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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.

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 blood[1]. There is increasing evidence that the expression of some genes associated with insulin resistance could be affected by high levels of testosterone (T) in PCOS[2]. 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 interlukin-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 resistance[7]. High levels of androgens are thought to impact on adipose tissue function and distribution in women with PCOS[8]. 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 androgens[9-12]. However, some investigations have suggested that the role of adipokines in the pathogenesis of PCOS is more complicated[1]. 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.

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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/m^2. 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 cm^2 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 transcriptase 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 l.

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 (PPARg), CCAAT-enhancer-binding proteins (C/EBP alpha), 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 PPARg and C/EBP alpha, 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′)
PPARg	Re GAGGGAGTTGGAAGGCTCTTC
	Fw GATCCAGTGGTTGCAGATTACAA
C/EBPa	Re CGCACATTCACATTGCACAA
	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 TGTACTCCCGAACCCATTTCTT
	Fw ACCCAGCAGTCGTCTTTGTCA
Leptin	Re TGAGGGTTTTGGTGTCATCTTG
	Fw TGGCTTTGGCCCTATCTTTTC
Visfatin	Re CCAGGACTGAACAAGAATAGTCTCAAT
	Fw TGTTCCTGAGGGCTTTGTCAT
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 patients 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 work[18] comparing people with high sensitivity and low sensitivity to insulin. By contrast, Carmina et al[19] 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

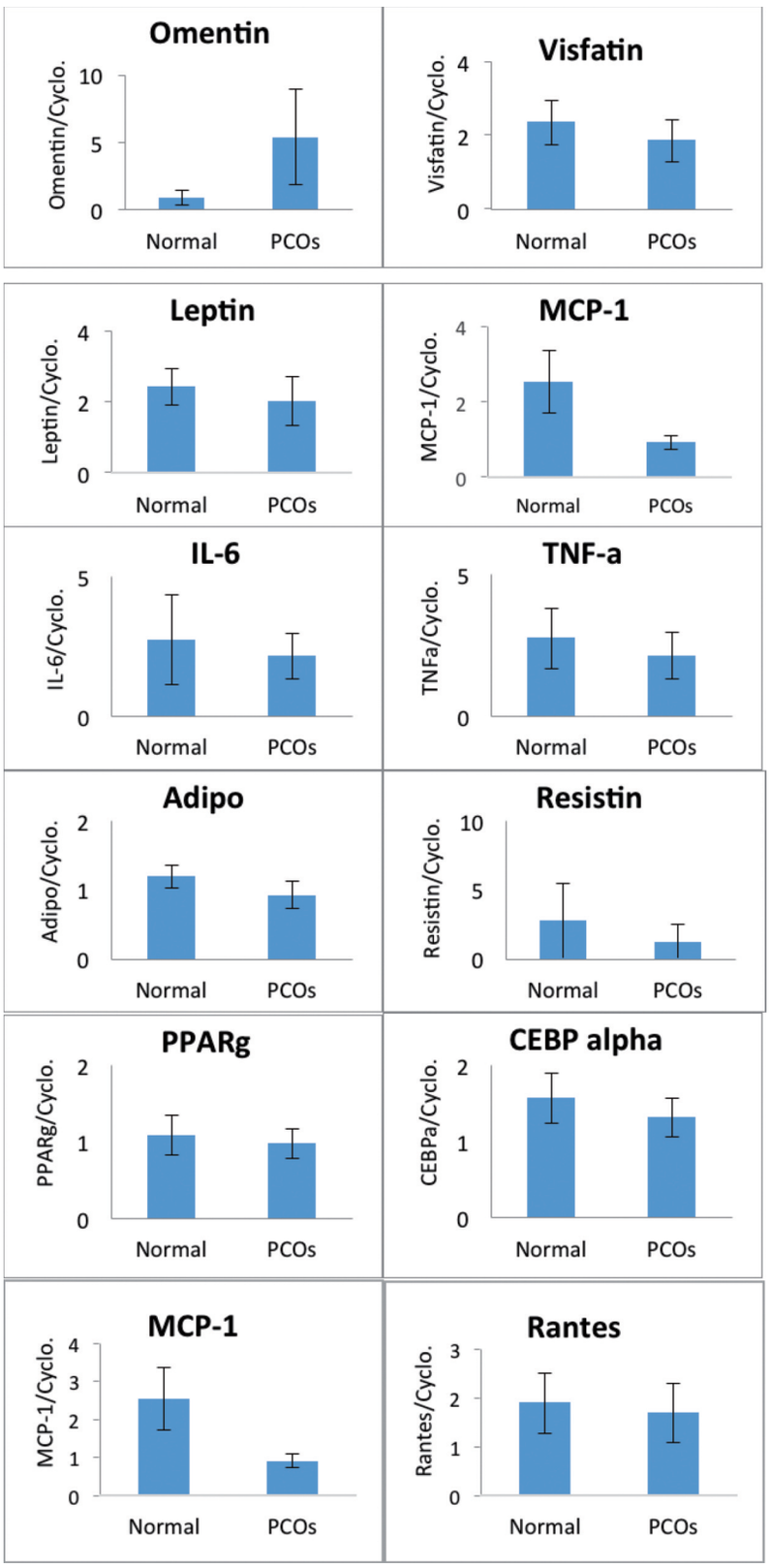

Figure 1. Levels of mRNA for inflammatory substances (mean ± SD) in abdominal superficial subcutaneous adipose tissue from obese patients with PCOS (n = 8), and obese control subjects (n = 12). Levels of mRNA are relative to the cyclophilin gene (Cyclo), which was used to normalize data.

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 previously[24]. The results also do not agree with another study[16], 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 another[26], 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 results[2]. However, the amount of IL-6 mRNA in VAT is 10 fold more than in SAT[27] 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 study[19] implied that expression was lower in both omental and SAT obtained from PCOS women, whereas others[28-30] 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 mass[33]. Moreover, Seow et al[16] 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 markers[34-36].

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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