

DECLARATION.

This is to declare that the work contained in this dissertation is mine and all studies quoted have been referenced appropriately.

SIGNED

Signed by candidate

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POLYCYSTIC OVARY SYNDROME: THE EFFECT OF WEIGHT LOSS ON INTRAVENOUS TRIGLYCERIDE CLEARANCE AND LIPID PROFILE.

INTRODUCTION AND LITERATURE REVIEW

The polycystic ovary syndrome occurs in up to 10% of the women of reproductive age (1;2).

It is a heterogeneous condition with considerable variation in presentation, which has led to considerable controversy as to its exact definition.

In 1935 Stein and Leventhal first described the syndrome as a clinical condition characterized by amenorrhoea, obesity, infertility, hirsutism and bilateral enlarged ovaries(3).

The development of radio-immunoassays in the 1960s facilitated the investigation of the endocrinological abnormalities of this condition, while the availability of pelvic ultrasonography in the 1980s made the non-invasive assessment of ovarian morphology possible. As a consequence the condition has been extensively evaluated over the past two decades and it is recognized as a condition characterized by reproductive dysfunction and metabolic disorders.

Polycystic ovary syndrome is associated with hyperandrogenemia, hyperinsulinemia, insulin resistance and altered lipid profiles which predisposes to metabolic complications such as type 2 diabetes and cardiovascular disease (4).

DIAGNOSIS

Stein and Leventhal diagnosed their patients on clinical criteria and performed ovarian wedge resections on a selected group of patients for histological confirmation and in some cases as a treatment modality. (3)

More recently the diagnosis of this common condition hinges on a combination of clinical findings, biochemical abnormalities and ultrasound.

Polycystic ovaries are defined by their ultrasound image and demonstrate the presence of 12 or more follicles [2 - 9 mm in diameter] and/or an increased ovarian volume [$>10 \text{ cm}^3$](5)

When the typical ultrasound findings co-exist with clinical or biochemical abnormalities, polycystic ovary syndrome is diagnosed.

Until recently controversy existed regarding the diagnosis of this condition. Because of the different manifestations and varying endocrine/metabolic features, clinicians and researchers had differing opinions on the criteria for diagnosis.

One school of thought was that a typical ultrasound appearance was central to diagnosis while another was that the absence of other endocrine diseases along with the presence of chronic anovulation and hyperandrogenism was sufficient to make a diagnosis.

In 2003, the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) arranged a polycystic ovary syndrome consensus workshop group in Rotterdam. The group was tasked with revising the guidelines for the diagnosis and management of PCOS, which the National Institute of Health (NIH) had developed in 1990.

The recommendation from the Rotterdam group was that, for the diagnosis of PCOS, a patient had to have 2 of 3 features, (1) Irregular or absent ovulation, (2) clinical or biochemical evidence of hyperandrogenism, (3) PCO morphology on ultrasound (6;7).

The study group recommended that women with PCOS be investigated for metabolic syndrome, including physical measurements for abdominal obesity, assessment of lipid and glucose metabolism as well as hypertension.

The metabolic syndrome is a common finding in PCOS and both contribute to a significantly increased risk of cardiovascular disease. Glueck and his co-workers reported the presence of the metabolic syndrome in 46% of women with confirmed PCOS compared with 23% of the general female population (8). The diagnostic criteria for defining the metabolic syndrome include components such as centripetal obesity, hypertension, fasting hyperglycaemia and dyslipidaemia. The recommendation for the diagnosis of the metabolic syndrome in women with PCOS is the presence of three or more of the following criteria: (i) abdominal obesity as reflected by a waist circumference > 88 cm (ii) fasting serum triglyceride (TG) levels of 1.8 mmol/L or greater, (iii) high density lipoprotein-cholesterol (HDL-C) levels < 1.30 mmol/L, (iv) fasting and 2 hour glucose following an oral glucose tolerance test of 6.1 to 7.0 mmol/L (fasting) and/or 7.8 – 11.1 mmol/L at 2 hours (v) blood pressure $\geq 130/ \geq 85$ mmHg(6).

Biochemical abnormalities, associated with the condition, include elevated concentrations of luteinizing hormone (LH) which is reported in 45-75% of cases (9). In a cohort of 300 patients with PCO morphology in a clinic in London, Franks and colleagues demonstrated a mean LH concentration more than double that of controls(10).

Raised total testosterone concentrations are present in up to 80% of patients and decreased sex hormone binding globulin (SHBG) level is a common finding, the latter resulting in an increase in free bioactive testosterone.

CLINICAL PRESENTATION

The clinical manifestation of PCOS includes a wide spectrum of abnormalities, ranging from the asymptomatic woman with a typical ultrasound appearance to the woman with severe endocrine and reproductive disorders.

In a review of 300 patients with the ultrasound diagnosis of polycystic ovaries attending a gynaecological endocrine clinic in the United Kingdom, Franks et al reported hirsutism in 64%, acne 27%, infertility 42% and oligomenorrhoea in 52%(10).

Polycystic Ovary syndrome is the commonest cause of anovulatory infertility(11)

At Groote Schuur Hospital in Cape Town a review of 800 women with polycystic ovaries on ultrasound revealed that 82% had menstrual dysfunction (including primary and secondary amenorrhoea and cycle irregularities), 65% hirsutism, 37% acne and 70% complained of infertility, (unpublished data-personal communication).

Patients with PCOS have an increased risk of pregnancy loss and because of their pre-existing hyperinsulinemia and insulin resistance also have an increased risk of gestational diabetes.

In a cohort of 2199 women with recurrent miscarriage assessed in a London tertiary centre, PCO morphology (ultrasound) was found in 859 cases (40.7%). This is a significantly higher prevalence than found in the general population (23%). The live-birth rate in subsequent pregnancies was similar to that of women with normal ovarian morphology. (60.9% Vs 58.5%.)

The authors suggested that the search for a specific endocrine abnormality that can divide women with PCO into those with good and those with poorer prognosis for ongoing pregnancy should continue (12).

