The Polycystic Ovary Syndrome a comparison of the presentation in adolescents compared to women aged 35 years and older attending the Gynaecological Endocrine clinic at Groote Schuur Hospital.

A dissertation submitted in part fulfilment of the requirements for the degree MMed (O&G).

Candice Morrison

Department of Obstetrics and Gynaecology

University of Cape Town

Supervisor: Professor Z M van der Spuy

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DECLARATION BY APPLICANT

I, Candice Morrison, declare that the work contained in this dissertation is my original work and work by others has been acknowledged as such.

The study was carried out while a registrar in the Department of Obstetrics and Gynaecology at the University of Cape Town as required for the MMed (O&G).

Name of the Applicant: Candice Jane Morrison

Signature of Applicant:

Date: 11/03/2015

DECLARATION BY SUPERVISOR

I have supervised the research which Dr CJ Morrison has undertaken and the presentation of this dissertation.

I am satisfied that this is Dr Morrison's original work and that this dissertation should be submitted as part of the requirements for the MMed (O&G)

Supervisor: Professor Zephne M van der Spuy

Signature:

Date: 11/03/2015

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Abstract

A comparison of the presentation of the polycystic ovary syndrome (PCOS) in adolescents compared to women aged 35 and older at Groote Schuur Hospital

INTRODUCTION: PCOS is the commonest endocrinopathy occurring in women of reproductive age. This study aimed at comparing the presentation of adolescents to that of women ≥ 35 years presenting to the gynaecological endocrine clinic with a diagnosis of PCOS.

METHODS: This was a descriptive cohort study. Since 1996 all women with PCOS have their clinical, metabolic and endocrine data entered into a database. We compared the initial presentation of adolescents and women aged 35 and above.

RESULTS: A total of 1549 patients were included in our database. Of these 146 patients were ≥35 years at initial presentation and 186 were adolescents. At presentation, menstrual dysfunction and acne was more common in adolescents than the older women ($p<0.0001$). Hirsutism was a common clinical complaint both among adolescents (79%) and older women (77%) (NSS). Among the older women 55% (n=67) who had attempted to conceive complained of infertility. The study population was mainly overweight or obese with only 67 adolescents and 10 adults having a BMI in the normal or underweight range. This tended to worsen with increasing age and waist hip ratio was >0.85 in 71% of adults and 46% of adolescents ($p<0.0001$). There was no significant difference in serum testosterone levels and the free androgen index between the two groups. The majority of women had some indication of biochemical hyperandrogenism. Most women in both groups had evidence of insulin resistance. Acanthosis nigricans was more common in the adult women (68%) ($p <0.026$). The glucose-insulin ratio was similar in the two groups. The majority of our patients have at least one lipid abnormality and this was more pronounced in the older women ($P < 0.0001$). The criteria for the diagnosis of the metabolic syndrome was fulfilled in 31 of the adults and 12 of the adolescents.

CONCLUSION: Our study demonstrates that even young women presenting with PCOS have metabolic dysfunction. This progresses over the years and the metabolic disorders were more pronounced in the older women. Early diagnosis and appropriate treatment of PCOS in adolescence may well improve the prognosis in terms of long term health.

LIST OF ABBREVIATIONS

17 α OHP	17-α-Hydroxy progesterone
ASRM	American Society for Reproductive Medicine
BMI	Body mass index
DHEAS	Dehydroepiandrosterone sulphate
ESHRE	European Society for Human Reproduction and Embryology
FAI	Free androgen index
FSH	Follicle stimulating hormone
GSH	Groote Schuur Hospital
HDL	High density lipoprotein
HOMA	Homeostatic model assessment of insulin resistance
IGF-1	Insulin-like growth factor 1
IGT	Impaired glucose tolerance
LDL	Low density lipoprotein
LH	Luteinizing hormone
NIDDM	Non- insulin dependent diabetes mellitus
NIH	National Institute of Health
OGTT	Oral glucose tolerance test
PCO	Polycystic ovary
PCOS	Polycystic ovary syndrome
SHBG	Sex hormone binding globulin
TG	Triglyceride
WHO	World Health Organisation
COC	Combined oral contraception

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Normal values and reference ranges in women:

BMI	19-25kg m^2
Waist circumference	68-80cm
WHR	<0.80
BP	≤120/80
SHBG	18-144nmol/L
DHEAS	Adolescents: 1.8-10umol/L
	Adult women: 1.7-9.25umol/L
17α OHP	0.7-14.2 nmol/L
Fasting Glucose	<6.1 mmol/L
Insulin	5-15 umol/ml
HOMA	<2.5
Total Cholesterol	< 5.2 mmol/L
HDL-C	>1.6 mmol/L
LDL-C	2.6-3.3 mmol/L
Triglycerides	<1.7 mmol/L

Chapter 1: Introduction and Literature review:

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy in women of reproductive age, affecting 6-10% of women in the general population.[1] PCOS often clusters in families which suggests a genetic basis to the syndrome.

The diagnosis of PCOS is made using a combination of clinical, ultrasonographic and biochemical criteria. The 1990 National Institute of Health Conference on PCOS recommended that the criteria include evidence of hyperandrogenism and ovarian dysfunction but no assessment of ovarian morphology was included.[2]

The 2003 ESHRE/ASRM Rotterdam consensus meeting redefined the diagnostic criteria for PCOS to include the presence of two out of three of the following criteria:

1. Oligo-and/or anovulation
2. Hyperandrogenism (clinical and/or biochemical)
3. Polycystic ovaries on ultrasound

Other etiologies for androgen excess including congenital adrenal hyperplasia, androgen- secreting tumours and Cushing's syndrome must be excluded before the diagnosis of PCOS is made.[2]

In 2009 the Androgen Excess Society suggested a new definition that considers PCOS primarily as a disorder of clinical and/or biochemical androgen excess plus either chronic oligo-anovulation and/or polycystic ovaries. This excludes women who do not have signs of androgen excess.[3]At present most units are using the Rotterdam criteria for diagnosis of PCOS and not the Androgen Excess Society definition. It was important that a consensus was reached with regard to the diagnosis of PCOS as this impacts clinical trials and audits.

PCOS is associated with multiple metabolic abnormalities including an increased risk of developing impaired glucose tolerance (IGT), type 2 diabetes and gestational diabetes. The prevalence of IGT and type 2 diabetes is substantially higher in women with PCOS when compared to age and weight matched women without PCOS. The conversion of IGT to type 2 diabetes is accelerated in PCOS.[4]

There is a close association between obesity and PCOS. Obesity amplifies insulin resistance and exacerbates the development of impaired glucose tolerance and type 2 diabetes. [5]

Women with PCOS, both lean women and those with elevated BMIs, are found to have an increased incidence of insulin resistance and compensatory hyperinsulinaemia.[3] Insulin acts through multiple sites to increase endogenous androgen levels. Increased peripheral insulin resistance results in a higher insulin concentration. Excess insulin binds to the IGF-1 receptors which enhances the theca cells androgen production in response to LH stimulation. Hyperinsulinaemia also decreases the synthesis of SHBG by the liver with a resultant increase in serum free testosterone concentration and increased bio-availability of testosterone. [3]

Features of insulin resistance are common in women with PCOS. Hyperinsulinaemia is thought to contribute directly to reproductive dysfunction in PCOS.[5]

