Endometriosis is associated with aberrant metabolite profiles in plasma

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Objective: To identify metabolites that are associated with and predict the presence of endometriosis.
Design: Metabolomics study using state-of-the-art mass spectrometry approaches.
Setting: University hospital and universities.
Patient(s): Twenty-five women with laparoscopically confirmed endometriosis (cases) and 19 women with laparoscopically documented absence of endometriosis (controls). None of the women included in this study had received oral contraception or GnRH agonists for a minimum of 1 month before blood collection.
Intervention(s): Plasma collection.
Main Outcome Measure(s): Metabolite profiles were generated and interrogated using multiple mass spectrometry methods, that is, high performance liquid chromatography coupled with negative mode electrospray ionization tandem mass spectrometry, UPLC-MS/MS, and ultra performance liquid chromatography-electroSpray ionization-quadrupole time-of-flight (UPLC-ESI-Q-TOF). Metabolite groups investigated included phospholipids, glycerophospholipids, ether-phospholipids, cholesterol-esters, triacylglycerol, sphingolipids, free fatty acids, steroids, eicosanoids, and acylcarnitines.
Result(s): A panel of acylcarnitines predicted the presence of endometriosis with 88.9% specificity and 81.5% sensitivity in human plasma, with a positive predictive value of 75%. However, due to data limitations the outcome of the receiver operating characteristic curve analysis was not significant.
Conclusion(s): A diagnostic model based on acylcarnitines has the potential to predict the presence and stage of endometriosis. (Fertil Steril® 2017;107:699–706. ©2017 by American Society for Reproductive Medicine.)
Key Words: Diagnosis, endometriosis, metabolomics, acylcarnitines

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Endometriosis is an estrogen-dependent disease that is characterized by the presence of functional endometrial tissue outside of the uterine cavity. It affects 5%–10% of women of reproductive age and is characterized by inflammation, pelvic pain, and infertility (1–3). Endometriosis can appear as peritoneal lesions, ovarian superficial implants or endometriotic cysts (endometriomas), and/or deeply

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infiltrating disease extending to bowel, bladder, and ureter, often associated with pelvic adhesions (4). Endometriosis is frequently associated with chronic abdominal pain, cyclic pelvic pain, dysmenorrhea, dyspareunia, dysuria, dyschezia, and impaired fertility (5, 6). Endometriosis-associated pain can be caused by peritoneal inflammation, adhesion formation, and specific innervation of the endometrium and endometriotic lesions and is correlated with the presence of deeply infiltrating disease (7–10). So far, it has not been possible to accurately predict the presence of endometriosis based on symptoms, clinical examination, imaging techniques, or blood tests, except laparoscopy. As a consequence it takes on average approximately 8–11 years (11) between onset of symptoms and diagnosis. At present, the gold standard for diagnosis of endometriosis is laparoscopic inspection with histologic confirmation (11). However, laparoscopy is a surgical procedure with rare but significant potential risks for the patient (12) and is only performed when endometriosis is suspected, which is often not the case.

According to our current understanding, endometriotic stromal cells are important drivers of endometriotic lesion growth and survival. At least two classes of small metabolites are known to be involved, that is, estrogens and prostaglandins (13). Estrogens are known to enhance the growth and invasion of endometriotic tissue, whereas prostaglandins and cytokines mediate pain, inflammation, and infertility. Numerous attempts have been made to elucidate whether any or a combination of these or other systemic biomarkers, metabolites, or cellular products can provide information regarding the presence and state of endometriosis (14).

Metabolomics is a promising approach to identify metabolite biomarkers in blood (15) as the metabolome is a reflection of phenotypic changes in an organism in response to the presence of disease, genetic changes, and nutritional, toxicological, environmental, and pharmacological influences (15). Several metabolomics studies employing nuclear magnetic resonance (NMR)-based approaches (metabolic fingerprinting) and mass spectrometry–based approaches (targeted metabolomics) have been performed in patients with endometriosis in an attempt to find disease biomarkers and gain more insight into the disease pathophysiology (16–22). Even though metabolites have been identified that show significant association with the presence of disease, none of the diagnostic candidates have been confirmed in independent metabolomics studies in other research labs nor have they been validated in prospective randomized clinical trials.

In the current pilot study, depending on the metabolites of interest, we used high performance liquid chromatography coupled with negative mode electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), ultraperformance LC (UPLC-MS/MS), and ultra performance liquid chromatography-electroSpray ionization-quadrupole time-of-flight (UPLC-ESI-Q-TOF) to interrogate a variety of metabolite classes in the plasma of patients with endometriosis that are known or suspected to have an association with the pathophysiology of endometriosis, that is, lipids, acylcarnitines, steroids, and eicosanoids. Vouk and coworkers (21) performed a similar study and evaluated 148 lipids and acylcarnitines, of which 109 passed measurement quality control, in the plasma of women with and without endometriosis. Eight lipid metabolites were identified as endometriosis-associated biomarkers. A model consisting of a combination of individual metabolites and ratios between pairs of metabolite concentrations showed the best diagnostic performance, 90% sensitivity and 84.3% specificity. In this study, we were able to enhance the scope of the metabolic diagnostic platforms by allowing the targeted analysis of 241 lipids and 43 acylcarnitines. Using nuclear magnetic resonance metabolomics, Vicente-Muñoz and coworkers (23) studied metabolites in the urine of women with endometriosis and observed that these women exhibited higher concentrations of N^1-methyl-4-pyridone-5-carboxamide, guanidinosuccinate, creatinine, taurine, valine, and 2-hydroxyisovalerate and decreased concentrations of lysine compared with healthy women.

Eicosanoids and steroids have not yet been thoroughly explored in the plasma of patients with endometriosis using mass spectrometry–based methods. Keski-Rahkonen and coworkers (24) developed LC-MS/MS methods for the quantitative analysis of seven steroid hormones in serum and analyzed serum samples in patients and controls for the purpose of validating the method but not to assess the predictive value of these metabolites. Using the same technology, Ray et al. (25) evaluated eicosanoids in the peritoneal fluid (PF) of patients with endometriosis in an attempt to find a plausible explanation for the chronic pelvic pain many women with endometriosis experience. While these investigators reported increased amounts of PGE_2, PGD_2, and 12- and 15-HETE in the PF of women with endometriosis, no inferences were made with regard to their diagnostic potential. In the present study, we report the development of two new platforms capable of detecting 17 different steroid hormones and 120 eicosanoids.

Using four high-performance targeted metabolomics platforms to search for (combinations of) metabolites with potential as diagnostic biomarkers, we report here that a selection of acylcarnitines was significantly associated with the presence of disease.