Jakubowicz et al from the University of Venezuela, hypothesized that hyperinsulinaemic insulin resistance, hyperandrogenism and obesity are risk factors for recurrent pregnancy loss in PCOS patients (13). In a retrospective analysis this collaboration found that PCOS patients with recurrent

pregnancy loss treated with metformin had a lower chance of losing their pregnancies than those on no treatment, 11.1% vs 58.3%. There were 36 women in the metformin group and 12 in the control group(13).

Obesity is present in at least 50% of patients with PCOS and this exerts an additive and synergistic effect on the manifestations of PCOS. It independently impacts on insulin sensitivity, risk of diabetes and adverse cardiovascular profile(14).

PREVALENCE

Different studies have attempted to assess the prevalence of polycystic ovaries in the normal population. Polson et al found 23%(15), and Clayton et al 22%(16) in the women they studied. In the latter study, postal invitations were sent to women born in the years 1952 to 1969 from a patient list of a single group practice. Three hundred and fifty three of the 1065 women invited agreed to participate and 190 (18%) completed the study. They concluded that the prevalence of polycystic ovaries is high but in their cohort there were *minimal clinical manifestations* compared to women with normal ovaries. They suggested that the presence of polycystic ovaries on ultrasound might be a variant of normal(16).

In the audit by Polson et al, 257 healthy volunteers were recruited and pelvic ultrasonography was performed. These women had not sought medical intervention for menstrual irregularities, hirsutism or acne. Ninety-nine women were using oral contraceptives and of the 158 not on oral contraception, 18% had irregular cycles. Of the women with PCO, 76% had irregular cycles and 6 of the 8 with regular cycles were hirsute. In this study, the true prevalence of menstrual irregularities was masked by the use of oral contraception.

In the study by Farquhar et al, in New Zealand, twelve hundred women chosen randomly from electoral rolls were invited. Of the 255 women who agreed to recruitment, 183 women completed the study. These women had an ultrasound and blood tests between day 5 and 9 of their cycles. Thirty-

nine (21%) had polycystic ovaries. Hirsutism and irregular menstrual cycles were commoner in the PCO group. Fifty-nine percent of women with PCO had elevated Ferriman Gallwey scores (> 7) or irregular periods or both (17).

The main criticism of these studies is the prevalence of gynecological complaints such as irregular periods and hirsutism in this group of so called normal women. Ideally a study should be undertaken among women who have no gynecological disorders, if the prevalence among "normal" women is to be established. It is however extremely difficult to identify and recruit such a group.

In a study conducted in Cape Town by van der Spuy et al, a total of 194 healthy university students were assessed and on abdominal ultrasound 7.7% had PCO morphology. A quarter of them however had increased ovarian volume, and may have had PCO, which was not definitively confirmed because of poor ultrasound resolution (personal communication).

LONG TERM SEQUELAE

Women with PCOS have an increased chance of developing diabetes mellitus, hypertension and cardiovascular disease.

Conn et al from Middlesex Hospital London studied the prevalence of polycystic ovaries in pre-menopausal women presenting with type 2 diabetes. Eighty-two percent had polycystic ovaries on ultrasound. Of these women 52% had cutaneous manifestations of hyperandrogenism with or without menstrual disturbance (18).

Wild and co-investigators from the University of Oklahoma compared cardiovascular mortality and morbidity in middle-aged women previously diagnosed with PCOS with age matched controls. Women with PCOS were found to have higher levels of severe cardiovascular risk factors, including diabetes, hypertension, hypercholesterolaemia, hypertriglyceridaemia and

increased waist:hip ratio (OR 2.2, 1.4 and 3.2 respectively for diabetes, hypertension and hypercholesterolaemia) (19).

The same investigator had previously studied 102 pre- and post-menopausal women undergoing cardiac catheterization for chest pain (in the same institution) and found just over half of them had arterial lesions. These women were more likely to report hirsutism, diabetes mellitus, hypertension and previous coronary artery disease (20).

In a similar cohort of patients in New Zealand, patients undergoing cardiac catheterization and coronary angiography had pelvic ultrasound. Of the 143 women studied, Birdsall et al found that women with PCO had more extensive coronary artery disease(21).

Although these are retrospective studies, a strong association between PCOS and cardiovascular disease seems to exist. Despite the existence of cardiovascular disease risk factors in these patients, studies demonstrating increased mortality are lacking. A follow-up study by Wild failed to demonstrate an increase in mortality due to circulatory or cardiovascular disease in patients with PCOS (22). This has led to speculation that some protective mechanisms may exist in these patients, such as prolonged exposure to unopposed estrogens and elevated levels of vascular endothelial growth factor VEGF (23).

Pierpoint et al working at the London school of Hygiene and Tropical Medicine traced and followed a total of 786 women with a diagnosis of PCOS made between 1930 and 1979. Standardized mortality ratios (SMR) were calculated to compare the death rates in these women with the national rates. There were 15 deaths from circulatory disease, 13 of which were from ischaemic heart disease. They concluded that patients with polycystic ovary syndrome do not have markedly higher than average mortality rates from circulatory disease. The characteristic endocrine profile of women with PCOS may protect them against circulatory disease(24).

In a paper published in 2002 by Dahlgren et al, 28 women with PCOS diagnosed 25 to 34 years before (histological diagnosis from wedge resections) were compared with 56 controls. They found a strong positive correlation between serum concentrations of triglyceride, basal insulin and

abdominal obesity on the one hand and plasminogen activator inhibitor, fibrinogen and von Willebrand factor on the other in both groups of patients. Mean values of most haemostatic variables studied did not differ between the 2 groups. Therefore, women with altered metabolic profile were also found to have affected haemostatic factors but PCOS in itself did not seem to influence the haemostatic factors (25).

The presence of chronic anovulation in these women coupled with the increased action of aromatase in the adipose tissue of obese patients leads to exposure to unopposed estrogens, predisposing to the development of endometrial hyperplasia and carcinoma. In the retrospective study by Wild and colleagues in 2000, 786 women with the diagnosis of PCOS in the United Kingdom before 1979 were traced. In this study women with PCOS were not at increased risk of breast cancer but were at increased risk of endometrial cancer(26).

