



## Research Paper

# Comprehensive assessment of expression of insulin signaling pathway components in subcutaneous adipose tissue of women with and without polycystic ovary syndrome



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## ABSTRACT

**Objective:** Insulin resistance is a common feature of polycystic ovary syndrome (PCOS). The insulin signaling pathway consists of two major pathways, the metabolic and the mitogenic cascades. The many components of these pathways have not been comprehensively analyzed for differential expression in insulin-responsive tissues in PCOS. The goal of this study was to determine whether the core elements of the insulin signal transduction cascade were differentially expressed in subcutaneous adipose tissue (SAT) between PCOS and controls.

**Materials/methods:** Quantitative real-time PCR for 36 insulin signaling pathway genes was performed in subcutaneous adipose tissue from 22 white PCOS and 13 healthy controls.

**Results:** Genes in the insulin signaling pathway were not differentially expressed in subcutaneous adipose tissue between PCOS and controls ( $P > 0.05$  for all). Components mainly of the mitogenic pathway were correlated with both androgens and metabolic phenotypes. Expression levels of five genes (*MKNK1*, *HRAS*, *NRAS*, *KRAS*, and *GSK3A*) were positively correlated with total testosterone level ( $\rho > 0$ ,  $P < 0.05$ ). Inverse correlation was found between expression of six genes (*HRAS*, *MAP2K2*, *NRAS*, *MAPK3*, *GRB2*, and *SHC1*) and metabolic traits (body mass index, fasting glucose, fasting insulin, and HOMA-IR) ( $\rho < 0$ ,  $P < 0.05$ ).

**Conclusions:** Differential expression of core insulin signaling pathway components in subcutaneous adipose tissue is not a major contributor to the pathogenesis of PCOS. Correlation between clinical phenotypes and expression of several genes in the mitogenic limb of the insulin signaling pathway suggests mitogenic signaling by insulin may regulate steroidogenesis and glucose homeostasis.

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**Abbreviations:** polycystic ovary syndrome, PCOS; subcutaneous adipose tissue, SAT; single nucleotide polymorphism, SNP; quantitative real-time PCR, qRT-PCR; MAP kinase, MAPK; phosphatidylinositol-3-kinase, PI3K; homeostasis model assessment, HOMA; body mass index, BMI.

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## Introduction

Polycystic ovary syndrome (PCOS), characterized by hyperandrogenism, polycystic ovaries and oligo-ovulation, affects 7–10% of reproductive age women [1]. Women with PCOS disproportionately manifest insulin resistance, obesity, diabetes mellitus and cardiovascular disease. Approximately 50–70% of all PCOS women demonstrate some degree of insulin resistance, as determined by a variety of methods [1]. As a consequence of insulin resistance, PCOS patients often present with compensatory hyperinsulinemia, which exacerbates hyperandrogenemia by promoting ovarian androgen

production and suppressing sex-hormone binding globulin levels [1,2]. That many features of PCOS improve on treatment with insulin sensitizing medications suggests that insulin resistance may be integral to the pathogenesis of PCOS [3].

Given that both PCOS and insulin resistance are heritable [4], the insulin signaling pathway provides a number of candidate genes that might illuminate the pathogenesis of PCOS. Only a handful of the many possible genes coding for components of the insulin signaling pathway were initially investigated in single nucleotide polymorphism (SNP) association studies [5]. We extensively interrogated the entire regions of ~30 genes comprising the core components of the classic metabolic limb of the insulin signaling pathway as well as the novel CAP/Cbl pathway, identifying several SNPs associated with PCOS in a discovery cohort; association with PCOS of one SNP, rs2252673, in the insulin receptor (*INSR*) gene was confirmed in a replication cohort [6]. Until the latter study, the majority of association studies of *INSR* in PCOS had focused only on a single variant [7]. Several studies describing association with PCOS of the D19S884 microsatellite provide further support of a potential role of *INSR* in the inheritance of PCOS [8,9]. Most convincingly, a recent genome-wide association study identified eight new risk loci for PCOS, including rs2059807, a SNP within an intron of the *INSR* gene [10]. This confirms the significance of *INSR*, and suggests a possible effect on the downstream insulin signaling events that follow binding of insulin in PCOS pathogenesis.

