Differences in Expression of Genes Related to Steroidgenesis in Abdominal Subcutaneous Adipose Tissue of Pregnant Women with and without PCOS

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Research

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Abstract

Background: It was hypothesized that steroid-related gene expressions in the adipose tissue (AT) of women are different between women with and without polycystic ovary syndrome (PCOS). However, little information exists regarding pregnant women suffering from PCOS when interrelationships among maternal PCOS, patient's quality of life and offspring's health is a consideration issue in health and diseases. Our objectives were to compare the mRNA expression levels of key steroid-converting enzymes in subcutaneous AT of PCOS *vs*. non-PCOS pregnant women.

Methods: The subjects were 48 pregnant women (12 PCOS and 36 non–PCOS women) undergoing cesarean section, from whom abdominal subcutaneous AT samples were retrieved. Expressions of fifteen genes related to steriodogenesis were investigated using quantitative real-time PCR.

Results: No significant differences were found with respect to age, BMI (before pregnancy and at delivery day), gestational period and parity among non-PCOS and PCOS pregnant women. Most of the sex steroid-converting genes except 17β-Hydroxysteroid dehydrogenases2 (*17BHSD2*), were highly expressed on the day of delivery in subcutaneous AT. PCOS patients showed significantly higher mRNA levels of steroidgenic acute regulator (*STAR; P<0.001*), cytochrome P450 monooxygenase (*CYP11A1; P<0.05*), 17α-hydroxylase (*CYP17A1; P<0.05*), and 11β-Hydroxysteroid dehydrogenase (*11BHSD1-2; P<0.05*). The expression of steroid 21-hydroxylase (*CYP21)* in non-PCOS women was 4-fold higher than for PCOS women ($P < 0.001$). There were no significant differences between relative expression of aromatase cytochrome P450 *(CYP19A1),* 3β-hydroxysteroid dehydrogenase1-2 *(3BHSD1-2)*, and *17BHSD* family (1, 3, 5, 7, and 12) between the two groups.

Conclusion: The expression levels of genes related to sex steroids metabolism were similar in age-matched and BMI- matched non-PCOS and PCOS pregnant women at delivery day. However, the alterations in gene expressions involved in glucocorticoids and mineralocorticoid metabolism was shown which warrants further studies regards functional activity. More attention should be given to AT of PCOS mothers that was previously ignored.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disease, occurring in 4–18% of adolescents and women of childbearing age depending on factors such as adiposity and diagnostic criteria [1]. Women suffering from PCOS have several health problems such as metabolic syndrome and androgen imbalance. In conditions of female androgen excess such as PCOS, androgens exert metabolically deleterious effects on adipose tissue (AT) function in a depot-specific manner [2]. Most recently, it has been suggested that AT may contain the steroidogenic machinery necessary for the initiation of steroid biosynthesis *de novo* from cholesterol [3].

The composition and the concentration of steroids released into circulation from AT or removed therefrom, are affected by alterations in fat mass and AT functionality [3, 4]. Androgen and cortisol can

modulate adipocyte function and possibly affect the size and distribution of AT [5]. The role of AT in steroid hormone metabolism has been addressed in research driven by the worldwide human epidemic of adiposities and related disturbances in health and fertility e.g. PCOS [6, 7]. Although the amount of androgen or estrogen derived from AT is not large, the abnormal conversion of androgen precursors to both active and inactive forms at the receptor level is important for the pathogenicity of PCOS [8, 9]. Moreover, pathologically increased circulating cortisol concentration is associated with obesity, insulin resistance and PCOS. It is also responsible for the generation of cortisol in AT by the enzyme 11beta-hydroxysteroid dehydrogenase type 1 (*11BHSD1*), which converts inactive cortisone to active cortisol [10].

Uniquely, Quinkler et al. suggested that high levels of *17* β-hydroxysteroid dehydrogenase 5 *(17 βHSD5*: responsible for the conversion of androstenedione to testosterone) in AT may lead to overweight and hyperandrogenism in PCOS women [11]. However, a surprising observation by Mackenzie et al. 2008, was that *de novo* syntheses of sex steroids, cortisol and aldosterone from cholesterol are not possible in pregnant women on the day of delivery because of the absence of key steroidogenic mRNAs in the AT [12]. Likewise, Lim et al. [1] showed that women with PCOS have increased risk of metabolic syndrome, which is associated with obesity and metabolic features but not with indices of hyperandrogenism. Overall, selectively targeting intra-adipose androgen activation may therefore represent a promising novel therapeutic strategy for women with PCOS [2].

There is increasing understanding on the roles of subcutaneous AT in PCOS women. More recently, Schiffer et al. suggested that adipose androgen generation is increased in subcutaneous AT in women with PCOS [2]. In this regard, it has been reported that only upper body not lower body obesity is associated with menstrual disorders [13]. The critical roles of subcutaneous AT in women was confirmed by Kuchenbecker *et al.* who showed that the subcutaneous abdominal AT and not the intra-abdominal fat compartment is associated with anovulation in women with obesity and infertility [14]. Moreover, Yucel et al. reported that free testosterone level is positively correlated with subcutaneous fat mass in the arms in women with PCOS [15]. Overall, previous studies support the “adipose tissue expandability” hypothesis, which proposes that overfilling of subcutaneous AT leads to the lipotoxic state, characterized by elevated free fatty acids, hypertriglyceridemia and unfavorable adipocytokine profile[16]. However, few studies have shown the effective roles of genes involved in AT’s steroidogenesis in PCOS women and to our knowledge, the question of whether gene expression in AT of PCOS pregnant women may differ from that of non-PCOS pregnant women has not been addressed yet. One of the underlying hypotheses was expecting to see is difference in expression of *HSD11B1* and *HSD11B2* at the time of delivery to confirm the importance of cortisol metabolism at delivery day in PCOS mother. With this background, the current study aims to investigate the mRNA levels of fifteen steroids genes in subcutaneous AT in pregnant women.