Women with PCOS have increased risk factors for cardiovascular disease including increased body mass index, central adipose deposition, raised insulin and triglyceride levels, decreased HDL and increased total cholesterol and fasting LDL.[16] These risk factors are often already present at an early age in PCOS and these women should be monitored for early detection and appropriate intervention to prevent cardiovascular disease. [7]

PCO also increases risk factors for endometrial cancer through chronic anovulation and unopposed oestrogen stimulation of the endometrium.[8]

PCOS is associated with menstrual abnormalities and is the most common cause of anovulatory infertility. These women have reduced ovulation rates and thus decreased pregnancy rates and may require treatment for infertility. [9] When pregnancy does occur it is often associated with higher incidence of adverse outcomes including gestational diabetes, fetal macrosomia, gestational hypertensive disorders (such as pre-eclampsia and pregnancy induced hypertension), and birth of small-for-gestational age babies.[9]

PCOS has a negative impact on the health-related quality of life (HRQoL) in women with the condition. Patients with PCOS are at higher risk for developing psychological difficulties such as depression and anxiety and body dissatisfaction. In addition they are at risk for developing eating disorders and sexual and relationship dysfunction. [10]

Early diagnosis of the syndrome and close long-term follow-up and screening for diabetes and cardiovascular disease are important. There is an opportunity for preventative therapy, which should improve the reproductive, metabolic, and cardiovascular outcomes.[11]

It is recognised that adolescents who are diagnosed with PCOS may not yet have developed the full spectrum of the disorder and it may be important to assess them differently to the older woman with PCOS.

Ultrasound assessment:

Ultrasonography has widened the clinical spectrum of PCOS by its sensitivity and it is important to assess the ultrasound features of the ovary in all clinical presentations of PCOS together with the appropriate endocrine, biochemical and metabolic tests.

Pelvic ultrasound scan should be performed by appropriately trained personnel and state of the art equipment is required. Transvaginal approach should be performed where possible and especially in obese patients unless the patient declines or in girls or women who are virgo intacta. Women with a regular menstrual cycle should be scanned in the follicular phase (days 3-5). Women with oligo-/amenorrhoeic should be scanned either at random or day 3-5 after a progesterone-induced bleed. If there is evidence of a dominant follicle > 10mm or a corpus luteum, the scan should be repeated in the next cycle. Calculation of the ovarian volume is performed using the simplified formula for an ellipsoid (0.5 x length x width x thickness). Follicle numbers should be estimated in both longitudinal, transverse and anterior- posterior cross section of the ovaries.[12]

The PCO should have at least one of the following: either 12 or more follicles measuring 2-9mm in diameter or increased ovarian volume >10cm ^{3} .Only one ovary fitting this definition is required to define the PCO. This definition does not apply to women taking the oral contraceptive pill as ovary size is reduced.

In our study the database had been reviewed and all patients fulfilled the Rotterdam criteria and most patients would have had polycystic ovaries and the few that didn’t had chemical or clinical hyperandrogenism and menstrual irregularities.

PCOS in adolescents compared with adult presentation:

Adolescence according to the WHO definition includes young women between the ages of 10 and 19 years. Diagnosis of PCOS is more difficult in adolescents as the manifestations of chronic anovulation and hyperandrogenism as defined for diagnosis in adult women are not as easily applied in the younger age group.[2] As a result PCOS may be underdiagnosed in the adolescent population.[11]

Appropriate screening in adolescents includes a thorough family history related to PCOS and associated conditions, such as diabetes and hypertension as well as including details of menstrual irregularities, infertility, hirsuitism, alopecia, cardiovascular disease and PCOS in female relatives. A careful physical examination is central to diagnosis and signs of clinical hyperandrogenism and insulin resistance should be documented.

Clinical markers of hyperandrogenism are present in two thirds of adolescents with PCOS.[13,14] These include hirsutism which is defined as terminal, coarse, dark hair on the face, back, abdomen, chest and inner thighs (scored using the Ferriman-Gallwey scoring system), acne (which is commonly seen during the adolescent years) or alopecia. In many adolescents the more convincing signs and symptoms of androgen excess often may not have yet developed at the time of initial presentation.[14,15]

The third Rotterdam criterium for diagnosis of polycystic ovaries on ultrasound is less applicable in adolescence as transabdominal scans are often performed rather than transvaginal scans and the resolution may be suboptimal, especially if the patient is obese. “Multicystic” ovarian morphology is often a feature of puberty and represents recruitment of multiple follicles without the selection and development of a dominant follicle and subsides with the onset of regular menstrual cycles and should not be confused with features of polycystic ovaries.[15] It is recognised that while transabdominal scans have limitations, the presence of large volume ovaries (10ml or greater) regardless of the resolution may, together with the clinical presentation, contribute to the Rotterdam diagnostic criteria.

Early diagnosis and intervention in young women potentially may prevent many of the long-term health consequences associated with PCOS.[13,16]

Obesity and PCOS:

Obesity has serious health implications and is associated with increased risk of psychosocial impairment, Type 2 diabetes, cardiovascular disease and uterine cancer.[16,17] Obesity is classified using body mass index (BMI) where a BMI > 25 is overweight and a BMI> 30 is obese. Another practical tool is waist –hip ratio and a ratio >0.85 in women indicates central adipose deposition and an increased risk for cardiovascular disease.

There is a close association between obesity and PCOS and a history of weight gain frequently precedes the onset of hyperandrogenism.[18] Fifty percent or more of adult women with PCOS have a body mass index over 30 (this varies between populations). Visceral or central fat is associated with a more adverse metabolic risk profile than subcutaneous fat and the prevalence of metabolic syndrome increases with obesity.

Obesity worsens the clinical manifestations of PCOS by increasing insulin resistance, decreasing the production of SHBG in the liver which impacts the bioavailability of testosterone and therefore potentiates the androgen manifestations of the syndrome. [18] Given the current epidemic of childhood obesity, early and accurate diagnosis of PCOS is vital as obesity increases the chances of associated metabolic and cardiovascular morbidities.[19]

The fat distribution in PCOS tends to be visceral or central even in lean patients and this is associated with greater insulin resistance which exacerbates the metabolic abnormalities of PCOS and increases the risk of developing Type 2 diabetes and the risk of cardiovascular disease. [21]

Lean PCOS patients may present with a different biochemical phenotype, with more androgen excess and less insulin resistance than obese PCOS patients. [22] In a study done at Columbia University (USA) in 2003 comparing 48 adolescents divided into three groups including 11 non obese PCOS adolescents, 22 obese adolescents with PCOS and 15 obese controls it was found that there is a more pronounced alteration in the hypothalamic-pituitary-adrenal axis in non-obese adolescents with PCOS and a more marked disregulation of insulin levels and impairment of insulin sensitivity in

their obese counterparts. Non obese adolescents had higher levels of LH, SHBG, DHEAS, androstenedione, dihydrotestosterone, free IGF-I and HDL and lower levels of LDL whereas obese PCOS adolescents had higher levels of fasting insulin and proinsulin and fasting glucose to insulin ratio.[22]

Insulin Resistance and PCOS:

