GLUCAGON IN HYPERANDROGENIC WOMEN:

RELATIONSHIPS TO ABNORMALITIES OF INSULIN.

by

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I declare that I have composed this thesis myself and that the work described has been my own, except as described in 'Acknowledgements'.

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PREFACE

This hypothesis that elevated concentrations of pancreatic glucagon are involved in the development and maintenance of insulin resistance in women with PCO, that there is a positive relationship between concentrations in serum of glucagon and androgens in women with PCO and that a decrease in the serum concentrations of androgens results in a reduced concentration of glucagon in the peripheral plasma have been examined in these studies. Thus, peripheral insulin resistance would be sustained as a result of increased plasma concentrations of glucagon in a counter-regulatory manner, but insulin would act, unopposed, upon the ovary to promote insulin-like growth factor-1 and androgen production.

ABSTRACT

In order to investigate whether glucagon is implicated in the reduced insulin sensitivity in hyperandrogenic women, concentrations of glucagon and insulin in serum were measured during a 75g oral glucose tolerance test (oGTT) in 24 obese (body mass index, BMI, $>25 kgm^{-2}$) and 20 non-obese women with polycystic ovary syndrome (PCO), and 10 obese and 13 non-obese control subjects. The oGTT was repeated during alteration of concentrations of endogenous androgens by administration of buserelin, spironolactone or a combination of cyproterone acetate and ethinyl oestradiol to women with PCO, and by administration of goserelin or danazol to control subjects. The relationships between glucose, insulin, C-peptide and glucagon values and those of testosterone, androstenedione, dehydroepiandrosterone sulphate and sex hormone binding globulin were examined before and during treatment. Additionally, the relationship between basal and glucose-stimulated glucose concentrations and that of haemoglobin A_1 (HbA_1) was examined to assess the value of HbA_1 estimation for monitoring glycaemic control in women with PCO.

Obese women with PCO had higher serum concentrations of insulin and glucose than did non-obese women with PCO and control subjects, but plasma concentrations of glucagon were greater in obese control women. There were no significant relationships between fasting concentrations of insulin or insulin responses to oral glucose and those of testosterone or androstenedione in either group of PCO subjects, but the glucagon response to oral glucose was significantly related to both testosterone and androstenedione in obese women with PCO. No significant relationship was demonstrated between change in serum concentrations of androgens as a result of treatment and those of insulin or glucagon in any of the groups. In no case was the HbA_1 concentration above normal laboratory values although a significant correlation between HbA_1 and summed glucose levels was apparent.

Glucagon does not appear to be implicated in the insulin resistance exhibited by women with PCO nor do androgens appear to directly effect concentrations of insulin or glucagon in normal women or in those with PCO. Measurement of HbA_1 is not sufficiently discriminatory for identification of impaired glucose tolerance in PCO.

INTRODUCTION

DEFINITION

The definition and pathogenesis of the polycystic ovary syndrome (PCO) have been subjects of controversy for more than 20 years. It encompasses a group of women with a heterogeneous collection of symptoms and signs of variable severity and which have included some of the following:

a) menstrual irregularity dating from the menarche;

b) oligomenorrhoea or amenorrhoea;

c) symptoms of hyperandrogenism (hirsutism, seborrhoea, acne, and occasionally male pattern hair loss);

d) reduction of fertility;

e) obesity;

f) bilaterally enlarged or normal sized sclerocystic ovaries;

g) elevation of serum concentrations of luteinising hormone (LH), an increased ratio of serum concentrations of LH to follicle stimulating hormone (FSH), elevation of serum concentrations of androgenic sex steroids and elevation of the ratio of serum concentrations of oestrone to oestradiol.

Sclerocystic ovaries in the human were described by Chereau in 1845 and partial resection of the sclerocystic ovary had been performed by the turn of the 20th century (Goldzieher 1981). Recognition of the association between the cluster of symptoms of menstrual irregularity, hirsutism and infertility with the presence of bilaterally enlarged ovaries is attributed to Stein and Leventhal (1935).

However, women with sclerocystic ovaries but without all, or indeed any, of this triad of clinical features were first reported by Goldzieher and Green (1962). Some of these women have hypothyroidism or Cushing's disease (Yen 1980), suggesting that the characteristic ovarian morphology may be a common end stage of the ovarian response to a variety of pathological stimuli.

The availability of radioimmunoassay has facilitated investigation of these women. Typical endocrine abnormalities include elevated concentrations in serum of LH, androstenedione (A) testosterone (T), dehydroepiandrosterone sulphate (DHEAS) and 17-alpha hydroxyprogesterone and more rapid oestrone production (Yen 1980). The origin of excess sex steroids has been investigated indirectly by selective stimulation or suppression of the adrenal (Kirschner et al 1976, Polson

et al 1988b) or ovary (Chang et al 1983a) and directly by catheterisation and sampling of the ovarian and adrenal veins (Kirschner and Jacobs 1971, Wajchenberg et al 1988). Whilst these studies have shown that the ovary is the major source of androgens in women with PCO, the relative contribution of the ovary and the adrenal to the pathogenesis and persistence of the syndrome continues to be debated (McKenna 1988).

More recently, high resolution ultrasonographic examination of the ovary has been applied in the investigation and management of PCO. The ovarian morphology in women with PCO, diagnosed by clinical and biochemical assessment, is heterogenous (Kim et al 1979, Orsini et al 1985). The ovary is defined as polycystic by ultrasound if multiple cysts (10 or more) 2-8 mm in diameter are arranged peripherally around a dense core of stroma or scattered through an increased amount of stroma, and is usually enlarged (Adams et al 1986). In a 'normal' population of women of reproductive age, up to 22% may have ultrasonic features of PCO, although a substantial proportion of these women have clinical symptoms and signs such as irregular periods or unwanted facial hair consistent with a mild form of PCO (Polson et al 1988a).

AETIOLOGY - POLYCYSTIC OVARY SYNDROME

Aetiological factors suggested for the development of the polycystic ovary syndrome are numerous and have included:

a) a primary ovarian abnormality (Jacobs 1987);

b) prepubertal adrenal dysfunction (Yen 1980);

c) sustained adrenal hypersecretion of androgens and then peripheral aromatisation resulting in increased serum concentrations of oestrone (McKenna 1988);

d) inheritance of a dominant trait with an increased rate of expression (Hague et al 1988) possibly related to an effect of maternal androgens upon gonadal development in utero;

e) differential regulation of the secretion of LH and FSH by androgenic inhibition of gonadotrophic releasing hormone (GnRH, Duignan et al 1975), partial pituitary desensitisation to GnRH (Waldstreicher et al 1988) or hyperoestronaemia (Chang et al 1982) inducing a deficiency of the adrenal and ovarian aromatase systems;

f) abnormal secretion of insulin (Taylor et al 1982, Barbieri et al 1986);

g) abnormalities of the secretion of insulin-like growth factors or other polypeptide growth factors (Franks 1989).

INSULIN AND ANDROGENS

An association between disturbed insulin and hyperandrogenism has been recognised for many years. As early as 1921, Achard and Thiers described the syndrome 'diabete a femmes de barbe', an association between hirsutism and non-insulin dependent diabetes mellitus (NIDDM). More recently an association between insulin resistance secondary to receptor abnormalities and hyperandrogenism of ovarian origin has been demonstrated (Kahn et al 1976, Taylor et al 1982, Poretsky and Kalin 1987), and in women with PCO, increased concentrations of insulin and androgen frequently coexist (Barbieri et al 1988).

Although Taylor et al (1982) suggested that hyperinsulinaemia may contribute to hypersecretion of androgens from the ovary in subjects with insulin resistance, other authors have suggested the converse, that excess production of androgens may induce hyperinsulinism in women with PCO (Shoupe et al 1983, Pasquali et al 1983). Alternatively, increased concentrations of insulin and androgen in women with PCO may reflect another, as yet uncharacterised, pathophysiological process.

GLUCAGON

Pancreatic glucagon is involved in the development and maintainance of abnormalities of carbohydrate metabolism (Unger 1978) including insulin resistance (DelPrato et al 1987). Insulin resistance and hyperglucagonaemia coexist in women with non-insulin dependent diabetes mellitus (Golay et al 1986, Reaven et al 1987), gonadal dysgenesis (Costin and Kogut, 1985) and a glucagon-secreting tumour (Boden et al 1978). Hyperglucagonaemia and insulin resistance also result from the administration of androgenic drugs to women (Williams et al 1985,1986).

CHAPTER 1: RELATIONSHIPS BETWEEN INSULIN AND OVARIAN FUNCTION

Ovarian steroid secretion and folliculogenesis are influenced by circulating concentrations of insulin. Epidemiological data from heterogeneous groups of hypo-insulinaemic women with untreated or inadequately treated diabetes mellitus have shown the age at menarche to be 1-2 years later than that of control subjects, that the age of the menopause appears to be earlier (Bergqvist 1954) and that amenorrhoea and oligomenorrhoea are more common (Joslin et al 1925). Menstrual abnormalities are often corrected by administration of insulin (Djursing et al 1982). Furthermore, in diabetic women administration of GnRH results in attenuated gonadotrophin responses (Distiller et al 1982), suggesting that pituitary secretion of gonadotrophins is impaired. Alloxan-treated rats which develop insulin-dependent diabetes have a lower mean ovarian weight than do control animals, and the ovarian weight is corrected by FSH only when administered together with insulin (Liu et al 1982).

The pattern of sex steroid hormone levels in women with diabetes is contentious. Anovulatory women with diabetes have lower serum concentrations of testosterone

and oestradiol in addition to gonadotrophins, compared with anovulatory non-diabetic women (Djursing et al 1982). However, the same authors have also reported increased serum concentrations of testosterone and androstenedione in insulin-treated diabetic women before the menopause (Djursing et al 1985) but increased levels of oestrogens and sex hormone-binding globulin (SHBG) after the menopause (Nyholm et al 1989), when compared with those in age and weight-matched controls. Interpretation of hormone values in diabetic women is complicated by the heterogeneous nature of the condition, particularly with respect to the sensitivity to insulin and insulin-like growth factor 1 (IGF-1) (Poretsky and Kalin 1987). It is apparent, however, that hyperglycaemia or other metabolic defects associated with hypo-insulinaemia may influence the hypothalamo-pituitary-ovarian axis independently of the effects of insulin deficiency.

INSULIN RESISTANCE AND OVARIAN FUNCTION

Insulin resistance is an attenuation of the effect of insulin upon its target tissue, which in the presence of a functioning pancreas results in hyperinsulinaemia (Olefsky 1981, Reaven 1988).

Obesity is the commonest clinical cause of hyperinsulinaemia and insulin resistance (Kissebah et al 1982, Peiris et al 1986, Olefsky and Kolterman 1981, Clark et al 1983, Dohm et al 1988). In obese subjects, the amplitude of each secretory pulse of insulin is increased (Polonsky et al 1988 a,b), there is a more rapid pancreatic response to glucose (Doeden and Rizza 1987) and hepatic clearance of insulin may be reduced (Polonsky et al 1988b). Obese subjects also have a reduced hypoglycaemic response to exogenous insulin (Clark et al 1983). Obesity is associated with loss of insulin receptors and with impairment of post-receptor events (Clark et al 1983, Felber et al 1987), particularly a reduction in insulin-mediated glucose uptake (Prager et al 1987). Hyperinsulinaemia, resulting from increased pancreatic secretion of insulin, does not fully compensate for insulin resistance in obese subjects, and thus hyperglycaemia results (Prager et al 1987). In addition, hyperglycaemia and hyperinsulinaemia may induce a degree of insulin resistance by receptor or post-receptor actions or desensitisation (Yki-Jarvinen et al 1987, Ratzmann et al 1983).

Obesity is often associated with amenorrhoea (Rogers and Mitchell 1952), although not all obese women have menstrual disturbances (Zhang et al 1984). Serum

concentrations of androgens are elevated in obese oligomenorrhoeic women compared with those in obese eumenorrhoeic women or in women of normal weight and matched for age (Friedman and Kim 1985). However, secretion and clearance rates of androgens are also increased in obese women (Samojlik et al 1984, Kurtz et al 1987). Although serum concentrations of free testosterone (free T) are elevated (Evans et al 1983, Wild et al 1983), those of androstenedione are reduced (Zhang et al 1984, Grenman et al 1986, Dunaif et al 1988) because of increased clearance by aromatisation to oestrone (Edman and MacDonald 1978, Kurtz et al 1987). In obese eumenorrhoeic women, concentrations of free T correlate with those of insulin (Dunaif et al 1987) and reduced hepatic clearance of insulin and reduced peripheral insulin sensitivity are mediated by increased concentrations of free T (Peiris et al 1987).

Between 35 and 60% of women with PCO are obese (Goldzieher and Green 1962, Franks 1989, Conway et al 1989) and women with PCO are commonly obese during adolescence (Yen 1980). However, obesity itself does not maintain the polycystic ovarian disorder nor does obesity affect gonadotrophin release (Dunaif et al 1988), but weight reduction in obese women with PCO is associated with a decline in concentrations of testosterone and

androstenedione and a resumption of ovulatory menstrual cycles (Bates and Whitworth 1982)

GLUCOSE TOLERANCE AND HYPERINSULINAEMIA IN WOMEN WITH PCO

Women with PCO have a similar fasting concentration of fasting glucose to that in age- and weight-matched normal women (Burghen et al 1980, Jailal et al 1987, Dunaif et al 1987). Obese women with PCO, however, have significantly greater plasma concentrations of glucose following administration of a glucose load (Burghen et al 1980, Dunaif et al 1987) and exhibit a 20% incidence of impaired glucose tolerance or diabetes (Dunaif et al 1987). Non-obese women with PCO have normal glucose tolerance (Chang et al 1983b, Bruno et al 1985, Dunaif et al 1987). Epidemiological studies of men and women have suggested that glucose intolerance is predictive of the later onset of diabetes mellitus (Medalie et al 1975), but data about long term glycaemic control in women with PCO are lacking.

Women with polycystic ovary syndrome (PCO) and elevated concentrations of circulating androgens are hyperinsulinaemic and exhibit insulin resistance when compared with age- and weight-matched control women (Burghen et al 1980, Chang et al 1983b, Shoupe et al 1983,

Pasquali et al 1983, Bruno et al 1985, Jailal et al 1987, Dunaif et al 1987, Dunaif et al 1989, Dunaif and Graf 1989, Peiris et al 1989a). PCO and obesity have synergistic deleterious effects upon glucose tolerance (Dunaif et al 1987, 1989). Both receptor and post-receptor defects in insulin action have been described (Shoupe et al 1983, Jailal et al 1987) but neither are related to the development of or presence of antibodies to insulin or its insulin receptor (Burghen et al 1980). Sathanandan et al (1987), however, were unable to demonstrate decreased sensitivity to insulin in women with PCO other than that due to obesity.

HYPERANDROGENISM AND GLUCOSE HOMEOSTASIS

An association between hyperinsulinism, insulin resistance and hyperandrogenism is well established. In women with PCO, fasting concentrations of insulin and insulin responses to an oral glucose load have been shown to correlate with serum concentrations of testosterone and androstenedione in peripheral (Burghen et al 1980, Chang et al 1983b, Shoupe et al 1983, Dunaif et al 1987) and ovarian venous blood (Nagamani et al 1986), although such relationships have not been consistently demonstrated (Alper and Garner 1987, Geffner et al 1986). Serum concentrations of insulin show an inverse correlation

with those of dehydroepiandrosterone sulphate (DHEAS) in women with PCO (Schriock et al 1988).

The role of androgens in inducing hyperinsulinaemia and insulin resistance or, conversely, the role of insulin in the causation of hyperandrogenaemia, have been widely investigated. Sex differences in insulin action have been attributed to higher concentrations of androgens in men (Yki-Jarvinen 1984, Hale et al 1985), although there is no difference in the rate of decline in glucose concentrations, nor concentrations of insulin or glucagon, during prolonged fasting in men and women (Clore et al 1989), and neither administration of DHEAS to normal men (Nestler et al 1988) nor suppression of ovarian secretion by analogues of GnRH, or surgical removal of hyperthecotic ovaries in hyperandrogenic women (Geffner et al 1986, Annos and Taymor 1981, Nagamani et al 1986, Leedman et al 1989) alters the insulin status.

Taylor et al (1982) and Barbieri et al (1988) proposed that insulin augments or stimulates the ovarian secretion of androgens. Addition of insulin in vitro to culture media of ovarian stromal cells obtained from hyperandrogenic women results in the increased secretion of testosterone and androstenedione (Barbieri et al 1986) whereas Smith et al (1987) showed that the insulin

response in vivo to an oral glucose load correlated with the incremental increase in concentrations of testosterone, androstenedione and dihydrotestosterone in those women with the greatest degree of insulin resistance, but not in control women nor in those with mild insulin resistance. During hyperinsulinaemic, euglycaemic clamp studies, however, concentrations of androstenedione, but not of testosterone, increased in women with PCO (Stuart et al 1987, Nestler et al 1987, Dunaif and Graf 1989), indicating that concentrations of testosterone do not rise acutely in response to insulin administration in vivo.

LH SECRETION

Ovarian production of androgens is dependent upon LH stimulation (Kirschner and Jacobs 1971, Givens et al 1974, Calogero et al 1987). In vitro, LH and insulin act independently to increase androgen output (Poretsky and Kalin 1987), whilst insulin potentiates LH-induced androgen secretion (Cara and Rosenfield 1988). Mean concentrations of LH in women with PCO are usually increased (Yen 1980, McKenna 1988, Franks 1989) as a result of greater amplitude and frequency (Waldstreicher et al 1988) or amplitude alone (Dunaif et al 1988) of LH secretory pulses compared with normal women. Lower

concentrations of LH have been reported in obese compared with non-obese women with PCO (Paradisi et al 1986) and it has been suggested that insulin may amplify LH-dependent secretion of androgens in these women (Pasquali et al 1987, Dunaif and Graf 1989). However, the concentrations of androgens in these two groups were also similar (Dunaif et al 1988) despite different concentrations of insulin.

