

Gene expression of inflammatory markers in adipose tissue between obese women with polycystic ovary and normal obese women

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Abstract. – OBJECTIVE: The pathogenesis of polycystic ovary syndrome (PCOS), a common endocrine disease and metabolic disturbance, is still unknown. The aim of the study was to investigate whether patients with PCOS display increased expression of inflammatory markers in adipose tissue.

PATIENTS AND METHODS: Two groups of women were investigated, those diagnosed with PCOS (n = 8) and age and BMI-matched normal women (n = 12). Their age was between 20-45 years and all subjects were apparently healthy and did not take any medications. Adipose tissue levels of mRNA of inflammatory markers were determined by use of real-time PCR.

RESULTS: There were no differences between obese patients and obese PCOS in levels of adipocytokines.

CONCLUSIONS: There were no effects of PCOS on the expression of any of the adipocytokines genes measured in subcutaneous adipose tissue.

Key Words:

Polycystic ovary syndrome, Insulin resistance, Inflammation.

genes associated with insulin resistance could be affected by high levels of testosterone (T) in PCOS². Many studies report that dysfunction of adipose tissue might be involved in the pathogenesis of PCOS^{3,4}. Patients with PCOS have low-grade inflammation characterized by high levels of tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6)^{5,6}. Recently, studies have focused on adipose tissue morphology in PCOS patients and tried to discover the link between adiponectin secreted from adipocytes and insulin resistance⁷. High levels of androgens are thought to impact on adipose tissue function and distribution in women with PCOS⁸. Previous researches have suggested that the molecules released by adipocytes, such as adiponectin, resistin, leptin, TNF- α and IL-6, are crucial factors in the pathology of PCOS and can be influenced by estrogens and androgens⁹⁻¹². However, some investigations have suggested that the role of adipokines in the pathogenesis of PCOS is more complicated¹. The effects of raised T levels in PCOS could be directly on the expression of adipokines secreted by adipocytes, such as adiponectin and resistin, which affect insulin resistance. These adipokines and cytokines are stimulated by other cytokines released by macrophages such as TNF- α and IL-6^{13,14}. We suggested that excess testosterone in the blood of women with PCOS alters the expression of adipocytokines involved in insulin resistance in subcutaneous fat tissue. To compare expression of adipokines that play an important role in insulin resistance in subcutaneous fat tissue from women suffering from hyperandrogenism (PCOS) and normal women at a similar age and BMI.

Introduction

The pathogenesis of polycystic ovary syndrome (PCOS), a common endocrine disease and metabolic disturbance, is still unknown. A link between disturbed adipokines secretions and type 2 diabetes in PCOS has previously been shown, and several studies indicate that the incidence of insulin resistance in PCOS women is related to the levels of adipokines in the blood¹. There is increasing evidence that the expression of some

Patients and Methods

Patients

Two groups of women were investigated, those diagnosed with PCOS (n=8) and age and BMI-matched normal women (n=12). Their age was between 20-45 years and all subjects were apparently healthy and did not take any medication. None of the subjects was postmenopausal and they all gave their consent to provide samples of subcutaneous adipose tissue. Height and weight were obtained and BMI calculated. The weight of collected samples did vary and was dependent on both the patients and the surgical conditions. All samples were immediately frozen in liquid nitrogen and stored at -80°C until total RNA isolation.

Criteria Considered For Screening and Diagnosis of Patients

This work was carried out with women of reproductive age, with BMI between 16.5 and 36.6 kg/m². Twenty hirsute women presenting oligo/amenorrheic cycles, increased levels of serum T or free androgen index (FAI), and/or polycystic ovaries, without other disorders causing hirsutism were enrolled.

Fat Biopsies

Following general anesthesia, 1.5 cm² of superficial subcutaneous of adipose tissue was excised through a 1.5 cm incision. The fat biopsies were placed into vials and snap frozen in liquid nitrogen and stored at -80°C until assayed.

