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Relationship of ovarian stromal volume to serum androgen concentrations in patients with polycystic ovary syndrome

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The aim of this study was to investigate of the relationship of ovarian stromal volume, measured using three-dimensional ultrasound, to serum androgen concentrations in patients with polycystic ovaries. Serum gonadotrophin, oestradiol and androgen concentrations and ovarian volume measurements were obtained in the early follicular phase from 100 women undergoing assisted conception treatment cycles. Group 1 contained 50 women with regular menstrual cycles and normal ovarian morphology, group 2 contained 24 women with regular menstrual cycles and polycystic ovaries seen on ultrasound scan and group 3 contained 26 women with polycystic ovary syndrome. Statistical analysis included analysis of variance, Scheffé's procedure and Pearson's correlation. Total ovarian volume (15.7–16.1 versus 11 ml, $P < 0.05$), stromal volume (14.5 versus 9.4 ml, $P < 0.05$) and thecal steroid concentrations were significantly greater in groups 2 and 3. Stromal volume was positively correlated with serum androstenedione concentrations ($r = 0.45$, $P = 0.0019$ in group 3) but was not correlated with any other endocrine parameter. It was concluded that polycystic ovaries are characterized by increased ovarian stroma with associated overproduction of theca-derived steroids, particularly androstenedione.

Key words: androgens/polycystic ovary/stromal volume

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder which has eluded definitive description because of the varied combination of clinical, biochemical and ultrasonographic features which may occur. The commonest association is of hyperandrogenism and chronic anovulation; recognition of characteristic ovarian ultrasound features together with clinical symptoms of oligomenorrhoea, hyperandrogenism, infertility or obesity is presently the preferred approach to diagnosis.

In early ultrasound studies of polycystic ovaries (PCO), the main diagnostic criterion was ovarian enlargement. Indeed, some authors regarded this as an absolute prerequisite for the condition (Parisi *et al*., 1982). The typical PCO was said to be two to five times larger than a normal ovary which has a volume of ~4–7 ml (Sample *et al*., 1977; Orsini *et al*., 1985). A mean ovarian surface area of 17 cm^2 (Parisi *et al*., 1982) and mean ovarian volume of about 12 ml (Swanson *et al*., 1981) were quoted for typical polycystic ovaries. The importance of ovarian size as an ultrasonographic diagnostic criterion of PCO lessened as various groups (Hann *et al*., 1984; Nicolini *et al*., 1985; Orsini *et al*., 1985) showed that about one-third of patients with PCOS had ovaries of normal volume.

Adams *et al*. (1985) refined the ultrasound diagnosis of PCO to include follicular number and stromal characteristics. The typical polycystic pattern was defined by the presence of $\geqslant$10 cysts measuring 2–8 mm in diameter arranged peripherally around a dense core of stroma or scattered through an increased amount of stroma. An important distinction was made from the multifollicular ovary, characteristic of normal puberty or recovering from weight-related amenorrhoea, in which no increase in the amount or echogenicity of the stroma was detected.

Recognition of stromal hyperechogenicity occurred at a time of growing interest in the physiological ovarian mechanisms regulated by the thecal interstitial cells (Erickson *et al*., 1985). Realization of their pivotal role in ovarian function has ensured that the ovarian stroma will be a focus of continued efforts to determine the pathogenic basis of PCOS since it is probably the source of the hyperandrogenaemia which is a consistent feature of this condition. Detection of stromal hyperechogenicity on ultrasound is an important criterion for diagnosis but the subjectivity of this parameter has hindered attempts to produce meaningful comparative analyses in PCOS.

