# The Role of Insulin Sensitising Agents in Polycystic Ovary Syndrome

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# Abstract

**Polycystic Ovary Syndrome (PCOS) is a common disorder of chronically abnormal ovarian function and hyperandrogenism whose clinical effects include oligomenorrhoea and hirsutism. It has been determined that the principal underlying defect is hyperinsulinaemia secondary to insulin resistance. Use of insulin sensitising agents in women with PCOS is becoming increasingly widespread. Trials with metformin, one such agent, enthusiastically report a wide range of benefits in metabolic, reproductive and clinical measures. We have shown that metformin treatment of women with polycystic ovary syndrome has the following effects;**

- **It may be more effective than Dianette in the treatment of hirsutism.**
- **It is effective in reducing weight and improves lipid profiles in obese women with oligomenorrhea or a raised free androgen index.**
- **It reduces serum mullerian inhibiting substance in the circulation after protracted treatment (> 4months).**
- **It has effects upon adrenal androgen biosynthesis manifested by a reduction in circulating basal concentrations of 17OHP.**

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# Presentations

## Poster Presentations

- West of Scotland Endocrine Group meeting (Glasgow, UK) (10/2001)
- Blair Bell Research Meeting (London, UK) (12/2001)
- 21st Meeting of the British Endocrine Societies (Harrogate, UK) (4/2002)
- Blair Bell Research Meeting (Sheffield, UK) (10/2002)- Second prize winner
- 193rd Society for Endocrinology (London, UK) (11/2002)
- Scottish Society for Experimental Medicine (Edinburgh,UK) (11/2002)
- The Royal Medico-Chirurgical Society of Glasgow (Glasgow, UK) (11/2002)
- 19th Annual Meeting of the European Society Human Reproduction and Embryology (ESHRE) (Madrid, Spain) (7/2003) (2 posters)

## Oral Presentations

- West of Scotland Endocrine Group meeting (Glasgow, UK) (10/2001)
- Verity UK (PCOS support group) (Edinburgh, UK) (5/2002)
- 18th Annual Meeting of the European Society Human Reproduction and Embryology (ESHRE) (Vienna, Austria) (7/2002)
- American Society Reproductive Medicine (ASRM) (Seattle, USA) (10/2002)
- 22nd Meeting of the British Endocrine Societies (BES) (Glasgow, UK) (3/2003)
- The Combined NSW/QLD Annual Scientific Meeting (RANZCOG) (Sydney, Australia) (6/2003)

- 5th Annual Scientific Meeting of RANZCOG (Auckland, New Zealand) (9/2003)

# Publications

## Published Abstracts

- Metformin or dianette treatment of hirsutism with polycystic ovarian syndrome. Harborne L, Fleming R, Lyall H, Norman J. (European Society Human Reproduction and Embryology). Human Reproduction. 2002. Vol 17. Abstract Book 1. Annual Meeting Supplement. Page 45.
- A randomised controlled trial comparing the use of metformin and dianette in hirsutism associated with PCOS. Harborne L, Fleming R, Lyall H, Norman J. (21st Joint meeting of the British Endocrine Societies). Endocrine Abstracts. 2002. Vol 3. P218.
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# Summary

Polycystic Ovary Syndrome (PCOS) is a common disorder of chronically abnormal ovarian function and hyperandrogenism. It has been determined that the principal underlying defect is insulin resistance. Use of insulin sensitising agents (ISAs), in women with PCOS is becoming increasingly widespread. Trials with metformin, an insulin sensitising agent, enthusiastically report a wide range of benefits in metabolic, reproductive and clinical measures. We wished to determine the answer to the following questions in relation to the role of metformin in the treatment of women with polycystic ovary syndrome.

- **The efficacy of metformin compared with Dianette in the treatment of hirsutism (chapter 3).**
- **If different doses of metformin reduce weight and improve lipid profiles in obese and morbidly obese women (chapter 4).**
- **If metformin reduces serum mullerian inhibiting substance levels (chapter 5).**
- **If metformin directly influences adrenal androgen biosynthesis (chapter 6).**

**Chapter 3: Metformin treatment results in a greater reduction of hirsutism in women with PCOS when compared with Dianette.** In this study we tested the hypothesis that reduction of insulin challenge by metformin treatment may reduce hirsutism in women with PCOS. This was explored by: 1) does metformin have an effect on hirsutism in women with PCOS, and 2) is metformin more or less effective than the 'gold standard' treatment of Dianette.

Patients (n=52) were randomised to receive either metformin (500mg tds) or Dianette® (ethinyl estradiol, 35ug; cyproterone acetate, 2mg) treatment for a duration of 12 months with assessments before treatment, at 6 months, and at 12 months. Both objective and subjective methods of evaluating hirsutism were used and, in addition, patient perceptions were examined. The results show that metformin is potentially an effective treatment for moderate to severe hirsutism in women with PCOS. They also suggest that, in some respects (Ferriman Gallwey score, and patient self-assessment), it is more efficacious than the standard treatment (Dianette®). The objective evaluation of hair diameter, showed that both treatments were moderately effective in reducing mean diameters at multiple anatomical sites. Dianette reduced hyperandrogenaemia significantly, and metformin reduced hyperinsulinaemia significantly. This suggests that hirsutism may be effectively treated by reducing hyperinsulinaemia.

**Chapter 4: Reduction of weight and improvement of lipid profiles using metformin in obese and morbidly obese women with PCOS**

The aim of this study was to determine whether different doses of metformin (1500 or 2550mg per day) could have different effects upon weight reduction, and circulating hormone and markers of inflammation and lipid profile parameters. Eighty two patients categorised as obese (Ob, BMI 30 to<37kg/m$^2$; n= 42) and morbidly obese (Mob, BMI $\geq$37kg/m$^2$; n=41) started treatment and 68 (82%) completed the course with assessments at the start, and at 4 and 8 months. Both the degree of weight reduction and the degree of suppression of circulating androstenedione in obese women (the Ob group) were dose related, but no similar relationship was established for the Mob group. Although generally beneficial changes in cardiovascular risk markers were recorded, there was no

relationship with either BMI category or metformin dose. Weight loss is a feature of protracted metformin therapy in women with PCOS, with greater weight reduction potentially achievable with higher doses of metformin.

**Chapter 5: Reduction of Mullerian Inhibiting Substance using metformin in women with PCOS**

The objective of this trial was to assess ovarian responses to metformin treatment in obese women with PCOS by randomization to 2 different doses of metformin treatment. Eighty-two obese women with polycystic ovary syndrome (PCOS) were recruited and markers of ovarian function were assessed after 4 and 8 months. The main outcome measures were reproductive hormone changes over time, primarily being those of androgens and mullerian inhibiting substance. Our results showed that there was no difference in the reproductive hormone changes between the doses of metformin. Significant responses to treatment were recorded for menstrual frequency and androstenedione (reduction) within the first 4 months of treatment. However, suppression of the elevated circulating MIS concentrations required protracted treatment, as no change was observed in the first 4 months – only in the second 4 month assessment period. This may be secondary to the development of a cohort of follicles, which underwent initial recruitment in an environment of reduced insulin stimulation.

**Chapter 6: Adrenal androgen biosynthesis directly influenced by metformin treatment in women with PCOS**

This study aims, by suppressing ovarian function using a gonadotrophin releasing hormone analogue (GnRHa), to measure adrenal steroid output alone in

response to a provocation test (short synacthen test) and in this way determine the effect of metformin on adrenal androgen output. A total of 18 hyperandrogenic women with PCOS, and 9 controls (women without PCOS) completed the trial. Assessments were performed at baseline (T0), two weeks (T2), and four weeks (T4). The main outcome measures were reproductive hormone changes, primarily being those of adrenal androgens. Our results suggest that GnRH-A suppression of ovarian activity had little effect upon the responses of 17OHP to standard synacthen tests or upon basal circulating concentrations of 17OHP of adrenal origin in controls or women with PCOS. However, in women with PCOS, combined GnRH-A and metformin induced reductions in both the 17OHP_AUC and also circulating basal concentrations of 17OHP. This implies that metformin treatment was responsible for suppression of both basal adrenal biosynthesis of 17OHP and its responses to exogenous ACTH stimulation. This suggests that the activity P450-17α hydroxylase may be abnormal in women with PCOS in the adrenal as well as the ovary, and that excessive insulin stimulation is the underlying controlling factor.

# Conclusion

The collection of trials contained in this thesis attempts to further elucidate the role of insulin sensitising agents in women with polycystic ovary syndrome. Metformin has been shown to be effective treatment for hirsutism, reduce serum mullerian inhibiting substance after protracted time, potentially have an effect of reducing weight in the obese when used in increased doses, and to induce reductions in both the 17OHP_AUC and also circulating basal concentrations of 17OHP suggestive of an abnormality in the enzyme P450-17α hydroxylase in the adrenal gland. This is in addition to beneficial effects on menstrual cyclicity, lipids, and reproductive hormones possibly by amelioration of insulin resistance.

Chapter 1

# Introduction

## 1.0 General Introduction

### 1.0.1 Polycystic ovary syndrome, insulin resistance and insulin sensitising treatments

Polycystic Ovary Syndrome (PCOS) is a common disorder of chronically abnormal ovarian function and hyperandrogenism affecting 5-10% of the female population of reproductive age (1). The primary aetiology remains unclear and historically there has been no consensus on absolute defining features of the phenotype. At the National Institutes of Health Conference in 1990, three key features of PCOS were generally agreed as oligomenorrhoea, hyperandrogenism (clinical or laboratory evidence), and the absence of other endocrine disorders (congenital adrenal hyperplasia, hyperprolactinemia, thyroid dysfunction and androgen secreting tumours) (2). The presence of polycystic ovaries, determined by ultrasound evaluation, was not included in this definition but this feature is mandatory in many centres. More recently, at the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop in 2003, it was concluded that PCOS is a syndrome of ovarian dysfunction along with the cardinal features hyperandrogenism and polycystic ovary (PCO) morphology. As PCOS remains a syndrome, no such single diagnostic criterion (ie hyperandrogenism or PCO) is sufficient for clinical diagnosis (3). Correspondingly, the agreed definition now rests upon demonstration of at least 2 of the following 3 criteria: oligo- or anovulation, clinical and/ or biochemical signs of hyperandrogenism, polycystic

ovaries determined by ultrasound examination, and exclusion of other etiologies. Patients with PCOS tend to present at clinics complaining of infertility, menstrual disturbance or hirsutism, with or without acne. They are therefore seen by gynaecologists, primary care physicians, endocrinologists and dermatologists.

A link between perturbed insulin action and PCOS was first highlighted in 1980 (4). Subsequent studies over two decades have convincingly shown insulin resistance to be an integral pathogenic feature of PCOS, particularly in obese individuals (5, 6). Current data indicate insulin resistance in both adipose tissue and skeletal muscle in PCOS (7). The associated hyperinsulinaemia may directly promote ovarian androgen secretion, and abnormal follicular development, which ultimately leads to dysfunctional ovarian and menstrual activity (8).

Androgens are carried in the circulation bound to sex hormone binding globulin (SHBG) with high affinity. Conditions of increased androgen concentrations and hyperinsulinaemia are associated with reduced circulating SHBG, which results in high levels of free androgen activity (9). Thus, clinical manifestations of androgen activity (hirsutism, acne and alopecia) may depend upon the SHBG activity as well as the total circulating androgen concentrations.

Other metabolic abnormalities commonly linked to insulin resistance are also evident in patients with PCOS and they include dyslipidaemia (10), increased concentrations of tissue plasminogen activator (t-PA) (11), and low-grade chronic inflammation (12). In line with these metabolic features is emerging evidence that patients with a history of PCOS, or its surrogate marker oligomenorrhoea, have an increased risk of diabetes and cardiovascular disease later in life (8, 10, 13, 14).

Until recently treatments in PCOS have been targeted towards the symptoms/signs of the disorder: ovulation induction for infertility, and anti-androgen therapy for hirsutism. In clinical terms, the most important consequence

of recognising the role of insulin resistance in PCOS is the possibility of therapeutic intervention using insulin sensitising agents (ISAs) at a more fundamental level (Figure 1.1). As a result, numerous trials of ISAs in PCOS have been reported since 1994. Non-randomised trials with metformin enthusiastically report a wide range of benefits in metabolic, reproductive and clinical measures. However, close inspection of results from the adequately controlled studies using metformin in PCOS are much more modest. This is reflected in the meta-analysis in the Cochrane Library Review by Lord et al (15) and also our systematic review (16). These reviews of randomised controlled trials using insulin sensitising agents in women with PCOS found that metformin, an insulin sensitising agent is an effective treatment for anovulation in women with PCOS. This introduction is a summary of the data from what we consider to be the most robust trials.

## 1.1 Insulin Sensitising Agents

Insulin sensitising agents, products that enhance tissue sensitivity to insulin action *in vivo*, have been used in type 2 diabetes for many years, and the ISA used most commonly in clinical practice is metformin. It is an oral biguanide antihyperglycaemic drug used for many years in Europe and is now also widely employed worldwide. Newer agents include the thiazolidinedione group of drugs of which the most widely used in the recent past is troglitazone. Unfortunately its hepatic toxicity has led to its removal, but a newer generation is now available including rosiglitazone (Glaxo SmithKline (GSK)) and pioglitazone (Takeda). Finally, D-chiro-inositol has been used with some success as an ISA in women with PCOS (17) but other clinical data are lacking.

## 1.1.1 Metformin

Metformin is thought to have primary effects on increasing peripheral glucose uptake in response to insulin, perhaps via effects at the post-receptor level, with some reduction in basal hepatic glucose production (18). However, it also lowers adipose tissue lipolysis and improves insulin sensitivity in muscle (19). Recent data suggest a unifying role of AMP-activated protein kinase in all the mechanisms of metformin action (20). It does not provoke hyperinsulinaemia and as such does not cause hypoglycaemia. It is now recommended as first line therapy in overweight patients with diabetes by most leading clinical associations (e.g. SIGN guidelines, Diabetes UK). It is also inexpensive.

### 1.1.1.1 Non-randomised trials of metformin in PCOS

Early ISA trials in PCOS, between 1994 and 1997 were mainly with metformin (21). The majority were cohort design and showed an improvement in insulin metabolism and a reduction in circulating androgen concentrations (22-32). In most cases, small reductions were seen in either BMI and/or waist-hip ratio measurements, and improvements in menstrual cyclicity (presumed ovulation) were also determined. Only one of these trials examined the effect of metformin on hirsutism and there were no data pertaining to acne (30). In general, the results were encouraging, but all trials involved small patient numbers, most were of short duration, and limited in design by not having a control or placebo arm (22-32). More importantly, none of these trials assessed ovulation incidence directly using frequent hormonal measurements.

More recent uncontrolled studies have confirmed the observations that metformin treatment effectively lowered insulin and androgen concentrations, but provided more specific evidence to support an improvement in menstrual cyclicity with

metformin (33-41). The range of perceived benefits in uncontrolled studies is considerable, with trials achieving normal menstrual frequency from 16% (n=4 of 24 cases) (30) to over 90% (39 of 43 cases) (34) of women with PCOS.

### 1.1.1.2 Results from controlled studies

Presented in this chapter are seven published metformin studies that have included some form of randomisation (i.e. control arm with or without placebo), of which five have also been double blind in design (42-48). The details of these are summarised in Table 1.1. Clearly, most attention should be focused on these studies due to the potential for bias in uncontrolled investigations. The data show that measures of obesity (e.g. BMI, waist to hip ratio) were reduced in 6 out 7 studies and insulin action and the SHBG / androgen axis improved in 5 out of the 7 studies with active treatment. Table 1.2 shows detailed analyses of the pre- and post- treatment data for BMI, fasting insulin concentration, SHBG and androgen measures in the principal studies. It demonstrates that they examined a wide range of patients with respect to pre-treatment fasting insulin levels and BMI. The most consistent findings are reductions in BMI of around 4% and in androgen measures of around 20% relative to placebo. The data on improvements in insulin level and, in particular SHBG are less convincing when considered together with placebo data. These observations show the potential for confounding effects during any prospective studies, re-emphasising the importance of control in study design.

#### 1.1.1.2.1. Ovulation

With respect to ovulation, the significant observations include i) that the interval from start of treatment to first ovulation is significantly reduced and ii) menstrual

or ovulation cyclicity is increased with the metformin groups compared to placebo, and that iii) these improvements are variable and relatively modest (Table 1.3). Table 1.3 shows a compilation of comparable ovulation data extracted from five of the seven controlled publications, all of which present their original data in different formats. However, they show that, on average, one additional ovulation is attained every five months with metformin treatment. Specifically, the increase is from 1 ovulation per 5-month interval to 2 in 5. It should be emphasised that these results relate to relatively short-term observations (up to 6 months), and that protracted treatment combined with weight loss may result in a higher frequency of normal ovarian function. Lord et al also found that metformin is an effective treatment for anovulation in women. In its ability to induce ovulation, metformin versus placebo showed an odds ratio of 3.88 (CI 2.25 to 6.69) (15).

#### 1.1.1.2.2. Time-scale of responses and ovulation

Spontaneous ovulation can occur rapidly and normal menstrual rhythm can be achieved within 3 months of starting therapy (42, 45). In a number of studies, an increased ovulation rate occurred with no change in weight suggesting that this effect is independent of weight loss (33, 35, 38, 42, 43). More recently, a randomised controlled double blind trial by Baillargeon et al found an increase in ovulatory frequency (measured by weekly progesterone levels) and amelioration of hyperandrogenaemia using insulin sensitising agents (metformin 850mg bd/ pioglitazone 4mg bd/combination/ placebo) in nonobese women with PCOS who appeared to have normal insulin sensitivity (49), again suggesting that metformin has actions independent of those of weight loss.

#### 1.1.1.2.3 Metformin in infertility: alone and in conjunction with clomiphene, FSH and in IVF

Metformin does not require intensive monitoring and current data and logic indicate a negligible risk of ovarian hyperstimulation and multiple gestation when used alone (47). It thus has potential merit as the first line treatment for ovulation induction (15).

Infertile women tend to demand a rapid resolution to their problems. If ovulation has not occurred within 12 weeks, then the anti-estrogen clomiphene citrate treatment may be added. When clomiphene citrate was used after metformin pre-treatment (42, 48) ovulation rates were higher than those achieved with placebo and clomiphene citrate (86% of patients, compared with 64%, respectively) (Table 1.4). Thus, metformin as co-treatment with clomiphene citrate appears to be successful, perhaps by sensitising follicles to FSH. Therefore, it has been proposed that metformin, in the first instance alone, and subsequently in combination with clomiphene citrate, be used in a sequential treatment programme prior to the use of gonadotrophin therapy for ovulation induction in infertile women with PCOS (50, 51). As per Lord et al the odds ratio for metformin and clomiphene citrate versus clomiphene alone for induction of ovulation was 4.41 (CI 2.37-8.22) (15).

Metformin has also been shown to possibly enhance/improve responses to ovulation induction with exogenous FSH stimulation. De Leo et al randomised 20 women with clomiphene-resistant PCOS to receive either FSH alone or FSH following 1 month pre-treatment of metformin 500mg tds (52). Compared to FSH alone, FSH plus metformin resulted in fewer dominant follicles (2.5 vs 4.5, $P<0.01$), a lower peak oestradiol and a lower cycle cancellation rate (0% vs 32%, $P<0.05$). Similarly, metformin treatment of PCOS patients undergoing in vitro

fertilisation may appear to improve outcome. Forty-six women with clomiphene-resistant PCOS, received daily metformin (1000 to 1500 mg) in half of 60 cycles prior to gonadotrophin treatment. In patients treated with metformin, the total number of follicles on the day of hCG treatment was decreased but there were more mature oocytes and both the fertilisation rate and the clinical pregnancy rate were significantly higher (Table 1.4) (40). George et al compared sequential 6 months combined treatment with metformin and clomiphene citrate with low dose gonadotrophin in clomiphene citrate-resistant PCOS women (53). Forty-five percent of women in the metformin group had improved menstrual and ovulatory function, whilst pregnancy rates were equal (16.7 vs 23.3%). However, more recently, Kjotrod et al in a prospective randomised double blind study found that pre-treatment with metformin (1000mg bd) prior to conventional IVF/ICSI in 73 women with PCOS did not improve stimulation or clinical outcome (54).
Although there are issues of gonadotrophin dose and variable sensitivity in these observations, metformin pre-treatment may allow a more orderly follicular growth in response to exogenous gonadotrophins. Table 1.4 shows that the evidence currently supporting this proposal whilst encouraging, has significant limitations. Thus, future prospective definitive studies should be undertaken (Table 1.5).
In summary, metformin appears to facilitate follicular growth and maturation in spontaneous and induced cycles leading to improved ovulation and pregnancy rates, whether given alone, or in conjunction with clomiphene citrate, or exogenous FSH.

#### 1.1.1.2.4 Metformin treatment continued into pregnancy

Consistent with improved ovulation rates is an improvement in the spontaneous pregnancy rate in several trials (35, 38, 40). With respect to pregnancy,

metformin is a category B agent, i.e. no evidence exists of animal or human fetal toxicity or teratogenicity (50, 55). Current conservative practice would recommend removal of treatment once pregnancy has been established. Two retrospective analyses (56, 57) of metformin treatment continued through the first trimester have suggested reduced rates of pregnancy loss, but data from a more recent prospective study did not support this effect (58). This area requires much more work before any recommendations can be made.

It is not known whether this apparent beneficial effect of metformin treatment is due to improved oocyte / embryo developmental potential, or improved implantation. If the latter were the case, then continuation of therapy into pregnancy would be advised, while the former would lead to no change in the advice to withdraw treatment at establishment of pregnancy. These elements demand formal prospective clarification, as do a number of clinical issues associated with metformin and pregnancy, including the well-being of the mother through pregnancy, and also the neonate (Table 1.5). Metformin has been used to treat diabetes in the second and third trimester in pregnancy (after the principal teratogenic period) and have suggested no increased perinatal morbidity although an increase in the incidence of neonatal jaundice was noted (59). A recent report of 118 diabetic pregnancies that involved use of an oral hypoglycaemic agent suggested an association of metformin treatment in the third trimester with a significantly higher perinatal mortality and prevalence of pre-eclampsia (60). However, compared to the reference group of women treated with insulin or sulphonylurea, women given metformin had significantly higher pre-pregnancy BMI (24.8 & 22.8 vs 31.2 kg/m$^2$, respectively) and were older. This difference in baseline characteristics, rather than metformin per se, might have accounted for the study findings.

#### 1.1.1.2.5 Caveat: metformin and pregnancy in obese women

Many patients with PCOS are obese, and pregnancy complications are linked to maternal obesity. Therefore pregnancies achieved in women with PCOS using metformin may lead to a greater proportion of adverse obesity-related pregnancy complications. If metformin is used prior to and continued through pregnancy, then carefully designed prospective and ideally randomised trials of metformin in pregnancy are required to determine pregnancy outcomes in mother and neonates (Table 1.5). There is also the ethical issue of assisting grossly obese women to achieve pregnancy when the mother and baby are at increased risk under normal circumstances (61) in the absence of a weight loss programme prior to conception.

#### 1.1.1.2.6 Metformin and ovulation: prediction of who will benefit

There is little published evidence concerning this important issue. It is predicted that metformin should have best results on ovulation rates in infertile women who are most insulin resistant (provided the dose schedule is satisfactory). However, the picture is not entirely clear. Moghetti et al found that higher BMI, plasma insulin, lower serum androstenedione, and less severe menstrual abnormalities were baseline predictors of clinical efficacy measured by improved menstrual cyclicity (43). In contrast, sub-group analysis in the large randomised study by Fleming et al revealed that BMI and insulin measures did not predict the ability to establish normal ovarian function, but high SHBG and lower free androgen index did (47). However, in the meta-analysis by Lord et al, it is suggested that it would be potentially beneficial to investigate those who have the greatest initial hyperandrogenaemia and/or hyperinsulinaemia, as the review found no effect on weight loss or change in body fat distribution as assessed by waist:hip ratio (15).

One of the factors to be resolved at this stage is the relationship between dose and body mass. It is also not correct to assume that lean women with PCOS will not respond to metformin treatment. Nestler and Jakubowicz have shown significant reductions in insulin response to glucose load and androgen levels in lean PCOS women (mean BMI of 21.7 $kg/m^2$) with metformin 500mg tds despite no change in BMI but WHR was reduced in the active group alone (6). More recently this is supported by Maciel et al in a randomised controlled study of 29 obese and non-obese patients with PCOS treated with metformin (1.5g/day) for 6 months (62). In this trial, non-obese women demonstrated increased responsiveness with regard to androgen levels, menstrual cyclicity, and metabolic features. However, they did not employ interval progesterone levels to objectively assess ovulation.

### 1.1.1.2.7 Metformin use for women wishing cycle regulation but not pregnancy?

The data discussed above indicates that metformin alone will not restore normal menstrual cyclicity in every woman with PCOS, at least in the short term. In fact, achieving normal ovarian function appears to occur in less than half of those women prescribed metformin at current doses. Thus, in women not wishing to become pregnant, the oral contraceptive pill may be a better agent to regulate cycles. However, these women tend to be insulin resistant, which is a state that is exacerbated by oral contraception (63), although the clinical relevance of such effects requires clarification. The use of the combined oral contraceptive is cautioned in obese women (BMI>30$kg/m^2$) and contraindicated in morbidly obese women (BMI>37$kg/m^2$). This should be taken into account when addressing this issue.

#### 1.1.1.2.8 Ovarian function, metformin and mullerian inhibiting substance

Mullerian Inhibiting Substance (MIS), a dimeric glycoprotein of the TGFβ super-family, is believed to act in a paracrine fashion after birth to regulate granulosa cell and oocyte function, and it may be an important regulator of follicular recruitment. Maximal expression is seen at the small antral follicle stage where there are also a large number of granulosa cells. Correspondingly, MIS has been found to be raised in the circulation of women with PCOS. A recent small trial by la Marca et al found that the HOMA score (a measure of insulin resistance) was positively related to serum MIS levels (64). However, there exists no other current data relating insulin and MIS levels. The effect of ISAs on circulating concentrations of MIS in PCOS has so far not been explored. In this thesis we undertook a trial to determine the effect of metformin on the relationship between MIS and other reproductive parameters in obese women with PCOS.

#### 1.1.1.2.9 Metformin and adrenal androgens

High circulating androgen concentrations may be produced by either the ovary or by the adrenal gland. About 25% to 60% of women with hyperandrogenism display excessive adrenal androgen levels (65, 66). One of the effects of metformin (in the majority of studies) is to reduce the circulating androgen concentrations. However, it is difficult to detect by analysing serum androgen concentrations whether metformin is exerting its effects at the level of the ovary or the adrenal gland, or both. It has been hypothesized that the dysregulation of cytochrome P450-17α hydroxylase activity may be responsible for the abnormalities seen in both the ovary and the adrenal gland (67). Studies have shown that metformin treatment can influence the ovarian production of 17

hydroxyprogesterone (17OHP) through the activity of cytochrome P450-17α hydroxylase activity in women with PCOS (24, 67). However, the possibility of a contributory effect of adrenal origin was not explored. The study contained in this thesis attempts to examine whether suppression of ovarian function using a gonadotrophin releasing hormone agonist (GnRHa), and provoking adrenal function in the absence and presence of metformin, may reveal an effect of metformin upon adrenal steroid output.

#### 1.1.1.2.10 Metformin and hirsutism

Six trials, other than our own have examined the treatment of hirsutism with metformin (1.5g to 1.7g daily) (30, 33, 36, 43, 45, 68). In only one of these was hirsutism the primary end-point (68). Three of the studies showed no change (30,33,43) and three demonstrated modest but statistically significant reductions in the Ferriman Gallwey (FG) score after metformin treatment. It is important to note that most of the trials were only of 3-6 months duration, and included relatively small numbers. In addition, no breakdown of anatomical site was included. Because of the duration of the average hair growth cycle, a response to any treatment is generally not seen within 6 months. At least 12 months should be allowed to optimise potential effects of treatment upon hair growth, so future trials need to be more focused, and of considerably longer duration. It is also important to point out that the mean FG scores pre-treatment in the studies published so far represented only moderate hirsutism (between 8 and 15). It may be that a larger effect may be seen with higher pre-treatment scores consistent with more severe hirsutism. In this thesis are presented the results of a trial we undertook to compare metformin with an established therapy. This was performed in a randomised manner, and an objective method of hair growth was employed.

Prior to our study being undertaken, there was no evidence to support a clinically significant effect of metformin on hirsutism.

#### 1.1.1.2.11 Metformin and acne

Other than our own study, described in detail later in this thesis, to date there is only one trial published addressing acne as a specific end-point. Kolodziejczyk et al found a statistically significant decrease in acne score from 1.45 to 1.14 ($p<0.001$) (36). However, this trial was not placebo-controlled and the clinical significance of the decrease is uncertain. More data are required (Table 1.6).

#### 1.1.1.2.12 Metformin in adolescents

A recent small (n=11) non-randomised study suggests that metformin (1.5 to 2.55 g/day) can achieve normal menstrual frequency in teenage girls in association with weight loss and reductions in testosterone concentration (69). The data are consistent with results in adult women with PCOS, but more rigorous, randomised studies are required.

#### 1.1.1.2.13 Cardiovascular risk factors

Women with PCOS show increased cardiovascular risk factors compared to weight matched controls with normal ovarian function (10). The evidence is broadly consistent for deranged lipids and greater glucose intolerance in women with PCOS. Evidence of hypertension was less consistent. There are also preliminary data now for altered haemostatic (tissue plasminogen activator antigen) (11) and inflammatory (C-reactive protein concentrations) (13) factors. A recent trial by Boulman et al found that 36.8% women with PCOS had C-reactive protein (CRP) levels above 5mg/liter, and only 9.6% of controls exhibited high

CRP levels (70). These subjects included 116 PCOS patients and 94 body mass index-matched controls. CRP is a strong independent predictor of future cardiovascular disease and/ or stroke. In line with such data, invasive and non-invasive tests have reported greater atherosclerotic burden in PCOS, in different vascular beds (12). Moreover, a recent prospective study has linked menstrual irregularity, approximately 80% of which is due to PCOS, to elevated risk of fatal CHD (14).

In clinical terms, all women with PCOS should have an assessment of fasting glucose to screen for diabetes. However, determination of lipid profiles will not alter management, according to current data, until these women reach the age of 35 - 50, unless there are significant other risk factors, such as obesity or family history.

#### 1.1.1.2.14 Cardiovascular parameters and metformin

Four of the studies in Table 1.1 (43, 44, 46, 47) examined circulating lipid profiles before and after 4 to 6 months treatment, and in comparison with controls. Two showed an increase in HDL-cholesterol concentration of around 0.10 mmol/L with metformin, and a trend towards lower LDL-cholesterol levels was also noted in one. One reported a significant reduction in free fatty acid concentrations (44). These changes are in keeping with improvements in insulin sensitivity and also reduced body mass. Lord et al found that metformin had a significant effect on not only fasting insulin levels but also blood pressure and low density lipoprotein cholesterol (LDL) (15). However, larger and more focused studies are needed, examining established and novel risk factors for coronary heart disease in women with PCOS, such as inflammatory and clotting parameters. The potential effect of metformin on factors associated with chronic inflammation would be particularly

important to establish, since a pro-inflammatory phenotype has now been linked to risk for diabetes and coronary heart disease in men and women (71, 72). Presented in this thesis are the results of a randomised trial we undertook, employing two differing doses of metformin to examine the effects on not only weight loss but also a variety of parameters designed to examine risk factors including lipids and C-reactive protein.

If women with PCOS are proven to be at excess risk for CHD and type 2 diabetes, then there may be a case for long term treatment with metformin. The United Kingdom prospective diabetes study (73) showed that metformin treatment was more effective in reducing CHD risk than either insulin or sulphonylurea. More recently, the Diabetes Prevention Program Research Group examined the effects of metformin, life-style modification or placebo, during a 4 year prospective programme, upon the incidence of onset of type 2 diabetes in non-diabetic persons with elevated glucose concentrations (74). They showed that metformin treatment resulted in a 31% reduction in the incidence of diabetes, compared with placebo. Interestingly, they demonstrated that intensive life-style intervention, involving at least a 7% weight loss and at least 150 minutes of physical activity per week, was associated with a much greater (58%) reduction in the incidence of diabetes onset. A similar reduction in BMI in PCOS women can improve ovarian function and fertility (75, 76) and thus, there is a strong basis to emphasise the enormous benefits women with PCOS could achieve with lifestyle measures alone. In particular, the fact that such benefits can exceed effects of any pharmaceutical intervention should be strongly stressed.

#### 1.1.1.2.15 Side effects with metformin

Side effects of metformin like nausea, vomiting, and diarrhoea may cause difficulties initially, leading to problems with compliance (47). Many physicians use a graduated dosing system, increasing dose on a weekly basis to attempt to ameliorate this problem. A reduced frequency of dosing may be preferred when attempting to optimise patient compliance and as a result some advocate switching from a dosage regimen of 500mg tds to 850mg bd. However, the use of 850mg tablets in the study by Fleming et al (47) showed a three-fold higher dropout rate in the metformin group compared to placebo. This drawback has not been reported in other studies. Finally, a sustained release form of metformin (Glucophage XR) is now available with a reported side effect profile similar to placebo.

