Proteomics
Clinical Applications

www.clinical.proteomics-journal.com

Polycystic Ovary Syndrome and Insulin Physiology: An Observational Quantitative Serum Proteomics Study in Adolescent, Normal-Weight Females

*Antigoni Manousopoulou, Nasser M. Al-Daghri, Shaun Sabico, Diana J. Garay-Baquero, Jie Teng, Amal Alenad, Majed S. Alokail, Nikos Athanasopoulos, Efthymios Deligeoroglou, George P. Chrousos, Flora Bacopoulou, and Spiros D. Garbis**

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with insulin resistance, even in the absence of overweight/obesity. The aim of the present study is to examine the global serum proteomic profile of adolescent, normal-weight females with PCOS in order to gain novel insight in the association of this endocrine disorder with insulin physiology and to identify novel circulating markers that can guide intervention protocols.

Methods: Non-depleted serum from normal-weight (BMI: 18–23 kg m^{-2}), adolescent females (13–21 years old) with PCOS ($n = 20$) is compared to BMI- and age-matched healthy controls ($n = 20$) using our 3D quantitative proteomics methodology. Serum samples from study participants are randomly pooled to form four biological replicates of females with PCOS and four of healthy controls ($n = 5$ per sample pool).

Results: One-hundred and twenty-six proteins are differentially expressed in females with PCOS compared to controls. Gene ontology analysis shows significant enrichment for terms related to inflammatory immune response, metabolism and insulin-like growth factor receptor signaling pathway. Circulating levels of IGF-1 and -2 and IGFBP-2, -3, and -4 are found to be lower in females with PCOS compared to healthy controls.

Conclusions: The present serum proteomics study provides insight into the pro-inflammatory status and insulin dysregulation in young females with PCOS and identifies potential serological markers that can guide early intervention protocols.

A. Manousopoulou[+], D. J. Garay-Baquero, J. Teng, A. Alenad, S. D. Garbis
Institute for Life Sciences
University of Southampton
Southampton, UK
E-mail: sgarbis@caltech.edu

N. M. Al-Daghri, S. Sabico, M. S. Alokail
Chair for Biomarkers of Chronic Diseases
Biochemistry Department
King Saud University
Kingdom of Saudi Arabia

N. Athanasopoulos, E. Deligeoroglou, G. P. Chrousos, F. Bacopoulou
Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care
First Department of Pediatrics
Medical School
National and Kapodistrian University of Athens
Aghia Sophia Children's Hospital
Athens, Greece

S. D. Garbis
Cancer Sciences
Faculty of Medicine
Southampton General Hospital
University of Southampton
UK

S. D. Garbis
Proteome Exploration Laboratory
Beckman Institute
Division of Biology and Biological Engineering
California Institute of Technology
Pasadena, CA, USA

[+]Present address: Department of Immuno-Oncology, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA, USA

DOI: 10.1002/prca.201800184

1. Introduction

Polycystic ovary syndrome (PCOS) is a very common endocrine disorder that affects approximately 10% of females in the reproductive age worldwide.[1] Patients with PCOS usually symptoms related with menstrual irregularities, hirsutism, and infertility.[2] In adolescence, the diagnosis of the syndrome is based on the adult criteria, that is, hyperandrogenism, menstrual irregularities, and enlarged ovaries, that may overlap normal pubertal findings.[3] PCOS is not merely a reproductive but mainly a metabolic disorder that has its origins very early in life with insulin resistance playing a major role in its evolution and persistence in adulthood.[4–6]

A recent study described that women with PCOS have a similar "metabolic inflexibility" to patients with type 2 diabetes. "Metabolic inflexibility" in this context was defined as the inability to switch from oxidizing fat during fasting to carbohydrate after eating.[7] It is reported that women with the syndrome have disordered insulin action due to decreased insulin receptor signaling, with selective insulin resistance in the classic insulin target tissues and the ovaries.[4]

Insulin resistance and hyperinsulinemia are intrinsic to PCOS not only in adults but also in adolescents, lean or obese. Along these lines, adolescent girls at risk for PCOS were found to have lower peripheral insulin sensitivity than controls, when assessed by the gold-standard hyperinsulinemic-euglycemic clamp.[8]

Nevertheless, the precise link between PCOS and insulin physiology remains unclear. In the case of non-overweight/obese adolescent females, understanding how PCOS can lead to insulin resistance is important in terms of early intervention as well as identifying novel therapeutic approaches. The aim of this study is to examine the global serum proteomic profile of adolescent females with PCOS in order to gain novel insight in the association of this endocrine disorder with insulin physiology and to identify potential serological markers that can guide early intervention protocols.