The advent of sophisticated new technology in the form of computerized three-dimensional (3D) ultrasound systems such as the Combison 530 (Kretztechnik AG, Zipf, Austria) means that, for the first time, the transverse plane of the pelvis can be visualized and direct measurement of ovarian and stromal volume can be made. The present prospective study was designed to investigate the relationship between ovarian volume and serum androgen concentrations in women with PCOS. In previous studies, we have validated the technique of 3D ultrasound measurement of ovarian follicular volume and demonstrated a high degree of reproducibility (Kyei-Mensah *et al*., 1996a) and significantly increased precision compared with conventional two-dimensional (2D) ultrasound volume measurements (Kyei-Mensah *et al*., 1996b). The advantage of using a 3D ultrasound system lies in its ability to provide follicular measurements which can be subtracted from the total volume to give a more accurate estimation of stromal volume. Several investigators have carried out similar studies using conventional ultrasound but were limited to

measurement of total ovarian volume only (Puzigaca *et al*., 1991; Balen *et al*., 1995). This is because 2D ultrasound has no facility for the visualization of follicles simultaneously in three planes which is essential if duplication of follicle numbers and measurements is to be avoided. For the first time, 3D ultrasound permits the relative contributions of follicles and stroma to the total volume to be estimated and correlation with various endocrine parameters should provide a sound basis for comparative studies and may improve our understanding of the underlying pathophysiological mechanisms in PCOS.

Materials and methods

This study involved 100 women complaining of infertility who had been consecutively referred to The London Women's Clinic for treatment by assisted conception. The study was approved by the Ethical Committee of the clinic and all patients gave informed consent. The subjects were divided into three groups using clinical and ultrasonographic criteria. Group 1 consisted of a control group of 50 women with regular spontaneous menstrual cycles, no hyperandrogenism and normal ovaries on transvaginal ultrasound scan. Most of these women had suffered tubal damage or there was a male factor accounting for their infertility. Group 2 consisted of 24 women who were similar to group 1 except that on baseline transvaginal ultrasound scan they had PCO, i.e. $\geqslant$10–15 cysts of 2–10 mm diameter arranged around a dense echogenic stroma or scattered through an increased amount of stroma. This was the PCO group. Group 3 consisted of 26 women who complained of menstrual irregularity (oligomenorrhoea), had clinical features of hyperandrogenism (hirsutism or acne) and who were found to have PCO on transvaginal ultrasound. This was the PCOS group.

None of the women studied had a history of any other endocrine disorders such as diabetes mellitus, hyperprolactinaemia, thyroid dysfunction, Cushing's syndrome or androgen-secreting tumour and none had received any form of hormonal treatment in the preceding 3 months. All had undergone a pelvic ultrasound in the luteal phase of the preceding cycle to exclude significant uterine or ovarian pathology such as endometrial polyps or ovarian cysts which would preclude their commencement on an assisted conception treatment cycle. Age was not a selection criterion in this study.

Patients attended the clinic between 11:00 and 14:00 h for evaluation in the early follicular phase of the menstrual cycle (day 2–4). In those women with oligomenorrhoea, withdrawal bleeding had been induced by administration of oral medroxyprogesterone acetate (Provera®; Upjohn Ltd, West Sussex, UK) 5 mg twice daily for 7 days. Details were recorded on the presence and regularity of the menstrual cycle, and any complaints of hirsutism or acne. Height and weight were recorded for determination of the body mass index (BMI, normal range 19–25 kg/m^2).

Hormonal measurements

Blood was drawn from the antecubital vein for measurement of serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, dihydroepiandrosterone sulphate (DHEA-S), oestradiol, androstenedione and 17-hydroxyprogesterone (17-OHP) concentrations. Serum LH and FSH concentrations were determined by specific immunoradiometric assay (IRMA) using ^{125}I-labelled monoclonal antibody to LH and FSH (Omnia IRMA; IDS Ltd, Boldon, Tyne and Wear, UK). Each assay was standardized against international reference preparations (IRP); the second International Standard IS 80/552 for LH and IRP 78/549 for FSH. Serum androgen concentrations were determined by radioimmunoassay following ether extraction. In the testosterone assay, the intra-assay coefficient of variation (CV) was 4% and inter-assay CV was 11%. Cross-reactivity was 20% with dihydrotestosterone and $<$0.1% with androstenedione, dehydroepiandrosterone, oestradiol, progesterone and cortisol. 17-OHP was measured by radioimmunoassay using Guildhay 17-OHP antiserum (Ref. No. G638; Southampton University Hospitals NHS Trust, UK) the intra-assay CV was 9% and inter-assay CV was 15%. Androstenedione was measured using the DSL Active Androsterone Solid Phase RIA Kit (IDS Ltd); the intra-assay CV was 4% and inter-assay CV was 12%. Cross-reactivity was $<$0.1% with dehydroepiandrosterone, oestradiol, progesterone and cortisol. Serum oestradiol concentrations were measured by radioimmunoassay using a commercial kit (Incstar UK, Wokingham, Berks, England) the intra-assay CV was 5% and inter-assay CV was 16%.