ADRENAL ANDROGENS

Dehydroepiandrosterone (DHEA) is the most abundantly secreted adrenal steroid. It has a rapid turn-over rate and is converted to DHEA sulphate (DHEAS) by sulphatases in all tissues. DHEA is a potent non-competitive inhibitor of glucose-6-phosphate dehydrogenase; a lack of DHEA may facilitate more rapid synthesis of dihydronicotinamide adenine dinucleotide phosphate (NADPH) and therefore encourage NADPH-dependent lipogenesis.

DHEA and DHEAS, used therapeutically, have beneficial effects upon diabetes and obesity in experimental models. DHEA reduces hyperinsulinaemia in genetically obese, diabetic mice by inhibiting pancreatic secretion of insulin and increasing tissue insulin sensitivity (Coleman et al 1982). It also reduces body fat and low density lipoprotein lipid values in normal men (Nestler et al

1988) and is believed to slow the development of atherosclerosis (Gordon et al 1988), the serum concentrations of DHEAS being inversely correlated to mortality from cardiovascular disease in men over 50 years (Barrett-Connor et al 1986).

Adrenal secretion of DHEA and responsiveness to ACTH are increased in some women with PCO (Lachelin et al 1979, Alper and Garner 1987, Devesa et al 1987, McKenna 1988).

Fasting concentrations of insulin and DHEAS are inversely correlated independently of BMI in hyperandrogenic hyperinsulinaemic women (Smith et al 1987, Schriock et al 1988) and those of DHEAS decrease during prolonged experimental hyperinsulinaemia (Nestler et al 1987), although no relationship between DHEAS and insulin was demonstrated in hyperandrogenic women by Alper and Garner (1987). DHEAS may enhance insulin binding to red blood cells in contrast to the inhibitory effects of testosterone (Schriock et al 1988).

SEX HORMONE BINDING GLOBULIN (SHBG)

The concentration of free testosterone in serum is determined by both the total concentration of testosterone and of SHBG.

Serum concentrations of SHBG are predominantly regulated by sex steroids, thyroid hormones and IGF-1 (Anderson 1974, Suikkari et al 1988, Plymate et al 1988). They are also influenced by dietary lipids (Reed et al 1988). SHBG levels are reduced in obesity (Plymate et al 1981, Wild et al 1983), and insulin has been postulated to inhibit the synthesis of SHBG (Plymate et al 1988). The relationship between concentrations of insulin and SHBG is independent of obesity and the androgen levels (Peiris et al 1989b).

Oestrogen and testosterone stimulate (Lee et al 1987) whilst both insulin and IGF-1 inhibit synthesis of SHBG from cultured human hepatoma cells (Plymate et al 1988, Hamilton-Fairley et al 1989).

In non-obese women with PCO, concentrations of SHBG are inversely correlated to those of insulin (Shoupe and Lobo 1984, Dunaif et al 1987). Consumption of a low calorie diet results in an increase in SHBG values and a reduction in concentrations of insulin and IGF-1 (Kiddy et al 1989) while reduction in insulin concentrations by administration of diazoxide is accompanied by a rise in those of SHBG (Nestler et al 1989).

GLUCAGON AND GLUCOSE HOMEOSTASIS

Several counterregulatory hormones, including adrenaline, cortisol and growth hormone, impair insulin-mediated metabolism of glucose and have been implicated in deterioration of glucose tolerance. In contrast, relatively few data concerning the effects of hyperglucagonaemia on insulin action, particularly upon peripheral tissues, have been reported.

Glucagon may contribute to abnormalities of insulin action (Unger 1978). Hyperglucagonaemia is associated with a modest impairment of glucose disposal in insulin-sensitive peripheral tissues in normal subjects (Rizza et al 1979, Bajorunas et al 1986, DelPrato et al 1987), and induces resistance to the suppressive effects of insulin on basal hepatic glucose output by the liver, primarily by an inhibition of insulin-mediated glycogen synthesis (DelPrato et al 1987). In contrast, in subjects with diabetes and hypoglucagonaemia secondary to pancreatectomy or pancreatic disease, tissue sensitivity to insulin is increased (Nosadini et al 1982).

Insulin is a potent inhibitor and regulator of glucagon secretion (Maruyama et al 1983). During experimental hyperinsulinaemia, pancreatic release of

glucagon is suppressed less than in that of insulin, possibly as a result of impaired intra-islet regulation (Wu and Ho 1987).

Insulin resistance is associated with hyperglucagonaemia in subjects with gonadal dysgenesis (Costin and Kogut 1985), obesity (Borghi et al 1984), a glucagon-secreting pancreatic tumour (Boden et al 1978) or non-insulin dependent diabetes mellitus (Reaven et al 1987). Administration of danazol or oxymetholone (Williams et al 1985, 1986) also results in hyperinsulinaemia and insulin resistance, and these effects have been attributed to hyperglucagonaemic actions of these drugs (Williams et al 1985, 1986).

Peripheral concentrations of glucagon are a reliable indicator of glucagon secretion by the pancreas under euglycaemic conditions (Herold and Jaspan 1986), although the liver is an important organ for glucagon extraction from the portal vein (Jaspan et al 1984). The portal-to-peripheral ratio for the biologically active 3,500 Dalton fraction of plasma glucagon is 2.7:1 in the basal state (Jaspan et al 1984). There is marked intra-subject variability in basal plasma concentrations of glucagon (Jaspan et al 1981).

Glucagon concentrations in androgenised women with PCO have not previously been studied.

ENDORPHIN

Beta-endorphin is an important regulator of the release of GnRH and thus of gonadotrophins, modulating androgenic inhibition of GnRH release in normal women (Vermesh et al 1987, Cumming et al 1984, Jewelewicz 1984). It has been suggested that women with PCO may have an aberration of opioidergic control of GnRH secretion (Cumming et al 1984), serum concentrations of beta-endorphin are elevated in obese hirsute women with PCO (Givens et al 1980, Aleem and MacIntosh 1984) and a positive correlation was demonstrated with body weight, although not in normal women (Aleem and McIntosh 1984). In addition, concentrations of beta-endorphin in ovarian follicular fluid are greater than those in plasma, particularly in fluid obtained from women with polycystic ovaries (Aleem et al 1987).

Beta-endorphin has been isolated from pancreatic islets (Bruni et al 1979) where it stimulates the release of insulin and glucagon (Reid and Yen 1981, Reid et al 1984). Intravenous infusions of beta-endorphin induces a

marked rise in peripheral concentrations of insulin and glucagon in obese subjects, whereas non-obese subjects responded with an increase in concentrations of glucagon only (Giugliano et al 1987a,b, Paolisso et al 1987).

PROLACTIN

Hyperprolactinaemia, which induces mild glucose intolerance and hyperinsulinaemia also decreases glucose transport in cultured adipocytes from pregnant (ie hyperprolactinaemic) rats without affecting insulin receptor binding (Ryan and Enns 1988) and has, therefore, been implicated in the development of insulin resistance associated with pregnancy (Ryan et al 1985).

Concentrations of prolactin are increased in 7-15% in women with PCO (Franks 1989). Prolactin may thus contribute to impaired glucose tolerance and hyperinsulinaemia in some women with PCO.

INSULIN-LIKE GROWTH FACTOR 1 (IGF-1)

IGF-1 has a structural homology to insulin and stimulates target cells by binding to insulin or IGF-1 receptors. IGF-1 activity is modulated by IGF-binding protein (IGF-BP, placental protein 12), circulating levels

of which are insulin dependent (Suikkari et al 1988) and which are reduced in women with PCO (Pekonen et al 1989).

High-affinity IGF-1 receptors have been demonstrated in human ovaries (Bayer et al 1986). In vitro studies have shown that IGF-1 stimulates accumulation of testosterone and androstenedione from the ovarian stroma of a woman with hyperandrogenism as efficiently as does insulin, whereas relaxin and IGF-2 had no effect (Barbieri et al 1986), and IGF-1 augments LH-induced secretion of androgens from the ovary (Cara and Rosenfield 1988). Barbieri et al (1988) and Dunaif et al (1989) have proposed that insulin may stimulate ovarian production of androgens in hyperandrogenic women by cross-reacting with IGF-1 receptors. In rats, however, sustained hyperinsulinaemia did not increase androgen concentrations, although IGF-1 binding sites increased in number suggesting that insulin may influence ovarian IGF-1 receptors (Poretsky et al 1988).

SUMMARY

A close relationship between ovarian function and insulin has been demonstrated. In some subjects with increased concentrations of androgens and insulin resistance, hyperglucagonaemia also coexists, and has

been proposed to be responsible for abnormalities of insulin action. Concentrations of beta-endorphin in obese women with PCO are increased, suggesting a possible effect upon the pancreas to increase glucagon secretion.

The study was initiated to investigate the role of glucagon in insulin resistant hyperandrogenic states, and to establish whether alteration of androgen values influenced those of insulin and glucagon. Chapter 2 describes the methodology used and subjects recruited for the study, whilst Chapters 3 and 4 describe the basal concentrations and glucose-stimulated responses of glucose, insulin, C-peptide and glucagon in relation to those of gonadotrophins and sex steroids. In Chapter 5, the changes in concentrations of gonadotrophin and sex steroids in response to administration of antiandrogens, androgens and antigonadotrophins are described, whilst the effects of these changes upon basal and stimulated glucose, insulin, C-peptide and glucagon values are examined in Chapter 6. The hypothesis is discussed and conclusions are drawn in Chapter 7.

CHAPTER 2: MATERIALS AND METHODS

SUBJECTS WITH PCO:

Forty-four women aged 20-33 years and with a diagnosis of PCO (the "PCO group", subjects 1-44; Table 2.1) based upon the presence of four out of five of the following criteria, were recruited for study:

a) Amenorrhoea or oligomenorrhoea, with the history of menstrual disturbance usually dating from the menarche.

b) Ultrasonographic features of PCO as defined by the criteria of Adams et al (1986). In 8 subjects, where the ultrasonographic appearances were not wholly typical (Orsini et al 1985) inspection of the ovary during operative laparoscopy confirmed the presence of enlarged sclerocystic ovaries, although biopsy and histological examination were not performed.

c) Clinical evidence of hyperandrogenism indicated by the presence of hirsutism or acne. Hirsutism was defined as hair more profuse, coarser, darker or longer than normal, affecting the sex hormone dependent areas of face,

TABLE 2.1: AGE, FERRIMAN-GALLWEY (FG) SCORE, OVARIAN VOLUME, 17HYDROXY PROGESTERONE (17OH PROGESTERONE) CONCENTRATIONS BEFORE AND AFTER ACTH STIMULATION, AND BODY MASS INDEX (BMI) IN WOMEN WITH PCO. (nr, not recorded; nd, not done)

NUMBER	AGE (years)	FG SCORE	MEAN OVARIAN VOLUME (ml)	17OH PROGESTERONE PRE (nmol/l)	17OH PROGESTERONE POST (nmol/l)	BMI (kg/m^2)
1	21	17	nr	nd	nd	31.7
2	27	7	nr	nd	nd	31.7
3	20	10	23.5	6.0	12.0	25.7
4	25	14	20.8	nd	nd	23.2
5	27	1	14.5	nd	nd	26.8
6	24	28	nr	nd	nd	23.1
7	25	15	13.0	7.0	9.0	28.1
8	25	9	nr	1.5	6.0	26.7
9	26	3	nr	nd	nd	20.7
10	21	4	10.0	2.0	8.0	23.2
11	27	6	11.5	4.0	12.0	35.4
12	24	16	15.3	4.4	7.0	19.3
13	27	7	18.4	3.5	9.0	30.6
14	25	10	nr	nd	nd	34.0
15	32	14	13.6	2.1	8.0	24.6
16	28	8	7.8	9.0	17.0	20.5
17	29	12	13.8	1.4	3.0	29.1
18	30	8	20.7	3.4	7.5	31.1
19	24	5	nr	3.0	5.0	41.5
20	23	10	7.4	1.8	5.0	24.7
21	18	18	15.5	4.0	8.0	32.8
22	34	6	nr	nd	nd	19.5
23	25	12	14.9	2.5	6.0	23.9
24	33	14	nr	10.0	18.0	34.2

TABLE 2.1: Continued:

NUMBER	AGE	FG SCORE	MEAN OVARIAN VOLUME	17OH PROGESTERONE		BMI
				PRE	POST	
	(years)		(ml)	(nmol/l)		(kg/m^2)
25	27	17	16.1	3.0	4.5	24.1
26	24	19	11.1	2.5	7.0	26.1
27	24	16	16.9	3.2	12.0	21.5
28	32	13	20.5	0.4	5.0	33.0
29	27	17	nr	nd	nd	34.8
30	34	21	nr	4.5	14.0	29.9
31	22	10	11.8	4.5	17.0	24.4
32	20	9	10.0	3.0	4.0	33.5
33	22	11	8.4	5.0	12.0	22.9
34	29	11	nr	nd	nd	33.4
35	33	29	14.1	2.5	11.0	37.8
36	29	8	18.5	3.5	8.0	38.8
37	18	32	19.0	6.1	10.0	19.6
38	21	5	29.2	6.0	11.0	19.1
39	24	19	13.2	10.6	11.1	24.5
40	22	20	nr	2.7	7.5	22.1
41	29	5	14.0	1.5	3.4	22.4
42	26	16	14.2	2.9	5.6	28.8
43	26	5	17.4	8.0	14.0	24.9
44	28	10	nr	nd	nd	43.9
n=	44	44	30	33	33	44
Median	26	11	14.9	3.5	8.0	26.4
Range	(18-34)	(3-32)	(7.4-29.2)	(0.4-10.6)	(3.0-18.0)	(19.1-43.9)

proximal limbs, abdomen, chest or back, and quantified by the criteria of Ferriman and Gallwey (1961). Each area was assessed scored up to 4 points to provide a maximum score of 36. Acne was defined as the presence of a typical pustular rash affecting face or upper thorax.

d) Elevation of the serum concentration of luteinising hormone (LH) on two occasions one week apart taken without regard to the timing of the menses, with a normal concentration of follicle stimulating hormone (FSH).

e) Elevation of one or more of serum concentrations of testosterone (T), androstenedione (A) or dehydroepiandrosterone sulphate (DHEAS).

All women underwent clinical, gynaecological and biochemical assessment and women with ovarian or adrenal tumours or Cushing's syndrome were excluded. Two women had acanthosis nigricans of the neck and axillae (Matsuoka et al 1987) and serum prolactin was mildly elevated in two women (Subjects Nos. 8 and 21) at 912 and 751 IU/L respectively (normal range 125-600 IU/L).

Thirty three women underwent a one hour adrenocorticotrophic hormone (ACTH) stimulation test (New

et al 1983) with the concentration of 17-hydroxyprogesterone (17OHPo) measured immediately before and 60 minutes after intramuscular injection of 250 micrograms Synacthen (Ciba Laboratories, Horsham, Sussex). The first sample was obtained between 0800 and 0900 h., without regard to the timing of the menses. In four of the 11 women who did not undergo the Synacthen test, congenital adrenal hyperplasia due to 21-hydroxylase deficiency had been excluded previously by a similar Synacthen stimulation test.

These clinical and biochemical details are shown in Table 2.1. All subjects were euthyroid and none was known to have any abnormality of glucose tolerance or blood pressure. No medication known to affect glucose tolerance or sex steroid levels had been taken in the three months preceding the study.

CONTROL SUBJECTS

Sixteen women with laparoscopically diagnosed mild endometriosis, ie, an American Fertility Society score less than 15 (American Fertility Society, 1985) and presenting with infertility and/or pelvic pain, and 7 women with uterine leiomyomata awaiting hysterectomy

served as control subjects (the "control group", subjects 45-67; Table 2.2). All had a regular menstrual cycle of length 23-36 days and elevated concentrations of progesterone greater than 25 nmol/l, consistent with ovulation, during the luteal phase. Ultrasonography in the follicular phase of the menstrual cycle or laparoscopy confirmed normal ovarian morphology. None of these subjects showed clinical evidence of androgen excess nor acanthosis nigricans. All subjects had normal serum concentrations of testosterone, androstenedione, prolactin and thyroxine. None of the control subjects was known to have any abnormality of glucose tolerance or blood pressure and no medication known to affect glucose tolerance or sex steroid levels had been taken in the three months preceding the study.

Subjects were weighed in indoor clothing after an overnight fast and those with a body mass index (BMI, body weight in kg/height in m^2) greater than 25.0 kg/m^2 were defined as obese. This index of obesity was used because it correlates well with the percentage of body fat and is the most satisfactory simple index of obesity based upon body weight and height (Reid and Van Vugt 1987). Where indicated, obese subjects were subdivided into those "overweight" with a BMI between 25.0-29.9 kg/m^2 and those more obese with a BMI greater than 30.0 kg/m^2.

TABLE 2.2: AGE, FERRIMAN-GALLWEY (FG) SCORE AND BODY MASS INDEX (BMI) IN CONTROL WOMEN.

NUMBER	AGE (years)	FG SCORE	BMI (kg/m^2)
45	23	2	22.8
46	22	2	24.8
47	28	1	21.6
48	30	0	21.4
49	33	5	28.4
50	23	2	18.9
51	28	0	36.9
52	28	5	18.5
53	26	0	19.6
54	36	4	23.8
55	34	0	32.9
56	44	3	28.9
57	26	0	28.0
58	33	1	24.0
59	21	0	31.0
60	34	3	21.5
61	32	4	18.6
62	39	2	19.2
63	37	1	28.0
64	26	1	28.0
65	27	2	18.2
66	45	4	31.8
67	33	0	28.1
n=23	23	23	23
Median	30	2	24.0
Range	(21-45)	(0-5)	(18.2-36.9)

ETHICAL APPROVAL

The study was approved by the hospital ethical committee and all subjects gave written, informed consent to participation.

CLINICAL DETAILS.

The mean age in each of the control groups was greater than that of the women with PCO. Twenty-four of the 44 women with PCO (58%) were obese (BMI >30 kg/m2 in 16 women) and 10 of the 23 control subjects (43%) were obese (BMI >30 kg/m^2 in 4 women). The BMI in the corresponding obese study and control groups were equally matched, as were those for the non-obese groups.