Clinical Relevance

Polycystic ovary syndrome (PCOS) is a very common endocrine disorder, affecting approximately 10% of females in the reproductive age worldwide. PCOS is not only associated with gynecological but also with metabolic implications, the most important being insulin resistance. Furthermore, the metabolic implications of PCOS can exist even in the absence of overweight/obesity. There is a clinical need for the identification of novel circulating markers in young, normal-weight females with PCOS that indicate metabolic dysregulation and can guide early intervention, through lifestyle changes or pharmacologic treatment. The study identified 126 differentially expressed serum proteins in normal-weight females with PCOS compared to controls. Gene ontology analysis showed significant enrichment for terms related to inflammatory | immune response, metabolism, and insulin-like growth factor receptor signaling pathway. Circulating levels of IGF-1 and -2 and IGFBP-2, -3, and -4 were found to be lower in females with PCOS compared to healthy controls. The identified serological proteins can be further validated in larger cohorts for their specificity and sensitivity in the diagnosis and treatment of metabolic dysregulation in PCOS.

2. Experimental Section

2.1. Recruitment of Participants and Intervention

The study received ethics approval from the Ethics Committee of the First Department of Pediatrics, Medical School, National and Kapodistrian University of Athens. Reporting of the present observational study adheres to the STROBE and broader EQUATOR guidelines.[9] All participants or their legal guardians (if younger than 18 years) signed informed consent forms.

Forty participants were recruited from the Centre for Adolescent Medicine and UNESCO Chair on Adolescent Health Care of the First Department of Pediatrics, in Athens, Greece, between November 2015 and February 2016 ($n = 20$ females with PCOS and $n = 20$ healthy controls). The inclusion criteria were: adolescent females (13–21 years old), post-menarcheal for at least 2 years, with normal BMI (18–23 kg m^{-2}), nondiabetic or chronically ill, nonpregnant, not using contraceptive pills or other medication. PCOS was diagnosed based on the most recent AE-PCOS Society 2006 criteria [clinical or biochemical hyperandrogenism and ovarian dysfunction: oligomenorrhea (menstrual cycle of more than 45 days) and/or polycystic ovaries on ultrasound (ovarian volume > 10 mL in at least one ovary)].[10]

The modified version of the Ferriman-Gallwey (FG) system[11] was used to assess hyperandrogenism clinically. Other disorders with similar presentation (hyperprolactinemia, thyroid disorders, late-onset congenital adrenal hyperplasia, androgen-secreting ovarian or adrenal tumors, and Cushing syndrome) were excluded. Transabdominal sonography and measurement of serum fasting glucose (mmol L^{-1}), fasting insulin (pmol L^{-1}), FSH (IU L^{-1}), LH (IU L^{-1}), estradiol (E_2) (pg mL^{-1}), testosterone (T) (ng mL^{-1}), free-testosterone (free-T) (ng dL^{-1}), Δ4-androstenedione (Δ4-A) (ng dL^{-1}), dehydroepiandrosterone sulfate (DHEAS) (μg dL^{-1}), 17-hydroxyprogesterone (17-OH P) (ng mL^{-1}), and sex-hormone binding globulin (SHBG) (nmol L^{-1}) concentrations were performed in all participants. The free androgen index (FAI) was calculated by the concentration of T (ng mL^{-1}) and SHBG (nmol L^{-1}) using the formula FAI = 100 × T/SHBG. The mean ovarian volume (MOV) represents the mean volume of both ovaries for each study participant. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the formula HOMA-IR = fasting glucose (mg dL^{-1}) × fasting insulin (μIU mL^{-1}) / 405 (Glucose–mmol L^{-1} to mg dL^{-1}: multiply by 18; Insulin–pmol L^{-1} to μIU mL^{-1}: divide by 6).

2.2. Hormonal Analyses

Serum concentrations of FSH, LH, E2, T, Δ4-A, DHEAS, and SHBG were measured by an Immulite 2000 analyzer (Siemens Healthcare Diagnostics Products Ltd., UK) using two-site chemiluminescent immunometric assays (analytical sensitivities for FSH 0.1 IU L^{-1}, LH 0.05 IU L^{-1}, E2 15 pg mL^{-1}, T 0.15 ng mL^{-1}, Δ4-A 0.3 ng dL^{-1}, DHEAS 3 μg dL^{-1}, and SHBG