The insulin signaling cascade is initiated when insulin binds to the  $\alpha$ -subunit of the insulin receptor, activating the tyrosine kinase activity in the  $\beta$ -subunit, which results in phosphorylation of the intermediates phosphatidylinositol-3-kinase (PI3K) and MAP kinase (MAPK) (Fig. 1). Activation of PI3K leads to a cascade of downstream signals (termed the PI3K pathway or metabolic pathway) that promote metabolic effects such as glucose uptake

**Table 1**

Clinical characteristics of PCOS and control subjects

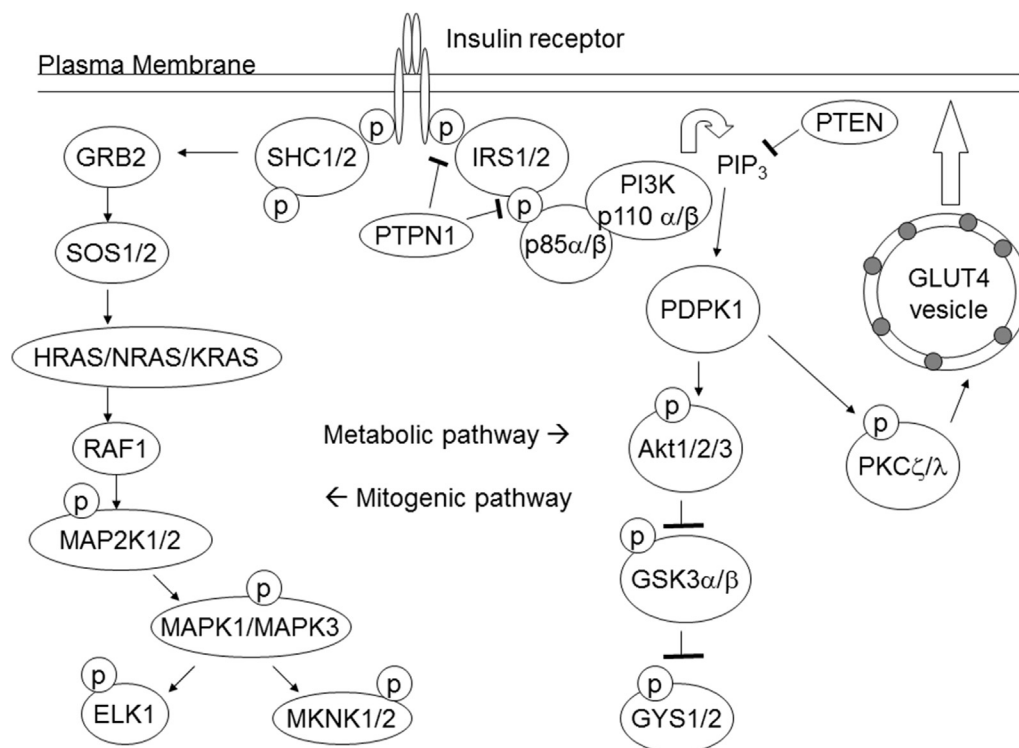
	Control (n = 13)	PCOS (n = 22)	P value
Age (yr)	33.0 (13.0)	28.0 (3.3)	0.042
BMI (kg/m <sup>2</sup> )	25.5 (8.7)	31.0 (8.0)	0.23
Insulin (pmol/l)	57.0 (48.0)	54.0 (69.0)	0.46
Glucose (mmol/l)	4.14 (1.28)	4.97 (0.55)	0.030
HOMA-IR	1.09 (0.46)	1.01 (1.08)	0.99
HOMA-%B	122.4 (75.3)	89.0 (44.6)	0.17
Total testosterone (nmol/l)	0.97 (0.29)	1.11 (0.99)	0.079
Free T (nmol/l)	0.080 (0.074)	0.18 (0.14)	0.0013
DHEAS ( $\mu$ mol/l)	5.41 (4.32)	6.60 (3.68)	0.038
mFG	0 (1)	7 (5)	<0.0001

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-%B, homeostasis model assessment of beta-cell function (insulin secretion); mFG, modified Ferriman-Gallwey score to evaluate hirsutism in women.