2. Materials And Methods

2.1. Subjects and Adipose tissue biopsies

After obtaining permission from Royan Institute Ethics Committee, Tehran, Iran (IR.ACECR.ROYAN.REC.1398.087), samples and demographic data were collected from three hospitals in Tehran, Iran. The subjects were 48 Iranian pregnant women undergoing cesarean section, including 12 PCOS and 36 non–PCOS women, from whom subcutaneous AT samples were retrieved from the abdominal area. Signed informed consent was obtained from all subjects. During sampling, the following information was collected: age, weight (before pregnancy and at delivery day), height, gestational information, and habits such as smoking, specific nutritional practices and alcohol consumption using a written questionnaire. According to the guideline for the assessment and management of PCOS [17], the diagnostic traits of PCOS are the presence of two or more significant signs of syndrome and patients must met two out of three signs. Diagnosis of PCOS in the enrolled subjects was the responsibility of the medical practitioner related to Royan Institute. Intake of any medication affecting glucose, lipid metabolism and diabetes, alcoholic consumption and smoking were defined as exclusion criteria. Reasons for delivery by caesarean section included maternal choice (< 10%), previous caesarean delivery and diagnosis by the medical practitioner.

At the time of caesarean section, the surgeon excised 3–4 g of subcutaneous AT upon exposing the abdominal cavity. The respective biopsies were immediately washed in isotonic saline solution, segmented and floated in liquid nitrogen, placed in cryovial tubes and snap-frozen. Samples were stored at liquid nitrogen (-196° C) until analysis [12] .

2.2 RNA extraction, cDNA synthesis and qPCR procedures

Total RNA were extracted for all samples, using the RNeasy lipid tissue mini kit (QIAGEN, Germany) according to the manufacturer's instructions. The integrity of the extracted RNA was evaluated using 1% agarose gels and stained with gel red, and the quality of extracted RNA was evaluated in terms of the A260/280 ratio using a spectrophotometer (Bio-Rad, Stanford, USA). Then, cDNA synthesis was performed on RNA samples using the PrimeScript RT Reagent Kit (TAKARA, Japan) according to the manufacturer's instructions, in the way that all experiments included RT controls and negative controls (without cDNA). PCR products were analyzed by gel electrophoresis. mRNA quantification was performed by qRT-PCR on the Step-One RT-PCR system (Applied Biosystems, USA). Each reaction was run in duplicate. Primer design was performed for all fourteen steroids target genes using the Perl primer Software (version1.1.21) and the NCBI primer Blast. The primer sets, product size, and NIH GenBank accession numbers are listed in Table 1. To verify primer specificities, melting curve analyses were performed. Standard curves were obtained for each gene to evaluate primer efficiency using the logarithmic dilution series of total cDNA. The housekeeping gene *GAPDH* was used for normalization and messenger RNA expression levels of all target genes were analyzed by quantitative real-time PCR ($2^{-\Delta\Delta CT}$).

Table1. Sequences of the primers used for the quantification of the target genes (F: Forward; R: Revers)

Gene	NIH Gen Bank accession No.	Product length (bp)	Primer sequence 5´- 3´
STAR	NM:000349.2	114	GCCCAAGAGCATCATCAAC(F)
			GCTGGTCTTCAACACCTG (R)
HSD11B1	NM:001206741.1	180	GCATTGTTGTCGTCTCCTCT(F)
			TGGCTGTTTCTGTGTCTATGAG(R)
HSD11B2	NM:000196.3	162	GCTGTGAACTCCTTCCCT(F)
			CGATGTAGTCCTTGCCGT(R)
HSD17B2	NM:002153.2	136	TCTGCCTGCTCATCCTGT(F)
			CAATCACCACCTGTCACCA(R)
HSD17B3	NM:000197.1	138	ATGCTTCCAAACCTTCTCCC(F)
			GAGACCTTTCTGCCTTGATTCC(R)
AKR1C3	NM:001253908.1	151	CTCCAGAGGTTCCGAGAA(F)
			CTCTTCACACTGCCATCTG(R)
HSD17B7	NM:001304512.1	112	TCATCTGTGTTTGGCGTG(F)
			GTTGCTGACATCCACCTG(R)
HSD17B12	NM:016142.2	113	CCTACCTAGCCCTGCGTATT(F)
			ACCTGTGACAACTGCCCA(R)
HSD3B1	NM:000862.2	185	CTTGGTGAAGGAGAAGGAGC(F)
			AGGCGGTGTGGATGATGA(R)
HSD3B2	NM:000198.3	173	TGCCAGTCTTCATCTACACC(F)
			TAGATTCCACCCATTAGCCG(R)
CYP17A1	NM:000102.3	154	GATAACCACATTCTCACCACC(F)
			GGCTGAAACCCACATTCTG(R)
CYP11A1	NM:000781.2	169	CTTCCTTTCTGTCTCAATTCCC(F)
			TCTACCAGATGTTCCACACC(R)
CYP21A2	NM:000500.8	103	TGAAGCAGGCCATAGAGAAG(F)
			ATGTAGTCCATCATGTCCCTC(R)
HSD17B1	NM:000413.3	234	TTCAGATCCATCCCAGAGC(F)
			TTGATGTCCCTTACGTCCAG(R)

GAPDH	NM: 001256799.3	134	TGAGAAGTATGACAACAGCCTC (F)
			TGATGGCATGGACTGTGGT (R)

2.2. Statistical analysis

Data were initially tested for normal distribution using Kolmogorov-Smirnov test. Data with normal distribution were analyzed using *t* test. Data that did not have normal distribution were analyzed using Mann–Whitney U test. All analyses were conducted in SAS. The differences were considered significant at $P \leq 0.05$.