In all the women with PCO, the basal concentration of 17OHPo was within the normal range (<10 nmol/L) and in none was the maximum concentration of 17OHPo in response to ACTH stimulation in excess of 20 nmol/L (Table 2.1).

ULTRASONOGRAPHY

All ultrasound examinations were performed by the author using the full bladder technique with a Diasonics

DS1-RF sector scanner with a 3.5MHz general purpose probe (Adams et al 1986).

The number of follicles seen in the greatest longitudinal plane and the maximum follicular diameter were recorded. The stromal density was assessed subjectively on a scale 0,+ or ++. The diameters of each ovary were measured in three planes, and the ovarian volume calculated from the formula for a prolate ellipsoid (Orsini et al 1985) (volume = D_1 x D_2 x D_3 x 0.5233 cm^3), where D_1, D_2 and D_3 are the three maximum longitudinal, antero-posterior and transverse diameters. In 7 subjects, only one ovary, and in 2, neither ovary, were seen.

The mean (SD) ovarian volume in subjects with PCO (n=30) was 15.7 (4.7) mm^3 (range, 7.4-29.2 mm^3) (Table 2.1). Measurements of ovarian volume were not made in 12 women with PCO and no measurements were possible in 2 further women. The mean ovarian volume in non-obese, normal women during the early follicular phase of the menstrual cycle, using the same method of measurement has been found to be 6.7 (2.3) mm^3 (n=22) (G. Shaw, P.J. Macrow, personal communication), similar to that of Conway et al (1989).

PROTOCOL

All women consumed a weight maintaining diet containing approximately 300g carbohydrate for 3 days before the study.

An oral glucose tolerance test (oGTT) was performed by the author using a 75g glucose load, commencing between 08.30 and 09.30 h after an overnight fast of 12h. The glucose was consumed over 10 minutes as a flavoured drink in 300ml water. The test was undertaken in the early to mid-follicular phase of the cycle (days 1-10) in control subjects, but without regard to the menses in the subjects with PCO.

Venous blood samples were obtained through an indwelling catheter sited in an antecubital vein. Samples of 20 ml. were obtained 30 and 15 mins and immediately prior to the ingestion of the glucose load and further samples were obtained 30, 60, 90, 120 and 150 min thereafter.

Patency of the venous catheter was maintained by flushing with 0.5ml N.saline containing 25u/ml sodium heparin after each blood sampling. On completion of the oGTT, the catheter was withdrawn.

Blood for measurement of glucose and HbA_1 was collected into commercial sodium fluoride tubes and assayed the same day. LH, FSH, T, A, DHEAS and SHBG concentrations were measured in pooled equal volumes of fasting serum. Serum was separated within one hour and stored at -20C until assayed. The free testosterone index (free T) was calculated from the equation:
free T = (total T/SHBG) x 100 (Pollock and Ratcliffe 1987).

Serum for measurement of insulin and C-peptide concentrations was separated within 1h and stored at -20C until assayed. Blood for measurement of pancreatic glucagon concentrations was drawn into ice-cold lithium heparin tubes containing 0.04 ml trasylol, 400 KIU/l (Bayer, Newbury, Berks). The plasma was separated by centrifugation, frozen within 7 minutes, and stored at -20C until assayed. Insulin, C-peptide and glucagon concentrations were measured in all samples taken from time 0 to the completion of the oGTT, except in 10 women in whom glucagon was measured at times 0 and 120 min only.

TREATMENT PROTOCOLS

After completion of the oGTT, women in the PCO group were allocated non-randomly to one of three treatment

groups, in accordance with their therapeutic requirements as described in Chapter 5. Women in the control groups were allocated by computerised randomisation to one of two treatment groups (Chapter 5). The treatment regimes were designed to alter serum gonadotrophins and/or sex steroids in order to determine the effects of these upon insulin, glucose and glucagon homeostasis. An oGTT was performed in an identical manner to that described above.

ASSAY METHODS

Plasma glucose was measured by the glucose oxidase method described by Brunsman (1973). HbA_1 was measured by a cation exchange analysis at 415nm (Parker et al 1981).

Insulin was measured by the radioimmunoassay (RIA) method of Hales and Randall (1963), with an intra-assay coefficient of variation (CV) of 11%. Results are expressed in mU/l of the first World Health Organisation human insulin standard.

C-peptide was measured by a commercial RIA kit (Medgenix, Fleurs, Belgium). The intra-assay CV was 15% at 0.35pmol/l and 7% at 1.36pmol/l.

Glucagon was measured in unextracted plasma using a recently available commercial RIA kit (Serono Laboratories, Welwyn Garden City). The inter- and intra-assay CV at 125pmol/l were 11% and 7.4% respectively.

Serum testosterone was measured by solid phase double antibody RIA after chromatographic extraction and ether extraction (Intra-assay CV 5%) (Dowsett 1985, Vaughan Williams et al 1989).

Concentrations of SHBG were measured by the immunoradiometric assay method (IRMA) of Hammond et al (1985).

Concentrations of DHEAS were measured in unextracted serum by RIA using an assay kit obtained from Amersham (Amersham, UK). The inter-assay CV was between 8-12%.

Androstenedione was measured in extracted serum by RIA using ^{125}I-labelled androstenedione 3cm-oxime/histamine produced in Hope Hospital, Salford, and sheep anti-A(6-conjugate) obtained from Guildhay AntiSera. The inter-assay CV was between 9-13%.

LH and FSH were measured by RIA using ^{125}I-labelled LH and FSH respectively obtained from Chelsea Hospital for

Women, London, and rabbit anti-LH (F87/2) and anti-FSH (M93/2) antiserum obtained from Professor Butt, Birmingham. The inter-assay CV was 4-12% for LH and 7-15% for FSH.

Samples from each subject were measured in the same batch to eliminate interassay variation.

STATISTICAL METHODS

Results are presented as the mean (SD) concentration, although graphs are given with mean (SE) concentrations for clarity, as results for values of glucagon exhibited a large variation in SD.

Cumulative totals of glucose, insulin, C-peptide and glucagon concentrations after oral glucose administration are the sum of all values from 0 to 150 mins.

Changes in glucose, insulin and glucagon concentrations over the test period were evaluated using an analysis of variance (ANOVA) with repeated measures on one factor (Winer 1981), the factor being study group. Differences in the HbA_1 level between the study groups were examined using a single factor ANOVA. The

relationship between HbA_1 and glucose levels (fasting and summed) was assessed for each study group separately using Pearson's correlation coefficient.

Comparison of group data for insulin, C-peptide and glucagon were analysed by Van der Waerden 1-way non-parametric tests. Pearsons correlation coefficient was used to compare these values with T, A, DHEAS and SHBG for any association. ANOVA (F-test) was used to examine differences between study groups and their interaction.

Changes in T, A, DHEAS, SHBG and free T over the test period were evaluated by Van der Waerden non-parametric tests.

Data were anaylsed using the PC/SAS version 6.03 statistical package.

Significance was assumed at the conventional <5% level.

DISCUSSION

In the investigation of hyperinsulinaemia and insulin resistance associated with PCO, a number of experimental

methods have been utilised, including measurement of insulin concentrations when fasting and in response to glucose administered orally (Burghen et al 1980, Chang et al 1983b, Bruno et al 1985, Jailal et al 1987, Dunaif et al 1987, Smith et al 1987) and intravenously (Billiar et al 1986), the insulin/glucose ratio (Pasquali et al 1983), C-peptide values in relation to those of insulin (Pasquali et al 1982, Bruno et al 1985) and hyperinsulinaemic euglycaemic clamp studies (DeFronzo et al 1979, Stuart et al 1987, Peiris et al 1989a, Dunaif et al 1989). The latter is required for measurement of hepatic and peripheral insulin sensitivity, whilst the assessment of insulin secretion rates and hepatic extraction by measurement of peripheral concentrations of C-peptide is subject to a number of assumptions, the validity of which has not been established (Polonsky and Rubenstein 1988).

In studies of the relationships between insulin, glucagon and androgens, the methodology used for the measurement of peripheral glucagon levels is of considerable importance.

Measurement of glucagon in peripheral plasma is complex. Glucagon circulates in low concentrations and earlier immunoassays were poorly specific with glucagon exhibiting molecular heterogeneity (Jaspan et al 1981,

Valverde et al 1974). The portal-to-peripheral ratio for immunoreactive glucagon is 1.58, whilst that for the biologically active 3500 molecular weight fraction is 2.77 (Jaspan et al 1984), although under conditions of increased glucagon stimulation, greater hepatic extraction of the 3500 MW fraction is seen. The new assay used in this study is specific for the 3500 MW fraction and exhibits minimal cross-rectivity for entero-glucagon and other glucagon-like activity detected by earlier assays (I. Laing, personal communication).

In 10 subjects, measurements of glucagon were taken at times 0 and 120 minutes only. Thereafter, glucagon assays were performed in blood samples taken at each time interval. This change was introduced to enable a better understanding of changes of glucagon values with time after oral glucose loading, and to allow appropriate comparison with insulin to be made. Excluding the glucagon data from the first 10 subjects did not significantly affect the mean values of glucagon at times 0 and 120 minutes, and they have therefore been included in the full analysis.

The study was designed to investige the insulin and glucagon responses to changes in the androgenic environment induced by various drug therapies, and thus

the marked inter-subject variability in concentrations of glucagon did not influence the interpretation of data. As no attempt to infer absolute sensitivity to insulin or glucagon in liver or peripheral tissues was necessary, the euglycaemic technique was not utilised for the investigation.

It was not possible to measure C-peptide values in the whole study population. To avoid analytical errors in applying complex correlation coefficients to small numbers, C-peptide data were used only to confirm trends in insulin values between groups.

BODY MASS INDEX (BMI)

Obesity itself influences concentrations of insulin, androgens and glucagon in peripheral plasma (Borghi et al 1984), and both PCO and control groups were therefore divided into obese (BMI >25kg/m^2) and non-obese subgroups. However, those with a BMI between 25 and 30kg/m^2 are "overweight" whilst those with a BMI greater than 30kg/m^2 are truly "obese", and further limited analyses of data in Chapters 3 and 4 using these subdivisions of BMI were undertaken, although some of these subgroups comprised small numbers of subjects, increasing the possibility of analytical errors.

The BMI value at which any division is set is arbitrary. Studies reporting variation in hormone values attributable to obesity have used masssively obese women for investigation (Grenman et al 1986). In studies of women with PCO, those with a BMI >25kg/m2 (Conway et al 1989, Franks 1989), >27kg/m2 (Pasquali et al 1987), >28kg/m2 (Pasquali et al 1986, Nestler et al 1989) and >29kg/m^2 (Bruno et al 1985) have been included in the "obese" sub-groups, whilst in most North American reports, a body weight >120% of normal (equivalent to BMI >30kg/m2) has been used (Dunaif et al 1987, 1988).

It has been shown that body morphology has effects upon insulin resistance, glucose tolerance and sex hormone secretion (Evans et al 1983). Most studies have not included waist-hip measurements as an index of body morphology and insufficient numbers of subjects in the current study were assessed using these measurements.

CHAPTER 3: BASAL STUDIES IN NORMAL WOMEN AND IN WOMEN WITH PCO.

INTRODUCTION.

Women with PCO have fasting concentrations of glucose similar to those in age and weight-matched normal women, but are comparatively hyperinsulinaemic and exhibit insulin resistance (Burghen et al 1980, Chang et al 1983b, Shoupe et al 1983, Pasquali et al 1983, Bruno et al 1985, Jailal et al 1987, Dunaif et al 1987, Dunaif et al 1989, Dunaif and Graf 1989, Peiris et al 1989a). Elevated serum concentrations of insulin and insulin resistance may be associated with excessive secretion of androgens from the ovary (Barbieri et al 1986).

In women with PCO, the fasting concentration of insulin and concentrations of A and T may be related (Burghen et al 1980, Chang et al 1983b, Shoupe et al 1983, Dunaif et al 1987) although these relationships have not been demonstrated by others (Alper and Garner 1987, Geffner et al 1986). Ovarian production of androgens is dependent upon LH stimulation (Kirschner and Jacobs 1971, Givens et al 1974, Calogero et al 1987) but insulin itself may have a gonadotrophic function also (Poretsky and Kalin

1987). Mean LH concentrations in women with PCO, which are usually elevated (Yen 1980, McKenna 1988, Franks 1989), may be weight-dependent (Paradisi et al 1986) although this has been disputed (Dunaif et al 1988, Franks 1989, Conway et al 1989).

Hyperglucagonaemia is associated with insulin resistance in subjects with gonadal dysgenesis (Costin and Kogut 1985), obesity (Borghi et al 1984), glucagonoma (Boden et al 1978) and non-insulin dependent diabetes mellitus (Reaven et al 1987) but glucagon concentrations in women with PCO have not previously been investigated.

Measurement of glycosylated haemoglobin (HbA_1) has been used to monitor glycaemic control (Jovanovic and Peterson 1981) and assist in the diagnosis of diabetes mellitus (Bolli et al 1980). Elevated levels of HbA_1 in diabetes mellitus are correlated with the development of diabetic complications (Knuiman et al 1986).

This chapter describes gonadotrophin and androgen values in the women with PCO and those in age- and weight-matched control subjects, and relates these to basal concentrations of glucose, insulin, C-peptide and glucagon. The relationship between basal concentrations of glucose and those of HbA_1 was examined to assess the

value of HbA_1 estimation for monitoring glycaemic control in women with PCO.

METHODS

Details of the subjects recruited and methodology used are described in Chapter 2. Clinical details for the study groups are summarised in Table 3.1.

RESULTS

GONADOTROPHINS AND ANDROGENS

Concentrations of LH in serum in obese and non-obese women with PCO did not differ but, as expected, were greater than those in the control groups ($p<0.01$) (Table 3.2). Concentrations of FSH were similar in all groups. In all women with PCO, the ratio between the concentrations of LH and FSH was 3.0:1 compared with a ratio of 1.6:1 in control women ($p<0.001$, Chi-Squared test). The ratios were similar in obese and non-obese women with PCO, as were those in obese and non-obese control women.

TABLE 3.1: MEDIAN AND RANGE OF AGE, BODY MASS INDEX (BMI) AND FERRIMAN-GALLWEY (FG) SCORE IN THE STUDY GROUPS.

	PCO SUBJECTS		CONTROL SUBJECTS	
	NON-OBESE	OBESE	NON-OBESE	OBESE
n	20	24	13	10
MEDIAN AGE (years)	24	27	28	33
Range	(18-34)	(18-34)	(22-39)	(21-45)
FG SCORE	11	11	2	1
Range	(3-32)	(1-29)	(0-5)	(0-5)
BMI (kg/m^2)	23.1	33.0	21.4	28.9
Range	(19.1-24.9)	(25.7-43.9)	(18.2-24.8)	(26.0-36.9)

	PCO SUBJECTS		CONTROL SUBJECTS	
OBESE SUBGROUP	>25 to <30	>30	>25 to <30	>30
n	8	16	6	4
BMI (kg/m^2)	27.7	34.9	28.1	32.4
Range	(25.7-29.9)	(30.6-43.9)	(28.0-28.9)	(32.4-36.9)

TABLE 3.2: MEAN (SD) BASAL CONCENTRATIONS OF GONADOTROPHINS, ANDROGENS AND SHBG IN THE STUDY GROUPS.

	PCO SUBJECTS		CONTROL SUBJECTS	
	NON OBESE	OBESE	NON-OBESE	OBESE
n	20	24	13	10
LH (iu/l)	12.4 (6.1)#	13.2 (5.6)#	7.8 (2.7)	8.3 (4.7)
FSH (iu/l)	4.3 (1.4)	5.0 (2.1)	5.9 (4.0)	4.7 (1.2)
LH:FSH	3.1#	2.8#	1.6	1.7
T (nmol/l)	3.8 (1.4)#*	3.0 (1.1)#	2.0 (0.6)	2.3 (0.7)
A (nmol/l)	12.7 (3.5)#	13.8 (5.7)#	6.9 (2.7)	8.3 (1.7)
DHEAS (umol/l)	9.3 (3.3)+	6.4 (2.8)	4.9 (3.8)	5.3 (2.4)
SHBG (nmol/l)	36 (15)*	24 (10)	63 (22)##	41 (32)
Free T (units)	11.4 (4.7)#	14.3 (7.4)#	3.5 (1.2)	5.1 (2.2)

PCO groups > control groups, $p<0.01$ ## Non-obese control group > other groups, $p<0.01$
+ Non-obese PCO group > obese PCO group and control groups, $p<0.05$
* Non-obese PCO group > obese PCO group, $p<0.05$

As expected, concentrations of T, free T, and A were greater in women with PCO than in control women ($p<0.01$) (Table 3.2). Non-obese women with PCO exhibited higher concentrations of T, DHEAS and SHBG than did obese women with PCO ($p<0.05$), but concentrations of free T and A were similar (Table 3.2).

BLOOD GLUCOSE, SERUM INSULIN, C-PEPTIDE, GLUCAGON AND HbA_1.

Fasting concentrations of glucose were similar in all groups (Tables 3.3 and 3.4). The fasting concentration of insulin was greater in obese women with PCO than in control subjects ($p<0.05$), although only in those with BMI $>30kg/m^2$. Those in non-obese and "overweight" women with PCO were similar to the control values (Table 3.4).

The ratio between insulin and glucose values was greater in obese women with PCO than in the control subjects ($p<0.05$), whereas the ratios were similar in non-obese women with PCO and in control subjects (Table 3.3).

C-peptide data were available in 8 non-obese and 9 obese women with PCO and in 10 non-obese and 6 obese control subjects. The fasting concentrations of C-peptide

TABLE 3.3: FASTING VALUES OF BLOOD GLUCOSE, SERUM INSULIN, C-PEPTIDE, GLUCAGON AND GLYCOSYLATED HAEMOGLOBIN (HBA_1) IN WOMEN WITH PCO AND CONTROL SUBJECTS.

