

Lipidomics Analysis of Human Follicular Fluid Form Normal-weight Patients With Polycystic Ovary Syndrome

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Research

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Abstract

Background

The polycystic ovary syndrome (PCOS) is the most common endocrine associated with insulin resistance, even in the absence of overweight. The global lipid profile of the follicular fluid in PCOS with normal weight as yet has not been investigated. The objection of this study was to explore the changes of lipids in the follicular fluid of PCOS with normal weight.

Methods

Follicular fluid samples were collected from patients who underwent IVF, including normal women (control group, n=10) and normal weight women with PCOS (PCOS group, n=8). A lipidomic analysis was performed by high performance liquid chromatography/ mass spectrometry (HPLC-MS). Multidimensional statistical analysis was performed to disclose the global differences between the two groups. Further, differential lipid analysis between the two groups was performed by Fold Change Analysis (FC Analysis) and T-test to screen potential markers.

Results

All 812 species of 32 subclasses of lipids were identified by lipidomics analysis. 108 kinds of lipids were considered as the potential candidate differential metabolites with the score of variable importance in the project (VIP) more than 1 by the orthogonal partial least squares discriminant analysis. 32 lipids were significantly different between the PCOS group and the control group simultaneously with $FC > 1.5$ or $FC < 0.67$, $p\text{-value} < 0.05$ and VIP value > 1 . These differential metabolites were the lipid subclasses including triglycerides (TG), phosphatidylethanolamines (PE) and phosphatidylinositols (PI).

Conclusion

The identified differential lipids in the follicular fluid may be considered as candidate biomarkers as well as therapeutic targets of PCOS with normal weight.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders with a prevalence ranging from 5 to 10% among women of reproductive age. Clinical manifestations include menstrual irregularities, anovulatory infertility, signs of androgen excess, metabolic and psychological disorders. PCOS is the most important cause of anovulation infertility, with a high incidence of 18%[1]. In addition, the presence of low oocyte quality is common when these patients undergo in vitro fertilization (IVF) treatment in PCOS patients[2]. Follicular fluid (FF) serves as the complex microenvironment for oocyte growth, follicular maturation, and germ cell-somatic cell communications[1]. It accumulates all metabolisms during oocytes growth. Hence, the changes in the metabolites of FF have been linked to impaired oocyte quality and outcomes of IVF in PCOS. Metabolomics is a high-throughput approach for the detection of extensive small-molecule metabolites in various biological samples that can provide useful information related to diagnosis biomarker and pathogenesis mechanism of diseases. Currently, metabolomics analysis of FF found that lipid metabolites are significantly changed in PCOS patients[3, 4]. Lipidomics was one of the metabolomics approach that focuses on lipids and was a promising technique for overviewing lipid profiles in body fluids blood and tissues. Currently, there are a few published lipidomics studies on PCOS in plasma[5, 6]. However, the lipidomics of PCOS in FF which directly reflect the microenvironment for oocyte has not been investigated previously.

Women with PCOS are often overweight or obese. However, due to the diversity of clinical and biochemical manifestations of PCOS, 30%-50% of patients are with normal weight[7]. The presence or absence of obesity is one of the most important factors influencing PCOS phenotypes. The incidence of insulin resistance and metabolic syndrome in obese PCOS patients was higher than that in non-obese PCOS patients, but the latter also had metabolic abnormalities[8]. The non-overweight PCOS women were found higher LH and FSH levels in plasma than normal women, and basal insulin, total cholesterol, LDL, VLDL, and triglyceride levels in the overweight group were found to be significantly higher. But there were no difference in triglyceride and total cholesterol in plasma between the normal weight PCOS women and normal women[9]. Until now, the lipids in FF from normal weight PCOS have never been studied. In the present study, we used lipidomics technology based on high performance liquid

chromatography/ mass spectrometry (HPLC-MS) platform to obtain a comprehensive picture of the lipid alternations that occur in FF of PCOS patient with normal weight.

2. Experimental

2.1. Subjects

We designed a case-control study, including FFs from 8 cases of normal-weight (body mass index (BMI) < 25 kg/m²) women who were diagnosed PCOS and 10 cases of healthy women. This study was performed at the First Affiliated Hospital of Chongqing Medical University, and designed conformed to the ethics guidelines given in the Declaration of Helsinki. The FFs were recruited who underwent IVF or intracytoplasmic sperm injection (ICSI) in our reproductive center between January 2019 and December 2019. Written informed consents were obtained from all subjects and all experimental protocols were approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University. The enrollment criteria for PCOS were based on the presence of any two or three features – hyperandrogenism, menstrual irregularity, and polycystic ovary morphology, according to the Rotterdam consensus criteria. None of the women had the abnormal levels of the serum androgen hormones. The control group included women who seek treatment for tubal infertility or male factors with normal ovarian reserve (regular menstrual cycles, and the AMH(anti-Mullerian hormone) concentration of ≥ 1.1 ng/mL) and normal BMI. Women with endometriosis, glucose metabolism, cancer, or other medical disorders that could affect folliculogenesis were excluded. The gonadotropin-releasing hormone antagonist protocol was used for controlled ovarian stimulation. When the follicle reached an average diameter of ≥ 18 mm the urinary human chorionic gonadotropin (u-HCG, Lizhu, Zhuhai) was administered; then ultrasound-guided FF samples were collected after 36 h by using an 18-gauge single-lumen aspiration needle.