#### 1.1.1.2.16 Contraindications to metformin treatment

As metformin is contraindicated in patients with hepatic or renal impairment caution must be taken prior to commencing patients on this treatment. Thus, liver and renal function testing is necessary in advance of prescription and thereafter yearly testing on treatment in indicated. Treatment is also contraindicated in conditions that predispose to lactic acidosis.

## 1.1.2 Thiazolidinediones

The thiazolidinediones sensitize peripheral tissues to insulin. They are synthetic ligands that bind to an orphan nuclear receptor called peroxisome proliferator-activated receptor gamma (PPARγ) and affect the expression of a number of genes. Thiazolidinediones bind to the orphan nucleotide receptor PPAR gamma which after dimerisation with the retinoid X Receptor (RXR) enters the nucleus.

The complex of receptors interacts with peroxisome proliferator responsive elements (PPRE) which activate the transcription of a number of genes involved in lipid and glucose metabolism. Observed effects of thiazolidinediones include increased glucose transporter expression (GLUT 1 and GLUT 4), decreased hepatic glucose output, decreased free fatty acid levels, and increased differentiation of preadipocytes into adipocytes. Troglitazone, which is now unavailable, due to reports of hepatic toxicity, is the most researched agent of the thiazolidinediones in the context of PCOS.

Initial trials with this agent by Dunaif et al (22) and Ehrmann et al (23) showed improvements in insulin metabolism, androgen, SHBG, LH, and oestrogen concentrations, and an improvement in reproductive function in women with PCOS.

As a follow up to the above data, an exemplary, large multicentre, dose-determining study with troglitazone has been carried out (77). The trial included 410 women with PCOS in a multicentre, double blind study over a 44-week period randomised to either placebo or 3 different dosages of troglitazone. Three hundred and five (74.4%) patients completed the study. They found that ovulation rates were significantly higher for patients treated with 300mg and 600mg troglitazone daily than for those receiving placebo. Fifty seven percent ovulated over 50% of the time in the 600mg troglitazone arm compared with 12% of placebo treated patients. Although not an entry criterion to this study, it was found that approximately 50% of the patients had a Ferriman Gallwey (FG) score of 6 or more prior to treatment, representing normal to moderate hirsutism. The mean basal FG scores were of the order of 14 in each arm. There was a dose-related decrease of 15% in the FG score by 20 weeks therapy that was only statistically significant with a dose of troglitazone 600mg. The authors also found that 600mg troglitazone reduced circulating insulin by 53%, and the insulin response to an

oral glucose challenge by 45%. There was also a decrease in circulating free testosterone concentrations, and a rise in SHBG.

Clearly, the above data pave the way for studies with newer PPARγ activators, such as rosiglitazone and pioglitazone in women with PCOS. These agents appear to avoid the hepatic side effects reported for troglitazone and are increasingly used in the management of patients with type 2 diabetes. They also appear to reduce surrogate risk factors for vascular disease and large prospective multicentre trials are underway to determine whether they lessen CHD risk and incidence of diabetes type 2. More recently, Legro et al studied 398 women with polycystic ovary syndrome and dyslipidaemia associated with insulin resistance (78). Troglitazone (3 different doses) or placebo were randomly assigned and taken for 44 weeks. There was no significant response of any of the circulating lipids to treatment with either placebo or one of the troglitazone arms. Further treatment and surveillance may be required.

### 1.1.2.1 Caveats of thiazolidinedione treatment

There are two notes of caution when considering the use of these agents in PCOS, however. Firstly, like troglitazone, they tend to increase body mass, and as many women with PCOS are already overweight then this can be an unwelcome effect. Secondly, and more importantly, although pregnancy has been reported in a woman with PCOS following treatment with rosiglitazone (79) these are pregnancy class C drugs which means that there is evidence of teratogenicity in animal studies including lethality. Specifically, fetal growth restriction has been noted and attributed to a reduction in the maternal hyperinsulinaemia and insulin resistance that occurs during pregnancy thereby

reducing the availability of metabolic substrates for fetal growth. Clearly, these findings mean that extreme caution must be taken with human studies.

## 1.2 Conclusion

We performed our own review of what we considered the best available evidence on the use of metformin in women with PCOS (Table 1.6 presents a summary of findings). There was considerable variation in the patients examined and the methods of assessment used, but a consistent yet moderate improvement in ovarian function was established. It is evident, that on average, women treated with metformin have one more ovulatory event and menstrual period in every five months – from one ovulation per five months to two. Thus, ovulation frequency is by no means restored to normal in every woman with metformin use. The accumulated data also suggests that metformin reduces weight by around 4% in women with PCOS. Lord et al found no effect on body mass index or waist:hip ratio.

Patients with symptoms related to PCOS are increasingly demanding treatment with metformin, regarding it as the panacea for their symptoms. They are receiving treatment for unlicensed indications, and often outside the research environment. The knowledge base supporting these actions does not exist, with the exception of ovulation induction.

To date, all studies reported have been investigator led, and existing evidence is of insufficient validity for pharmaceutical companies to proceed to licensing procedures. Therefore, it is important that clinicians counsel women appropriately prior to commencement of metformin therapy.

Many aspects of ISAs and PCOS remain to be determined definitively including those of ovulation induction and infertility treatment. These include the

complications and outcomes of pregnancy in obese women, and metformin dose and its relation to body mass. Prior to this thesis data on effects on hirsutism and acne were limited, such that no recommendations could be made. In this thesis we have attempted to address the existing knowledge base to provide some answers to further elucidate the action of metformin in women with PCOS.

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The aims of this thesis were to address the dearth of information in the following areas:

**1) To determine the efficacy of metformin compared to Dianette in the treatment of hirsutism in women with polycystic ovary syndrome.**

**2) To examine the effects of different doses of metformin in obese women with PCOS: roles in weight reduction and improvement in lipid profiles.**

**3) To investigate the role of Mullerian Inhibiting Substance in obese women with PCOS, and to determine the effects of metformin treatment.**

**4) To determine if metformin directly influences adrenal androgen biosynthesis in women with polycystic ovary syndrome.**

These examinations were all intended to provide more substantial evidence in the area of insulin metabolism and PCOS, at the same time as compounding the information regarding the use of metformin in PCOS. We would have expected to produce many questions from these explorations, and indeed they have been forthcoming, but at the same time, I believe that some important questions have been answered.

**Figure 1.1**

The postulated role for insulin sensitising agents in the scheme describing the range of consequences linked to insulin resistance in women with PCOS.

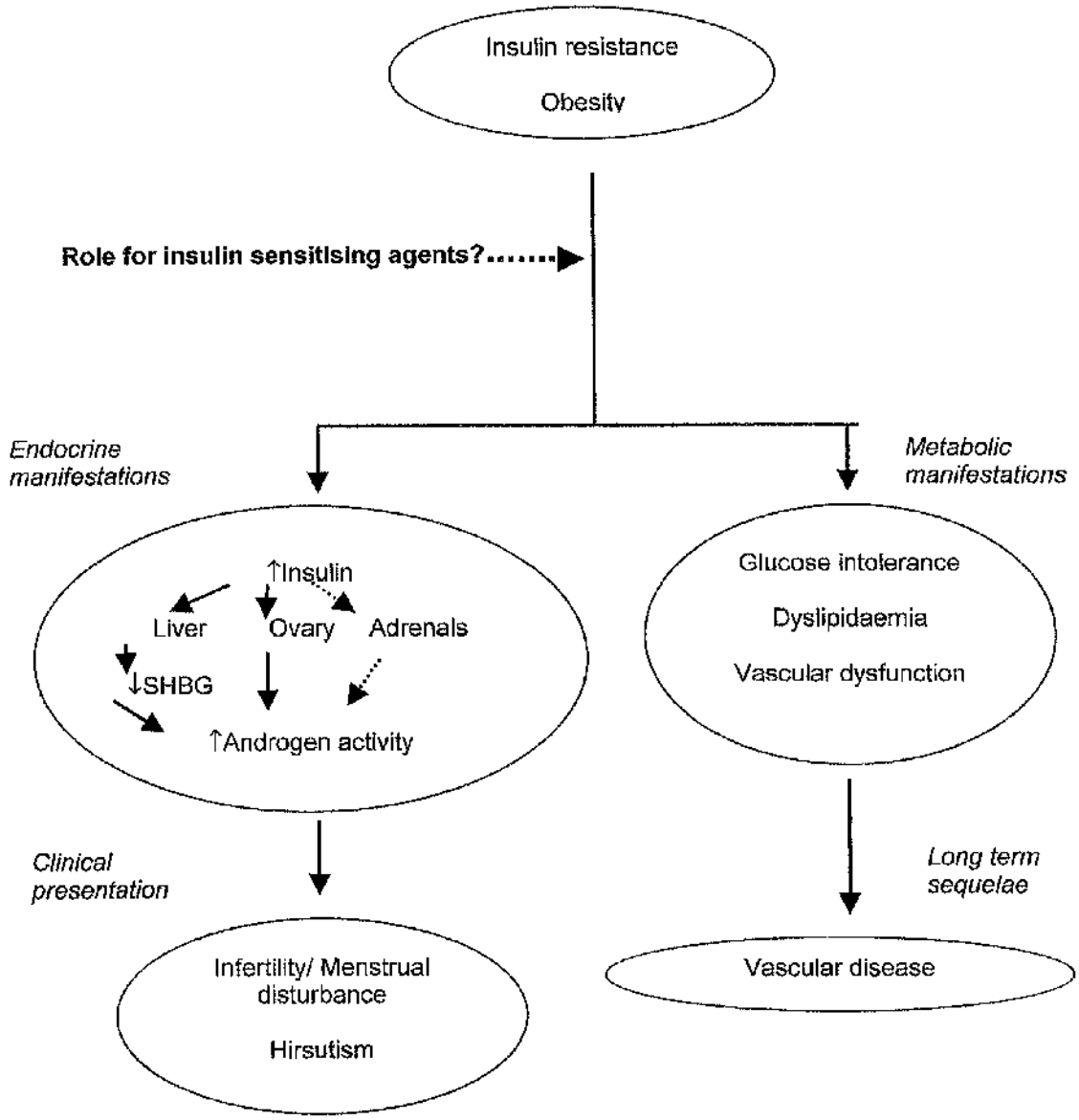


**Table 1.1**

Summary of findings from trials of metformin in PCOS that included a randomised component

| | STUDY CHARACTERISTICS | | | | Δ IN KEY STUDY PARAMETERS | | | |
|---|---|---|---|---|---|---|---|---|
| | **No.** | **Design** | **Dose** | **Mean basal BMI** | **Δ Adiposity measures** | **Δ Insulin resistance measures** | **Δ Testosterone SHBG** | **Δ Lipids** |
| Nestler et al[38] | 35 (M) 26 (P) | RPCT (35 days) | 500 tds intially then + clomiphene | 32 | Y | Y | Y | - |
| Moghetti et al[39] | 23 | RPCT-DB (6 months) | 500mg tds | 27(M) 33(P) | N | Y | Y | ↑HDL-C |
| Morin-Papunen et al[40] | 32 | RCT vs Diane Nova (D) (6 months) | 500 bd then 1g bd | 32·5 | Y | Y | N | ↓FFA's |

(Table 1.1 cont.)

| | No. | Design | Dose | Mean basal BMI | Δ Adiposity measures | Δ Insulin resistance measures | Δ Testosterone SHBG | Δ Lipids |
|---|---|---|---|---|---|---|---|---|
| Pasquali et al[41] | 20 | RPCT-DB (6 months) | 850mg bd | 39·8 | Y | Y | Y | - |
| Ng et al[42] | 40 | RPCT-DB (3 months) | 500mg tds | 24·1 (M) 23·8 (P) | Y | N | Y | No change in lipids |
| Fleming et al[43] | 94 | RPCT-DB (4 months) | 850mg bd | 35·3 | Y | N | N | ↑HDL-C; trend ↓LDL-C |
| Kocak* et al[44] | 56 | RPCT-DB 2 cycles | 850mg bd cycle one; 2nd cycle clomiphene added | M, 31·9 P, 30·8 | Y | Y | Y | - |

RCT= randomised controlled trial; RPCT = randomised placebo controlled trial; DB= double-blind; M=metformin; P=placebo; LDL=low density lipoprotein, HDL=high density lipoprotein; FFAs= Free fatty acids.

**Table 1.2**

Detailed changes in insulin, SHBG and BMI in controlled PCOS metformin studies

| | BMI (kg/m$^2$) | | | | Insulin (IU/mL) | | | | SHBG (nmol/L) | | | | Androgen measures | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Metformin | | Placebo | | Metformin | | Placebo | | Metformin | | Placebo | | Metformin | | Placebo | |
| | Pre | %Δ | Pre | %Δ | Pre | %Δ | Pre | %Δ | Pre | %Δ | Pre | %Δ | Pre | %Δ | Pre | %Δ |
| Nestler et al[38] | 32·3 | NA | 32·2 | NA | 19 | NA | 22 | NA | 2·0# | +35 | 2·7# | +33 | 1·0[a] | -20 | 0·8 | -20 |
| Moghetti et al[39] | 27·1 | -4·0 | 32·6 | -2·1 | 15·2 | -33 | 20·1 | +4 | 35·6 | +25 | 33·5 | +3 | 11·6[a] | -16 | 10·7 | -3 |
| Morin-Papunen et al[40] | 32·5 | -3·6 | NA | NA | 99* | -26 | NA | NA | 32·0 | -17 | NA | NA | 11·5[c] | -15 | NA | NA |
| Pasquali et al[41] | 39·8 | -8·5 | 39·6 | -4·0 | 43 | -49 | 33·5 | -43 | 18·7 | -11 | 16·0 | -14 | 0·68[b] | -28 | 0·51 | -8 |
| Ng et al[42] | 24·1 | -3·7 | 22·7 | +1·8 | 10·8 | -25 | 12·1 | -40 | 28·7 | -7 | 36·6 | -10 | 1·8[b] | -33 | 1·5 | +13 |
| Fleming et al[43] | 35·2 | -2·0 | 35·3 | +0·9 | 16·8 | -2 | 18·4 | -5 | 29·2 | +4 | 28·1 | +9 | 10·3[c] | -5 | 13·7 | +4 |
| Kocak et al[44] | 31·9 | -4·4 | 30·8 | +1·0 | 28·1 | -24 | 21·3 | +5 | NA | NA | NA | NA | 1·5[b] | -29 | 1·3 | 0 |
| Average (% change) | | **-4·4** | | **-0·5** | | **- 27** | | **- 16** | | **+5** | | **+4** | | **-21** | | **-2** |

The placebo data are omitted from study by Morin-Papunen et al, because it was treatment with Diane Nova®, not placebo. Unit discrepancies: *pmol/L, #µg/dl. For testosterone measures, a=free testosterone, b=total testosterone and c=free androgen index - please refer to individuals papers for relevant units. NA = result not available.

**Table 1.3**

Examination of ovulation/cyclicity data in relevant trials including, where possible, extraction of cycles per hundred patient months during placebo and metformin phases.

| | | Cycles per hundred patient months | | |
|---|---|---|---|---|
| | **Δ in ovulation parameters** | **Placebo rates** | **Metformin rates** | **Difference** |
| Nestler et al[38] | ovulation 34% M, 4% P<br>+ clomiphene: 90% M, 8% P | 4 | 34 | 30 |
| Moghetti et al[39] | ↑0.35 cycles/month<br>↑cycles 50% women (M), no Δ P | 22 | 59 | 37 |
| Morin-Papunen et al[40] | time between periods<br>M=98 to 69 days, D=88 to 28 | 32* | 44 | 12 |
| Pasquali et al[41] | ↑cycle frequency<br>M>P | 29 | 39 | 10 |
| Ng et al[42] | Median ovulation rates 0% in both M and P groups | NA | NA | NA |
| Fleming et al[43] | normalised cycles M=30%, P=18%<br>Days to ovulation M=23, P=42 | 18 | 30 | 12 |
| Kocak et al[44] | Ovulations over 2 cycles:<br>M 78%, P 14% | NA | NA | NA |
| ***Averages*** | | **21** | **41** | **20** |

M=metformin; P=placebo; D=Diane Nova

For a normally menstruating woman the cycles per hundred patient months column would =100.

*compared to baseline pre-treatment value as no placebo group.

NA – data not available as either effect of metformin use alone[44] or mean ovulation rates[42] were not reported.

**Table 1.4**

Summary of benefits of metformin use in ovulation induction and early pregnancy

| | **Strength of Evidence** | **Magnitude Of Benefit Noted** |
|---|---|---|
| Spontaneous ovulation | + | Non-randomised trials: up to 90% improvement<br>Randomised trials: 0-35% improvement |
| With clomiphene | + | Ovulation rates 64-88% of cases higher than placebo |
| With FSH and Prior to IVF | + | (compared with FSH alone)<br>↓ dominant follicles<br>↓ peak oestradiol<br>↓cycle cancellation rate (0% vs 32%)<br>-<br>more mature oocytes<br>↑fertilisation rate (64% vs 43%)<br>↑clinical pregnancy rate (70% vs 30%) |
| Pregnancy Loss | +/- | 73% prior to treatment Vs 10% with metformin<br><br>42% control group Vs 13% with metformin<br><br>35% spontaneous abortion rate in series of PCOS women given metformin up to 12 weeks gestations |

**Table 1.5**

Areas where more (prospective) data is required

| **Areas for future studies** |
|---|
| Dose studies and effects on ovulation |
| Effects on stimulated ovarian function |
| Lean women with PCOS |
| Ethnic variability of response |
| Compliance issues |
| Acne and Hirsutism |
| Early pregnancy loss |
| Pregnancy outcome |
| Neonatal outcome |
| Effects on surrogate risk factors for CHD |
| Longer term risk for diabetes & CHD |
| Effects of prolonged treatment |
| New insulin sensitising agents (ISAs) |
| Determination of baseline factors predicting benefit to ISAs |

**Table 1.6**

Summary of evidence for benefit in key areas

| Key area | Summary of evidence |
|---|---|
| Ovulation | Metformin treatment achieves only a modest improvement in ovulation rate: on average increasing from a baseline of one ovulation every five months to two. |
| Ovulation induction | Metformin enhances ovarian function when used in conjunction with clomiphene, but more data are needed. |
| Weight and androgens | Metformin achieves modest reductions in body mass and androgenicity. |
| Pregnancy | More precise controlled data are required on pregnancy and neonatal outcome. In particular, caution may need to be taken with ovulation induction in obese women with PCOS. |
| Hirsutism and acne | There exists insufficient evidence to warrant metformin for first line therapy for treatment of hirsutism and acne. |

Chapter 2

# Patients and Methods

## 2.0 Summary

This chapter provides a description of the general protocols for the clinical and laboratory techniques used in the studies described in this thesis. All patient information sheets and data recruitment forms for the relevant trials are included in the Appendix.

## 2.1 Location

Studies were performed at the Royal Infirmary (Glasgow) in the areas of Ward 33, the Assisted Conception Unit, and the University Department of Obstetrics and Gynaecology, Division of Developmental Medicine.

## 2.2 Patients

Patients with PCOS and controls were recruited from the Reproductive Endocrinology and Assisted Conception Unit clinics at the Royal Infirmary, North Glasgow University NHS Trusts. The North Glasgow University Trust Ethics Committee approved all studies. All patients gave informed consent for inclusion in each respective trial.

## 2.2.1 Patients with PCOS

All patients with PCOS met the criteria defined below, and those who also had co-morbidities, including the specific disorders of adult onset congenital adrenal hyperplasia, hyperprolactinaemia, thyroid dysfunction, diabetes mellitus and androgen secreting tumours were excluded. The tests for these were performed using standard laboratory testing for 17αhydroxyprogesterone, thyroid function tests, fasting glucose, and total testosterone. Other exclusion criteria were those of medication for treatment of dyslipidaemia, hirsutism, or reproductive causes. Patients were required to fulfil all entry criteria prior to providing informed consent. Organisation, recruitment, clinical assessments and blood sampling for all trials were performed by myself, while the hormone assays were carried out by MM, DL, EM, and NS in the university department gynaecology laboratory, and the university department of vascular biochemistry. Statistical advice and analyses were performed by RF and NS (see acknowledgements).

## 2.2.2 Definition of PCOS

The parameters used in this thesis are based on the criteria set by the National Institutes of Health conference held in Bethseda, Maryland, USA in 1990, with the addition of ultrasound criteria. At this conference the following diagnostic criteria were put forth: clinical or biochemical evidence of hyperandrogenism, chronic anovulation, and exclusion of other known disorders (2). The patients included in this thesis were defined as having polycystic ovary syndrome based on the following: raised free androgen index (>7.9) +/- oligo/amenorrhea (<6 cycles/year), +/- polycystic ovaries on ultrasound (80).

Subsequent to the finalisation of work included in this thesis was the ESHRE/ASRM-sponsored PCOS consensus workshop group conference held in

Rotterdam, The Netherlands, in 2003. The consensus on defining criteria from this international conference are as follows (2 out of 3): oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries determined by ultrasound examination, and exclusion of other etiologies (3). Our defining parameters for PCOS included in this thesis are consistent with this most recent consensus.

## 2.2.3 Definition of PCO

Echographic diagnosis of polycystic ovaries in this thesis was carried out according to the published criteria by Adams et al (80). Measured were follicular diameter of the largest follicle present and total number of follicles (TFN) (at least 10 follicles) present in one mid-point 'slice' through each ovary, with diameters (FD) of 2 - 9mm. An ovarian volume of >10cm$^3$ was deemed as being consistent with PCO.

Following finalisation of the work included in this thesis a description of the morphology of the PCO was generated by the ESHRE/ASRM-sponsored PCOS consensus workshop group conference held in Rotterdam, The Netherlands, 2003 (81). It was decided that the criteria fulfilling sufficient specificity and sensitivity to encompass this definition should have at least one of the following: either 12 or more follicles measuring 2-9mm in diameter, or increased ovarian volume (>10cm$^3$). Thus, our ultrasound criteria for PCO included in this thesis corresponds to this most recent definition, the only difference being the TFN consistent with PCO (>10 in this thesis, as opposed to 12 or more in most recent published definition).

## 2.2.4 Body weight

Body weight was measured in kilograms to the nearest 0.1kg of weight. All patients had heavy clothing and shoes removed. The same set of scales was used for all studies (Weylux Scales; Model 424; England, UK)). The scales were maintained and calibrated by the hospital's medical physics department.

## 2.2.5 Body height

Height was measured in centimetres to the nearest 0.5cm (Weylux Scales; Model 424; England). All patients removed their shoes.

## 2.2.6 Body mass index

Body mass index (BMI) was calculated using the equation:

$$BMI = \frac{\text{Body weight (kg)}}{(\text{Height (m)})^2}$$

## 2.2.7 Waist/ hip ratio

Waist and hip circumferences were measured to the nearest centimetre with a soft 1cm wide tape as per WHO criteria. Generally, the waist measurement was ascertained as being the minimum value between the iliac crest and the lateral costal margin, and the hip circumference was determined to be the maximum area around the buttock region.

### 2.2.8 Blood pressure

All blood pressure measurements (both systolic and diastolic) were performed with a manual sphygmomanometer in a standard clinical method. The heart sound Korotkoff 4 was taken to ascertain diastolic measurement. Where appropriate a large cuff was used. This was to ensure that the cuff had a width approximately 40% of the circumference of the arm and the inflation bladder encircled the arm to apply even occlusive pressure over the brachial artery. All measurements were performed with the patient in a semi- recumbent position after 10 minutes rest.

## 2.3 Hair measurement

### 2.3.1 Ferriman-Gallwey score

Hirsutism was assessed by measurement with the modified Ferriman-Gallwey score (FG score) (82) (see Appendix). Specifically, the eleven body sites containing hormone-sensitive hairs are graded from 1 (minimal terminal hair) to 4 (frank virilization), and the grades in each of these areas are summed. A score of 8 or more indicates hirsutism. Hirsutism was classified as mild, moderate and severe when the FG score was 7-9, 10-14 and >15 respectively. The FG score is semi-quantitative, with inter-observer and some intra observer variation. However, it has been shown to be reproducible to a level of 3 points (83). FG score was always evaluated by the same physician (LH). On calculating the FG score for an individual patient's hirsutism on 4 separate occasions there was no difference noted for intra-measure coefficient of variance.

## 2.4 Transvaginal ultrasound

Transvaginal ultrasound was performed with an ultrasound imaging system (Sonoline Sienna; Siemens Medical Systems Inc.) and a 6.5MHz vaginal transducer (6.5EV13) with 160 degrees sector angle focused at 3cm. The value for ovarian volume was calculated automatically by the ultrasound system after the measurements for length, width, and depth were entered. All examinations were performed using the same ultrasound system. The formula for ovarian volume as set on this particular system used the simplified formula for ellipsoids: 0.5233 x length x width x depth (84).

## 2.5 Questionnaires

In the hirsutism trial, patients were asked to assess their own status of hirsutism and acne, and the effects of treatment at baseline (T0), 6 months (T6) and 12 months (T12). This was estimated in a quantitative manner using a mark on a visual analogue sliding scale. In the same questionnaire, they were asked to assess change in hair quality, and their need for use of cosmesis (ie methods of hair removal) at T6 and T12, using Boolean operators (see Appendix).
As part of the weight loss trial, patients were required to complete a comprehensive diet and exercise questionnaire obtained from the Department of Dietetics and Nutrition at the Royal Infirmary, Glasgow. This was in an attempt to ascertain if the effect of weight loss was independent of the factors of diet and/or exercise thus being wholly due to metformin treatment alone. Patients were asked to complete these questionnaires at baseline (T0), 4 months (T4), and 8 months (T8) (see Appendix).

## 2.6 Side Effect Profiles

In the hirsutism trial, a side effect profile questionnaire was completed after 2 months (T2), at 6 months (T6) and 12 months (T12), and in the weight loss trial at 1 month (T1), 2 months (T2), 4 months (T4) and 8 months (T8) to assess worsening of symptoms compared with baseline, using Boolean operators. Issues covered included, reduced appetite, nausea, vomiting, diarrhoea, headache, breast tenderness and depression (see Appendix).

## 2.7 Laboratory Methods

Hormone concentrations were assayed in plasma or serum that were separated from blood cells by centrifugation within 45 minutes of collection. Samples were stored at –20°C and in most cases they were assayed in patient-specific batches to eliminate the effect of inter assay drift.

### 2.7.1 Reproductive Hormones

Testosterone was assessed using the semi-automated 'Immulite' technology (DPC, Los Angeles, USA) (intra-assay CV<7.5%), while sex hormone binding globulin, and DHEAS were assessed by the Immulite 2000 analyser (DPC, Los Angeles, USA) (intra-assay CV <6% and <12% respectively).

Free androgen index was calculated as total testosterone divided by the concentration of sex hormone binding globulin x 100. The upper limit of normal for FAI was 7.9.

Estradiol, progesterone, LH, and FSH were assessed using the semi-automated 'Immulite' technology (DPC, Los Angeles, USA) (their intra-assay coefficient variants were < 10%, 8%, 6%, and 7% respectively).

Androstenedione and 17OHP were measured by 'Coat-A-Count' technology (DPC, Los Angeles, USA) (intra-assay CV<5% and <7% respectively) for the adrenal androgen trials. However, for the MIS and weight loss trials, androstenedione was assayed using the semi-automated 'Immulite' technology (DPC, Los Angeles, USA). Intra assay coefficient of variation was < 8%.

For the hirsutism and weight loss trials the 'in house' radioimmunoassay for 17OHP (intra assay CVs <12%) was used. For androstenedione the radioimmunoassay was used as described in (85). Reference range in women aged 18-40 yrs was 0.6 -8.8 nmol/l. The within batch and between batch CV's are consistently below 10% for low, medium and high concentration serum pools (3,9 and 16nmol/l.)

IGF-1 was assessed using the semi-automated 'Immulite' technology (DPC, Los Angeles, USA) (the intra-assay coefficient variant was <7%). IGFBP-3 was assessed using the semi-automated 'Immulite' technology (DPC, Los Angeles, USA) (the intra-assay coefficient variant was <9%). IGFBP-1 was assessed using the 'Bridge' technology (BioChem ImmunoSystems Italia S.P.A., Bologna, Italy) (the intra-assay coefficient variant was <5%). Inhibin-B was measured using a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (Oxford Bio-Innovation Ltd.,Upper Heyford, Oxfordshire, UK) . The inter plate and intra plate coefficient of variations were <7%, and the limit of sensitivity was 15pg/ml. Vascular endothelial growth factor (VEGF) was performed using Quantikine (Human VEGF Immunoassay) (R&D Systems Europe Ltd., Abingdon, Oxon, UK).

The intra assay coefficient variant was<6.5%, and the inter assay coefficient variant <5.8%. For MIS assay details see Chapter 5.

Plasma leptin was measured by an in-house radioimmunoassay validated thoroughly against the commercially available Linco assay (86). The intra assay and inter assay coefficient variations were <7% and <10% respectively over the sample range. The detection limit of the assay was 0.5ng/ml.

## 2.7.2 Glucose and Insulin

Fasting plasma glucose was measured using the glucose oxidase method (Bayer Advia 1650 Chemistry System) (Bayer Corporation, Tarrytown NY, USA), intra-assay CV <2%).

Insulin was measured using two different assays. For the chapters concerned with the weight loss during metformin treatment and also the dynamics of MIS concentrations, a competitive radioimmunoassay (Coat-A-Count® I, DPC, Los Angeles, USA) (the intra-assay coefficient of variant < 6%) was used. Fasting plasma insulin was measured using a competitive radioimmunoassay ('in house'; intra-assay CV<8%) for the hirsutism, and adrenal androgen trials. Following immediate collection the insulin sample was placed in crushed ice for transportation to the laboratory and then centrifuged at 3000rpm at 4 degrees Celsius for 10minutes.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from the fasting concentrations of insulin and glucose using the following formula: HOMA-IR = fasting serum insulin (μU/ml) x fasting plasma glucose (mmol/liter) / 22.5.

Glycosylated hemoglobin (HbA1C) was measured using high performance liquid chromatography (HA8121 analyser; Menarini Diagnostics, Berkshire, UK).

## 2.7.3 Lipids

Venous blood was taken after an overnight fast for analysis of lipids and lipoproteins. Plasma total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol were determined by a modification of the standard Lipid Research Clinics Protocol (87) using the Bayer Advia 1650 Chemistry System (Bayer Corporation, Tarrytown NY, USA) the intra assay and inter assay coefficients of variation for all lipid measures were <3%.

## 2.7.4 Inflammatory Markers

C-reactive protein (CRP) concentrations were measured using an in-house sensitive double antibody sandwich ELISA as described previously by (88). The assay was linear up to 5mg/l and logarithmic thereafter, and had a lower detection limit of 0.10 mg/l. The inter- and intra-assay coefficients of variation were less than 10% across the range of measured results.

## 2.7.5 Screening Tests

Standard 'in-house assays' for urea, creatinine and electrolytes, thyroid function tests, prolactin, liver function tests, and urate were performed in the routine hospital laboratories prior to recruitment to the respective studies.

# 2.8 Medications

## 2.8.1 Metformin Hydrochloride BP

Also known by the trade name Glucophage™. This insulin sensitising agent is marketed by Merck, West Drayton, Middlesex UB7 7QG, UK. In the trials included in this thesis two doses of 500mg and 850mg tablets were used. All patients were screened as to absolute and relative contra-indications and advised about side effects/ potential adverse outcomes. Patients were then recruited accordingly. Patient information leaflet was also provided. Medication was obtained through the pharmacy at the Royal Infirmary. Contact was made to ascertain side effects on the medication.

## 2.8.2 Cyproterone acetate and ethinylestradiol

This medication was used in the hirsutism trial. It is marketed by Schering Health Care Limited, Burgess Hill, West Sussex RH15 9NE, UK. The trade name is known as Dianette™, and comprised cyproterone acetate 2mg and ethinylestradiol 35mcg. All patients were screened as to absolute and relative contraindications and advised about side effects/ potential adverse outcomes. Patients were then recruited accordingly. Also specifically, patients with a BMI ($kg/m^2$) >38 were excluded from recruitment. Patients included in the hirsutism trial were also provided a patient information booklet. Medication was obtained through the pharmacy at the Royal Infirmary. Contact was made to ascertain side effects on the medication.

## 2.8.3 Tetracosactrin

This medication was used in the adrenal androgen trial. It is also known as Synacthen™ (Alliance). Intravenous administration of Tetracosactrin 250mcg occurred each 2 and 4 week patient visit after obtaining informed consent for the study on adrenal androgens. This medication was the normal ward stock of the Assisted Conception Unit, Royal Infirmary, Glasgow.

Patients were informed of possible side effects prior to administration of the medication. Administration was via a Venflon™ inserted into the cubital fossa of each patient recruited as per product administration recommendations. Samples of serum for hormonal testing were obtained at baseline (T0), 30minutes (T30), 60 minutes (T60), and 90 minutes (T90).

## 2.8.4 Leuprorelin acetate

This medication was used in the adrenal androgen trial. Also known as Prostap (Wyeth), this GnRH analogue was administered to the patients in the adrenal androgen trial in order to down regulate ovarian reproductive hormonal output so that only adrenal hormonal production could be measured. Dosage was 3.75mg by intramuscular injection.