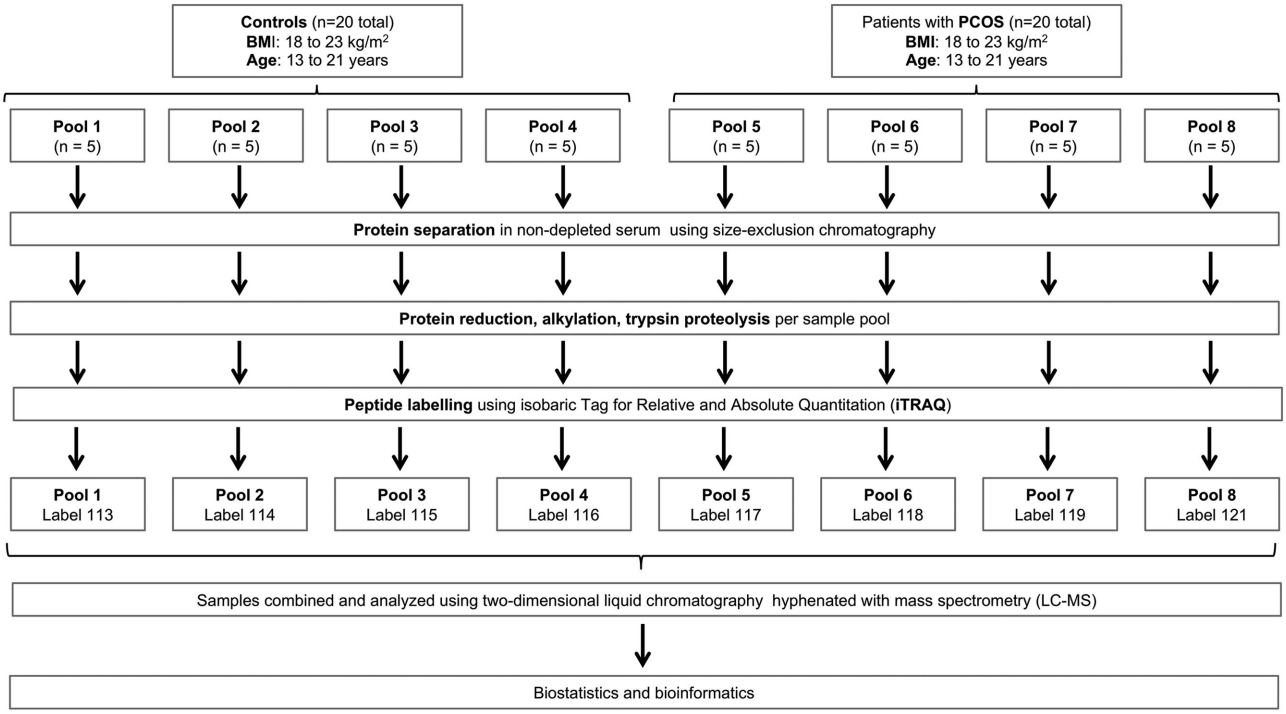

Figure 1. Serum proteomics pipeline.

0.02 nmol L^{-1}; intra-assay and inter-assay precision CVs range 2.9–7.9% for FSH, 3.0–7.1% for LH, 6.7–16% for E2, 5.1–11.7% for T, 3.5–13.2% for Δ4-A, 4.9–13% for DHEAS, and 2.3–6.6% for SHBG). Serum concentrations of 17-OHP were determined by RIA (DIAsource Immunoassays SA, Belgium; intra-assay CV $< 7\%$, inter-assay CV $< 10\%$; analytical sensitivity 0.02 ng mL^{-1}). ACTH stimulation testing was performed in adolescents with elevated serum 17-OHP, to exclude late-onset congenital adrenal hyperplasia.

2.3. Clinical Data Analysis

Clinical data were analyzed using SPSS (Version 25). An unpaired, two-tailed Student *t*-test was applied to compare the clinical characteristics of the PCOS versus control groups. Parameters are presented as mean ± standard deviation. A *p*-value less than 0.05 was considered significant.

2.4. Serum Procurement and Proteomic Analysis

Procurement and handling of serum samples was applied in accordance to the recommendations of the Standard Operating Procedure Integration Working Group (SOPIWG).[12] Serum samples from females with PCOS and healthy controls were pooled to adjust for inter-individual differences. Individual 50 μL aliquots from five patients with PCOS and five healthy controls were randomly pooled using the randomization function of Excel (Microsoft Office 2011) to form four biological replicates for each study group (PCOS and control).

Unprocessed serum was then subjected to 3D quantitative proteomic analysis using the previously published methodology.[13–16] The iTRAQ labeling scheme used was the following: 113 = Control-Sample pool 1; 114 = Control-Sample pool 2; 115 = Control-Sample pool 3; 116 = Control-Sample pool 4; 117 = PCOS-Sample pool 5; 118 = PCOS-Sample pool 6; 119 = PCOS-Sample pool 7; 121 = PCOS-Sample pool 8. The study pipeline is presented in **Figure 1**.