2.2. Preparation of samples

Clear FF samples with no macroscopic blood contamination were included. After oocyte isolation, the FF samples were centrifuged at 800 g for 10 min to remove particulates. The FF supernatant was stored at -80 °C until analysis. Before analysis, the FF samples were thawed in a 4 °C water bath. Lipids were extracted according to MTBE (methyl tert-butyl ether) method. Briefly, samples were homogenized with 200 μ L water and 240 μ L methanol. Then 800 μ L of MTBE was added and the mixture was ultrasound 20 min at 4 °C followed by sitting still for 30 min at room temperature. The solution was centrifuged at 14,000 g for 15 min at 10 °C and the upper organic solvent layer was obtained and dried under nitrogen. The samples were reconstituted in 200 μ L 90% isopropanol/acetonitrile and centrifugation at 14,000 g for 15 min at 10 °C, finally 3 μ L of sample was injected to the LC-MS system. A pooled FF sample from all healthy controls and PCOS patients was used as quality control (QC) and undergo the same sample extraction procedures.

2.3. Chemicals and reagents

MS-grade methanol, MS-grade acetonitrile, HPLC-grade isopropanol were purchased from ThermoFisher (Thermo Fisher Scientific Co., Waltham, Massachusetts, USA). HPLC-grade formic acid, HPLC-grade ammonium formate and MTBE were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, Missouri, USA).

2.4 UPLC-MS experiments

The UPLC-MS experiments were settled using the Q-Exactive Plus mass spectrometer (Thermo Scientific) system equipped with a UHPLC Nexera LC-30A (SHIMADZU). Mass spectrometer performed in either ESI positive or negative mode. ESI parameters were optimized and preset for all measurements as follows: Source temperature, 300 °C, Capillary Temp, 350 °C, the ion spray voltage was set at 3000V, S-Lens RF Level was set at 50% and the scan range of the instruments was set at m/z 200–1800. Reversed phase chromatographic separation was performed on a Waters ACQUITY UPLC CSH C18 column (2.1 mm \times 100 mm, 1.7 μ m) maintained at 45 °C. Solvent A was acetonitrile–water (6:4, v/v) with 0.1% formic acid and 0.1mM ammonium formate and solvent B was acetonitrile–isopropanol (1:9, v/v) with 0.1% formic acid and 0.1mM ammonium formate. The initial mobile phase was 30% solvent B at a flow rate of 300 μ L/min. It was held for 2 min, and then linearly increased to 100% solvent B in 23 min, followed by equilibrating at 5% solvent B for 10 min.

The sample was analyzed in random order. To test the reproducibility of the sample preparation procedure and LC-MS analyse, QC samples were injected at the beginning of the run and after every eight real samples. Each of the prepared QC samples was analyzed only once.

2.5. Data processing

The raw data were processed by LipidSearch software version 4.1 (Thermo Scientific™) for peak identification, lipid identification by MS/MS, peak extraction, peak alignment, and quantitative. The parameter was set as following: precursor tolerance: 5 ppm product tolerance: 5 ppm, product ion threshold: 5%. Then the extraction data was normalized by total peak area after deleted the lipid with RSD>30% among samples.

2.6. Statistical analysis.

The software simca-p 14.1 (Umetrics, Umea, Sweden) was used for pattern recognition. After pareto-scaling pretreatment, multidimensional statistical analysis was performed, including unsupervised principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA). One-dimensional statistical analysis by SPSS Statistics v17.0.0 (SPSS, Inc., Chicago, USA) includes Student's t-test and variance multiple analysis. R software was used to draw volcano map, hierarchical clustering analysis map and correlation analysis map. Comparisons of parameters between two groups were performed by independent-Sample T test ,depending on the distribution (normal or not) of the examined variables.

3. Results

3.1. Clinical characteristics

The selected clinical baseline characteristics of these participants in our study are summarized in Table 1. The cases and controls were well matched on age and BMI. There were no significant differences in LH (Luteinizing Hormone), FSH (follicle-stimulating hormone), LH/FSH, E2 (Estradiol), T (testosterone) levels, blood glucose, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fertilization rate, cleavage rate and embryos top-quality rate between normal-weight PCOS patients and the controls. AMH (anti-Mullerian hormone) and No. of oocyte retrieve in PCOS were significantly higher than the control group.

Table 1 Main clinical characteristics of the study groups.