About one third of overweight or obese women with PCOS have either impaired glucose tolerance or type 2 diabetes by the age of thirty.[23]In 2007 the Androgen Excess Society position statement recommended that all patients with PCOS should be screened for IGT with a two hour oral glucose tolerance test and those with IGT should be screened annually for development of type 2 diabetes.[24] Family history of diabetes, increased BMI, and a history of GDM is associated with an increased risk of glucose intolerance in women with PCOS.[4]

Hyperinsulinaemia plays a role in the hyperandrogenism of PCOS by stimulating androgen production in the theca cells of the ovary and indirectly by suppressing hepatic synthesis of sex hormone binding globulin (SHBG) which leads to increased tissue availability of circulating testosterone.[25]

There is a high frequency of IGT and NIDDM among women with PCOS and insulin resistance is a risk factor for type 2 diabetes. In a study done in Adelaide (Australia) in 2001 assessing 67 women with PCOS there was shown to be a high incidence of adverse change in glucose metabolism and over a period of 6.2 years 54% of women with IGT at baseline developed NIDDM.[26]

Clinical surrogates for insulin resistance include truncal obesity, skin tags and acanthosis nigricans which is a velvety rash, on the back of the neck, axilla and groin and is a cutaneous manifestation of insulin resistance.[20]

Metabolic syndrome and PCOS:

Adolescent girls with PCOS have a 4.5 times increased risk of developing metabolic syndrome compared with the general adolescent population.[3,25] One third of adolescents with PCOS meet criteria for metabolic syndrome. [19] Metabolic syndrome is associated with insulin resistance, glucose intolerance, dyslipidaemia, hypertension and central obesity. [25] In a study done in the USA at Yale University by Weiss and his co-workers reviewing the metabolic syndrome in adolescents the prevalence of metabolic syndrome was high amongst obese children and adolescents and increased with the severity of obesity reaching close to 50% in severely obese adolescents. [19,26]

Those PCOS phenotypes with the combination of hyperandrogenism and oligomenorrhea are most at risk for developing metabolic syndrome. [17] It is important that once the diagnosis of PCOS has been made in an adolescent girl, she should be followed up long term and regularly screened for metabolic abnormalities.[19]

Table 1: Criteria for the diagnosis of metabolic syndrome in adult women with PCOS requires 3 out of 5 criteria.[20]

Risk Factor	**Cut Off**
1. Abdominal obesity (waist circumference)	>88cm
2. Triglycerides	> 1.695mmol/L
3. HDL-C	< 1.295mmol/L
4. Blood pressure	≥130/≥85 mm Hg
5. Fasting and 2 hour glucose from OGTT	Fasting:6.105-6.993mmol/L 2 hour glucose: 7.77-11.0445mmol/L

The diagnostic criteria for metabolic syndrome in adolescents were modified from the adult criteria by lowering the TG and HDL cutoff points and using the 90th percentile for age, gender, and height for blood pressure as cutoff points.[25] This takes into account the fact that many of the abnormalities worsen with age and the adolescent may therefore initially be less affected than her older counterpart.

Table 2: The recommended diagnostic criteria for metabolic syndrome in adolescents: three out of five of these criteria must be present.[26]

Risk Factor	Cut-off
1. Waist circumference	>90th percentile for age and sex
2. Triglycerides	>1.24mmol/L
3. HDL-C	<1.036 mmol/L
4. Blood Pressure	>90th percentile of age and sex
5. Fasting blood glucose	>5.55 mmol/L

Hyperandrogenaemia, in addition to obesity and insulin resistance, is an important risk factor for metabolic syndrome and it is thought that early identification of adolescent girls with PCOS and metabolic syndrome may allow early intervention to decrease the metabolic effects such as the risk of developing diabetes and cardiovascular disease.[25] Levels of LDL cholesterol and triglycerides have been shown to be higher and HDL lower in adolescents with PCOS compared with unaffected adolescents and when left untreated the abnormalities continued to worsen.[7]

Hyperandrogenism in adolescents with PCOS:

Hyperandrogenism is seen clinically as acne, hirsuitism, male pattern balding, diffuse alopecia, seborrhoea or hyperhidrosis.[14] Hirsuitism is seen in two thirds of adolescents with PCOS and is graded with the Ferriman Gallwey scoring system with a score of 8 or more indicating hirsuitism.[52] The hirsuitism of PCOS is often

slowly progressive and adolescents may not yet have developed clinically significant symptoms.[3]

The history of hirsuitism should include the timing, location, and rate of progression and recent changes in the amount of hair. It is also important to ask about methods used to remove unwanted hair including shaving, waxing, bleaching, electrolysis and laser hair removal and how this changes after treatment is initiated. Although testosterone is the biochemical marker that is frequently used to define hyperandrogenism it is recognised that this is not an accurate reflection of bioavailable androgens.[14] The free androgen index (which is calculated) as testosterone (nmol/l) × 100 ÷ SHBG (nmol/l) gives a better indication of bioavailability.

Menstrual Disturbances:

Menstrual dysfunction is seen in two thirds of adolescents with PCOS and includes primary/secondary amenorrhoea, oligomenorrhoea, dysfunctional uterine bleeding and anovulatory regular menses.[3] Menstrual irregularities are common in unaffected women in the years following menarche and up to 85% of menstrual cycles are anovulatory in the first year following menarche and 59% are still anovulatory three years after the onset of menarche. [17]

Quality of life in adolescents with PCOS:

Symptoms of PCOS develop around menarche including acne, weight gain, hirsuitism and menstrual problems and have been shown to have a negative impact on quality of life of affected adolescents in a health related quality of life study. Adolescents with PCOS often have low self-esteem, self-consciousness, poor body

image and struggle with weight control. Anorexia, depression and anxiety in adolescents has been linked to PCOS.[28]

Treatment:

Untreated PCOS is a progressive syndrome and there is a need for early diagnosis, intervention and treatment including lifestyle modification and weight loss.[27]Treatment is aimed at the underlying pathophysiology and the presenting symptoms of the patient.[3]

Goals of treatment include management of irregular menses, protection of the endometrium from unopposed oestrogen stimulation (and development of endometrial hyperplasia and endometrial), decrease in hirsuitism and acne, decrease in the risk of developing diabetes mellitus or dyslipidaemia and improved quality of life.[29]

1. **Life style modifications:**

Even modest weight loss of 5-10% of body mass has been shown to improve the impact of PCOS and improve menstrual function .[30] A serious attempt at weight loss is first line treatment for overweight or obese adolescents with PCOS. Lifestyle modifications with weight loss and exercise have been shown to decrease the incidence of diabetes even more effectively than when using metformin therapy.[31]

Weight loss decreases the circulating androgen levels and decreases serum testosterone and increases SHBG and, as a consequence, decreases free androgen index.[34]

2. Oral Contraceptive Agents:

The oestrogen in the COC suppresses LH and thus ovarian androgen production and increases hepatic production of SHBG. This results in less free testosterone.[2]

Usually a combined oral contraceptive that provides 30 to 35 micrograms of ethinyl estradiol is used cyclically, is tricycled or is given continuously.[28]

3. Progestins:

Cyclical progestins can be used to provide regular menses and endometrial protection but they do not treat the insulin resistance or the androgen excess effectively.[2]

4. Anti-androgens:

A range of antiandrogens can be used to control hair growth and are often used with COC's. Spironolactone, an aldosterone antagonist, is used in USA for its antiandrogen properties. In a Cochrane review it was shown to improve hirsuitism and decrease the Ferriman-Gallwey score.[29] Spironolactone is usually used in combination with a combined oral contraceptive preparation. A Cochrane review in 2003 comparing the effectiveness of cyproterone acetate with or without ethinyl estradiol with other anti-androgens found that cyproterone acetate when combined with estradiol caused a subjective improvement in hirsuitism and has similar beneficial effects to other anti-androgenic agents.[30]

5.Insulin Sensitizing Agents:

Treatment with insulin sensitizing agents such as metformin in adolescents and adult women improves ovulatory dysfunction, hyperandrogenemia, insulin resistance and possibly hirsuitism as well as decreasing total cholesterol, triglycerides and LDL cholesterol.[30]

PCOS remains a challenge and requires management throughout reproductive life. A major impact on long term health is seen in older women and in theory if management is started in adolescence it may prevent the long term sequelae associated with PCOS.