MATERIALS AND METHODS

Patient Information and Sample Collection

The women participating in the pilot study (n = 44) were recruited from the Leuven University Fertility Center of the University Hospital Gasthuisberg in Leuven, Belgium, and the Department of Obstetrics and Gynecology at the University Hospital Saint Luc in Brussels, Belgium. They were scheduled for laparoscopic surgery for the diagnosis and treatment of endometriosis or other gynecological diseases because of pelvic pain (n = 14/44 or 32%), infertility (n = 14/44 or 32%), or combined pelvic pain and infertility (n = 16 or 36%). None of these women had received oral contraceptives, GnRH analogues, or any other hormone treatment. Demographic, clinical, and menstrual cycle characteristics are shown in Table 1. All women gave their written informed consent to participate in this study, and the study was approved by the Commission for Medical Ethics of the Leuven University Hospital (approval number KU Leuven: ML6282) and of the

TABLE 1

Demographic, clinical, and menstrual cycle characteristics for endometriosis patients and controls.

Characteristic	Controls without endometriosis (n = 19)	Cases with endometriosis (n = 25)	Total group (n = 44)	*P* value by group
Age, mean ± SD				
Total	41 ± 14	32 ± 7	35 ± 12	.415
<26	21	21 ± 5	21 ± 4	.542
27–30	28 ± 1	27 ± 1	27 ± 1	.472
31–35	35	33 ± 1	34 ± 1	.398
36–40	37 ± 1	38 ± 1	38 ± 1	.423
>41	58 ± 13	42 ± 1	50 ± 9	.421
Smoking (%)				
No	73 (14)	100 (25)	89 (39)	.07
Yes	26 (5)	0	11 (5)	.345
Infertility (%)	47 (9)	72 (18)	61 (27)	.114
Pelvic pain (%)	10 (2)	80 (20)	50 (22)	.05
BMI, kg/m^2, mean ± SD				
Underweight <18.5	18.5	0		
Normal 18.6–24.9	23 ± 1	21 ± 2	22 ± 2	.643
Overweight 25–29.9	27 ± 2	26	27 ± 2	.361
Obese >30	35 ± 2	36 ± 2	36 ± 2	.286
Total	26 ± 5	24 ± 6	25 ± 5	.345
Stage of menstrual cycle (%)				
Luteal phase	47 (9)	28 (7)	36 (16)	.123
Follicular phase	53 (10)	72 (18)	63 (28)	.153
Endometriosis stage (American Society for Reproductive Medicine)				
IV	None	7	7	
III	None	9	9	
II	None	6	6	
I	None	3	3	

Note: P values are based on Student's *t*-test (*P*< .05).

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University Hospital Saint Luc in Brussels, both in Belgium (Université Catholique de Louvain: B40320107889).

Twenty-five women had laparoscopically confirmed endometriosis (cases), stage according to the classification of the American Society for Reproductive Medicine (26) (Table 1) as minimal (n = 3), mild (n = 6), moderate (n = 9), or severe (n = 7) endometriosis. The other 19 women had laparoscopically documented absence of endometriosis (controls), including both women with a normal pelvis (n =16) and women with the presence of uterine myoma (two with subserosal myoma, one with submucosal myoma) as nonendometriotic pelvic pathology (n = 3).

The collection of peripheral blood samples was carried out based on a protocol from the Leuven University Fertility Center in all patients just before induction of anesthesia for laparoscopic surgery (27). After blood collection, the heparin tubes were gently inverted 10 times to prevent the formation of blood clot. The heparin tube was centrifuged at 1,400 *g* for 10 minutes at 4°C; 300 μL aliquots of heparin plasma were prepared in labeled Eppendorf tubes and stored at −80°C until further analysis.

Metabolite Measurements

In the next section, a short description is given of the platforms used in this study, and more details on these protocols are available in the Supplemental Material (available online).

Eicosanoids were detected by HPLC-ESI-MS/MS (Acquity, Ultra performance LC, Waters), using the modifications of a previously reported method (28). In addition, a subcohort of the samples was used for the screening of F3-isoprostanes using an LC-MS/MS approach. F3-isoprostanes are a measure of oxidative damage resulting from lipid peroxidation by reactive oxygen species (29). Forty-five eicosanoids passed the quality control criteria and were included in the analyses.

For steroid profiling in human plasma, a new standardized UPLC-MS/MS (Acquity, TQMS Xevo, Waters) assay in kit format (SteroIDQ kit BIOCRATES Life Sciences AG) was used for routine determination of 17 steroid hormones: aldosterone, androstenedione, androsterone, corticosterone, cortisol, cortisone, 11-deoxycorticosterone, 11-deoxycortisol, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) 17β-estradiol (E_2), estrone (E_1), etiocholanolone, 17α-hydroxyprogesterone, progesterone, testosterone, and dihydrotestosterone (30).

To assess the lipid profile in human plasma, an adapted method from Hu et al. (31) was used. The lipidomics platform based on the UPLC-ESI-Q-TOF (Agilent 6530) high-resolution mass spectrometer was employed in both positive and negative detection mode to be able to detect 186 lipids including bile acids, free fatty acids, phospholipids such as phosphocholine (PC) and lyso-phosphocholine (LPC), phosphoethanolamines (PE) and lyso-phosphoethanolamines (LPE), glycerophospholipids, and sphingolipids such as ceramides and sphingomyelins, diglycerols, cholesterol esters, and triglycerides (TG).

The acylcarnitine platform covers acylcarnitines like trimethylamine-N-oxide, choline, betaine, deoxycarnitine, and carnitine. An Acquity UPLC system with autosampler

(Waters) was coupled online with a Xevo tandem quadrupole mass spectrometer (Waters) operated using Masslynx data acquisition software (ver. 4.1; Waters). Twenty-four acylcarnitine metabolites met the quality control criteria and were included in the analyses.

Statistical Analysis

All the metabolite data were checked for normal distribution. Acquired data were evaluated using TargetLynx software (Waters), by integration of assigned multiple reaction monitoring (MRM) peaks and normalization using proper internal standards. The closest-eluting internal standard was employed. Blank samples were used to correct for background, and in-house developed algorithms were applied using the pooled quality control (QC) samples to compensate for shifts in the sensitivity of the mass spectrometer over the batch. Metabolites that did not show a normal distribution of their values were log-transformed before statistical analysis.