	Controls(n=ten)	PCOS(n=eight)	P-value
Age(years)	28.80±2.74	27.12±3.40	0.26
BMI(kg/m ²)	20.96±1.99	19.96±1.77	0.28
LH (mIU/mL)	4.20±2.61	5.57±2.48	0.22
FSH (mIU/mL)	5.86±1.64	6.66±2.21	0.39
LH/FSH	0.76±0.63	0.93±0.49	0.28
E ₂ (pg/mL)	44.3±40.69	68.75±39.91	0.22
T (ng/mL)	0.49±0.14	0.53±0.11	0.52
Glucose (mmol/l)	4.83±0.42	5.19±0.46	0.52
Cholesterol (mmol/L)	4.25±0.25	4.12±0.32	0.77
triglyceride(mmol/L)	0.87±0.28	0.88±0.40	0.96
HDL-C(mmol/L)	1.47±0.28	1.56±0.54	0.64
LDL-C(mmol/L)	2.40±0.71	2.02±0.41	0.17
AMH(ng/mL)	3.52±1.12	12.97±3.42	0.00*
No.of Oocyte retrieve	13.2±5.59	22.3±5.99	0.00*
Normal fertilization Rate(%)	87.03±0.18	79.24±0.16	0.40
Cleavage Rate(%)	95.08±0.57	87.76±0.11	0.07
Embryo top-quality Rate(%)	43.44±0.21	53.42±0.27	0.43

Data presented as mean ± standard deviation (SD).

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E₂, estradiol; T, testosterone; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AMH, anti-Mullerian hormone.

normal fertilization rate=no. oocytes with 2PN and 2PB/no. COC inseminated×100%; cleavage rate=no. cleaved embryos on Day 2/no. 2PN/2PB oocytes on Day 1×100%; embryo top-quality rate=top quality embryos/no. normally fertilized oocytes×100%.

3.2. Quality control

Four QC samples were analyzed by both ESI+ and ESI- scan models, described in Section 2.4. Then the base peak chromatogram were extracted and overlapped to compare the response intensity and the retention time of the peak. The results showed that the response intensity and retention time of the peak of each QC sample basically overlapped (showed in figure 1 of supplementary material). Then the Pearson correlation analysis was applied to the four QC samples of the response intensity of the extracted peaks. The results showed that the correlation coefficients were more than 0.9 (showed in figure 2 of supplementary material). The peaks of all experimental samples and QC samples were extracted to perform principal component analysis (PCA) after pareto-scaling. The results showed that QC samples were closely packed together. Multivariate Control Chart (MCC) was used to monitor the stability of the method. The Y-axis is the variance of the first principal component of PCA model in all QC samples and the X-axis is the loading order the QC samples. The variance was no more than two fold of standard deviations, indicating that the analysis system was stable and all the data were under control (Figure 1). The relative standard deviation (RSD) of peaks in the QC samples is an important indicator of data quality. The ratio of peaks with RSD no more than 30% was more than 80% in the four QC samples. All these results indicated that the method had good repeatability, reliability, and stability for lipidomics analysis.

3.3. Identification of lipid compounds

Data obtained from positive and negative scan models of HPLC–MS were analyzed by LipidSearch. All 812 species of 32 subclasses of lipids were identified (Figure 2). The differential between the two groups in subclasses of lipid was showed in figure 3 of supplementary material.

3.4. Data modeling

In this study, PCA of FF was constructed to disclose the global differences of lipid between PCOS and healthy controls. In PCA model, the clusters were not distinctly separated in the scores plots, which might due to the complexity and variation of clinical samples (Fig 3A). Green box represented healthy controls, and the blue dot represented PCOS patients. Then OPLS-DA was used finally chosen as an effective approach to filter the unrelated variations. In contrast, the two-dimensional scores of OPLS-DA plot showed a clearer separation between the healthy controls and the PCOS patients (Fig 3B).

3.5. Identification of Potential Biomarkers

Potential differential metabolites were defined according to the variable importance in the projection (VIP) values based on OPLS-DA. Usually, lipid molecules with VIP more than 1 are considered to have significant contributions in model interpretation. As a result, 108 kinds of lipids were considered as the potential candidate differential metabolites with VIP scores more than 1. Among these lipids, 36 lipids were significantly different between the PCOS group and the control group analyzed by T-test/non-parametric test ($P < 0.05$), shown in table 1 of supplementary material. Differential lipids between the two groups were performed by Fold Change Analysis (FC Analysis) and T-test to screen potential biomarkers. Usually, the screening criteria is $FC > 1.5$ or $FC < 0.67$ and $P \text{ value} < 0.05$. The information of the differential expression multiples, P values and VIP values of lipid molecules were displayed in the form of volcano plot, as shown in figure 4. The purple dots in the volcano plot were the differential lipid molecules that were simultaneously with $FC > 1.5$, $P \text{ value} < 0.05$ and $VIP \text{ value} > 1$, and the blue dots were the lipid molecules simultaneously with $FC < 0.67$, $P \text{ value} < 0.05$ and $VIP \text{ value} > 1$. The area of the dots represents the VIP value. The larger the bubble area showed in the plot, the greater the VIP value. As a result, 32 lipids were simultaneously with $FC > 1.5$, $P \text{ value} < 0.05$ and $VIP \text{ value} > 1$ (showed in table 2).