2.5. Database Searching

Unprocessed raw files were submitted to Proteome Discoverer 1.4 for target decoy searching against the SwissProt homo sapiens database (v2015-11-11), allowing for up to two missed cleavages, a precursor mass tolerance of 10 ppm, a minimum peptide length of six and a maximum of two variable (one equal) modifications of; oxidation (M), deamidation (N, Q), or phosphorylation (S, T, Y). Methylthio (C) and iTRAQ (K, N-terminus) were set as fixed modifications. Peptide FDR confidence threshold was set at over 95% ($q < 0.05$). Reporter ion ratios from unique peptides only were used for the quantitation of the respective protein. The threshold of percent co-isolation used to exclude peptides from quantitation was set at 50. Quantification ratios were median normalized and log2 transformed. All mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD011656.

A one-group *t*-test was used to identify proteins differentially expressed in females with PCOS compared to healthy controls.

A $p \leq 0.05$ was considered significant. In adherence to the Paris Publication Guidelines for the analysis and documentation of peptide and protein identifications,[17] only proteins identified with at least two unique peptides were subjected to bioinformatics analysis. DAVID (https://david.ncifcrf.gov/) was used for gene ontology term enrichment analysis. A Fisher-exact *p*-value < 0.05 was considered significant.

3. Results

3.1. Proteomic Analysis

The participants' clinical characteristics are presented in **Table 1**. With regard to the serum proteomics analysis, in total 516 proteins were fully quantified in all groups ($q < 0.05$ at the peptide level). Of these, 126 were differentially expressed in females with PCOS compared to healthy controls (Table S1, Supporting Information).

Gene ontology (GO) enrichment analysis of the differentially expressed proteins (DEPs) using DAVID showed that terms related to inflammatory | immune response, metabolism, and insulin-like growth factor receptor signaling pathway were significantly enriched (**Figure 2**A). The proteins mapping to these GO terms are presented in heatmap format in Figure 2B. Of particular relevance to inflammatory response, protein S100A8 (S100A8 or calgranulin A) and monocyte differentiation antigen CD14 (CD14) were expressed at higher levels in patients with PCOS compared to controls whereas serum uteroglobin levels were lower in patents with PCOS versus controls. With regard to insulin physiology, insulin-like growth factor (IGF) -1 and -2 as well as insulin-like growth factor binding protein (IGFBP) -2, -3, and -4 were analyzed to be expressed at lower levels in females with PCOS compared to controls (**Figure 3**).

Table 1. Clinical characteristics of participants.

Parameters	PCOS	Control	*p*-value
N	20	20	
Age [year]	18.2 ± 2.9	17.3 ± 2.1	0.29
BMI [kg m^{-2}]	21.3 ± 1.6	21.0 ± 2.0	0.79
Fasting glucose [mmol L^{-1}]	4.7 ± 0.5	4.3 ± 0.3	0.04
Fasting insulin [pmol L^{-1}]	50.5 (49.3)	44.0 (38.9)	0.65
HOMA-IR	1.5 (1.6)	1.2 (1.0)	0.47
QUICKI	0.35 ± 0.05	0.37 ± 0.03	0.24
FG score	14.2 ± 4.8	4.3 ± 1.3	<0.0001
FSH [IU L^{-1}]	4.7 ± 1.4	5.8 ± 1.1	0.02
LH [IU L^{-1}]	10.1 ± 5.1	4.9 ± 2.9	0.001
E2 [pg mL^{-1}]	40.7 ± 16.2	29.7 ± 12.1	0.04
T [ng mL^{-1}]	0.7 ± 0.3	0.3 ± 0.1	<0.0001
Free T [ng dL^{-1}]	0.8 ± 0.4	0.5 ± 0.2	0.01
Δ4-A [ng dL^{-1}]	453.3 ± 253.6	187.9 ± 75.1	0.0009
DHEAS [μg dL^{-1}]	290.5 ± 106.9	181.7 ± 69.9	0.002
SHBG [nmol L^{-1}]	37.9 ± 22.6	46.1 ± 20.6	0.35
17-OH P [ng mL^{-1}]	1.5 ± 0.4	0.9 ± 0.4	0.001
FAI	8.5 ± 5.2	3.1 ± 1.5	0.0004
MOV [mL]	13.6 ± 3.3	6.4 ± 1.4	<0.0001

Data are presented as mean ± SD or median (IQR). BMI: body mass index; HOMA-IR: homeostatic model assessment for insulin resistance; QUICKI: quantitative insulin sensitivity check index; FG: Ferriman-Gallwey score; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol; T: testosterone; SHBG: sex-hormone binding globulin; FAI: free androgen index; Δ4-A: Δ4 androstenedione; DHEAS: dehydroepiandrosterone sulfate; 17-OHP: 17-hydroxyprogesterone; MOV: mean ovarian volume; SHBG: sex-hormone binding globulin.