Objectives:

The purpose of this study is to compare adolescents with PCOS with women aged 35 years and upwards who presented for the first time to the Gynaecological Endocrine clinic at Groote Schuur Hospital with PCOS and to compare the presentation, metabolic and endocrine profiles of the two groups.

Chapter 2: Methodology and study design:

This is a descriptive cohort study. The study population were patients who presented to the Gynaecological Endocrine Clinic with a diagnosis of PCOS as defined by the Rotterdam 2003 criteria and entered into our database, with their consent.

Inclusion criteria:

- Consent to be included in the PCOS database at first presentation.
- Any adolescent women presenting to the Gynaecological Endocrine Clinic with a diagnosis of PCOS.
- Women who presented at 35 years of age and older with a diagnosis of PCOS.

Exclusion criteria:

- Any women in our clinical service who declined to have their information included in the PCOS database.

Recruitment and enrolment:

Patients have prospectively been entered into the PCOS database since 1996 and their data were reviewed. Of the 1549 patients recorded in the database to date, 183 are adolescents and 146 presented at 35 years or older. At presentation to our clinic a detailed history was taken, which included enquiry into menstrual patterns, reproductive function, signs of clinical hyperandrogenism (hirsuitism and acne), personal and family medical history, the use of any medication, contraceptive use and past treatments for PCOS.

All subjects underwent a physical examination including, weight, height, waist and hip circumference, assessment for clinical features of hyperandrogenism, hirsuitism and hyperinsulinaemia (acanthosis nigricans). Blood for endocrine and metabolic assessment was taken after an overnight fast.

Information entered into the database includes:

- Age
- Ethnic Group
- Height and weight and BMI (kg/m2)
- Waist-Hip ratio
- Menstrual history
- Hirsuitism and acne
- Fertility problems
- Presence of acanthosis nigricans
- Blood pressure (mmHg) the mean of three readings taken in recumbent position 5 minutes apart

The database includes the results of fasting blood samples:

- FSH, LH, Estradiol
- Testosterone, DHEAS, 17α OHP
- SHBG
- Glucose, Insulin
- Lipid profile including cholesterol, triglycerides, HDL and LDL
- FAI calculated
- Glucose:insulin ratio calculated
- HOMA (calculated using the following formula: HOMA= [Insulin (uU/ml) × Glucose (mmol/L)] ÷ 22.5.

The endocrine profile was not assessed in women using hormonal treatment at the time of assessment. If this was considered essential, hormone therapy was stopped for 4 weeks in the case of the COC and longer after injectable progestogens before assessment. The reference ranges for testosterone and FAI changed in July 2010 and because of this testosterone was recorded either as raised or not raised rather than giving a value.

Data Safety and monitoring:

The data is anonymized and strict confidentiality is maintained and only the research team has access to the database.

Data Analysis:

Clinical data recorded from the clerking sheets by members of the Reproductive Medicine Unit were captured using Microsoft Excel and was entered into our PCOS database by members of our research team. For this subanalysis data was extrapolated for adolescents and women 35 years and older.

Statistical analysis was performed with assistance from Katya Mauff a statistician from the department of Statistical Sciences at the University of Cape Town.

Informed consent:

Informed consent or assent in underage women was taken when the patients presented to the Gynaecology Endocrine Clinic. Subsequently the Human Research Ethics Committee has given us permission to maintain the database, provided patients are informed about the addition of new data.

Research Ethics Committee Approval:

Approval to undertake this sub analysis was granted by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. **(HREC REF 085/1996 & 068/2009)**

Chapter 3: Results

Summary demographics for study population:

From 1996 until 2013, a total of 1549 patients were included in the PCOS database of these 146 patients were 35 years or older and 183 were between the ages of 10 and 19 years old. The clinical data was collected and captured using Microsoft excel. The mean age of the older women was 37± 3.0 (35-46) and of the adolescents was 17 ± 2.0 (12-19). The majority of the patients were of coloured ethnicity 86% of the adolescents (n=161) and 84% (n=122) of the older women were from this ethnic group.

BMI:

Many of the study population was overweight and the majority had an increased BMI but there was a statistically significant difference in BMI between the two groups. The older women were more likely to be overweight or obese the mean BMI being 35.7± 7.5 (19.9-53.6) and BMI for the adolescent group was 29.4± 8(16.4-60.9). Only 6.8% (n=10) of the older women had a normal BMI compared with 32.2% (n=67) of the adolescent group.

Table 3: BMI

		Group		
		Adolescent	Adult	Total
BMI	Underweight	7	0	7
	Normal	60	10	70
	Overweight	33	28	61
	Obese	66	67	133
	Obese Class 3*	20	41	61
Total		186	146	332

χ^2= 16.786 df[1] , p<0.0001 (*Obese class 3 = BMI ≥ 40)

Waist-hip ratio:

The mean waist hip ratio was 0.9 ± 0.1 in both the women aged 35 years and older and the adolescents. But a greater proportion of the older women 71.3% (n=102) compared to 46.2% (n=84) of adolescents had a waist-hip ratio greater than 0.85.

Table 4: Waist- hip ratio

		Group		
		Adolescent	Adult	Total
WHR	WH<=0.85	98	41	139
	WH>0.85	84	102	186
Total		182	143	325

$\chi^2 = 20.735$ df^1, $p < 0.001$.

Blood Pressure:

The mean blood pressure in the adult group was 128/78mmHg and in the adolescent group was 116/69 mmHg. The prevalence of hypertension as defined as having a systolic blood pressure greater than 140mmHg and diastolic blood pressure greater than 90mmHg was 28% (n=41) in adult group and 8% (n=15) in the adolescent group. (χ^2= 23.3741, df^1, p<0.0001.)

Table 5: Blood Pressure

Crosstab

Count

		Group		
		Adolescent	Adult	Total
BP	<140/90	179	116	295
	≥140/90	15	41	37
Total		186	146	332

Forty adolescents (21.5%) and 73 (50%) of adults had a systolic blood pressure greater than 130mmHg and a diastolic blood pressure greater than 85. Which is the

cut-off for BP as a criterium for diagnosing the metabolic syndrome. ($\chi 2$= 29.580, df^1, p< 0.0001). More of the older women had demonstrated a blood pressure in the range for diagnosis of the metabolic syndrome. (see table 6)

Table 6: Blood Pressure for the diagnosis of Metabolic Syndrome

BP≥(130/85)	Over 35	Under 20	Total
<130/85	73	146	219
≥130/85	73	40	113
Total	146	186	332

Menstrual cycle:

Menstrual irregularities were reported in 167 (89.7%) adolescents at recruitment and 105 (72.4%) of women 35 years and older. There is a statistically significant difference between the two groups. In both groups oligomenorrhoea was the commonest menstrual abnormality reported by 48.9% (n=71) of older women and 47.3% (n=88) of the adolescents. In the adolescent group only 10.2% (n= 19) reported a regular menstrual cycle whereas in the older women 27% (n=40) reported a regular menstrual cycle.