Univariate Analysis

To assess the association between the metabolites and the presence of disease, pelvic pain, and infertility, Student's *t*-test was used at a significance level of 5%. To account for multiple testing, we applied the Benjamini-Hochberg false discovery rate correction procedure (32) at a significance level of 5%. A general linear model analysis adjusted for age, body mass index (BMI), and phase of menstrual cycle was used to evaluate the association between metabolite levels and both the presence and classification of endometriosis. One-way analysis of variance (ANOVA) followed by Bonferroni corrections was used to determine whether these differences were significant. Receiver operative characteristics (ROC) curve was used for determination of diagnostic value. $P<.05$ was considered statistically significant. For maximum diagnostic value (sensitivity of 100% and specificity of 100%) and corresponding confidence intervals, the area under the ROC curve is 1; and in the minimum diagnostic value, that the diagnosis is totally by chance, the area under the curve is 0.5. For the univariate analysis, the IBM/SPSS statistical software package (ver. 20) was used.

Multivariate Analysis

Principal component analysis (PCA) was performed to visualize the variation of metabolite levels of each platform on the classification of endometriosis (both cases and controls). Data were always autoscaled before PCA analysis. Partial least squares discriminant analysis (PLSDA) included double cross validation as well as the "leave one out" method and was applied to discriminate cases from controls (33). Multivariate analysis was based on SOLO (Eigenvector Research Inc.), a stand-alone Chemometrics Software based on MATLAB.

RESULTS

Out of 421 potential targeted metabolic analytes, 290 were detected in the plasma of control subjects and women with endometriosis. Of these, 272 passed the quality control: 186 lipids, 24 acylcarnitines, 17 steroid hormones, and 45 eicosanoids.

Eicosanoid Profiling

Examples of the eicosanoid profiling and identification in plasma of patients with endometriosis and controls are presented in Supplemental Figure 1. Multiple cyclooxygenase, lipoxygenase, and cytochrome P450 metabolite products were identified including arachidonic acid derivatives (44%), with a minority being derived from the oxidation of linoleic acid (29%) or from eicosapentanoic acid (12%) and a small percentage being derived from α-linoleic acid (6%), docosahexanoic acid (6%), and dihomo-γ-linolenic acid (3%).

Univariate analysis did not reveal any statistical association between the endometriosis stage and all individual eicosanoid metabolites. Furthermore, neither multivariate analysis nor univariate analysis based on the F3 isoprostane profile revealed any significant difference between cases and controls (data not shown).

The two-component PCA model of eicosanoid profile explains 46% of the total variation in the data set (cases and controls; Supplemental Fig. 4A), however, participants were distributed in the eicosanoids space without separation between controls and patients. A bi-plot, a visualization of the relationship between the participants (cases versus controls) and the eicosanoids in the reduced two-dimensional space, revealed that cases tended to be associated closer to eicosanoids derived from cyclooxygenase (COX) or lipoxygenase (LOX) pathways, while controls tended to be associated closer to eicosanoids derived from the cytochrome P-450 (CYP) pathway (Supplemental Fig. 5A).

Steroid Profiling

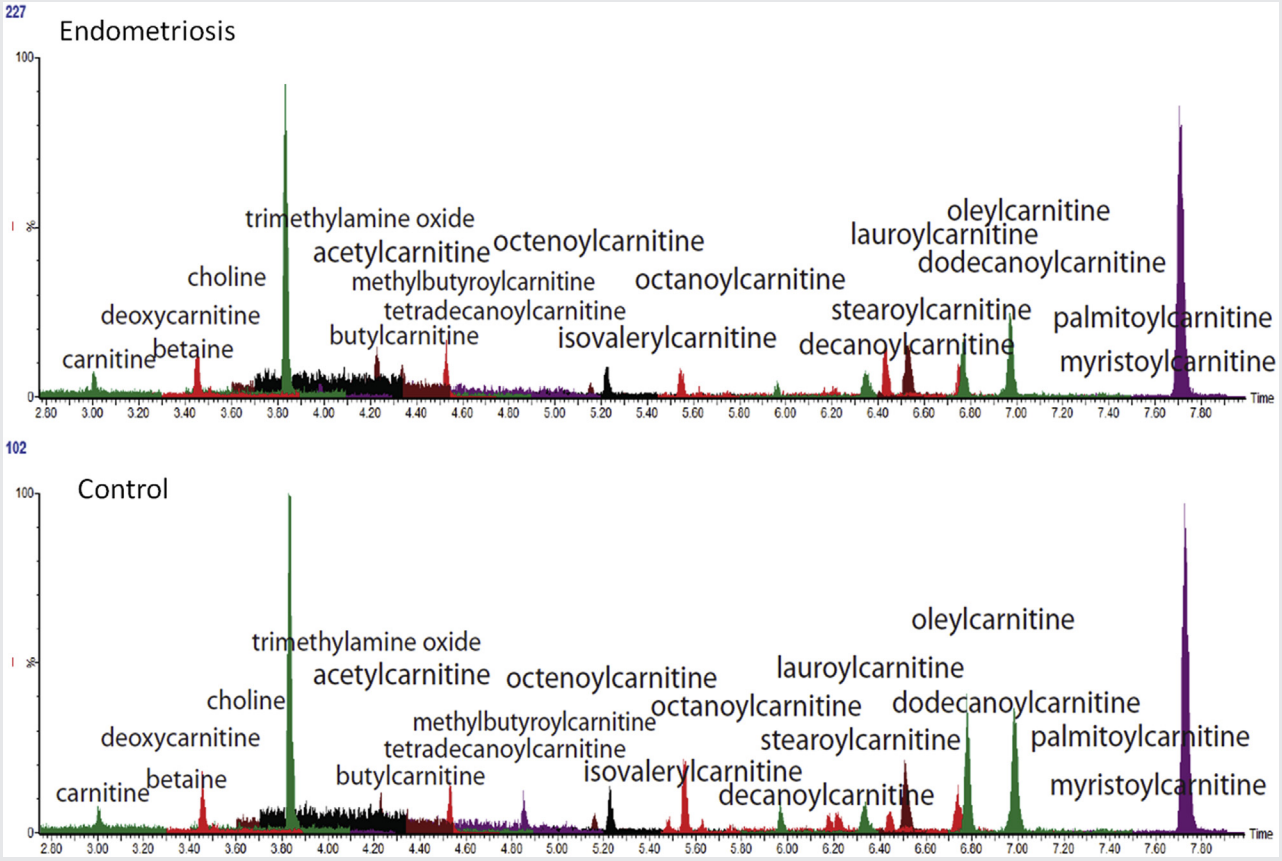
Examples of the steroid profiling and identification in the plasma of patients with endometriosis and controls are presented in Supplemental Figure 2. The identified steroids belonged to four different groups of steroids: progestagens, glucocorticoids, estrogens, and androgens. Univariate analysis did not reveal any statistically significant differences between cases and controls.

The two-component PCA model of steroid profiles explained 43.6% of the total variation in the data set (Supplemental Fig. 4B). As for the eicosanoids, no separation was observed between cases and controls with regard to the steroid profiles; this was confirmed in the bi-plot visualization (Supplemental Fig. 5B).