## 2.8.5 Menotrophin

This medication was used in Chapter 8. Also known as Menogon (follicle stimulating hormone purified from human urine) (Ferring) this was used for ovarian stimulation treatment as part of an assisted conception cycle.

Chapter 3

# Metformin compared with Dianette in the treatment of hirsutism in women with PCOS

## 3.0 Summary

Hirsutism is a common and distressing symptom frequently encountered in women with polycystic ovary syndrome (PCOS), who also show relative insulin resistance. The aim of this trial, where treatment of hirsutism was the primary endpoint, was to compare the efficacy of the insulin sensitising agent metformin with an established treatment, combined ethinyl estradiol and cyproterone acetate.

Patients (n=52) were randomised to receive either metformin (500mg tds) or Dianette® (ethinyl estradiol, 35μg; cyproterone acetate, 2mg) treatment for a duration of 12 months with assessments before treatment, at 6 months and at 12 months. Both objective and subjective methods of evaluating hirsutism were used and, in addition, patient perceptions of their own condition were examined.

The results show that metformin is potentially an effective treatment for moderate to severe hirsutism in women with PCOS. They also suggest that, in some respects (Ferriman Gallwey score, and patient self-assessment), it is more efficacious than the standard treatment (Dianette®). The objective evaluation of hair diameter, showed that both treatments were moderately effective in reducing mean diameters at multiple anatomical sites.

Dianette®treatment was responsible for profound suppression of androgen activity, in contrast to metformin, which induced negligible change. However metformin did reduce markers of insulin resistance. The data suggest that hirsutism may be effectively treated by reducing hyperinsulinaemia. Correspondingly, the efficacy of a combined approach should be explored.

# 3.1 Introduction

Hirsutism is excessive hair growth in a male pattern distribution in women. It is not only distressing to patients, but also presents a challenging clinical management dilemma. In approximately 90% of women with hirsutism, the underlying disorder is either polycystic ovary syndrome (PCOS) with its intrinsic hyperandrogenism, or it is idiopathic, probably related to increased tissue sensitivity to androgens (89). In fact, a high proportion of patients with idiopathic hirsutism demonstrate polycystic ovaries (90), suggesting that these 2 forms may not be easily distinguished.

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterised by chronic anovulation, hyperandrogenism and hyperinsulinaemia, secondary to reduced insulin sensitivity. The increased secretion of ovarian androgens is considered to be due to increased insulin stimulation of ovarian steroid secreting cells (mainly thecal cells) by LH promoted by insulin, and also insulin stimulated growth factors, including insulin-like growth factor (IGF-1), and perhaps decreased IGF-binding protein activity (91). Hyperandrogenism commonly manifests itself as hirsutism (60-83%), acne (11-43%) (92), seborrhoea and alopecia.

Androgens have been shown to be at least partly responsible for promoting the anagen phase (growth phase) of the hair cycle, leading to larger hair follicles (93), and bringing about a change from vellus to terminal hair status. In vitro studies show that the active androgen is 5αdihydrotestosterone, produced locally by the action of 5α reductase enzyme on testosterone (89). The anagen phase has also been shown to be influenced by the growth factor, IGF-1. IGF-1 is carried in the circulation, predominantly by specific IGF binding proteins, but it is also produced locally by the dermal papilla where it acts on both the dermis and epidermis (94, 95). The activity of these growth factors depends on a number of factors, including local and circulating binding proteins, which in turn are also influenced by the actions of insulin. Thus, women with PCOS may demonstrate abnormalities in the metabolism of both the major factors responsible for hirsutism- androgens and insulin / growth factors.

Women with PCOS also suffer from a high incidence of acne, which has also been linked with raised serum androgen (96), insulin (97), and free IGF-1 (98, 99) concentrations in the circulation.

The use of insulin sensitising agents (ISAs), predominantly metformin and the thiazolidinediones, in the treatment of women with PCOS, has been shown to improve insulin sensitivity and ovarian function (16). Treatment with the ISA metformin reduced circulating insulin, luteinising hormone, androstenedione, and testosterone concentrations in a number of studies, and protracted treatment has resulted in improvements in BMI, menstrual cycle regulation, spontaneous ovulation rates, and spontaneous and assisted pregnancy rates (16, 42, 48). It has been hypothesised that by reducing circulating insulin concentrations, leading to decreased free androgen concentrations, ISAs may ameliorate hirsutism. In fact, examination of the literature shows that most, but not all,

controlled studies achieved modest reductions in circulating free androgens using metformin (16). A recent study in lean women with PCOS showed significant reductions of circulating testosterone, even though they were only modestly elevated prior to treatment (100). However, changes in insulin and possibly IGF metabolism, justify further examination of this therapeutic approach, since, as described above, changes in the growth factor environment may also be important in the treatment of hirsutism.

Some recent reports have addressed the use of ISAs in hirsutism (36, 43-45, 77, 101). However, in none of them was hirsutism a primary outcome measure, and no objective measure of hair growth was undertaken. There has been one very small study reporting the effect of metformin upon hirsutism as a primary end-point measure, and using an objective measure of hair growth (68). The results suggested that metformin may show benefit compared with placebo.

The aim of this trial, where hirsutism is the primary endpoint, was to firstly elucidate if metformin does have an effect on hirsutism in women with PCOS, and secondly, to compare its efficacy with an established treatment for hirsutism, combined ethinylestradiol and cyproterone acetate. In doing so we have used objective techniques and also validated subjective methods of assessment of hirsutism, and also incorporated patient perception measures.

# 3.2 Subjects and methods

## 3.2.1 Study population

Women with PCOS (n=52), whose primary complaint was hirsutism (Ferriman-Gallwey score >8) were recruited from the Reproductive Endocrinology clinic at

the Royal Infirmary, Glasgow, UK. The diagnosis of PCOS was described in chapter 2. Exclusion criteria included; contraindications to either metformin or Dianette (including BMI >38kg/m$^2$), and use of COCP or metformin within the previous 3 months. Thyroid dysfunction, hyperprolactinaemia, diabetes mellitus, or late-onset congenital adrenal hyperplasia were excluded as described. Women taking medication known to affect gonadal or adrenal function, or carbohydrate or lipid metabolism were also excluded. Women were also advised to use barrier contraception if randomised to metformin.

The study was conducted at the Royal Infirmary, Glasgow, UK, following approval from the ethics committee of the North Glasgow Hospitals University NHS Trust, and informed consent was obtained from each woman.

## 3.2.2 Study design

### 3.2.2.1 Treatments

Patients were block (n=10 per block) randomised in a 1:1 ratio to receive either ethinyl estradiol 35mcg and cyproterone acetate 2mg (Dianette; Schering AG, Germany) or metformin (Glucophage; Merck, West Drayton, UK) for a 12 month treatment course. Randomisation was by random number tables. The patient number treatment codes were held by a third party and were allocated individually following written consent. A list of codes was kept by a third party and patient names were checked after completion of the trial. Medication was commenced one week following written consent. The Dianette was administered in the recommended regime (ethinyl estradiol 35mcg + CPA 2mg; 21 days/month followed by a 7-day pill-free period). Metformin (metformin hydrochloride) was administered orally at a dose of 500mg tds.

### 3.2.2.2 Assessment programme

At baseline (T0), 6 months (T6), and 12 months (T12) all patients underwent clinical and hormonal assessments. These included anthropometric measurements of height, weight, BMI, waist and hip (WHR), blood pressure, and hirsutism using the Ferriman-Gallwey score and hair diameter measurements. These assessments were performed by the same observer (LH). Sebum excretion rate (SER) was also assessed at each time point, and a side effect profile was also performed at 2, 6 and 12 months. Assessment of patient perception was recorded at 0, 6 and 12 months for hirsutism and acne. Circulating concentrations of insulin, glucose, testosterone, SHBG, androstenedione, DHEAS, 17αOH progesterone, total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), IGF-1 and IGFBP-3 were also determined in a fasting blood sample taken at the T0, T6 and T12.

## 3.2.3 Methods

Most of the methods used were described in Chapter 2, but those specific to this study are described below. All patients completed assessments for side effects of medication, Ferriman Gallwey score, visual analogue scales for hirsutism and acne and a qualitative assessment of hirsutism (see Appendix).

### 3.2.3.1 Hair diameter

Samples of terminal hair were collected from each patient from anatomical sites of the chin, lower abdomen, anterior mid-thigh and forearm at baseline (T0), T6

and T12 for measurement of hair diameter. This method was described by Falsetti et al (102). Women were asked to allow at least 3 days growth of hair prior to removal. This time period was determined as subjects were unwilling to allow a longer hair growth interval for the face. The samples were removed using a stitch cutter blade (Swann-Morton™; Swann-Morton Ltd, Sheffield, UK) to cut the cutaneous base. Hair samples were then aligned on a microscopy slide with the hair base towards the frosted end, and all were fixed using the same roll of selotape™ placed over the hair. Thus, the area of most recent growth was adjacent to the frosted end of the microscopy slide.

Hairs were examined with a bright field microscope set up precisely for Koëhler illumination. All condenser and illumination settings were kept constant. Digital images of hairs were captured with a three chip analogue camera (SBCKY55, JVC, London) connected to a frame grabber (Snapper, Data Cell, Finchampstead, UK).

Hair diameter was determined using digital image analysis software (Image Pro Plus, Media Cybernetics, Silverspring MD). One investigator (LH), made all hair diameter measurements whilst blind to the treatment group.

An objective lens was used (x40) (field width = 242.32 μm), with the exception of certain hairs that were too large to be viewed at this magnification and in these cases a x20 objective lens was used (field width = 487.50μm). The system was calibrated using a 10μm stage micrometer (Graticules Ltd UK, Tonbridge).

Hairs were aligned horizontally on screen and with the segment immediately next to the cut end in view. The hair diameters were measured by drawing a single line across the hair shaft perpendicular to the long axis of the hair. The optical measurement was effected immediately adjacent to the cut end in order to

estimate the diameter of most recent growth. Hair diameter measurements were calibrated in micrometers to 2 decimal points, and repeated measurements (n=18) of the same hair demonstrated that the technology was consistent and precise to <2% variation.

Initially, as the precision of the method was deemed to be precise, one terminal hair from each site was measured. However, the protocol was changed part way through the trial, after testing revealed significant biological variation between terminal hairs within the same location. Thereafter, six hairs from each site were measured and a mean value calculated. Table 3.1 shows the variation in hair diameters according to location.

The numbers of patients who had 6 hairs obtained at T0, T6 and T12 were 14 (includes one patient who withdrew prior to T12), at T6 and T12 were 4, and only at T12 were 6. All other patients had only one hair obtained from each anatomical site (n=14).

### 3.2.3.2 Sebum excretion rate

The method of SER was performed as described by Rademaker et al (103). An area on the forehead was wiped clean with 4 absorbent papers (Rizla™cigarette papers), each of 7.0cm x 2.5cm=17.5cm². A pad of 4 papers were prepared and placed on the delineated forehead, and held in place by gauze sponges and a bandana. After 15 mins, another pad of 4 papers and gauze sponges was prepared and placed in situ for 1.5 hours. The papers were then removed and placed on tin foil for laboratory processing. All handling of the papers was by sterilised forceps. In the laboratory the papers were triple extracted with analar grade diethyl ether (20ml). Volumes used were 8ml, 6ml, and 6ml. The weighed

boats were evaporated to dryness and reweighed. Control papers (same size) were extracted as well. Results are expressed in mg sebum excreted per sq cm per hour, and the limit of sensitivity was 0.01mg/sqm/h.

### 3.2.3.3 Statistics

Changes in parameters over time within patients were assessed using repeated measures analyses of variance (ANOVA), and differences between groups were evaluated using a non-parametric test (Mann-Whitney). Comparisons between 2 time points within the same patient were effected using paired t tests. Proportions of patients responding were compared using contingency table analyses. Statistical analyses were performed using GraphPad Prism (San Diego, CA) software.

## 3.3 Results

### 3.3.1 Patients and randomisation

The baseline characteristics of the two treatment groups were similar. Means of the base line characteristics are shown in table 3.2. They showed similar ages and degrees of hirsutism. Normal (non-hirsute) values for the Ferriman-Gallwey score lie below 8, so the degree of hirsutism in both groups was considerable. The patients were generally obese and the proportions of patients in each group with BMI >29 kg/m$^2$ were: Dianette, 20 of 26 and metformin, 14 of 26. Elevated fasting insulin concentrations in the circulation were also observed (laboratory upper limit of normal = 13.9mU/L; and the proportions of patients showing elevated fasting insulin were: Dianette, 8 of 26, and metformin, 9 of 26. Fasting

glucose concentrations were within the normal range. The free androgen index (normal upper limit = 7.9) was elevated in both groups, and the proportions of patients in each group with FAI >7.9 were: Dianette, 16 of 26 (62%) and metformin, 14 of 26 (54%). Acne was generally not a profound problem amongst the groups.

Figure 3.1 shows the process of patients from recruitment and randomisation and through the 12 month treatment programme. Ten patients discontinued Dianette, while 8 stopped taking metformin (including 3 pregnancies).

## 3.3.2 Hirsutism

Figure 3.2 shows that Ferriman Gallwey score was significantly reduced following treatment in both groups, using repeated measures ANOVA. The degree of reduction in FG score was significantly greater ($p<0.01$, Mann-Whitney test) in the metformin group (approximately 25%) compared with the Dianette group (approximately 5%). Twelve months treatment with metformin resulted in 5 patients with severe hirsutism (FG score $\geq 15$, at T0) achieving a FG score of <15 (ie 'moderate / mild' hirsutism) out of a total of 22, while only 1 did so after Dianette treatment (out of n = 25: Chi-square, $p=0.08$).

The mean hair diameters were significantly reduced ($P \leq 0.001$; repeated measures ANOVA) in both groups during the treatment programme (figure 3.3), and to a similar degree (Dianette, 17% reduction; metformin, 12%; difference between groups, $p = 0.15$). Table 3.3 shows changes in hair diameter according to anatomical site. In fact, the changes in hair diameter appeared to be dependent upon either anatomical site and / or baseline hair diameter in both treatment groups. It can be seen that hairs on the forearm were relatively fine at

T0 and underwent negligible change, while hairs on the chin and abdomen were relatively coarse at T0 and apparently underwent considerable change (both groups).

## 3.3.3 Patient self assessments

Table 3.4 shows that patient self assessment (visual analogue scale) of both hirsutism (figure 3.4) and acne underwent significant reduction in both groups (ANOVA). There was no difference between the treatment groups at T0, but at T12 the metformin patients scored their hirsutism significantly lower than the Dianette group (Mann Whitney, $p = 0.01$).

Table 3.5 demonstrates changes in patient perception of specific qualitative aspects of their hirsutism. More than half the patients in both treatment groups assessed their hair quality to be 'finer' after treatment, and there was no difference between the treatments in this parameter. Approximately half of the metformin group recorded that their hair growth rate was reduced at T6. This proportion was significantly ($p<0.05$) greater than in the Dianette group. Half the metformin treated patients responded with a reduced requirement for the use of cosmesis (T6 and T12). This proportion was not significantly greater than the Dianette group at either time point. The 'overall appearance' as a description specific to hirsutism was improved in more than 50% at both T6 and T12 in the metformin group. This was a significantly greater proportion than the Dianette group at both time points.

## 3.3.4 Acne and sebum excretion

The degree of acne in general was low (secondary outcome measure), but both groups considered that acne improved significantly (table 3.4) by self

assessment. There was no difference between the treatment groups in the responses recorded (p=0.36).

The sebum excretion rates underwent modest improvement ($p<0.05$) during Dianette treatment, but no change during metformin treatment. (Dianette, from 0.14 (+/- 0.11 sd) $\mu g/m^2/h$ at T0 to 0.08 (+/- 0.04 sd) at T12, $p = 0.04$; metformin, 0.15 (+/- 0.08 sd) $\mu g/m^2/h$ at T0 to 0.12 (+/- 0.08 sd) at T12, p=0.18).

## 3.3.5 Hormone changes

The effects of Dianette treatment upon hormone profiles at 6 months and 12 months were profound (table 3.6), with reduced total androgens in the circulation and an increase in the SHBG, effectively reducing free androgens to values below the normal range. Similar changes were recorded in circulating 17αOH-progesterone and DHEAS. However there was no effect upon glycaemic parameters, and the BMI did not change over the 12 month course of treatment. In contrast, metformin treatment showed negligible effects upon circulating total androgens, SHBG, or free androgen index, or 17αOH-progesterone. Although a significant (p=0.02) increase in the circulating DHEAS was observed. However, metformin treatment resulted in a significant decrease in the glucose/insulin ratio and the logHOMA-IR (log transformed to normalise the distribution), suggesting improved efficiency of utilisation of glucose, secondary to improved insulin sensitivity. There was no change in the circulating IGF1, IGF-BP1 or IGF-BP3 during metformin treatment.

## 3.3.6 Blood pressure and circulating lipid profiles during metformin treatment

Table 3.6 shows that Dianette treatment was not associated with changes in blood pressure, while the patients treated with metformin showed a clinically insignificant increase in diastolic blood pressure. There was no change in the systolic blood pressure in either treatment group. The circulating lipid profiles were normal and showed non-significant improvements during treatment with metformin (table 3.6).

## 3.3.7 Changes in hirsutism and metformin treatment

Metformin treatment was associated with changes in the FG scores, BMI, and improved indices of insulin action. There was little correlation between these specific changes. Those individuals who lost more than 1 $kg/m^2$ in BMI (n=11) showed no greater reduction in FG score (reduction of 3.8 FG units) compared with those who lost less weight (n=7, reduction of 6.6 FG units: P = 0.13). Similar analyses with the glucose / insulin ratio showed that those showing the greatest improvement in the ratio reduced their FG scores to the same degree as those showing a relatively inferior response (mean FG reductions, 4.8 and 5.0 respectively).

The changes in FG score showed poor correlations with changes in glucose/insulin ratio and logHOMA-IR value (Glu/Ins, $r^2 = 0.01$; change in HOMA-IR, $r^2 = 0.004$) indicating that changes in hirsutism were relatively independent of changes in both measures of insulin sensitivity. The correlation of change in FG score with change in BMI ($r^2 = 0.17$) was not significant ($p = 0.08$).

The population median BMI was 34 kg/m$^2$, and responses were examined according to the 2 BMI subgroups (n = 9, each) lying either side of this value. The data suggest that BMI may be a relevant factor with respect to treatment efficacy as the leaner sub-group showed a tendency (p = 0.08) to greater improvement in FG score (6.8 units, 95%CL, 3.3 – 10.3) than the more obese sub-group (3.0 units, 95%CL, 0.2 – 6.2). The same sub-grouping revealed that the change in the free androgen index was significantly greater (p = 0.03) in the leaner sub-group.

A similar examination of changes in FG score in relation to hyperandrogenaemia prior to treatment (FAI > 7.9) failed to establish any relationship, as both groups showed similar FG scores at T0 and T12, with similar degrees of benefit.

## 3.3.8 Side effects

Table 3.7 shows the results of side-effect recordings by those patients who continued on each treatment, despite side effects. It shows that the side effect profiles of both treatments were moderate, and there was little difference between the treatment groups. Gastro-intestinal problems (including reduced appetite) affected approximately half of the patients on metformin in the first 6 months, contrasting with the Dianette group. Headache and breast tenderness were features in both treatment groups, but there was no difference between them.

## 3.4 Discussion

The results of this prospective randomised, study show that metformin is an effective treatment for moderate to severe hirsutism in women with PCOS. The data also suggest that, in some respects (FG score, and patient self-assessment), it is more efficacious than the 'gold standard' treatment, combined estrogen and anti-androgen (cyproterone acetate), Dianette®. The objective evaluation of one component of hirsutism, hair diameter, showed that both treatments were effective in reducing mean diameters at multiple anatomical sites. However, this reduction was modest and probably within the known biological variation in both treatment groups.

To my knowledge, this is the first comparative, randomised, controlled trial of sufficient duration and patient numbers to address the issue of efficacy and acceptability of metformin, in the treatment of hirsutism in women with PCOS, as a primary outcome measure. Furthermore, the use of an objective measure of one aspect of hair growth is an important addition to the assessment, since subjective evaluations may be influenced by many factors.

Previous trials addressing the use of ISAs for the treatment of hirsutism in women with PCOS, were not unanimous, but four of them suggested that metformin treatment would be efficacious if addressed directly. However, the studies generally suffered from small patient numbers, patients who were only mild-moderately hirsute (assessed by FG score), short therapeutic duration, and only one study employed an objective measure of hair analysis. In none of the trials was treatment acceptability or assessment of response explored.

Our trial indicates that the recorded improvement in hirsutism also equated with patient perception of improvement, which was strongly in favour of metformin

compared with Dianette. The acceptability of metformin as a treatment for hirsutism appeared to be high. In addition, although side effects were similar in the two treatment groups there was a trend towards increased compliance in the metformin group as evidenced by a lower side-effect motivated dropout rate (excluding pregnancies). This is important, as high patient compliance is essential for optimal treatment effect, given the length of time of the hair bio-cycle.

Overall, the results present a counter-intuitive profile in the observations of a limited change in circulating total and free androgens at the same time as considerable improvements in hirsutism. Hirsutism is a result of end-organ sensitivity as well as direct androgen stimulation, and this tissue sensitivity is known to be controlled by factors other than androgens, such as insulin and IGF-1 activity. In our study, metformin treatment showed significant improvement in the glucose / insulin ratio and the logHOMA-IR, but it had negligible impact upon circulating androgens. In contrast, Dianette virtually eliminated free androgens from the circulation, but in fact showed little effect upon severe hirsutism, as has been recorded previously (92). Taken together, these data suggest that addressing insulin insensitivity may be a more effective therapeutic approach to hirsutism in women with PCOS, than aggressive suppression of androgens, in the form of anti-androgen therapy. Thus hirsutism and hyperandrogenism may be related through a common underlying mechanism, in addition to a direct androgen stimulant – response aetiology.

The activity of IGF1 is related to both absolute circulating concentrations and also those of its carrier proteins, such as IGF-BP3, which effectively reduce IGF potency. Women with PCOS may have raised circulating free IGF1, mediated mainly through reduced IGF-BPs, suggesting increased growth factor stimulation

(104, 105). However, we did not demonstrate any change in the circulating concentrations of either IGF1 or IGF-BP3 or IGF-BP1 after metformin treatment, thus we may hypothesise that the beneficial effect of metformin is unlikely to be due to an effect upon circulating growth factor stimulation. Correspondingly, benefit may be due to a mechanism involving local growth factor action at the dermal papillae. The inability of metformin to modify serum IGF-1 concentrations has been reported previously (40, 106).

The failure of metformin to influence circulating SHBG concentrations beyond placebo or control, is another surprising observation which has been recorded previously (16). The pre-treatment values would be considered low and representative of a cohort of obese women with PCOS. The failure to significantly change these values with protracted treatment may reflect the confounding effect of obesity in the patient cohort, and the complex nature of SHBG control mechanisms. Body mass has a profound influence upon SHBG secretion, and the weight loss during the programme was modest, and the patients remained generally obese. It may be that a greater degree of weight loss is needed to effect a substantial increase in SHBG, such that higher doses of metformin should be used in obese women.

We observed poor correlations between reduction in FG score and changes in BMI, or measures of insulin sensitivity, suggesting that neither of these changes is directly responsible for improvement in FG score. Further suggesting that beneficial effects may be due to endocrine changes not so far determined, such as the evolution of growth factor and binding protein complexes at local tissue level, secondary to induced changes in insulin sensitivity.

The degree of acne in our patient cohort was generally low, and it is difficult to extract useful conclusions from the data, as there was no absolute difference

between the two treatment groups for sebum excretion over the 12 months. This is probably related to the fact that acne was not a primary complaint in our study.

The examination of the impact of morbid obesity upon the responses to metformin therapy suggest that in such women metformin at a dose of 500mg tds may have reduced efficacy, compared with leaner women. A similar observation was recorded with morbidly obese women with PCOS in aspects of improving ovarian function, weight reduction and circulating lipids (47). This observation suggests that either morbidly obese women are refractory to metformin therapy or quite simply that the current dose is insufficient.

Life-style change and weight loss have been shown to be effective means of treating many of the abnormalities associated with PCOS (76, 107), and hirsutism may also respond to this approach. Crave et al (30) suggested that metformin may confer no additional advantage over weight loss, which contrasts with the analyses presented above.

In summary, the results of this study open the prospect of a realistic treatment for a large number of women with hirsutism and PCOS, and possibly also idiopathic hirsutism, a large proportion of whom (>90%) have polycystic ovaries (90). The beneficial effects do not appear to be mediated by suppression of circulating androgens, which opens the possibility that hyperinsulinaemia or related metabolic pathways may be important determinants of end-organ responses at the level of the hair follicle. Future work should address this therapeutic approach through examining optimal doses of ISAs, either alone or in combination with anti-androgen treatment.

**Table 3.1**

Mean biological variation and standard deviations in hair diameters recorded within a single site

| | Mean Diameter (μm) | Standard Deviation (μm) | Variation (%) |
|---|---|---|---|
| Abdomen | 87.9 | 15.1 | 19.4 |
| Chin | 103.7 | 16.6 | 23.3 |
| Forearm | 58.8 | 8.7 | 14.9 |
| Thigh | 73.5 | 11.9 | 16.2 |

**Table 3.2**

Standard deviations (in parentheses) and means of baseline characteristics between the two treatment groups.

| | Dianette | Metformin | P Values |
|---|---|---|---|
| Age (yrs) | 31.65 (8.2) | 31.30 (6.9) | 0.88 |
| Hirsutism (FG score>8) | 22.8 (5.9) | 20.3 (5.0) | 0.24 |
| BMI (kg/m$^2$) | 31.8 (5.6) | 31.7 (6.0) | 0.86 |
| WHR | 0.81 (0.051) | 0.85 (0.067) | 0.17 |
| Fasting insulin (mU/ml) | 19.0 (23.7) | 15.8 (11.1) | 0.92 |
| Fasting glucose (mmol/L) | 5.0 (0.55) | 5.4 (1.4) | 0.36 |
| Gluc/Insulin | 0.4 (0.16) | 0.45 (0.21) | 0.73 |
| Testosterone (nmol/L) | 3.5 (1.4) | 3.2 (1.7) | 0.65 |
| FAI (>7.9) | 15.8 (13.5) | 14.1 (13.1) | 0.46 |

**Table 3.3.**

Changes in mean hair diameters and standard deviations (in parentheses) according to anatomical site during 12 months treatment with Dianette or metformin. The data were compared using paired t test analyses.

| | Dianette | | | | Metformin | | | |
|---|---|---|---|---|---|---|---|---|
| | **T0** | **T12** | **%** | **p** | **T0** | **T12** | **%** | **p** |
| Chin (μm) | 115 (41) | 102 (42) | 11.3 | 0.06 | 103 (41) | 91 (37) | 12 | 0.09 |
| Abdomen (μm) | 100 (18) | 75 (19) | 25.0 | 0.0004 | 93 (22) | 72 (24) | 22 | 0.002 |
| Mid-thigh (μm) | 89 (21) | 71 (21) | 20.0 | 0.0001 | 67 (23) | 62 (19) | 7.5 | 0.10 |
| Forearm (μm) | 63 (10) | 58 (9.2) | 7.9 | 0.03 | 53 (13) | 52 (11) | 1.9 | 0.38 |
| Combined (μm) | 92 (16) | 76 (15) | 17 | 0.001 | 79 (18) | 69 (17) | 13 | 0.004 |

**Table 3.4**

Comparison of patient own quantitative self-assessments of hirsutism and acne through and at the end of 12 months treatment with either Dianette or metformin. (Standard deviations are in parentheses.)

| | **Dianette** | | | | **Metformin** | | | | ***Mann-Whitney*** |
|---|---|---|---|---|---|---|---|---|---|
| | **T0** | **T6** | **T12** | ***ANOVA (P)*** | **T0** | **T6** | **T12** | ***ANOVA (P)*** | **Rx Groups T12** |
| Hirsutism | 7.4 (1.8) | 7.1 (1.7) | 6.6 (1.9) | 0.005 | 7.0 (2.1) | 5.0 (2.) | 3.9 (3.1) | <0.0001 | 0.01 |
| Acne | 2.0 (2.3) | 1.2 (1.9) | 1.0 (1.3) | 0.002 | 3.4 (2.5) | 2.9 (2.6) | 1.9 (2.4) | 0.005 | 0.36 |

**Footnote to table 3.4**

The values derive from patients own assessment of their degree of hirsutism and acne according to a visual analogue scale graded from 0 (nil) to 10 (grossly abnormal). See appendix.

**Table 3.5**

Numbers of patients (proportions presented as %) reporting changes in qualitative aspects of hirsutism after 6 months (T6) and 12 months (T12) treatment with Dianette or metformin.

| | T6 Nos. of patients (%) | | | T12 Nos. of patients (%) | | |
|---|---|---|---|---|---|---|
| | **Dianette** | **Metformin** | **P** | **Dianette** | **Metformin** | **P** |
| Finer hair quality | 10/17 (59) | 13/20 (65) | ns | 9/16 (65) | 11/18 (61) | ns |
| Reduced rate of hair growth | 4/17(24) | 13/20 (65) | 0.02 | 9/16 (56) | 8/18 (44) | ns |
| Reduced need for cosmesis | 3/17 (18) | 10/20 (50) | 0.08 | 4/16 (25) | 9/18 (50) | ns |
| Overall improved appearance (hirsutism) | 1/17 (6) | 11/20 (55) | 0.002 | 4/16 (25) | 11/18 (61) | 0.002 |

**Table 3.6**

Changes in mean (SDs) circulating hormone concentrations and anthropomorphic features over 6 and 12 months' treatment with either Dianette or metformin. The statistical power (*P*) represents changes within each group assessed by repeated measures ANOVA.

| | Dianette | | | | Metformin | | | |
|---|---|---|---|---|---|---|---|---|
| | **T0** | **T6** | **T12** | **P** | **T0** | **T6** | **T12** | **P** |
| Fasting Insulin (mU/L) | 16.5 (23.1) | 14.5 (10.7) | 15.0 (8.9) | 0.7 | 15.8 (11.1) | 12.0 (7.8) | 11.3 (8.8) | 0.07 |
| Glucose (mmol/L) | 5.0 (0.55) | 4.8 (0.36) | 4.8 (0.39) | 0.3 | 5.4 (1.38) | 5.2 (1.14) | 5.3 (1.42) | 0.5 |
| HOMA-IR | 0.49 (0.32) | 0.39 (0.32) | 0.44 (0.34) | 0.35 | 0.49 (0.30) | 0.37 (0.26) | 0.32 (0.32) | 0.03 |
| Gluc/Insulin | 0.40 (0.16) | 0.50 (0.31) | 0.40 (0.16) | 0.2 | 0.45 (0.21) | 0.55 (0.26) | 0.68 (0.40) | 0.004 |
| Testosterone (nmol/L) | 3.52 (1.4) | 2.38 (1.3) | 2.68 (1.3) | 0.006 | 3.19 (1.7) | 3.34 (1.9) | 2.82 (1.3) | 0.39 |
| SHBG (nmol/L) | 31.4 (17.9) | 141.1 (63) | 117.4 (72) | <0.0001 | 30.4 (15.6) | 29.6 (11.9) | 28.8 (11.5) | 0.90 |
| FAI | 15.5 (12.7) | 2.0 (1.1) | 3.2 (2.2) | <0.0001 | 14.1 (12.8) | 15.5 (16.7) | 12.9 (12.0) | 0.4 |
| DHEAS (μmol/L) | 7.2 (3.7) | 4.8 (2.3) | 4.4 (1.9) | 0.0002 | 6.8 (3.6) | 7.9 (3.6) | 7.4 (3.4) | 0.02 |
| Androstene dione (ng/ml) | 11.6 (3.5) | 7.7 (3.0) | 8.2 (3.7) | 0.0001 | 11.6 (6.6) | 11.5 7.9() | 10.4 (6.2) | 0.3 |
| 17αOH Progesterone (nmol/L) | 5.4 (3.3) | 2.3 (0.9) | 3.1 (2.4) | 0.0008 | 4.2 (2.2) | 5.2 (2.7) | 5.8 (2.9) | 0.2 |
| BMI (kg/m²) | 31.8 (5.6) | 31.1 (5.6) | 31.3 (5.8) | 0.15 | 31.7 (6.0) | 30.3 (6.2) | 30.1 (6.0) | 0.001 |
| WHR | 0.81 (0.051) | 0.81 (0.059) | 0.81 (0.060) | 0.69 | 0.85 (0.067) | 0.84 (0.053) | 0.85 (0.057) | 0.59 |

(Table 3.6 cont.)

| | Dianette | | | | Metformin | | | |
|---|---|---|---|---|---|---|---|---|
| | **T0** | **T6** | **T12** | **P** | **T0** | **T6** | **T12** | **P** |
| BP: diastolic (mmHg) | 73.1 (10.1) | 73.4 (10.0) | 72.8 (9.3) | 0.97 | 74.4 (10.6) | 76.4 (10.4) | 80.3 (9.3) | 0.01 |
| BP: systolic (mmHg) | 118.8 (14.1) | 116.3 (17.5) | 116.3 (15.3) | 0.58 | 119.1 (19.4) | 117.2 (17.3) | 120.4 (20.6) | 0.71 |
| Cholesterol (Total: mmol/L) | 4.90 (1.1) | 5.05 (1.0) | 4.75 (1.4) | 0.35 | 4.98 (0.9) | 4.87 (0.7) | 4.79 (0.8) | 0.32 |
| Triglycerides (mmol/L) | 1.68 (1.2) | 1.68 (0.9) | 1.54 (0.9) | 0.72 | 1.48 (0.6) | 1.24 (0.6) | 1.27 (0.6) | 0.19 |
| LDL Cholesterol (mmol/L) | 2.81 (0.6) | 2.43 (1.2) | 2.55 (1.2) | 0.40 | 3.08 (0.9) | 2.94 (1.2) | 2.85 (1.0) | 0.39 |
| HDL Cholesterol (mmol/L) | 1.41 (0.30) | 1.64 (0.33) | 1.51 (0.51) | 0.01 | 1.29 (0.31) | 1.27 (0.27) | 1.31 (0.27) | 0.52 |
| IGF-1 (ng/ml) | | | | | 267 (117) | 250 (75) | 250 (98) | 0.90 |
| IGF-BP1 (ng/ml) | | | | | 21.6 (19.6) | 32.2 (35.5) | 25.9 (29.6) | 0.30 |
| IGF-BP3 (ng/ml) | | | | | 6.6 (1.4) | 6.5 (1.1) | 6.4 (1.2) | 0.50 |

**Table 3.7**

Record of side effects considered attributable to either treatment with Dianette or metformin at 2 months (T2), 6 months (T6) and 12 months (T12).

| | Time | Dianette N (%) | Metformin N (%) | P |
|---|---|---|---|---|
| Reduced appetite | T2 | 2/26 (8) | 12/26 (46) | 0.004 |
| | T6 | 0/17 (0) | 12/20 (60) | 0.001 |
| | T12 | 0/16 (0) | 6/18 (33) | 0.001 |
| Nausea | T2 | 7/26 (27) | 6/26 (23) | ns |
| | T6 | 1/17 (6 ) | 5/20 (25) | ns |
| | T12 | 0/16 (0) | 3/18 (17) | ns |
| Vomiting | T2 | 2/26 (8) | 2/26 (8) | ns |
| | T6 | 217 (12) | 0/20 (0) | ns |
| | T12 | 0/16 (0) | 0/18 (0) | ns |
| Diarrhoea | T2 | 2/26 (8) | 13/26 (50) | 0.002 |
| | T6 | 1/17 (6) | 9/20 (45) | 0.01 |
| | T12 | 0/16 (0) | 6/18 (33) | 0.02 |
| Headache | T2 | 8/26 (30) | 4/26 (16) | ns |
| | T6 | 3/17 (18) | 2/20 (10) | ns |
| | T12 | 3/16 (19) | 3/18 (17) | ns |
| Breast tenderness | T2 | 10/26 (38) | 4/26 (15) | ns |
| | T6 | 6/17 (35) | 6/20 (30) | ns |
| | T12 | 4/16 (25) | 3/18 (17) | ns |
| Depression | T2 | 6/26 (23) | 2/26 (8) | ns |
| | T6 | 4/17 (24) | 4/20 (20) | ns |
| | T12 | 1/16 (6) | 3/18 (17) | ns |

**Figure 3.1**

Randomisation and process of patients from recruitment to completion of treatment after 12 months with Dianette or metformin.