Data are median (interquartile range). P values are derived from the Mann–Whitney test.

and glycogen synthesis. The activation of the MAPK cascade promotes gene expression and cell growth, comprising the mitogenic pathway [11] (Fig. 1).

Women with PCOS often have abdominal obesity, and gene expression profiling experiments suggest dysfunction of visceral fat plays an important role in PCOS [12]. However, visceral fat is not the only determinant of insulin resistance, and a substantial proportion of lean PCOS women are insulin resistant as well [13]. In addition to visceral adipose tissue, dysfunction of subcutaneous adipose tissue (SAT) also contributes to insulin resistance and obesity; SAT mass and capacity for fat storage influence insulin sensitivity, independent of visceral fat [14,15]. Investigators have successfully utilized SAT to demonstrate adipose tissue dysfunction in PCOS. A



**Fig. 1.** Core components of the insulin signaling pathway. The metabolic (PI3K) and mitogenic (MAPK) limbs of the insulin signaling pathway were studied. The products of the 36 genes whose expression was analyzed are indicated. Names containing a slash indicate isoforms encoded by different genes. Flat arrows indicate inhibition. p = phosphate group; PIP<sub>3</sub> = phosphatidylinositol (3,4,5)-trisphosphate. Full gene names are listed in Supplemental Table 1.

microarray study found significant gene expression alterations in SAT from non-obese PCOS, revealing differentially expressed genes involved in inflammation, lipid metabolism and Wnt signaling [16]. SAT from non-obese PCOS women had increased expression of the genes for CD11c (a proinflammatory macrophage marker), leptin, and tumor necrosis factor alpha and decreased expression of peroxisome proliferator-activated receptor gamma [17,18]. Compared to body mass index (BMI)-matched controls, PCOS women had increased SAT on magnetic resonance imaging, which correlated negatively with insulin sensitivity [17]. The above findings highlight the importance of SAT in PCOS and insulin resistance.

To test the hypothesis that the insulin signaling pathway may be disrupted in PCOS, we analyzed the core elements of the insulin signal transduction cascade in SAT of PCOS women and controls, looking for differential gene expression.

## Materials and methods

### Subjects

For mRNA analysis, subcutaneous lower abdominal adipose tissue was obtained from 22 white PCOS and 13 white control subjects (clinical characteristics are shown in Table 1); we have previously conducted expression studies in some [19] or all [20] of these subjects. The protocol for acquiring and processing SAT was previously described [21]. Cases were premenopausal, non-pregnant, on no hormonal therapy, including oral contraceptives, for at least 3 months, and met the National Institutes of Health (NIH) 1990 criteria for the diagnosis of PCOS. Parameters for defining hirsutism, hyperandrogenemia, ovulatory dysfunction, and exclusion of related disorders have been reported previously [22]. Controls were healthy women with regular menstrual cycles, no evidence of hirsutism, acne, alopecia, or endocrine dysfunction, and had not taken hormonal therapy (including oral contraceptives) for at least 3 months. Controls were recruited by word of mouth and advertisements calling for healthy women.

Fasting blood was obtained in cycle days 3–8 (follicular phase) after a spontaneous menstrual cycle or a progesterone-induced withdrawal bleed. Homeostasis model assessment of insulin resistance (HOMA-IR) and beta cell function (HOMA-%B) were calculated using the web tool at <http://www.dtu.ox.ac.uk/homacalculator/index.php>.

### Ethical approval

All subjects gave informed written consent, according to the guidelines of the institutional review board at Cedars-Sinai Medical Center.