Neutral Lipid Profiling

Examples of the neutral lipid profiling in the plasma are shown in Supplemental Figure 3A. Univariate analysis (Student's *t*-test) showed that triacylglycerol levels (TG(51:4), $P=.026$; TG(56:8), $P=.03$) were significantly lower in cases than in controls, whereas levels of phosphatidylcholine (PC)-(O 34:1) ($P=.04$), PC-(O 34:2) ($P=.02$), and PC-(O 38:4) ($P=.03$) were significantly higher in cases than in controls. However, these differences were no longer statistically

FIGURE 1



Representative chromatograms of acylcarnitines detected with UPLC-MS/MS in plasma collected from a control subject and a patient with endometriosis.

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significant after correction for false discovery rate using the Benjamini and Hochberg method.

The two-component PCA model explained 51% of the variation in the data set describing neutral lipids abundances in plasma (Supplemental Fig. 4C). Again, no separation was observed between cases and controls based on the neutral lipid profiles. In line with this, a bi-plot (Supplemental Fig. 5C) showed that the various lipids cluster together, while the participants (cases and controls) were spread around this space.

Polar Lipid Profiling

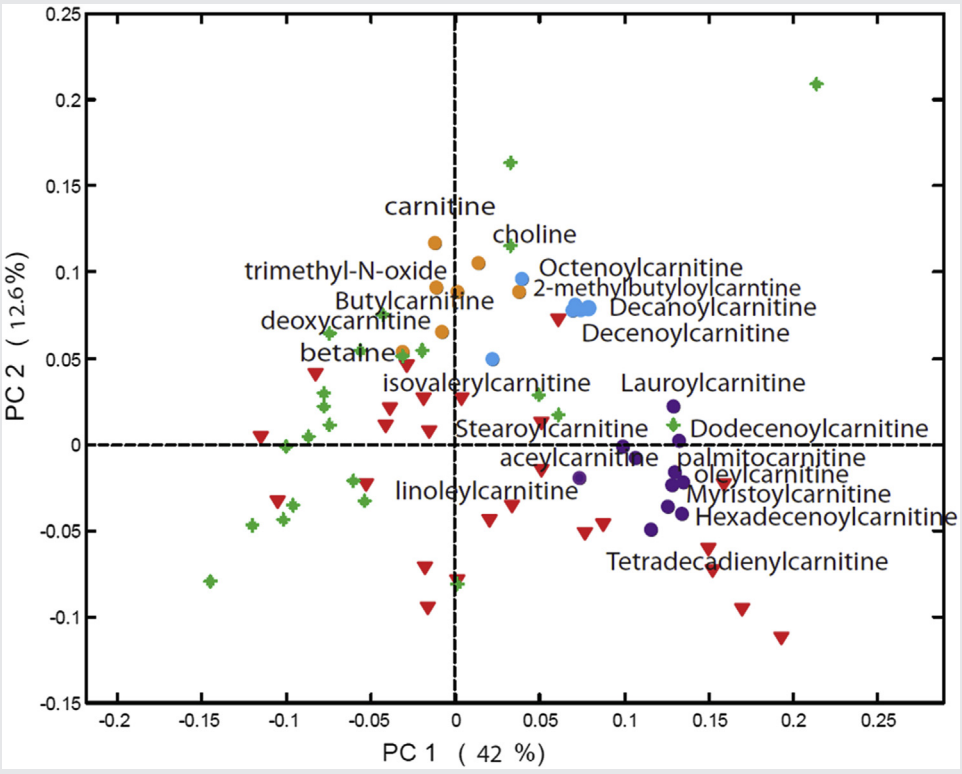
Polar lipids were profiled separately from the neutral lipids; examples of the metabolite profiles are presented in Supplemental Figure 3B. According to univariate analysis, plasma levels of FA (20:3) were significantly higher in cases ($P=.04$) than in controls, but this difference was not apparent after correction for a false discovery rate using the Benjamini and Hochberg method.

The two-component PCA model explained 62% of the variation in the data set describing polar lipids abundances in plasma, but no separation was observed between cases and controls based on the polar lipid profiles. This was confirmed in the bi-plot (not shown).

Acylcarnitine Profiling

All carnitines were used for both univariate or multivariate analysis, but only significant results are presented. Examples of the acylcarnitine profiling results are presented in Figure 1. Univariate analysis revealed that lauroylcarnitine (patients with endometriosis: 0.2–0.5 μg/L, controls: 0.08–0.09 μg/L; $P=.013$, $q = 0.05$), oleylcarnitine (patients with endometriosis: 0.4–0.7 μg/L, controls: 0.1–0.25 μg/L; $P=.010$, $q = 0.046$), myristoylcarnitine (patients with endometriosis: 0.07–0.1 μg/L, controls: 0.02–0.04 μg/L; $P=.009$, $q = 0.052$), tetradecenoylcarnitine (patients with endometriosis: 0.5–0.6 μg/L, controls: 0.09–0.12 μg/L; $P=.005$, $q = 0.038$), and hexadecenoylcarnitine (patients with endometriosis: 0.09–0.12 μg/L, controls: 0.03–0.05 μg/L; $P=.002$, $q = 0.023$) were increased in the plasma of patients with endometriosis when compared with control subjects, even after correction for multiple testing using the Benjamini and Hochberg method. In contrast, trimethylamine-N-oxide levels were significantly decreased in cases (0.2–0.6 μg/L;

FIGURE 2



Bi-plots visualizing the relation between controls (*green*), cases (*red*). and acylcarnitines (short-chain acylcarnitines: *orange*; medium-chain acylcarnitines: *light blue*; long-chain acylcarnitines: *purple*).

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$P=.001$, $q = 0.023$) compared with controls (0.8–2 μg/L). In relation to this, ANOVA analysis revealed that the above significant differences were related to stage III endometriosis only and not to other stages (I, II, IV). These differences remained significant after a general linear models analysis adjusted for age, BMI, and phase of menstrual cycle (follicular or luteal phase). However, owing to data limitations, the outcome of the ROC curve analysis was not significant.

The two-component PCA model based on the acylcarnitines profile in plasma explained 56% of the total variation in the data set (Supplemental Fig. 4D) and a better separation was observed between controls and cases compared with the previously tested metabolites. The bi-plots (Fig. 2) clearly showed that the cases group were closer to medium- or long-chain acylcarnitines, while controls group were closer to the short-chain acylcarnitines.

Analysis using the PLSDA model followed by double validation revealed 11 out of 47 misclassifications, resulting in a specificity of 88.9%, a sensitivity of 81.5% (Supplemental Fig. 6), and a positive predictive value of 75%.