4. Discussion

The present study provides evidence of an altered serum proteomic profile in adolescent, normal-weight females with PCOS compared to controls. The identified DEPs are related to inflammatory | immune response, metabolism, and insulin-like growth factor receptor signaling pathway. The molecular portraits presented can help increase our understanding of the link between PCOS and insulin resistance in normal-weight females, also highlighting the involvement of inflammatory response in this common endocrine disease. The identified proteins can be further examined as novel circulating markers of dysregulated insulin physiology, thus allowing early lifestyle (nutrition, physical activity) or pharmacological intervention.

Our serum proteomic results show an increased inflammatory status in females with PCOS. Serum levels of uteroglobin, a small globular protein with reported anti-inflammatory activity,[18] were lower in females with PCOS compared to healthy controls. Interestingly, experimental data show that estrogen transcriptionally controls the uteroglobin gene.[19,20] Further examining the correlation between estrogen and uteroglobin levels was beyond the scope of the present study and constitutes a future perspective.

Calgranulin A (S100A8) is a low molecular weight protein with calcium binding properties that is released by activated granulocytes and mediates inflammatory signaling pathways, such as the NF-kB pathway.[21] Serum S100A8 levels have been shown to increase in inflammatory disease, such as autoimmune disease (including rheumatoid arthritis and inflammatory bowel disease), various types of cancer, cystic fibrosis, and neurodegeneration.[22–24] In our study, serum S100A8 levels were higher in females with PCOS compared to controls.

Monocyte differentiation antigen CD14 (CD14) is a 55-kDa protein expressed in membrane anchored and soluble serum protein forms. CD14 is a co-receptor for bacterial lipopolysaccharide and mediator of the inflammatory response. Furthermore, CD14 has been shown to participate in adipose tissue related chronic inflammation, and the eventual development of insulin resistance as a result of chronic inflammatory signals.[25] Our study results showed that women with PCOS have increased CD14 levels compared to controls. This could reflect a chronic inflammatory status and increased risk of developing insulin resistance and type 2 diabetes mellitus.

The growth hormone | insulin-like growth factor axis, an evolutionarily conserved system, primarily controls growth and carbohydrate metabolism.[26] The insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2) components, hormones