Whether or not these patients are at increased risk of breast cancer is controversial. In the Pierpoint study quoted above breast cancer was the commonest cause of death. On the one hand chronic anovulation during the reproductive ages may potentially raise the risk in the post-menopausal age group from chronic estrogen exposure earlier in life, as some observational studies have suggested and on the other hand since early menarche and late menopause are associated with an increased risk of breast cancer the anovulatory state of PCOS and subsequent decreased exposure to progesterone may be protective (1).

It would appear that further studies are also needed to clarify whether ovarian cancer risks are increased in these patients. The Cancer and Steroid Hormone study reported an increased previous diagnosis of PCOS in patients presenting with ovarian cancer but this has not been confirmed by findings from longitudinal studies (1).

METABOLIC DISTURBANCES

An association between hyperinsulinaemia, insulin resistance and PCOS has been well described. A defect in insulin mediated receptor autophosphorylation has been described in a substantial proportion of PCOS women. This defect is unique to PCOS and is not unusual in other common states of insulin resistance.

The degree of hyperandrogenism correlates significantly with levels of hyperinsulinaemia, and this is probably mediated via a direct action of insulin on the ovary and on the sex hormone binding globulin (SHBG) levels (23).

Some studies have demonstrated that the use of insulin-lowering agents leads to a suppression in androgen levels. This suggests that insulin has an effect on ovarian androgens, most likely via the pituitary gland(23). Supporting this hypothesis is the demonstration of insulin receptors in the pituitary gland (27).

Dunaif and colleagues in Massachusetts in 2001 performed sequential euglycemic glucose clamp studies and skeletal muscle biopsies on PCOS patients and matched controls. They found insulin-mediated glucose disposal was significantly decreased in PCOS patients compared to controls and suggested that there is a physiologically relevant defect in insulin receptor signaling in patients with PCOS (28).

Nestler and colleagues proposed an insulin-mediated increase in ovarian cytochrome P450c17a activity as another possible mode of action of insulin in a subgroup of obese PCOS women (29).

Robinson et al in St Mary's Hospital London performed a cross sectional study of insulin sensitivity and lipids in a cohort of PCOS subjects (30). They investigated 19 lean and 55 obese patients with PCO and compared the results with those in 22 lean controls and 15 obese controls. They found HDL2-cholesterol was reduced in both lean and obese PCO patients. A

significant negative correlation was also found between body mass index and total HDL-cholesterol as well as HDL2-cholesterol levels both within the PCOS group and the control women.

Insulin resistance is associated with impaired glucose tolerance, type 2 diabetes, hypertension, abdominal obesity and adverse lipid profile, all features of the 'metabolic syndrome' (6).

Due to higher levels of circulating androgens, body fat distribution and hyperinsulinaemic insulin resistance, patients with PCOS would be expected to be at a higher risk of dyslipidemia (23). There have been a few studies in women with PCOS demonstrating an abnormal lipoprotein profile. This is characterized by raised triglycerides, marginally elevated low-density lipoprotein (LDL) cholesterol and reduced high-density lipoprotein (HDL) cholesterol (23).

OBESITY AND PCOS

The World Health Organization defines obesity as a body mass index of 30Kg/m^2 or greater. Due to the difference in diagnostic criteria for polycystic ovary syndrome, the reported prevalence of obesity in this condition varies and has been documented in 38-50% of women with PCOS (14;31). Hoeger and colleagues estimated that approximately 50% of individuals with PCOS are obese (14). Obesity, hyperinsulinaemia and hyperandrogenaemia all interact and impact on the presentation and long-term outcome of PCOS (32). Nestler et al hypothesized that obesity and the consequent development of hyperinsulinaemia might lead to the genesis of PCOS in susceptible individuals (33).

The mechanism of the increased prevalence of obesity in women with PCOS is not currently known, but it affects other stigmata of PCOS including sex hormone binding globulin (SHBG) levels and markers of insulin action. In a study done by Acien et al in Spain, 137 women with PCOS and 75 without were studied with respect to BMI, gonadotrophins, insulin, androgens (testosterone, androstenedione, DHEAS), 17 alpha-hydroxyprogesterone, SHBG, triglycerides as well as glycaemic and insulin response to a 100-g oral glucose load in 103 of the women.

They found insulin and metabolic indices were similar in lean women with and without PCOS but obese women with PCOS were more insulin resistant, hyperandrogenic and hypertriglyceremic (34).

In the work of Dunaif et al in 1987, 62 hyperandrogenic women were compared to 36 controls. Obese PCOS women were at risk of impaired glucose tolerance compared to their lean counterparts and the negative impact of PCO and obesity on insulin action was additive. (35)

The ovary possesses receptors for both insulin and IGF-1 and these hormones have similar biological activity. Hyperinsulinaemia stimulates both insulin and IGF-1 receptors. The effect of increased IGF-1 receptor stimulation on the ovary is an increase in testosterone and androstenedione biosynthesis via the cytochrome P450c-17alpha hydroxylase pathway (36).

Insulin-like growth factor binding protein -1 (IGFBP-1) concentrations correlate inversely with insulin concentrations. In obese compared to non-obese women with PCOS, IGFBP-1 is significantly lower with resultant increased free IGF-1 and an exaggerated impact on androgen production (36)

It has been suggested that the reduction of SHBG and the consequent increase of percentage free testosterone may lead to inhibition of follicular maturation and consequently, induce anovulation and altered ovarian morphology, thus starting the series of events which could lead to the development of PCOS (37).

The fact that hyperinsulinemia and insulin resistance are invariably associated with obesity (particularly with abdominal obesity), represents the basis of the hypothesis linking obesity itself to the development of hyperandrogenism in women with PCOS (37)

It seems that in obese women, hyperinsulinaemia is a secondary disorder (secondary to insulin resistance) resulting in a decreased SHBG and IGFBP-1 levels and therefore causing an increase in active androgen levels. The free androgens are in turn converted to estrone in adipose tissue.

In a study population of 263 consecutive women presenting to the gynaecological endocrine clinic at St Mary's Hospital in the United Kingdom with hirsutism and/or anovulation and found to have polycystic

ovaries on ultrasound, 35% were obese (BMI > 25 Kg/m²). There was a higher prevalence of hirsutism and menstrual abnormalities in this group compared with their lean counterparts with PCOS.

Patients in the obese group also demonstrated lower SHBG levels, higher free testosterone levels as well as higher concentrations of androsterone glucuronide, a marker for 5 alpha reductase activity (38).