Table 7: Menstrual cycle

		Group		
		Adolescent	Adult	Total
Menstrual cycle	Prim Amen	5	0	5
	Sec Amen	42	19	61
	Oligomen	88	71	159
	Regular	19	40	59
	Irregular	26	10	36
	Menorrhagia	5	2	7
	Other	1	3	4
Total		186	145	331

Table 8: Menstrual cycle dysfunction

	Group		
	Adolescent	Adult	Total
Menstrual dysfunction	167	105	272
Regular cycle	19	40	59
Total	186	145	331

$\chi 2 = 16.786$ df [1], $p < 0.0001$

Clinical Hyperandrogenism:

1.Hirsuitism:

The presence of hirsutism or unwanted hair was a common presenting problem in both groups. There was no significant difference noted between the two groups. Hirsutism either mild, moderate or severe was reported in 79.3% (n=146) of the adolescents and in 76.6% (n=109) of the adults.

Table 9: Hirsutism

		Group		
		Adolescent	Adult	Total
Hirsutism	Nil	38	37	75
	Mild	71	47	118
	Moderate & Severe	75	62	137
Total		184	146	330

$\chi 2 = 1.776$ df[2], $p = 0.411$

2.Acne

Acne was seen more commonly in the adolescent group, 107 (57%) had some degree of acne. In this group mild acne was present in 36.5% (n=68) of the adolescents and moderate and severe acne in 20.9% (n=39) whereas in the group of women aged 35 years and older only 29.8% (n=43) of women had either mild, moderate or severe acne and 70.1% (n=101) had no acne documented. There was a significant difference between the two groups ($\chi 2$= 25.056, df^1, p<0.0001).

Fertility

In the adolescent group only 13(6.9%) patients had attempted to conceive and of these only 5 (38.4%) had been successful. In the women 35 years and older 123(84.2%) had attempted fertility and of these 67 (54.5%) reported that they had problems conceiving with 31 (25.2%) reporting primary infertility and 36 (29.2%) reporting secondary infertility.

Table 10: Fertility

		Group		
		Adolescent	Adult	Total
fert1	Attempted	13	123	136
	Never Attempted	173	23	196
Total		186	146	332

Table 11: Primary versus Secondary Infertility

		Group		
		Adolescent	Adult	Total
FERTILITY	Primary Infertility	8	31	39
	Secondary Infertility	0	36	36
Total		8	67	75

$\chi 2$= 8.266, df^1, p= 0.004.

Metabolic parameters:

There was no significant difference in the mean fasting insulin level, HOMA and glucose: insulin ratio between the two groups. In older women the mean fasting insulin was 23.9 mmol/L and in adolescent group it was 24.4mmol/L. This is raised in both groups as normal fasting insulin is usually around 5mmol/L. HOMA-IR is an evaluation of insulin resistance by analysing fasting glucose and insulin levels in the blood. A HOMA score > 2.5 is an indication of insulin resistance and this was found in 114(78%) of the older women and 129(69.3%) in the adolescent group ($\chi 2$= 3.175, df^1, p=0.075). Mean HOMA score in the older women was 6.6 ± 6.4 and in the adolescents HOMA score was 5.9 ± 8.2. (NS)

Table 12: HOMA

		Group		
		Adolescent	Adult	Total
homa	HOMA<=2.5	57	32	89
	HOMA>2.5	129	114	243
Total		186	146	332

A glucose:insulin ratio of < 0.45 indicates insulin resistance. This was low in both groups. In both the adult group 74.8% (n=107) and the adolescent group 78.8% (n=145) had a low G:I ratio less than 0.45. ($\chi 2$= 0.721, df^1, p=0.396 (NS)

Table 13: GI-ratio

		Group		
		Adolescent	Adult	Total
girat	GIratio>=0.45	39	36	75
	GIratio<0.45	145	107	252
Total		184	143	327

Normal fasting glucose measurement should be between 4-6mmol/L and in the older women this was raised, the mean fasting glucose in adult women was 6.0 mmol/l compared to 5.0mmol/l in adolescent women (normal range).

The clinical feature of hyperinsulinaemia including trucal obesity and acanthosis nigricans were seen more frequently in adult women compared to adolescents, with 67.8% (n=97) of adult women and only 55.7% (n=102) of adolescents presenting with acanthosis nigricans. (χ^2= 4.938. df[1], p=0.026).

Table 14: Acanthosis Nigricans:

		Group		
		Adolescent	Adult	Total
ACANNIG	Yes	102	97	199
	No	81	46	127
Total		183	143	326

Endocrine changes

Serum Androgens:

There was no statistically significant difference in testosterone levels or free androgen index between the two groups. In the adolescent group 76.7% and in the adult group 82.5% had raised testosterone levels. (χ2= 1.595, df[1] , p=0.207).

Table 15:Testosterone

		Group		
		Adolescent	Adult	Total
testo	NOT RAISED	42	25	67
	RAISED	139	118	257
Total		181	143	324

Free androgen index is calculated by total testosterone× 100/ SHBG and this value gives an indication of the bioavailable testosterone level. Slightly more of the older women 39% (n=55) had a raised FAI compared with 30% (n=54) of the adolescent group. ($\chi 2$= 3.443, df[1], p=0.064 NS).

Table 16: FAI

		Group		
		Adolescent	Adult	Total
FAI	NOT RAISED	128	84	212
	RAISED	54	55	109
Total		182	139	321

SHBG:

SHBG may be low in women with PCOS. Normal range for SHBG in both age groups is 18-144nmol/L, in the adolescent group 28.5% (n=53) and in the adult group 17% (n=25) had SHBG levels less than 18 nmol/L.(X^2= 5.3398, df[1], P = 0.021)

Table 17: SHBG

SHBG	Over 35	Under 20	Total
>=18	115	130	245
<18	25	53	78
Total	140	183	323

DHEAS:

DHEAS may be raised in PCOS as a result of increased adrenal androgen production. In unaffected populations the normal range for adolescents is 1.8-10 umol/L and in adults is 1.7-9.2 umol/L. DHEAS and 17α-OHP in our study was only recorded after 2008 and therefore there are no values available from 1996 to 2008. Using the available data it was found that 14% (n=5 out of 36) of the older women and 18% (n=15 out of 81) of the adolescents had elevated DHEAS levels. (X^2 = 0.3769, df [1], p = 0.539) (NS).