DISCUSSION

Small metabolites such as estrogens and prostaglandins are considered to be the fuel of endometriotic cells (1, 2) and at the same time may reflect changes in the phenotype of the women due to the presence of endometriotic tissue. As such they could potentially serve as noninvasive biomarkers for the presence and or stage of the disease. In this pilot study, we analyzed a total of 272 metabolites related to inflammation, steroid metabolism, apoptosis, and cellular energy homeostasis in plasma obtained from women with and without endometriosis using mass spectrometry–based methods. Increased levels of acylcarnitines including lauroylcarnitine, oleylcarnitine, myristoylcarnitine, hexadecenoylcarnitine, and tetradecenoylcarnitine combined with decreased levels of trimethylamine-N-oxide predicted the presence of endometriosis with 88.9% specificity and 81.5% sensitivity. In contrast, no correlations with the presence of disease or disease state were found for the steroid hormones, eicosanoids, and neutral and polar lipids.

Acylcarnitines are composed of a fatty acid esterified to a carnitine molecule and are intermediate oxidative metabolites with proinflammatory properties (34). The role of the carnitine is to transport the long-chain fatty acids across the mitochondrial membrane for oxidization. Under certain circumstances, when the fatty acids are not completely metabolized and pools of long-chain fatty acid CoA accumulate, this drives conversion to acylcarnitines, which can be exported out of the mitochondria and cells (34). Aberrant acylcarnitines levels therefore are a reflection of a dysbalance

in the energy production and/or oxidative state of cells known to be associated with the presence of endometriosis (18, 35).

Our findings are supported by earlier reports of increased plasma levels of long-chain acylcarnitines and higher ratios of long-chain acylcarnitine variants over medium-chain acylcarnitine C8:1 (21) in cases with ovarian endometriosis when compared with controls. In addition, the investigators in one report found that five out of eight of the tested ratios of long-chain acylcarnitines over C8:1 were positively correlated with the number of leukocytes in the blood of patients with endometriosis, pointing also towards a proinflammatory environment.

As there are many conditions or situations that can lead to increased levels of long-chain acylcarnitines (i.e., fatty acid oxidation disorders, ischemia, type 2 diabetes mellitus), it is not likely that by using this class of metabolites alone sufficient specificity will be achieved with regard to predicting the presence of disease. However, we also found significantly reduced levels of trimethylamine-N-oxide in the plasma of women with endometriosis. Trimethylamine-N-oxide is the product of the conversion of trimethylamine in the liver. The trimethylamine itself is formed from precursors such as phosphatidylcholine and carnitine found in food products such as meat and eggs (36). Elevated trimethylamine-N-oxide levels have been associated with reduced renal function, increased systemic inflammation, and a higher risk of developing cardiovascular disease (37), which could be related to its potential to increase vascular inflammation (38). More important, however, is probably the role of trimethylamine-N-oxide as natural organic osmolyte (39). Osmolytes are critical to help cells and organisms adapt to perturbations that can cause structural changes in cell volume and proteins. Osmolytes act as chaperones of proteins by increasing the thermodynamic stability and keeping them in a functionally active conformation. Reduced levels of trimethylamine-N-oxide may indicate suboptimal cellular conditions that can lead to disruption of biologically important processes. Reduced levels of trimethylamine-N-oxide were also found in patients with inflammatory bowel disease (36). PLSDA revealed that combining the selected long-chain acylcarnitines with the trimethylamine-N-oxide yielded a specificity of 88.9% and a positive predictive value of 75%.

When compared with previously reported known diagnostic biomarkers, the performance of this panel is fairly good (14). However, recent studies in which the investigators used NMR-based metabolomic fingerprinting approaches on serum samples (19, 22) particularly revealed changes in amino acids as well as in metabolites that are evident of impaired glucose metabolism and mitochondrial respiration. With pattern recognition analysis of profiles of metabolites, a clear separation between endometriosis and controls was achieved, reaching a positive predictive value of 95%. These metabolites apparently better reflect the impact of the presence of endometriotic lesions on homeostasis in women. The same can be said for proteins that have been identified recently in mass spectrometry–based proteomics approaches and that show impressive predictive power with regard to the presence of disease (20, 40). A logical next step towards a reliable diagnostic set of biomarkers would be to perform a validation study in which the combined discriminating power of the identified disease-related metabolites and proteins is tested, since this is a pilot study and its confirmation has to come from a follow-up study.