In a review article published in 2004 by Hoyt and Schimdt, hirsutism and menstrual irregularities was found to be more prevalent in obese patients with PCOS compared to their non-obese counterparts (1).

Holte and colleagues in a series of studies in Sweden compared gonadotrophin and sex steroid concentrations in women with PCOS (n = 67) with a control group (n = 59) and assessed the effects of obesity in both groups. They found an association between PCOS and increased testosterone levels across the BMI range but with obesity having a synergistic effect in the PCOS group and no effect of obesity on testosterone levels in the control group. Similar findings were recorded for the free androgen index (FAI)(39). DHEAS levels were higher by about 30% in the obese PCOS group compared to obese controls (BMI of 30 Kg/m² or more). Further findings in this study include the confirmation from other studies that the negative effects of obesity and PCOS on SHBG levels are independent of each other (39).

The distribution of fat is also important when assessing cardiovascular risk as well as endocrine changes. An increased waist to hip ratio (WHR) has been identified as an independent risk factor for arterogenesis and poor metabolic parameters. Pasquali and colleagues found a positive correlation between the WHR and increased LH, androstenedione and estrone concentrations as well as higher levels of insulin (fasting and stimulated), triglycerides, very-low-density-lipoprotein (VLDL) and apolipoprotein B. The levels of high density lipoprotein (HDL) cholesterol were inversely proportional to the WHR(40). Obesity contributes to the morbidity of PCOS as well as aggravating insulin resistance and hyperlipidemia.

The presence of excess adipose tissue in the obese woman leads to an increase in aromatase activity and enhanced conversion of estrogens to androgens and vice versa. The development of symptoms may be linked to the development of obesity in some patients and weight loss often leads to a return to normal menstrual cyclicality and fertility (41).

In a study from St Mary's Hospital medical school in London, 24 obese women with PCOS were treated for 6-7 months on a 1000 Kcal, low fat diet. Thirteen subjects lost more than 5% of their starting weight. These subjects demonstrated a marked increase in concentrations of SHBG and a reciprocal decrease in free testosterone levels. There was also a reduction in fasting serum insulin levels and the insulin response to 75g of oral glucose. Eleven of these women also showed an improvement in reproductive function and 5 of them got pregnant.

In a similar study, Pasquali and colleagues looked at a less well-defined group of patients. They reviewed obese, hyperandrogenic, amenorrhoeic women with or without PCO morphology on ultrasound. Weight loss was beneficial in terms of decreased serum insulin levels, tendency towards more regular menstrual periods and improved hirsutism scores (42).

There is considerable evidence that in the long term, PCOS, predisposes to the development of type 2 diabetes and this is more likely in the obese patient. The increased aromatase activity in the obese woman also increases the chances of endometrial hyperplasia and carcinoma from increased estrogen concentrations.

The incidence of morbidity such as stroke, ischaemic heart disease and diabetes is three to four times greater in female subjects with body mass index (BMI) of 28 or more when compared to the general population (14).

Bilenka and colleagues in New Zealand have also identified obesity in the absence of PCO as a risk factor for decreased fertility and pregnancy loss. (43).

The early identification and appropriate management of risk factors such as hypertension, dyslipidaemia, hyperglycaemia and central obesity will contribute to the reduction in long-term morbidity and cardiovascular risk (44). Weight loss is therefore a critical part of the management of this

condition, however achieving this is difficult especially in the absence of adequate exercise facilities, affordable low calorie diet and support groups.

GENETICS

Studies of first-degree relatives of women diagnosed with PCOS reveal familial clustering of the disease. A prospective study of first-degree relatives of ascertainable sisters of women with PCOS found that 46% of them were hyperandrogenaemic and these studies have suggested a dominantly inherited trait controlling androgen levels(45). Studies on cultures of human theca cells derived from follicles isolated from the ovaries of PCOS and normal women demonstrated that PCOS theca cells produce greater amounts of testosterone, 17alpha-hydroxyprogesterone, and progesterone than normal theca cells. This is probably as a result of multiple alterations in the steroidogenic machinery such as elevated expression of the CYP11A, 3BetaHSD2, and CYP17 genes, leading to an up-regulation of hormone production in these women. Genetic studies have suggested that a possible site responsible for this may be chromosome 19p13.3(45).

The lack of universal diagnostic criteria, heterogeneity of the condition and the possible different ethnic presentations makes determining the genetics of PCOS and possible associations with obesity difficult.

MANAGEMENT

The management of PCOS depends to a large extent on the presenting complaints of the patient. Probably the one mode of management common to all aspects of PCOS is the advice on lifestyle modification including a sensible dietary regime and regular exercises especially in the obese woman.

In a study already described, weight loss of 5% or more of the initial body weight improved biochemical parameters as well as clinical features of the disease(41). The main aims of treatment are, control of symptoms and prevention of long-term sequelae.

The medical modalities for managing this condition vary from the use of ovulation inducing agents like clomiphene citrate and gonadotrophins in patients presenting with anovulatory infertility to the use of antiandrogen such as cyproterone acetate and spironolactone in patients presenting with hirsutism and acne.

The use of insulin sensitizing agents such as metformin in selected patients to manage insulin resistance and in so doing improve the response to ovulatory agents(46) and to complement weight loss regimens has been extensively described(47). Glueck et al in Cincinnati, Ohio, compared the development of gestational diabetes in PCOS women on metformin throughout pregnancy to that in PCOS women not taking metformin and found a 10 fold decrease in the incidence of gestational diabetes in the women on metformin(48). In 2003 Barbieri from the Brigham and Women's Hospital, Harvard Medical School, Massachusetts published an article reviewing the use of metformin for the treatment of polycystic ovary syndrome. The findings from this review (of 94 English language articles published after 1966) were that 3 clinical trials showed metformin plus clomiphene is more effective than clomiphene alone in inducing ovulation. In addition metformin may restore ovulatory menses in women with irregular menses (not trying to conceive) and that in obese women, metformin plus a low-calorie diet was more effective at achieving weight loss than a low-calorie diet alone(49). Stadtmayer et al from Norfolk USA, (2002) advocated the use of insulin sensitizing agents such as metformin in the reduction of miscarriage rates, as well as in the reduction of the risks associated with coronary artery disease, gestational diabetes and obesity in women with PCOS(50). Data on the long-term effect of this therapy are not presently available.