3. Results

3.1. Characteristics of PCOS patients and non-PCOS pregnant women

No significant differences were found with respect to age, BMI (before pregnancy and at delivery day), gestational period and parity among non-PCOS and PCOS pregnant women (Table 2).

Table 2
General characteristics of study participants (PCOS: polycystic ovary syndrome and non-PCOS) (mean ± sd) (ns.; Non-significant; p > 0.05)

	non-PCOS (n = 36)	PCOS (n = 12)	P -Value
Age (year)	32 ± 5.7	31.6 ± 3.6	ns.
BMI (kgm^{-2}) before pregnancy	25.4 ± 6.06	26.2 ± 6.11	ns.
BMI (kgm^{-2}) at delivery day	31 ± 5.13	30.5 ± 5.74	ns.
Gestation period (Day)	264 ± 13	267 ± 5	ns.
Parity (no.)	1.4 ± 0.62	1.4 ± 0.51	ns.

3.2. Profiling of the key steroidogenic genes in subcutaneous AT

The expression profile of key steroidogenic enzyme was compared in human AT obtained from PCOS and non-PCOS pregnant women (Table 3). mRNAs of *STAR* ($P < 0.001$) and *CYP11A1* ($P < 0.05$) – the genes involved in the initial steps of steroidogenesis – were significantly more abundant in the subcutaneous AT of PCOS women, than in those of non-PCOS women (Fig. 1). In addition, *CYP17A1* ($P < 0.05$) mRNA changed in the PCOS pregnant women, being significantly higher than that of non-PCOS pregnant women (Fig. 2). The mRNA encoding *11BHSD1* ($P < 0.05$) and *11BHSD2* ($P < 0.01$), the genes related to

glucocorticoids metabolism, were more abundant in PCOS than non-PCOS women (Fig. 3). In contrast, the expression of CYP21A2, the gene involved in the mineralocorticoids metabolism, was 4-fold higher in non-PCOS group than that in the PCOS group (Fig. 4; $P < 0.001$). There were no significant differences between relative expression of *3BHSD1* and *3BHSD2* (Fig. 2). There were also no differences between the groups in the expressions of genes related to sex steroid metabolism such as the *CYP19A1*, and *17BHSD* families (1, 3, 5, 7, and 12) (Fig. 5). Expression of *17BHSD2* was undetectable in AT of both groups with the protocol used herein.

Table 3
Levels of mRNA in subcutaneous adipose tissue samples of PCOS (n = 12) relative to non-PCOS (n = 36) (ns.: non-significant)

Gene	Relative level	Fold difference	P-Value
STAR	PCOS > non-PCOS	1.5	< 0.001
CYP11A1	PCOS > non-PCOS	1.3	< 0.05
HSD11B1	PCOS > non-PCOS	1.5	< 0.05
HSD11B2	PCOS > non-PCOS	1.63	< 0.01
CYP17A1	PCOS > non-PCOS	1.2	< 0.05
CYP21A2	non-PCOS > PCOS	4	< 0.001
HSD17B1	PCOS > non-PCOS	1.03	ns.
HSD17B3	PCOS > non-PCOS	1.05	ns.
AKR1C3	non-PCOS > PCOS	2.2	ns.
HSD17B7	non-PCOS > PCOS	1.02	ns.
HSD17B12	PCOS > non-PCOS	1.09	ns.
HSD3B1	PCOS > non-PCOS	1.13	ns.
HSD3B2	PCOS > non-PCOS	2	ns.
CYP 19	PCOS > non-PCOS	1.02	ns.
HSD17B2	undetectable	-	-

4. Discussion

It was reported that AT is equipped with a steroidogenic machinery for *de novo* synthesis from cholesterol, initiated by *STAR* and *CYP11A1* [3]. Not only the total amount of steroids within AT was found to be 40–400 times greater than in plasma, but there also was a positive gradient between tissue and plasma [7]. It was suggested that both *STAR* and *CYP11A1* are rate-limiting factors for steroidogenesis as they produce crucial precursors [3, 18]. Previous studies have shown the association

between *CYP11A1* and PCOS [19]. For the first time in 2008, MacKenzie et al. identified *STAR* and *CYP11A1* gene expression in paired visceral and subcutaneous AT from the lower abdominal region were taken from eight women undergoing caesarean section [12]. Likewise, Wang et al. (2012) showed expression of *CYP11A1* in abdominal subcutaneous AT taken from non-pregnant women [7]. Although Alizadeh et al. reported that *CYP11A1* mRNA was not detectable in retroperitoneal and subcutaneous dairy cattle AT [20], they showed that the amount of *STAR* mRNA in subcutaneous AT on the day of delivery was 3-fold higher than before calving. The up-regulation of genes involved in initial steps of steriodogenes on the day of delivery in PCOS mothers indicate an increase capacity for cholesterol uptake to the inner mitochondrial membrane and steroidogenesis stimulation. These data provide evidence that PCOS women may have more functional and effective sources of steroid metabolism pathways in subcutaneous AT on the day of delivery than age- and BMI-matched non-PCOS women.