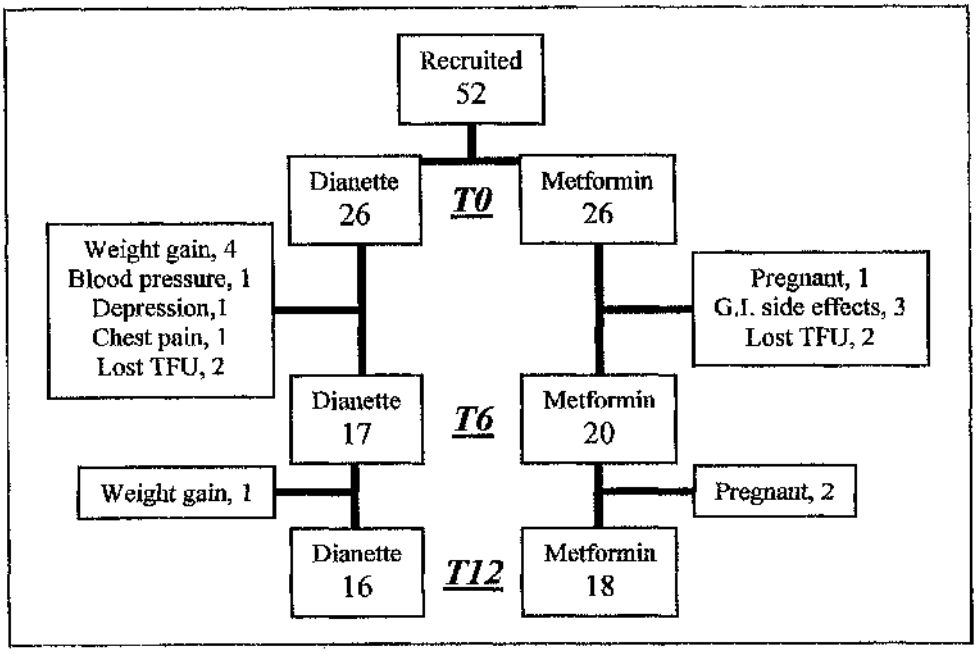


**Figure 3. 2**

Responses of Ferriman Gallwey score to treatment with Dianette or metformin over 12 months. The broken line represents the upper limit of normal values.

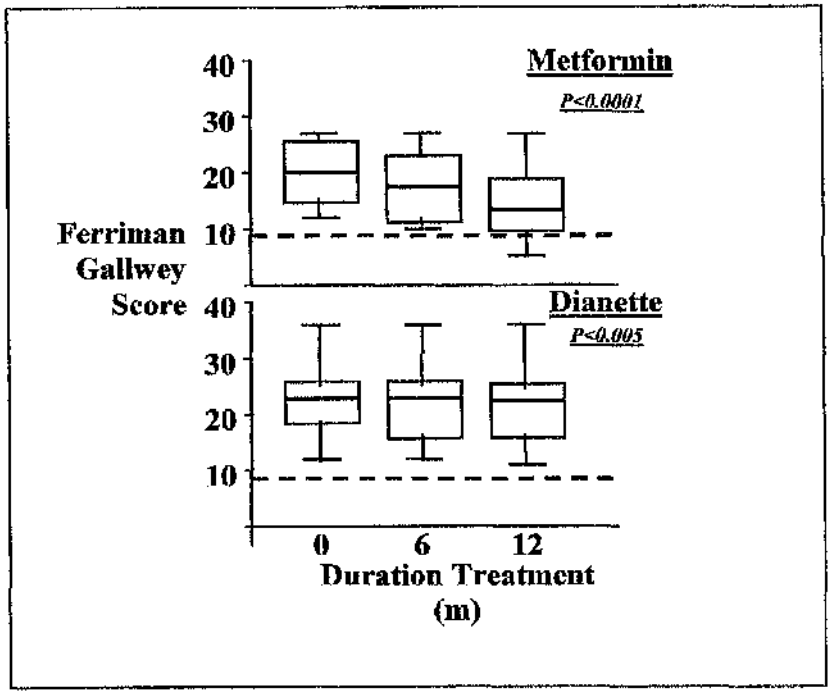


**Figure 3.3**

Mean terminal hair diameter values through the programme of treatment with Dianette or metformin over 12 months.

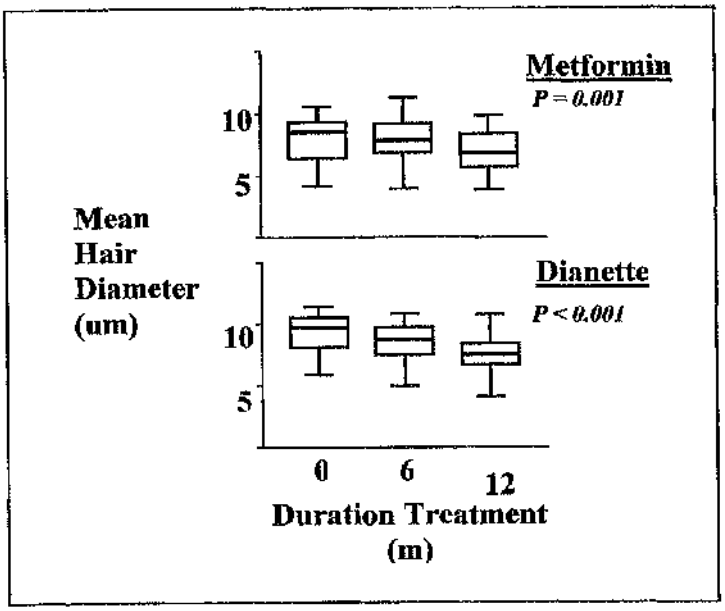
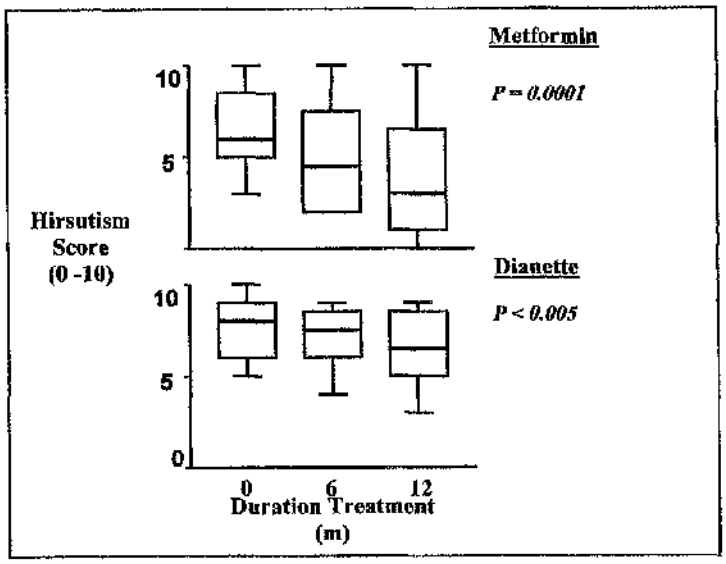


**Figure 3.4**

Responses of self-assessment score to treatment with Dianette or metformin over 12 months.



Chapter 4

# To examine the effects of different doses of metformin in obese women with PCOS: roles in weight reduction and improvement in lipid profiles

## 4.0 Summary

The widespread use of metformin in the treatment of women with polycystic ovary syndrome (PCOS) is based upon a limited, but growing evidence base. There has been a widespread patient population examined in previous studies, from morbidly obese to lean patients with PCOS, and weight reduction has been reported in some, but not all reports. Some studies have suggested that most immediate benefits may be achieved with lean patients, but no examination of different doses in different body mass categories has been reported. The aim of this study was to determine whether different doses of metformin (1500 or 2550mg per day) could have different effects upon clinical features associated with PCOS; specifically weight reduction, circulating hormone changes, markers of inflammation and lipid profiles.

Patients (n=82) categorised as obese (Ob, BMI 30 to<37 kg/m$^2$; n= 40) and morbidly obese (Mob, BMI $\geq$37 kg/m$^2$; n=42) started treatment and 68 (82%) completed the course with assessments at the start, and after 4 and 8 months treatment.

Both the degree of weight reduction and the degree of suppression of circulating androstenedione in obese women (the Ob group) were dose related, but no

similar relationship was established for the Mob group. Although generally beneficial changes in cardiovascular risk markers were recorded, there was no relationship with either BMI category or metformin dose.

We conclude that weight loss and suppression of androgens are features of protracted metformin therapy in obese women with PCOS, with greater weight reduction, and more profound suppression of androgens potentially achievable with higher doses of metformin.

# 4.1 Introduction

Following the first publications of treatment of women with PCOS with ISAs, (6, 25, 30, 31) there has been an enthusiastic subsequent exploration of effects. Recent objective reviews (16, 108) determined that the effects of metformin upon ovarian activity were beneficial, and that a number of other potential benefits were observable in appropriately controlled studies. These include improvements in lipid profiles, and reductions in the circulating concentrations of androstenedione, if not always testosterone. However, in the Cochrane database review (108), despite a number of reports observing weight loss during treatment, the meta-analysis of current studies did not confirm that weight loss was a significant factor. This contrasts with the comprehensive data provided by the use of metformin in patients with glucose intolerance in the Diabetes Prevention Program (DPP) (74), where significant and prolonged weight loss was recorded. Furthermore, there had been debate over whether some of the apparent improvement in ovarian function associated with metformin treatment, such as circulating androgen and lipid profiles, may be directly related to changes in body constitution (30).

One placebo controlled trial of metformin in women with PCOS (47) observed that the biochemical effects and reductions in body mass were more consistent in the lean and obese patients than in the morbidly obese group (Mob; body mass index, BMI > 37kg/m$^2$). This paper suggested that such (Mob) cases are either refractory to the effects of metformin, or that they may require increased doses. One recent study (62) further substantiated the view that obesity may reduce the benefit of metformin treatment. Many of the studies with metformin in women with PCOS have included large proportions of obese and morbidly obese women, and this may be a confounding feature in the conclusion of the Cochrane database review (108). Furthermore, the relationships between metformin and dose, and weight changes, endocrine changes and cardiovascular risk factors such as lipid profiles (78) and C-reactive protein (CRP) (109) have only been sparsely examined.

The aim of this study was to examine the efficacy of metformin at 2 different doses (1500mg per day, and 2550mg per day) in obese and morbidly obese women. The primary endpoint was weight loss, with the secondary intent to examine effects of treatment or weight loss upon cardiovascular risk factors, as these latter measures may be an important part of the case for extended use of ISAs in women with PCOS.

# 4.2 Methods

## 4.2.1 Study population

Women with PCOS (n=82) were recruited from the Reproductive Endocrinology/Assisted Conception clinic at the Royal Infirmary, Glasgow, UK, and surrounding hospitals. The diagnosis of PCOS was as described in methods

(chapter 2) (3). Amongst the presenting symptoms were hirsutism, menstrual disturbance and weight gain. Women whose primary complaint was obesity were invited to take part in this study. Women taking medication known to affect weight loss, gonadal or adrenal function, or carbohydrate or lipid metabolism, were excluded. Women were also advised to use barrier contraception, and were excluded if interest were expressed in immediate pregnancy.
Informed consent was obtained from each woman, and the study was conducted at the Royal Infirmary, Glasgow, UK, following approval from the ethics committee of the North Glasgow Hospitals University NHS Trust. The study was passed by the hospital ethics committee and supported by a grant from the Chief Scientist Office (CZG/4/1/47).

## 4.2.2 Power calculation

This was based on the concept that the morbidly obese may respond to the higher dose of metformin in a similar way to patients with BMI <37kg/m² responded to 850mg bd in the study by Fleming et al (47). This precedent data showed that the effect upon weight loss was significant at $p<0.02$ (BMI declined from 29.4 to 28.5kg/m², representing approximately 2kg reduction after 14 weeks) in 15 patients in whom the BMI <37kg/m². There was no change in the weight of those whose BMI was >37kg/m² (BMI from 42.5-42.3 kg/m²), while the placebo group showed a significant weight increase. Thus, it was calculated that efficacy should be detectable with 15 cases in each arm, requiring a total of 60 patients. The group in whom the BMI is >37kg/m² on the lower dose would be expected to show no change after 4 months, but the trial will determine whether changes at 8 months can be achieved.

## 4.2.3 Study design

The study was prospective, with dose block randomisation in 2 groups of patients with PCOS, defined as obese (Ob; BMI $\geq$30 <37kg/m$^2$) and morbidly obese (Mob; BMI $\geq$37kg/m$^2$). The patient number treatment codes were held by a third party and were allocated following individually written consent. Metformin (Glucophage; Merck, West Drayton, UK) doses were 500mg tds and 850mg tds. Patients were not blinded to treatment dose. Medication was commenced in a titrated fashion by taking one tablet a day for the first week, then one tablet twice a day for the second week, and then one tablet three times a day thereafter. Tablets were either 500mg or 850mg. Study assessments were performed prior to treatment (T0), after 4months (T4) and 8 months (T8) and included anthropometric measurements (weight, BMI, waist-hip ratio), ovarian structure, circulating hormones, and fasting glucose.

All patients were given the same advice concerning the benefits of life-style modification through diet and exercise. No further advice or framework to assist weight reduction was given.

## 4.2.4 Assessment programme

At T0, T4, T8 all patients underwent clinical and hormonal assessments. These included anthropometric measurements of height, weight,BMI, waist and hip (WHR), and also blood pressure, menstrual cyclicity, and hirsutism using the modified Ferriman-Gallwey score (see Appendix) (82). Ultrasound assessments (all performed by the same observer, LH) were also performed at 0, 4 and 8 month intervals to assess ovarian morphology and follicular growth (ovarian volume, numbers of follicles with diameter (FD) <10mm, and the diameter of the largest follicle.

Circulating blood samples taken at each assessment point were analysed for fasting insulin, glucose, LH, FSH, estradiol (E2), testosterone, sex hormone binding globulin (SHBG), free androgen index (FAI), DHEAS, androstenedione, 17αOHProgesterone, high sensitivity C-reactive protein (CRP), total cholesterol, triglycerides, LDL-C, HDL-C, leptin, IGF-1 and IGFBP-3. Also performed were liver function tests, basic blood biochemistry and thyroid function tests. Although there was no specific diet or exercise regime advice given as part of this trial, patients were asked to complete an exercise and dietary questionnaire at each time interval (T0, T4 and T8) (see Appendix). A side effect profile was also performed at 1, and 2 months (see Appendix).

## 4.2.5 Techniques

These were described in chapter 2.

## 4.2.6 Statistics

Where distributions were normal, group statistical evaluations were compared with unpaired t-tests, with Welch correction for unequal variances where necessary, and when distributions were non-Gaussian the Mann-Whitney U test was applied. Changes in variables during treatment within groups were evaluated using repeated measures ANOVA on values pre-treatment (T0) and at 4 months and 8 months treatment. Simple linear correlation examinations were effected to examine relationships between changes in weight and other parameters. Statistical analyses were performed using GraphPad Prism (San Diego, CA) software.

## 4.3 Results

The randomisation resulted in similar cross-sectional data within the body mass groups (nil significant difference between the dose categories), and table 4.1 shows the characteristics of the Ob and Mob groups starting treatment on the 2 doses of metformin (1500 mg/d and 2550 mg/d). They generally showed abnormal ovarian activity, with only 19% of the patients showing normal menstrual rhythm.

Of the 82 patients embarking on the study, 68 (82%) completed the course to the final 8 month assessment point. There was no difference in the proportions of patients completing the study in each group (Ob500, 18/20; Ob850, 17/20; Mob500, 18/21; Mob850, 15/21). Subsequent evaluations were effected on those patients completing the study.

Table 4.2 shows that the Ob and Mob groups differed in a number of the factors examined prior to treatment. The fasting insulin concentrations were significantly lower ($p=0.025$) in the Ob group, and the glucose/insulin ratios ($p=0.003$) were significant higher in the Ob group, while the HOMA-IR measure of insulin resistance was borderline lower ($p=0.06$) in the Ob group. The concentrations of IGF-1 were higher in the Ob group and leptin was significantly lower in the Ob group, as were circulating CRP and VEGF concentrations. The lipid profiles also differed between the groups, with the expected relationships: the Ob group showed significantly lower triglycerides and higher HDL-C concentrations, while there was no difference in the LDL-C between the groups. Broadly, table 4.2 shows that the markers of insulin sensitivity indicated increased resistance in the Mob group. Markers of endothelial function, and chronic inflammation were increased, while the lipid profiles tended to indicate increased proportion of

patients with undesirable balance of HDL to LDL and total cholesterol values in the Mob group.

## 4.3.1 Effects of metformin treatment

### 4.3.1.1 Weight loss

Figure 4.1 shows the analyses of the weight changes in all patients completing the 8 months metformin treatment (n=68). This revealed that there was a highly significant reduction from a mean BMI of 37.1kg/m$^2$ with 95% confidence limits (CL) of 35.6 to 38.6 kg/m$^2$ at the start (T0) to a mean BMI of 35.7kg/m$^2$ (CL: 34.2, 37.3 kg/m$^2$). This was a considerable (3.8%) and highly significant reduction in body mass (repeated measures ANOVA, P<0.0001).

**4.3.1.1.1 Dose of metformin.** Table 4.3 examines the effects of the different doses of metformin on the body mass of the patients and reveals that both dose groups lost significant weight over the 8 month assessment period under both dosage regimes. At 8 months, the 1500mg group (n=36) lost an average of 2.8 kg, (mean BMI from 37.9 to 36.8 kg/m$^2$), while the 2550mg group (n=32) lost 4.8 kg (mean BMI from 36.3 to 34.5 kg/m$^2$). The higher dose of metformin appeared to have a more consistent effect upon weight loss as evidenced by the degree of significance in all cases and in both obesity categories. However, with all cases combined, there was no statistical difference between the 2 dose groups with respect to the total weight lost (1500 mg group = 2.84 kg, 2550 mg group = 4.70 kg: unpaired t test [Welsh correction for unequal variances]; p = 0.19).

**4.3.1.1.2 Obesity category.** The Ob sub-group showed a statistically significant dose relationship with respect to weight loss, as those taking the higher dose lost an average of 4.2 kg (CL 3.4, 5.9) while those taking the lower dose lost an average of 1.6 kg (CL –0.2, 2.4). The difference between these 2 values was significant (P= 0.03).

In contrast, the Mob group showed similar mean losses on both doses: 4.4 kg on the lower dose, and 5.3 kg on the higher dose.

Figure 4.2 shows that the percentage decline in weight for the 1500mg dose groups, both Ob and Mob groups, was around 2%, while it was greater than 5% for both high dose groups. However, the difference between the doses was statistically valid only for the Ob group (P=0.03). The error bars represent the standard error of the mean.

## 4.3.1.2 Ovarian function as represented by menstrual frequency

There was effectively a doubling of the frequency of menses during metformin treatment in those patients with oligomenorrhoea in both dosage groups. In the 1500 mg group the mean frequency increased from 3.8 menses per year to 6.7 ($p<0.0001$, paired t-test), and in the 2550 group the increase was from a mean of 3.8 to 6.8 menses per year ($p<0.0001$).

There was no difference apparent between either the dose or obesity sub-groups in the menstrual responses to treatment. The proportions of patients with oligomenorrhoea who achieved normal menstrual rhythm ($\geq$10 menstrual cycles per year) during treatment were 36% in the 1500 mg group and 48% in the 2550 mg group ($p = 0.41$). There was also no difference between the Ob and Mob groups in the proportions attaining normal menstrual rhythm.

### 4.3.1.3 Blood analytes and metformin doses

Markers of insulin resistance (fasting insulin, glucose/insulin ratio and logHOMA-IR) revealed modest improvements associated with treatment in the high dose group only (table 4.4). Although there were significant changes in the glucose/insulin ratio and HOMA-IR in the 2550mg dose group, which were not observed in the 1500mg dose group, absolute values at 8 months did not differ significantly between the 2 dose groups (unpaired t tests: fasting insulin, $p = 0.29$; glucose/insulin ratio, $p = 0.47$; HOMA-IR, $p = 0.15$).

Table 4.4 also shows that circulating leptin concentrations indicated significant reductions in fat mass in both dose groups, and reductions in leptin and androstenedione appeared greater in the 2550 mg group (6.9 ng/ml) than the 1500mg group (4.6 ng/ml). However, absolute values at 8 months did not differ significantly between the 2 dose groups (unpaired t test: leptin, $p = 0.12$ androstenedione, $p = 0.81$). The increase in DHEAS recorded in the 1500mg group was not replicated in the 2550mg group, and there was no difference between the groups in the final concentrations of DHEAS at T8 ($p=0.37$).

The circulating concentrations of LH showed no indication of change in either treatment group. This was despite the changes in ovarian activity evidenced by menstrual frequency noted above. Analysis of the LH values in those cases undergoing the change from oligomenorrhoea (<9 menses per year) to normal menstrual rhythm (>9 menses per year) revealed that these individuals ($n = 21$) showed marginal reduction in circulating LH ($p = 0.042$, paired t-test) with absolute values at T0 (12.2 IU/L) evolving through 11.4 IU/L at 4 months down to 7.6 IU/L at 8 months. This latter value would be considered to represent the 'high / normal' range.

Table 4.4 also shows the changes in lipid and inflammatory marker profiles observed during treatment with the 2 doses of metformin. Significant reductions in total cholesterol and LDL-C were observed in both dosage groups, but there was no dose effect apparent. The changes in lipids, and leptin as a marker of total fat mass in both groups were not reflected in changes in circulating CRP which showed no significant change in either dose group, and reductions in VEGF were significant only in the 1500mg dose group.

### 4.3.1.4 The role of weight change

Changes in parameter values were examined in respect of weight change over the 8 month treatment period, irrespective of obesity category and dose of metformin. There were simple linear correlations between the change in weight (kg) and change in total testosterone ($r^2 = 0.09$, $P = 0.01$), androstenedione ($r^2 = 0.06$, $P = 0.04$), SHBG ($r^2 = 0.11$, $P = 0.006$) and FAI ($r^2 = 0.10$, $P = 0.01$), and also total triglycerides ($r^2 = 0.19$, $P = 0.0002$) and leptin ($r^2 = 0.25$, $P < 0.0001$). Changes in fasting insulin and HOMA-IR, as well as cholesterol, LDL-c, and HDL-c showed no relationship with weight change. There was also no correlation with CRP.

## 4.4 Discussion

We believe this is the first systematic, randomised study of the effects of different doses of metformin in obese women with PCOS. The data reveal that women with PCOS respond to metformin in a manner related to both the dose and their body mass. Both the degree of weight reduction and the degree of suppression of circulating androstenedione in obese women (the Ob group) were dose related, while neither showed such consistent changes in the morbidly obese

(Mob) group. These observations imply that the degree of obesity impacts upon responses to metformin, and that maintained excessive obesity had a confounding effect upon responses to treatment. Notwithstanding this, ovarian function appeared to show general improvement in all sub-groups. It is interesting to record that although measures of insulin resistance changed only in the high dose group, some markers of cardiovascular risk showed apparent improvements with both metformin doses and both BMI groups, while others remained unchanged in all circumstances.

The recent Cochrane Library review and its summary (108) concluded that metformin treatment of women with PCOS was effective in increasing ovulation rates and suppressing androstenedione in women with PCOS, but it could not confirm effects upon weight reduction. Many of the studies quoted in the review, included relatively small numbers, were of generally short duration, and most of the primary end-points were related to ovarian function and fertility. All of these characteristics may reduce the validity of the conclusions with respect to the non-ovulation end-points. That conclusion also contrasts with the finding of the diabetes prevention study (74), with large numbers of individuals (not PCOS) in whom weight loss was consistent and long lasting. The study reported here was designed to address weight loss during metformin treatment amongst PCOS patients given standard weight reducing advice, and it shows convincing evidence supporting a pharmacological effect.

The effects of metformin upon lipid profiles and markers or risk factors for vascular disease were complex, generally beneficial, and appeared to be unrelated to metformin dose, degree of obesity, or weight change. Total cholesterol showed a general decrease, mainly through a decrease in LDL-cholesterol, and there was a trend towards increased HDL-cholesterol. There appeared to be no dose differential in either BMI category. There was no

consistent change in total triglycerides. A large series of women with PCOS treated with 3 doses of troglitazone similarly showed no change in circulating triglycerides (78), and furthermore only non-significant trends in reduction of LDL-cholesterol and increase in HDL-cholesterol were recorded. The review (108) of studies using metformin for variable duration revealed consistent changes in LDL-cholesterol only, in accord with the observations above.

It is acknowledged that the absence of a placebo group of patients limits the validity of observations of weight changes, but a previous study with placebo in similar patients given identical advice showed a modest but significant weight increase over 16 weeks (47).

Although relative insulin resistance, and its compensatory hyperinsulinaemia, or insulin hypersecretion, are thought to underpin the aetiology of PCOS, the origins of the ovarian disorder (excessive, primary and small antral follicular development) may be established at birth through excessive primordial follicular development at birth (110). An alternative explanation accounting for at least some cases include environmentally induced increased primary and more advanced follicular development. Correspondingly, until means of influencing the rate of initial follicle recruitment and survival to the antral stage can be elucidated, the prospect of a 'cure' for PCOS is a distant concept. However, the effects of increasing body mass and hyperinsulinism appear to have a profound impact upon ovarian function and fertility in women with (and perhaps without) PCOS, and metformin appears to be defining a role for itself in this specific area. This study appears to suggest that the doses used hitherto in obese and morbidly obese women with PCOS may be sub-optimal.

It is likely that most of the immediate symptoms of PCOS will continue to be treated using a symptom-specific approach, but the role of insulin sensitising agents in the treatment of these disorders remains to be determined either alone

or as an adjunctive medication. With the possible exception of fertility issues, the principle symptoms for considering treatment generally require protracted treatment, often in conjunction with other medications.

Optimal doses of metformin in these circumstances have not been elucidated, but the indication from this study is that more obvious benefit down stream from changes in hormones, lipids and weight change may be obtained with doses higher than those previously explored – particularly in patients with excessive body mass.

We conclude that weight loss is a feature of protracted metformin therapy in women with PCOS, with greater weight reduction potentially achievable with higher doses of metformin. Correspondingly, future studies should examine whether higher metformin doses yield greater clinical benefit, although this study suggests that large numbers of patients will be required to show convincing differences in many parameters.

**Table 4.1**

Profiles of the groups of patients (obese, Ob, and morbidly obese, Mob) with PCOS before treatment with metformin at 2 different doses. The doses were 500 mg tds (1500 mg/d) and 850 tds (2550 mg/d). Standard deviations are in parentheses.

| **Group** | **Ob** | | **Mob** | |
|---|---|---|---|---|
| **Dose (mg/d)** | **1500** | **2550** | **1500** | **2550** |
| N | 20 | 20 | 21 | 21 |
| Amen (n) | 9 | 7* | 6 | 8 |
| Oligomenorrhoea (n) | 8 | 9 | 10 | 10 |
| Normal Menses (n) | 4 | 4 | 4 | 3 |
| Mean BMI (kg/m²) | 32.9 (2.7) | 32.1 (2.2) | 42.6 (5.3) | 41.2 (3.6) |
| Waist/Hip Ratio | 0.84 (0.075) | 0.85 (0.048) | 0.86 (0.065) | 0.87 (0.043) |
| Ovarian Volume (ml) | 10.6 (4.9) | 10.3 (4.0) | 8.6 (2.6) | 10.9 (7.1) |
| Ovarian Follicles (FD2-9, n) | 14.7 (5.4) | 14.4 (5.3) | 12.3 (4.5) | 12.9 (4.6) |
| Androstenedione (nmol/L) | 11.1 (3.8) | 12.7 (5.5) | 11.2 (4.4) | 10.0 (4.1) |
| Testosterone (nmol/L) | 5.2 (1.8) | 6.8 (3.2) | 5.8 (2.2) | 5.8 (2.9) |
| LH (IU/L) | 10.5 (5.1) | 10.8 (6.4) | 11.2 (6.6) | 9.2 (5.6) |
| FAI | 8.9 (6.6) | 11.5 (8.4) | 11.8 (5.3) | 9.9 (6.9) |
| Fasting Insulin (mIU/L) | 15.9 (13.1) | 14.1 (6.5) | 19.2 (7.0) | 22.1 (11.7) |
| Glucose/Insulin Ratio | 0.45 (0.25) | 0.41 (0.15) | 0.31 (0.16) | 0.28 (0.12) |
| HOMA-IR | 3.8 (3.8) | 3.1 (1.4) | 4.4 (1.9) | 4.8 (2.4) |
| DHEAS (µmol/L) | 5.9 (1.8) | 6.7 (2.7) | 5.9 (3.3) | 6.1 (2.8) |
| Leptin (ng/ml) | 56.0 (16.9) | 56.8 (34.4) | 81.7 (21.1) | 77.4 (25.2) |
| CRP (mg/L) | 7.0 (6.9) | 4.8 (6.4) | 7.0 (5.1) | 9.0 (9.5) |

*1 patient had a levonorgestrel intrauterine system *in situ* and could not provide a reliable estimate of her menstrual history.