Probably the only role for surgery in the management of PCOS is in the treatment of the infertile woman where laparoscopic laser or diathermy ovarian drilling has been described as a method of inducing ovulation. This

is thought to reduce serum androgen concentrations, normalizing cycle lengths and restoring ovulatory cycles(51).

The advantage of ovarian drilling over medical therapy includes a decreased risk of ovarian hyperstimulation and of multiple pregnancy in subsequent cycles(17). In a Cochrane Database systematic review by Farquhar et al in 2005, it was suggested that there was no difference in live births and cumulative ongoing pregnancy rates between laparoscopic ovarian drilling (6-12 months follow up) and 3-6 cycles of ovulation induction using gonadotrophins in the primary treatment of anovulatory patients with PCOS. There was a reduction in multiple pregnancy rates in the ovarian drilling group but concerns regarding long-term ovarian function remain(52). Even though this is an attractive option in the management of these patients, there is a small but significant risk of ovarian adhesion formation and premature ovarian failure, which must be considered.

AIM

The aim of this study was to investigate the effects of weight loss on markers of insulin resistance, triglyceride clearance rate and lipid profile in obese patients with PCOS.

PATIENTS AND METHODS

Patients were recruited from the Gynaecological Endocrine Clinic at Groote Schuur Hospital. The Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town approved the protocol. Detailed written informed consent was obtained from every participant.

The inclusion and exclusion criteria for the study participants are shown in table 1.

TABLE 1: INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA	EXCLUSION CRITERIA
<ul style="list-style-type: none">• BMI > 30 kg/m²• Confirmed PCOS• Willing to use contraception for up to 8 months	<ul style="list-style-type: none">• Pregnancy• Need for immediate fertility treatment• Poorly controlled hypertension (DBP > 100 mmHg)• Creatinine > 200 µmol/L• Severe hepatic dysfunction• Marked hypertriglyceridaemia (fasting > 5 mmol/L)• Previous CVA• Poor compliance

Abbreviations:

BMI – Body mass index, DBP= Diastolic blood pressure, CVA= Cerebrovascular accident.

Study design

The trial was an un-blinded, non-placebo controlled study in which every participant acted as her own control over the 6 months study period.

At visit 1, a detailed clinical evaluation was done and the nature of the study was explained to the patients. A trained dietician instituted dietary modification from visit 2 and the same dietician reviewed participants at regular (2 monthly) intervals during the trial (4 visits). Patients who wished to participate in the study and who were sexually active were commenced on an oral contraceptive agent.

At the second visit anthropometric measurements included height, mass (in light clothing on an electronic balance that was accurately calibrated), waist and hip measurement. Fasting blood samples for lipid and endocrine profiles were taken. Glucose and insulin (fasting), sex hormone binding globulin (SHBG) and testosterone were measured and intravenous fat tolerance test was done.

Triphasil®, a combined oral contraceptive [containing ethinyl estradiol 30 µg + levonorgestrel 50 µg (6 tablets), ethinyl estradiol 40 µg + levonorgestrel 75µg (5 tablets), ethinyl estradiol 30 µg + levonorgestrel 125 µg (10 tablets) and 7 placebo tablets] (Acromed, South Africa) was prescribed for 19 of the 22 subjects. Two subjects had never been sexually active and did not require contraception and one patient had a sterilization procedure before recruitment.

Sibutramine (Reductil® Abbott Laboratories South Africa) was commenced (to assist in dietary control) from visit 2. This is a centrally acting weight management agent that inhibits the re-uptake of noradrenaline and serotonin, enhancing satiety and thereby leading to less food intake and subsequent loss of weight. It has been shown that subjects on Sibutramine lose an average of 7% of their body weight in six months. Wide inter-individual variability exists but up to 15% of patients lose more than 10% body weight on a dose of 10mg daily (53-55). It is a registered product with a reasonable side effect profile. Side effects that have been documented include headache, dry mouth, loss of appetite and constipation.

Physiological effects of the drug include a mild increase in heart rate (3 beats/minute) and an increase in blood pressure (1.5 mmHg).

Sibutramine is not licensed for use in pregnancy.

Compliance with medication (sibutramine and triphasil) was checked by pill count at every visit from visit 3. A nursing sister who was part of our research team assessed each patient every four weeks. Patients with concerns or complaints were seen at unscheduled visits.

The anthropometric measurements and blood tests were repeated after six months at visit 8.

The study design and visit schedule is presented in table 2.

TABLE 2: STUDY DESIGN

	Visit 1	Visit 2	Visit 3-7	Visit 8
• Clinical assessment	+			+
• BMI, WHR	+	+	+	+
• Pulse	+	+	+	+
• Blood pressure	+	+	+	+
• Dietary reinforcement	+	+	+	+
• Blood tests including IVFTT		+		+

Visit 1 = Screening, Visit 2 = Initiation of treatment

Abbreviations: WHR = Waist-hip ratio, BMI = Body mass index, IVFTT = Intravenous fat tolerance test.

Laboratory methods

Fasting plasma triglyceride, total cholesterol and glucose were determined with conventional enzymatic assays (Roche platform). HDL-cholesterol was determined after precipitation of apolipoprotein B- containing lipoproteins. The LDL-cholesterol was calculated using Friedewald's formula (56) The non-denaturing gradient gel electrophoresis for determining LDL particle size was done according to Blom et al(57) This technique separates LDL particles according to size which is reproducibly categorized from large to small in 5 steps, but classified for this study into large (A), intermediate (I) and small (B) particles.

Serum testosterone was measured by competitive chemiluminescent immunoassay on the ACS (Ciba-Corning). SHBG was measured in as an IRMA (immunoradiometric assay) from Orion Diagnostica and insulin in an RIA (radioimmunoassay): DPC-Coat-a-Count. The intra-assay variables for glucose, cholesterol, TG, HDL, SHBG, insulin and testosterone were 0.9%, 0.8%, 1.5%, 0.9%, 1.7-3.6%, 3.1-9.3% and 4.5-11.3% respectively and the respective inter-assay variables were 1.8%, 1.7%, 1.8%, 1.85%, 4.4-8.2%, 4.9-10.0% and 5.2-13.8%.