	PCO SUBJECTS		CONTROL SUBJECTS	
	NON-OBESE	OBESE	NON-OBESE	OBESE
n	20	24	13	10
Glucose (mmol/l)	4.1 (0.4)	4.4 (1.0)	4.2 (0.5)	4.5 (0.3)
Insulin (iu/l)	8.1 (7.2)	11.4 (7.7)#	4.6 (1.8)	6.4 (1.7)
Insulin:glucose ratio	1.9 (1.6)	2.6 (1.7)#	1.1 (0.5)	1.4 (0.4)
Glucagon (pmol/l)	68.5 (22.7)	69.3 (18.8)	61.5 (24.3)	70.4 (21.3)
n	8	9	10	6
C-peptide (nmol/l)	0.63 (0.26)	1.24 (0.56)*	0.39 (0.16)	0.53 (0.20)
n	13	14	8	9
HbA_1 %	5.2 (1.0)	5.0 (0.9)	5.5 (1.0)	5.0 (0.9)

#Obese women with PCO>controls ($p<0.05$) *Obese women with PCO>other groups ($p<0.05$)

TABLE 3.4: FASTING VALUES OF BLOOD GLUCOSE, SERUM INSULIN AND GLUCAGON IN OBESE WOMEN WITH PCO AND OBESE CONTROL SUBJECTS.

	PCO SUBJECTS		CONTROL SUBJECTS	
OBESE SUBGROUP BMI	>25 to <30	>30	>25 to <30	>30
n	8	16	6	4
Glucose (mmol/l)	4.1 (0.4)	4.6 (0.7)	4.5 (0.3)	4.5 (0.6)
Insulin (IU/l)	6.1 (2.2)	14.1 (3.0)	6.1 (1.6)	6.7 (1.2)
Glucagon (pmol/l)	69.4 (15.0)	69.3 (11.6)	72.2 (21.4)	67.8 (21.9)

were greater in obese than in non-obese women with PCO ($p<0.05$) and than those in control women ($p<0.05$, Table 3.3).

Fasting concentrations of glucagon were similar in women with PCO and control subjects and in obese and non-obese subjects (Table 3.3), and in women with a BMI between 25-29.9 kg/m^2 and in those with a value >30 kg/m^2 (Table 3.4).

Glycosylated haemoglobin was measured in 27 of the women with PCO (14 obese and 13 non-obese) and in 17 of the control group (9 obese and 8 non-obese, Table 3.3). In no case was the HbA_1 concentration above the upper limit of normal for the laboratory (7.2%) and HbA_1 concentrations did not differ between the groups. No significant correlation was found between HbA_1 and fasting glucose values ($r=-0.18$, $p=0.28$).

RELATIONSHIP BETWEEN INSULIN AND GLUCAGON AND OTHER HORMONAL VALUES.

Analyses are presented for the study subjects as a whole and for each of the four groups of women (Tables 3.5 and 3.6).

In the study population as a whole, fasting insulin values correlated with the BMI ($r=0.38$, $p<0.01$) and free T values ($r=0.40$, $p<0.005$), and inversely with values of SHBG ($r=-0.35$, $p<0.01$, Table 3.5). There were no significant relationships between concentrations of insulin and those of T, A, DHEAS or LH.

In non-obese women with PCO, the concentrations of fasting insulin were related to those of DHEAS ($r=0.55$, $p<0.01$). No relationship was shown between insulin levels and those of T, free T or A in these women. No relationship was demonstrated between the fasting concentrations of insulin and other measurements in these women.

Similarly, no relationship was demonstrated between insulin and BMI or between insulin and any hormone measurements in obese women with PCO, nor in either non-obese or obese normal women; in particular there was no relationships demonstrated between values of insulin and those of glucagon in any group, or by division of obese groups into those with BMI less than and greater than 30 kg/m^2.

Fasting values of glucagon were inversely related to total T ($r=-0.63$, $p<0.05$) and A ($r=-0.67$, $p<0.01$) only in

TABLE 3.5: RELATIONSHIP BETWEEN FASTING VALUES OF INSULIN AND THOSE OF OTHER MEASUREMENTS IN NORMAL WOMEN AND IN WOMEN WITH PCO.

	ALL SUBJECTS		PCO				CONTROL			
			NON-OBESE		OBESE		NON-OBESE		OBESE	
n	67		20		24		13		10	
	r	p	r	p	r	p	r	p	r	p
BMI	0.38	<0.01*	-0.01	0.98	0.39	0.06	0.46	0.11	0.01	0.98
T	0.09	0.44	0.22	0.35	-0.23	0.29	-0.14	0.65	-0.17	0.63
A	0.22	0.07	0.39	0.09	-0.19	0.38	0.11	0.69	0.38	0.27
DHEAS	0.18	0.15	0.55	0.01*	-0.14	0.52	0.13	0.66	-0.02	0.96
SHBG	-0.35	<0.01*	-0.12	0.60	-0.38	0.07	-0.13	0.67	-0.29	0.41
free T	0.40	<0.005*	0.32	0.18	0.18	0.41	0.06	0.85	-0.15	0.15
LH	-0.01	0.98	-0.19	0.43	-0.24	0.27	0.29	0.33	0.29	0.41
Glucagon, t=0	0.24	0.04*	0.40	0.07	0.19	0.37	0.16	0.59	-0.11	0.74

*$p<0.05$

TABLE 3.6: RELATIONSHIP BETWEEN FASTING VALUES OF GLUCAGON AND THOSE OF OTHER MEASUREMENTS IN NORMAL WOMEN AND IN WOMEN WITH PCO.

	ALL SUBJECTS		PCO				CONTROL			
			NON-OBESE		OBESE		NON-OBESE		OBESE	
n	67		20		24		13		10	
	r	p	r	p	r	p	r	p	r	p
BMI	0.13	0.27	0.32	0.16	0.01	0.99	0.14	0.63	0.13	0.72
T	0.02	0.87	-0.05	0.18	-0.30	0.17#	0.39	0.18	0.36	0.29
A	-0.06	0.61	-0.09	0.70	-0.33	0.13#	0.04	0.88	0.03	0.91
DHEAS	-0.12	0.33	0.09	0.70	-0.44	0.04*	-0.33	0.25	-0.03	0.93
SHBG	0.02	0.86	0.15	0.51	-0.36	0.09	0.18	0.55	0.42	0.22
Free T	0.11	0.39	-0.16	0.49	0.16	0.45	0.18	0.55	-0.41	0.73
LH	-0.13	0.31	-0.29	0.22	-0.05	0.22	0.02	0.93	-0.42	0.23
Insulin, t=0	0.24	0.04*	0.40	0.07	0.19	0.37	0.16	0.59	-0.11	0.74

*$p<0.05$

In the obese subgroup with BMI >30kg/m^2, with T, r=-0.62, $p<0.02$; with A, r=-0.67, $p<0.01$).
In the obese subgroup with BMI >25 to <30kg/m^2, with T, r=-0.51, p=0.19; with A, r=-0.33, p=0.4

those obese women with PCO with BMI greater than 30 kg/m^2, but were unrelated to the BMI and to serum concentrations of androgens, SHBG and gonadotrophins in all other groups (Table 3.6).

For the study population as a whole, the concentration of LH was closely related to those of A ($r=0.41$, $p<0.001$), free T ($r=0.35$, $p<0.01$), SHBG ($r=-0.36$, $p<0.005$), and weakly to total T values ($r=0.25$, $p<0.05$) (Table 3.7). However, there was no such correlation in either group of women with PCO, nor in normal women.

The relationship between concentrations of LH and fasting insulin values in women with PCO is shown in Figure 3.1. In only 4 subjects were values of both LH and insulin greater that 2SD above the mean, whilst, in the remaining subjects, values of one or both were within the normal range. The relationship between concentrations of LH and glucagon in women with PCO is shown in Figure 3.2. An inverse relationship between values of LH and BMI was demonstrated in obese women with PCO, but not in the other groups (Table 3.7).

TABLE 3.7: RELATIONSHIP BETWEEN VALUES OF LH AND THOSE OF OTHER MEASUREMENTS IN NORMAL WOMEN AND IN WOMEN WITH PCO.

	ALL SUBJECTS		PCO				CONTROL			
			NON-OBESE		OBESE		NON-OBESE		OBESE	
n	67		20		24		13		10	
	r	p	r	p	r	p	r	p	r	p
T	0.25	0.04*	0.10	0.68	0.14	0.54	-0.37	0.20	0.12	0.73
A	0.41	<0.001*	0.28	0.24	0.24	0.28	-0.18	0.55	0.49	0.15
DHEAS	0.12	0.32	-0.16	0.51	0.01	0.99	0.24	0.43	0.21	0.57
SHBG	-0.36	<0.005*	-0.22	0.36	0.12	0.59	-0.41	0.16	-0.41	0.24
Free T	0.35	<0.01*	0.24	0.31	0.05	0.80	0.03	0.91	0.01	0.99
BMI	-0.01	0.99	-0.11	0.65	-0.43	<0.05*	-0.30	0.30	-0.12	0.73
Insulin, t=0	0.24	0.04*	0.40	0.07	0.19	0.37	0.16	0.59	-0.11	0.74
Glucagon, t=0	-0.13	0.31	-0.29	0.22	-0.05	0.22	0.02	0.93	-0.42	0.23

*$p<0.05$

Figure 3.1: Relationship between serum concentrations of LH and those of insulin in women with PCO. The mean+2SD concentrations of LH and insulin for control women are indicated by dotted lines.

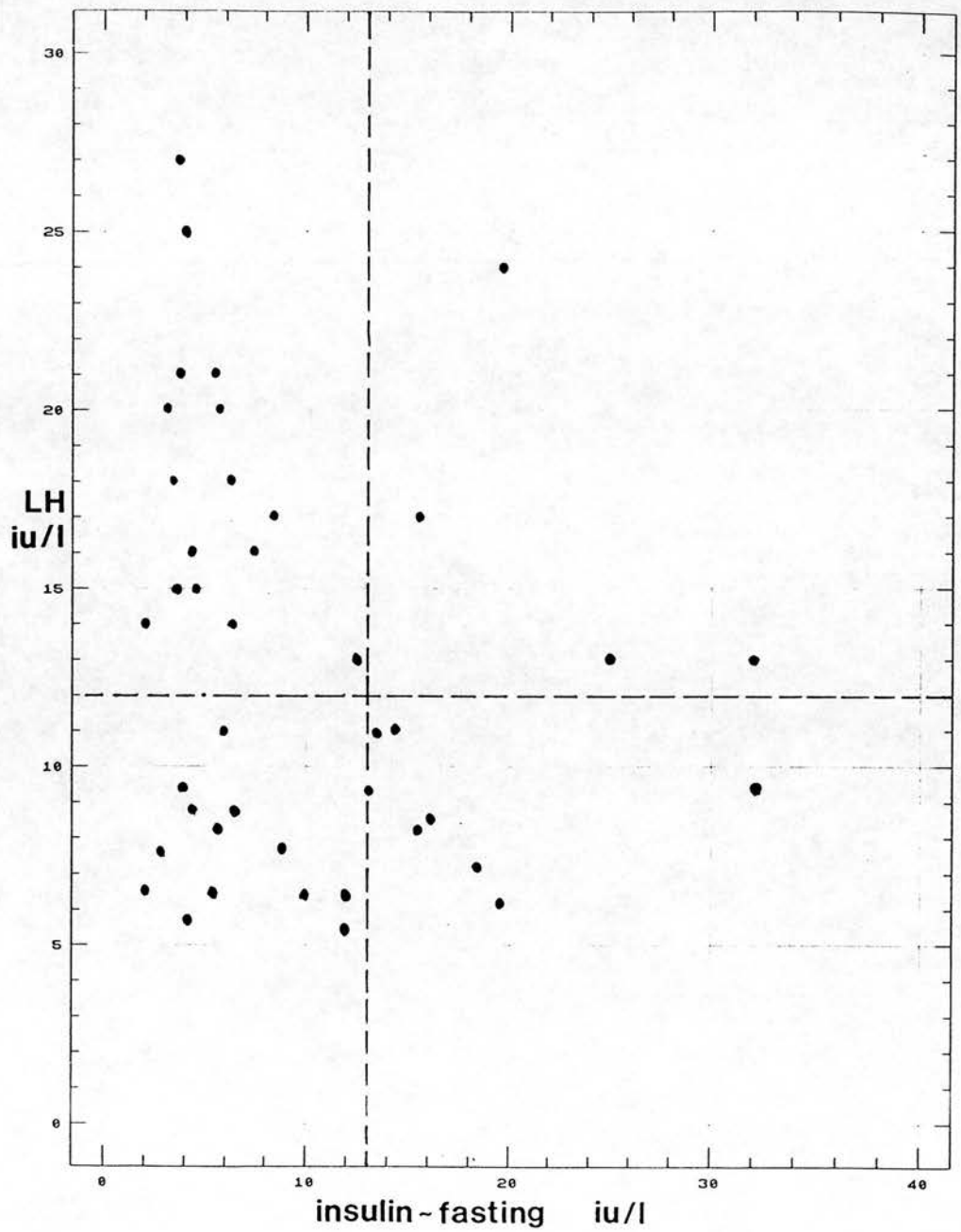
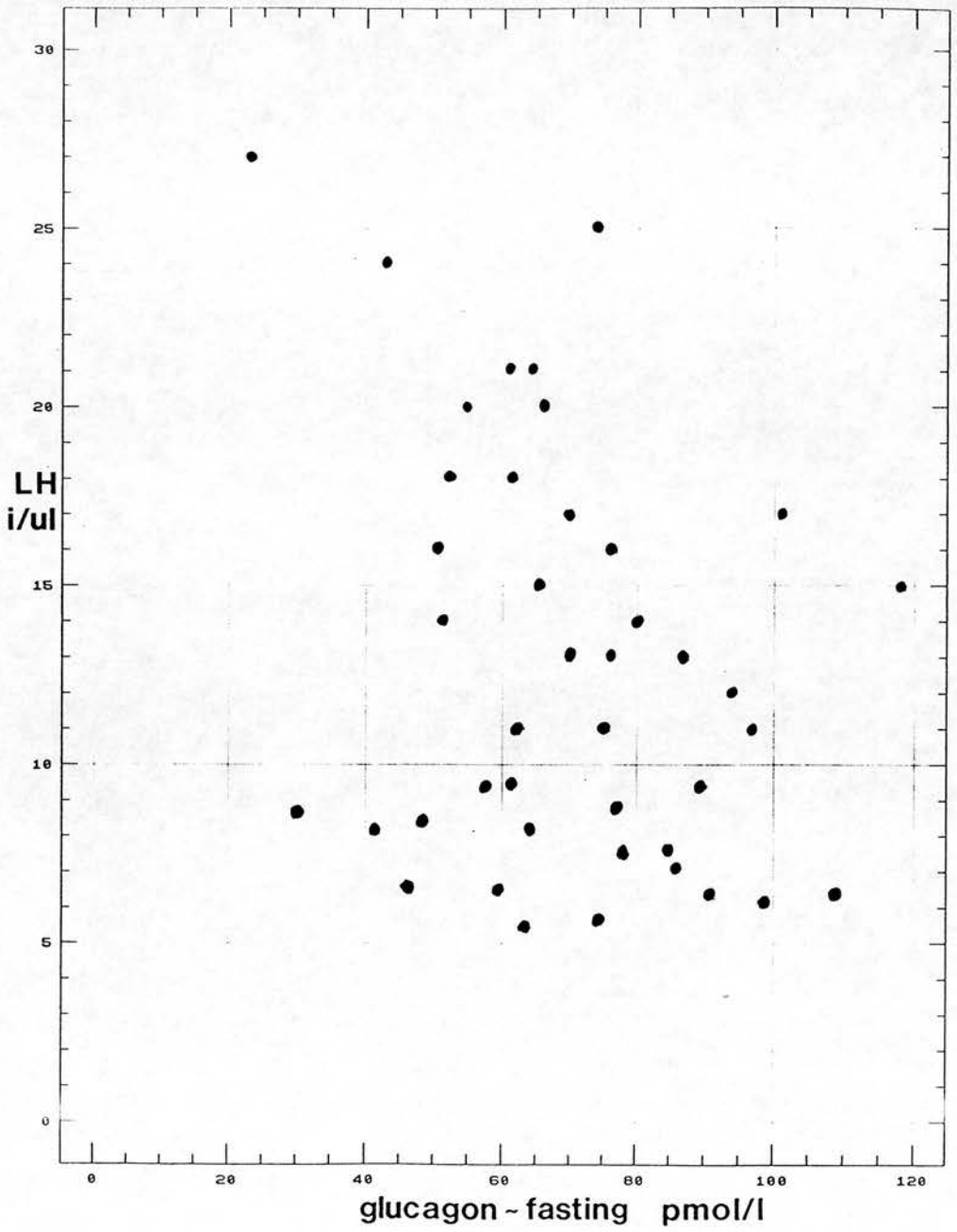


Figure 3.2: Relationship between serum concentrations of LH and fasting concentrations of glucagon in women with PCO.



DISCUSSION.

The variability of clinical and biochemical features of women with PCO is well recognised (Yen 1980, Goldzieher 1981, Franks 1989, Conway et al 1989).

Despite a careful selection procedure, the endocrine results indicate that the study group of women with PCO were not a uniform population. The serum concentration of LH in women with PCO varies rapidly as a consequence of wide pulse amplitude (Waldstreicher et al 1988, Dunaif et al 1988) such that a single sample may not accurately reflect the mean concentration of LH. Of women with ultrasonically-diagnosed PCO, 44-51% will have a random basal LH value within the normal range, although a higher proportion will have increased mean LH values if sampled frequently over an 8 hour period because of the greater amplitude of LH pulses (Waldstreicher et al 1988, Franks 1989, Conway et al 1989). The method chosen in this study for determining mean LH and androgen values, from pooled samples obtained three times over a period of 30 minutes, may not thus accurately reflect mean LH values. LH concentrations were within the normal range (less than 2SD above the mean for the control women) in 17 (38.6%) of the 44 women with PCO on the day of sampling, all of whom had previously demonstrated an elevated basal LH value on at

least one occasion. The sampling frequency, however, represented a compromise between a single basal result and multiple sampling over several hours.