RNA Extraction

Total RNA was extracted from subcutaneous adipose tissue (100-150 mg) using Trizol (Invitrogen Corp., Carlsbad, CA, USA), followed by tested the quality of RNA. cDNA was synthesized from 500 ng of total RNA and prepare real time poly chain reaction (RT-PCR) steps. For cDNA synthesis, the pellets were dried for 10 min then RNA quality was assessed with a nanodrop machine. The absorbance of 1.5 µL from each sample was determined spectrophotometrically at 260 nM and 280 nM against a water blank at 260 nM. The ratio of purity of RNA was between the absorbance at 260 and 280 nM ranged from 1.8 to 2. 100 ng/µL for each sample was calculated by adding RNase-free water. To complete cDNA synthesis, random primer, H₂O were added and incubated at 70°C for 5 min in PCR machine. The following mixture MMLV reverse transcrip-

tase buffer, Nucleotides, RNase inhibitor, MMLV reverse transcriptase, and RNase water were added. All of them were mixed and spined down then incubated at room temperature for 10 min then returned into PCR machine to incubate them at 42°C for 60 min. All the mRNA of treated cells was investigated by RT-PCR. All cDNA samples were mix with SYBR Green I master (containing HotStarTaq DNA polymerase, QuantiTectSYBR Green PCR Buffer, dNTP mix and SYBR Green I) and mixed with 1.25 µL of each primer. The sequences of all genes investigated are listed on Table I.

Statistical Analysis

To compare the effects of PCOS on gene expression of Inflammatory Markers in adipose tissue, one-way ANOVA was performed by SPSS 21 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SEM and considered significant where $p < 0.05$.

Results

All women with PCOS have polycystic ovaries, and the majority also had clinical or biochemical hyperandrogenism among overweight patients with PCOS compare to normal patients. Baseline characteristics of the study population are shown in Table II. Both the overweight patients with PCOS and normal patients were at a similar age. In comparison with normal patients, patients with PCOS had increased serum concentrations of free androgen index, LH, and testosterone (p -value = 0.042, < 0.001, 0.004 respectively).

Expression of almost of genes involved in insulin resistance directly or indirectly such as adiponectin, resistin, TNF- α , IL-6, Peroxisome proliferator-activated receptor gamma (PPAR γ), CCAAT-enhancer-binding proteins (C/EBP α), rantes, omentin, visfatin, MCP-1 and leptin were done by RT-PCR in the subcutaneous adipose tissue. There is no significant difference between normal patients compare with PCOS patients in all genes mentioned above. In term of adipogenesis markers PPAR γ and C/EBP α , both are similar in two groups. There were no differences between obese patients with obese PCOS in levels of adipose tissue mRNA for adiponectin and as well as the Figure 1 shows that a big variation between the groups in gene

Table I. Primer Sequences Used for RT-PCR.

Gene	Primer sequence (5'→3')
PPAR γ	Re GAGGGAGTTGGAAGGCTCTTC Fw GATCCAGTGGTTGCAGATTACAA
C/EBP α	Re CGCACATTACATTGCACAA Fw CAAATATTTTGCTTTATCAGCCGATA
Adiponectin	Re CTTAGGACCAATAAGACCTGGATCTC Fw GGCCTGCACAGGTTGGAT
Resistin	Re GGATCCTCTCATTGATGGCTTCT Fw GCGCCTGCAGGATGAAAG
IL-6	Re CGTCAGCAGGCTGGCATT Fw CTGCAGAAAAAGGCAAAGAATCTAG
TNF- α	Re GGTTTGCTACAACATGGGCTACA Fw CCCAGGGACCTCTCTCTAATCA
MCP-1	Re GCCTCTGCACTGAGATCTTCCT Fw GCTCAGCCAGATGCAATCAA
Rantase	Re TGTACTCCCGAACCCATTCTT Fw ACCCAGCAGTCGTCTTTGTCA
Leptin	Re TGAGGGTTTTGGTGTCTCTTG Fw TGGCTTTGGCCCTATCTTTTC
Visfatin	Re CCAGGACTGAACAAGAATAGTCTCAAT Fw TGTTCTTGAGGGCTTTGTCTCAT
Omentin	Re GGAAAGTATCCTCCTCCACCAA Fw GCAGCCAACGCCTTGTGT
Cyclophillin	Re CGTAGTGCTTCAGTTTGAAGTTCTCA Fw GGAGATGGCACAGGAGGAAA