### Quantitative real-time PCR (qRT-PCR) gene expression analysis

Total RNA was isolated from SAT after rapid thaw with the AllPrep DNA/RNA/protein minikit (QIAGEN, Valencia, CA, U.S.A.) and was quantified and checked for quality (260/280 and 260/230 ratios) on a Nanodrop-1000 (Nanodrop, Wilmington, DE, U.S.A.). The integrity of RNA was checked by Bioanalyzer (RIN = 7.6–9.8). cDNA was created using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, U.S.A.). According to the manufacturer's protocol, 10 µl of RNA at 5 ng/µl was used to generate cDNA. A pre-amplification protocol was then performed using the Taqman PreAmp Master Mix (Life Technologies) and a custom pool of primers for 38 gene expression targets following the manufacturer's directions. The genes consisted of two housekeeping genes (*GAPDH*, *GUSB*), plus genes for 36 insulin signaling elements (*AKT1*, *AKT2*, *AKT3*, *ELK1*, *GRB2*, *GSK3A*, *GSK3B*, *GYS1*, *GYS2*, *HRAS*, *IRS1*, *IRS2*, *INSR*,

**Table 2**

Relative gene expression levels and fold change for core factors in the insulin signaling pathway of PCOS and control subjects

Gene name	Controls (ΔCt)	PCOS (ΔCt)	ΔΔCt	Fold change	P value
<i>AKT1</i>	0.557 (1.367)	0.487 (0.935)	−0.042	1.03	0.99
<i>AKT2</i>	−0.371 (1.681)	−0.708 (1.24)	−0.011	1.01	0.93
<i>AKT3</i>	1.998 (0.544)	2.171 (0.96)	0.199	0.87	0.25
<i>ELK1</i>	2.851 (0.795)	2.675 (0.734)	0.184	0.88	0.93
<i>GRB2</i>	0.856 (0.619)	0.869 (0.405)	−0.148	1.11	0.33
<i>GSK3A</i>	−0.0135 (0.628)	0.0149 (0.987)	0.174	0.89	0.80
<i>GSK3B</i>	1.256 (0.518)	1.312 (0.369)	0.035	0.98	0.57
<i>GYS1</i>	1.745 (0.831)	1.231 (1.168)	−0.233	1.18	0.27
<i>GYS2</i>	5.356 (1.638)	5.456 (1.965)	0.089	0.94	0.67
<i>HRAS</i>	1.600 (1.134)	1.202 (1.009)	−0.123	1.09	0.24
<i>INSR</i>	1.171 (0.719)	1.425 (0.649)	0.245	0.84	0.094
<i>IRS1</i>	1.630 (0.876)	1.817 (1.129)	0.309	0.81	0.44
<i>IRS2</i>	0.301 (1.233)	0.406 (0.771)	0.159	0.90	0.37
<i>KRAS</i>	4.136 (0.661)	4.111 (0.583)	−0.028	1.02	0.77
<i>MAP2K1</i>	1.416 (0.62)	1.416 (0.301)	−0.006	1.00	0.80
<i>MAP2K2</i>	1.778 (0.592)	1.453 (0.58)	−0.183	1.14	0.20
<i>MAPK1</i>	0.673 (0.617)	0.876 (0.482)	0.160	0.90	0.13
<i>MAPK3</i>	0.353 (0.709)	0.307 (0.539)	0.055	0.96	0.67
<i>MKNK1</i>	1.655 (0.713)	1.926 (0.895)	0.223	0.86	0.69
<i>MKNK2</i>	−0.563 (0.736)	−0.637 (0.923)	0.277	0.83	0.30
<i>NRAS</i>	1.101 (0.552)	0.893 (1.059)	0.039	0.97	0.53
<i>PDPK1</i>	1.224 (0.752)	1.371 (0.985)	0.213	0.86	0.77
<i>PIK3CA</i>	1.566 (0.861)	1.912 (0.623)	0.267	0.83	0.14
<i>PIK3CB</i>	2.095 (0.653)	2.222 (0.645)	0.136	0.91	0.30
<i>PIK3R1</i>	−1.646 (1.288)	−1.456 (0.916)	0.006	1.00	0.93
<i>PIK3R2</i>	2.245 (0.372)	1.962 (0.533)	−0.188	1.14	0.14
<i>PRKCI</i>	7.222 (0.76)	7.392 (0.816)	0.387	0.77	0.37
<i>PRKCZ</i>	6.173 (1.082)	6.962 (1.236)	0.655	0.64	0.082
<i>PTEN</i>	0.519 (0.852)	0.55 (0.579)	0.209	0.87	0.75
<i>PTPN1</i>	1.799 (1.059)	1.879 (0.833)	0.052	0.96	0.85
<i>RAF1</i>	3.533 (0.624)	3.383 (0.485)	0.072	0.95	0.96
<i>SHC1</i>	−0.183 (1.085)	−0.676 (0.98)	−0.041	1.03	0.28
<i>SHC2</i>	2.513 (0.766)	2.858 (0.816)	0.023	0.98	0.88
<i>SLC2A4</i>	0.803 (1.715)	0.0649 (1.686)	−0.065	1.05	0.75
<i>SOS1</i>	0.589 (0.612)	0.544 (0.62)	−0.222	1.17	0.21
<i>SOS2</i>	1.630 (0.43)	1.714 (0.641)	0.065	0.96	0.77