Nevertheless, the mean concentrations of LH, total and free testosterone and androstenedione were significantly greater in the PCO groups than those in control women and compatible with the biochemical profile of PCO (Yen 1980). Thus, the women selected for the PCO groups represented an androgenised study population.

Although Orsini et al (1984) described four types of ovarian ultrasonographic appearance in women with PCO diagnosed on biochemical criteria, Adams et al (1986) based the diagnosis of PCO on typical morphological appearances of the ovary, and showed a close relationship with menstrual and clinical features associated with the syndrome (Franks 1989). The ultrasound investigation of ovarian morphology of obese women with PCO is difficult because of interposition of adipose tissue. Vaginal ultrasonography, in which the distance between the ovary and transducer is reduced, overcomes the problem, but was not available to the author. Assessment of ovarian morphology in the obese women was, therefore, less accurate than in the non-obese. However, as the study was of biochemical and endocrinological features of PCO, and

not the morphological changes in the ovary in response to treatment, less importance was placed upon the morphological compared with the biochemical characteristics for the fulfilment of recruitment criteria to the study.

The control subjects formed a heterogenous group. Most of these women had been shown, laparoscopically, to have minimal degrees of endometriosis, whilst the remainder were awaiting surgery for menstrual disorders secondary to leiomyomata. However, the biochemical profiles of this group were normal except for reduced concentrations of SHBG in the obese subjects, although associated non-hormonal effects of endometriosis such as immunological changes (El-Roeiy et al 1988) might have had unrecognised influences upon the results.

The groups were not matched for age, which has effects upon insulin resistance (Reaven 1988). However, as the control women were older than the women with PCO, any differences demonstrated in insulin values between the two groups would have been expected to be even greater. Nevertheless, there may be potential confounding effects of age upon glucagon levels in the control groups that cannot be assessed on the basis of data obtained in this study. The obese and non-obese PCO groups were of similar

age, however, and the comparison between these groups remains valid.

Fasting and glucose-stimulated hyperinsulinaemia have been consistently described in women with PCO (Barbieri et al 1988) affecting those women with associated acanthosis nigricans most severely (Dunaif et al 1987, Kahn et al 1976). Women with PCO and acanthosis nigricans are considered to be a subgroup of PCO but do not have a distinct endocrine disorder (Dunaif et al 1987), and have therefore been included in the PCO group in this study.

For women with PCO, mean fasting insulin levels and the ratio between insulin and glucose were elevated only in the obese group compared with weight-matched women, indicating resistance to insulin in obese women with PCO. The fasting concentration of C-peptide demonstrated a similar pattern to that of insulin. These was no difference between the groups in the fasting concentration of glucagon.

Elevated serum values of insulin have been demonstrated in a number of studies to correlate with the serum concentrations of total T and A (Burghen et al, Chang et al 1983b, Shoupe et al 1983, Bruno et al 1985, Jailal et al 1987, Pasquali et al 1986, Dunaif et al 1987,

Schriock et al 1988) and free T (Shoupe and Lobo 1984). However, no relationships were demonstrated between the fasting concentrations of insulin and concentrations of T or A, in contrast to these reports. Similarly, there were no correlations between the concentrations of LH and those of T, A, free T or SHBG in any of the groups of women. This is discussed further in Chapter 4.

An inverse relationship was demonstrated between fasting concentrations of glucagon and those of testosterone and androstenedione in obese (BMI >30kg/m^2) women with PCO, but not in any other group. This result is discussed further in Chapter 4 after presentation of oGTT data.

CHAPTER 4: RESPONSE TO ORAL GLUCOSE IN NORMAL WOMEN AND WOMEN WITH PCO.

INTRODUCTION

Women with PCO have similar basal concentrations of glucose to age and weight-matched normal women (Burghen et al 1980, Jailal et al 1987, Dunaif et al 1987, Chapter 3), but obese women with PCO have increased glucose levels following a glucose load (Burghen et al 1980, Dunaif et al 1987) and PCO and obesity may have synergistic deleterious effects upon glucose tolerance (Dunaif et al 1987, 1989). Several epidemiological studies have suggested that glucose intolerance is predictive of the development of diabetes mellitus (Kannel et al 1979, Knuiman et al 1986). A glucose load provides a stimulus to insulin release and suppression of release of glucagon in normal subjects (Borghi et al 1984) thereby permitting an examination of the relationships between the summed values of glucose, insulin and glucagon and the serum concentrations of androgens and gonadotrophins.

The insulin response to oral glucose may be related to serum concentrations of testosterone and

androstenedione in a similar manner to fasting concentrations of insulin (Burghen et al 1980, Chang et al 1983b, Dunaif et al 1987, Chapter 3). The suppression of plasma concentrations of glucagon in response to an oral glucose load is inhibited in insulin resistant obese subjects (Borghi et al 1984), but the response of glucagon to a similar glucose load in women with PCO has not been examined.

In order to determine whether glucagon is implicated in the development of hyperinsulinaemia and insulin resistance associated with PCO, glucose, insulin and glucagon responses to an oral glucose load were investigated in two groups of women with PCO. Responses were also related to circulating concentrations of gonadotrophins, androgens and C-peptide in an attempt to determine the role of glucagon and insulin in the hyperandrogenism associated with PCO.

METHODS

Obese and non-obese women with PCO and weight-matched control women, in the same groups as described in Chapters 2 and 3, underwent an oral glucose tolerance test (oGTT) as described in Chapter 2. Clinical details of these

women are given in Tables 2.1 and 2.2. The subjects in obese groups were further classified into those with a BMI less than or greater than 30 kg/m^2 where indicated.

Cumulative totals of glucose, insulin, C-peptide and glucagon concentrations after oral glucose administration are the sum of all values from time 0 to 150 minutes. These are described in Table 4.1.

RESULTS

BLOOD GLUCOSE, SERUM INSULIN, C-PEPTIDE, GLUCAGON AND HBA_1.

Glucose reponses to oral glucose loading in the four groups of women are shown in Figures 4.1 and 4.2, and summed values in Table 4.1. In obese women with PCO, the glucose concentration 60, 90 and 120 minutes after glucose loading and the summed glucose concentration during the oGTT were greater than the corresponding values in non-obese women with PCO ($p<0.05$) and those in the control groups, although division of the obese groups revealed that this applied only to those women with PCO and BMI greater than 30 kg/m^2 (Figure 4.3, Table 4.2). Two obese women with PCO were found to exhibit impaired

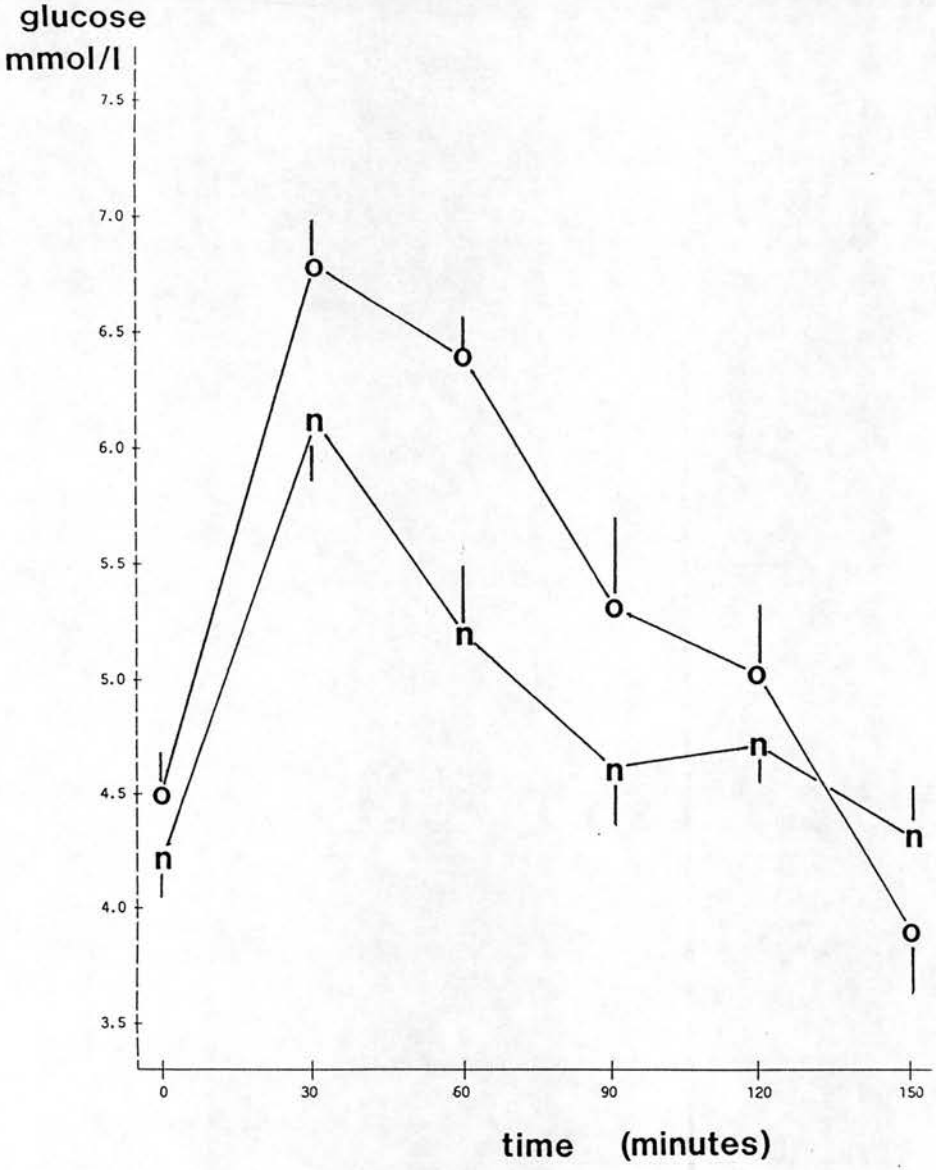


Figure 4.1: Mean (SE) glucose responses to oral glucose in obese (o) and non-obese (n) control women.

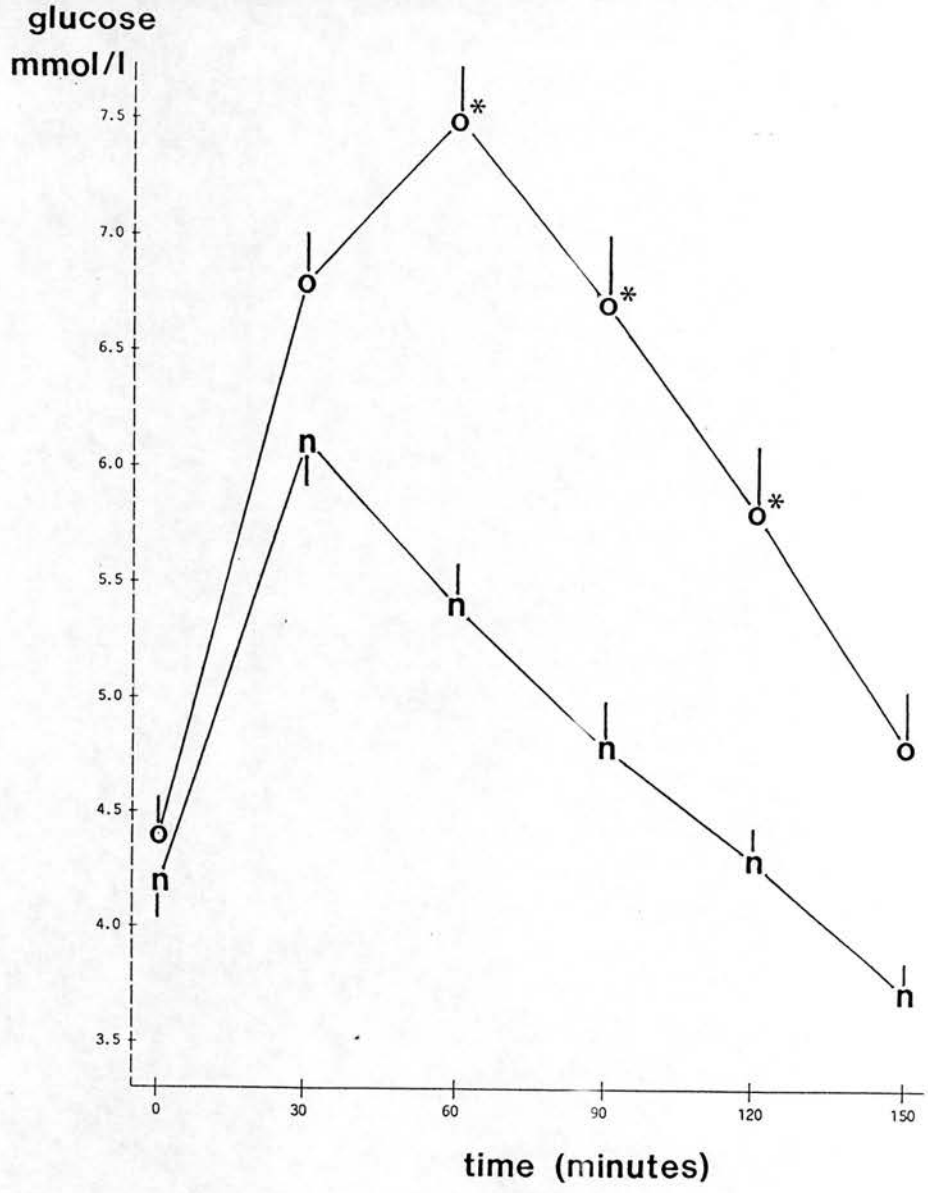


Figure 4.2: Mean (SE) glucose responses to oral glucose in obese (o) and non-obese (n) women with PCO. x, $p<0.05$ v. non-obese control women and control women.

TABLE 4.1: SUMMED VALUES OF GLUCOSE, INSULIN, GLUCAGON, INSULIN:GLUCOSE RATIO AND C-PEPTIDE DURING oGTT IN NORMAL WOMEN AND WOMEN WITH PCO.

	PCO SUBJECTS		CONTROL SUBJECTS	
	NON-OBESE	OBESE	NON-OBESE	OBESE
n	20	24	13	10
Glucose (mmol/l)	28.5 (6.8)	36.0 (12.8)#	29.2 (4.5)	31.9 (5.5)
Insulin (iu/l)	247.6 (128.2)*	416.7 (327.0)#	162.4 (64.7)	212.6 (87.4)
Insulin:glucose	8.8 (4.2)*	12.9 (9.5)#	5.5 (2.0)	6.7 (2.8)
Glucagon (pmol/l)	278.5 (169.7)	305.9 (115.0)	286.5 (127.4)	399.3 (133.1)+
n	8	9	10	6
C-peptide (nmol/l)	10.7 (7.3)	20.7 (9.2)*	8.0 (3.0)	11.2 (3.0)

#Obese PCO > non-obese PCO and control subjects ($p<0.05$) *non-obese PCO > control ($p<0.05$)

+obese control > PCO subjects and non-obese control ($p<0.05$)

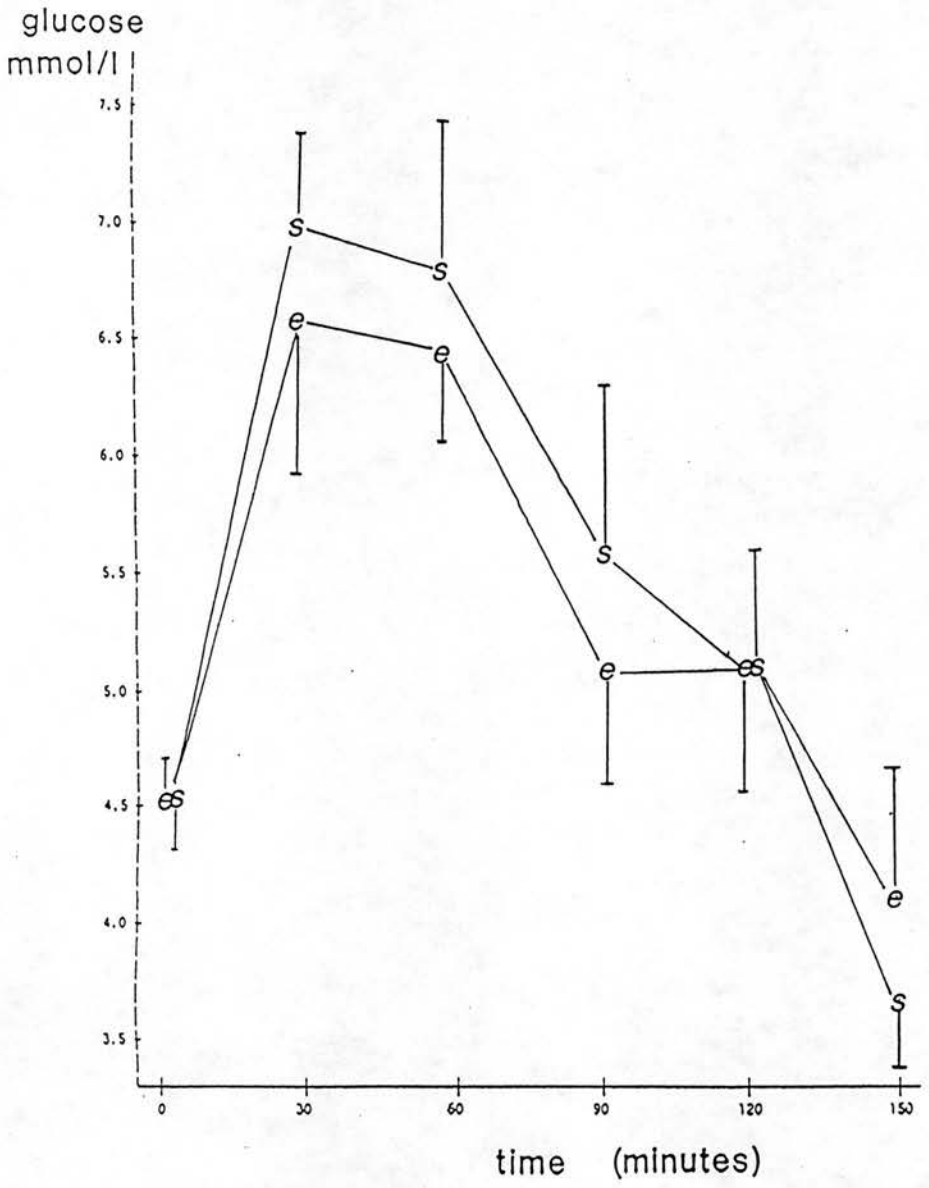


Figure 4.3(a):
Mean (SE) glucose responses to oral glucose in obese subgroups of control women.
s, subgroup obese women with BMI > 30 kg/m^2
e, subgroup obese women with BMI > 25, < 30 kg/m^2