**Table 4.2**

The mean values (with 95% confidence limits) and differences in anthropometric, endocrine and circulating lipids of the Ob (n=42) and Mob (n=41) groups of women with PCOS determined prior to treatment.

| | Ob | Mob | P |
|---|---|---|---|
| BMI (kg/m²) | 32.5 (31.7, 33.3) | 41.9 (40.5, 43.3) | |
| Age (years) | 30.5 (28.3,32.7) | 30.5 (28.7, 32.3) | NS |
| Fasting Insulin (mIU/L) | 15.0 (11.5, 18.6) | 20.5 (17.2, 23.8) | 0.025 |
| Glucose/Insulin | 0.43 (0.36, 0.50) | 0.30 (0.25, 0.35) | 0.003 |
| Homa-IR* | 3.45 (2.46, 4.44) | 4.61 (3.87, 5.36) | 0.063 |
| IGF-1 (ng/ml) | 224 (191, 257) | 158 (131, 184) | 0.002 |
| CRP (mg/L) | 5.92 (3.63, 8.21) | 7.92 (5.3, 10.5) | 0.010 |
| Leptin (ng/ml) | 56.4 (47.3, 65.5) | 79.8 (71.7, 87.8) | 0.0002 |
| VEGF (pg/ml) | 19.0 (9.1, 28.8) | 24.5 (18.4, 30.6) | 0.002 |
| Triglycerides (mmol/L) | 1.46 (1.15, 1.77) | 2.05 (1.65, 2.45) | 0.0004 |
| HDL-Cholesterol (mmol/L) | 1.16 (1.05, 1.28) | 0.91 (0.84, 0.97) | 0.0002 |
| LDL-Cholesterol (mmol/L) | 3.03 (2.77, 3.30) | 3.50 (3.20, 3.80) | 0.021 |

*Data log-transformed for comparative analyses

**Table 4.3**

The mean BMI (kg/m²) values before and after 8 months treatment with 2 doses of metformin (with standard deviations in parentheses). The changes (significance levels, P) were assessed using values taken at T0, 4 months and 8 months using repeated measures ANOVA.

| | 1500mg/d | | | 2550mg/d | | |
|---|---|---|---|---|---|---|
| | T0 | 8m | P* | T0 | 8m | P* |
| All cases | 37.9(6.5) | 36.8 (6.9) | 0.0022 | 36.3 (5.6) | 34.5 (5.7) | <0.0001 |
| Ob group | 33.0 (2.8) | 32.4 (3.5) | 0.0577 | 31.9 (2.3) | 30.4 (3.0) | <0.0001 |
| Mob group | 42.7 (5.5) | 41.3 (6.7) | 0.0268 | 41.2 (3.7) | 39.2 (4.1) | 0.0027 |

*repeated measures ANOVA of BMI values at T0, T4 and T8.

**Table 4.4**

Changes in concentration of individual analytes during treatment with metformin in the 2 dose groups of patients (Ob and Mob patients combined) treated with 1500mg or 2550mg per day. The absolute values are shown for pre-treatment (T0) and at 8 months (8m), and the statistical value (P) represents the repeated measures ANOVA assessment for values at T0, 4 months and 8 months. (Standard deviations are represented in parentheses.)

| | 1500mg/d | | | 2550mg/d | | |
|---|---|---|---|---|---|---|
| | **T0** | **8m** | **P** | **T0** | **8m** | **P** |
| Fasting Insulin (mU/L) | 17.6 (10.5) | 18.6 (16.1) | 0.746 | 17.7 (10.0) | 15.1 (10.9) | 0.084 |
| Glucose/Insulin Ratio | 0.38 (0.22) | 0.39 (0.23) | 0.644 | 0.35 (0.15) | 0.43 0.25) | 0.039 |
| HOMA-IR | 4.10 (3.0) | 4.37 (4.3) | 0.711 | 3.92 (2.1) | 3.13 (2.3) | 0.039 |
| IGF1(ng/ml) | 187 (102) | 186 (104) | 0.856 | 197.0 (79) | 183.1 (78) | 0.373 |
| Testosterone (nmol/L) | 5.54 (2.0) | 5.83 (1.8) | 0.57 | 6.40 (3.1) | 6.05 (3.6) | 0.078 |
| Androstenedione (ng/ml) | 11.18 (4.2) | 9.34 (2.7) | 0.016 | 11.97 (5.0) | 9.14 (4.1) | <0.0001 |
| FAI | 10.45 (6.3) | 12.04 (8.7) | 0.158 | 11.5 (8.1) | 11.1 (8.4) | 0.870 |
| LH (IU/L) | 11.07 (5.7) | 9.43 (7.2) | 0.605 | 10.3 (6.4) | 9.5 (5.6) | 0.305 |
| DHEAS (uml/L) | 5.86 (2.7) | 6.53 (3.4) | 0.023 | 6.81 (2.9) | 7.28 (3.4) | 0.133 |
| Triglycerides (mmol/L) | 1.63 (0.96) | 1.54 (0.66) | 0.684 | 1.87 (1.1) | 1.70 (0.9) | 0.374 |
| Total Cholesterol (mmol/L) | 5.16 (0.96) | 4.82 (0.83) | 0.0002 | 5.04 (0.90) | 4.70 (0.87) | 0.004 |
| HDL-C (mmol/L) | 1.09 (0.30) | 1.15 (0.36) | 0.072 | 0.99 (0.28) | 0.99 (0.28) | 0.807 |

(Table 4.4 cont.)

| | 1500mg/d | | | 2550mg/d | | |
|---|---|---|---|---|---|---|
| | T0 | 8m | P | T0 | 8m | P |
| LDL-C (mmol/L) | 3.33 (0.76) | 2.97 (0.76) | <0.0001 | 3.17 (0.91) | 2.88 (0.87) | 0.003 |
| Leptin (ng/ml) | 68.9 (22.9) | 57.1 (25.8) | <0.0001 | 66.5 (31.7) | 48.3 (19.8) | <0.0001 |
| CRP (mg/L) | 7.02 (6.0) | 5.51 (8.1) | 0.15 | 6.74 (8.2) | 5.71 (7.4) | 0.485 |
| VEGF (pg/ml) | 26.0 (29.5) | 16.6 (8.6) | 0.016 | 16.8 (14)0 | 15.6 (8.0) | 0.762 |

**Figure 4.1**

Weight changes in all patients completing the 8 months metformin treatment revealed that there was a highly significant reduction from a mean BMI of 37.1 to 35.7 kg/m² (CL: 34.2, 37.3 kg/m²).

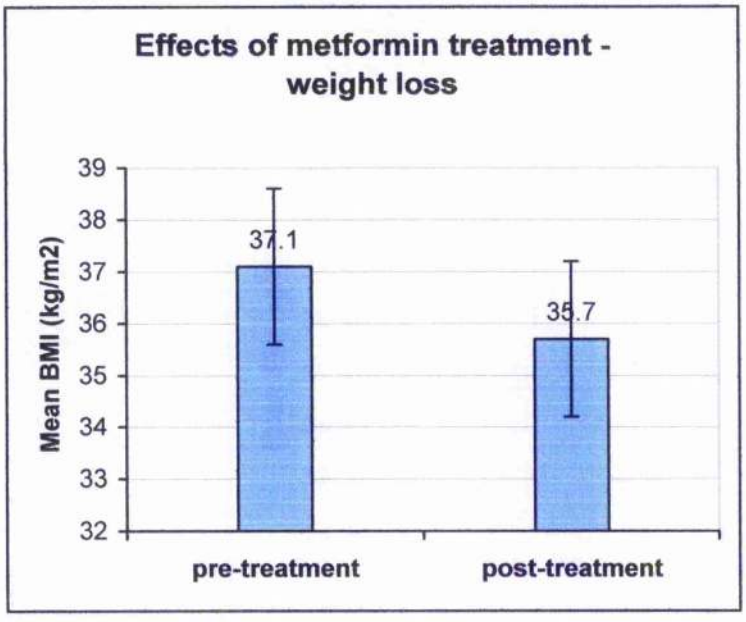
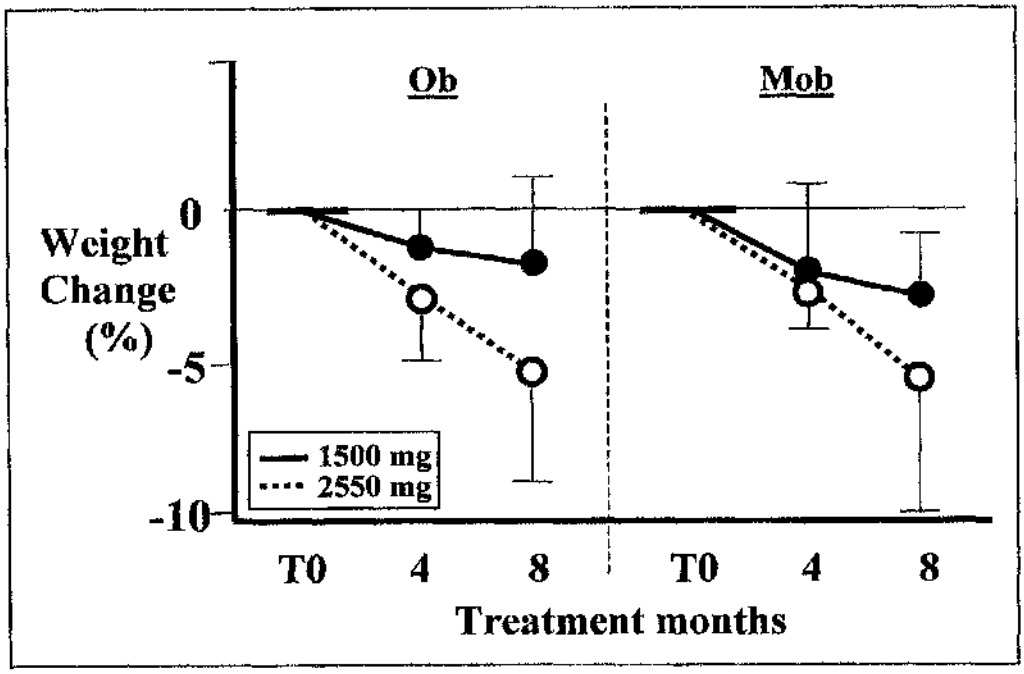


**Figure 4.2**

Percentage weight changes during 8 months completed metformin therapy amongst the Ob and Mob groups of women taking 1500mg and 2550mg doses per day.(Error bars represent the standard error of the mean.)



Chapter 5

# The investigation of Mullerian Inhibiting Substance in obese women with PCOS, and determination of the effects of metformin treatment

## 5.0 Summary

The objective of this study was to assess the role of Mullerian inhibiting substance (MIS) in obese women with PCOS and investigate changes in response to metformin treatment. The study was a prospective element based upon the investigation into the effects of metformin upon body mass (chapter 4), whereby sample aliquots were stored for assessment of MIS. The study included randomization to 2 different doses of metformin treatment.

Eighty-two obese women with polycystic ovary syndrome (PCOS) were recruited to the trial at the Royal Infirmary, Glasgow, through the University Department of Obstetrics and Gynaecology. Markers of ovarian function were assessed at T0 and after 4 and 8 months. The main outcome measures were reproductive hormone changes over time, primarily being those of androgens and MIS.

The results showed that there was no difference in the reproductive hormone changes between the doses of metformin (including MIS), and all data were combined for analyses.

Significant responses to metformin treatment were recorded for menstrual frequency (increased) and androstenedione (reduction) within the first 4 months

of treatment. However, suppression of the elevated circulating MIS concentrations required protracted treatment, as no change was observed in the first 4 months – only in the second 4 month assessment period. Thus the initial ovarian changes took place in the absence of changes in MIS.

In conclusion, women with PCOS show high concentrations of circulating MIS which are correlated with the number of small antral follicles in their ovaries. Metformin treatment of obese women with PCOS lead to rapid suppression of androstenedione and improved menstrual frequency, while suppression of MIS showed a delayed response. We may construct a hypothesis suggesting that the delayed effect may be secondary to the development of a cohort of follicles, which underwent initial recruitment in an environment of reduced insulin stimulation under the influence of metformin, and it takes in excess of 4 months for that cohort to be represented at the more advanced stages.

# 5.1 Introduction

Following the advances in the understanding of the metabolic / endocrine disturbances seen in polycystic ovary syndrome (PCOS) (111), recent work has addressed the ovarian abnormality seen in this common disorder. Webber et al (110) showed that the proportions of primitive follicular categories differed between normal and PCOS ovaries, also concluding that the number of primordial follicles present at birth was greater in PCOS. Jonard et al (112) showed that there are important differences in categories of antral follicles definable by their size. The number of smaller visible follicles (follicle diameter [FD] between 2 and 5 mm; FD 2-5), is significantly raised in PCOS compared with normal, and they represent the category responsible for the high circulating

androgen concentrations, while the number in the larger category (FD 6-9) reflects the degree of insulin resistance, and is closely linked to the frequency of follicular maturation, ovulation and infertility. These 2 follicular categories represent later stages of development, prior to the pre-ovulatory stage, and whose development from the primordial stage requires many weeks growth (113). Thus in PCOS, rates of initial recruitment and progress through developmental stages appear to differ from normal. The raised androgens in the circulation of women with PCOS probably derive from the enlarged cohort of small follicles at the androgen secreting stage (112) as well as increased direct stimulation by insulin and LH, perhaps involving stromal tissue as well (114).

Mullerian inhibiting substance (MIS), a dimeric glycoprotein, also known as anti-mullerian hormone (AMH), is a member of the transforming growth factor beta super-family of growth and differentiation factors, and its concentration is raised in the circulation of women with PCOS (64, 115, 116). MIS is believed to act in a paracrine fashion after birth to regulate granulosa cell and oocyte function (117), and it may be an important regulator of follicle recruitment (118). In humans, MIS is not seen in primordial follicles, but it is expressed from the primary stage through to small antral follicles where maximal expression is seen (119). It is this latter category of follicle, with maximal granulosa cells, that is likely to be responsible for much of the MIS in the circulation.

A study by la Marca et al found higher serum MIS levels in women with PCOS compared with controls. They also found that the level of MIS was higher in those subjects with amenorrhea compared with oligomenorrhea, thus indicating a possible role for MIS in PCOS related anovulation. A simple measure of insulin sensitivity, the HOMA score, was found to positively relate to serum MIS levels. However, there exists no literature relating insulin and MIS levels (64).

As discussed previously, women with PCOS show relative insulin resistance and compensatory hyperinsulinaemia (120), which may be critical to the underlying disorder. Treatment with ISAs in women with PCOS results in reduced androgenicity (suppression of total androstenedione) and increased frequency of follicular development and ovulation as described in Chapter 1. It is not known whether the reduced circulating androgen concentrations reflects simply reduced insulin driven secretion of androgens per follicle, or a reduced follicle cohort size (or both). The effect of ISAs on circulating concentrations of MIS in PCOS has not been explored, and its elucidation is important for furthering the understanding the ovarian effects of ISA treatment.

The aims of this study were to explore the relationship between MIS and other reproductive parameters in obese women with PCOS, and also to test the hypothesis that reducing the degree of insulin stimulation to the ovaries in these women, using protracted metformin therapy, would reduce the circulating concentrations of MIS. The relationships of changes in all parameters were explored and revealed some critical observations.

## 5.2 Methods

Most of the methods used were described in Chapter 2, but those specific to this study are described below. Details addressing study population, study design, assessment programme and techniques are provided in Chapter 4.

## 5.2.1 Serum MIS assay

Serum MIS was measured by an enzyme linked immunosorbent assay as described in earlier studies (121). There is no cross reactivity with other members of the TGF beta gene family, and is specific for primate MIS. The intra-assay coefficient of variation was 9%, the inter-assay coefficient of variation was 15%, and the limit of sensitivity was 0.3 ng/ml. MIS values that fell below the limit of sensitivity for the assay (<0.3 ng/ml) were considered undetectable. Mean serum MIS values in female infants, ages two to twelve months, were reported to be 0.7 ng/ml (range <0.3-1.9 ng/ml), increasing minimally to prepubertal values of 0.9 ng/ml (range <0.3-3.9 ng/ml) by 18 months. During the prepubertal years, MIS gradually increased to pubertal and adult values of 2.9 ng/ml (range <0.3-8.9 ng/ml). The upper limit of normal ($95^{th}$ centile) was taken to be 7ng/ml.

## 5.2.2 Statistical analyses

Comparison of variables between groups were effected using unpaired t tests where distributions were normal, and using Mann-Whitney U tests for analyses involving the glucose/insulin ratio and MIS concentrations which showed non-Gaussian distributions. Changes of variables within patients during metformin treatment, with values at T0 and 4 and 8 months, were assessed using repeated measures ANOVA. In the cross-sectional analyses prior to treatment, correlation analyses were effected using Pearson correlation test where data showed normal distributions, and using the Spearman rank test when the data showed non-Gaussian distributions. The software package employed for these analyses, and the multiple regression model involving the factors shown to demonstrate significant correlations with MIS, was SPSS (version 8; SPSS UK Ltd, Woking, UK).

# 5.3 Results

## 5.3.1 Patients prior to treatment

Table 5.1 shows the characteristics of the patients participating in the study. They were obese (BMI > 29 kg/m$^2$) with indications of insulin resistance determined by high fasting insulin (laboratory normal maximum $\geq$ 13 nmol/dl), and a raised HOMA-IR. The total follicle number (TFN) was high (diagnosis of polycystic ovary demanded a minimum TFN of 10 follicles), and circulating androgen and LH concentrations were elevated. The mean circulating concentration of MIS was elevated, with a value of 7.9 ng/ml, which is above the laboratory normal upper limit described previously as 7.0 ng/ml.

Table 5.2 shows that there was no relationship between MIS and BMI or fasting insulin concentrations or HOMA-IR values. Of the clinical markers of ovarian function, ovarian volume showed no clear relationships with circulating MIS. However, there was a positive linear correlation between circulating MIS and TFN, and also between MIS and androstenedione and a negative correlation with age.

When the 3 variables (TFN, androstenedione and age) showing significant individual correlation with circulating MIS as the dependent variable, were entered into a multiple regression model, only TFN remained significant ($p = 0.022$). The predictive capacity (r squared) for the model was 0.18, and the significance of the combined model was $p = 0.011$.

## 5.3.2 Effects of metformin treatment

### 5.3.2.1 Ovarian function and MIS

During metformin treatment, there were numerous changes recorded over the 8 month treatment period, but in respect of factors related to ovarian function, there was no significant difference between the 2 doses employed. Correspondingly, the data from both dose schedules were combined for further analyses.

There was an increase in the presumed ovulation rate, evidenced by an increase in the frequency of menstrual events. The mean frequency of menses per year increased from 5.6 (+/- 0.5, standard error) to 8.3 (+/- 0.5, standard error), which represents (paired data) a highly significant increase ($p<0.0001$) with 95% confidence intervals of the difference between 3.7 to 1.7 events.

Analysis of all cases using repeated measures ANOVA showed a highly significant reduction in MIS during 8 months metformin treatment ($p=0.0005$, table 5.3). The absolute suppression of MIS concentrations in both dosage groups during 8 months metformin treatment were similar (1500mg group, -1.98 ng/ml +/- 6.3; 2550mg group, -1.60 ng/ml +/- 2.8: $P=0.78$, Mann-Whitney Test).

Table 5.3 also shows that there were significant overall (repeated measures ANOVA) reductions in circulating androstenedione and VEGF, and significant increases in DHEAS, while there was no change in total testosterone concentrations.

Table 5.3 also shows that there was no change in the total ovarian volume, which remained above the normal upper limit of 9 ml, and there was a negligible change in the TFN ($P=0.07$, repeated measures ANOVA) over the 8 month treatment period.

Metformin treatment afforded limited change in markers of energy metabolism, with no significant change in fasting insulin or HOMA-IR. However, there was a highly significant reduction in BMI, representing a 4.3% reduction in weight in the 8 month period. Correspondingly, there was a highly significant reduction in leptin.

### 5.3.2.2 The chronology of responses to metformin

Table 5.3 shows the sequences of changes during metformin treatment. The data were examined using repeated measures ANOVA for the whole treatment period and paired t tests for examination of differences between 2 time points. It was clear that some factors responded rapidly (within 4 months) whilst other factors appeared to be delayed to between the $4^{th}$ and $8^{th}$ month of treatment.

While fasting insulin and HOMA-IR showed negligible change during treatment, the BMI showed significant reductions in both intervals. In contrast the reduction in leptin was significant only over the first 4 months. Similarly, the increase in DHEAS was restricted entirely to the first 4 months. The main change in VEGF also appeared to be in the early stages of treatment, but statistical significance was achieved in neither individual time frame. Androstenedione showed a rapid decline over the first 4 months and a further, more moderate, decline between months 4 and 8.

In contrast to those 'rapid' effects of metformin treatment, although there were highly significant reductions in MIS, table 5.3 shows that the reduction in concentration was restricted entirely to the 4m to 8m interval. Figure 5.1 shows the contrast in responses between androstenedione and MIS. The TFN showed no changes overall, but they did show a significant reduction between 4m and

8m. The apparent delayed reduction in LH concentrations (months 4 to 8) did not achieve statistical significance.

## 5.4 Discussion

These data demonstrate, for the first time, that treatment of women with PCOS with metformin results in a highly significant reduction in the circulating concentrations of MIS. Furthermore, they show that the suppressive effect occurs only after protracted treatment of the time scale suggestive of effects upon initial follicular recruitment. The changes in circulating MIS contrast with those of androstenedione whose suppression is rapid, and observed mainly within the first few weeks. Thus, the testing of the hypothesis that insulin resistance (HOMA-IR) and MIS are directly linked did not confirm any such relationship.

The cross-sectional data from these women with PCOS prior to treatment with metformin support the previous demonstrations (115, 116, 122) that women with PCOS have elevated circulating concentrations of MIS. In accord with previous observations, there was no relationship between circulating MIS and markers of insulin resistance, and there were positive correlations with TFN and circulating androgen concentrations and a negative correlation with age. The multiple regression model revealed that only TFN showed a significant relationship with circulating MIS. Conversely, in the trial by la Marca et al, a significant positive correlation was found between MIS and HOMA index. However, it should be noted that this trial was of small patient numbers (14 women with PCOS and 15 women as controls), and there were no ultrasound parameters included as an outcome measure.

The effects of metformin treatment on circulating concentrations of androstenedione, with an immediate suppression, and MIS, with no change within the first 4 months, indicates a dissociation between the 2 follicular products and their specific sources and control mechanisms. Furthermore, the fact that metformin increases the ovulation rate, represented by menstrual frequency in the data above, and shortens the duration to first ovulation (47) within the first 4 months of treatment during which there is no change in MIS, suggests that circulating MIS activity is not responsible for the blockade to follicular maturation commonly seen in PCOS.

The eventual suppression of MIS, occurring after 4 months therapy, suggests that follicular profiles change during protracted metformin therapy, in the absence of any indication that its secretion per follicle is promoted by any of the factors influenced by metformin therapy. The protracted delay in the suppression of MIS (approximately 4 months) indicates that the eventual change in follicle profiles is independent of short term changes in metabolic and gonadal steroid profiles.

These observations lead to the hypothesis that the critical ovarian factor being influenced by protracted metformin treatment, and represented by the delayed changes in MIS, is initial follicle recruitment, a key abnormality in PCOS (110). The data suggest that changes in MIS require the replacement by the whole follicle cohort present at T0 by a cohort recruited under a reduced degree of insulin (and perhaps androgen) stimulation. The reduction in TFN between months 4 and 8 may provide some circumstantial support for this concept. According to previous observations (113), it requires approximately 3 months from initial recruitment to achieve the antral stage of follicular development ready to undergo final maturation, and it is not until a new cohort has grown and replaced the original cohort (recruited under conditions of insulin resistance and

high androgen concentrations), that we see changes in MIS. This subsequent follicle cohort is smaller, because it was recruited under a "normalized" insulin (and perhaps androgen) drive, showing the importance of insulin metabolism at this early stage. The apparent discrepancy between the accepted 3 month interval and the minimum of 4 months observed above may also indicate a key difference in PCOS, or the delay between the antral stage and the MIS secreting phase, or alternatively, a weakness in the hypothesis. The results indicate that more detailed observations are required, including determination of whether the same effect is seen in lean women with PCOS.

This hypothesis would demand that all insulin resistant states, with compensatory hyperinsulinaemia, would lead to increased follicular initial recruitment, cystic ovaries and high concentrations of MIS. The cystic ovaries, and raised androgen levels have been observed in a case of an insulinoma prior to surgical removal (123), although the MIS concentrations under these circumstances are unknown. During adolescence, there is a recognized state of insulin resistance, and its abnormal ovarian morphology often observed at this time (124) may fit this criterion. It may be advantageous to explore further the link between PCOS and adolescence.

Other factors examined during metformin treatment revealed similar data to previous publications, as metformin treatment resulted in negligible change in fasting insulin concentrations or HOMA-IR, although weight loss and reductions in circulating leptin were highly significant. The metformin treatment resulted in significant reductions in circulating VEGF and a significant increase in DHEAS, all within the first 4 months, while there was no change in circulating total testosterone values. The pattern of changes in androgens (suppression of androstenedione and negligible effect upon testosterone) is consistent with the

combined observations of Lord et al (15) and some other single studies (125). This implies that improving insulin sensitivity in PCOS has a differential effect upon different follicle categories. The role of MIS in this context is complex, since previous evidence indicates that high MIS values should suppress androgen biosynthesis (126), and a reduction in MIS under the influence of metformin should lead to an increase in androgen production. Both predictions are contradicted by the present observations, suggesting that the change in follicle category and cohort size may be more important than the quantitative effect upon individual follicles studied in vitro.

Irrespective of the speculation over an active role for MIS in the pathogenesis of PCOS, evidence is accumulating supporting further investigations of MIS in women with PCOS. As a marker of ovarian follicular activity, knowledge of the circulating MIS concentrations may have important repercussions in a number of areas, including assisted reproduction where it may be used to predict the response of patients to ovarian stimulation in normal and poor responder patients (116, 127-129).

In summary, metformin treatment of women with PCOS resulted in rapid and maintained responses of ovarian function under direct insulin control, while circulating MIS concentrations required 4 months treatment before responding. This suggests that MIS reflects the cohort size of follicles undergoing initial recruitment approximately 4 months previously.

**Table 5.1**

Mean background data of the women with PCOS (n=82) prior to treatment, set against laboratory normal data for reference.

| | **Mean** | **95% CI** | **Upper limit of laboratory normal range** |
|---|---|---|---|
| Age (y) | 30.2 | 28.7 – 31.8 | - |
| BMI (kg/m$^2$) | 37.1 | 35.6 – 38.6 | - |
| Fasting Insulin (nmol/dl) | 17.7 | 15.2 – 20.2 | 12.9 |
| HOMA-IR | 4.0 | 3.4 – 4.6 | 3.8 |
| Glucose / insulin ratio | 0.37 | 0.32 – 0.41 | 0.4* |
| IGF-1 (ng/ml) | 192 | 170 – 214 | 330 |
| | | | |
| Menses per year (n) | 5.5 | 4.5 – 6.5 | 10* |
| TFN (n) | 13.8 | 12.5 – 15.1 | 9 |
| | | | |
| Testosterone (nmol/L) | 5.9 | 5.3 – 6.5 | 4.9 |
| Androstenedione (nmol/L) | 11.6 | 10.4 – 12.7 | 8.9 |
| LH (IU/L) | 10.7 | 9.2 – 12.1 | 9.9 |
| Inhibin-B (pg/ml) | | | 200 |
| VEGF (pg/ml) | 20.2 | 16.1 – 24.3 | 55 |
| MIS (ng/ml) | 7.9 | 6.2 – 9.6 | 6.9 |

*the limit of these factors is the lower value of the normal range

**Table 5.2**

Correlation assessments between circulating MIS and anthropometric, metabolic and endocrine factors in obese women with PCOS.

| | R | 95% CI | P |
|---|---|---|---|
| BMI ($kg/m^2$) | 0.037 | -0.274 to 0.203 | 0.761 |
| Fasting Insulin (nmol/dl) | 0.006 | -0.233to 0.245 | 0.959 |
| HOMA-IR | 0.002 | -0.247 to 0.243 | 0.988 |
| Age (years) | -0.241 | -0.454 to -0.003 | 0.047 |
| Total Follicle Number (TFN) (n) | 0.384 | 0.145 to 0.582 | 0.002 |
| Ovarian Volume ($cm^3$) | 0.006 | -0.261 to 0.251 | 0.966 |
| Androstenedione (nmol/L) | 0.239 | 0.287 to 0.662 | 0.049 |
| Testosterone (nmol/L) | 0.000 | -0.221 to 0.256 | 0.881 |

**Table 5.3**

Mean values of endocrine and other factors before treatment (T0) and after 4 and 8 months treatment with metformin. The statistical evaluations refer to change throughout the period of examination (repeated measures ANOVA) and also for the individual 4 month intervals (paired t tests). Standard deviations are represented in parentheses.

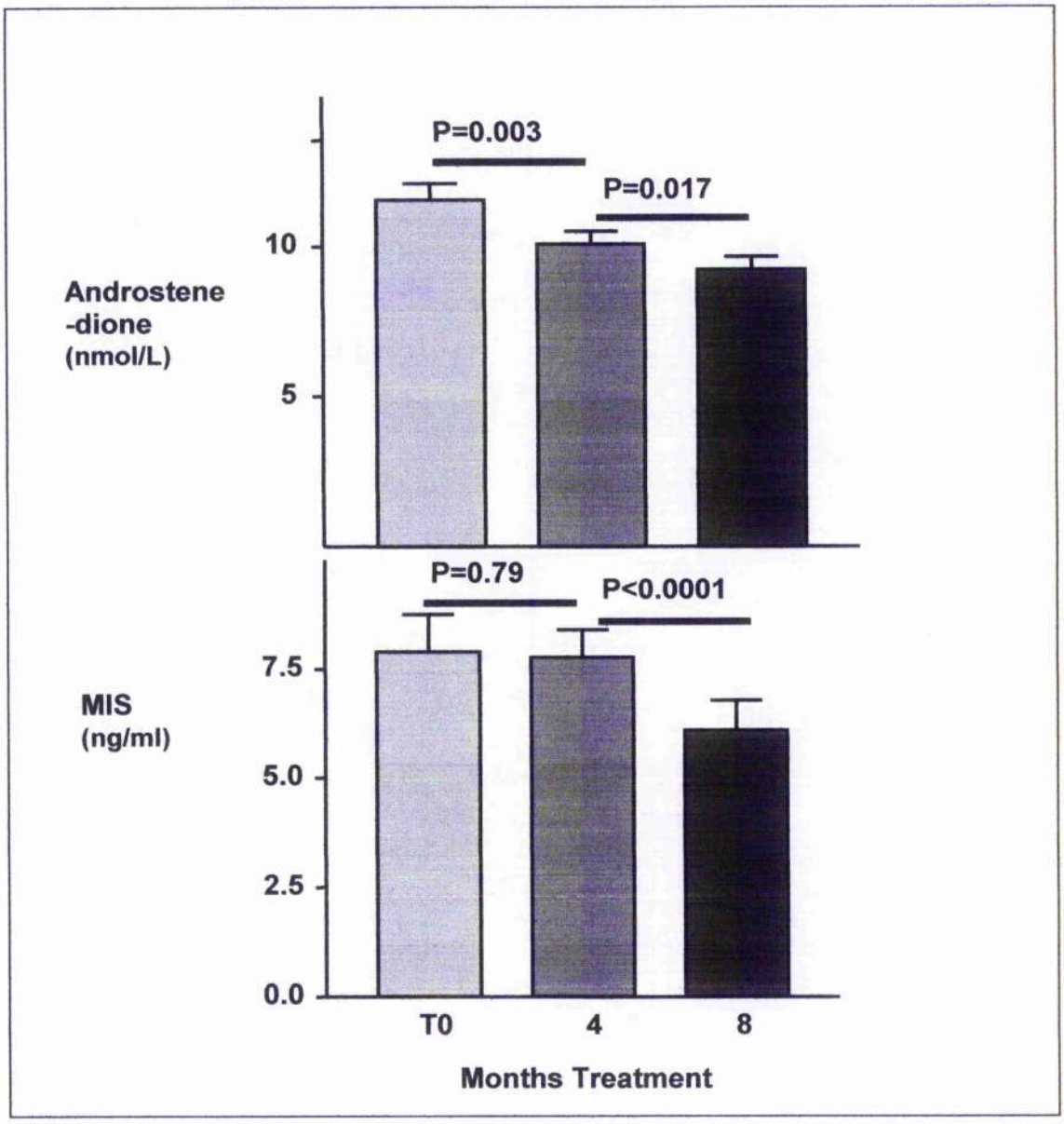
| | | | | Significance | | |
|---|---|---|---|---|---|---|
| **Factor** | **T0** | **4m** | **8m** | **Repeated Measures Anova** | **Paired t-test T0 – 4m** | **Paired t-test 4m – 8m** |
| Fasting Insulin (nmol/dl) | 17.7 (10.2) | 18.9 (12.1) | 17.1 (13.9) | 0.81 | 0.74 | 0.57 |
| HOMA-IR | 4.02 (2.6) | 4.3 (3.6) | 3.8 (3.5) | 0.50 | 0.44 | 0.32 |
| BMI ($kg/m^2$) | 37.1 (6.1) | 36.3 (6.2) | 35.7 (6.4) | <0.0001 | <0.0001 | 0.0002 |

(Table 5.3 cont.)

| | | | | Significance | | |
|---|---|---|---|---|---|---|
| **Factor** | **T0** | **4m** | **8m** | **Repeated Measures Anova** | **Paired t-test T0 – 4m** | **Paired t-test 4m – 8m** |
| Leptin (ng/ml) | 67.7 (27) | 58.6 (23) | 53.0 (23) | <0.0001 | 0.04 | 0.160 |
| DHEAS (µmol/L) | 6.31 (2.8) | 6.94 (3.1) | 6.88 (3.4) | 0.005 | 0.002 | 0.77 |
| Androstene-dione (nmol/L) | 11.6 (4.6) | 10.1 (3.4) | 9.3 (3.4) | <0.0001 | 0.003 | 0.017 |
| Testosterone (nmol/L) | 5.9 (2.6) | 5.5 (2.3) | 5.8 (2.8) | 0.15 | 0.10 | 0.46 |
| VEGF (pg/ml) | 20.2 (17) | 16.8 (9.6) | 16.1 (8.3) | 0.03 | 0.08 | 0.55 |
| MIS (ng/ml) | 7.9 (7.1) | 7.7 (5.3) | 6.1 (5.7) | 0.0005 | 0.79 | <0.0001 |
| TFN (n) | 14.0 (5.0) | 15.1 (5.6) | 13.2 (4.8) | 0.07 | 0.18 | 0.023 |
| Ovarian volume (ml) | 10.5 (5.4) | 10.9 (4.3) | 11.1 (4.0) | 0.59 | 0.72 | 0.76 |
| LH (IU/L) | 10.9 (6.1) | 11.7 (12.0) | 9.5 (6.4) | 0.24 | 0.48 | 0.12 |

**Figure 5.1**

The changes in the mean concentrations of androstenedione and MIS during metformin treatment of women with PCOS. The significance values refer to changes between the start of treatment (T0) and at 4 months, and between the 4 and 8 month intervals.