Table 18: DHEAS

DHEAS	Over 35	Under 20	Total
Raised	5	15	20
Norm	31	66	97
Total	36	81	117

17- α-Hydroxy Progesterone

Normal range for 17- OHP for both groups is 0.7-14.2nmol/L. In this study there are only 17-OHP values available for 33 women older than 35 and 78 adolescents. We found that 17-OHP was only increased in 3.8% (n=3 out of 78) of the adolescents and 3% (n=1 out of 33). Pearson (χ^2= 0.0444, df^1 , p = 0.833) (NS)

Table 19: 17α-OHP

17-OHP	Over 35	Under 20	Total
outside norm	1	3	4
norm	32	75	107
Total	33	78	111

Serum Lipids

Serum total cholesterol, LDL cholesterol and triglyceride levels were significantly higher in the adult group than the adolescents. In the adult group 13.7% (n=20) of women the values of all four lipids were abnormal compared with 3.2% (n=6) of adolescents. In the adult group only 17.8% (n=26) had no lipid abnormalities compared with 41.2% (n=77) of adolescents.

Mean cholesterol level in adults was 5.1mmol/L ± 1.0 versus 4.6mmol/L ± 0.9 in adolescents. In the adult group 48 (33.5%) compared with 30 (16.6%) adolescents

had a triglyceride level greater than 1.5mmol/L. HDL-C was lower in the adult group than the adolescents and 66.3% (n=120) of adolescents versus 53.2% (n=75) of adults had an HLD-C level greater than 1.2 mmol/L.

Metabolic syndrome:

The prevalence of metabolic syndrome in the adolescent group was 12.7% (n=23) when applying the adult criteria. There is no difference in prevalence of metabolic syndrome when the criteria for diagnosing metabolic syndrome in adolescents was used. In the adult group 37% (n=54) of women met the criteria for metabolic syndrome. This difference is statistically significant ($\chi 2 = 28.527$, df^1, $p<0.001$).

Table 20: Adult criteria for metabolic syndrome:

		Group		
		Adolescent	Adult	Total
Adult Criteria	Criteria not satisfied	158	87	280
	Criteria satisfied	23	54	43
Total		180	143	323

Table 21: Adolescent criteria for metabolic syndrome:

		Group	
		Adolescent	Total
Adolescent	Criteria not satisfied	157	157
	Criteria satisfied	23	23
Total		180	180

Summary of results

Group	Women 35 years and older	Adolescents
Total number studied	N=146	N=186
Age (mean±SD)	37 ±3.0	17±2.0
BMI (mean±SD)	35.7±7.5	29.4±8.0
WHR (mean± SD)	0.9±0.1	0.9±0.1
Systolic BP (mean±SD)	128±17.0	116±14.0
Diastolic BP (mean±SD)	78.0±11.0	69.0±9.0
SHBG (mean±SD)	34.6±23.6	37.1±34.0
DHEAS (mean±SD)	4.7±2.5	6.3±2.9
17α-OHP (mean±SD)	4.8±3.8	6.6±4.5
Fasting Glucose (mean±SD)	6.0±2.3	5.0±1.3
Fasting Insulin (mean±SD)	23.9±18.4	24.4±21.6
HOMA (mean±SD)	6.6±6.4	5.9±8.2
Total Cholesterol (mean±SD)	5.1±1.0	4.6±0.9
Triglycerides (mean±SD)	1.6±1.2	1.1±0.9
HDL-C (mean±SD)	1.3±0.3	1.4±0.4
LDL-C (mean±SD)	3.0±1.0	3.0±1.0
Total with menstrual dysfunction	N=105 (72.4%)	N=167(89.7%)
Total with hirsutism	N=109(76.6%)	N=146 (79.3%)
GI-ratio< 0.45	N=107 (74.8%)	N=145(78.8%)
Acanthosis Nigricans present	N=97 (67.8%)	N=102(55.7%)
Raised Testosterone	N=118 (82.5%)	N=139 (76.7%)
Raised FAI	N=55 (39%)	N=54(30%)
Metabolic Syndrome	N=54 (37%)	N=23 (12.7%)

Chapter 4: Discussion

This study demonstrates that even young women with PCOS have signs of metabolic dysfunction and this seems to progress over time and is more pronounced in the older women.

The study population was mainly overweight and in the adolescent group 63.9% were classified as overweight, obese or obese class 3 compared to 93% of older women. This is higher than the numbers reported by Balen et al in the UK where 40% of their study population were overweight and in Amsterdam in the Netherlands Elting et al found that 44% of 300 women with PCOS had a BMI>25.[35,36] In a report from New York City Dunaif et al reported obesity rates of 66% in women with PCOS.[37]

Obesity tended to worsen with increasing age and the mean waist: hip ratio among the adolescents in our study was 0.9± 0.1. This was greater than the mean hip waist ratio reported by Mastorakos et al in Athens, which was 0.77 ± 0.02 in their adolescent group.[44] Huang et al reported a mean waist hip ratio of 0.8 in their study population in South China.[45] A waist: hip ratio > 0.85 indicates central obesity and a visceral distribution of adiposity putting these women at increased risk of cardiovascular disease.

Weight loss has been shown to have beneficial effects in improving menstrual cycle frequency, restoring ovulation and normalising biochemical indices especially insulin resistance. [31]Pasquali et al studied 20 obese hyperandrogenic women and showed that after weight reduction, insulin values in the OGTT were lower and similar to that reported in normal-weight women. [38]. A study from Adelaide, Australia by Clark et al reviewing 67 women with PCOS who lost an average of 10.2kg± 4.3kg, reported that 60 out of 67 women who were previously anovulatory, resumed ovulation after weight loss and 52 of the 67 women achieved pregnancy.[39] Early life style interventions in the adolescent group and targeting weight loss is vital in reducing androgen excess and improving reproductive functioning.[3]

Our study showed that at presentation, menstrual dysfunction was very common in the adolescent group, seen in 89.7% of the adolescent group and 72.5% in the older women. This is higher than the rates suggested by Hickey et al from Melbourne, Australia who reported menstrual irregularities in 51% of their adolescent PCOS population and Carmina et al who reported menstrual abnormalities in 40-50% of adolescents in their study.[32, 15]

Hirsuitism is a clinical indicator of androgen excess and was a common clinical complaint both among adolescents (79%) and older women (77%) which is higher than that reported by Warren-Ulanch et al in their study, where hirsuitism was present in two thirds of adolescents with PCOS and Azziz et al reported hirsuitism in approximately 60% of adult women.[2,40]

Acne was not a common presenting complaint among the adult women with only 30% presenting with acne compared to 57% in the adolescent group. This suggests that acne maybe a transient occurrence associated with puberty and not a reliable sign of androgen excess.

In our study serum testosterone was raised in many of the patients but there was no significant difference in the testosterone levels between the two groups. Measurement of FAI is a better indication than total testosterone of the bioavailability of testosterone and was raised in a greater percentage of the adult women (40%) compared with (30%) of the adolescent group.

DHEAS was raised in more of the adolescent (18%) compared to the older women (14%) and this is lower than expected Azziz et al reported that approximately 20-30 % of patients will demonstrate elevated levels of DHEAS and in 10% of patients this may be the sole abnormality of circulating androgens.[53] DHEAS and FAI data was incomplete and so we cannot draw a conclusion from these results.