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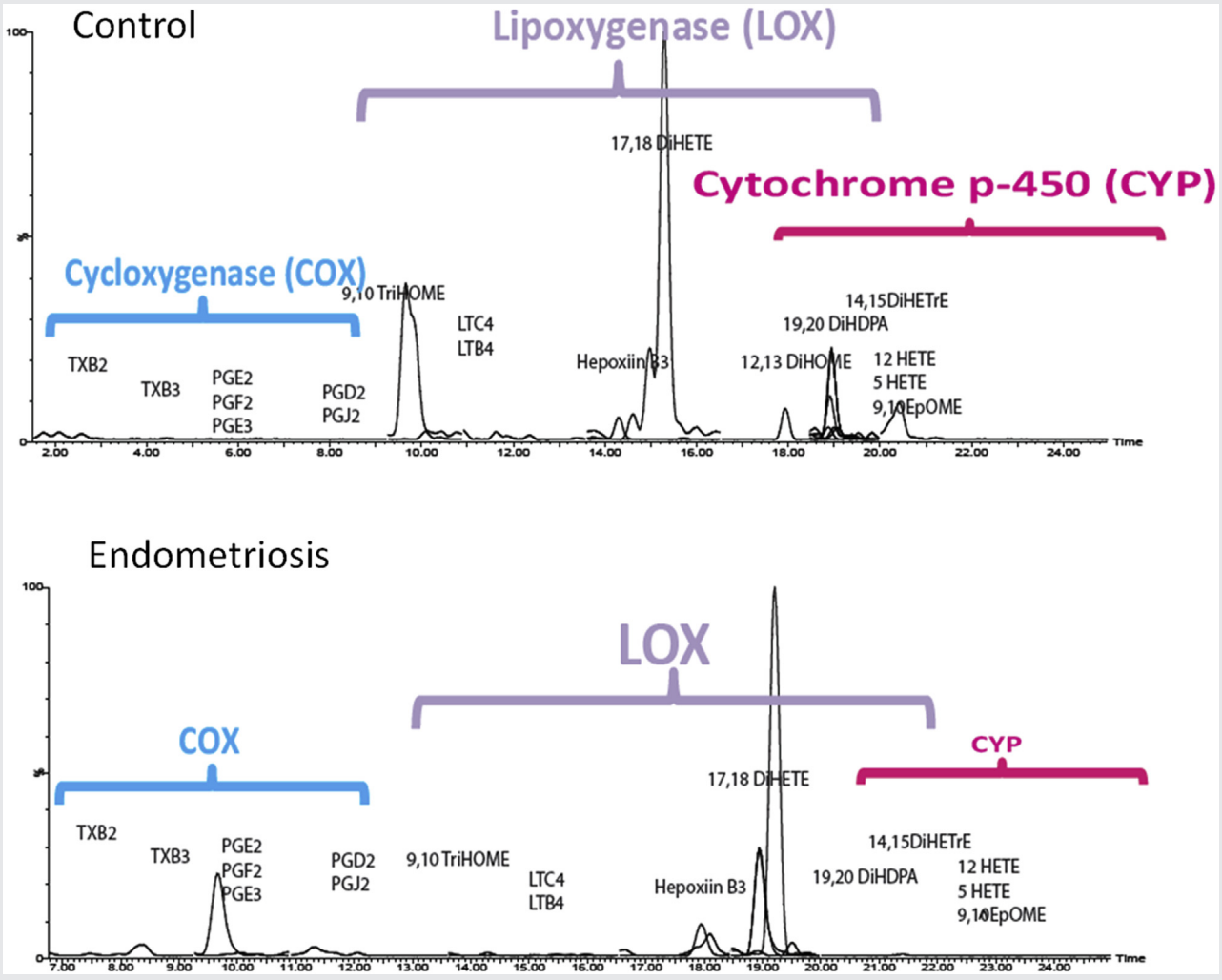
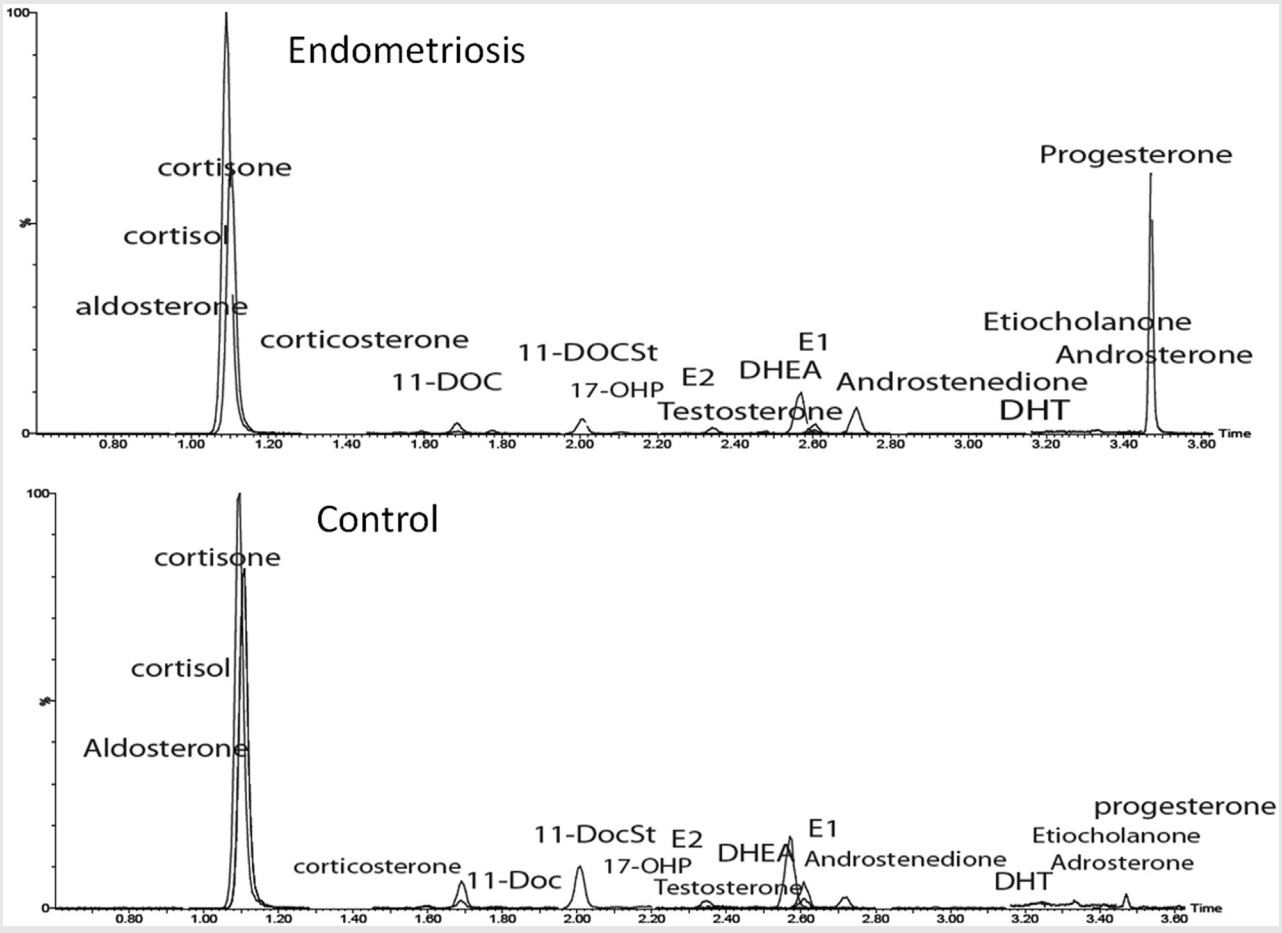
SUPPLEMENTAL FIGURE 1



Representative chromatograms of eicosanoids detected with LC-ESI/(selected reaction monitoring)/MS in plasma collected from a control subject and a patient with endometriosis.

Letsiou. Metabolite profiling in endometriosis. Fertil Steril 2017.