Chapter 6

# To determine if metformin directly influences adrenal androgen biosynthesis in women with PCOS

## 6.0 Summary

Raised androgen concentrations in women with PCOS may be a result of increased production by the ovary, adrenal gland, or both. It is hypothesised that the dysregulation of cytochrome P450 - 17α hydroxylase activity may be a factor in androgen production from not only an ovarian source but also from the adrenal gland. The activity of this enzyme, found in both ovarian and adrenal tissues, is influenced by insulin drive. Metformin treatment has been found to influence the ovarian production of 17αhydroxyprogesterone (17OHP) through the activity of cytochrome P450 - 17α hydroxylase activity in women with PCOS. The aim of this study was to examine whether suppression of ovarian function using a gonadotrophin releasing hormone agonist (GnRHa), and provoking adrenal function in the absence and presence of metformin, may reveal an effect of metformin upon adrenal steroid output in 18 women with PCOS and 8 control patients. Our results suggest that GnRH-A suppression of ovarian activity had little effect upon the responses of 17OHP to standard synacthen tests or upon basal circulating concentrations of 17OHP of adrenal origin in controls or women with PCOS. However, in women with PCOS, combined GnRH-A and metformin induced reductions in both the 17OHP_AUC and also circulating basal concentrations of 17OHP. This implies that metformin treatment was responsible for suppression of both basal adrenal biosynthesis of 17OHP and its responses

to exogenous ACTH stimulation. This suggests that the activity P450-17α hydroxylase may be abnormal in women with PCOS in the adrenal as well as the ovary, and that excessive insulin stimulation is the underlying controlling factor.

# 6.1 Introduction

Polycystic ovary syndrome is a common cause of anovulation and infertility, and is associated with insulin resistance and hyperandrogenism. High circulating androgen concentrations may be produced by either the ovary or by the adrenal gland. About 25% to 60% of women with hyperandrogenism display excessive adrenal androgen levels (65, 66).

There is evidence that the insulin sensitising drug metformin can improve menstrual cyclicity and fertility in hyperandrogenic women with PCOS. One of the effects of metformin (in the majority of studies) is to reduce the circulating androgen concentrations. However, it is difficult to detect by analysing serum androgen levels whether metformin is exerting its effects at the level of the ovary or the adrenal gland, or both.

Studies have shown that metformin treatment can influence the ovarian production of 17 hydroxyprogesterone (17OHP) through the activity of cytochrome P450 - 17α hydroxylase activity in women with PCOS (24, 67). However, these have been effected in women with different patterns of endogenous ovarian activity, and the possibility of a contributory effect of adrenal origin was not explored. It was considered that the investigation into the role of the adrenal in these tests should be explored further, in the absence of ovarian activity, removing any influence of changes in ovarian androgens.

This study aimed to examine whether suppression of ovarian function using a gonadotrophin releasing hormone agonist (GnRHa), and provoking adrenal

function in the absence and presence of metformin, may reveal an effect of metformin upon adrenal steroid output.

# 6.2 Subjects and methods

## 6.2.1 Study population

A total of 33 pre-menopausal women were recruited from the Reproductive Endocrinology and Assisted Conception Unit clinics at the Royal Infirmary, Glasgow, UK. Patients were recruited if they were found to have a diagnosis of PCOS (described earlier in chapter 2) and a raised free androgen index. Other characteristics of patients recruited included being of reproductive age (range 19-39 years; mean for PCOS patients 32 and for control patients 33 years), no limitation of weight (range 52-111kg), BMI (21-44 $kg/m^2$; mean for PCOS patients 35 and for control patients 30 $kg/m^2$), WHR (range 0.68-1.04; mean for PCOS patients 0.88 and for control patients 0.78), and variable severity of hirsutism, acne, alopecia and acanthosis nigricans. Twenty four of these women were diagnosed as having hyperandrogenic PCOS, and 9 were controls (women without PCOS). Eighteen women with PCOS and 8 controls completed all 3 visits (see Figure 6.1a/b). Exclusion criteria were: contraindications to metformin, or metformin within last 4 months. None had thyroid dysfunction, hyperprolactinaemia, diabetes mellitus, or late-onset congenital adrenal hyperplasia. Women taking medication known to affect gonadal or adrenal function, or carbohydrate or lipid metabolism were also excluded.
Informed consent was obtained from each woman, and the study was conducted at the Royal Infirmary, Glasgow, UK, following approval from the ethics committee of the North Glasgow Hospitals University NHS Trust.

## 6.2.2 Study design

Recruitment patients were down regulated using a GnRH agonist (Prostap). Two weeks later they then underwent the first synacthen test. They were then randomised to either metformin or no metformin. The patients randomised to metformin commenced the medication at a dose of 500mg tds for two weeks. After treatment or no treatment for two weeks all volunteers then returned for the second synacthen test.

The study was a prospective randomised controlled trial and power was calculated to detect a 15% difference AUC. The PCOS patients and controls were randomly assigned a treatment number (computer aided random number generation) and patients (controls and PCOS) with odd numbers received metformin while those with even numbers did not.

## 6.2.3 Assessment programme

Assessments were performed at baseline (T0), two weeks (T2), and four weeks (T4). Obtained at baseline was clinical history, including the date of the last menstrual period, menstrual frequency, symptoms of hirsutism/ acne/ acanthosis nigricans/ alopecia, and anthropometric data including measurements of height, weight, waist and hip (WHR). Hormonal assessments at baseline included those of E2, progesterone, testosterone, SHBG, and LH. All patients (PCOS and controls) then received a depot injection of the GnRH analogue Prostap. Two weeks later they then underwent a Synacthen test (Tetracosactrin 250mcg IV) to assess their adrenal function (fasting; Test 1). Blood analytes were performed at half hourly intervals including baseline, for 90 minutes, for E2, testosterone, progesterone, androstenedione, 17OHP, DHEAS, LH, FSH, and SHBG. Initial sample prior to Synacthen being given also included HbA1C, insulin, and

glucose. All patients were then randomised to receive metformin 500mg tds or no medication for 14 days. After this time interval the patients returned to have a repeat Synacthen test performed (Test 2). The same time interval blood analytes were obtained.

At each assessment (T0, T2, T4) a transvaginal ultrasound was performed for ovarian morphology, ovarian volume, and size of the largest ovarian follicle in each respective ovary.

### 6.2.4 Methods

In addition to those covered in Chapter 2 are:

*Acne* was assessed by the presence of inflammatory follicular sebaceous units at any one time.

*Acanthosis nigricans* was noted by the presence of flexural hyperpigmented skin thickening.

*Alopecia* was noted by the presence of balding of the head in a male pattern distribution.

## 6.3 Results

### 6.3.1 Limitations of the hormone analyses

Unfortunately, the GnRH-A suppression of ovarian function reduced the circulating concentrations of both testosterone and androstenedione to close to the limits of assay sensitivity, and it was decided to make no use of these data. Correspondingly, the analyses are restricted to the adrenal product 17OHP and

its responses to the synacthen tests before and after GnRH-A suppression, with and without metformin treatment.

### 6.3.2 Circulating 17OHP concentrations prior to ovarian suppression

Table 6.1 shows that the circulating concentrations of 17OHP were not significantly higher in the PCOS group prior to the first synacthen test, and that the greater response to stimulation (AUC) seen in the PCOS group was also not statistically significant ($p = 0.06$).

### 6.3.3 Influence of GnRH-A suppression and metformin upon responses of 17OHP to synacthen

Table 6.2 shows that the AUC of 17OHP following synacthen stimulation was not influenced by GnRH-A suppression of ovarian function as there was no difference in the 17OHP_AUC between test 1 and test 2 for either Controls No Metformin or PCOS No Metformin. In contrast, there was a highly significant ($p=0.005$) reduction in the 17OHP_AUC by combined GnRH-A and metformin demonstrated in the 17OHP Metformin group. There were insufficient values for assessment of the responses of the control group to GnRH-A and metformin.

### 6.3.4 Influence of GnRH-A suppression and metformin upon circulating concentrations of 17OHP.

Table 6.3 shows that although there appeared to be a suppression of circulating 17OHP (at T0) by GnRH-A in the small number of cases in the Controls No Metformin group, no effect was seen in the PCOS ($p = 0.12$). In contrast, the

PCOS Metformin group showed a significant (P = 0.04) reduction in circulating concentrations of 17OHP. There was no difference between PCOS no metformin and PCOS metformin baseline 17OHP values prior to treatment.

There was no difference in the absolute concentrations of 17OHP or 17OHP_AUC values between the PCOS and control groups afforded by GnRH-A treatment alone or combined with metformin. However, the results for the PCOS group treated with metformin showed a statistically significant result (see Figure 6.2).

## 6.4 Discussion

These data demonstrate that GnRH-A suppression of ovarian activity had no effect upon the responses of 17OHP to standard synacthen tests or basal circulating concentrations of 17OHP of adrenal origin in controls or women with PCOS. However, in women with PCOS, combined GnRH-A and metformin induced reductions in both the 17OHP_AUC and also circulating basal concentrations of 17OHP. This implies that metformin treatment was responsible for suppression of both basal adrenal biosynthesis of 17OHP and its responses to exogenous ACTH stimulation. This suggests that the activity P450-c17 hydroxylase may be abnormal in women with PCOS in the adrenal as well as the ovary.

'Dysregulation' of cytochrome P450c17α has been proposed to result in the exaggerated secretion of ovarian androgens in PCOS (130). Both 17α hydroxylase and 17, 20 lyase activity arise from the action of the enzyme P450c17α which is expressed in both adrenocortical and ovarian tissue (131). Thus, it has also been hypothesised that this 'dysregulation' of cytochrome P450c17α may also be occurring in the adrenal gland (4).

Hyperinsulinaemia and subsequent raised androgen concentrations from either an ovarian or adrenal source have been found to be a prominent feature of the polycystic ovary syndrome. Lanzone et al demonstrated that hyperinsulinaemia in women with PCOS affects adrenal androgen production (132), and there are also in vitro and in vivo studies showing that insulin potentiates the response of adrenal steroidogenesis to ACTH. Moghetti et al found that insulin infusion in hyperandrogenic women potentiates ACTH-stimulated 17-hydroxypregnenelone and 17-hydroxyprogesterone responses. This effect was likely due to stimulation of the P450c17α leading to an increase of the 17α hydroxylase and 17,20 lyase activity, with the former being enhanced markedly more than the letter (133). Martikainen et al also showed that the selective catheterisation of the adrenal gland in hyperinsulinaemic hyperandrogenic women resulted in significant correlations with insulin and androgen concentrations (134). Studies have shown that metformin treatment can influence the ovarian production of 17 hydroxyprogesterone (17OHP) through the activity of cytochrome P450c-17α hydroxylase activity in women with PCOS (24, 67). This raises the question of the potential for ISAs to address adrenal androgen production possibly via the same route.

La Marca et al found that the administration of metformin to unselected women with PCOS led to a reduction in the adrenal steroidogenesis response to ACTH (37). This was in the form of reduced response of 17OH and androstenedione to ACTH, equating to a reduction in adrenal cytochrome P450 activity. Metformin presumably brought about a reduction in 17α hydroxylase and 17,20 lyase activity by reducing insulin levels, however, proof of hyperinsulinaemia was not an inclusion criteria. This result was in agreement with the findings by Arslanian et al that found that metformin treatment of obese adolescents with PCOS and impaired glucose tolerance was beneficial in improving glucose tolerance and

insulin sensitivity, in lowering insulinaemia, and in reducing elevated androgen levels (135). They found that metformin therapy was associated with attenuation of the adrenal steroidogenic response to ACTH with androstenedione, 17OH, and 17hydroxypregnenelone lower after metformin treatment. Recently, pioglitazone (an ISA) administration was found to reduce the response of 17OH and androstenedione to ACTH, most likely due to an inhibition of cytochrome P450c17α (136).

Contrary to these findings 2 studies found that the dysregulation hypothesis of cytochrome P450c17α was unlikely to be a factor in adrenal androgen production. Azziz et al found that the steroidogenic profile in a population of 92 hyperandrogenic women before and after ACTH stimulation was not consistent with dysregulation of cytochrome P450c17α. However, in this trial there was no diagnosis of PCOS made prior to inclusion, and no use of ISAs employed (137). Also, Unluhizarci et al also found that the treatment of insulin resistance with metformin did not improve adrenal cytochrome P450c17α enzyme dysregulation in PCOS with no reduction in 17OH and androstenedione (138). This was a small trial (15 pts with PCOS and 10 controls) and only 500mg BD was used possibly reflecting suboptimal dosing. Our results suggest that there may be an abnormality in the action of cytochrome P450c17α in the adrenal gland as well as the ovary. It should also be noted that in none of the trials in the literature did they employ down regulation of ovarian function in an attempt to decrease background ovarian androgen 'noise'.

It is unknown if this proposed dysregulation of cytochrome P450c17α is genetic or acquired. A genetic defect in serine phosphorylation in PCOS may lead to enhanced 17,20 lyase activity in both the ovary and adrenal gland (139), a great deal further work in this area is required to elucidate if the 'dysregulation' of

cytochrome P450c17α hypothesis may be correct, and if so, then the evolution of this abnormality whether it be genetic or acquired.

**Table 6.1**

The concentrations at T0 and the AUC of 17OHP at synacthen test 1 in controls and PCOS patients at the start of the experiment.

| | **17OHP at T0** | | **17OHP AUC** | |
|---|---|---|---|---|
| | **Test 1** | | **Test 1** | |
| | **Mean (ng/ml)** | **CLs** | **Mean (ng/ml)** | **CLs** |
| Controls (All, n = 8) | 1.08 | 0.46 – 1.69 | 10.7 | 7.58 – 13.8 |
| PCOS (All, n = 18) | 1.53 | 0.98 – 2.07 | 14.4 | 12.0 – 16.7 |
| P (unpaired t test) | 0.303 | | 0.06 | |

**Table 6.2**

Responses of circulating 17OHP (AUC) in the 3 groups of patients to their 2 synacthen tests. The responses to the two tests were compared within groups using paired t tests.

Test 1 refers to the first Synacthen test prior to randomisation using metformin.

Test 2 refers to synacthen testing after randomisation using metformin.

| | | 17OHP_AUC | | | | |
|---|---|---|---|---|---|---|
| | | Test 1 | | Test 2 | | P |
| | n | Mean (ng/ml) | CLs | Mean (ng/ml) | CLs | Paired t test |
| Controls No Metformin | 5 | 10.1 | 7.08 – 13.1 | 9.79 | 7.28 – 12.3 | 0.75 |
| Controls Metformin | 3 | 11.7 | | 11.4 | | |
| PCOS No Metformin | 10 | 13.9 | 11.3 – 16.5 | 14.0 | 10.7 – 17.2 | 0.94 |
| PCOS Metformin | 8 | 14.9 | 9.75 – 20.1 | 12.4 | 7.75 – 17.0 | 0.005 |

**Table 6.3**

Mean concentrations of 17OHP at the start of the 2 synacthen tests. Within group comparisons between test 1 and test 2 were effected using paired t tests.

| | | 17OHP_T0 | | | | |
|---|---|---|---|---|---|---|
| | | Test 1 | | Test 2 | | P |
| | n | Mean (ng/ml) | CLs | Mean (ng/ml) | CLs | Paired t test |
| Controls No Metformin | 5 | 0.81 | 0.28 – 1.34 | 0.57 | 0.18 – 0.95 | 0.02 |
| Controls Metformin | 3 | 1.52 | - | 1.43 | - | |
| PCOS No Metformin | 10 | 1.36 | 0.75 – 1.97 | 1.06 | 0.61 – 1.52 | 0.12 |
| PCOS Metformin | 8 | 1.73 | 0.58 – 2.88 | 0.96 | 0.38 – 1.54 | 0.04 |

**Figure 6.1a**

Recruitment to the adrenal androgen trial for the patient group: PCOS

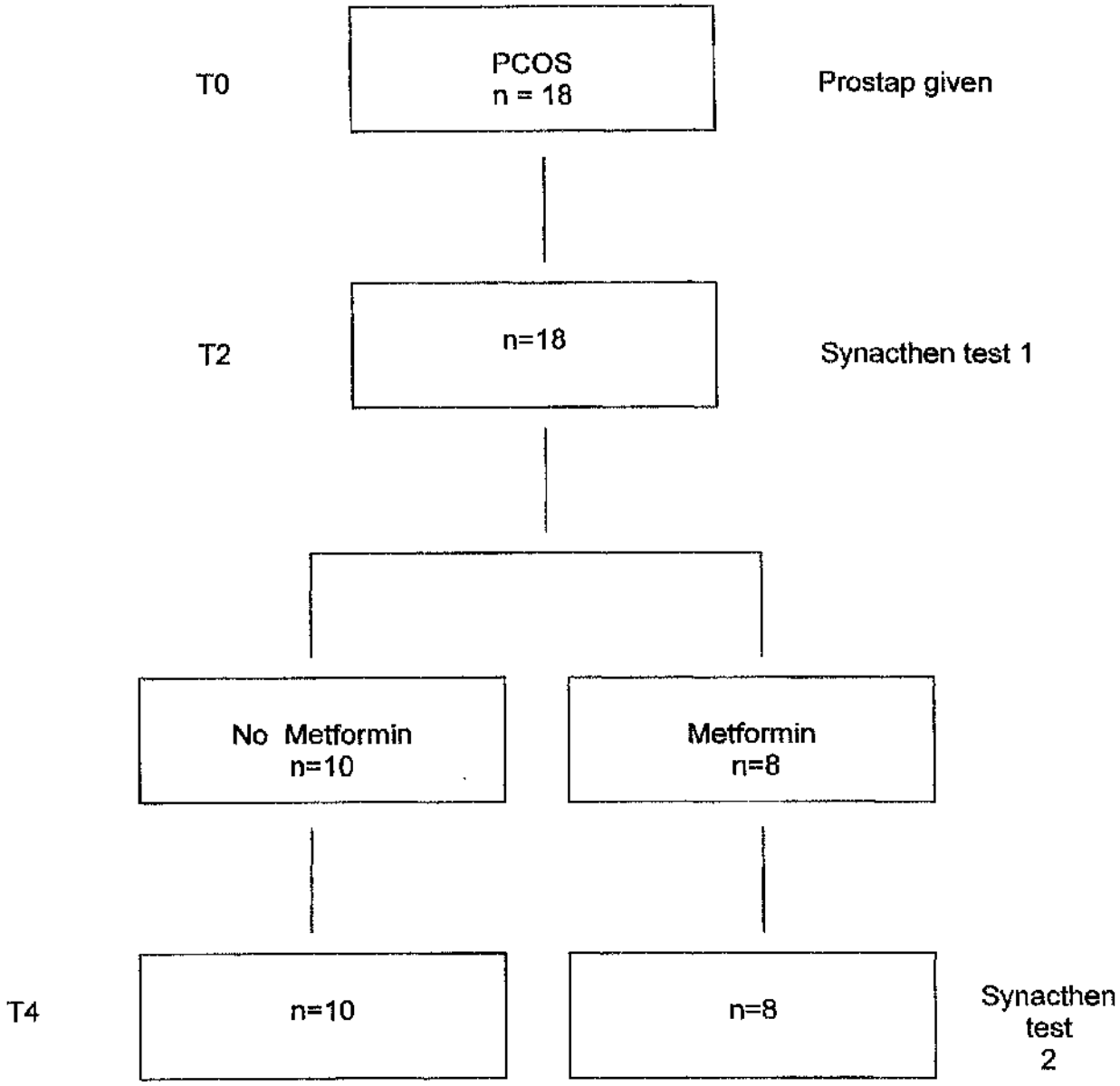


**Figure 6.1b**

Recruitment to the adrenal androgen trial for the patient group: controls

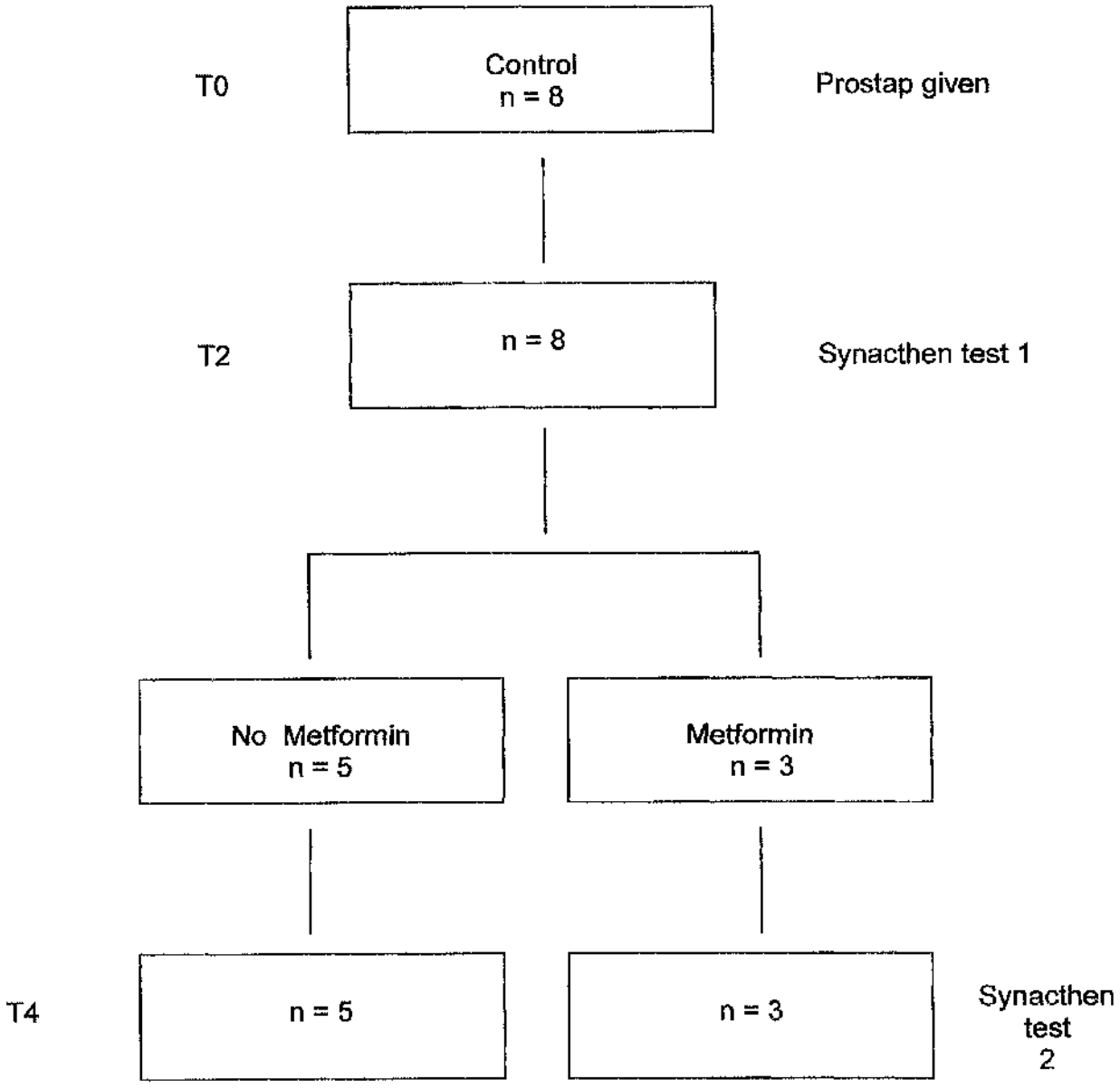
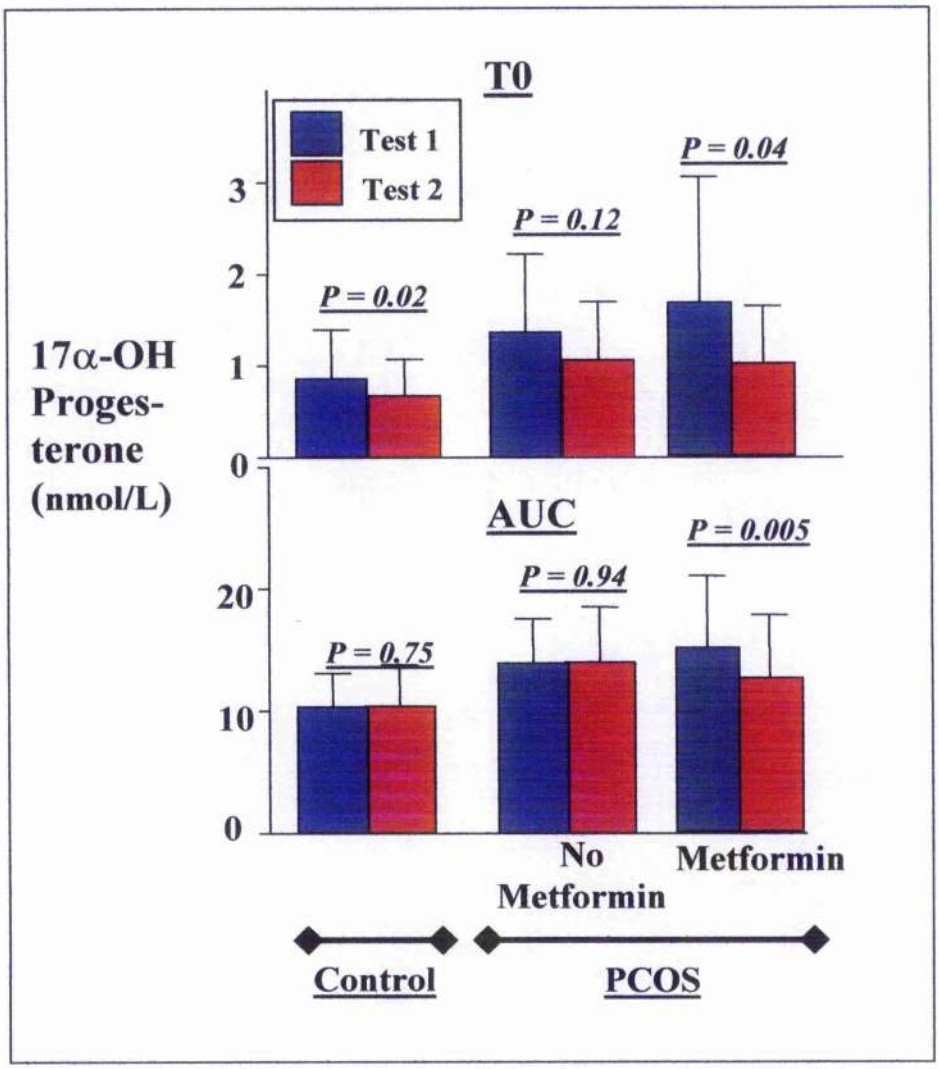


**Figure 6.2**

Comparisons of mean concentrations of circulating 17OHP (AUC) and basal circulating 17OHP for all three groups to their two synacthen tests.



Chapter 7

# Case Report:

## Increased dosing of metformin treatment may result in greater weight loss in a woman with PCOS

The insulin sensitising agent, metformin has been used for many years in patients with glucose intolerance and has been associated with either weight reduction, or weight maintenance while control groups increase weight. Although weight loss is evident in a number of studies, many of which have been of short duration it is generally of a modest degree (42, 44-48). This is in contrast to the results presented in the metanalysis by Lord et al (15) that states that there is no significant reduction in weight using metformin.

In an attempt to determine whether the morbidly obese are refractory to metformin or whether they may respond to either higher doses or more protracted treatment we performed the trial described in Chapter 5. Generally, we found a 5-6% reduction in weight following treatment for 8 months. Azziz et al also found a dose response to weight loss using Troglitazone, another insulin sensitising agent (77). Some women show little or no weight loss, while others show clear and rapid responses.

Here we highlight a case of a morbidly obese woman treated with a high dose of metformin (2550 mg/d), which resulted in a dramatic reduction in weight. The aim was to attempt to determine why this case may have been exceptional, and whether there were other, perhaps endocrine, differences associated with this case.

# 7.0 Case report

A 30 year old caucasian female presented to the Reproductive Endocrinology Unit at the Royal Infirmary, Glasgow, UK, complaining of primarily morbid obesity, but also of menstrual disturbance and significant hirsutism. Table 7.1 shows that her weight was recorded as120.8kg, with a BMI of 44 kg/m² and waist/hip ratio of 0.871 (waist measurement 121cm, hip measurement 139cm). Gastric banding 8 months prior to presentation had failed to result in any weight loss. She had a history of amenorrhea, her last menses being in 1995, and she had never conceived despite a lack of contraceptive use. At the time of the trial she was not sexually active. She was also severely hirsute (Ferriman Gallwey score = 25). A reproductive hormone concentration screen in peripheral plasma revealed that she had a modestly raised free androgen index of 9.4 (upper limit of normal = 7.9), low sex hormone binding globulin (18nmol/l), a normal total testosterone of 1.7nmol/l and also a normal LH concentration of 6.8 IU/L. The patient had a classic appearance of PCO on ultrasound scan, and a diagnosis of PCOS was made.

There was no evidence of thyroid dysfunction, diabetes mellitus, hyperprolactinaemia or congenital adult onset adrenal hyperplasia (DHEAS, 2.8 μmol/l; 17αOHProgesterone, 3.0 nmol/L). Not surprisingly, she was found to be significantly insulin resistant with a raised fasting insulin of 41 IU/ml (laboratory upper limit of normal = 13 IU/ml) with a fasting glucose of 5.0mmol/L (Table 7.2)

Table 7.2 also shows that other measurements prior to treatment including HOMA-IR, leptin, CRP, total cholesterol, triglycerides, HDL, LDL and VLDL were

unexceptional for an individual displaying such obesity. She was taking no medication at the time of commencement of metformin.

As part of the ongoing trial examining the weight reducing effects of metformin (Chapter 5), the patient was randomised to metformin 850mg tds following informed consent. It should be stressed that there was no specific instruction as to diet and exercise. The medication was found to be extremely well tolerated at one and two months follow up. However, interestingly, she complained of feeling constantly warm regardless of the ambient temperature, which was associated with significant sweating.

The patient re-presented at the scheduled intervals of 4 and 8 months. Table 7.1 shows the changes in BMI, weight, WHR, waist and hip measurement at baseline (T0), 4 (T4) and 8 (T8) months. It shows that WHR showed negligible change as weight was lost at both the waist and the hips. At the 4 month clinic appointment her weight had undergone a dramatic reduction such that she had never previously experienced. Her weight had reduced by 21.8kg, and by the 8 month appointment she had lost a total of 27kg in weight. Accordingly, her BMI decreased 10 $kg/m^2$.

Correspondingly, she showed changes in most other parameters at 4 months and then again at 8 months: Table 7.2 shows changes for endocrine, metabolic and lipid parameters. There was negligible change in LH, or LDL. Table 7.3 shows changes over the time points for ultrasound parameters.

An important observation throughout the trial was that this patient experienced significant and distressing diaphoresis. No attempt at objective measure was made of this however.