Insulin resistance plays an important role in the pathogenesis of PCOS and evidence of insulin resistance is seen in both groups. In our study the incidence of insulin resistance with HOMA > 2.5 was 78% in older women and 69% in the adolescent group and this is higher than numbers reported by Begum in a study done in South East Asia where only 43% of the adult women had raised HOMA levels.[42] Studies in

the USA by Ehrman et al show that the prevalence of IGT and NIDDM is higher in women with PCOS when compared to age and weight matched controls and report that 45% of women with PCOS in their study had an abnormal OGTT with an incidence of IGT in these women of 35% and NIDDM in 10% of women .[41]

Acanthosis nigricans is a marker of insulin resistance and was seen more commonly in the older group (68%) ($p<0.026$) this is in keeping with the rate of acanthosis nigricans reported by Sharquie et al of 64% amongst women with PCOS presenting with primary infertility in Bagdad, Iraq.[43] The glucose-insulin ratio was similar between the adolescents and older women ($p= 0.396$).

Serum SHBG is a surrogate marker of androgen dynamics and levels are often lower in women with PCOS due to decreased liver production under the influence of hyperinsulinaemia. In our study there was a significant difference between the two groups with a greater percentage of the adolescents having low SHBG level despite the finding that insulin resistance and a HOMA score >2.5 were more common in the adult population.

Our study shows that insulin resistance is a core component of PCOS and is present from early on in the disease process. Affected adolescents are at increased risk of developing impaired glucose tolerance and type 2 diabetes. A study by Norman et al over 6.2 years demonstrated that the change in glycaemic control from baseline is frequent and 9% of patients who were normoglycaemic at baseline developed IGT and 8% moving from normoglycaemia to NIDDM and of the patients with IGT at baseline 54% had progressed to NIDDM at follow up.[26]

Lewy et al showed that correction of hyperinsulinaemia in adolescents with PCOS either through weight loss or pharmacological treatments, possibly a combination of metformin and the COC, is indicated in the women with insulin resistance, this leads to lowering of the androgen levels and improvement of ovulatory function and may delay the progression to type 2 diabetes.[54]

In our study there were more older women who had insulin resistance but this was not statistically significant despite the fact that more of the older women had increased BMI. Obesity influences the development of insulin resistance but there

are other contributing factors. Up to 75% of lean PCOS patients are found to have insulin resistance. Possibly women who presented in adolescence may have had more severe metabolic disturbances typical of PCOS leading to their early presentation and this may have been influenced by genetic factors.[26]

PCOS is the most common cause of anovulatory infertility. In our study 54% of the older women who had attempted fertility, reported either primary infertility (25%) or secondary infertility (29%). In the adolescent group of the thirteen adolescents who had attempted to conceive 62% reported primary infertility. Weight loss and use of oral contraceptive pill in adolescence may improve menstrual function and prevent primary infertility.

In our study total cholesterol, LDL cholesterol, and triglyceride levels were significantly higher in the adult group compared to the adolescents. Some form of dylipidaemia was present in in 59% of the adolescents in our study and this is higher than rates reported by Huang et al in South China where dyslipidaemia was present in 22.7% of adolescents with PCOS.[45] The lipid abnormalities in our study population tended to worsen with age, and in the group of older women only 13% had no lipid abnormalities compared with 41% of the adolescent group.

The prevalence of hypertension amongst the older women (28%) in our study was higher than the prevalence of hypertension in all South African women which is reported to be 21%.[49] This is higher than figures reported in a Brazilian study by Barcellos et al which revealed a hypertension prevalence of 20.3% amongst women with PCOS.[51]

Amongst the adolescent group the prevalence of hypertension was 8% which is lower than that reported by Bradshaw et al amongst non PCOS overweight South African adolescents (11%). [50] These findings suggest that hypertension is not a common finding in PCOS adolescents and is more common in the older women suggesting that it develops over a passage of time.

The prevalence of metabolic syndrome amongst the adolescent group was 12.7% when applying both the adult criteria and the adjusted criteria for diagnosis in adolescence. This number is lower than the finding reported by Rossi et al in

Rochester, USA who found that when the adolescent criteria was used 53% of the adolescents in their study population had metabolic syndrome but by the adult criteria only 26% of the PCOS adolescents met the diagnostic criteria for metabolic syndrome.[47] Our findings were similar to those reported in Pennsylvania, USA by Roe et al who found that 10.8% of their adolescents fulfilled the criteria for metabolic syndrome. [19] Our results were in keeping with a study done in Western Australia by Hart et al where the criteria for metabolic syndrome were met in 11.8% of the adolescent girls with PCOS, this was influenced by BMI and where the BMI was raised 53% of adolescents in the study met the criteria for metabolic syndrome.[46] This highlights the importance of early intervention and lifestyle changes including weight loss.

Among the older women in our study population only 38% satisfied the criteria and were classified as having metabolic syndrome. This is similar to the findings by Essah et al in Virginia, USA suggesting that metabolic syndrome in adults with PCOS is approximately 40%. [48] This is higher than the rates of metabolic syndrome reported among the adolescents which shows that the disease progresses with age.

Conclusion:

The results of our study clearly demonstrate that the adolescents share many of the clinical and metabolic abnormalities as the adult population. In this study abnormalities progress over the years and the metabolic disorders, especially dyslipidaemia and hypertension, infertility and metabolic syndrome were more pronounced in the older women.

PCOS is chronic lifelong condition requiring long term management. Early diagnosis and a multidisciplinary approach to treatment of PCOS and an increased awareness of the need for assessment of co-morbidities such as hypertension, dyslipidaemia and IGT, is required to allow for earlier detection and treatment in adolescence and then continued into adult years and this may improve the prognosis and have long term health benefits.

If diagnosed early and managed properly with lifestyle modification and pharmacological treatments, the onset of type 2 diabetes mellitus and the risk of coronary artery disease may be delayed or prevented.

References:

1. The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1): 41-47.

2. Warren-Ulanch J, Arslanian S. Treatment of PCOS in adolescence. Best Practice & Research Clil Endocrinol & Metabol 2006;20(2):311-330.

3. O'Brien R, Emans S. Polycystic ovary syndrome in adolescents. J Pediatr Adolesc Gynecol. 2008;21(3):119-128

4. Ehrmann DA, Barnes RB, Rosenfield RL et al. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999;22(1):141-146.

5. Dunaif A. Insulin action in the polycystic ovary syndrome. Endocrinol & Metabol Clinics of North America. 1999;28(2):341-359.

6. Du Toit M, Siebert T. Polycystic ovary syndrome (PCOS)–the long-term implications. S Afr J Obstet Gynaecol. 2009;15(2): 48-53.

7. Talbott E, Guzick D, Clerici A et al. Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol. 1995;15(7):821-826.

8. Hardiman P, Pillay OS, Atiomo W. Polycystic ovary syndrome and endometrial carcinoma. Lancet. 2003;361(9371):1810-1812.

9. Norman, RJ. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008; 23(3):462-477

10. Himelein MJ, Thatcher SS. Polycystic ovary syndrome and mental health: A review. Obstet and Gynaecol Survey. 2006;61(11):723-732.

11. Lobo RA, Carmina E. The importance of diagnosing the polycystic ovary syndrome. Ann Int Med. 2000;132(12):989-993.

12. Balen A, Laven J, Tan S et al. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod. 2003; 9 (6): 505-514

13. Fingert SB, Shah B, Kessler M et al. Evaluation of adolescents for polycystic ovary syndrome in an urban population. J Clin Research Paediatr Endocrinol. 2009;1(4):188-193.