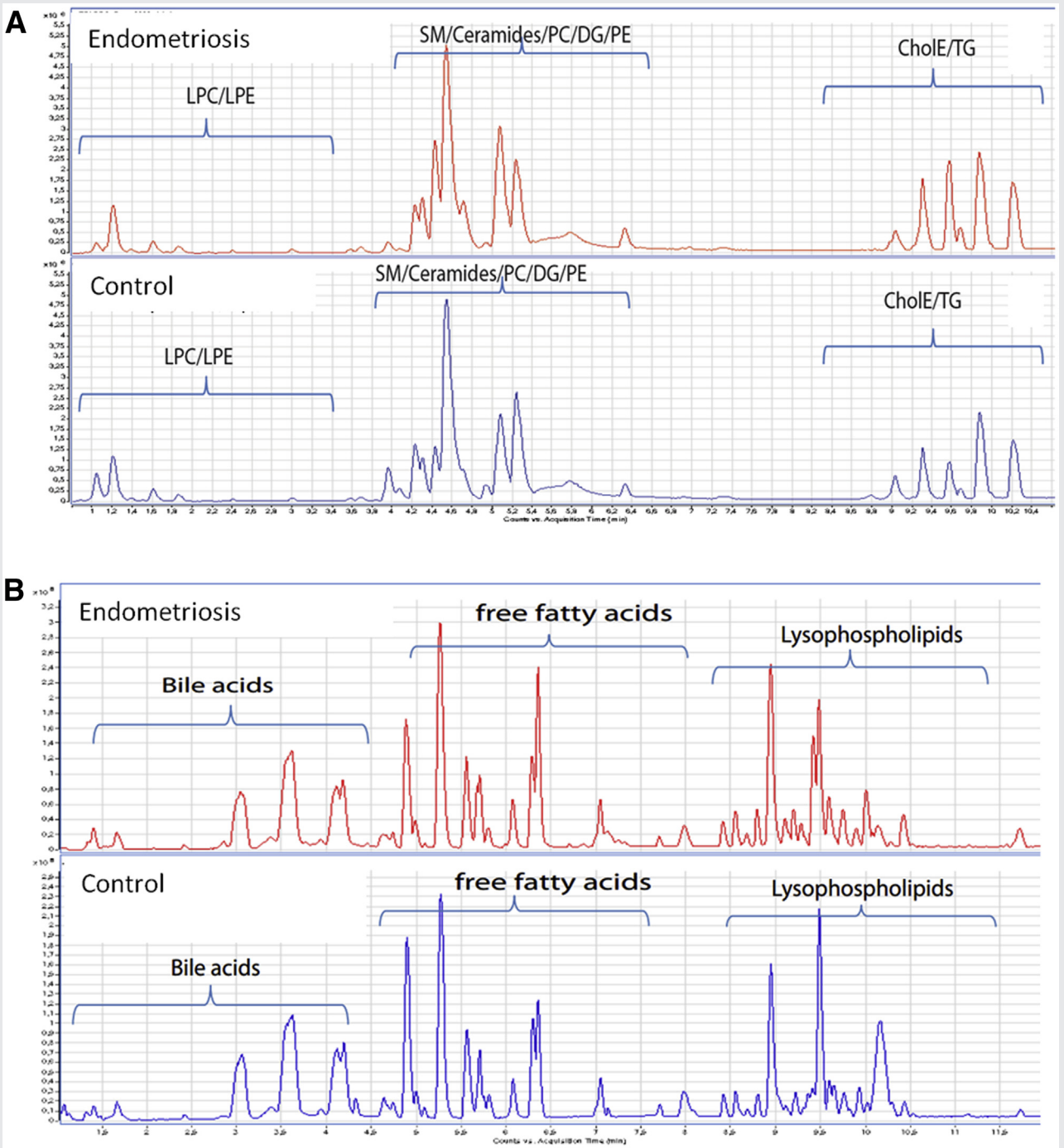
SUPPLEMENTAL FIGURE 2



Representative chromatograms of steroid hormones detected with UPLC-ESI/(SRM)/MS in plasma collected from a control subject and a patient with endometriosis.

Letsiou. Metabolite profiling in endometriosis. Fertil Steril 2017.

SUPPLEMENTAL FIGURE 3



Representative chromatograms of neutral (**A**) and polar (**B**) lipids detected with UPLC-TOF/MS in plasma collected from a control subject and a patient with endometriosis.

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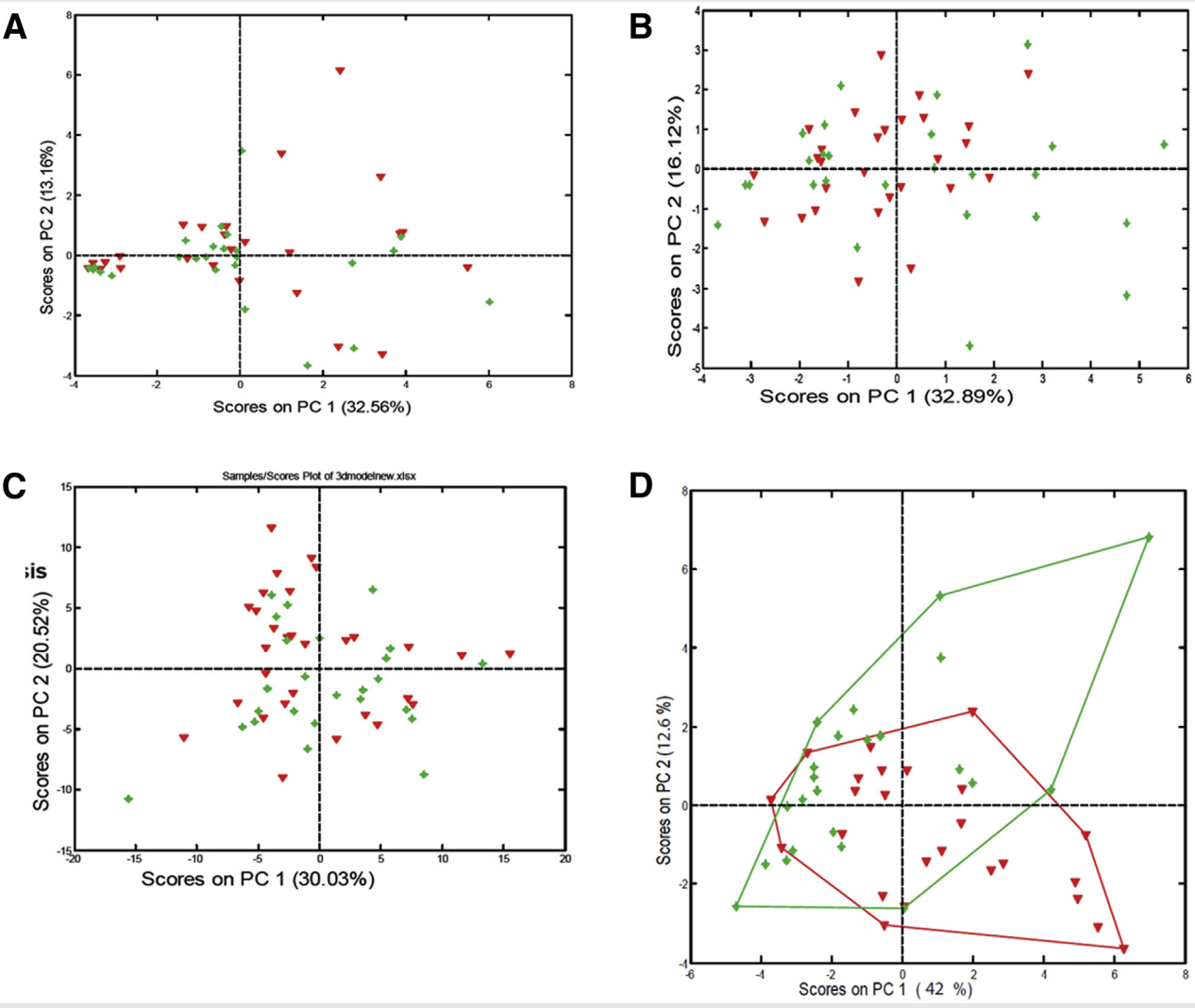
SUPPLEMENTAL FIGURE 4



PCA scores plots for the eicosanoids (**A**), steroid hormones (**B**), neutral lipids (**C**), and acylcarnitines (**D**). Red triangles = cases, green cross = controls.