Ultrasound investigation

All 3D ultrasound scans were obtained by A.A.K.-M. using the Combison 530 system (Kretztechnik AG, Zipf, Austria) with a Voluson transvaginal 7.5 MHz volume transducer (Kretztechnik AG). This system also provides conventional two-dimensional (2D) ultrasound. Each patient underwent systematic examination of the morphology of the uterus and ovaries as previously described. The 3D facility was engaged by switching into 'volume mode'. A mobile sector appeared and the ovary was centralized within this 'region of interest'. The patient was instructed to remain very still as the volume acquisition setting was activated. The transducer crystal then rotated through 360° for ~8 s. During this time, the resulting sections were stored sequentially in the computer memory. The scanned ovarian volume was displayed on the screen in three orthogonal planes and then stored digitally on an 88 Mbyte removable cartridge hard disc (SyQuest®; Technology Inc, Fremont, CA, USA) for subsequent analysis. The left and right ovarian volumes were scanned and stored for each patient.

In order to analyse the stored ovarian volumes, the plane which gave the clearest view of the ovarian outline was chosen for measurement and highlighted within a green box. Any contour changes were now restricted to this planar view. The ovarian contour was outlined in 10 serial sections using a rollerball cursor and the total ovarian area and volume were displayed on the screen after completion of the last slice. Individual cyst volumes were then measured using two serial sections for the smallest cysts of $<$4 mm diameter and four serial sections for those of 4–10 mm diameter. For each patient the total ovarian volume was expressed as the sum of the left and right ovarian volumes. The total stromal volume was calculated by subtracting the cyst volume from the total ovarian volume in each case. The intra-observer coefficient of variation for ovarian volume was 8% and the intraclass correlation coefficient for ovarian volume was 0.95.

Statistical analysis

All data were analysed on a personal computer using the SPSS for Windows statistical package. Data are expressed as median (range) where $n = 50$, 24 and 26 for groups 1, 2 and 3 respectively. Ovarian cyst volume, total volume, serum oestradiol and 17-OHP concentrations were not normally distributed. Logarithmic transformation of skewed data was carried out prior to parametric analysis and geometric means are presented for these variables. Analysis of variance (ANOVA) was used to demonstrate whether any differences in endocrine and ovarian parameters existed between the three groups and then Scheffé's procedure, which is a multiple comparison technique, was used to specify significant differences between each group. $P < 0.05$ was considered significant. Correlation analysis was performed using Pearson's correlation.

Table I. Aetiology of infertility in the three study groups

Cause	Group 1 Normal	Group 2 PCO	Group 3 PCOS
Number	50	24	26
Male factor	26	14	14
Tubal damage	9	2	3
Anovulation	2	0	9
Endometriosis	2	1	0
Unexplained	6	4	0
Multiple factors	5	2	0
Other causes	0	1	0

PCOS = polycystic ovaries; PCOS = polycystic ovary syndrome.

Table II. Clinical and endocrine parameters in the three study groups

	Group 1 (normal)	Group 2 (PCO)	Group 3 (PCOS)
Number	50	24	26
Age (years)	35.4 (17.7)	34.0 (19.0)	31.8 (25.5)
BMI (kg/m^2)	22.8 (16.7)	22.4 (17.7)	22.9 (15.5)
Androstenedione (nmol/l)	5.0 (10.3)	5.65 (5.9)[c]	6.85 (7.9)[bc]
Testosterone (nmol/l)	1.6 (2.1)[b]	1.65 (2.0)	2.05 (2.6)[b]
17-OHP (nmol/l)	1.2 (2.9)[b]	1.3 (1.6)	1.7 (3.8)[b]
DHEA-S (nmol/l)	4.05 (8.1)	4.45 (7.2)	3.75 (6.6)
Oestradiol (pmol/l)	118 (864.0)	117.3 (174.0)	114 (2398.0)
FSH (IU/l)	7.4 (14.2)[ab]	6.75 (3.2)[a]	6.25 (9.0)
LH (IU/l)	6.15 (9.7)[b]	7.5 (9.8)	8.5 (24.2)[b]