Table 2 The candidate lipids which as potential biomarkers to separate normal weight PCOS women to control women

LipidIon	Class	IonFormula	CalMz	RT-(min)	Fold Change	P-value	VIP
PE(16:0/22:6)+H	PE	C43 H75 O8 N1 P1	764.522484	10.2352508	2.1364297	0.01170255	1.2827738
TG(16:0/14:0/18:1)+NH4	TG	C51 H100 O6 N1	822.754516	20.7435549	2.82786013	0.00768395	1.04024184
TG(16:1/16:1/18:1)+NH4	TG	C53 H100 O6 N1	846.754516	19.714067	2.85935032	0.00103754	2.07802736
TG(16:0/16:0/18:2)+NH4	TG	C53 H102 O6 N1	848.770166	20.8812114	2.84645308	0.00753119	3.11342089
TG(16:0/16:0/18:1)+NH4	TG	C53 H104 O6 N1	850.785816	21.7834094	2.9164798	0.00508461	3.14637718
TG(16:1/18:1/18:2)+NH4	TG	C55 H102 O6 N1	872.770166	19.859873	2.51669633	0.00941933	4.83457578
TG(16:0/18:1/18:2)+NH4	TG	C55 H104 O6 N1	874.785816	20.8954062	2.26683859	0.00796182	7.04217237
TG(16:0/18:1/18:2)+NH4	TG	C55 H104 O6 N1	874.785816	19.8521076	2.42105627	0.00117044	1.21618584
TG(16:0/18:1/18:1)+NH4	TG	C55 H106 O6 N1	876.801466	21.7866945	2.51369634	0.00910841	6.11421082
TG(18:0/16:0/18:1)+NH4	TG	C55 H108 O6 N1	878.817116	22.584629	2.22068302	0.0275356	1.3333522
TG(18:1/18:2/18:3)+NH4	TG	C57 H102 O6 N1	896.770166	19.4825832	2.55170831	0.00642044	1.03093598
TG(18:2/18:2/18:2)+NH4	TG	C57 H102 O6 N1	896.770166	18.8771548	2.24487784	0.00535694	1.51541463
TG(16:0/18:1/20:4)+NH4	TG	C57 H104 O6 N1	898.785816	20.5420673	2.42182935	0.00972723	1.68243907
TG(18:1/18:2/18:2)+NH4	TG	C57 H104 O6 N1	898.785816	19.884727	2.00402129	0.00849049	2.6606902
TG(18:1/18:1/18:2)+NH4	TG	C57 H106 O6 N1	900.801466	20.9160682	1.83056246	0.01101498	3.33870883
TG(18:0/18:1/18:2)+NH4	TG	C57 H108 O6 N1	902.817116	20.8947311	1.52937154	0.03751919	1.62092888
TG(18:0/18:1/18:2)+NH4	TG	C57 H108 O6 N1	902.817116	21.8017114	2.30938518	0.01129426	3.83179623
TG(16:0/18:1/20:1)+NH4	TG	C57 H110 O6 N1	904.832766	22.5836836	2.22447076	0.02719304	1.57736708
Cer(d18:1/16:0)+HCOO	Cer	C35 H68 O5 N1	582.510298	11.3804774	1.65187002	0.02982499	1.19827027
PE(16:0/18:2)-H	PE	C39 H73 O8 N1 P1	714.507931	10.7122963	2.02509893	0.00623164	2.06847352
PE(16:0/18:1)-H	PE	C39 H75 O8 N1 P1	716.523581	11.4653225	1.87622225	0.00103417	1.36389091
PE(16:0/20:4)-H	PE	C41 H73 O8 N1 P1	738.507931	10.5345784	1.94530888	0.01049121	2.08653155
PE(18:0/18:2)-H	PE	C41 H77 O8 N1 P1	742.539231	11.6753321	1.59339478	0.03091878	2.34017355

PE(18:0/18:1)-H	PE	C41 H79 O8 N1 P1	744.554881	12.413823	1.66288434	0.00777766	1.2000378
PE(16:0/22:6)-H	PE	C43 H73 O8 N1 P1	762.507931	10.2584914	2.08517498	0.01442886	3.08345178
PE(18:1/20:4)-H	PE	C43 H75 O8 N1 P1	764.523581	10.6214751	1.71023624	0.0148648	1.68604552
PE(16:0/22:5)-H	PE	C43 H75 O8 N1 P1	764.523581	10.9153492	2.03051326	0.01237958	1.17476918
PE(18:0/20:4)-H	PE	C43 H77 O8 N1 P1	766.539231	11.5051781	1.55899537	0.02358573	2.91621258
PE(18:1/22:6)-H	PE	C45 H75 O8 N1 P1	788.523581	10.323258	1.98966059	0.02424517	1.17590614
PE(18:0/22:6)-H	PE	C45 H77 O8 N1 P1	790.539231	11.2313382	2.08883459	0.01561633	2.20426914
PI(16:0/18:2)-H	PI	C43 H78 O13 N0 P1	833.518557	9.29326139	1.56851322	0.01842781	1.53643448
PI(16:0/18:1)-H	PI	C43 H80 O13 N0 P1	835.534207	10.0911401	1.56876413	0.0223933	1.66545385