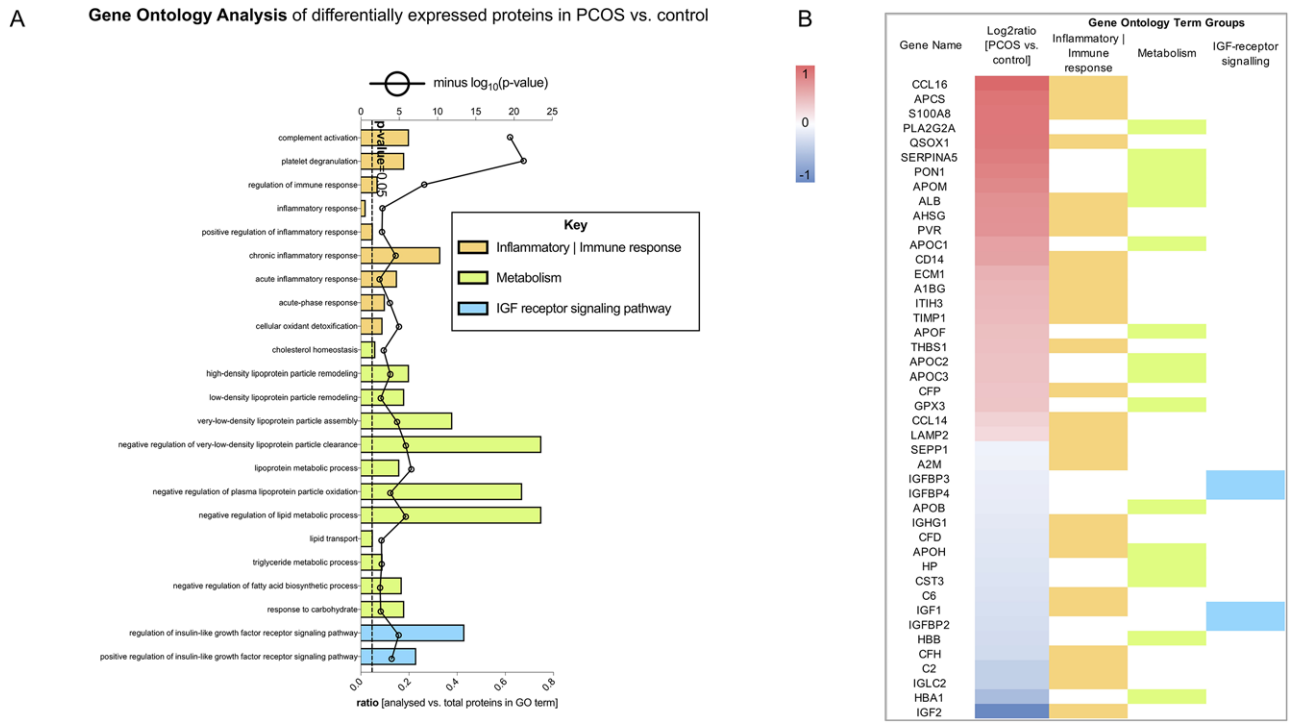

Figure 2. A) Gene ontology enrichment analysis of the differentially expressed proteins in PCOS versus controls, B) Heatmap of the proteins mapping to the gene ontology term groups.

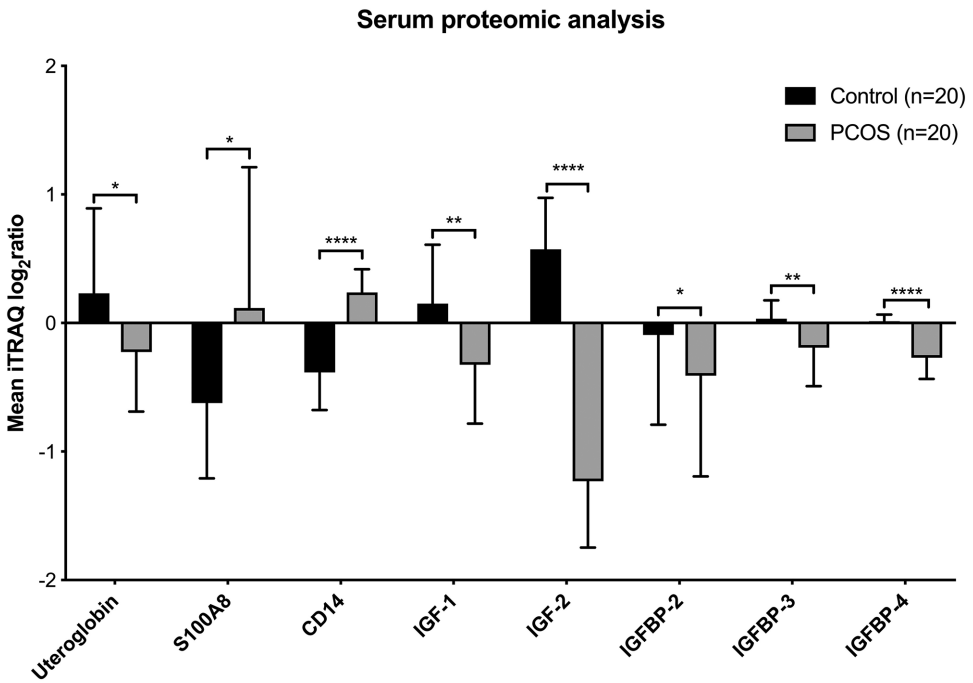

Figure 3. Untargeted serum proteomic analysis shows upregulation of S100A8 and CD14 and downregulation of uteroglobin, IGF-1, IGF-2, IGFBP-2, -3, and -4 in young normal-weight females with PCOS compared to age- and BMI-matched controls.

Received: November 15, 2018
Revised: February 12, 2019
Published online:

with well-established growth promoting and insulin-like effects, are carried in the systemic circulation by the insulin-like growth factor binding proteins (IGFBPs).[27] In agreement with our findings of reduced serum IGF-1 and IGF-2 levels in females with PCOS compared to controls, a study has shown that intrafollicular levels of IGF-1 and IGF-2 are lower in women with PCOS compared to healthy controls.[28] An observational study among nondiabetic adults showed that low circulating IGF-1 levels were associated with increased risk of insulin resistance and cardiovascular disease.[29]

The IGFBP family has six members (numbered 1 through 6) with a molecular weight that varies from 24 to 45 kDa.[30] The IGFBPs play an important role in extending the half-life of IGFs in plasma.[31] Studies have shown that IGFBPs can also inhibit the binding of IGF-1 and IGF-2 to their respective receptors.[31,32] Interestingly, our results indicate that females with PCOS have lower levels of IGF1, and IGF2 as well as IGFBP-2, -3, and -4 compared to healthy controls. We have previously shown that IGBP-2 and -3 are positively associated with vitamin D status in a sex-specific manner.[33] Future randomized control studies can examine whether vitamin D supplementation improves the clinical manifestations of PCOS in relation to insulin physiology.