PCO = polycystic ovaries; PCOS = polycystic ovary syndrome; BMI = body mass index; 17-OHP = 17-hydroxyprogesterone; DHEA-S = dihydroepiandrosterone sulphate; FSH = follicle stimulating hormone; LH = luteinizing hormone.
Values are medians with range in parentheses.
[a]Group 1 versus group 2: $P < 0.05$.
[b]Group 1 versus group 3: $P < 0.05$.
[c]Group 2 versus group 3: $P < 0.05$.

Results

There were no significant differences in the median age or BMI among the three groups of women. Table I shows the aetiology of infertility in the three study groups. Male factor was the predominant cause of infertility (54%), followed by tubal disease (14%) and unexplained infertility (10%). Nine out of eleven women with anovulatory infertility were in the PCOS group and in the remaining two women, despite normal ovarian ultrasound appearances, early follicular phase serum FSH concentrations were consistently raised at 11–15 IU/l leading to ovarian resistance and anovulation. Endometriosis and male factor provided the main combined causes (4%) of infertility and there were individual cases of weight-related amenorrhoea and psychosexual problems.

On comparing the endocrine data from the three study groups, serum FSH concentrations were significantly higher in group 1 ($P = 0.0008$). Serum testosterone and LH concentrations were significantly higher in group 3 compared with group 1 ($P = 0.0018$; $P = 0.01$ respectively) but serum testosterone and LH concentrations in group 2 did not differ significantly from group 1 or 3 (Table II). Theca-derived concentrations of androstenedione and 17-OHP were found to be significantly higher in group 3 compared with group 1 ($P = 0.0006$; $P = 0.0055$, Table II). Serum androstenedione concentrations were also significantly higher in group 3 compared with group 2, although serum 17-OHP was not significantly different between the two groups. Serum oestradiol and DHEA-S concentrations did not differ significantly between the three groups.

Table III. Combined ovarian volume measurements for the three study groups

	Group 1 (normal)	Group 2 (PCO)	Group 3 (PCOS)
Number	50	24	26
Total ovarian volume (ml)	9.6 (20.4)[ab]	15.0 (18.9)[a]	16.7 (23.1)[b]
Stromal volume (ml)	8.6 (20.0)[ab]	13.4 (18.6)[a]	15.5 (20.9)[b]
Cyst volume (ml)	1.6 (5.2)	1.1 (3.0)	1.5 (6.2)

PCO = polycystic ovaries; PCOS = polycystic ovary syndrome.
Values are medians with range in parentheses.
[a]Group 1 versus group 2: $P < 0.05$.
[b]Group 1 versus group 3: $P < 0.05$.

The total ovarian volume and stromal volume were not significantly different between groups 2 and 3 (Table III). The volumes in both groups were, however, significantly greater than group 1 ($P < 0.05$). Stromal volume was positively correlated with serum androstenedione concentrations in group 3 only ($r = 0.45$, $P = 0.018$). No correlation was found between stromal volume and serum LH, 17-OHP or testosterone concentrations in any of the three groups. There was no significant difference in cyst volume among the three groups and no correlation was found between any of the ovarian parameters with age.

Discussion

Successive advances in ultrasound technology have resulted in changes of emphasis regarding the importance of total ovarian size, follicle number and stromal features in the ultrasound diagnosis of PCO. Studies investigating the ovarian stroma using conventional ultrasound systems have been hindered by the highly subjective nature of stromal assessment. The latest computerized ultrasound systems, e.g. the Combison 530, producing area and volume measurements of the ovary, can provide much-needed objective quantitative data. Stromal quantification may reduce the importance of follicular numbers in the diagnosis of PCO in the future. A recent study (Robert *et al*., 1995) involving ovarian stromal area assessment using a computerized ultrasound system in 69 patients complaining of hyperandrogenism and 48 normal ovulatory women demonstrated increased specificity of computer-assisted analysis compared with visual analysis (96% versus 84%).