ΔCt data are median (interquartile range). P values were derived from Mann–Whitney tests comparing ΔCt values between women with PCOS and controls.

*KRAS*, *MAP2K1*, *MAP2K2*, *MAPK1*, *MAPK3*, *MKNK1*, *MKNK2*, *NRAS*, *PDPK1*, *PI3KCA*, *PI3KCB*, *PI3KR1*, *PI3KR2*, *PRKCI*, *PRKCZ*, *PTEN*, *PTPN1*, *RAF1*, *SHC1*, *SHC2*, *SLC2A4*, *SOS1*, *SOS2*) (gene names and assay numbers are listed in Supplemental Table 1); these 36 genes were selected because they are integral to the metabolic and mitogenic limbs of the insulin signaling pathway (Fig. 1). Quantitative RT-PCR analysis was performed using the OpenArray platform and Taqman gene expression assays (Life Technologies). Samples were run in technical triplicate with a no template control carried forward from cDNA generation. The housekeeping genes *GAPDH* and *GUSB* were used for signal normalization. The results were analyzed using the ΔΔCt relative quantification method [23]. Relative mRNA expression levels of each transcript in PCOS tissues were compared by fold change to the control samples; average cycle threshold (Ct) values of each group were adjusted by subtracting the average Ct values of the housekeeping genes, yielding ΔCt. The ΔΔCt for each transcript across groups was calculated as  $\Delta\Delta Ct = \Delta Ct^{PCOS} - \Delta Ct^{controls}$ . The fold change of PCOS relative to controls was calculated as  $2^{(-\Delta\Delta Ct)}$ .

### Statistical analysis

Mann–Whitney tests were used to compare clinical characteristics between cases and controls. Data are presented as median (interquartile range). The ΔCt for PCOS versus controls from qRT-PCR was subjected to Mann–Whitney tests. Logistic regression was used to adjust for age and BMI, to avoid their potential effects on expression of genes coding for components of the insulin

**Table 3**  
Correlation between clinical traits and gene expression levels ( $\Delta$ Ct)

Traits	Gene symbol	Spearman $\rho$	P value
<b>Androgens</b>			
Total testosterone	<i>MKNK1</i>	0.43	0.025
Total testosterone	<i>HRAS</i>	0.43	0.027
Total testosterone	<i>NRAS</i>	0.42	0.030
Total testosterone	<i>KRAS</i>	0.39	0.042
Total testosterone	<i>GSK3A</i>	0.39	0.046
DHEAS	<i>RAF1</i>	-0.40	0.044
<b>Metabolic traits</b>			
BMI	<i>GRB2</i>	-0.42	0.013
BMI	<i>HRAS</i>	-0.44	0.0078
BMI	<i>MAP2K2</i>	-0.37	0.031
BMI	<i>NRAS</i>	-0.39	0.022
Glucose	<i>MAPK3</i>	-0.50	0.013
Glucose	<i>NRAS</i>	-0.42	0.043
HOMA-IR	<i>GRB2</i>	-0.56	0.017
HOMA-IR	<i>MAPK3</i>	-0.64	0.0043
Insulin	<i>GRB2</i>	-0.43	0.048
Insulin	<i>HRAS</i>	-0.46	0.030
Insulin	<i>MAPK3</i>	-0.58	0.0050
Insulin	<i>NRAS</i>	-0.52	0.014
Insulin	<i>SHC1</i>	-0.53	0.012