expression of resistin. TNF- α and IL-6 expression did not differ between the groups and as well as Leptin and Visfatin, whereas MCP-1 gene expression was decreased in PCOS patient compared to normal patients but not significant. In contrast, Omentin-1 was increased 5 folds in a normal patient, but due to a big standard error it is not significant Figure 1.

Discussion

The aim of this study was to investigate the effect of high level of free testosterone in the blood on insulin resistance caused by an imbalance between adipocytokines in subcutaneous fat tissue obtained from PCOS patients and normal pa-

tients at the same BMI and age. The stimulus for the study to compare between PCOS and normal patients is that most of the PCOS patients have insulin resistance which could be as a result of hyperandrogenemia^{1,2,7}. This report demonstrates that the gene expression pattern of a number of genes in abdominal superficial subcutaneous tissue relating to insulin resistance and markers of inflammation were similar in overweight women with and without PCOS.

In terms of Adiponectin, levels of adiponectin mRNA in SAT from the two groups are consistent with recent scholars^{2,15-17} and agrees with another work¹⁸ comparing people with high sensitivity and low sensitivity to insulin. By contrast, Carmina et al¹⁹ suggested that expression of adiponectin and leptin mRNA in subcutaneous samples obtained

Table II. Clinical characteristics of women in a study of markers of adipose tissue inflammation in relation to PCOS.

Subject characteristics	Normal atients (n = 12)	PCOS patients (n = 8)	p-value
Age(years)	34.6 (28-45)	35.7 (22-45)	0.699
Testosterone nmol/l	1.14 (0.6-1.5)	2.1 (1.4-2.9)	0.004
FSH iu/l	6.86 (4.7-10.0)	6.7 (4.5-9.6)	0.884
FAI	0.8 (0.4-1.3)	1.78 (1.0-3.0)	0.042
LH iu/l	2.4 (1.2-3.6)	14.122 (6.3-21.0)	< 0.001