The intravenous fat tolerance test (IVFTT) is a non-steady state, provocative test to evaluate the clearance of triglyceride-rich lipoproteins. After an overnight fast, a standardised dose (0.5ml/kg body mass) of a commercially available lipid emulsion (Lipovenous Fresenius[®] South Africa) was administered by a bolus intravenous infusion and blood was sampled at -5, 0, 5, 10, 15, 20, 25, 30, 35, 40, 60 and 90 minutes for triglyceride assay by spectrophotometry. The plasma triglyceride concentration should increase by about 3mmol/L, and the subsequent declining triglyceride (TG) concentrations are analysed with first order kinetics using non-linear regression (58) The triglyceride clearance constant (k) was calculated using the formula $TG = D \cdot e^{-kt} + c$, where D is the rise of TG over the baseline, c is the baseline concentration and t is time in minutes.

There is evidence that delayed postprandial triglyceride clearance is a marker of insulin resistance and is also associated with a cluster of pro-atherogenic metabolic disturbances (small dense LDL, reduced HDL₂, increased plasminogen-activator inhibitor type 1)(59).

Statistical methods

At the onset of the trial a cohort size of 20 was assessed as adequate to demonstrate a difference in lipid parameters, and was similar to that of other studies investigating endocrine parameters in PCOS patients⁽¹⁷⁾ No formal calculation of cohort size could be done for changes in LDL species and TG clearance owing to a lack of studies in this area.

The data were captured and processed using commercially available software (Excel®, GraphPad® Prism and Instat®) to provide descriptive statistics. Repeated measures analysis of variance as well as paired t-tests were used for comparisons. Chi-squared analysis was done for categorical data. A probability (p-value) of <0.05 was taken as statistically significant.

RESULTS

A total of 28 women were recruited to the study, 22 of whom completed the study satisfactorily and were included in the final analysis. Five women were discontinued for non-compliance and 1 had side effects from the medication that she could not tolerate. No results from these 6 patients are presented.

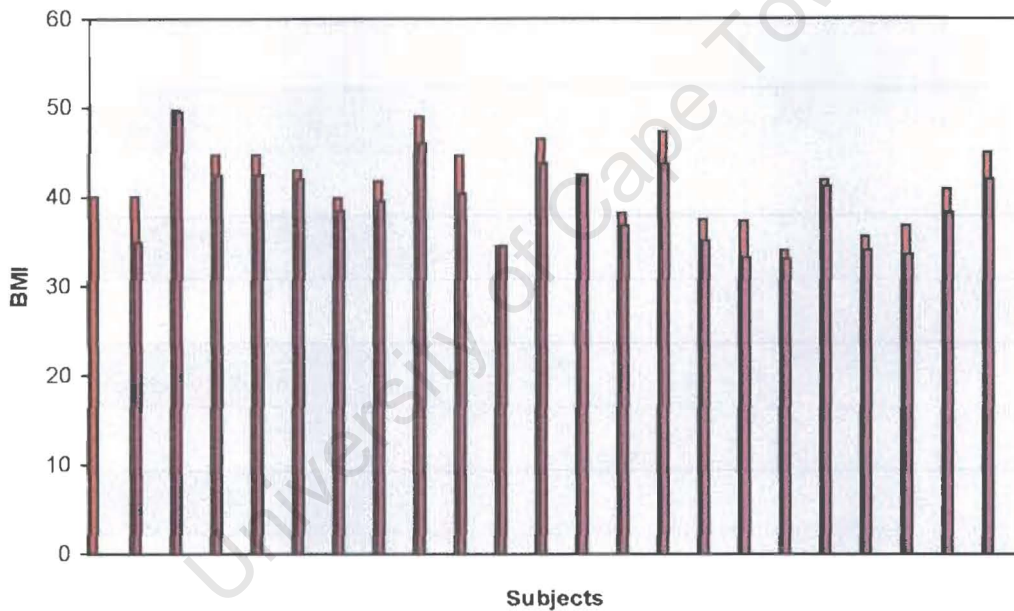
Anthropometric measurements

Of the 22 subjects analyzed, 16 (73%) lost more than 5% of their initial body mass, five lost less than 5% and one subject gained weight by the end of the study. The mean body mass index \pm SD at recruitment was 41.6 ± 4.56 kg/m² compared to 39.4 ± 4.57 kg/m² at the end of the study ($p < 0.0001$). The mean waist-hip ratio was 0.86 ± 0.06 at the beginning and 0.85 ± 0.05 at the end of the study ($p = 0.09$). There was no statistically significant

difference in mean mass and waist diameter- 109.48 ± 12.8 and 102.95 ± 12.8 ($\text{kg} \pm \text{SD}$) and 114 ± 7.7 and 108 ± 6.4 ($\text{cm} \pm \text{SD}$) at the beginning and the end of the study respectively. Separate analysis excluding the patient who gained weight did not affect the results.

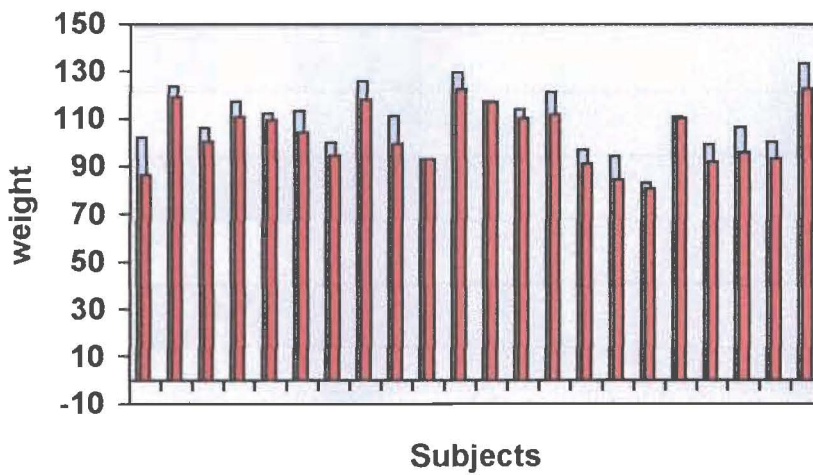
Figure 1 and 2 shows the BMI and weights of the patients at the beginning and at the end of the study.