P values were derived from Spearman's rank correlation.

signaling pathway. Spearman's rank was used to test correlation between gene expression levels ( $\Delta$ Ct) and quantitative traits in the entire cohort. All statistical tests were performed in JMP 9.0 (SAS Institute Inc., Cary, NC, U.S.A.), and significance was taken as  $P < 0.05$ .

## Results

PCOS women and controls were similar in BMI; however, PCOS women were slightly but statistically significantly younger than controls (Table 1). Expression levels of all core factors in the insulin signaling pathway were examined by qRT-PCR. Table 2 displays the median (inter-quartile range) of the  $\Delta$ Ct for each transcript, as well as fold change. No genes were differentially expressed between PCOS and controls. Adjustment for age and BMI did not modify these results.

We conducted exploratory analyses wherein we evaluated correlations between gene expression levels and quantitative phenotypes. The exact correlation coefficient  $\rho$  values are shown in Table 3. For androgens, positive correlation was found between expression of five genes (*MKNK1*, *HRAS*, *NRAS*, *KRAS*, and *GSK3A*) and total testosterone level ( $\rho > 0$ ,  $P < 0.05$ ). In contrast, metabolic traits, including BMI, fasting insulin, HOMA-IR, and fasting glucose, were inversely correlated with expression of several genes (*HRAS*, *MAP2K2*, *NRAS*, *MAPK3*, *GRB2*, and *SHC1*) ( $\rho < 0$ ,  $P < 0.05$ ). No gene expression was correlated with HOMA-%B. All genes (except *GSK3A*) whose transcripts were correlated with clinical traits belong to the mitogenic limb of the insulin signaling pathway (Fig. 1). Of note, these exploratory correlation analyses were conducted in the entire cohort to maximize sample size. We also performed correlation analyses stratified by PCOS diagnosis; several of the inverse correlations seen in the entire group between mitogenic gene expression and metabolic traits were also observed in the PCOS group. This pattern was not clear in the control group, possibly due to reduced sample size (Supplemental Table 2). Thus, we cannot definitively comment on whether the observed correlation results are specific to PCOS or to women in general.

## Discussion

In women with and without PCOS, we analyzed the gene expression of 36 elements of the insulin signal transduction

cascade, from the insulin receptor through the distal effectors downstream, which result in metabolic (PI3K pathway) and mitogenic (MAPK pathway) effects (Fig. 1). We observed no differences in expression in any of the insulin signaling pathway elements, although there was a trend towards significance for *INSR* ( $P = 0.094$ ), with PCOS women demonstrating decreased *INSR* expression versus controls. Interestingly, among insulin signaling pathway genes, polymorphisms at the *INSR* locus have the best evidence of association with PCOS [6,8–10]. Exploratory correlation analysis between gene expression in SAT and clinical traits found that several components of the mitogenic pathway were correlated with both androgens and metabolic phenotypes.

Consistent with our results, prior microarray studies in adipose tissue have not revealed differential mRNA expression of core insulin signaling pathway components in women with PCOS compared to controls [12,16]. Very few studies have examined mRNA levels of specific insulin signaling pathway components; one found no difference in expression of the gene for the p85 subunit of PI3K [24] and another found decreased expression of *GLUT4* mRNA [19]. The scarcity of such studies highlighted the need for the current study, which examined mRNA levels for genes for all of the central elements in the metabolic and mitogenic insulin signaling pathways.