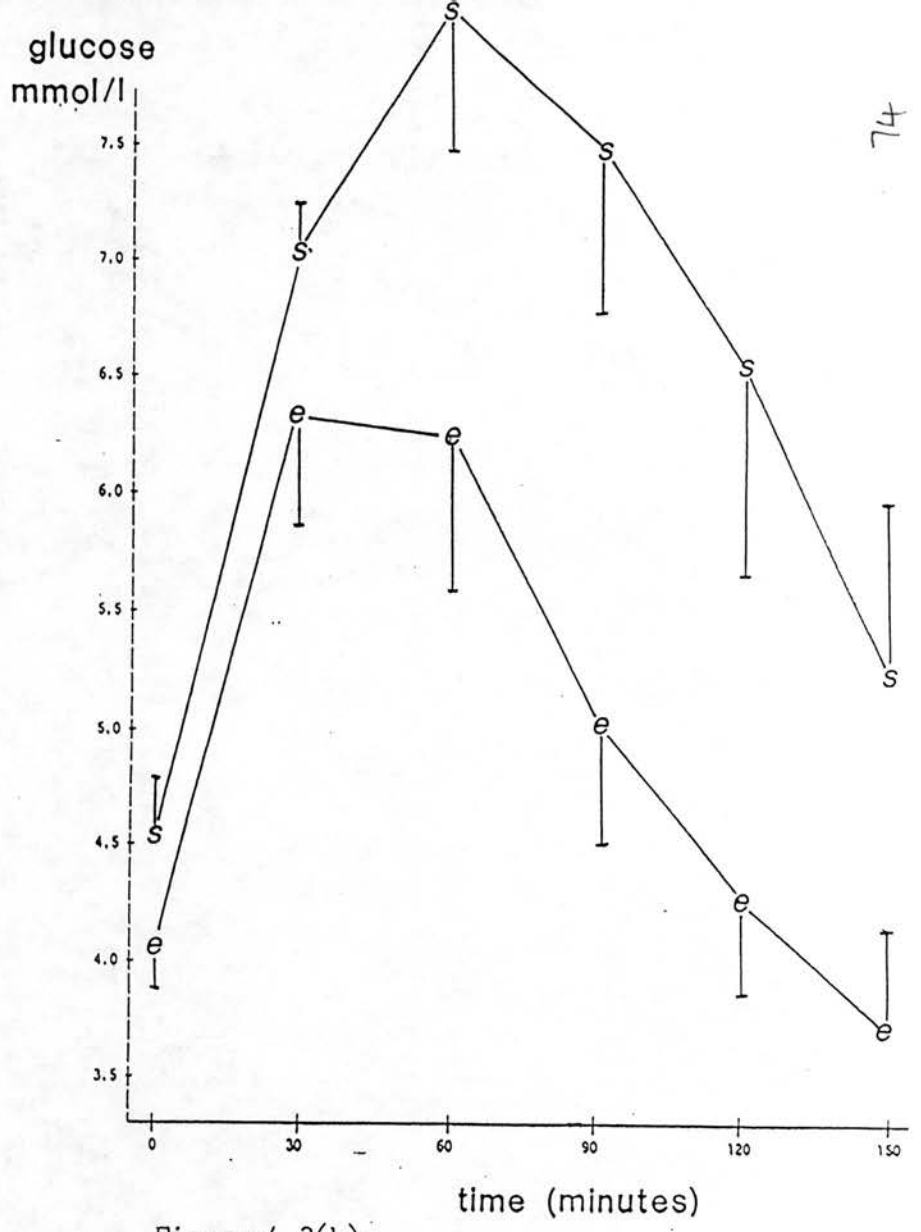


Figure 4.3(b):
Mean (SE) glucose responses to oral glucose in obese subgroups of women with PCO.
s, subgroup obese women with BMI > 30 kg/m^2
e, subgroup obese women with BMI > 25, < 30 kg/m^2

TABLE 4.2: SUMMED VALUES OF BLOOD GLUCOSE, SERUM INSULIN AND GLUCAGON IN OBESE WOMEN WITH PCO AND OBESE CONTROL SUBJECTS.

	PCO SUBJECTS		CONTROL SUBJECTS	
OBESE SUBGROUP BMI	>25 to <30	>30	>25 to <30	>30
n	8	16	6	4
Glucose (mmol/l)	29.9 (2.8)	39.2 (4.2)	31.4 (1.6)	32.4 (3.1)
Insulin (IU/l)	265 (116)	492 (373)	195 (91)	238 (86)
Glucagon (pmol/l)	254 (125)	322 (104)	399 (163)	401 (93)

glucose tolerance, as defined by the criteria of the National Diabetes Data Group (1979).

Insulin concentrations in obese women with PCO 30, 60, 90 and 120 minutes after glucose loading and the summed insulin value were greater than in non-obese women with PCO ($p<0.05$) and in weight-matched control subjects ($p<.05$)(Figures 4.4 and 4.5). The summed insulin value was also greater in non-obese women with PCO than in non-obese control subjects ($p<0.05$) (Table 4.1). Further analysis showed insulin concentrations were greatest in women with PCO and BMI >30kg/m^2 (Figure 4.6, Table 4.2).

The ratio between summed values of insulin and those of glucose was greater in the PCO groups than in weight-matched controls ($p<0.05$) and was greater in obese than in non-obese women with PCO ($p<0.05$).

The number of subjects in whom C-peptide was measured was too small for analysis at each time point. C-peptide values in control women and in women with PCO are shown in Figures 4.7 and 4.8 respectively. The summed C-peptide value was greater in obese women with PCO than that in non-obese women with PCO ($p<0.05$) and that in the control group ($p<0.05$, Table 4.1).

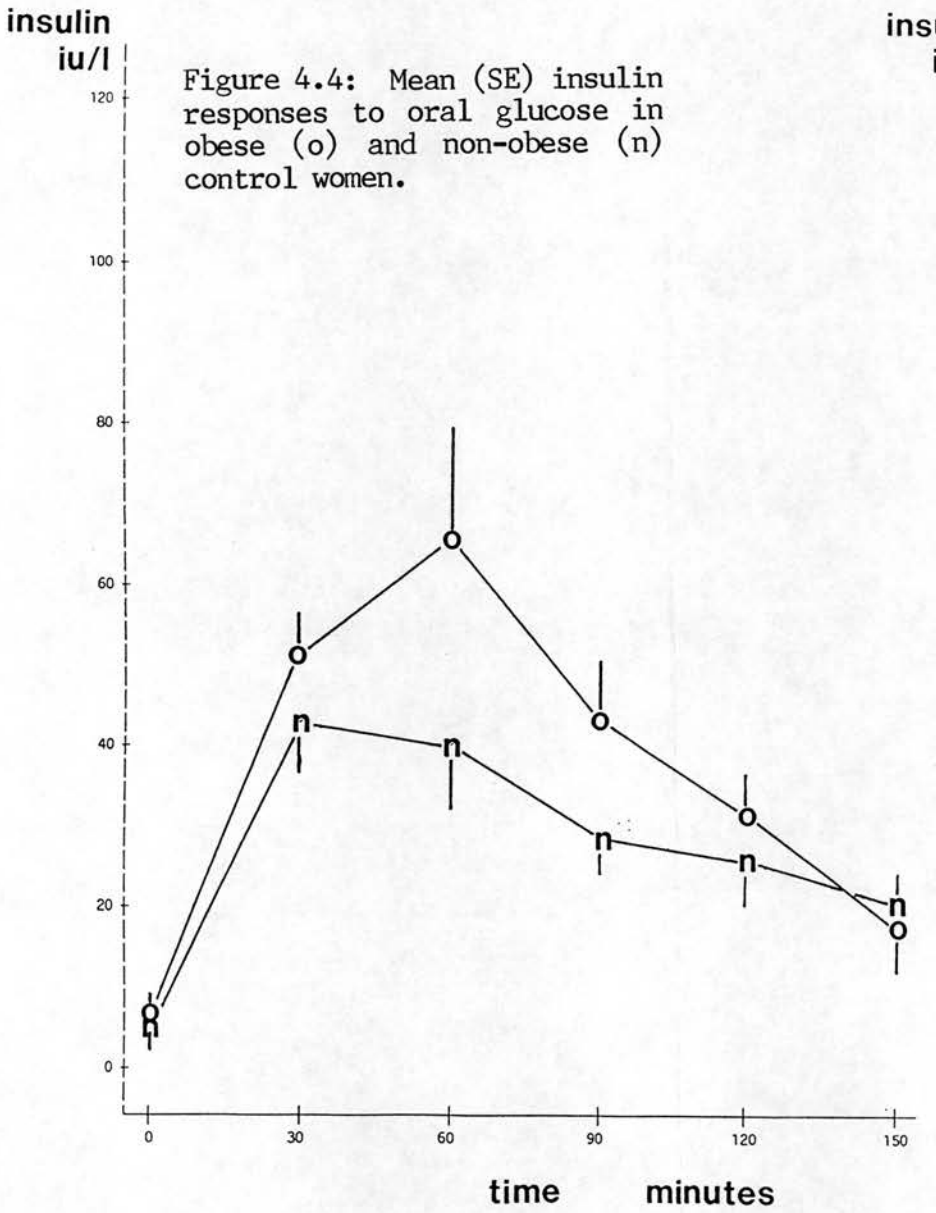


Figure 4.4: Mean (SE) insulin responses to oral glucose in obese (o) and non-obese (n) control women.

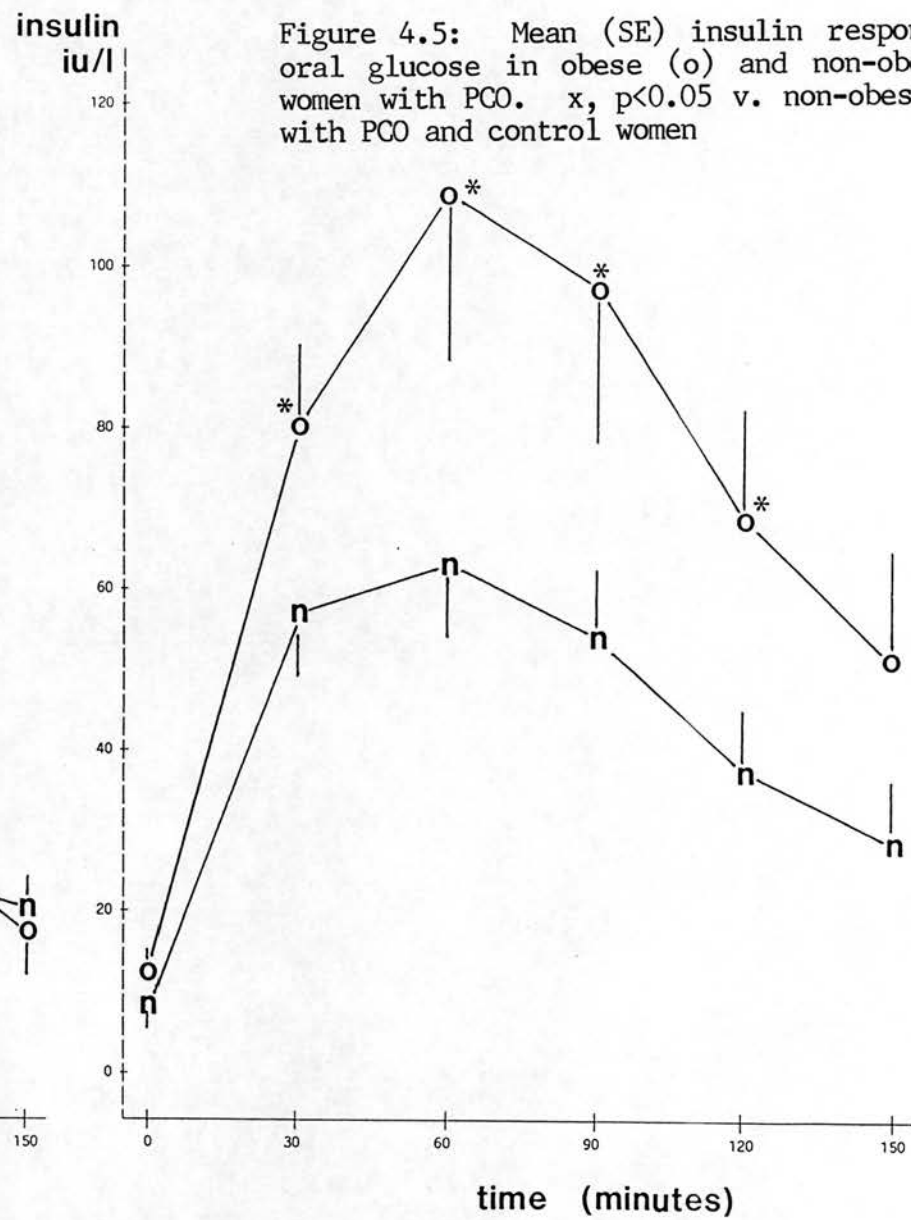


Figure 4.5: Mean (SE) insulin responses to oral glucose in obese (o) and non-obese (n) women with PCO. x, $p<0.05$ v. non-obese women with PCO and control women

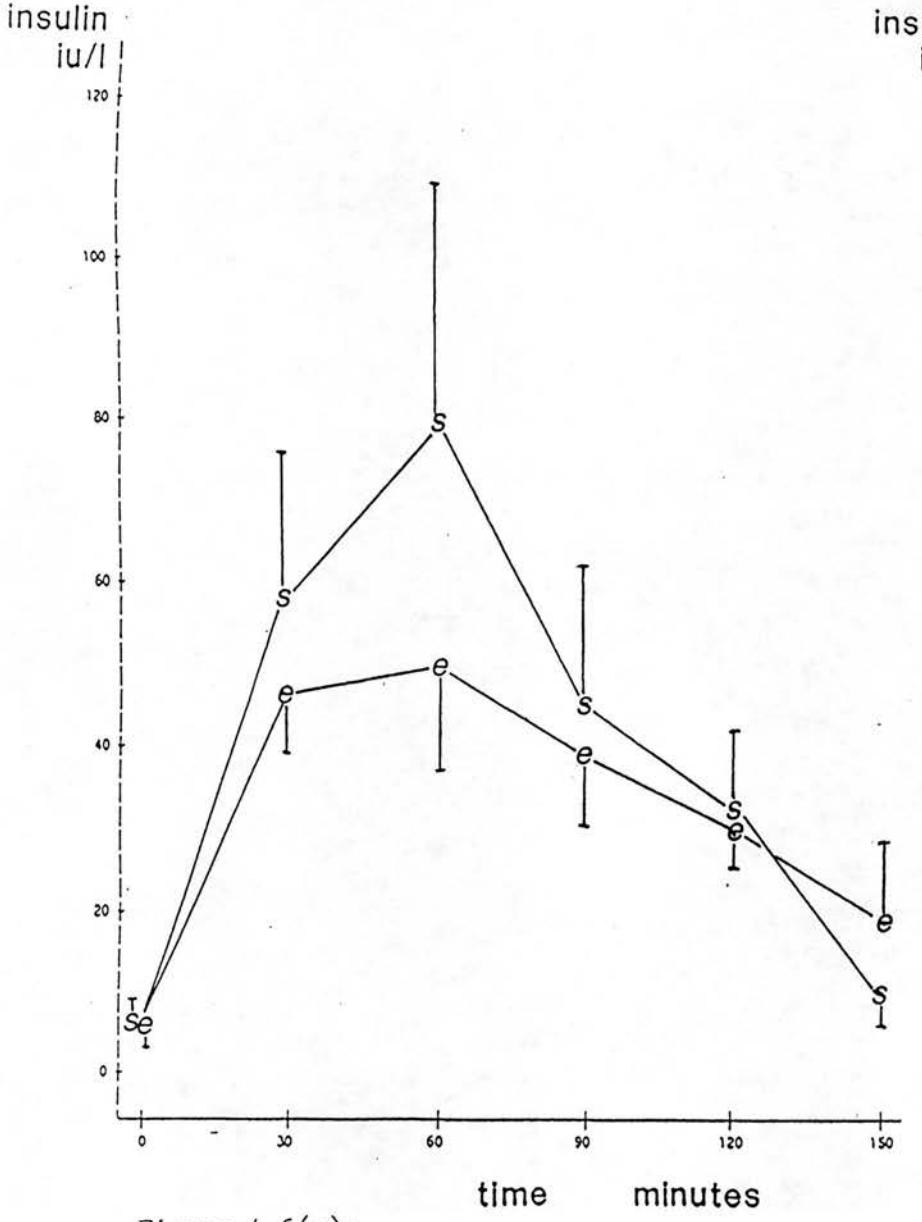


Figure 4.6(a):
Mean (SE) insulin responses to oral glucose in obese subgroups of control women.
s, subgroup obese women with BMI > 30 kg/m^2
e, subgroup obese women with BMI > 25, < 30 kg/m^2