In conclusion, the results of the present serum proteomic study highlight a pro-inflammatory status and dysregulated insulin physiology in normal-weight, adolescent females with PCOS compared to age and BMI-matched healthy controls. The identified proteins can be further examined as candidate circulating markers that can inform and guide early intervention protocols in young females with PCOS.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

A.M. and N.M.A. have contributed equally to this work. Concept and clinical design: A.M., N.M.A., G.P.C., F.B., and S.D.G.; Proteomics analysis: A.M., D.J.G., J.T., A.A., and S.D.G.; Sample procurement and initial evaluation: G.P.C. and F.B.; Data interpretation: A.M., G.P.C., F.B., and S.D.G.; Guided the design and performance of specific aspects of the experiments: M.S.A., N.A., E.D., G.P.C., and F.B.; Wrote the manuscript: A.M., F.B., and S.D.G.; all authors contributed to its revision and final form. This study was financially supported by the Deanship of Scientific Research, Chair for Biomarkers of Chronic Diseases, in King Saud University, Riyadh, Saudi Arabia; the Wessex Cancer Trust, Wessex Medical Research; EU-FP7/HELLENIC NSRF (BIOEXPLORE); Annual Adventures in Research—University of Southampton; and the EU-Excellence II—Systems Biology Framework FRA-SYS (Grant 4072).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

inflammation, insulin resistance, iTRAQ, non-depleted serum, PCOS, proteomics

[1] S. M. Sirmans, K. A. Pate, *Clin. Epidemiol.*, **2013**, *6*, 1.
[2] B. C. Fauser, B. C. Tarlatzis, R. W. Rebar, R. S. Legro, A. H. Balen, R. Lobo, E. Carmina, J. Chang, B. O. Yildiz, J. S. Laven, J. Boivin, F. Petraglia, C. N. Wijeyeratne, R. J. Norman, A. Dunaif, S. Franks, R. A. Wild, D. Dumesic, K. Barnhart, *Fertil. Steril.*, **2012**, *97*, 28.
[3] S. F. Witchel, S. Oberfield, R. L. Rosenfield, E. Codner, A. Bonny, L. Ibáñez, A. Pena, R. Horikawa, V. Gomez-Lobo, D. Joel, H. Tfayli, S. Arslanian, P. Dabadghao, C. Garcia Rudaz, P. A. Lee, *Horm. Res. Paediatr.* **2015**, *83*, 376.
[4] E. Diamanti-Kandarakis, A. Dunaif, *Endocr. Rev.* **2012**, *33*, 981.
[5] M. C. Amato, R. Vesco, E. Vigneri, A. Ciresi, C. Giordano, *J. Endocrinol. Invest.* **2015**, *38*, 1319.
[6] K. E. Salley, E. P. Wickham, K. I. Cheang, P. A. Essah, N. W. Karjane, J. E. Nestler, *J. Clin. Endocrinol. Metab.* **2007**, *92*, 4546.
[7] N. T. Broskey, C. S. Tam, E. F. Sutton, A. D. Altazan, J. H. Burton, E. Ravussin, L. M. Redman, *Nutr. Metab.* **2018**, *15*, 75.
[8] V. D. Lewy, K. Danadian, S. F. Witchel, S. Arslanian, *J. Pediatr.* **2001**, *138*, 38.
[9] E. von Elm, D. G. Altman, M. Egger, S. J. Pocock, P. C. Gøtzsche, J. P. Vandenbroucke, STROBE Initiative, *Lancet* **2008**, *370*, 1453.
[10] R. Azziz, E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, H. F. Escobar-Morreale, W. Futterweit, O. E. Janssen, R. S. Legro, R. J. Norman, A. E. Taylor, S. F. Witchel, *Fertil. Steril.* **2009**, *91*, 456.
[11] R. Hatch, R. L. Rosenfield, M. H. Kim, D. Tredway D, *Am. J. Obstet. Gynecol.* **1981**, *140*, 815.
[12] M. K. Tuck, D. W. Chan, D. Chia, A. K. Godwin, W. E. Grizzle, K. E. Krueger, W. Rom, M. Sanda, L. Sorbara, S. Stass, W. Wang, D. E. Brenner, *J. Proteome Res.* **2009**, *8*, 113.
[13] S. D. Garbis, T. I. Roumeliotis, S. I. Tyritzis, K. M. Zorpas, K. Pavlakis, C. A. Constantinides, *Anal. Chem.* **2011**, *83*, 708.
[14] N. M. Al-Daghri, O. S. Al-Attas, H. E. Johnston, A. Singhania, M. S. Alokail, K. M. Alkharfy, S. H. Abd-Alrahman, S. L. Sabico, T. I. Roumeliotis, A. Manousopoulou-Garbis, P. A. Townsend, C. H. Woelk, G. P. Chrousos, S. D. Garbis, *J. Proteome Res.* **2014**, *13*, 5094.
[15] N. M. Al-Daghri, M. S. Alokail, A. Manousopoulou, A. Heinson, O. Al-Attas, Y. Al-Saleh, S. Sabico, S. Yakout, C. H. Woelk, G. P. Chrousos, S. D. Garbis, *Eur. J. Clin. Invest.* **2016**, *46*, 1031.
[16] A. Manousopoulou, E. Scorletti, D. E. Smith, J. Teng, M. Fotopoulos, T. I. Roumeliotis, G. F. Clough, P. C. Calder, C. D. Byrne, S. D. Garbis, *Clin. Nutr.* **2018**, https://doi.org/10.1016/j.clnu.2018.07.037.
[17] S. Carr, R. Aebersold, M. Baldwin, A. Burlingame, K. Clauser, A. Nesvizhskii, *Mol. Cell. Proteomics*, **2004**, *3*, 531.
[18] G. Antico, M. Aloman, K. Lakota, L. Miele, S. Fiore, S. Sodin-Semrl, *Mediators Inflammation* **2014**, 876395.
[19] A. Acosta-Montesdeoca, T. Zariñán, A. Ulloa-Aguirre, R. Gutiérrez-Sagal, *Gen. Comp. Endocrinol.* **2014**, *199*, 94.
[20] A. Acosta-Montesdeoca, T. Zariñán, H. Macías, M. A. Pérez-Solís, A. Ulloa-Aguirre, R. Gutiérrez-Sagal, *Mol. Reprod. Dev.* **2012**, *79*, 337.
[21] F. Shabani, A. Farasat, M. Mahdavi, N. Gheibi, *Inflammation Res.* **2018**, *67*, 801.
[22] T. Nilsen, K. Sunde, A. Larsson, *J. Inflammation* **2015**, *12*, 45.
[23] A. Poullis, R. Foster, M. A. Mendall, M. K. Fagerhol, *J. Gastroenterol. Hepatol.* **2003**, *18*, 756.
[24] N. Rumman, M. Sultan, K. El-Chammas, V. Goh, N. Salzman, D. Quintero, S. Werlin, *BMC Pediatr.* **2014**, *14*, 133.
[25] J. M. Fernández-Real, S. Pérez del Pulgar, E. Luche, J. M. Moreno-Navarrete, A. Waget, M. Serino, E. Sorianello, A. Sánchez-Pla, F.