Figure 1- BMI in kg/m^2



Red bar- visit 1, brown bar-visit 8

Figure 2- Weight in Kg



Blue bar-visit1, Red bar –visit 8

Metabolic and endocrine indices

There were no statistical significant difference in metabolic and endocrine indices measured at the start and end of the study, as detailed in table 3

TABLE 3

Table 3: Metabolic and endocrine indices.

	Visit 1(Mean ± SD)	Visit 8(Mean ± SD)	P Value
Testosterone (nmol/l)	1.96 ± 0.7	1.89 ± 0.9	0.7
SHBG (nmol/l)	47.7 ± 27.1	43.7 ± 33.9	0.22
FAI	7.3 ± 12.7	7.6 ± 8.6	0.46
Fasting glucose (mmol/l)	4.9 ± 0.5	4.7 ± 0.5	0.32
Fasting insulin (mU/l)	15.9 ± 7.1	18.1 ± 11.4	0.46
Glucose/insulin ratio	0.45 ± 0.37	0.34 ± 0.12	0.28

Abbreviations

SHBG =Sex Hormone Binding Globulin, FAI = Free Androgen Index

LIPIDS

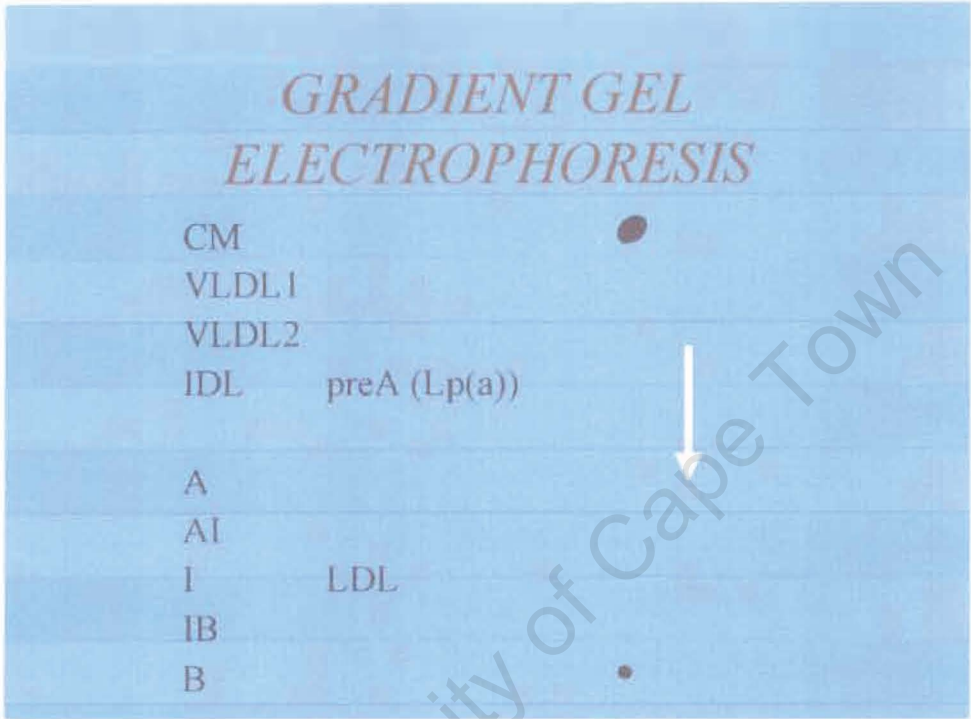
The lipid studies at the beginning (visit 1) and end (visit 8) of the study are shown in table 4. None of the parameters demonstrated a statistically significant difference. There was, however, a trend towards statistical significance in HDL levels and LDL particle size changes.

Only samples from 20 participants were analyzed for LDL particle size. Two subjects were excluded because in one case no final analysis was done, as the specimen was misplaced and in the other the lipoprotein profile did not yield defined LDL particles.

Chi squared analysis of the LDL particle sizes revealed a trend towards larger particles in visit 8 compared to visit 1 ($p=0.09$). Excluding the patient who gained weight from the analysis, demonstrated a statistical significant change in particle size distribution in the women who had lost weight ($p=0.037$). Separate analysis of patients who lost 5% or more of body mass did not show any statistical significance. The IVFTT yielded widely varying clearance constants but no significant change occurred during the study.

Gradient gel electrophoresis was performed on the samples obtained in an attempt to determine the concentrations of the different lipid particles. In figure 1, the pattern that emerges is demonstrated. Chylomicrons (CM) are the largest particles while LDL particle B is the smallest and therefore moves furthest from origin. This method separates the lipid particles based on their sizes and makes them identifiable on a plate depending on the distance traveled from origin.

Figure 3: Gradient Gel Electrophoresis



KEY:

- CM Chylomicron
- VLDL Very low-density lipoprotein
- IDL Intermediate-density lipoprotein
- LDL Low-density lipoprotein

The LDL species were divided into small, intermediate and large and the findings are shown in table 5.

TABLE 4: Fasting plasma lipids, lipoproteins and triglyceride clearance

	Visit 1 (Mean ± SD)	Visit 8 (Mean ± SD)	P Value
Triglycerides	1.32 ± 0.41	1.26 ± 0.59	0.46
Cholesterol	4.6 ± 0.98	4.5 ± 0.85	0.57
LDL-C (Derived)	2.96 ± 0.83	2.89 ± 0.73	0.52
HDL-C	1.03 ± 0.26	1.09 ± 0.3	0.09 (t)
LDL Size A:I:B	3:8:9	7:10:3	0.09 (t)
TG clearance constant	0.08 ± 0.09	0.09 ± 0.07	0.46

All values in mmol/litre.

Abbreviations

LDL-C = Low-density lipoprotein cholesterol, HDL-C = High-density lipoprotein cholesterol, TG = Triglyceride

LDL particle size is graded from large to small:

A = Large particles

I = Intermediate particles

B = Small particles.

t = trend.

TABLE 5: LDL PARTICLE SIZE AS DETERMINED BY GEL ELECTROPHORESIS.