# 7.1 Discussion

With a high dose of metformin treatment this morbidly obese patient underwent dramatic weight loss over 8 months. This was most marked in the initial four months. Associated with these changes was a negligible change in her waist hip ratio. The greatest reduction in her lipids was with triglycerides and VLDL. There was however, no change in her LDL level. HDL improved moderately. Weight loss was associated with a decrease in the inflammatory marker of CRP corresponding to an overall decrease in cardiovascular risk. Of note is that she had a resumption of menstruation once again most marked in the initial 4 months. Interestingly however, metformin showed significant improvement in the glucose / insulin ratio and the HOMA-IR, however, it had negligible impact upon circulating androgens. Also, an important observation in this individual case was the patient's significant ongoing complaint of excessive sweating. Despite this trial being conducted over the Scottish winter the patient found this to the point of being extremely distressing and embarrassing, as she had not experienced significant diaphoresis prior to being commenced on metformin. In this case study, it may be that the side effects of sweating and reported heat are due to a normalisation of postprandial thermogenesis often described in women with PCOS. Robinson et al (140) found that PCOS subjects have a reduced postprandial thermogenesis that related statistically to the reduced insulin sensitivity. They also hypothesised that the decreased postprandial thermogenesis may predispose women with PCOS to weight gain. Brooks et al (141) found that, in Type 2 diabetic patients given Acarbose, it improved energy expenditure and diet induced thermogenesis postprandially. However, Segal et al (142) although confirming blunted thermogenesis in obese patients, failed to demonstrate evidence of altered resting metabolic rate or postprandial

thermogenesis in women with PCOS compared with women of similar degrees of obesity. It should be noted however, that all three of these trials were of small numbers (<15 PCOS, <15 controls).

This patient showed distinct improvements in circulating fasting glucose, and perhaps more significantly in the Glu/Ins ratio. Before treatment she showed one of the poorest G/I ratios (marker of efficiency of insulin action) and she ended with one of the better one) – so maybe there is something critical about this case in that metformin improved glucose uptake more than in others. Thus, it may be that the beneficial effects of weight loss appear to be mediated primarily through glycaemic metabolic pathways rather than effects upon circulating androgens, and it may be that women with morbid obesity may respond better to higher doses of metformin, as discussed in chapter 5.

Quite why this patient showed such remarkable weight loss is not clear, but there were a number of distinctive features about this case. Despite her multiple ovarian cysts and enlarged ovarian volume, she showed an unremarkable androgen profile. Her FAI was elevated more because of the low SHBG than the total androgens.

It is well known that PCOS is characterised by a great variety of symptoms, and this case appears to be one whose symptoms are more related to abnormal glucose / insulin metabolism than to hyperandrogenism: the latter being a product of excessive insulin secretion.

The ability of metformin to increase glucose uptake appears to have corrected all the manifestations, including reduced lipid production and deposition, and maybe produced her thermogenesis.

It also supports the observation that metformin works best in women with least endocrine (androgen) disturbance.

Thus, it may be in this case, by correcting abnormal insulin metabolism rather than by significantly reversing hyperandrogenism, metformin resulted in this patient's dramatic weight loss.

(see Appendix re patient letter)

**Table 7.1**

Changes in anthropometric parameters, FG score and menses over the three time intervals (T0, T4, T8).

| | T0 | T4 | T8 |
|---|---|---|---|
| Weight (kg) | 120.8 | 99 | 93.8 |
| Waist (cm) | 121 | 112 | 108 |
| Hip (cm) | 139 | 127 | 121 |
| WHR | 0.871 | 0.882 | 0.893 |
| BMI ($kg/m^2$) | 44 | 35.9 | 34 |
| FG score | 25 | 25 | 18 |
| Menses (n) | 0 | 3 | 4 |

**Table 7.2**

Changes in endocrine, metabolic and lipid parameters over the three time intervals (T0, T4, T8).

| | T0 | T4 | T8 | % Change (T4 - T8) | % Change (T0-T8) |
|---|---|---|---|---|---|
| Testosterone (nmol/L) | 1.7 | 1.3 | 0.7 | 46 ↓ | 59 ↓ |
| SHBG (nmol/L) | 18 | 19 | 22 | 16 ↑ | 22 ↑ |
| FAI | 9.4 | 6.8 | 3.2 | 53 ↓ | 66 ↓ |
| Insulin (IU/ml) | 41 | 19 | 15 | 21 ↓ | 63 ↓ |
| Glucose (mmol/L) | 5.0 | 4.7 | 4.6 | 2 ↓ | 8 ↓ |
| HOMA- IR | 9.0 | 4.0 | 3.1 | 22 ↓ | 66 ↓ |
| Glu/Ins Ratio | 0.12 | 0.25 | 0.31 | 24 ↑ | 58 ↑ |
| IGF-1 (ng/ml) | 85 | 188 | 195 | 4↑ | 29↑ |
| Leptin (ng/ml) | 59.4 | 27.8 | 36.2 | 30 ↑ | 39↓ |
| CRP (mg/L) | 8.8 | 4.88 | 3.8 | 22 ↓ | 57 ↓ |
| Cholesterol (mmol/L) | 4.52 | 4.16 | 4.22 | 1 ↑ | 7 ↓ |
| TG (mmol/L) | 4.14 | 2.02 | 1.42 | 30 ↓ | 66 ↓ |
| HDL (mmol/L) | 0.53 | 0.86 | 0.98 | 14 ↑ | 85 ↑ |
| VLDL (mmol/L) | 1.89 | 0.92 | 0.65 | 29 ↓ | 66 ↓ |

**Table 7.3**

Changes for ultrasound parameters over the three times intervals.

| | T0 | T4 | T8 |
|---|---|---|---|
| Right ovarian volume ($cm^3$) | 14.7 | 12.2 | 16.3 |
| Left ovarian volume ($cm^3$) | 14.9 | 12.0 | 9.6 |
| Mean no. of cysts (n) | 16 | 15 | 13 |

Chapter 8

# Case Report:

## Metformin treatment may alter responses of mature follicles to the luteinising signal in polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a disorder of chronically abnormal ovarian function and hyperandrogenism affecting 5-10% of the female population of reproductive age (1). Insulin resistance / hypersecretion is an intrinsic feature underlying PCOS, particularly in obese individuals (5, 6, 120). The corresponding hyperinsulinaemia, in concert with increased LH exposure, is probably responsible for increased ovarian androgen secretion, and abnormal follicular development, which ultimately leads to dysfunctional follicular development and anovulation (143). We would presume that this primary function would be enacted through increased insulin/ LH drive to the thecal cells of follicles, since granulosa cells do not produce androgens. However, the granulosa cells of mature follicles acquire LH receptors in the latest stage of pre-ovulatory development, but there is little evidence indicating whether insulin can influence these phenomena as well.

Treatment of women with PCOS with ISAs has been shown to promote follicular growth (16). Notable observations include i) that the interval from start of treatment to first ovulation is significantly reduced (47, 77) and ii) menstrual or ovulation cyclicity is increased with metformin treatment compared to placebo (16). The mechanism underwriting these changes is presumed to reflect a

reduced insulin promotion of intrafollicular androgen biosynthesis, an excess of which may induce follicular atresia. However, troglitazone (23) and recently metformin (144) have also been shown to influence ovarian steroid biosynthesis directly.

Metformin treatment of PCOS patients undergoing controlled ovarian stimulation (COS) for in vitro fertilisation with co-treatment with metformin may also improve outcome (40, 52). These studies are generally poorly controlled, with issues of variable exogenous gonadotrophin dose, but metformin pre-treatment may allow a more orderly follicular growth in response to stimulation. A recent prospective study in assisted conception found no change in the ovarian responses to FSH afforded by metformin. This shows that short term metformin treatment (<4 months) failed to influence the number of follicles recruited by high FSH concentrations (145). The evidence pertaining to actions of insulin or hyperinsulinaemia is restricted to aspects of follicular growth and development – generally of immature follicles. We present here a case providing circumstantial evidence supporting the concept that the response to the luteinising signal may also be an area influenced by insulin or hyperinsulinaemia. Correspondingly, this would imply that the excessive insulin drive is acting at this stage through the granulosa cells.

# 8.0 Case report

A 30 year old female of Asian origin presented to the Assisted Conception Unit at the Royal Infirmary, Glasgow, UK, with a six year history of infertility. She had a history of oligomenorrhoea (cycle length; 6-20wk), and was severely hirsute (Ferriman Gallwey score = 34). A reproductive hormone concentration screen in peripheral plasma revealed that had a raised free androgen index of 13.0 (upper

limit of normal = 7.9), low sex hormone binding globulin (16nmol/l), and an elevated LH concentration (10 IU/L). Her BMI was 30 kg/m² with a weight of 70kg, and she was found to be insulin resistant with a raised fasting insulin of 25.8 IU/ml (laboratory upper limit of normal = 13 IU/ml. The patient had a classic appearance of PCO on ultrasound, and a diagnosis of PCOS was made. A 2hr fasting oral glucose tolerance test revealed a 2h glucose concentration of 8.6mmol/L, consistent with glucose intolerance, not NIDDM. There was no evidence of thyroid dysfunction, hyperprolactinaemia or CAH (DHEAS, 3.9 μmol/L; 17αOHProgesterone, 8.0 nmol/L).

Her partner's semen analysis showed a reduced sperm count, and the couple were recommended to undergo COS and intracytoplasmic sperm injection (ICSI) treatment.

The couple underwent 2 conventional cycles of COS with a standard long GnRH-agonist protocol (Chapter 2) for ICSI, using human menopausal gonadotropin (HMG) as the ovarian stimulant, and similar, but abnormal responses were observed. Table 8.1 shows that both treatment cycles progressed well with multiple follicular development and associated high estradiol (E2) concentrations. In the first 2 cycles the follicular phases were of 16 days and 14 days respectively, yielding circulating E2 concentrations of 9000 pmol/L and 6000 pmol/L, associated with more than 6 follicles in each cycle whose follicular diameter was greater than 15mm at the time of HCG. However, in both cycles, 37 hours after HCG, at the time of oocyte pick up, the large follicles had disappeared and no mature oocyte was recovered. A single immature oocyte was collected in the second cycle. There was no evidence of premature luteinisation as evidenced by progesterone concentrations on the day of HCG administration <3.0 nmol/L prior to HCG in both cycles.

At review, it was suggested that the use of metformin may reduce insulin promoted actions at the ovarian level, which may decrease androgen biosynthesis and allow normal follicular and oocyte maturation to proceed, perhaps avoiding precocious ovulation and follicular collapse.

The patient subsequently underwent COS as before (cycle 3 in table 8.1), except that on the same day as the GnRH-agonist was administered, daily metformin treatment was commenced at a dose of 500mg tds. After 2 weeks, HMG was commenced at a daily dose of 150IU/day (the same dose as previous cycles) until >3 follicles were observed at diameter >17mm. Table 8.1 shows that the responses through the follicular phase were broadly similar to the previous 2 cycles except that the follicular phase was markedly reduced in length. This cycle required only 8 days HMG treatment, and HCG was administered as before. Metformin treatment was continued until the day of oocyte retrieval (discontinued after the morning dose). Oocyte retrieval was performed 35h after HCG administration, and serial ultrasound examinations to monitor follicle size and number revealed no shrinkage of the follicles prior to oocyte pick-up, when 5 oocytes were obtained, 4 of which were deemed mature at ICSI. Two days culture resulted in 3 good quality embryos, 2 of which were transferred to the uterus. Unfortunately, the patient failed to conceive.

Table 8.1 also shows that fasting insulin concentrations at oocyte pickup were reduced (though still above the normal range) during the metformin treated cycle while circulating IGF-1 and IGF-BP3 were similar in all 3 cycles.

# 8.1 Discussion

Although the specific actions of insulin at the ovarian level are not clearly understood it is thought that insulin promotes androgen biosynthesis by direct

action on thecal cells in the presence of LH (146), and it may also play a role in granulosa cell function (147-149).

Restriction of follicular maturation, leading to amenorrhoea, may be incurred by a premature induction of LH receptors in granulosa cells. Thus, hyperinsulinaemia may be associated with multiple small follicle development because of abnormal control of follicular maturation in an environment of relatively raised LH and modestly reduced FSH, leading to a clinical picture of estrogenised anovulation consistent with PCOS. Induction of follicular growth with exogenous FSH often results in multiple follicular development, with apparently normal responses to HCG.

The main effect of metformin on ovarian function is thought to be by dint of reducing the degree of insulin stimulation of thecal androgen biosynthesis, and modifying the control mechanisms of follicular growth and maturation.

This case suggests that insulin may have a further role in ovarian function, previously unrecorded. The excessive hyperinsulinaemia in this case appears to have influenced the response of mature sized follicles to the pre-ovulatory, luteinisation signal, such that luteinisation, ovulation and follicular collapse (corpus luteum formation) occurred more rapidly than normal. The process appeared to be normalised by reducing the exposure to excessive insulin concentrations after metformin treatment. This concept would support the hypothesis that insulin promotes the actions of LH in both follicular cell types.

**Table 8.1**

Changes over the three treatment cycles for hormones, ultrasound parameters and responses to treatment and outcomes.

| | Cycle no. 1 | Cycle no. 2 | Cycle no. 3 (with metformin) |
|---|---|---|---|
| Fasting insulin (IU/ml) (at oocyte pickup) | 54 | 109 | 32 |
| IGF-1 (ng/ml) | 187 | 203 | 248 |
| Follicular phase (days) | 16 | 14 | 8 |
| Estrogen (pmol/L) | 9000 | 6000 | 4000 |
| Follicles at HCG (diameter >15mm) (n) | 7 | 8 | 9 |
| Follicles at HCG (diameter = 12-14mm) (n) | 6 | 5 | 2 |
| Follicles at OPU (diameter >15mm) (n) | 0 | 0 | 9 |
| Oocytes | 0 | 1 (immature) | 5 |
| IGF-BP3 (mg/L) | 5.1 | 4.9 | 5.5 |
| Embryos | 0 | 0 | 3 |

Chapter 9

# Discussion

## 9.0 Introduction

To date in the literature ISAs have been shown to have effects in women with PCOS both clinical and biochemical. However, there are a great many questions that still remain to be answered. This thesis comprises a number of studies in an attempt to answer certain questions that have arisen from the literature. We used the agent metformin due to its ready availability, good safety record, lack of expense and previous experience. Metformin on the whole was well tolerated by the patient cohorts.

We believe that the studies presented in this thesis have not only answered some existing questions, but raise further possibilities for the future exploratory use of metformin in women with PCOS.

## 9.1 Hirsutism

Hirsutism in women with PCOS has significant psychosocial sequelae and traditionally poses a difficult management dilemma. The existing treatments for hirsutism are time consuming (eg waxing), expensive (electrolysis, laser treatment), or not especially effective (COCP). The underlying hormonal basis for the condition is complex, but accepted to be based upon either excessive androgen exposure and/or sensitivity.

It has been hypothesised that by reducing circulating insulin concentrations, leading to decreased free androgen concentrations, ISAs may ameliorate hirsutism; and some trials have addressed their use in hirsutism. In none of these was hirsutism a primary outcome measure and in only one of these was an objective measure of hirsutism used. These trials also tended to be of too short a duration, the longest being 6 months and the patients were only mild to moderately hirsute. Thus, we determined there was a need to perform a simple, comparative randomised trial of significant power and sufficient duration where not only semi-subjective but also objective measures were employed, and hirsutism was a primary outcome measure.

In this trial, we attempted to compare the efficacy of metformin with the 'gold standard' treatment, Dianette (ethinylestradiol and cyproterone acetate). Dianette has been shown, however, to have only limited efficacy in women with severe hirsutism, thus there is a place for an acceptable and hopefully more efficacious treatment in the management of women troubled by this condition.

In this trial we found that there was a clear benefit of a standard dose of metformin treatment on moderate to severely hirsute women with PCOS. Metformin reduced hirsutism on both objective and semi-subjective measures. The objective measure of hair diameter however was modest and comparable with Dianette. However, the FG score reduced dramatically and patients' perception of an improvement in hirsutism was also positive. The medication was found to be tolerable to the majority of patients thus hopefully aiding compliance. The decrease in FG score was also independent of changes in BMI.

Hirsutism occurs as a result of end organ sensitivity and direct androgen stimulation. Tissue sensitivity is also controlled by insulin and IGF-1 activity, possibly through reduced IGF-BP's.

Contrary to the existing literature we did not see a significant decrease in androgenicity. Metformin treatment did however show significant improvement in the glucose/ insulin ratio and the logHOMA-IR, but no change in IGF-BP1, IGF-BP3 or IGF-1. Thus, addressing insulin sensitivity, possibly at the local tissue level may be a more effective approach to hirsutism in women with PCOS.

This trial has raised further questions for future work.

**Combined therapies**: given that hirsutism is a result of androgen stimulation and insulin metabolism influences androgens and growth factors, there is a role for a randomised placebo controlled trial that addresses different therapies and the combinations. Possibilities are ISA's including the biguanides and thiazolidinediones, the COCP +/- cyproterone acetate and the direct androgen blockers ie Flutamide and/or Finasteride.

**Tissue level insulin metabolism**: based on the results from this trial there is the suggestion that local tissue factors may play a role in the amelioration of hirsutism. Thus, a study to examine IGF's at the tissue level may further clarify this issue.

**Reduction of C19 steroid 5α reductase**: a recent paper by Tsilchorozidou et al (150) found that insulin seems to enhance 5α reduction of steroids in PCOS in vivo. They found that the increased 5α reductase activity in women with PCOS may be secondary to hyperinsulinaemia. Thus, potentially by decreasing hyperinsulinaemia there may be a reduction in 5α reductase activity and thus in turn reducing the conversion of testosterone to the more potent DHT, thereby ameliorating hirsutism. A placebo controlled trial addressing insulin metabolism in relation to 5α reductase would be appropriate.

## 9.2 Weight loss

Obesity is an extremely common feature of PCOS. This, like hirsutism, not only has significant psychosocial consequences, but also those of adverse reproductive and cardiovascular outcomes. The Cochrane database review did not confirm that weight loss was a significant factor in the use of metformin for weight loss in women with PCOS. This is contrary to a number of reports in the literature that note modest weight loss with metformin. It may be that significant obesity may reduce the benefit of metformin treatment and thus greater doses of metformin are required to observe an effect.

In this trial the aim was to determine whether different doses of metformin could have different effects upon features associated with PCOS, notably, weight reduction, circulating hormone changes, markers of inflammation and lipid profiles.

We found that women with PCOS respond to metformin in a manner related to both the dose and their body mass. Firstly, weight loss was a feature of protracted metformin therapy. Both the degree of weight reduction and the degree of suppression of circulating androstenedione in obese women were dose related, however, the morbidly obese group showed no such changes. This finding implies that the degree of obesity impacts upon responses to metformin. The effects of metformin upon lipid profiles were generally beneficial and appeared to be unrelated to metformin dose, degree of obesity, or weight change. Effect of metformin upon CRP showed a trend towards benefit without reaching statistical significance.

Our results raise the question as to why we did not see any significant results in the morbidly obese group. It may be simply that an even greater dose of medication is required given the degree of obesity. Alternatively, it may be that

the nature of insulin metabolism is more complex in the morbidly obese which requires further elucidation. Regardless, given the pharmaceutical restriction of maximum dosing (3g/day) there is scant leeway for further increasing the dose, especially in light of a lack of licensing for use in women with PCOS. Thus, it may be that the pathway for future work in this area should be directed towards other means of achieving weight loss in conjunction with metformin treatment.

**Lifestyle modification:** this must remain the mainstay of weight loss programmes with or without metformin treatment. This dogma was reinforced by the UKPDS study as mentioned previously, and the results from our study reiterate this. It may be that further weight loss medications may need to be added to the regime in an attempt to decrease weight by whatever means possible. By enabling weight loss we would expect a greater benefit on lipids as in our study we saw no significant benefit of a dose response with differing doses of metformin, and we would also expect an improvement in CRP levels thus reflecting a reduction in the long term risk of cardiovascular compromise.

## 9.3 MIS

Ovarian function in PCOS is characterised by an increased ratio of growing follicles to primordial stage follicles: the rate of initial recruitment of ovarian follicles and progress through developmental stages appears to differ from normal and result in excessive antral follicles. MIS is a product of granulosa cells and because there are excessive follicles present in PCOS, it has been found to be raised in women with PCOS. Its potential role in regulating follicular recruitment and growth has yet to be determined in humans. Prior to this study, the effect of ISAs on circulating concentrations of MIS in PCOS had not been explored. The aims of this study were to explore the relationship between MIS

and other reproductive parameters in obese women with PCOS, and also to test the hypothesis that reducing the degree of insulin stimulation to the ovaries in these women, using protracted metformin therapy, would reduce the circulating concentrations of MIS. This trial was undertaken as a result of sample aliquots stored from the weight loss trial and eventually the availability of a suitable assay for circulating MIS.

The results show that the treatment of women with PCOS with metformin resulted in a highly significant reduction in the circulating concentrations of MIS. They also show that the suppressive effects occur only after protracted treatment of the time scale suggestive of effects upon initial follicular recruitment. Thus, within the first 4 months, despite a rapid reduction of androgen concentrations, possibly as a result of the reduced insulin stimulation of the existing thecal cells, there was no observed effect on the number of ovarian follicles present or on the circulating MIS. However, when we continued treatment beyond the 4month time interval in an environment of continued insulin metabolism normalisation, there was a decrease in the number of ovarian follicles. This may represent a reduction in the total mass of granulosa cells, secondary to a reduction in the number of FSH responsive follicles evident at the 8 month point. There was some evidence of a reduction in the number of small follicles at this stage, although there was no change in ovarian volume, perhaps indicating that the existing follicles were growing to more advanced stages. It was not evident from our study if the emphasis was on reduction of androgen concentrations or on the normalisation of insulin metabolism to achieve these results, however, as it has been shown in the literature that hyperinsulinaemia facilitates raised androgen concentrations then by ameliorating hyperinsulinaemia we can attempt to achieve the same result. These results suggest that the existing literature of the effect of ISAs on ovarian function are too short in duration. Only after 4-8 months should the effects of

metformin on the underlying ovarian function be examined. Thus, **longer trials** are required to elucidate this further. Perhaps this also indicates a role for longer treatment of women with PCOS undergoing assisted conception cycles. A recent prospective study in assisted conception (145) found no change in the ovarian responses to FSH afforded by metformin. This shows that short term metformin treatment failed to influence the number of follicles recruited by high FSH concentrations. Correspondingly, the patients remained at risk of ovarian hyperstimulation. Our data imply that to achieve improved safety in this area, the metformin pre-treatment should be more of the order of 6 months.

## 9.4 Adrenal androgens

High circulating androgen concentrations may be produced by either the ovary or by the adrenal gland, and metformin has been shown to reduce androgen concentrations in women with PCOS. However, it is difficult to detect by analysing serum androgen levels whether metformin is exerting its effects at the level of the ovary or the adrenal gland, or both. Metformin treatment can influence the ovarian production of 17αhydroxyprogesterone (17OHP) through the activity of cytochrome P450 - 17α hydroxylase activity in women with PCOS. It has been found that the activity of this enzyme, found in both ovarian and adrenal tissues, is influenced by insulin drive. It was considered that this hypothesis of dysregulation of cytochrome P450 - 17α hydroxylase activity may also be a factor in hyperandrogenaemia from an adrenal source. It was decided that this should be explored further, and that this should be tested, as far as possible, in the absence of ovarian activity, removing any influence of changes in ovarian androgens.

This study aimed to examine whether suppression of ovarian function using a gonadotrophin releasing hormone agonist (GnRHa), and provoking adrenal function in the absence and presence of metformin, may reveal an effect of metformin upon adrenal steroid output. Despite the fact that this work remains incomplete (as we intend performing DHEA and DHEAS assays on the stored samples) this trial showed some very interesting results pertaining to 17OHP. The data suggest that GnRH-A suppression of ovarian activity had little effect upon the responses of 17OHP to standard synacthen tests or upon basal circulating concentrations of 17OHP of adrenal origin in controls or women with PCOS. However, in women with PCOS, combined GnRH-A and metformin induced reductions in both the 17OHP_AUC and also circulating basal concentrations of 17OHP. This implies that metformin treatment was responsible for suppression of both basal adrenal biosynthesis of 17OHP and its responses to exogenous ACTH stimulation. This suggests that the activity P450-17α hydroxylase may be abnormal in women with PCOS in the adrenal as well as the ovary, and that excessive insulin stimulation is the underlying controlling factor.

**Adrenal gland trials:** as the adrenal gland is a less complex organ compared with the ovary, there may exist a role for the adrenal gland to help us examine the dynamics of PCOS and efficacies of different treatments in an attempt to develop alternate therapeutic approaches. If this were to occur then it would be advisable to perform a trial in which ovarian down regulation is compared with non ovarian down regulation. The changes observed in basal levels of 17OHP may not have been evident in the absence of ovarian down regulation, because mature follicles and corpora lutea secrete significant quantities of 17OHP.

## 9.5 Conclusion

Widespread use of metformin in PCOS is based upon a broad consensus with a poor evidence base in a broad clinical environment. Perhaps because of the multiple symptoms of PCOS, there are many and complex reasons for actively treating women with ISAs in general and metformin in particular. Unfortunately, the evidence supporting this widespread use is playing catch-up with clinical practice, and alternative strategies such as exercise driven weight reduction appear to have less appeal, but may be more beneficial in the long run. Or perhaps there is a role for combined therapies.

We have been able to show that for women with moderate to severe hirsutism, metformin may provide a better treatment modality than the standard OCP Dianette. However, the main recommendation from this work would be that future studies should explore combined therapies.

Concerning the long term health indications of women with PCOS, the comparative dosing examination revealed that higher doses of metformin may be more beneficial in weight reduction and suppressing androgen concentrations under some circumstances, but the modest effects upon indicators of cardiovascular and diabetic risk would not support a high dosing regime.

Most of the original work with metformin in PCOS explored effects upon ovarian function. These studies, by dint of their duration, tended to be examinations of the effects of insulin upon the functions of LH (or thecal cells) in the excess of established follicles. We present novel information suggesting that prolonged treatment with metformin may result in a degree of correction of the principle underlying factor in PCOS – the excessive

incidence of initial recruitment and survival of ovarian follicles. Of course this is speculative, but it would be interesting to examine further.

The dysregulation of P450- 17α hydroxylase activity may also be a factor in hyperandrogenaemia from an adrenal source. Our study implies that metformin treatment suppressed basal adrenal biosynthesis of 17OHP and its responses to exogenous ACTH stimulation. This also requires further elucidation.

Thus, the studies presented in this thesis have not only answered some existing questions but also raise further possibilities for the elucidation of the use of metformin treament in women with PCOS.

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# APPENDIX

# THE ROLE OF ISAs IN PCOS

METFORMIN STUDY

Name Hospital number

Address Date of birth

Date of recruitment

Consent signed: Y N

GP informed: Y N

Inclusion criteria:

Diagnosis of PCO Y N

Hirsutism, FG score > 8 Y N

Exclusion criteria:

Contraindications to metformin: hepatic or renal impairment, heart failure, severe infection or trauma, alcohol dependence, pregnancy, breast feeding Y N

Contraindications to Dianette: risk factors for arterial disease, arterial or venous thrombosis, aged > 35 and a smoker Y N

BMI > 35 Y N

Use of COCP or metformin within last 3 months Y N

Presenting complaint

Hirsutism Y N

Oligomenorrhoea Y N

Amenorrhoea Y N

Previous obstetric history

Previous medical history

Drug history (within the last 6 months)

Social history

Smoker ?

Contraceptive use

*Return visit - 6 / 12 after starting*

Treatment allocation

Name

Date

Weight Height

BMI Waist

BP Hips

Woman's perception of hairiness

Very bad normal
Acne _____

Ferriman Galwey score (fill in on accompanying sheet)

Menstrual cycle over last 3 / 12 LNMP
(Menstrual diary Y / N)
Blood results
LH
FSH
Testosterone
SHBG
FAI
?DHAS
?17 OHP
? HbA1
? random glucose
Insulin
Lipids

| | | | |
|---|---|---|---|
| Finer Hair | Y | N |
| ↓ Rate Growth | Y | N |
| ↓ Need Cosmesis | Y | N |
| Improved Appearance | Y | N |
| ↑ Confidence | Y | N |

Hair removed from following areas and placed on slide (with base to frosted end)
Chin - C
Abdomen - A Sebum
Anterior midthigh - T
Forearm - F

Side effects:

| | | |
|---|---|---|
| Loss appetite | Y | N |
| Nausea | Y | N |
| Vomiting | Y | N |
| Diarrhoea | Y | N |
| Headache | Y | N |
| Breast tenderness | Y | N |
| Change in body weight | Y | N |
| Depression | Y | N |
| Skin reaction | Y | N |

Patient happy to continue? If so, review 6 / 12 months

Centre number:
Study number:
Patient identification number for this trial:

CONSENT FORM

Title of project: **A randomised trial to determine the efficacy of metformin in the treatment of hirsutism**

Name of researcher: Dr Jane Norman / Dr Helen Lyall

(Please initial each statement)

1. I confirm that I have read and understand the information sheet dated February 2000, version 1 for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I agree to take part in the above study.

| | | |
|---|---|---|
| Name of patient | Date | Signature |
| Name of person taking consent | Date | Signature |
| Researcher | Date | Signature |

TABLE 1. DEFINITION OF HAIR GRADINGS AT EACH OF 11 SITES

(GRADE 0 at all sites indicates absence of terminal hair.)

| SITE | GRADE | DEFINITION |
|---|---|---|
| 1. Upper Lip | 1<br>2<br>3<br>4 | A few hairs at outer margin.<br>A small moustache at outer margin.<br>A moustache extending halfway from outer margin.<br>A moustache extending to mid-line. |
| 2. Chin | 1<br>2<br>3 & 4 | A few scattered hairs.<br>Scattered hairs with small concentrations.<br>Complete cover, light and heavy. |
| 3. Chest | 1<br>2<br>3<br>4 | Circumareolar hairs.<br>With mid-line hair in addition.<br>Fusion of these areas, with three quarter cover.<br>Complete cover. |
| 4. Upper Back | 1<br>2<br>3 & 4 | A few scattered hairs;<br>Rather more, still scattered.<br>Complete cover, light and heavy. |
| 5. Lower Back | 1<br>2<br>3<br>4 | A sacral tuft of hair.<br>With some lateral extension.<br>Three-quarter cover.<br>Complete cover. |
| 6. Upper Abdomen | 1<br>2<br>3 & 4 | A few mid-line hairs.<br>Rather more, still mid-line.<br>Half and full cover. |
| 7. Lower Abdomen | 1<br>2<br>3<br>4 | A few mid-line hairs.<br>A mid-line streak of hair.<br>A mid-line band of hair.<br>An inverted V-shaped growth. |
| 8. Arm | 1<br>2<br>3 & 4 | Scarse growth affecting not more then a quarter of the limb surface.<br>More than this; cover still incomplete.<br>Complete cover, light and heavy. |
| 9. Forearm | 1,2 3,4 | Complete cover of dorsal surface, 2 grades of light and 2 of heavy growth. |
| 10. Thigh | 1 2.3,4 | As for arm. |
| 11. Leg | 1 2 3 4 | As for arm |

*Return visit 1 (2 /12 after recruitment)*

METFORMIN STUDY

Name

Hospital number

Address

Date of birth

Date of recruitment

Side effects:

| | | |
|---|---|---|
| Loss appetite | Y | N |
| Nausea | Y | N |
| Vomiting | Y | N |
| Diarrhoea | Y | N |
| Headache | Y | N |
| Breast tenderness | Y | N |
| Change in body weight | Y | N |
| Depression | Y | N |
| Skin reaction | Y | N |

**Waist**

**Hips**

**BP**

**Weight**

Any problems

Review in 4 /12

## Patient information sheet, version 1.2

A randomised trial to determine the efficacy of metformin in the treatment of hirsutism

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done, and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear of if you would like more information. Take time to decide whether or not you wish to take part.

We are doing a study to compare a new treatment for body and face hair (Metformin) with the standard treatment (Dianette). We believe the new treatment may be as good or better than the old treatment, but we need to do this study to find out if we are correct. We are asking you to help us because you have asked for treatment for face and / or body hair.

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

If you are willing to take part you will be allocated either to the new treatment (metformin), or to the standard treatment (Dianette). Neither you nor the doctor looking after you will be able to chose which treatment you have. The new treatment is in tablet form, and will involve taking one tablet three times each day. We will examine you and take 10 ml ( a small tube) of blood from you immediately before you start on the treatment, 6 months after you start on the treatment, and 12 months after you start on the treatment. We will also take a sample of hair at the beginning, middle and end of treatment to see if the thickness of the hair has changed. We will also ask you about any side effects which you experience. Possible side effects of metformin (the new treatment) include: decreased appetite, nausea, vomiting and diarrhoea.

We hope that the treatment will help you. However, this cannot be guaranteed. The information we get from this study may help us to treat future patients with hirsutism (excess hair growth) better.

Sometimes during the course of a research project, new information becomes available about the treatment/ drug that is being studied. If this happens, your research doctor will tell you about is and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form.

Also on receiving new information, your research doctor might consider it to be in your best interests to withdraw you from the study. He / she will explain the reasons and arrange for you care to continue.

If you are harmed by taking part in this research project, there are no special

compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action, but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

All information which is collected about you during the course of the research will be kept strictly confidential. Any information which leaves the hospital will have your name and address removed so that you cannot be recognized from it.

Your GP will normally be informed that you are participating in this research. If you do not agree to your GP being informed, you should decline to participate in this research.

The results of the research will be submitted for publication at the end of the study. If you wish to obtain a copy of these results you should inform the research doctor. You will not be identified in any report or publication.

The study is being organised by Glasgow Royal Infirmary. The study has been reviewed by the Ethics Committee at Glasgow Royal Infirmary.