Differential mRNA expression of core signaling elements represents only one potential mechanism of pathway dysfunction. Aberrant post-transcriptional or post-translational regulation may exist in PCOS adipose, resulting in alterations in concentration and/or phosphorylation of pathway proteins. Several studies have examined protein levels of specific insulin signaling components in adipose tissue from women with and without PCOS. Most of these did not observe differences in protein levels (Akt-1, Akt-2, c-Cbl associated protein (CAP), Cbl proto-oncogene, GLUT-4, insulin receptor  $\beta$  subunit, IRS-1, IRS-2, PKC- $\zeta$ , p85 subunit of PI3K) [24–26], while others found decreased GLUT-4 protein [19,27] and decreased protein levels and phosphorylation of IRS-1 [28]. Post-translational regulation may alter activity of insulin signaling components even in the absence of altered mRNA or protein levels, as has been seen for PI3K and GSK-3 $\beta$  in PCOS adipose [21,24]. The current study, focused on transcript levels, cannot evaluate these possibilities.

Interestingly, exploratory analyses found that expression levels of several genes from the mitogenic limb of the pathway (and *GSK3A*, which has effects on the cell cycle as well as metabolism [29]) were correlated with androgen levels. In general, MAPK signaling plays a role in processes such as cell differentiation, cell proliferation, and cell death [30]. Members of the MAPK family regulate steroid biosynthesis through effects on expression of steroidogenic acute regulatory protein (StAR) [31]. Testosterone in turn can activate MAPK signaling via the androgen receptor [32]. The cause-and-effect relationship underlying the positive correlation between testosterone levels and expression of MAPK components requires further investigation. This correlation raises the possibility of interaction between MAPK signaling and testosterone synthesis. These exploratory correlation analyses should be considered hypothesis-generating as statistical significance was not corrected for multiple testing.

Alternatively, we found inverse correlation between several MAPK signaling pathway components and metabolic traits. This is not in agreement with findings in PCOS skeletal muscle, in which activation of the MAPK pathway may cause insulin resistance via serine phosphorylation [33]. SAT may respond differently than skeletal muscle to MAPK signaling. Members of the MAPK family have effects on each step of adipocyte differentiation [34]. MAPKs may play a role in “adipose tissue expandability,” which hypothesizes that SAT has a limited capacity to expand [34,35]. Before the maximal point of SAT expansion is reached, SAT storage capacity prevents fat deposition in visceral adipose tissue, liver, and muscle,

protecting against insulin resistance and other metabolic complications [35]. We hypothesize that increased expression of genes in the mitogenic pathway contributes to increased amounts of SAT available to store excess body fat.

Our study has several strengths. Our sample size was larger than many prior expression studies in this field. Our cases and controls overlapped substantially in BMI (Table 1), precluding concerns that any observed expression differences might be due solely to differences in adiposity. Moreover, we investigated the expression of the full complement of insulin signal transduction elements, from proximal insulin receptor to distal metabolic and mitogenic elements within an insulin target tissue. However, although abdominal subcutaneous adipose significantly modulates total body insulin action [14,15], it is possible that alterations in insulin signaling gene expression may exist in visceral adipose tissue only or in other insulin target tissues, such as skeletal muscle and liver. Another limitation of our study is that, although larger than several prior studies, the number of samples studied is still relatively small. Therefore, correlation analyses stratified by PCOS diagnosis may generate unreliable results. In addition, one possible reason for the lack of expression differences is that our cases and controls were generally similar with respect to their insulin dynamics; only fasting glucose approached significance, whereas differences in fasting insulin, HOMA-IR and HOMA-%B were insignificant. Perhaps expression differences would have been detectable if our cases and controls had been more divergent in insulin sensitivity.

In conclusion, we contrasted the expression of genes involved in the insulin signaling pathways in SAT derived from women with PCOS and healthy controls. We did not find expression differences for any of the 36 components studied. This suggests that differential mRNA expression of core insulin signaling pathway components in this tissue is not a major contributor to the pathogenesis of PCOS. Further studies will be needed to determine whether this is the case for other insulin responsive tissues.

### Conflict of interest

The authors declare they have no conflicts of interest.

### Acknowledgments

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jcte.2015.06.002>.

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