondrial dysfunction in obese insulin-resistant rats. *Geroscience.* 2020;42:233–249.
11. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril.* 2016;106(1):6–15.
12. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod.* 2004;19(1):41–47.
13. Feng D, Shi B, Bi F, et al. Elevated serum mannose levels as a marker of polycystic ovary syndrome. *Front Endocrinol.* 2019;10:711. (Lausanne).
14. Shi B, Feng D, Sagnelli M, et al. Fructose levels are elevated in women with polycystic ovary syndrome with obesity and hyperinsulinemia. *Hum Reprod.* 2020;35(1):187–194.
15. Paris VR, Solon-Biet SM, Senior AM, et al. Defining the impact of dietary macronutrient balance on PCOS traits. *Nat Commun.* 2020;11(1):5262.
16. World Health Organization (WHO) International Obesity Task Force. *The Asia-Pacific Perspective: Redefining Obesity and Its Treatment. WHO Western Pacific Region.* Geneva, Switzerland: World Health Organization; 2000.
17. Parcha V, Heindl B, Kalra R, et al. Insulin resistance and cardiometabolic risk profile among nondiabetic American young adults: insights from NHANES. *J Clin Endocrinol Metab.* 2022;107(1):e25–e37.
18. Friedrich N, Thuesen B, Jørgensen T, et al. The association between IGF-I and insulin resistance: a general population study in Danish adults. *Diabetes Care.* 2012;35(4):768–773.
19. Kopin L, Lowenstein C. Dyslipidemia. *Ann Intern Med.* 2017;167: ITC81–ITC96.
20. Einhorn D, Reaven GM, Cobin RH, et al. American college of endocrinology position statement on the insulin resistance syndrome. *Endocr Pract.* 2003;9:237–252.
21. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology.* 1983;148(3):839–843.

22 Thakur M, Feldman G, Puscheck EE. Primary ovarian insufficiency in classic galactosemia: current understanding and future research opportunities. *J Assist Reprod Genet*. 2018;35(1):3–16.

23 Park JH, Choi TS. Polycystic ovary syndrome (PCOS)-like phenotypes in the d-galactose-induced aging mouse model. *Biochem Biophys Res Commun*. 2012;427(4):701–704.

24 Diao XH, Yao LJ, Wang YL, et al. Identification of critical miRNAs and mRNAs associated with polycystic ovary syndrome. *J Obstet Gynaecol Res*. 2021;47(4):1416–1424.

25 Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev*. 2016;37(5):467–520.

26 Kenawy S, Hegazy R, Hassan A, et al. Involvement of insulin resistance in d-galactose-induced age-related dementia in rats: protective role of metformin and saxagliptin. *PLoS One*. 2017;12(8):e0183565.

27 Stahel P, Kim JJ, Xiao C, Cant JP. Of the milk sugars, galactose, but not prebiotic galacto-oligosaccharide, improves insulin sensitivity in male Sprague-Dawley rats. *PLoS One*. 2017;12(2):e0172260.