CYP17A1 is a key branch point in steroid biosynthesis, driving the pathway to the direction of either mineralocorticoid and glucocorticoid production or sex steroid metabolism [21]. Wickenheisser et al. reported that *CYP17A1* mRNA was more abundant in ovarian theca cells of PCOS than non-PCOS women [21, 22]. In the current study, *CYP17A1* was transcribed higher in subcutaneous AT of PCOS than non-PCOS women. MacKenzie et al did not detect this gene in subcutaneous AT on the day of delivery, and neither did Valle et al in non-pregnant women and men [12, 23]. However, the presence of *CYP17A1* gene in PCOS women's subcutaneous AT was reported by Wang et al in 2012. Uniquely, Kinoshita et al. using an innovative liquid chromatography–mass spectrometry (LC-MS/MS)-based method could detect CYP17A1 at the protein level [24]. A potential for up-regulation of *CYP17A1* expression in AT was proposed, which may contribute to hyperandrogenism in women suffering from PCOS [25–27]. Therefore, our data for CYP17A1 support the hypothesis [27] that confirms the overexpression of this gene that plays a crucial role in PCOS pregnant women, which may be related to the regulation of glucocorticoid as well as mineralocorticoids metabolism .

Among fourteen genes that we detected and compared among both groups, relative expression of *CYP21* was 4-fold higher in non-PCOS than in PCOS. Similarly, Azziz et al. [28] and Witchel and Aston [29] showed the deficiency of 21-hydroxylase in hirsute women with PCOS. One of the main steps in adrenal and ovarian steroidogenesis is the conversion of 17-hydroxyprogesterone into 11-deoxycortisol, which is catalyzed by the 21-hydroxylase enzyme encoded by *CYP21*. The deficiency of this enzyme, which is inherited by an autosomal recessive trait, is responsible for most cases of congenital adrenal hyperplasia and increased serum 17-hydroxyprogesterone levels are correlated with its deficiency. Furthermore, patients suffering from heterozygote *CYP21* mutations as well as clinical symptoms exhibit PCOS-like phenotype [30]. Therefore, the changes in amounts of *CYP21* mRNA support the above reasoning.

Our data confirmed the findings of previous studies that *HSD11B1* and *HSD11B2* mRNA are present in both subjects and are also more in subcutaneous AT of PCOS than non-PCOS .In the same study, 42 women with PCOS with a broad range of BMI were studied and it was shown that in these women, there was increased *HSD11B1* enzyme activity with increasing central fat distribution [31]. This could indicate impaired *HSD11B1* activity in women with PCOS compared with control.

Due to cortisol peak and critical roles of cortisol around delivery day, more attention must be paid to cortisol metabolism. Previously, the expression of *HSD11B1* in human AT, particularly in visceral fat, has been confirmed [32]. Makenzie et al suggested high expression of *HSD11B1* gene in both subcutaneous and visceral AT on the day of delivery [12]. Based on the evidence for *HSD11B1* in human AT and bovine AT from different visceral and subcutaneous depots, AT likely affects glucocorticoid metabolism with consequences for both endocrine as well as auto/paracrine glucocorticoid effects locally within AT, the latter being supported by the presence of glucocorticoid receptors, also demonstrated in bovine AT [12, 33]. In this regard, Alizadeh et al (2016 b) studied the presence of *HSD11B1* mRNA in dairy cattle AT around calving day. They showed that the highest expression of *HSD11B1* in bovine AT was shown on the day of delivery rather the days before and after calving [34]. Therefore, the elevation of cortisol metabolism around parturition is normal; but our study clearly showed 1.5-fold elevation of *HSD11B1* mRNA levels in PCOS over non-PCOS. Similarly, Corto´n et al. reported that non-pregnant PCOS women had up regulation of *HSD11B1* expression in abdominal subcutaneous AT compared to non-PCOS ones, which is consistent with our data [35]. Although the elevation of *HSD11B1* in obesity has been reported, it was surprising to note that the alarming increase in BMI-matched subject incidence of AT importance is associated with an array of metabolic pathologies, including PCOS.

The presence of *CYP19* (aromatase) mRNA in human AT has already been well established by Corbould et al [36] and Makenzi et al [12]. Although it has been proposed that PCOS may result from reduced aromatase activity and subcutaneous AT have higher expression of *Cyp19a1* compared to visceral AT in morbidly obese men and premenopausal women [37], we cannot find difference in mRNA levels of *CYP19* between PCOS and non-PCOS women. Previously, the conversion of androstenedione to estrone in subcutaneous AT from the lower body of women (i.e., thighs and buttocks) was shown to be higher than that of in the subcutaneous AT of upper body fat (i.e., breast and abdomen), along with the highest level of *Cyp19a1* gene expression [38]. Altogether, the absence of changes in *Cyp19a1* suggest that delivery day or sampling area may influence *CYP19* expression than PCOS.

Enzymes with activities associated with various *17BHSD* isoforms are widespread, not only in classic steroidogenic tissues such as the testis, ovary, and placenta but also in a large number of peripheral sites including AT [39]. The estrogenic isoforms of *17BHSD* (types 1, 7 and 12) catalyze the conversion of estrone to estradiol [3] and they are similar in PCOS and non-PCOS groups in the current study. The *17BHSD* family genes involved in the estrogenic process in AT are much fewer than those of the androgenic one, inferring that subcutaneous AT may serve as a dominant activation site for androgens than estrogens. Bellemare et al. (2009) suggested that type 12 *17BHSD* is probably involved in the local conversion of estrone into estradiol in differentiated adipocytes and it has more activity than other isoforms [40]. Uniquely, Quinkler et al showed that *17BHSD5* has pivotal roles in women androgen metabolism in subcutaneous buttock AT [11]. Therefore, we expected that *17BHSD5* and type 12 *17BHSD* may differ between experimental groups; but our findings did not support the hypotheses regarding different sex steroid related gene expression in subcutaneous AT.