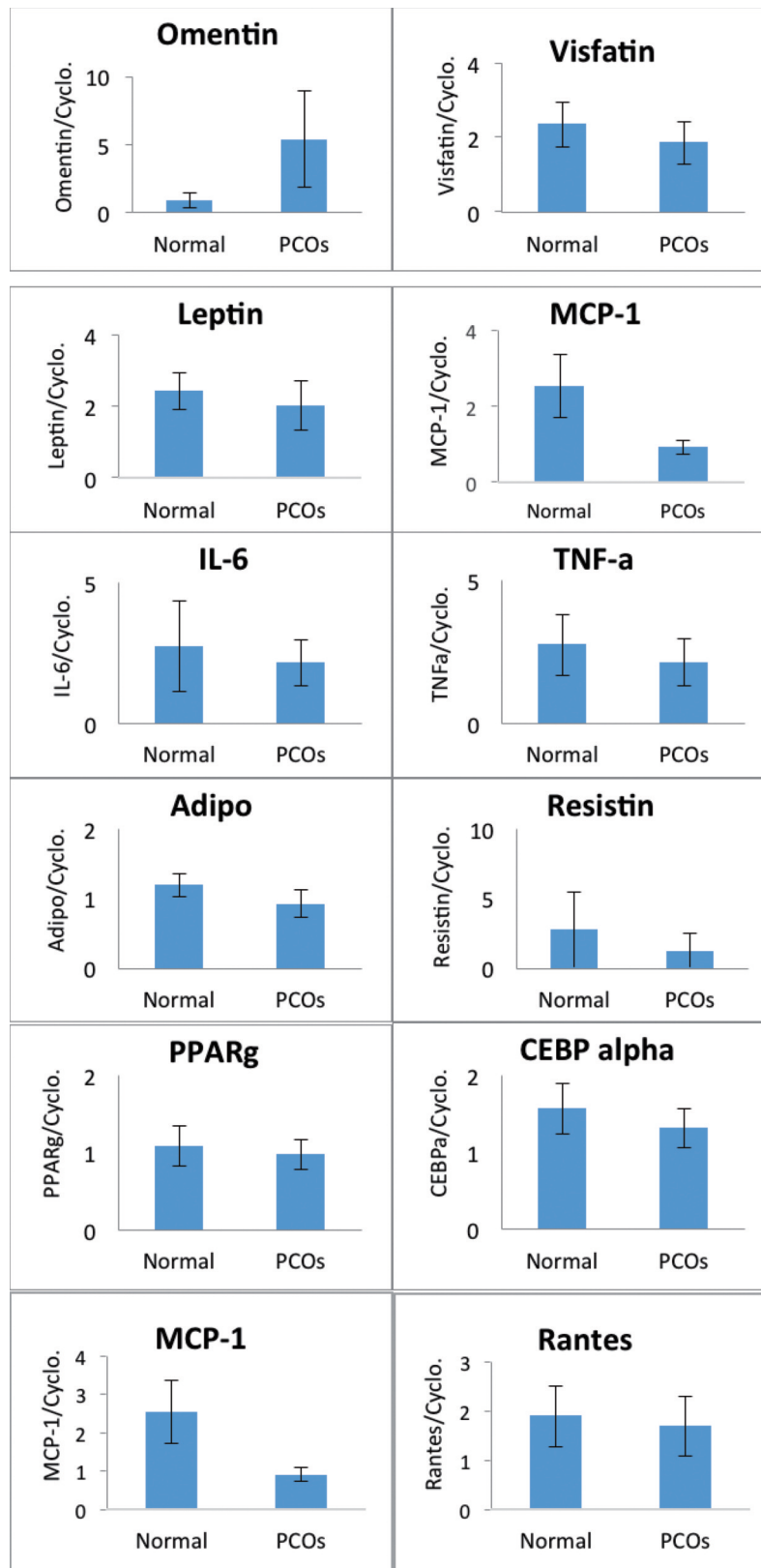


Figure 1. Levels of mRNA for inflammatory substances (mean ± SD) in abdominal superficial subcutaneous adipose tissue from obese patients with PCOS (n = 8), and obese control subjects (n = 12). Levels of mRNA are relative to the cyclophilin gene (Cyclo), which was used to normalize data.

from PCOS patients was low compared with normal women. On the other hand, some studies appeared to suggest that omental, not subcutaneous, adipose tissue secretes adiponectin and correlates negatively with body fat mass^{20,21}.

Resistin levels in PCOS have been sparsely investigated, and the findings have been conflicting^{22,23}. No difference in mRNA levels of resistin is not consistent with results produced previously²⁴. The results also do not agree with another study¹⁶, possibly due to different fat tissue (omental fat) being used.

Regarding TNF- α , no difference was noted between the two groups, which is consistent with 2 other studies^{2,25}, but disagrees with another²⁶, who suggest that levels of TNF-alpha mRNA in SAT are significantly higher in women with PCOS than those in BMI-matched controls. Various inflammatory proteins have been investigated in women with PCOS, including IL-6 and the data is suggestive of the presence of a chronic low-grade inflammatory state, especially in obesity, insulin resistance and hyperandrogenism. Figure 5.1 shows that levels of IL-6 gene expression are similar in both PCOS and normal patients. This data is supported by previous results². However, the amount of IL-6 mRNA in VAT is 10 fold more than in SAT²⁷ and the amount of IL-6 secreted by VAT explants is clearly higher than that secreted by SAT explants.