4. Discussion

PCOS is a highly heterogeneous disease. Women with PCOS the oocytes are often weak quality, which leads to lower fertilization, cleavage, implantation, and increase of miscarriage rates[10]. The oocyte microenvironment provides the necessary requirements for oocyte developmental competence. FF is the liquid that surrounds the oocyte, forms its microenvironment and plays a key role in its development[3]. So it is crucial to explore the FF metabolites pattern. Untargeted metabolomics, which focuses on the dynamic changes of all small molecules in response to the disturbance of the organism, can provide deep insights for the etiopathogenesis and the discovery of biomarkers for various diseases[11]. Several researches of metabolomics on PCOS in plasma found the lipids were significantly changed. Lipidomics as a branch of metabolomics is a high-throughput analytical technique that can systematically and efficiently analyze lipid composition and expression changes in various biological processes. At present, lipidomics analysis Technology is generally based on liquid chromatography-mass spectrometry (LC-MS) platform and mainly divided into untargeted and targeted analysis. The non-targeted lipidomics can systematic analysis of various types of lipids in the sample without bias, and the targeted lipidomics is mainly used for selective and specific quantitative analysis of specific lipids[12].

In present study, we are the first to comprehensively investigate lipid profile changes of PCOS with normal weight in FF using the untargeted lipidomics approach based on the ultra-high performance LC coupled to Q-Exactive MS. A number of lipids were detected in FF by this sensitive technique. We identified a series of differential lipids including triglycerides (TG), phosphatidylethanolamines (PE), phosphatidylinositols (PI) and etc. between women with PCOS and the healthy women.

TG was only the differentially represented subclass of lipid in FF in PCOS patients and normal women in this study (showed in figure 3 of supplementary material). TG is a combination of three fatty acids combined with glycerol which are the main source of energy. PCOS patients are often with dyslipidemia mainly includes high levels of low-density lipoprotein (LDL) and TG and low levels of high-density lipoprotein (HDL). Furthermore, lipid abnormalities are closely associated with obesity, insulin resistance and hyperandrogenemia in PCOS patients[13]. On the other hand, obesity has an important influence on the lipid metabolism [14]. Recently, a case-control study was conducted on 153 women with PCOS and 449 healthy women as controls to compare the serum lipid profile. Each group was divided into normal, overweight and obesity subgroups according to the BMI. Surprisingly, the TG in plasma was only found in the obesity group with significantly difference between the women with PCOS and healthy women. There was no difference of plasma TG in non-obesity women between the two groups[15]. As refer to FF, Liu et al. found reduced level of TG was highly related to the lower fertilization rate in PCOS[16]. However, increased BMI is associated with elevated TG in ovarian FF[17]. This research about the lipid profile in FF was enrolled PCOS women with average weight heavier

than normal women. To our knowledge the lipid metabolites profiles in FF of PCOS and normal women with weight matched were not been investigated previously. In this study, significantly increased TG levels in FF were found in PCOS compared with normal women. Increased TG might associate with low quality of oocyte in PCOS patients. A research found mouse cumulus-oocyte complexes (COCs) exposed to lipid-rich FF during their maturation had increased oocyte lipid content, induction of endoplasmic reticulum stress markers, and impaired oocyte nuclear maturation[17]. TG accumulation in the FF were also correlated to the levels of adipokines and proinflammatory cytokines in FF, implying inflammatory processes in the FF that are caused by high TG levels and may also attenuate oocyte development[18]. That was why PCOS patients always have the impaired oocyte development in ART.

In this study many phosphatidylethanolamines showed higher accumulation in FF of the PCOS patients. Considering the potential PE functions, it was found that PE is a major phospholipid in the membranes of eukaryotic cells and modulates the membrane fluidity[19]. PE also existed in FF. A lipidomic analysis of FF samples collected from patients who underwent IVF, including normal responder women who became pregnant (control group), women with PCOS and a hyper response to gonadotropins (PCOS group) and women with only hyper response to gonadotropins (HR group) found that some form of PEs were higher represented HR groups and lower represented in PCOS group compared with the control group[20]. Regarding our study, although many forms of PE were high presented in PCOS patients, the total PE level was found no significant difference between the two groups in this paper.