It appears that sampling area (buttock AT *vs.* abdominal AT) may influence these results; but the mRNA levels of *17BHSD5* were 2-folds higher in abdominal subcutaneous AT of PCOS women compared to the non-PCOS women [7]. So, this result may affect the status on the day of delivery, as shown by Mackenzie et al. [12]. They suggested that *de novo* synthesis of sex steroids from cholesterol is not possible in pregnant women on the day of delivery because of the absence of key steroidogenic mRNAs in the AT.

Finally, the survey on PCOS mother and offspring health is an exciting and emerging area for research and clinical studies. Uniquely, Kosidou et al. [41] reported that children of mothers with PCOS have an increased risk of developing autism spectrum disorder and their findings support that early life androgen exposure may be important for the development of autism in both sexes which it was supported by Katsigianni et al [42]. These reports alongside our data suggest further studies are warranted in order to shed more light on of AT's roles in PCOS mother.

Our study has a few limitations that must be considered when interpreting our results. AT samples are not easily available. Therefore, small sample size was the limitation of this study for age- and BMI- matched PCOS women. It would have been interesting to compare expression with non-pregnant women or across gestation and additional studies with larger sample sizes could confirm these results in pregnant and non- pregnant PCOS women alongside measured several hormone levels. Other factors such as fat distribution may be related to the differences observed.

5. Conclusion

In conclusion, our data showed that in AT of pregnant women suffering from PCOS, the genes related to initial steps of steroidogenesis (*STAR* and *CYP11A1*) were more expressed compared to age- and BMI-matched non-PCOS women. The expression levels of genes related to cortisol metabolism (*11BHSD1* and *11BHSD2*) were higher in PCOS women than age- and BMI- matched non-PCOS, the profile which may be due to the regulation of glucocorticoid metabolism. This finding opens the door to further assessment of the critical roles of AT in cortisol metabolism in PCOS mothers. Moreover, dramatic decrease of *CYP21* mRNA confirms gene expression disorders in mineralocorticoid metabolism for PCOS women. Altogether, the alterations in gene expressions involved in glucocorticoids and mineralocorticoid metabolism was shown which warrants further studies regards functional activity.

Abbreviations

AT: adipose tissue; PCOS: polycystic ovary syndrome; *17BHSD:* 17β-Hydroxysteroid dehydrogenases; STAR: steroidgenic acute regulator; *CYP11A1:* cytochrome P450 monooxygenase; *CYP17A1:* 17α-hydroxylase; *11BHSD*: 11β-Hydroxysteroid dehydrogenase; *CYP21*: steroid 21-hydroxylase; *CYP19A1:* cytochrome P450 aromatase; *3BHSD*: 3β-hydroxysteroid dehydrogenase.

Declarations

Ethical Approval and Consent to participate

After obtaining permission from Royan Institute Ethics Committee, Tehran, Iran (IR.ACECR.ROYAN.REC.1398.087), samples and demographic data were collected from three hospitals in Tehran, Iran. Signed informed consent was obtained from all subjects.

Consent for publication

This manuscript did not contains any individual person's data in any form (including any individual details, images or videos).

Availability of data and material

All data and material used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NE PhD. student**,** Main project contributor, Investigation, Data analysis, Writing- Original draft preparation, Visualization. **AM** Project advisor, Methodology and participants selection, Resources, Writing - review & editing. **PY** Project advisor, Investigation, Methodology, Writing - review & editing. **VA** Methodology, Data analysis, Writing - review & editing. **MS** Projects supervisor, Methodology, Validation, Writing and Reviewing, Visualization. **AA** Projects supervisor, Project management, Validation, Writing and Reviewing, Visualization. All authors have read and approved the final manuscript.