Leptin gene expression levels were the same in both groups, but one study¹⁹ implied that expression was lower in both omental and SAT obtained from PCOS women, whereas others²⁸⁻³⁰ suggested Leptin gene expression was higher in SAT obtained from PCOS women compared to normal patients. MCP-1 expression was also not different between the two groups and this result is consistent with all previous works on MCP-1 in PCOS women^{25,31}. Visfatin gene expression was similar between the two groups, which is supported by previous papers^{2,32,33}. There was no difference in the expression of Visfatin between visceral and SAT, and no correlation observed between plasma Visfatin and visceral fat mass³³. Moreover, Seow et al¹⁶ investigated the expression of visfatin mRNA in omental fat tissue and it was significantly higher in the women with PCOS than in the controls.

The main advantage of using subcutaneous fat is that the procedure for obtaining samples is less invasive than for visceral fat, but we could not determine the protein levels of the various adipokines due to limited sample volume.

Conclusions

There were no effects of PCOS on the expression of any of the adipocytokines genes measured in SAT, despite significantly higher levels of free testosterone in this group compared with control women. This may be explained by the fact that the plasma levels of free testosterone in our PCOS population, although above the normal range for females, was still only a tenth of the androgen levels in males, and thus did not reach the levels where they might be expected to have an effect on gene expression. Furthermore, there may be differences in androgen levels between PCOS populations due to differences in BMI and levels of insulin resistance and sex hormone-binding globulin, which may explain some of the discrepancies between our report and previous studies. Moreover, we believe that a limitation of our research may relate to the number of subjects observed, since other studies have had higher numbers in the two groups. Since visceral adiposity is thought to be the closest link to metabolic risk, it is unfortunate that we had only biopsies from the abdominal superficial SAT depot. It has recently been reported that the different adipose tissue depots have different profiles of inflammatory markers³⁴⁻³⁶.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

- 1) GLINIANOWICZ M, MADEJ P, NYLEC M, OWCZAREK A, SZANECKI W, SKALBA P, CHUDEK J. Circulating apelin level in relation to nutritional status in polycystic ovary syndrome and its association with metabolic and hormonal disturbances. *Clin Endocrinol (Oxf)* 2013; 79: 238-242.
- 2) SVENDSEN PF, CHRISTIANSEN M, HEDLEY PL, NILAS L, PEDERSEN SB, MADSBAD S. Adipose expression of adipocytokines in women with polycystic ovary syndrome. *Fertil Steril* 2012; 98: 235-241.
- 3) RYDÉN M, JOCKEN J, VAN HARMELEN V, DICKER A, HOFFSTEDT J, WIRÉN M, BLOMQUIST L, MAIRAL A, LANGIN D, BLAAK E, ARNER P. Comparative studies of the role