In the present study, the appearance of polycystic ovaries on ultrasound was associated with significantly greater total ovarian and stromal volume compared with normal ovaries. The cyst volume, however, was similar in normal and polycystic ovaries. A typical polycystic ovary may contain >20 small follicles of 4–5 mm in the early follicular phase (Fox *et al*., 1991), whereas a normal active ovary contains fewer, but larger follicles. Even a so-called inactive ovary (in a patient with normal ovarian appearance and a raised serum FSH concentration) may contain several small follicles which subsequently fail to respond to gonadotrophic stimulation. The

total follicular volume is similar in all these groups, with the main difference being its distribution between a greater number of small follicles in PCO.

This study confirms the findings of a previous histological study (Hughesdon, 1982) which showed that ovarian enlargement in PCO is mainly caused by stromal changes.

Hughesdon (1982) studied 34 full-thickness Stein–Leventhal ovarian wedges and 30 age-matched controls and demonstrated that Stein–Leventhal ovaries have an average cross-sectional area about twice that of controls and contain double the number of primary, secondary, small tertiary and subsequently atretic follicles together with increased ovarian stroma and thickening of the tunica. The doubled count of ripening follicles is responsible for the main visible 'polycystic' effect and persistent, excessive follicle maturation and atresia increases the bulk of the ovarian stroma. Subcortical ovarian stroma develops from retrogressed theca and granulosa of atretic follicles which initially forms a sharp halo around the follicle. This disperses in the later stages of atresia and the ensuing hyperplasia further increases the ovarian stroma.

The ovarian volume data in the present study have been confirmed in similar studies using 3D ultrasound (Watkin *et al*., 1996) and conventional 2D ultrasound (Puzigaca *et al*., 1991). In patients with PCOS and enlarged ovaries, Puzigaca *et al*. also found significantly higher serum androstenedione concentrations which showed significant positive correlation with ovarian volume. In contrast to the present study, Takahashi *et al*. (1994) found a significant positive correlation between the number of small cysts and serum androstenedione concentrations in patients with PCOS. The number, size and position of small cysts on transvaginal ultrasound correlated with the histopathological findings, which were identical to those described by Hughesdon. Histological examination of these small cysts showed that ~75% of them were degenerated atretic follicles with hypertrophied and luteinized inner thecal cell layers. The same histological pattern is exaggerated in the ovarian stroma of PCO. The common factor is the evidence of thecal cell overactivity, proved directly from wedge resection biopsy (Takahashi *et al*., 1994) and indirectly from the significantly increased stromal volume in the present study. These two studies demonstrate that thecal cell overactivity can be associated with an increased cystic appearance as well as with stromal hypertrophy.

Quantitative androgen receptor immunocytochemistry studies on the primate ovary have illustrated a possible mechanism for the regulatory role of androgens in folliculogenesis. Hillier *et al*. (1997) found intense immunostaining in the granulosa cells of healthy immature follicles in the ovary of the common marmoset monkey. Atretic follicles showed light staining only and there was little or no staining in theca, stroma or oocytes. They postulated that a development-related reduction in androgen receptor numbers can 'protect' granulosa cells from the inhibitory action of androgen, thereby promoting pre-ovulatory follicular dominance in primate ovarian cycles. It is interesting to speculate on whether the pathogenetic basis of the ovulatory disturbance characteristic of PCOS is a failure of down-regulation of these granulosa cell androgen receptors, leaving the granulosa cells exposed to the potentially deleterious effects of androgens on the mature follicle.