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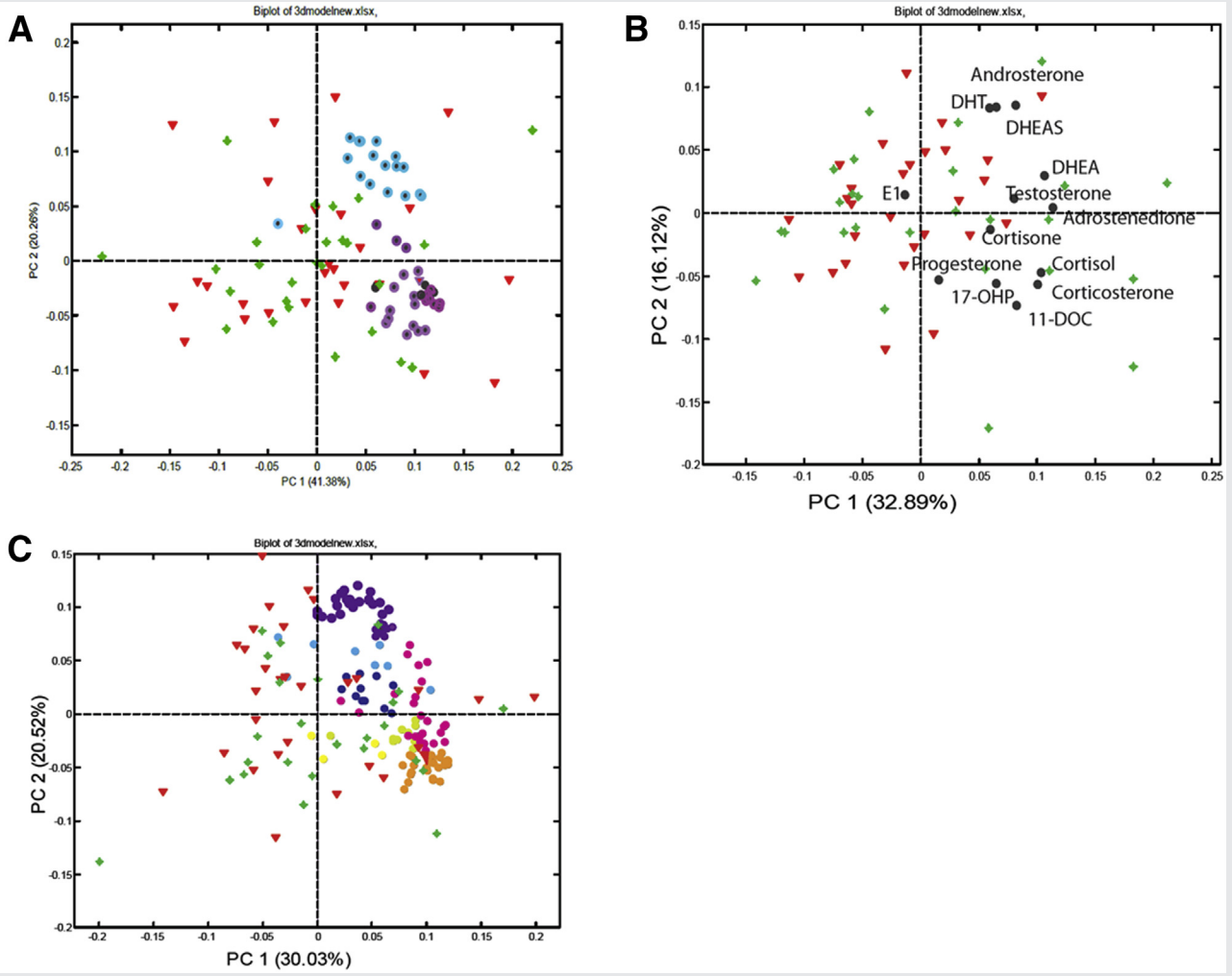
SUPPLEMENTAL FIGURE 5



Bi-plots visualizing the relation between controls (*green*), cases (*red*), and (**A**) eicosanoids (CYP pathway: *pink*; LOX derived: *purple*; COX-derived: *light blue*), (**B**) steroid hormones (*black*), and (**C**) neutral lipids (SM: *purple*; CM: *light blue*; PE: *dark blue*; PC: *pink*; LPC: *light green*; LPE: *yellow*; TG: *orange*).

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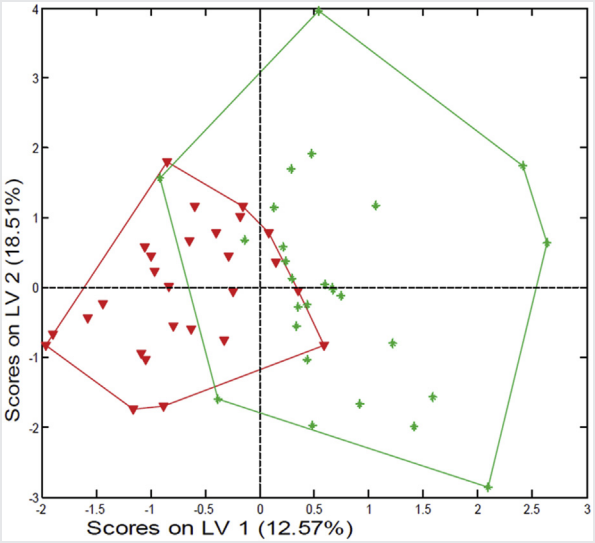
SUPPLEMENTAL FIGURE 6



PLSDA model for the acylcarnitines in plasma. We applied the cross validation as well as the "leave one out" method. Sensitivity, 81.5%; specificity, 88.9%. Cases (n = 25) and controls (n = 19).

Letsiou. Metabolite profiling in endometriosis. Fertil Steril 2017.