- of hormone-sensitive lipase and adipose triglyceride lipase in human fat cell lipolysis. *Am J Physiol Endocrinol Metab* 2007; 292: 1847-1855.
- 4) MORO C, PASARICA M, ELKIND-HIRSCH K, REDMAN LM. Aerobic exercise training improves atrial natriuretic peptide and catecholamine-mediated lipolysis in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2009; 94: 2579-2586.
- 5) ESCOBAR-MORREALE HF, LUQUE-RAMÍREZ M, GONZÁLEZ F. Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and meta-analysis. *Fertil Steril* 2011; 95: 1048-1058.
- 6) ESCOBAR-MORREALE HF, SAN MILLÁN JL. Abdominal adiposity and the polycystic ovary syndrome. *Trends Endocrinol Metab* 2007; 18: 266-272.
- 7) MANNERÅS-HOLM L, LEONHARDT H, KULLBERG J, JENNISCHE E, ODÉN A, HOLM G, HELLSTRÖM M, LÖNN L, OLIVECRONA G, STENER-VICTORIN E, LÖNN M. Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *J Clin Endocrinol Metab* 2011; 96: 304-311.
- 8) BLOUIN K, VEILLEUX A, LUU-THE V, TCHERNOF A. Androgen metabolism in adipose tissue: recent advances. *Mol Cell Endocrinol* 2009; 301: 97-103.
- 9) ORIO F JR, PALOMBA S, CASCELLA T, MILAN G, MIONI R, PAGANO C, ZULLO F, COLAO A, LOMBARDI G, VETTOR R. Adiponectin levels in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2003; 88: 2619-23.
- 10) KELLY CC, LYALL H, PETRIE JR, GOULD GW, CONNELL JM, SATTAR N. Low grade chronic inflammation in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2001; 86: 2453-2455.
- 11) HICKEY MS, ISRAEL RG, GARDINER SN, CONSIDINE RV, MCCAMMON MR, TYNDALL GL, HOUMARD JA, MARKS RH, CARO JF. Gender differences in serum leptin levels in humans. *Biochem Mol Med* 1996; 59: 1-6.
- 12) MERKI-FELD GS, IMTHURN B, ROSSELLI M, SPANAUS K. Serum concentrations of high-molecular weight adiponectin and their association with sex steroids in premenopausal women. *Metabolism* 2011; 60: 180-185.
- 13) MATSUZAWA Y, FUNAHASHI T, NAKAMURA T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann N Y Acad Sci* 1999; 892: 146-154.
- 14) GIRARD J. Is leptin the link between obesity and insulin resistance? *Diabetes Metab* 1997; 23: 16-24.
- 15) WANG L, LI S, ZHAO A, TAO T, MAO X, ZHANG P, LIU W. The expression of sex steroid synthesis and inactivation enzymes in subcutaneous adipose tissue of PCOS patients. *J Steroid Biochem Mol Biol* 2012; 132: 120-126.
- 16) SEOW KM, JUAN CC, HO LT, HSU YP, LIN YH, HUANG LW, HWANG JL. Adipocyte resistin mRNA levels are down-regulated by laparoscopic ovarian electrocautery in both obese and lean women with polycystic ovary syndrome. *Hum Reprod* 2007; 22: 1100-1106.
- 17) SEOW KM, TSAI YL, JUAN CC, LIN YH, HWANG JL, HO LT. Omental fat expression of adiponectin and adiponectin receptors in non-obese women with PCOS: a preliminary study. *Reprod Biomed Online* 2009; 19: 577-582.
- 18) HOFFSTEDT J, ARVIDSSON E, SJÖLIN E, WÄHLÉN K, ARNER P. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. *J Clin Endocrinol Metab* 2004; 89: 1391-1396.
- 19) CARMINA E, CHU MC, MORAN C, TORTORIELLO D, VARDHANA P, TENA G, PRECIADO R, LOBO R. Subcutaneous and omental fat expression of adiponectin and leptin in women with polycystic ovary syndrome. *Fertil Steril* 2008; 89: 642-648.
- 20) CNOP M, HAVEL PJ, UTZSCHNEIDER KM, CARR DB, SINHA MK, BOYKO EJ, RETZLAFF BM, KNOPP RH, BRUNZELL JD, KAHN SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 2003; 46: 459-469.
- 21) MOTOSHIMA H, WU X, SINHA MK, HARDY VE, ROSATO EL, BARBOT DJ, ROSATO FE, GOLDSTEIN BJ. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. *J Clin Endocrinol Metab* 2002; 87: 5662-5667.
- 22) PANIDIS D, FARMAKIOTIS D, ROUSSO D, KATSIKIS I, DELKOS D, PIOUSA A, GEROU S, DIAMANTI-KANDARAKIS E. Plasma visfatin levels in normal weight women with polycystic ovary syndrome. *Eur J Intern Med* 2008; 19: 406-412.
- 23) STEPPAN CM1, BAILEY ST, BHAT S, BROWN EJ, BANERJEE RR, WRIGHT CM, PATEL HR, AHIMA RS, LAZAR MA. The hormone resistin links obesity to diabetes. *Nature* 2001 409: 307-312.
- 24) CHU Y, CUI Q, FENG G, SONG Z, JIANG X. The expression of resistin in adipose tissues of patients with polycystic ovary syndrome and insulin resistance. *J Huazhong Univ Sci Technolog Med Sci* 2009; 29: 642-645.
- 25) LINDHOLM A, BLOMQUIST C, BIXO M, DAHLBOM I, HANSSON T, SUNDSTRÖM POROMAA I, BURÉN J. No difference in markers of adipose tissue inflammation between overweight women with polycystic ovary syndrome and weight-matched controls. *Hum Reprod* 2011; 26: 1478-1485.
- 26) UNLUTURK U, HARMANCI A, KOCAEFE C, YILDIZ BO. The Genetic Basis of the Polycystic Ovary Syndrome: A Literature Review Including Discussion of PPAR-gamma. *PPAR Res* 2007; 2007:49109.
- 27) VATIER C, KADIRI S, MUSCAT A, CHAPRON C, CAPEAU J, ANTOINE B. Visceral and subcutaneous adipose tissue from lean women respond differently to lipopolysaccharide-induced alteration of inflammation and glyceroneogenesis. *Nutr Diabetes* 2012; 2: e51.