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Figures

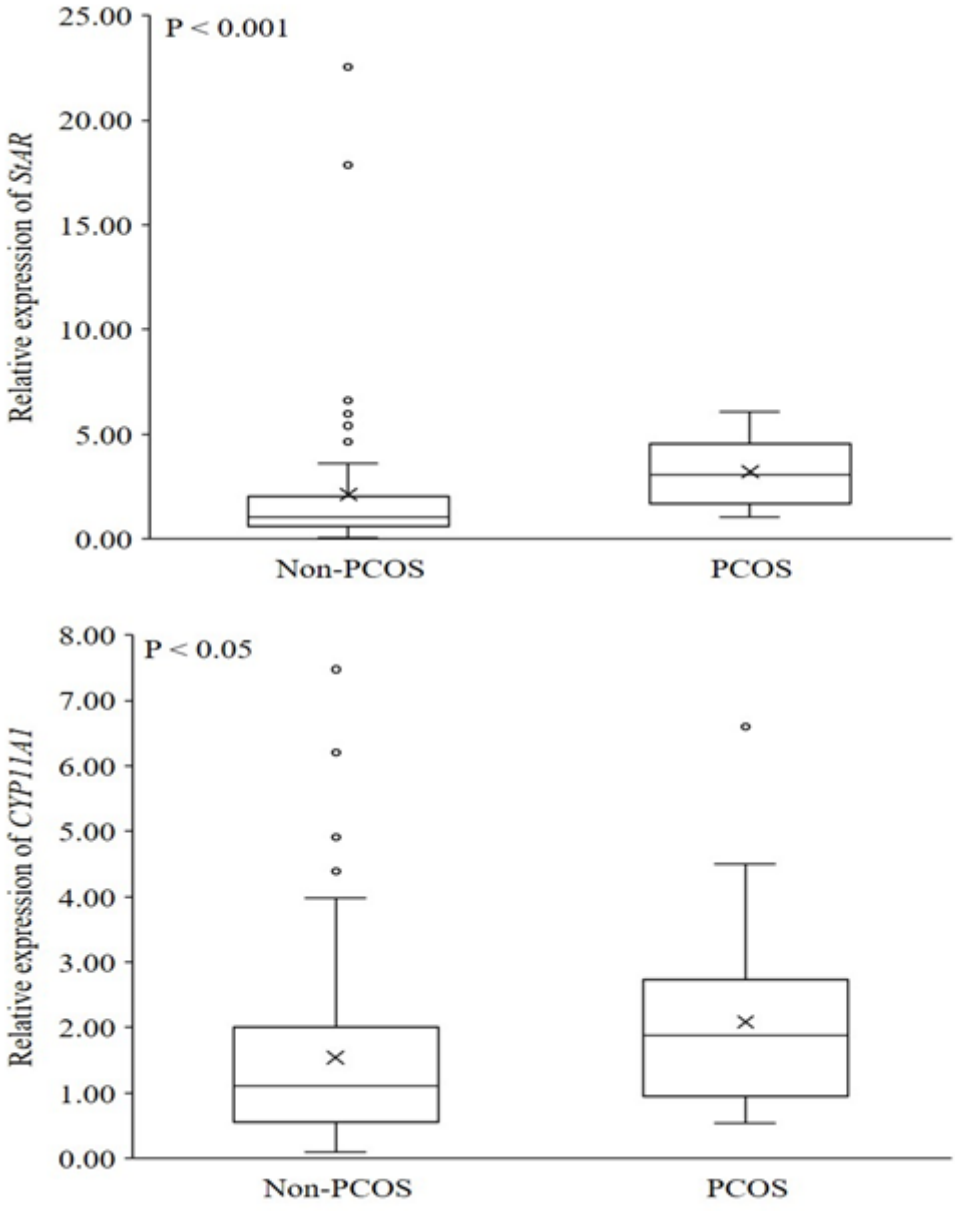

Figure 1

mRNA relative expression of the key enzymes involved in the initial steps of steroidogenesis in adipose tissue based on qRT-PCR in non-PCOS pregnant women (n=36) and PCOS (n = 12) pregnant women.

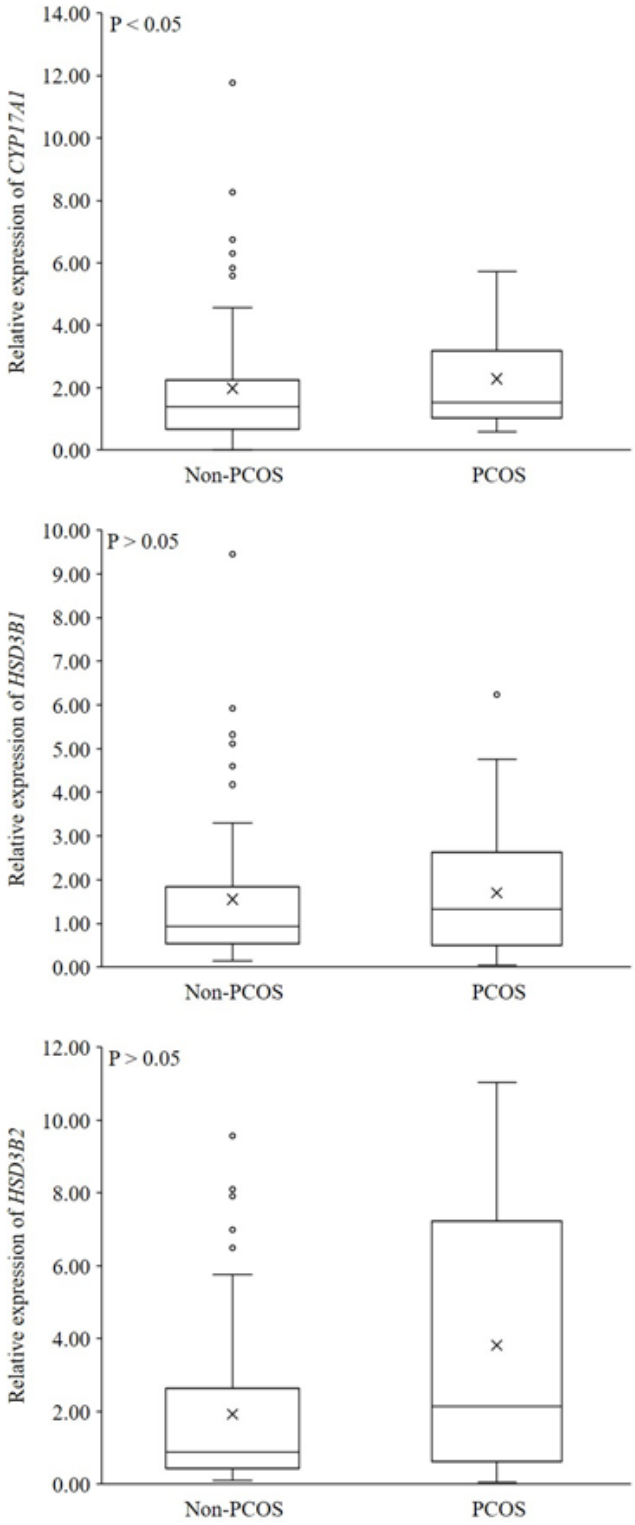

Figure 2

mRNA relative expression of CYP17, 3BHSD1 and 3BHSD2 in adipose tissue based on qRT-PCR in non-PCOS pregnant women (n=36) and PCOS (n = 12) pregnant women.