LDL SPECIES

	VISIT 1	VISIT 8
LARGE	3	7
INTERMEDIATE	8	10
SMALL	9	3

Chi square analysis of the LDL particle sizes revealed a trend towards larger particles in visit 8 compared to visit 1, p 0.0897. There was one patient of the 21 who gained weight and if that patient were excluded from the above analysis the change would have been significant, p 0.0372.

TRIGLYCERIDE CLEARANCE

The rate of triglyceride clearance (K) was calculated using the formula $TG = D \cdot e^{-kt} + c$.

Visit 1 k was 0.079 ± 0.091 while visit 8 K was 0.089 ± 0.067 . $p = 0.46$.

This indicates that there was no significant difference in the rate of triglyceride clearance at the beginning of the study when compared to the rate at the end of the study. Separate analysis, excluding the subject who gained weight did not affect the results.

Discussion

This study was designed to examine serum endocrine, lipid and lipoprotein changes in a small cohort of subjects with PCOS who attempted to lose mass with dietary information, counselling and sibutramine. The relatively small change in mass did not have a significant impact on the concentration of lipids and lipoproteins and thus it is not surprising that no change was found in TG kinetics. Of interest, however, was the finding that LDL species changed towards larger sizes in subjects who lost mass. To the best of our knowledge, this is the first report of LDL particle size responding to a relatively modest mass reduction in PCOS despite the persistence of obesity.

Polycystic ovary syndrome is associated with an increased risk of cardiovascular disease. Dahlgren et al in 1992 compared a risk factor model for myocardial infarct in 33 women with PCOS with 132 age-matched controls. Factors used in the risk factor model included age, triglycerides, WHR, diabetes mellitus and hypertension. They found a relative risk of 7.4 of developing myocardial infarction in the PCOS group compared to controls (60). They concluded that since the risk factors include variables correlated to obesity, advice on dietary restriction is an important part of treatment of these patients. In the same group of women, Dahlgren and colleagues demonstrated a marked increase in central obesity, higher basal serum insulin concentrations and a higher prevalence of diabetes mellitus and hypertension in the PCOS group compared to age-matched controls (61).

Abdominal obesity, a common finding in PCOS patients is a recognized risk factor for the development of cardiovascular disease and is one of the

markers of the metabolic syndrome. Central to the management of PCOS is weight reduction, in particular the reduction in waist diameter. Our subjects demonstrated a significant reduction in weight with more than 70% of them losing in excess of 5% body weight with a trend towards a reduction in waist- hip ratio following treatment.

There have been a few studies in women with PCOS, which reported an abnormal lipoprotein profile. This is characterized by raised triglycerides, marginally elevated low-density lipoprotein (LDL) cholesterol and reduced high-density lipoprotein (HDL) cholesterol(23). Studies by Dejager et al in France as well as Pirwany et al in Scotland described raised concentrations and proportions of atherogenic small, dense LDL-III in PCOS patients compared to body mass index-matched controls with normal menstrual cycles and normal ovarian morphology (62;63). Factors that are known to influence LDL species include age, gender, obesity, insulin resistance and diabetes, plasma triglyceride concentration, Cholesterol Ester Transfer Protein (CETP) activity and (hepatic and lipoprotein) lipase activity (64;65).

The favorable changes in lipids in our study following weight loss included a trend towards higher HDL-C levels and larger LDL-C particle sizes. Although associated with mass reduction the mechanism appears not to involve increased lipoprotein lipase activity but may relate to changes in lipoprotein production that are influenced by obesity or a combination of subtle changes in parameters including hepatic lipase that are influenced by androgens. As smaller LDL particles are associated with an increased cardiovascular risk, this could translate into a lower risk for cardiovascular disease in these patients.

Loss of 5% or more of body weight has been shown to improve endocrine and ovarian function in obese PCOS patients(41). In the study by Kiddy et al, 5 of the 9 women with improved menstrual function conceived. In our study, 2 of the women who lost more than 5% of their body weight conceived but not all of our patients desired fertility. Mass loss improves the cardiovascular risk profile and also results in improved reproductive capacity.

There was no statistical significant difference in the endocrine profile measured at the beginning and the end of the study including fasting glucose, fasting insulin, testosterone and sex hormone binding globulin. In Kiddy's study the mean mass was 91.5 kg and they demonstrated a decrease in insulin concentrations and reciprocal changes in SHBG(41). In our study the mean mass at the beginning of the study was 109.5kg and at the end 103Kg. The higher mass of our subjects may represent a more deranged endocrine milieu and may impair the demonstration of improvement with modest weight loss. Fleming et al in a study on women with PCOS, found that morbid obesity ($BMI > 37 \text{ kg/m}^2$) was associated with an attenuation of the positive effects of treatment (with metformin) on PCOS patients with oligomenorrhoea (66)

The outcome of weight loss on lipid studies and on waist measurement would suggest that weight loss is associated with a decrease in cardiovascular disease risk associated with obesity in PCOS patients. Beneficial changes are associated with modest changes in body mass. This may in turn translate into a decrease in cardiovascular disease that has been associated with PCOS(19;20)

The dietetic counselling during the study reinforced previous counselling for mass reduction in some patients and no specific exercise regimens were advocated. Therefore the lack of impact of the study on the lipid profile may thus be due to a previous response in dyslipidaemia to dietary advice and small changes in body mass in these women.

In conclusion, weight loss in this group of patients is difficult to achieve and management should ideally include exercise, continuous positive reinforcement and the use of group therapy (67;68). These interventions are important in maintaining achieved weight loss but are difficult in poorly resourced areas, as they are expensive and require considerable co-ordination. The use of pharmacological agents such as sibutramine as well as a more aggressive dietary and exercise regime may be of value in achieving the all-important aims of weight reduction and maintenance of weight loss and should be part of the armamentarium available to the clinician for the management of obese patients with PCOS.

In our study we found that moderate weight loss achieved with calorie restriction translated into a beneficial change (non significant trend) in the LDL particle size, even though the lipid profile and metabolic indices were not statistically changed. Given the results of our study, we suggest that further research on the impact of lifestyle and dietary modification in obese patients with PCOS in our population should be undertaken. This is planned for future research

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