Finally, Phosphatidylinositol (PI) was identified high presented in the PCOS patients with normal weight. PI is composed of a glycerol backbone, two acyl chains esterified and an inositol ring linked by a phosphate. Although PI constitutes only 5–10% of total cellular lipids in mammalian cells, it is the source for generating seven phosphorylated derivatives of PI which plays a major role in a vast array of cellular functions including signalling, membrane traffic, ion channel regulation and actin dynamics[21]. The PI subclass of lipid had detected in FF before. Thais et al. compared MALDI-MS lipid fingerprints in the FF of young poor responder women in comparison with normal responders. A lipid ion belonged to the PI subclass was found overrepresented in the poor ovarian response group[22]. Another lipidomic analysis of FF samples found some PIs was higher presented in normal responder women compared with women with PCOS and a hyper response to gonadotropins and women with only hyper response to gonadotropins. In this study, some forms of PI were high presented in PCOS patients, but the total PI level was found no significant difference between the two groups.

The results were obtained from a small set of sample because strict criteria were used to screen the patients enrolled in this study. This experimental design is a biomarker discovery study in general and the sample was used as a screening set, so the prospective biomarkers proposed in this work need to be confirmed in an independent cohort as the validation set. This study noted the lipid profiles of normal weight women with PCOS were different from the lipid profiles found in normal women. Furthermore, the potential lipid markers found in FF, highlighted by the relative increase in TG in the PCOS groups, contributed to improving the understanding of the molecular mechanisms involved in PCOS women without overweight. These biomarkers have demonstrated the lipids are related to molecular processes in the normal weight PCOS, such as inflammatory processes and endoplasmic reticulum stress in FF that are caused by high TG levels and may impaired oocyte nuclear maturation. That was why PCOS patients always have the impaired oocyte development in ART. Therefore, FF lipid profile analysis is an important tool for identifying a panel of potential biomarkers, because it reflects the ovarian microenvironment.

5. Conclusions

In conclusion, this is the first study using the untargeted lipidomics technology based on HPLC-MS to analyze the lipid subclasses alterations in normal weight PCOS focusing on the microenvironment of the oocyte. A pattern recognition technique allowed us to specifically discriminate normal weight patients with from the normal women during IVF, which described a comprehensive picture of the lipid alternations that occurred in PCOS. The identified dysfunction lipids of TG, PE and PI might function as an important diagnostic tool and closely related to alteration in FF of PCOS with normal weight.

Abbreviations

POCS: Polycystic ovary syndrome; IVF: In vitro fertilization; COC: Cumulus-oocyte complexes; FF: Follicular fluid; BMI: Body mass index; ICSI: Intracytoplasmic sperm injection; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E₂:Estradiol; T: Testosterone; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AMH: Anti-Mullerian hormone; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides; PE: Phosphatidylethanolamines; PI: phosphatidylinositols.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

YNB and LM contributed to the conception and design of the study; YNB, HYR and YC consulted the literature; YNB and YC enrolled the patients and collected the sample; LM performed the data acquisition and statistical analysis; YNB, HYR and YC wrote the first draft of the manuscript. LM contributed to the manuscript revision, The authors read and approved the submitted version.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved by the First Affiliated Hospital of Chongqing Medical University and all human biologic materials were collected after receiving written informed consent from patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

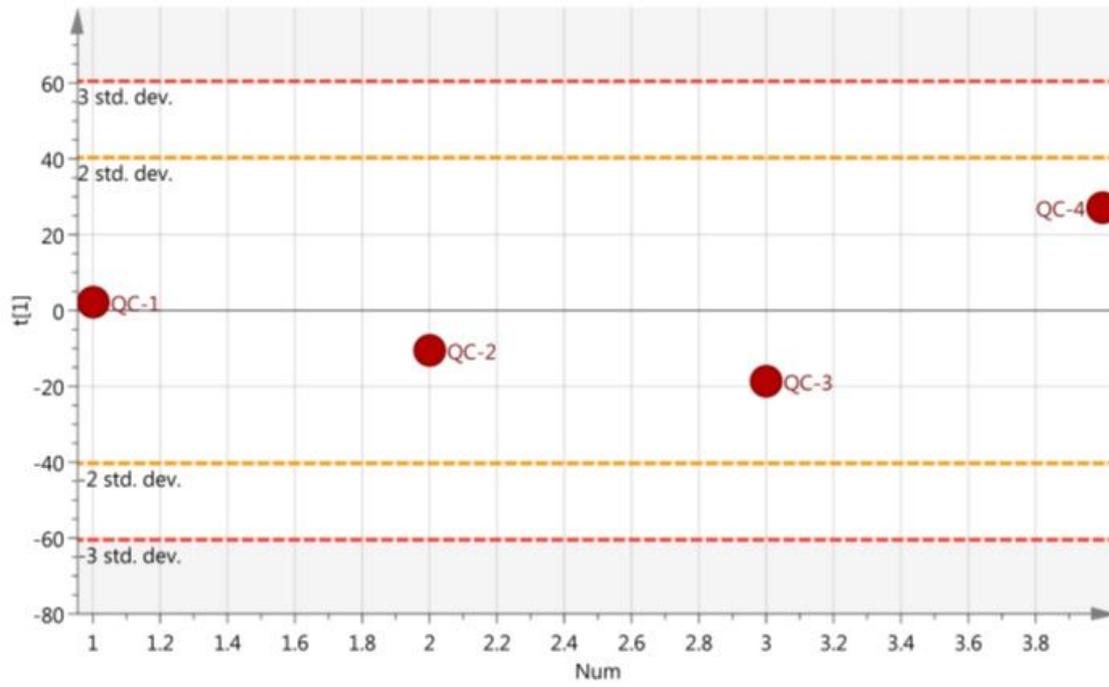


Figure 1

Quality control charts.