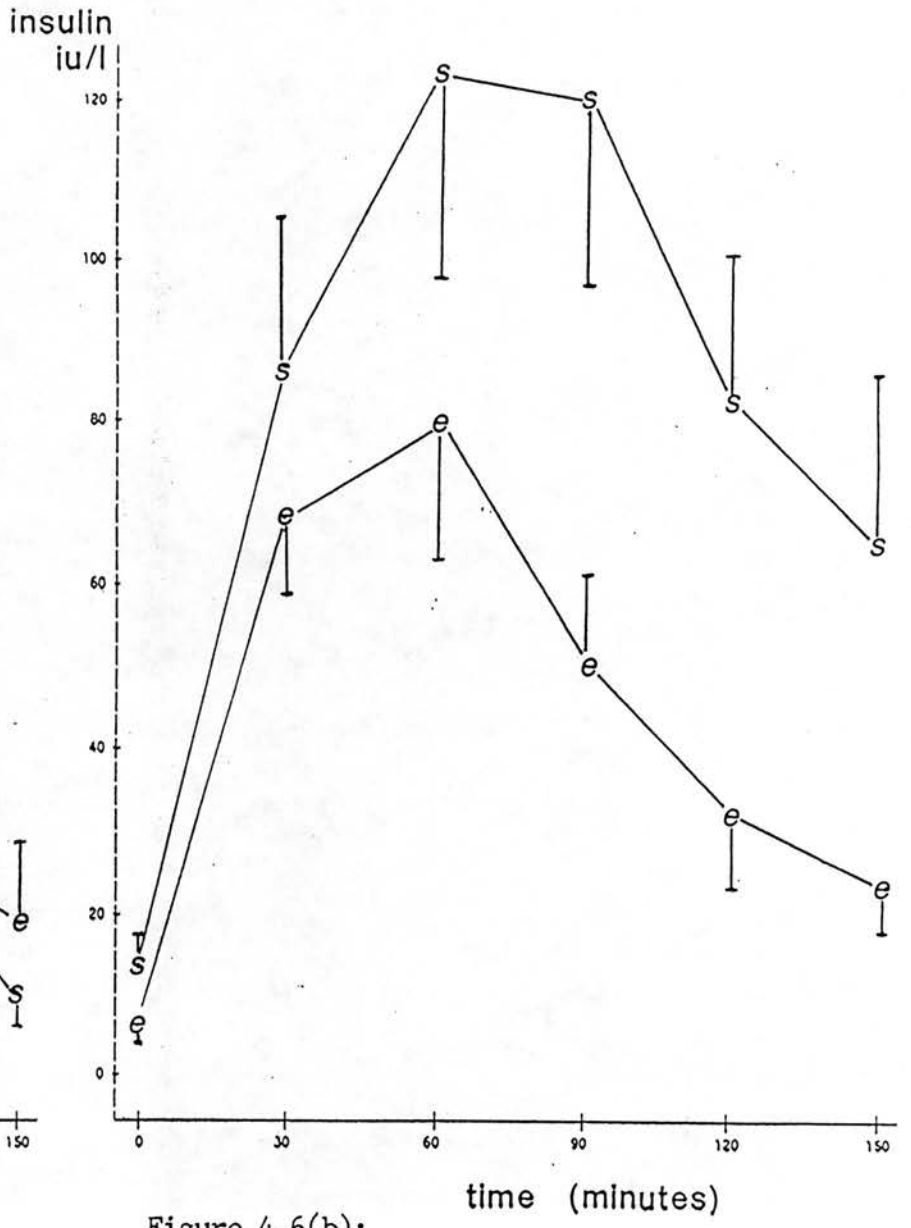
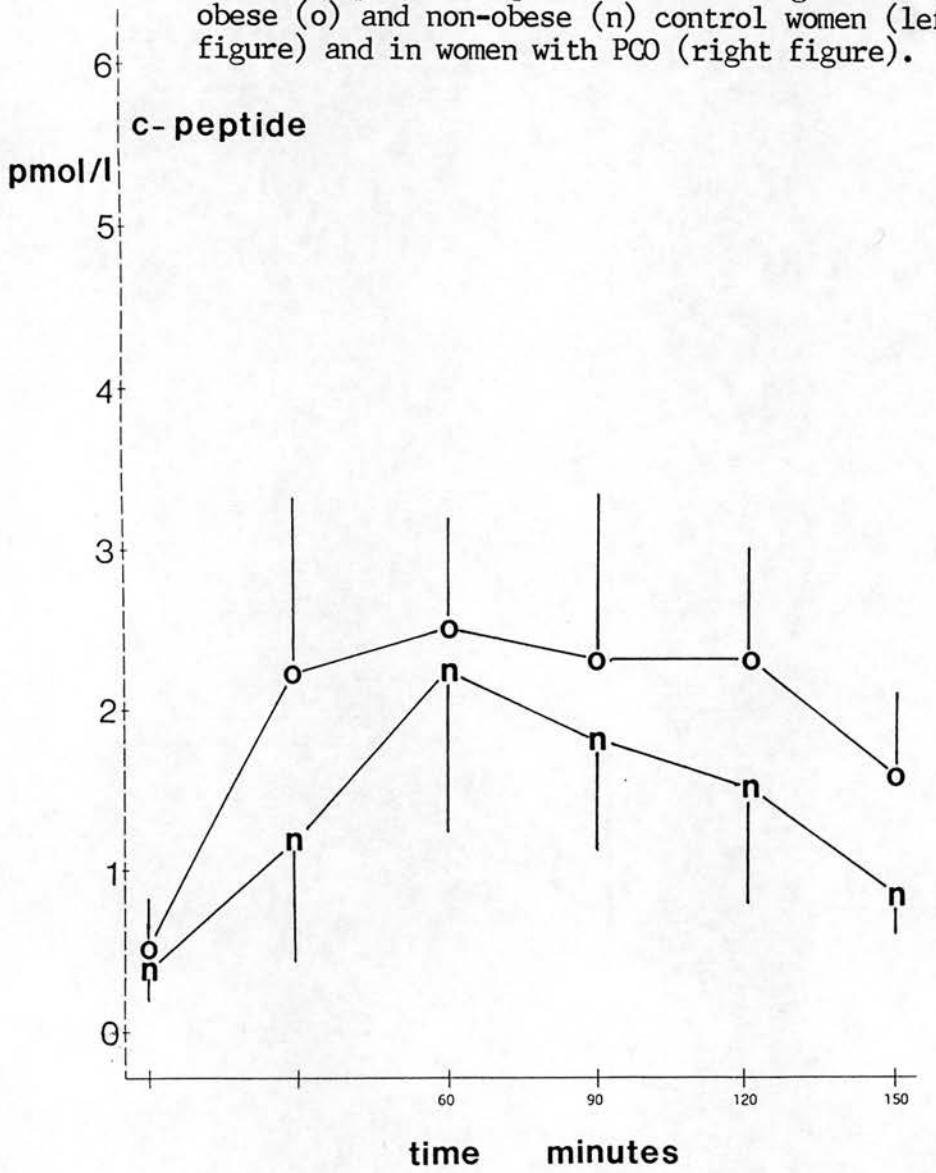
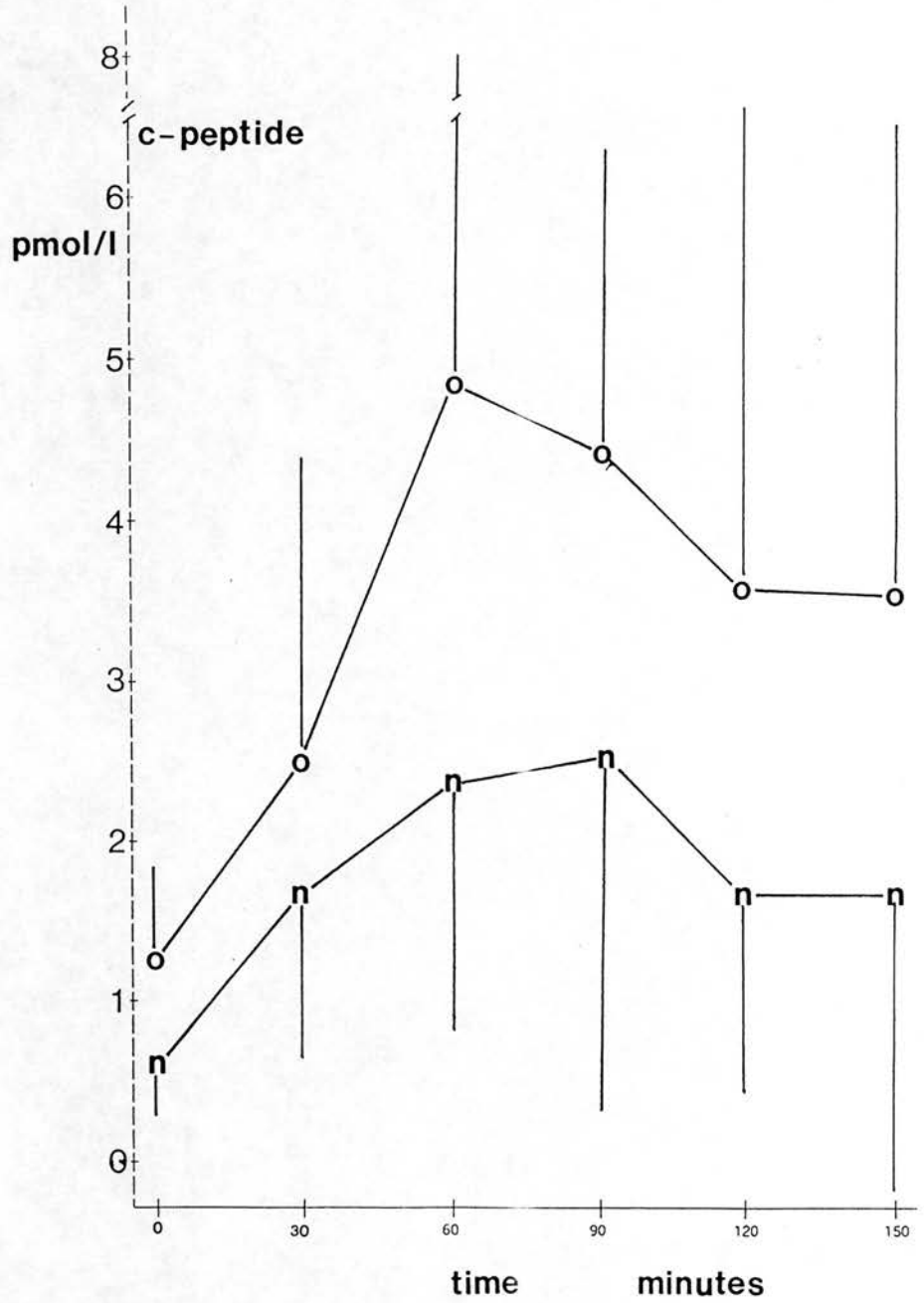


Figure 4.6(b):
Mean (SE) insulin responses to oral glucose in obese subgroups of women with PCO.
s, subgroup obese women with BMI > 30 kg/m^2
e, subgroup obese women with BMI > 25, < 30 kg/m^2

Figure 4.7 (left) and Figure 4.8 (right): Mean (SE) C-peptide responses to oral glucose in obese (o) and non-obese (n) control women (left figure) and in women with PCO (right figure).



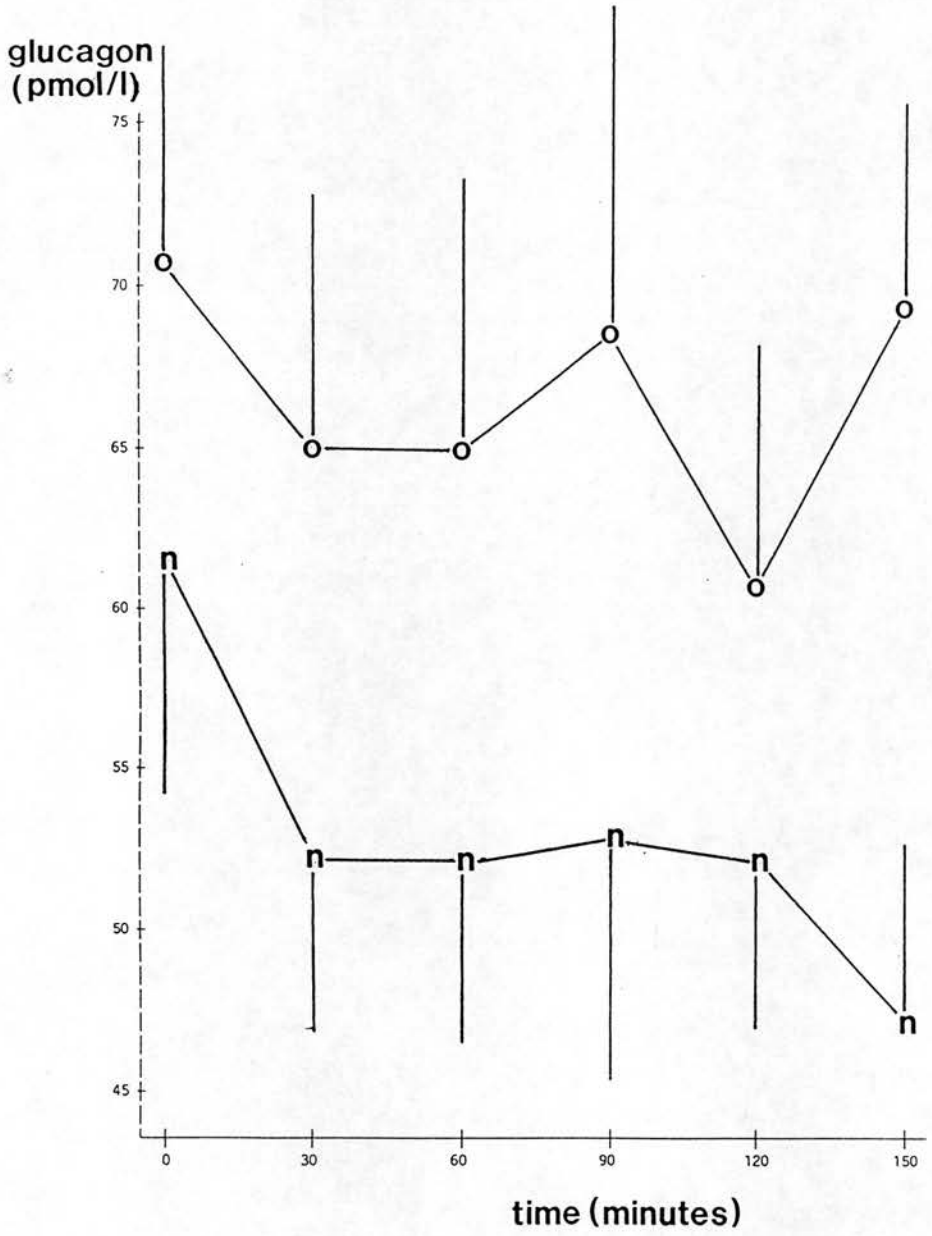


Obese women in the control group had a significantly greater glucagon response to oral glucose loading than did non-obese control subjects and women in both PCO groups ($p<0.05$) (Figures 4.9 and 4.10). However, there was a wide variation in the concentrations of glucagon within each of the study groups. Glucagon values at 60-150 minutes were significantly lower in subjects in PCO groups and in non-obese control subjects than those when fasting and 30 minutes after glucose loading ($p<0.05$). With further analysis, both obese control subgroups (less than and greater than 30 kg/m2) had greater values of glucagon at 60, 90 and 150 minutes than PCO groups and non-obese control women (Figure 4.11).

RELATIONSHIP BETWEEN SUMMED GLUCOSE, INSULIN AND GLUCAGON VALUES AND OTHER HORMONAL MEASUREMENTS.

Analyses are presented for the whole study population and for each of the four groups of women (Tables 4.3 and 4.4).

In the study group as a whole, summed insulin values correlated with the BMI ($r=0.47$, $p<0.001$) and free T values ($r=0.38$, $p<0.005$) and inversely with SHBG ($r=-0.35$, $p<0.005$). No correlations were seen between summed insulin values and those of T, A, DHEAS or LH.



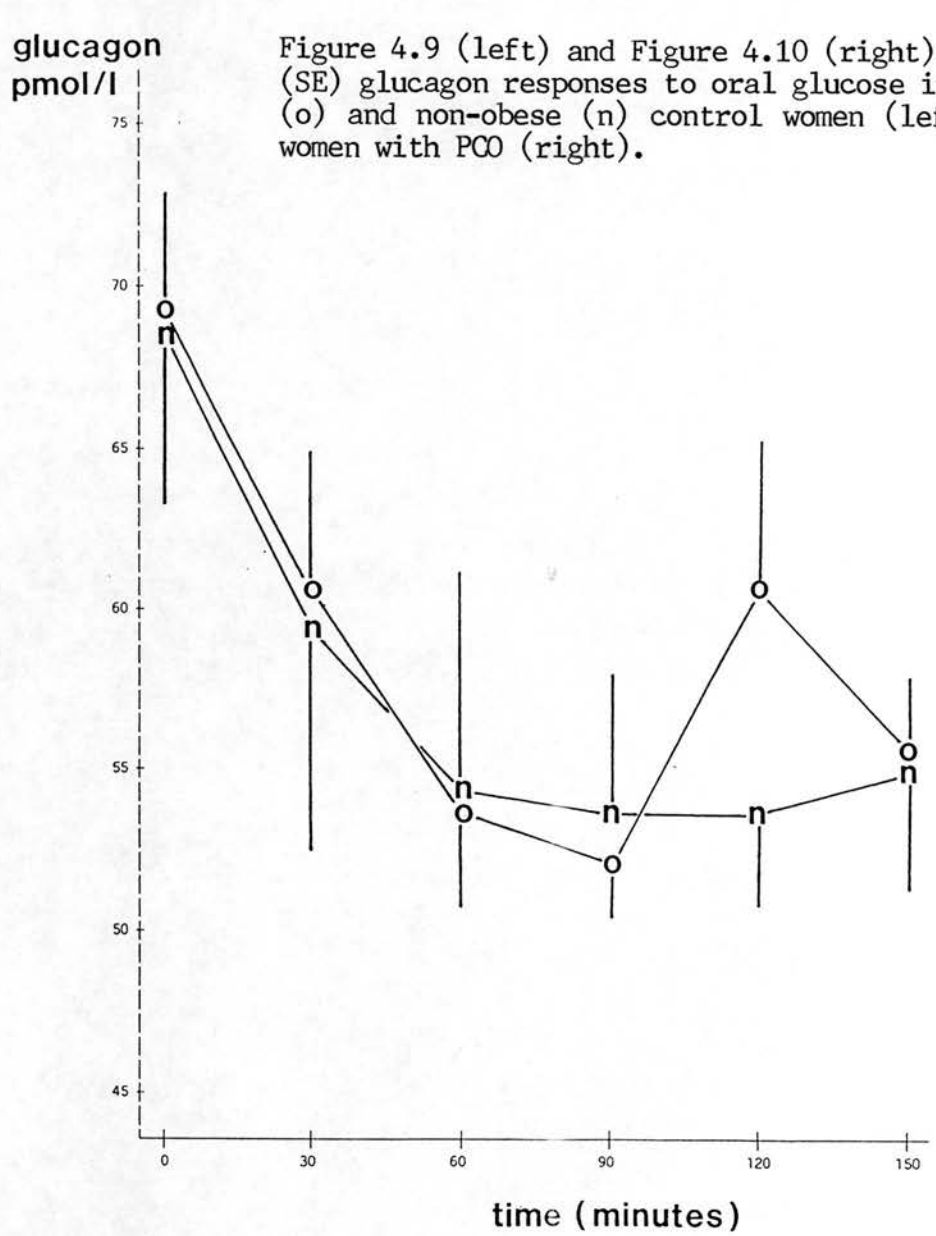


Figure 4.9 (left) and Figure 4.10 (right): Mean (SE) glucagon responses to oral glucose in obese (o) and non-obese (n) control women (left) and women with PCO (right).