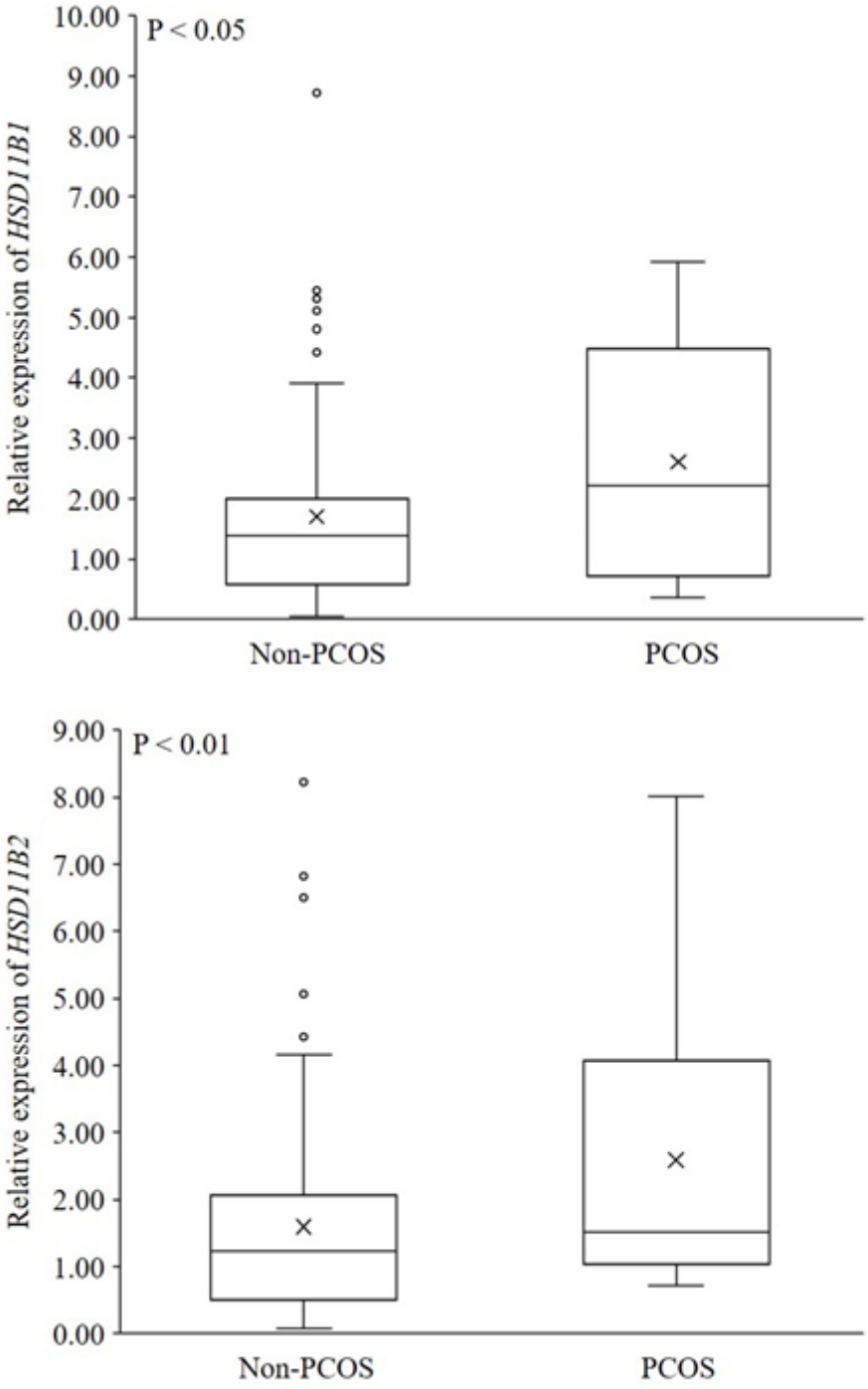

Figure 3

mRNA relative expression of 11BHSD1 and 11BHSD2 which related to glucocorticoids metabolism in adipose tissue based on qRT-PCR in non-PCOS pregnant women (n=36) and PCOS (n = 12) pregnant women.