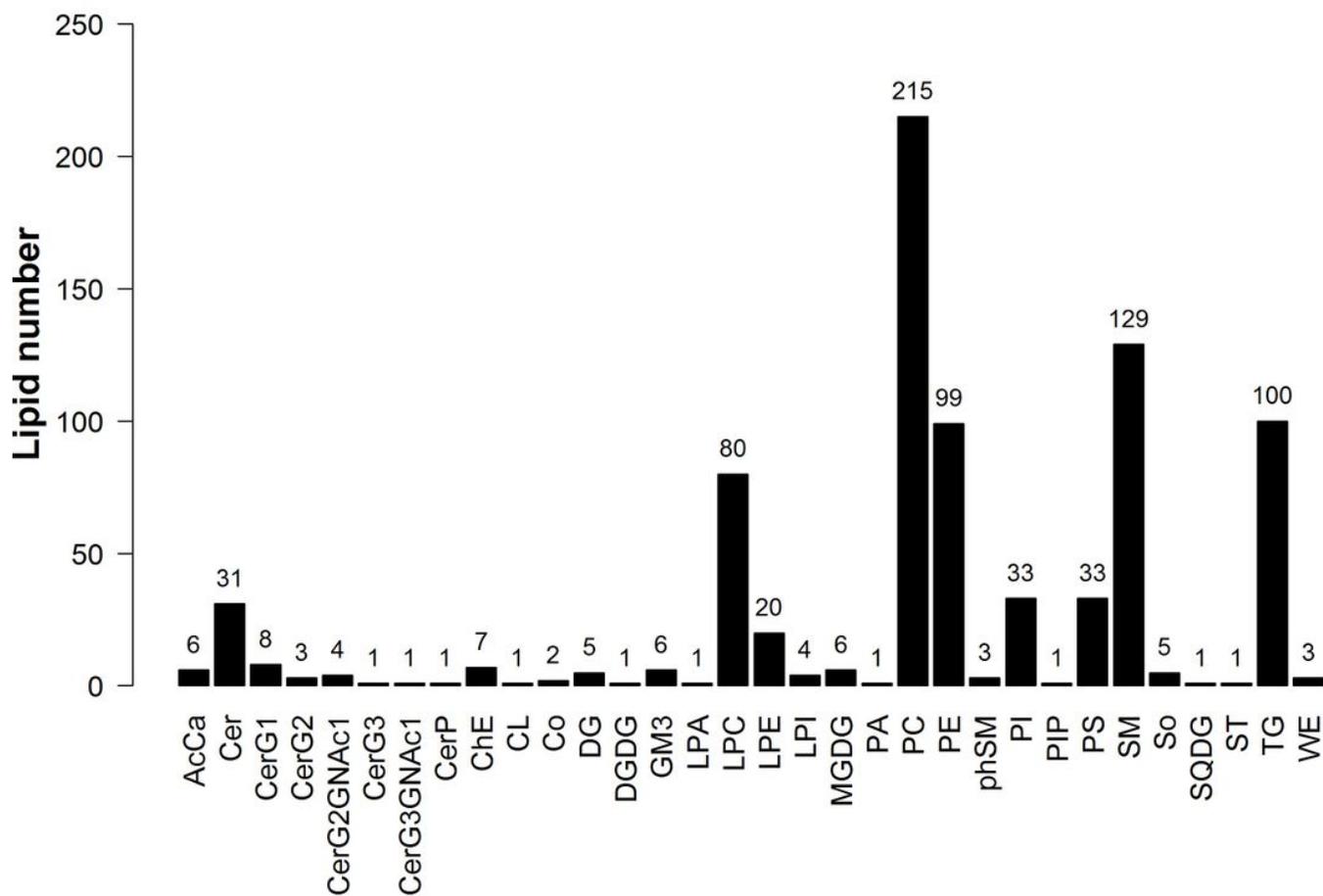


Figure 2

The classification of identified lipids

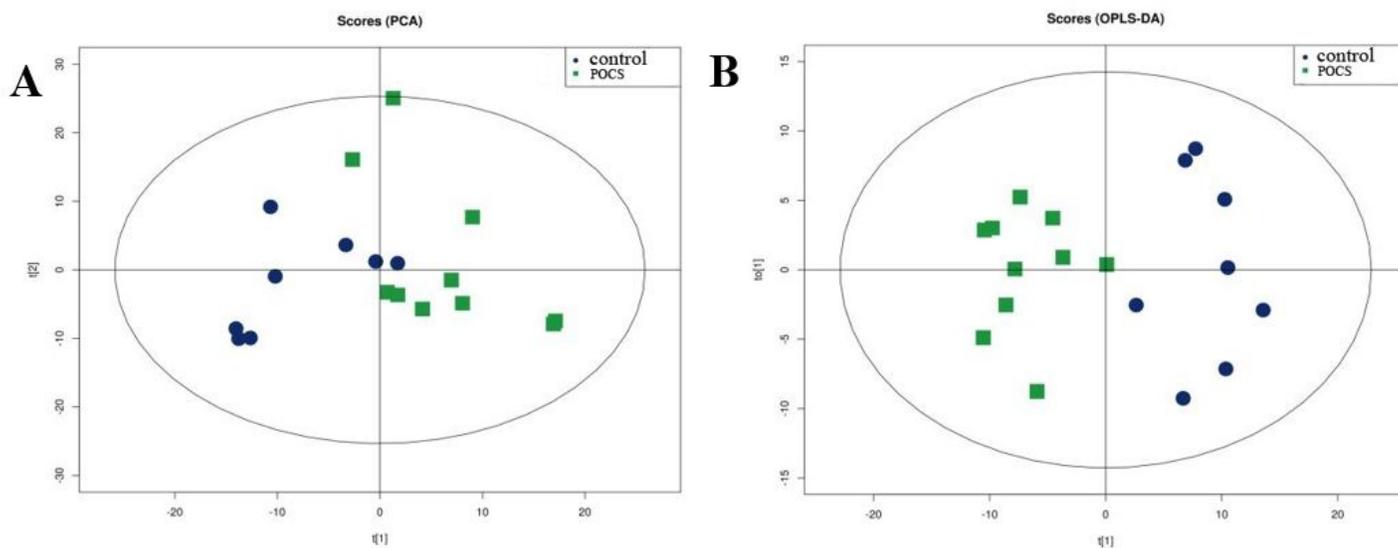


Figure 3

Score plots of PCA (A) and OPLS-DA (B) model separating normal weight PCOS women (blue circle) to control women (green square).

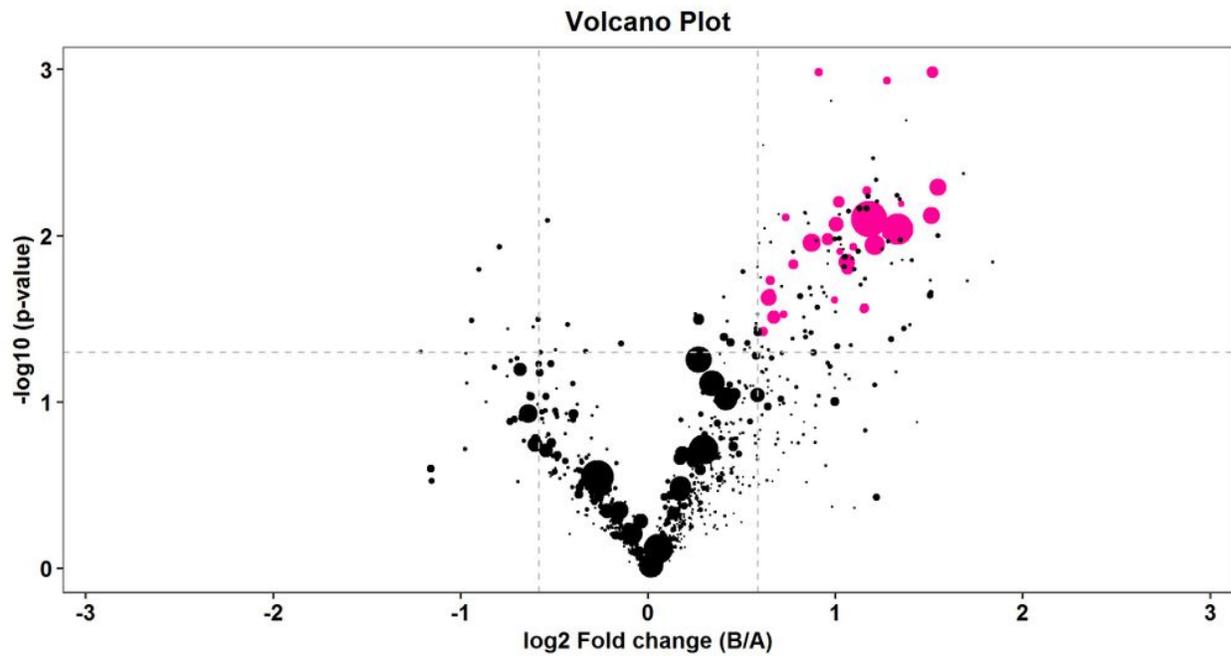


Figure 4

The volcano plot based on FC, VIP and P values of the differential lipid molecules. The purple dots in the volcano plot were the differential lipid molecules that were simultaneously with FC > 1.5, P value <0.05 and VIP value > 1, and the blue dots were the lipid molecules simultaneously with FC <0.67, P value <0.05 and VIP value > 1. The area of the dots represents the VIP value.

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