In the present study, we found that asymptomatic PCO patients were endocrinologically indistinguishable from their normal counterparts. Nevertheless, the detection of polycystic ovaries on ultrasound in a woman complaining of infertility is still significant even if the woman has spontaneous regular menstrual cycles, with no symptoms of hyperandrogenism and none of the biochemical features classically associated with PCOS. This is because of the increased sensitivity of women with PCO to gonadotrophin therapy (Shoham *et al*., 1992) and the consequent higher risk of ovarian hyperstimulation syndrome (MacDougall *et al*., 1993).

Serum concentrations of androstenedione were significantly higher in PCOS patients compared with those with PCO or normal ovaries, and serum 17-OHP concentrations were significantly higher in PCOS patients compared with those with normal ovaries. These findings concur with those of others (Barnes *et al*., 1989) who reported excessive production of androstenedione and 17-OHP in women with PCOS after nafarelin administration. Androstenedione and 17-OHP production is catalysed by 17α-hydroxylase and C17,20 lyase respectively. The activity of these enzymes is altered by fluctuations in serum LH concentrations during the ovarian cycle and they are regulated by a single cytochrome, P450c17. The consistency of the nafarelin response has led to speculation that P450c17 dysregulation is the 'cause' of PCOS (Rosenfield *et al*., 1990). The characteristic increase in serum testosterone and LH concentrations (Conway *et al*., 1989) and reduction in serum FSH concentrations (Yen *et al*., 1970; Holte *et al*., 1994) in PCOS was replicated in the present study, although the serum testosterone and LH concentrations in asymptomatic PCO patients were intermediate and did not differ significantly from normal or PCOS patients. This suggests that PCOS represents the end of a spectrum which includes stromal hypertrophy and androgen overproduction within the ovary. It is consistent with the concept of PCOS as primarily an ovarian disorder.

Androstenedione is particularly suitable as a marker of ovarian androgen synthesis since it is not protein-bound, and is therefore not affected by changes in sex-hormone binding globulin (SHBG) levels. Blood samples were obtained within a limited 4 h range in the late morning to early afternoon in order to minimize the effect of diurnal variation. At this time, the adrenal contribution to the overall serum A concentration was reduced. In the present study, stromal volume showed significant positive correlation with serum A concentrations, particularly in the PCOS group ($r = 0.45$, $P = 0.019$), but not with serum 17-OHP concentrations. Similar observations have been made by other investigators who found positive correlation of androstenedione with stromal area in a group of hyperandrogenic patients ($r = 0.47$, $P < 0.005$) and also a significant correlation with 17-OHP (Dewailly *et al*., 1994). Balen *et al*. (1995) investigated 1741 patients with PCOS and demonstrated a positive correlation between ovarian volume and serum concentrations of LH and testosterone. This was not confirmed by Dewailly *et al*. (1994) or the present study.

The ultrasound findings in the present study are consistent

with the observations of investigators who have used colour and pulsed Doppler ultrasound to investigate blood flow in the early follicular phase in the stroma of women with normal and polycystic ovaries undergoing in-vitro fertilization–embryo transfer cycles (Zaidi *et al*., 1995). Ovarian stromal blood flow velocity (V_{max}), was significantly greater in patients with PCO and PCOS compared with normal patients (16.88 versus 8.74 cm/s). Taken together, these studies build a useful morphological picture of the polycystic ovary on which to base possible theories of causation. In PCO, hypertrophy of the stroma is associated with increased stromal blood flow velocity. The latter may be caused by neovascularization or the activation of vasoactive factors which may in turn influence androgen synthesis within the ovary. Growth factors such as insulin-like growth factor-1, vascular endothelial growth factor and insulin are all possible candidates for a regulatory role in this fascinating condition. Further studies are needed to clarify their contribution to the pathogenetic process. The relationship of insulin and SHBG to stromal volume could be explored in a similar way to the present study.

In conclusion, women with PCO have significantly larger ovaries because of increased stromal volume. In PCOS, the serum androgen concentrations resulting from cytochrome P450c17-regulated enzyme activity in the theca are significantly increased, and serum androstenedione concentrations show positive correlation with stromal volume. 3D ultrasound has facilitated a more objective and approach to ovarian stromal assessment and should increase our ability to examine important structure–function relationships in the ovary.

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