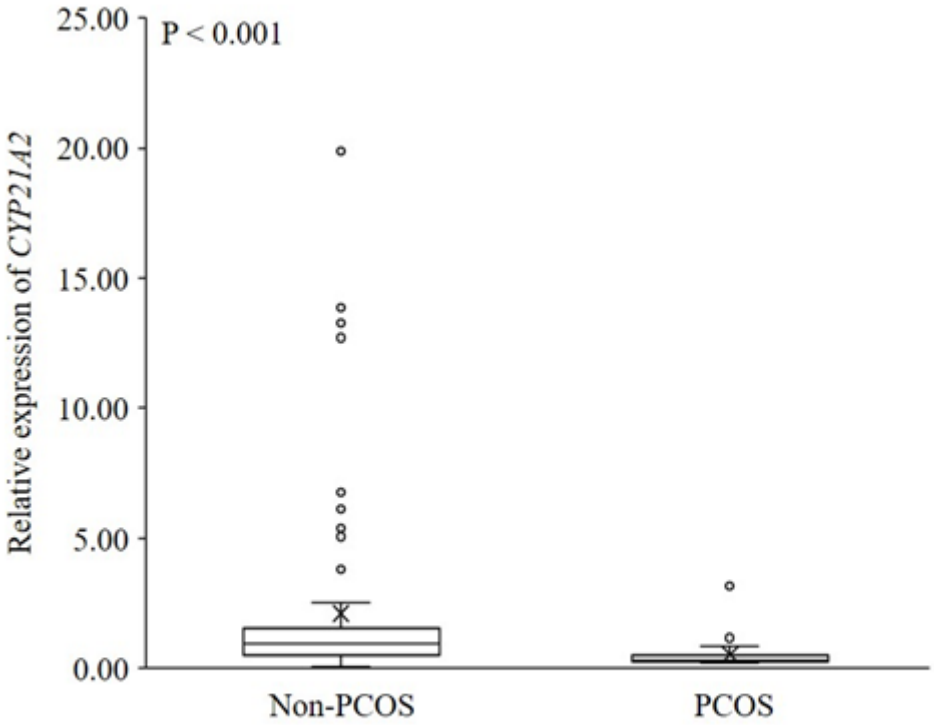

Figure 4

mRNA relative expression of CYP21 which related to mineralocorticoids metabolism in adipose tissue based on qRT-PCR in non-PCOS pregnant women (n=36) and PCOS (n = 12) pregnant women.

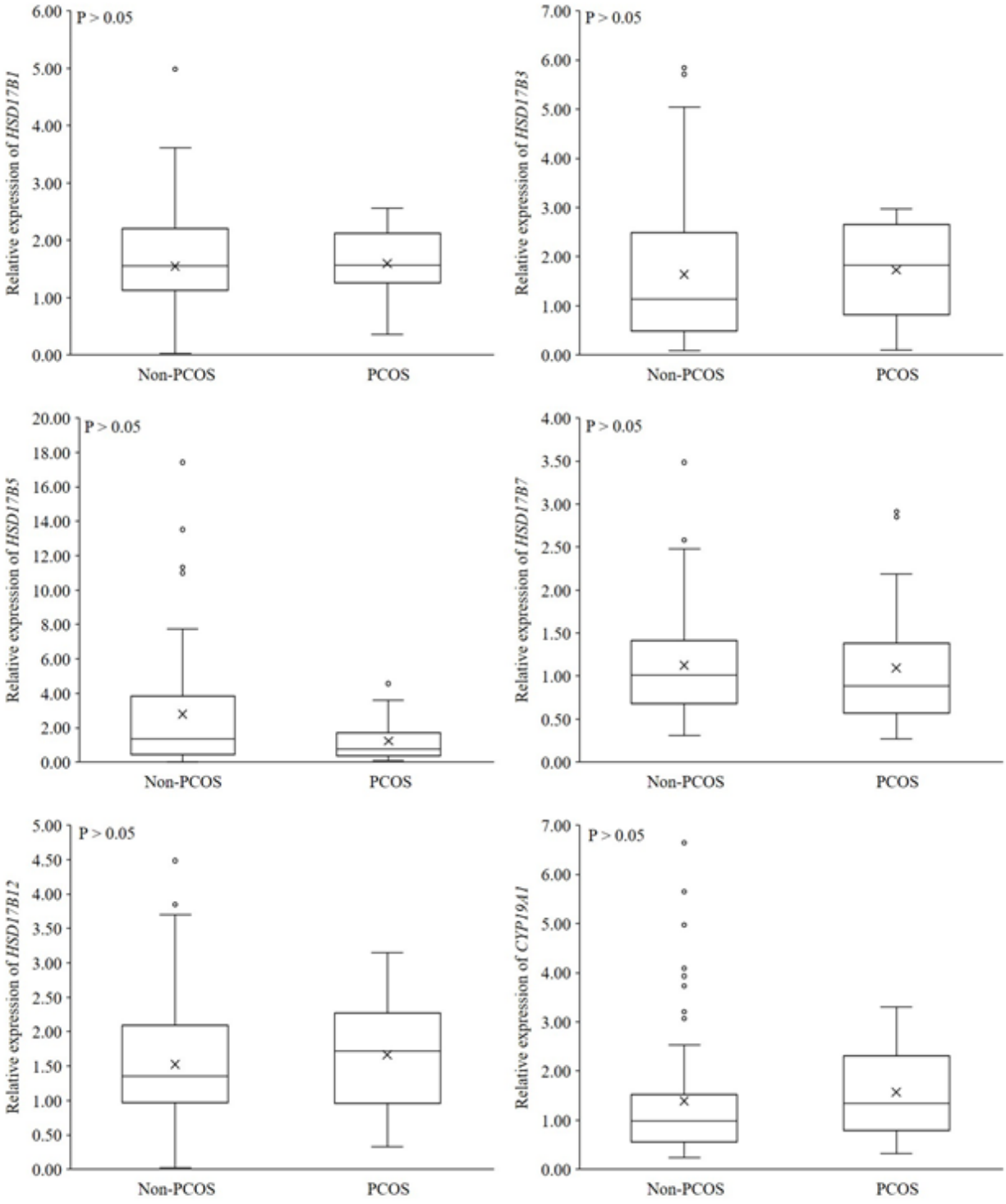

Figure 5

mRNA relative expression of genes involved in sex steroid metabolism in adipose tissue based on qRT-PCR in non-PCOS pregnant women (n=36) and PCOS (n = 12) pregnant women.