C. Pontaque, J. Vendrell, M. R. Chacón, W. Ricart, R. Burcelin, A. Zorzano, *Diabetes* **2011**, *60*, 2179.
[26] D. Le Roith, *N. Engl. J. Med.* **1997**, *336*, 633.
[27] S. Shimasaki, M. Shimonaka, H. P. Zhang, N. Ling, *J. Biol. Chem.* **1991**, *266*, 10646.
[28] A. Barreca, P. Del Monte, P. Ponzani, P. G. Artini, A. R. Genazzani, F. Minuto, *Fertil. Steril.* **1996**, *65*, 739.
[29] G. Sesti, A. Sciacqua, M. Cardellini, M. A. Marini, R. Maio, M. Vatrano, E. Succurro, R. Lauro, M. Federici, F. Perticone, *Diabetes Care* **2005**, *28*, 120.
[30] L. A. Wetterau, M. G. Moore, K. W. Lee, M. L. Shim, P. Cohen, *Mol. Genet. Metab.* **1999**, *68*, 161.
[31] V. Hwa, Y. Oh, R. G. Rosenfeld, *Endocr. Rev.* **1999**, *20*, 761.
[32] D. R. Clemmons, W. H. Busby, T. Arai, T. J. Nam, J. B. Clarke, J. I. Jones, D. K. Ankrapp, *Prog. Growth Factor Res.* **1995**, *6*, 357.
[33] N. M. Al-Daghri, A. Manousopoulou, M. S. Alokail, S. Yakout, A. Alenad, D. J. Garay-Baquero, M. Fotopoulos, J. Teng, O. Al-Attas, Y. Al-Saleh, S. Sabico, G. P. Chrousos, S. D. Garbis, *Nutr. Diabetes* **2018**, *8*, 54.