- 28) LECKE SB, MATTEI F, MORSCH DM, SPRITZER PM. Abdominal subcutaneous fat gene expression and circulating levels of leptin and adiponectin in polycystic ovary syndrome. *Fertil Steril* 2011; 95: 2044-2049.
- 29) O'CONNOR A, PHELAN N, TUN TK, BORAN G, GIBNEY J, ROCHE HM. High-molecular-weight adiponectin is selectively reduced in women with polycystic ovary syndrome independent of body mass index and severity of insulin resistance. *J Clin Endocrinol Metab* 2010; 95: 1378-1385.
- 30) LECKE SB, MORSCH DM, SPRITZER PM. Association between adipose tissue expression and serum levels of leptin and adiponectin in women with polycystic ovary syndrome. *Genet Mol Res* 2013; 12:4292-4296.
- 31) WU R, FUJII S, RYAN NK, VAN DER HOEK KH, JASPER MJ, SINI I, ROBERTSON SA, ROBKER RL, NORMAN RJ. Ovarian leukocyte distribution and cytokine/chemokine mRNA expression in follicular fluid cells in women with polycystic ovary syndrome. *Hum Reprod* 2007; 22: 527-535.
- 32) TAN BK, CHEN J, DIGBY JE, KEAY SD, KENNEDY CR, RANDEVA HS. Increased visfatin messenger ribonucleic acid and protein levels in adipose tissue and adipocytes in women with polycystic ovary syndrome: parallel increase in plasma visfatin. *J Clin Endocrinol Metab* 2006; 91: 5022-5028.
- 33) BERNDT J, KLÖTING N, KRALISCH S, KOVACS P, FASSHAUER M, SCHÖN MR, STUMVOLL M, BLÜHER M. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 2005; 54: 2911-2916.
- 34) HARMAN-BOEHM I, BLÜHER M, REDEL H, SION-VARDY N, OVADIA S, AVINOACH E, SHAI I, KLÖTING N, STUMVOLL M, BASHAN N, RUDICH A. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab* 2007; 92: 2240-2247.
- 35) HUBER J, KIEFER FW, ZEYDA M, LUDVIK B, SILBERHUMER GR, PRAGER G, ZLABINGER GJ, STULNIG TM. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. *J Clin Endocrinol Metab* 2008; 93: 3215-3221.
- 36) POULAIN-GODEFROY O, LECOEUR C, PATTOU F, FRÜHBECK G, FROGUEL P. Inflammation is associated with a decrease of lipogenic factors in omental fat in women. *Am J Physiol Regul Integr Comp Physiol* 2008; 295: 1-7.