If you wish further information, please contact your research doctor, Dr Jane Norman or Dr Helen Lyall at the Department of Obstetrics and Gynaecology, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER, telephone 0141 211 4702

Thank you for taking part in this study.

Information sheet, version 1.2, prepared March 2000

You will be given a copy of this information sheet and a signed consent form to keep.

## Metformin for Weight Loss Study

Name Hospital number

Address DOB

Date of recruitment

Consent signed Y N

GP informed Y N

Inclusion criteria

| | | | |
|---|---|---|---|
| BMI | 29-36.9 | Y | N |
| | >37 | Y | N |
| Oligomenorrhea | | Y | N |
| Amenorrhea | | Y | N |
| PCO on U/S | | Y | N |
| FAI | | Y | N |

Exclusion criteria

| | | |
|---|---|---|
| Contraindications to Metformin | Y | N |
| Desire to conceive | Y | N |
| Metformin in last 4 months | Y | N |
| COCP in last 2 months | Y | N |
| Nil CAH; Thyroid; NIDDM | Y | N |

Previous obstetric history

Previous medical history

Drug history (within last 6 months)

Smoker

Average daily caloric intake

Average weekly exercise

Date

Weight Waist

Height Hips

BMI BP

FG Score

Menstrual diary LNMP K

Blood Results

1) Main Lab
   -Red- Test/ SHBG/ FAI/ DHEAS/ 17OHP/ Andros/ TFT
   -Grey- Glucose
   -Yellow-U&E/LFT/GGT/urate

   Gyn Lab
   -Green- Insulin
   -Red- CRP/ Leptin etc
   -Purple- Lipids

Ultrasound (TVS) W x L x D W x L x D

Ovarian volume L R

FD Max

No. follicles <10mm

Average daily caloric intake

Average weekly exercise

PPARS

NEXT VISIT DATE:
DOSE:

**Organising body**
The study is being organised by the Glasgow Royal Infirmary. The study has been reviewed by the Ethics Committee at Glasgow Royal Infirmary.

If you wish further information, please contact your research doctor, **Dr. Lyndal Harborne** at the Department of Obstetrics and Gynaecology, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER,
Tel.: 0141 211 4000. Page No. 2262
**Dr Richard Fleming** at the Department of Obstetrics and Gynaecology, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER, Tel.: 0141 211 4703.

Thank you for volunteering to take part in this study.

You will be given a copy of this information sheet and a signed consent form to keep.

## CONSENT

1. I confirm that I have read and understood the information sheet dated 29th August 2001 (version 1.2) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study ☐

| Name of Patient | Date | Signature |
|---|---|---|
| Name of Person taking Consent | Date | Signature |

Patient information sheet: **version 3** 13/09/2001

## A trial to determine the efficacy of metformin in the treatment of weight loss in women with polycystic ovary syndrome



You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done, and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

**The study**

Metformin, a drug normally used in diabetes, has been shown to improve ovarian function, aid weight loss, and improve the profiles of fats (cholesterol) circulating in the blood in women with polycystic ovaries. Women who were above average weight did not respond as well as those with normal weight. We are now carrying out a study to find out if a higher dose of metformin, or if a longer course of treatment is effective for weight loss and blood cholesterol levels. We think that heavier women may need a higher dose than used previously, but we need to find out if we are correct. We are asking you to help us find out if, and if so, how much, weight you will lose by taking the treatment.

**Conditions**

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this patient information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

**Details about the study**

If you are willing to take part you will be allocated to either the higher or lower dose of Metformin. Neither you nor the doctor looking after you will be able to choose which dose you will have. The treatment for both dosages is in tablet form and will involve taking one tablet three times a day. We will examine you and take 30ml of blood from you immediately before you start on the treatment, 4 months after you start on the treatment, and 8 months after you start on the treatment. We will also measure your blood pressure, height and weight, and you will be asked to keep a diary of your menstrual periods for the 8 months. We will also ask you about any side-effects of Metformin that you may experience. This will involve phoning you each month for 4 months. Possible side effects include: decreased appetite, nausea, vomiting and diarrhoea.

We hope that this treatment will help you. However, this cannot be guaranteed. The information we get from this study may help us revise how we treat women for weight loss in the future.

**Developments during the study**

Sometimes during the course of a research project, new information becomes available about the treatment/ drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form.

Also on receiving new information, your research doctor might consider it to be in your best interests to withdraw you from the study. He/ she will explain the reasons and arrange for your care to continue.

**Risk**

Metformin has been used extensively to sensitise people to insulin as in diabetes. It has been shown to be a safe drug in practice. It does not lead to abnormal circulating sugar levels. It has not been implicated in the development of liver dysfunction as have some other insulin sensitising agents.

**Complaints**

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone`s negligence, then you may have grounds for a legal action, but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been treated or approached during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

**Confidentiality**

All information that is collected about you during the course of the research will be kept strictly confidential. Any information which leaves the hospital will have your name and address removed so that you cannot be recognised from it.

Your GP will normally be informed that you are participating in this research. If you do not agree to your GP being informed, you should inform us of this fact.

**On completion**

The results of the research will be submitted for publication at the end of the study. If you wish to obtain a copy of these results you should inform the research doctor. You will not be identified in any report or publication.

**Organising body**
The study is being organised by the Glasgow Royal Infirmary. The study has been reviewed by the Ethics Committee at Glasgow Royal Infirmary.

If you wish further information, please contact your research doctor, **Dr Lyndal Harborne** at the Department of Obstetrics and Gynaecology. Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER,
Tel.: 0141 211 4000. Page No. 2262
**Dr Richard Fleming** at the Department of Obstetrics and Gynaecology, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER, Tel.: 0141 211 4703.

Thank you for volunteering to take part in this study.

You will be given a copy of this information sheet and a signed consent form to keep.

## CONSENT

1. I confirm that I have read and understood the information sheet dated 29th August 2001 (version 1.2) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study ☐

| Name of Patient | Date | Signature |
|---|---|---|
| Name of Person taking Consent | Date | Signature |

# Synacthen Study in PCOS: Laboratory Data Sheet

**Sheet 1**

Pt. Sticky Label

**PCOS** (20;10,10) / **CONTROL** (20; 10,10) Random: YOB,odd=Rx, even=NoRx

**Visit 1** (Prostap Day) **Date:** **Last Menstrual Period:**

**Ultrasound Scan:**
Largest Follicle: **R:** **L:** Ovarian Volume:

PCO appearance **R: Y / N** **L: Y / N**

**ANTHROPOMETRIC DATA**

Kgs: Ht: Waist (cms): Hip (cms):

HIRSUTISM / ACNE / ACANTHOSIS NIGRICANS / ALOPECIA

**MENSTRUAL FREQUENCY** **K:**

NMR OLIGOMENORRHOEA POLYMENORRHOEA

**START Prostap: DATE:**

**Non Fasting Blood**

| Gyn.Lab. | E2 | |
|---|---|---|
| | Progesterone | |
| | Testosterone | |
| | SHBG | |
| | LH | |

# Synacthen Study in PCOS: Laboratory Data Sheet



## Visit 2 (14 days post prostap) **Date:** **Last Menstrual Period:**

Ultrasound Scan: Largest Follicle: R: L: Ovarian Volume:

PCO appearance R: Y / N L: Y / N

| **Fasting Bloods** | **Time of samples:** | |
|---|---|---|
| **Haematology** | HbIC | |
| **Biochemistry** | Insulin, | |
| | Glucose | |
| **Gyn.Lab.** | E2 | |
| | Testosterone | |
| | Progesterone | |
| | Androstenedione | |
| | 17OHProg | |
| | DHEAS | |
| | LH | |
| | FSH | |
| | SHBG | |

## Synacthen Test 1 (250ug tetracosactrin iv)

| Time | E2 | T | A4 | 17OHP | DHEAS |
|---|---|---|---|---|---|
| T0 | | | | | |
| T30 | | | | | |
| T60 | | | | | |
| T90 | | | | | |

START METFORMIN [500mg tds] (or NOT): Y / N

## Visit 3 (14 days post starting metformin) **Date:** **Last Menstrual Period:**

Ultrasound Scan: Largest Follicle: R: L: Ovarian Volume:

PCO appearance R: Y / N L: Y / N

## Synacthen Test 2 (250ug tetracosactrin iv)

| Time | E2 | T | A4 | 17OHP | DHEAS |
|---|---|---|---|---|---|
| T0 | | | | | |
| T30 | | | | | |
| T60 | | | | | |
| T90 | | | | | |

## Study to investigate the influence of metformin on adrenal androgen output.

CONSENT

I, (Name).....

of (Address).....

.....

agree to take part in the Research Project/Study Programme described above.

Dr/Mr ..... has explained to me what I have to do, how it might affect me and the purpose of the Research Project/Study Programme.

Signed ..... Date .....

Witness ..... Date .....

## Study to investigate the influence of metformin on adrenal androgen output.

Polycystic ovary syndrome (PCOS) is a common cause of failure to ovulate (produce an egg) each month. This may lead to difficulties in achieving a pregnancy. The cause of PCOS is poorly understood, but many women with PCOS have increased levels of androgens (the male type of hormone) in the blood. Androgens are produced normally in the ovary and also the adrenal gland (a gland which lies above each kidney).

Insulin (a hormone) in our body as well as other actions, controls our blood sugar (glucose). PCOS may be associated with resistance to the actions of insulin, which means the body is less sensitive to insulin. Metformin is a drug which has been widely used in the treatment of diabetic patients as it helps the body to use glucose and therefore helps insulin to work. There is evidence that metformin can improve menstrual cycle regularity and fertility in women with PCOS who have high blood levels of androgens by improving the action of insulin.

One of metformin's effects is to rapidly reduce the level of testosterone (one specific androgen hormone) in the blood. However it is impossible to detect by measuring androgen levels in the blood whether metformin is exerting its effect on the ovary or the adrenal gland. We aim to test whether androgens from the adrenal gland may be affected by metformin treatment. Metformin may cause you to feel off your food and may cause nausea, vomiting and diarrhoea, but these are usually short-lived.

In order to answer our question, we will suppress the function of the ovary using an injection (gonadotrophin releasing hormone analogue, GnRHa) which temporarily stops the ovaries producing hormones. This is similar to the buserelin (snuff) which you take during the course of your treatment. We will then stimulate the adrenal gland using a test widely used in medical practice. This is called a Synacthen test. You will have a blood sample taken then receive a small (250 microgram) injection of Synacthen (Tetracosactrin). Thirty and sixty minutes later further blood samples are taken. As this is such a small dose problems associated with the injection are very unlikely. However rarely, Synacthen can be associated with an allergic reaction.

We will ask you to attend the hospital for an injection of the GnRH analogue Prostap (leuprorelin acetate). Two weeks later you will have a Synacthen test. This involves a blood test then a small injection. Two further blood tests will then be taken 30 and 60 minutes after the injection.

Some of you (whether you have PCOS or are a person with a regular menstrual cycle) will then be asked to take metformin 500 mg three times daily for 14 days. A second synacthen test will then be performed which will be the same as previously.

Please note that if you agree to take part, this Research Project may be of little or no benefit to you but the results may help other patients in the future.

If you do not want to take part in the Research Project or if at any time you wish to stop taking part you may do so. The care which you are presently receiving will not be affected in any way.

If you do agree to take part in the Research Project, your own General Practitioner will be told and will be given details of information about any care which you are to receive.

# PHYSICAL ACTIVITY QUESTIONNAIRE

**The following questionnaire is a simple way of measuring the amount of physical activity you have done over the last week. The questionnaire is strictly confidential so try and answer all questions as honestly as you can. Obviously, the overall accuracy depends on the accuracy of individual answers. The questionnaire is not a test so there is no pass or fail.**

**May I take this opportunity to thank you for taking the time to fill out the questionnaire.**


(1) Do you consider yourself to be regularly active now ? YES | | NO | |
(Please tick one box)

If YES go to question (2). If NO, were you regularly physically active.

3 months ago ? YES | | NO | |

6 months ago ? YES | | NO | |

Now go to question (2)

(2) Please read through all the categories listed below and tick ONE box for the category which best describes how physically active you have been over the last six months.

(i) I am not regularly active and do not intend to be so in the next six months | |

(ii) I am not regularly physically active but am thinking about starting to do so in the next six months. | |

(iii) I do some physical activity but not enough to meet the description of regular physical activity given above. | |

(iv) I am regularly physically active but only began in the last six months. | |

(v) I am regularly physically active and have been for six months or longer. | |

(4) Was last week typical of the amount of physical activity you usually do ?
(Please tick one box)

| | |
|---|---|
| YES | [ ] |
| NO – I USUALLY DO MORE | [ ] |
| NO – I USUALLY DO LESS | [ ] |

*If you usually do more, normally how much more (in minutes) and of which activity ?*

| |
|---|
| |

If you usually do less, normally how much less (in minutes) and of which activity ?

| |
|---|
| |

(v) Cycling *for pleasure or to work* ?

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

(vi) Participating in a *sport*, leisure activity or training ?
*Do include* E.g. exercise classes, football, swimming, golf, jogging, athletics, etc.
*Do not include* E.g. darts, snooker/pool, fishing, playing a musical instrument, etc.

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

(vii) Any other physical activity if not already covered ? (please write in)

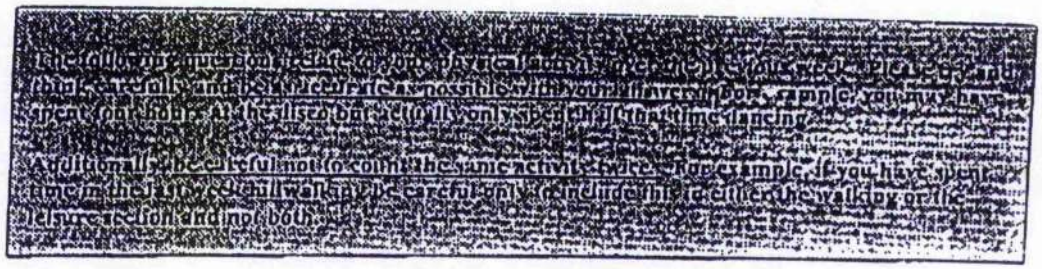
| |
|---|
| |

Please fill in below how many minutes you spent each day doing these other physical activities

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

(3) Please fill in below how many calories you burnt each day whilst wearing the Caltrac device.

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |



(2) In the past week how many minutes did you spend each day:

(i) Walking at work ?
E.g. . walking up or down stairs, walking to and from your desk, "doing the rounds", etc.

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

(ii) Walking outwith work ?
E.g. walking to the shops, walking your dog, walking to work, hillwalking, walking for pleasure, walking up and down stairs, etc.

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

(iii) Doing active housework ?
*Do Include* E.g. hoovering, scrubbing floors, bed making, hanging out washing, etc.
*Do not include* E.g. sewing, dusting, washing dishes, preparing food, etc.

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

(iv) Dancing ?
E.g. disco, line, country etc.

| MON | TUES | WED | THURS | FRI | SAT | SUN | TOTAL |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

## PERSONAL DETAILS

| |
|---|
| NAME: |
| TELEPHONE NUMBER : |
| AGE IN YEARS : |
| TODAY'S DATE: |

(1) Are you currently employed ? YES [ ] NO [ ]
(please tick one box)

(2) If NO, end of questionnaire. If YES, have you changed your employment or the kind of work you do in the last month ? YES [ ] NO [ ]
(please tick one box)

(3) If NO, end of questionnaire. If YES, has this resulted in an increase in the amount of physical activity you do ? YES [ ] NO [ ]

If NO, end of questionnaire. If YES, how and by how much ?

| |
|---|
| |

END OF QUESTIONNAIRE – THANK YOU

# FOOD INTAKE QUESTIONNAIRE

FOR OFFICE USE ONLY

Surname..... Subject ID

First Name(s) .....

Address ..... Questionnaire No

..... Group Code

Phone No. ..... Survey No

Male / Female

Date of Birth ..... Date of Survey .....

1-4 5-8 9 10 11-12 13 14-15 16-17 18-19/162-163 20-21 22-23 24-25/164-165

The following questions are about the foods you USUALLY eat.
Please indicate the number of days per week that you eat each item on average. Ring the answer as in these examples:

| | |
|---|---|
| If you eat the food every day, ring 7 | 7 6 5 4 3 2 1 F R |
| If you eat the food three days/week, ring 3 | 7 6 5 4 3 2 1 F R |
| If you eat the food once a fortnight, ring F | 7 6 5 4 3 2 1 F R |
| If you rarely or NEVER eat the food, ring R | 7 6 5 4 3 2 1 F R |

**PLEASE ANSWER EVERY QUESTION**

## BREAD

**How often do you eat the following breads and how many slices do you have per day?**

| | *No. days/week* | *No. slices or rolls per day* | *Size of slices or rolls* | |
|---|---|---|---|---|
| White or high fibre | 7 6 5 4 3 2 1 F R | ..... | Thick/medium/thin Large/small | 26-28 |
| Brown or wheatgerm | 7 6 5 4 3 2 1 F R | ..... | Thick/medium/thin Large/small | 29-31 |
| Wholemeal/chapatis | 7 6 5 4 3 2 1 F R | ..... | Thick/medium/thin Large/small Chapatis | 32-34 |
| Bread rolls | 7 6 5 4 3 2 1 F R | | White/brown/ wholemeal | 35-37 |
| Crispbread, Ryvita or cream crackers | 7 6 5 4 3 2 1 F R | ..... | | 38-39 |

**How often do you eat jam, marmalade or honey on bread?** 7 6 5 4 3 2 1 F R — 40

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## BREAKFAST CEREALS

**How often do you eat the following cereals?**

| | | |
|---|---|---|
| 1 | Cornflakes or Frosties | 7 6 5 4 3 2 1 F R |
| 2. | Sugar Puffs, Special K, Ricicles or Rice Crispies | 7 6 5 4 3 2 1 F R |
| 3. | Muesli or Fruit n' Fibre | 7 6 5 4 3 2 1 F R |
| 4. | Weetabix, Advantage or Shredded Wheat | 7 6 5 4 3 2 1 F R |
| 5 | Bran Flakes or Sultana Bran | 7 6 5 4 3 2 1 F R |
| 6 | Porridge or Ready Brek | 7 6 5 4 3 2 1 F R |
| 7 | All Bran | 7 6 5 4 3 2 1 F R |
| | Other Cereal | 7 6 5 4 3 2 1 F R |
| | Please specify brand | ... |
| **How many teaspoons of sugar/honey do you add?** | | ..... |
| **How often do you have wheat bran?** | | 7 6 5 4 3 2 1 F R |

## MEATS

**How often do you have the following meats?**
**Include all forms of each meat, eg use in stews, casseroles, lasagne, curry etc.**

| | | |
|---|---|---|
| Beef | 7 6 5 4 3 2 1 F R | |
| Lamb | 7 6 5 4 3 2 1 F R | |
| Pork | 7 6 5 4 3 2 1 F R | |
| Bacon | 7 6 5 4 3 2 1 F R | |
| Ham | 7 6 5 4 3 2 1 F R | |
| Chicken or other poultry | 7 6 5 4 3 2 1 F R | |
| Canned meat (e g corned beef) | 7 6 5 4 3 2 1 F R | |
| Sausages | 7 6 5 4 3 2 1 F R | |
| What type of sausages do you have? | 1 Pork<br>2 Beef<br>3 Pork and Beef<br>4 Turkey<br>5 Low Fat | |
| Meat pies/pasties - shop bought | 7 6 5 4 3 2 1 F R | |
| Meat pies/pasties - home made | 7 6 5 4 3 2 1 F R | |
| Liver/kidney/heart | 7 6 5 4 3 2 1 F R | |
| Do you usually eat the fat on meat | Yes / No | |

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## FISH

**How often do you eat the following fish?**

| | | |
|---|---|---|
| White fish (cod/haddock/plaice/fish fingers) | 7 6 5 4 3 2 1 F R | 60 |
| Kipper/herring/mackerel/trout (including canned) | 7 6 5 4 3 2 1 F R | 61 |
| Pilchards/sardines/salmon (including canned) | 7 6 5 4 3 2 1 F R | 62 |
| Tuna (including canned) | 7 6 5 4 3 2 1 F R | 63 |

## VEGETABLES & SAVOURY DISHES

**How often do you have the following vegetables or dishes?**

| | | |
|---|---|---|
| Potatoes - boiled or mashed | 7 6 5 4 3 2 1 F R | 64 |
| Potatoes - jacket | 7 6 5 4 3 2 1 F R | 65 |
| Chips - shop bought or 'oven chips' | 7 6 5 4 3 2 1 F R | 66 |
| Chips - homecooked | 7 6 5 4 3 2 1 F R | 67 |
| Potatoes - roast | 7 6 5 4 3 2 1 F R | 68 |
| Peas | 7 6 5 4 3 2 1 F R | 69 |
| Other green vegetables/salads | 7 6 5 4 3 2 1 F R | 70 |
| Carrots | 7 6 5 4 3 2 1 F R | 71 |
| Parsnips/swedes/turnips | 7 6 5 4 3 2 1 F R | 72 |
| Baked beans | 7 6 5 4 3 2 1 F R | 73 |
| Butter beans or broad beans | 7 6 5 4 3 2 1 F R | 74 |
| Lentils, Chick peas or Dahl | 7 6 5 4 3 2 1 F R | 75 |
| Onions (cooked/raw/pickled) | 7 6 5 4 3 2 1 F R | 76 |
| Spaghetti/other pasta | 7 6 5 4 3 2 1 F R | 77 |
| Rice (NOT pudding rice) | 7 6 5 4 3 2 1 F R | 78 |
| Quiche | 7 6 5 4 3 2 1 F R | 79 |
| Pizza | 7 6 5 4 3 2 1 F R | 80 |
| Vegetarian pasties | 7 6 5 4 3 2 1 F R | 81 |

## BISCUITS, CAKES & PUDDINGS

**How often do you eat the following items?**

| | | |
|---|---|---|
| Digestive biscuits/plain biscuits | 7 6 5 4 3 2 1 F R | 82 |
| Other sweet biscuits | 7 6 5 4 3 2 1 F R | 83 |
| Chocolate | 7 6 5 4 3 2 1 F R | 84 |

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| | | |
|---|---|---|
| Sweets | 7 6 5 4 3 2 1 F R | 85 |
| Crisps | 7 6 5 4 3 2 1 F R | 86 |
| Nuts | 7 6 5 4 3 2 1 F R | 87 |
| Ice cream | 7 6 5 4 3 2 1 F R | 88 |
| Low fat yogurt | 7 6 5 4 3 2 1 F R | 89 |
| Low calorie yogurt (eg Shape) | 7 6 5 4 3 2 1 F R | 90 |
| Other yogurts | 7 6 5 4 3 2 1 F R | 91 |
| Fruitcake/sponge cake - shop bought | 7 6 5 4 3 2 1 F R | 92 |
| Fruitcake/sponge cake - homemade | 7 6 5 4 3 2 1 F R | 93 |
| Fruit tart/jam tart - shopbought | 7 6 5 4 3 2 1 F R | 94 |
| Fruit tart/jam tart - home made | 7 6 5 4 3 2 1 F R | 95 |
| Milk pudding (eg. rice/tapioca/macaroni) | 7 6 5 4 3 2 1 F R | 96 |

What type of milk do you use for milk pudding?

1 Ordinary/whole
2 Semi-skimmed
3 Skimmed
4 Canned milk pudding - ordinary
5 Canned milk pudding - low fat

97

## FRUIT

| | | |
|---|---|---|
| How often do you have fruit canned in syrup? | 7 6 5 4 3 2 1 F R | 98 |
| How often do you have fruit canned in juice? | 7 6 5 4 3 2 1 F R | 99 |
| How many apples do you have per week? | | 100 |
| How many pears do you have per week? | | 101 |
| How many oranges/grapefruit do you have per week? | ..... | 102 |
| How many bananas do you have per week? | | 103 |

## EGGS & MILK PRODUCTS

How many eggs do you usually eat per week? ..... 104-105

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***Roughly how much milk do you drink in a day in tea/coffee/milky drinks/cereals?***

1 None
2 Half a pint or less
3 Between half and one pint
4 One pint or more

☐ 106

**What type of milk do you have?**

1 Whole
2 Semi-skimmed
3 Skimmed
4 More than one type

☐ 107

**How much cream do you use per week?**

(1 tablespoon=20g, small carton=150g, large carton=300g) . . . . . g ☐☐☐ 108-110

**How much cheese (excluding cottage cheese) do you usually eat per week?** . . . . . g ☐☐☐ 111-113

(Suggestion: divide amount bought for household by number of people in house)

**How often do you eat cottage cheese?** 7 6 5 4 3 2 1 F R ☐ 114

## FATS

**What do you usually spread on bread?**

1 Butter
2 Margarine - polyunsaturated
3 Margarine - other soft (tub)
4 Margarine - hard (block)
5 Low fat spread - polyunsaturated
6 Low fat spread - other
7 Lard, dripping, solid vegetable oil
8 Very low fat spread (25% fat)
0 Bread eaten dry

☐☐ 115-116

Brand name & description on packet/tub . . .

***How much butter/margarine/spread do you usually eat per week?***.. g ☐☐☐ 117-119

(One block or small tub = 250g. *Spread on one slice of bread*: Thinly=5g, Medium=8g; Thickly=13g.)

**How often do you have food which is shallow fried?**
(e.g., fish/onions/mushrooms/tomatoes/eggs) 7 6 5 4 3 2 1 F R ☐ 120

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**What BRANDS of fat do you use in cooking?**

| | | |
|---|---|---|
| Shallow Frying | solid/liquid | 121 |
| Chips | solid/liquid | 122 |
| Roast Potatoes | solid/liquid/eaten out | 123 |
| Home made cake | | 124 |
| Home made pastry | | 125 |

## DRINKS

| | | |
|---|---|---|
| **How many cups of tea do you have per day?** | | 126-127 |
| **How many teaspoons of sugar/honey per cup?** | | 128 |
| **How many cups of coffee do you have per day?** | | 129-130 |
| **How many teaspoons of sugar/honey per cup?** | | 131 |
| **How often do you have fruit juice/squash/fizzy drinks (NOT low calorie)?** | 7 6 5 4 3 2 1 F R | 132 |
| **Which of these do you usually have?** | 1 Natural Juice<br>2 Squash<br>3 Fizzy Drink<br>4 More than one | 133 |
| **How often do you have drinks containing alcohol?** | 7 6 5 4 3 2 1 F R | 134 |
| **When you drink, how many do you have?** | | |

**Please specify how many drinks of each type per occasion.**

| | | | |
|---|---|---|---|
| Beer/stout/cider | Number of pints | | 135-136 |
| Wine | Number of glasses | | 137 |
| Sherry/port/vermouth | Number of glasses | | 138 |
| Spirits | No. of single measures | | 139 |

## HEIGHT, WEIGHT & ACTIVITY

What is your height? ..... ft ..... ins OR ..... cm

What is your weight? ..... st ..... lbs OR ..... kg

140-143

144-147

How physically active is your occupation?

1 Not very active
2 Moderately active
3 Very active
4 Not working

148

How physically active is your leisure time?

1 Not very Active
2 Moderately active
3 Very active

149

Questions for women only.

Are you pregnant? Yes / No 150

Are you breast feeding? Yes / No 151

## ADDITIONAL QUESTIONS

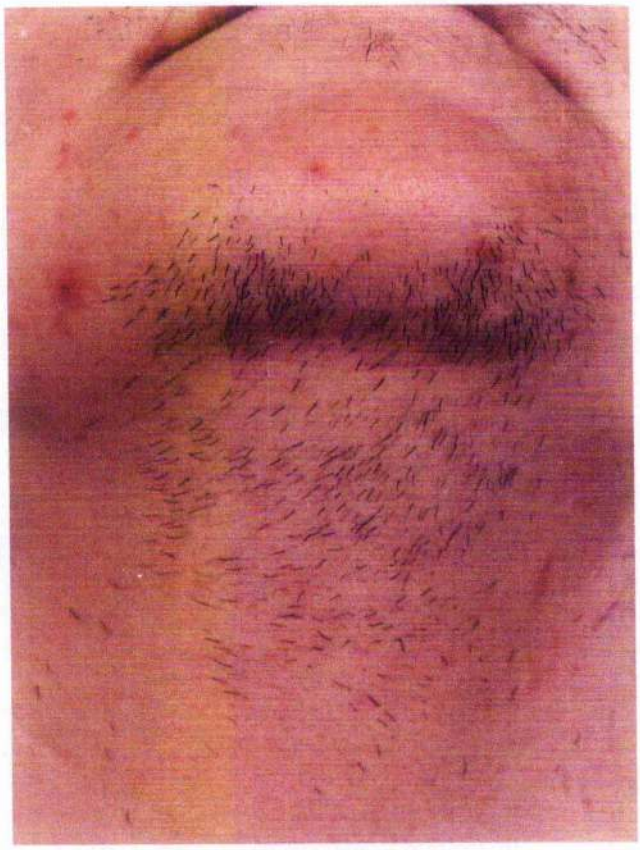
152-154

155-157

158-160

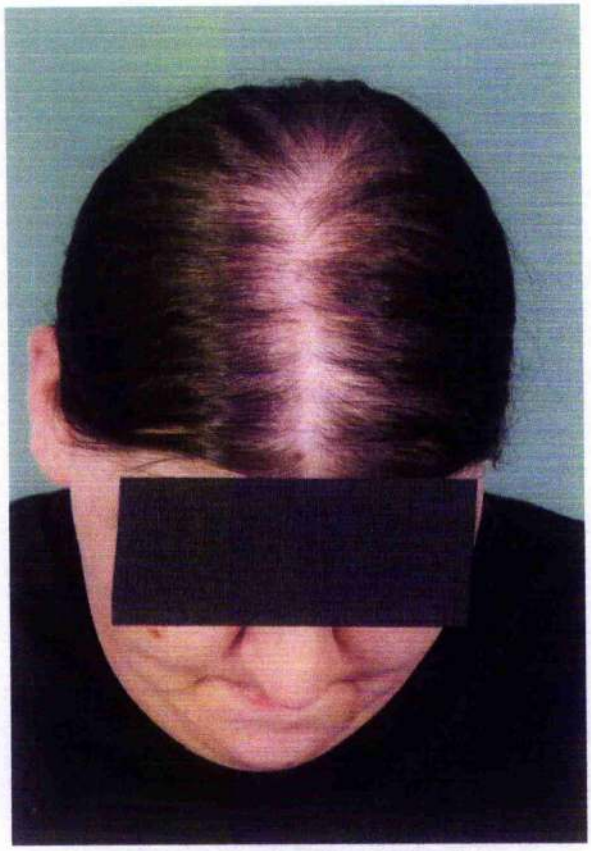
THANK YOU VERY MUCH FOR YOUR HELP

Diet Code 161

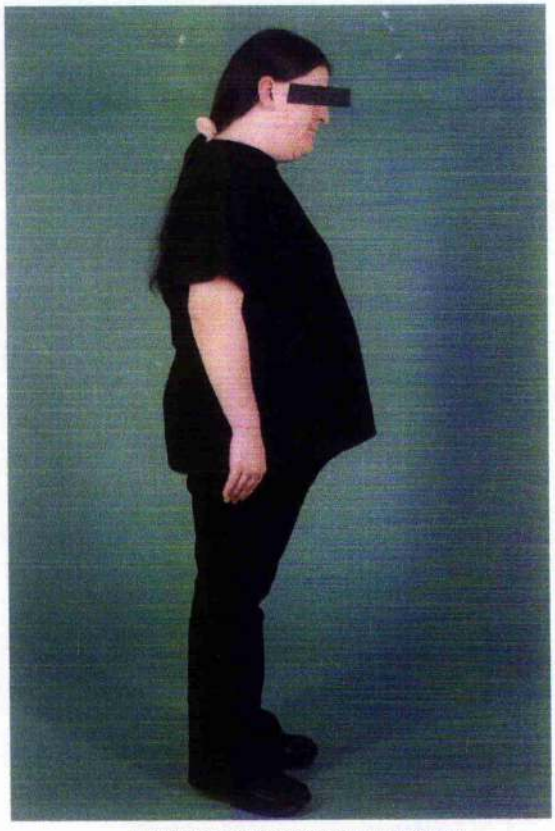


EXAMPLE OF FACIAL HIRSUTISM

EXAMPLE OF ABDOMINAL HIRSUTISM



EXAMPLE OF MALE PATTERN BALDING



EXAMPLE OF CENTRAL OBESITY

9th June 2003

Dear Lyndal,

Hope this letter finds you and your family well. I hope you don't mind me writing to you but I was shocked when I went to the Royal Infirmary back in January and they told me you went home. I'm so happy that you went home because I know how much you missed it. I was just sorry that you had left before I had the chance to thank you properly. You were the only person who believed me when I told you I was not a big eater and that meant a lot to me and you were fantastic and very caring during the metformin drug trial and that in itself has changed my life. The last time I saw you in July last year I was around 14 ½ stone, well now I am down to 9 stone 13 and am over the moon as I feel that I have a decent life now and am very happy and I owe it all to you so I hope you don't mind me writing to you but I just wanted to thank you for all you've done and I truly hope you are happy and settled being back home. Lots of love

I've enclosed a photo of me and my mum.