14. Hickey M, Doherty DA, Atkinson H et al. Clinical, ultrasound and biochemical features of polycystic ovary syndrome in adolescents: Implications for diagnosis. Hum Reprod. 2011;26(6):1469-1477.

15. Carmina E, Oberfield SE, Lobo RA. The diagnosis of polycystic ovary syndrome in adolescents. Obstet & Gynaecol. 2010;203(3):201-205.

16. Glueck CJ, Morrison JA, Friedman LA et al. Obesity, free testosterone, and cardiovascular risk factors in adolescents with polycystic ovary syndrome and regularly cycling adolescents. Metabolism. 2006;55(4):508-514.

17. Pasquali R, Casimirri F, Atenuccil D. Relationship between onset of obesity and onset of oligomenorrhea or amenorrhea in females with obesity and polycystic ovaries. In: Adolescence in females.Year Book Medical Publications, Chicago, USA. 1985:363-365.

18. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. J Clin Endocrinol & Metab. 2006;91(2):492-497.

19. Weiss R, Dziura J, Burger TS et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004;350(23):2362-2374.

20. Roe AH, Prochaska E, Smith M et al. Using the androgen excess-PCOS society criteria to diagnose polycystic ovary syndrome and the risk of metabolic syndrome in adolescents. J Pediatr. 2013; 162(5): 937-941.

21. Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. Hum Reprod Update. 2009;15(4):477-488.

22. Silfen ME, Denburg MR, Manibo AM et al. Early endocrine, metabolic, and sonographic characteristics of polycystic ovary syndrome (PCOS): Comparison between nonobese and obese adolescents. J Clin Endocrinol & Metab. 2003;88(10):4682-4688.

23. Nur MM, Newman IM, Siqueira LM. Glucose metabolism in overweight Hispanic adolescents with and without polycystic ovary syndrome. Pediatrics. 2009;124(3):496-502.

24. Salley KE, Wickham EP, Cheang KI et al. POSITION STATEMENT: Glucose intolerance in polycystic ovary syndrome—a position statement of the Androgen Excess Society. J Clin Endocrinol & Metab. 2007;92(12):4546-4556.

25. Stanley T, Misra M. Polycystic ovary syndrome in obese adolescents. Curr Opin Endocrinol, Diabet and Obesity. 2008;15(1):30-36.

26. Norman RJ, Masters L, Milner CR et al. Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. Hum Reprod. 2001;16(9):1995-1998.

27. The Amsterdam ESHRE/ASRM-Spnsored 3rd PCOS Consensus Workshop group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod. 2011;27(1): 14-24.

28. Jones GL, Hall JM, Lashen L et al. Health-Related quality of life among adolescents with polycystic ovary syndrome. J Obstet, Gynecol, Neonatal Nurs. 2011;40(5):577-588.

29. Homburg R,Lambalk CB. Polycystic ovary syndrome in adolescence a therapeutic conundrum. Hum Reprod. 2004;19(5):1039-1042.

30. Ornstein RM, Copperman NM, Jacobson MS. Effect of weight loss on menstrual function in adolescents with polycystic ovary syndrome. J Pediatr Adolesc Gynecol. 2011;24(3):161-165.

31. Lifshitz F, Hall JG. Reduction in the Incidence of type II Diabetes with Lifestyle Intervention or Metformin. J Pediatr. 2002;109:45-60.

32. Brown J, Farquhar C, Lee O et al. Spironolactone versus placebo or in combination with steroids for hirsutism and/or acne (review). Cochrane Database Systematic Review.2003; 4: CD00019:1-15.

33. Van der Spuy Z, Le Roux P, Matjila M. Cyproterone acetate for hirsutism. Cochrane Database Syst Rev. 2003;4(CD001125):1-28.

34. Franks S. Polycystic ovary syndrome in adolescents. Int Journal Obesity. 2008;32: 1035-1041.

35. Balen AH, Conway GS, Kaltsas G et al. Polycystic ovarian syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod.1995;10(8): 2107-11.

36. Elting MW, Korsen TJ, Schoenmaker J. Obesity rather than menstrual cycle pattern or follicular cohort size, determines hyperinsulinaemia, dyslipidaemia and hypertension in ageing women with PCOS. Clin Endocrinol .2001; 55(6): 767-776.

37. Dunaif A, Segal K, Shelly D et al. Evidence for distinctive and intrinsic defects in insulin action in Polycystic Ovary Syndrome. Diabetes.1992; 41: 1258-1262.

38. Pasquali R, Antenucci D, Casimirri F et al. Clinical and Hormonal characteristics of obese hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab. 1989; 68 (1): 173-176.

39. Clark AM, Thornley B, Tomilinson L et al. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod. 1998; 13 (6):1502–1505.

40. Azziz R, Carmina E, Dewailly D et al. Androgen Excess Society. Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society Guideline. J Clin Endocrinol Metab. 2006; 91: 4237-4245.

41. Ehrmann DA, Barnes RB, Rosenfield RL et al. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999; 22(1): 141-146

42. Begum, F. Clinical and Hormonal Profile of Polycystic Ovary Syndrome. South Asian Fed Obstet Gynaecol. 2009; 1 (2): 22-25

43. Sharquie KE, AL- Bayatti, A, AL-Bahar A & Al-Zaidi, Q. Acanthosis Nigricans as skin manifestation of PCOS in primary infertile females. Middle East J Fertil Soc. 2004; 9 (2): 136-139

44. Mastorakos G, Koliopoulos C, Creatsas G. Androgen and lipid profiles in adolescents with Polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. Fertil Steril. 2002; 77(5): 919-927

45. Huang J, Renmin N, Xioli C et al. Metabolic abnormalities in adolescents with Polycystic ovary syndrome in South China. Reprod Bio Endocrinol. 2010; 8: 142-149.

46. Hart R, Dohert D, Mori T et al. Extent of metabolic risk in adolescent girls with features of polycystic ovary syndrome. Fertil Steril. 2011; 95 (7): 2347-2353.

47. Rossi B, Sukalich S, Droz J et al. Prevalence of Metabolic Syndrome and related characteristics in obese adolescents with and without Polycystic Ovary Syndrome. J Clin Endocrinol Metabol. 2008; 93 (12): 4780-4786.

48. Essah PA, Nestler JE. The metabolic syndrome in polycystic ovary syndrome. Clin Obstet Gynaecol. 2007; 50 (1): 205-225.

49. Steyn K, Gaziano T, Thomas A et al. Hypertension in South African adults: results from the demographic and health survey. 1998; 19 (10) 1717-1725.

50. Bradshaw, Steyn K, Levitt N & Nojilana B. Non-communicable diseases- the race against time. Parow, South Africa: Medical Research Council (2010).

51.Barcellos C, Rocha M, Hayashida S et al. Impact of body mass index on blood pressure levels in patients with polycystic ovary syndrome. Arq Bras Endocrinol Metabol. 2007; 51(7): 1104-1109

52. Wild R, Vesely S, Whitsett T et al. Ferriman Gallwey Self Scoring 1: Performance assessment in women with PCOS. J Clin Endocrinol Metabol. 2005; 90(1): 4112-4114

53. Azziz R, Carmina E, Dewailly D et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009; 91 (2): 456-488

54.Lewy V, Danadian K, Witchel S & Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. J Paed. 2001;138 (1): 38-44