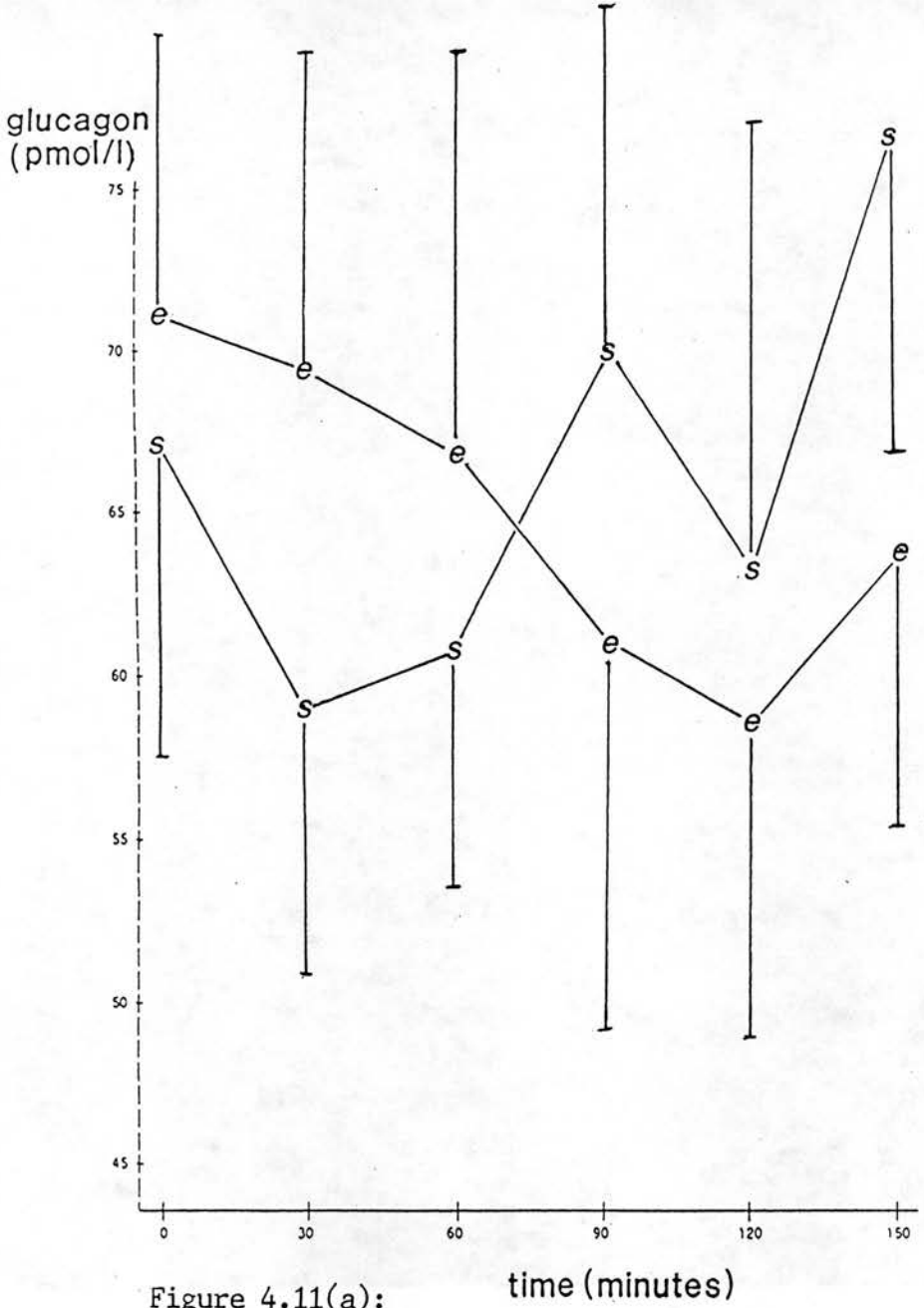


Figure 4.11(a):
Mean (SE) glucagon responses to oral glucose in obese subgroups of control women.
s, subgroup obese women with BMI > 30 kg/m^2
e, subgroup obese women with BMI > 25, < 30 kg/m^2

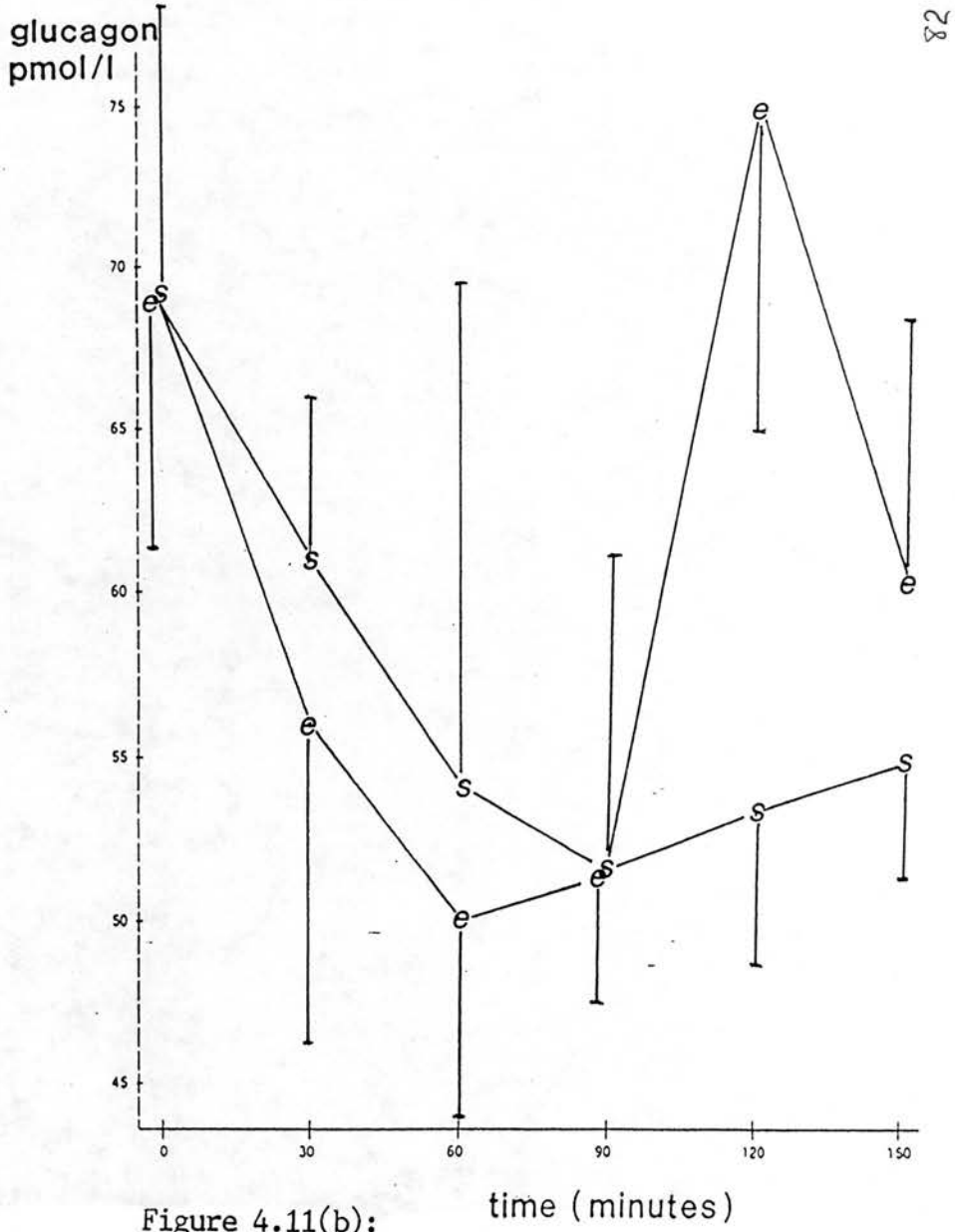


Figure 4.11(b):
Mean (SE) glucagon responses to oral glucose in obese subgroups of women with PCO.
s, subgroup obese women with BMI > 30 kg/m^2
e, subgroup obese women with BMI > 25, < 30 kg/m^2

TABLE 4.3: RELATIONSHIP BETWEEN SUMMED VALUES OF INSULIN AND THOSE OF OTHER MEASUREMENTS DURING oGTT IN NORMAL WOMEN AND IN WOMEN WITH PCO.

	ALL SUBJECTS		PCO				CONTROL			
			NON-OBESE		OBESE		NON-OBESE		OBESE	
n	67		20		24		13		10	
	r	p	r	p	r	p	r	p	r	p
BMI	0.47	0.001*	0.42	0.04*	0.14	0.56	0.29	0.33	0.01	0.97
T	-0.01	0.93	-0.27	0.22	0.14	0.55	-0.02	0.93	0.36	0.31
A	0.12	0.33	-0.27	0.22	0.41	0.07	0.31	0.30	0.11	0.74
DHEAS	-0.12	0.92	-0.26	0.23	0.42	0.07	0.39	0.19	-0.08	0.81
SHBG	-0.35	0.005*	-0.46	0.03*	-0.30	0.20	-0.06	0.83	0.28	0.42
Free T	0.38	0.004*	0.12	0.57	0.53	0.02*#	0.13	0.68	-0.49	0.17
LH	-0.04	0.17	-0.35	0.10	-0.28	0.24	0.19	0.53	0.43	0.21
Glucagon, t=0	0.16	0.17	0.21	0.37	0.21	0.31	-0.22	0.46	0.29	0.40
Glucagon, sum	0.03	0.78	-0.01	0.98	0.08	0.70	-0.23	0.45	0.45	0.19

*$p<0.05$

\# In the obese subgroup with BMI >25 to <30kg/m^2, with free T, $r=0.77$, $p<0.05$
in the obese subgroup with BMI >30kg/m^2, with free T, $r=0.14$, $p=0.64$

TABLE 4.4: RELATIONSHIP BETWEEN SUMMED VALUES OF GLUCAGON AND THOSE OF OTHER MEASUREMENTS DURING oGTT IN NORMAL WOMEN AND IN WOMEN WITH PCO.

	ALL SUBJECTS		PCO				CONTROL			
			NON-OBESE		OBESE		NON-OBESE		OBESE	
n	67		20		24		13		10	
	r	p	r	p	r	p	r	p	r	p
BMI	0.21	0.08	0.19	0.42	0.25	0.08	0.23	0.77	0.06	0.96
T	-0.09	0.44	0.12	0.60	-0.66	0.001*#	0.42	0.14	0.51	0.13
A	-0.21	0.09	-0.03	0.90	-0.59	0.005*#	0.19	0.51	-0.04	0.90
DHEAS	-0.11	0.34	0.17	0.49	-0.32	0.14	-0.30	0.32	0.12	0.73
SHBG	0.13	0.30	0.18	0.45	-0.09	0.66	0.09	0.76	0.53	0.10
Free T	-0.09	0.47	-0.06	0.79	-0.31	0.15	0.35	0.23	0.78	0.68
LH	-0.17	0.15	-0.08	0.75	-0.34	0.11	-0.39	0.19	0.08	0.80

*$p<0.05$

\# In the obese subgroup with BMI >30kg/m^2, with T, r=-0.68, p<0.01; with A, r=-0.72, p<0.005
In the obese subgroup with BMI >25kg/m^2, with T, r=-0.51, p=0.19; with A, r=-0.33, p=0.42

In non-obese women with PCO, however, summed insulin values correlated weakly with BMI ($r=0.47$, $p<0.05$) and were inversely related to SHBG values ($r=-0.46$, $p<0.05$) (Table 4.3). In obese women with PCO the summed insulin values correlated with those of free T ($r=0.53$, $p<0.05$), although this continued to hold only for women with BMI <30 kg/m^2 ($r=0.77$, $p<0.05$) but not for the more obese subgroup ($r=0.13$, $p=0.64$).

No relationship between summed insulin values and other measurements was demonstrated in the PCO nor in the two control groups and there was no relationship between insulin and summed glucagon values in any of the groups. The relationships between the LH values and summed values of insulin and of glucagon were similar to that for fasting values (Figures 4.12 and 4.13)

The summed value of glucagon was inversely related to total T ($r=-0.66$, $p<0.001$) and A concentrations ($r=-0.59$, $p<0.005$) in obese women with PCO but not in non-obese women with PCO or in the control groups (Table 4.4). These relations continued to hold for women with PCO with BMI >30 kg/m^2 (total T, $r=-0.63$, $p<0.02$; A, $r=-0.67$, $p<0.01$) but not in the less obese subgroup. No other relationships were seen between summed glucagon and other hormone measurements or BMI.

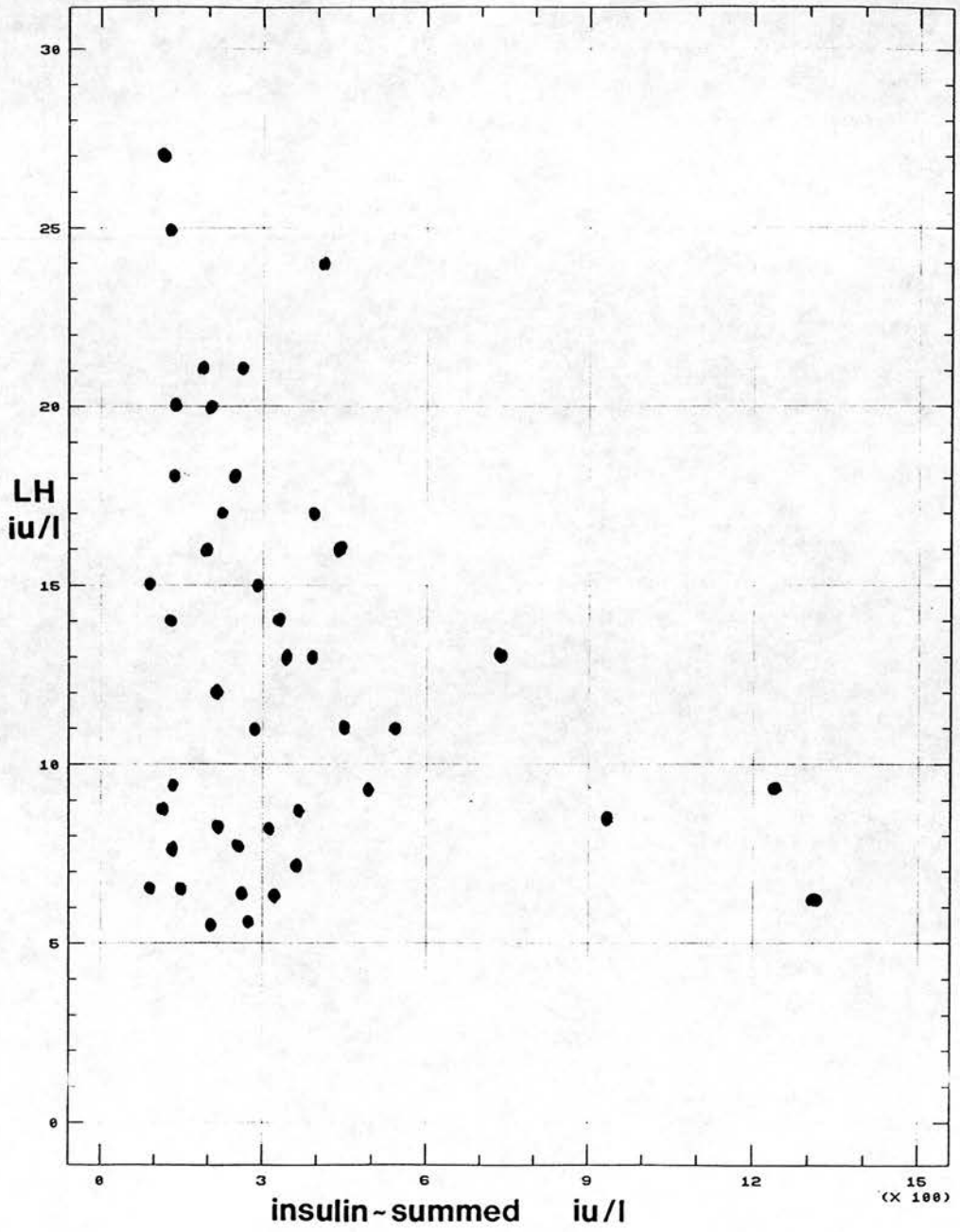


Figure 4.12: Relationship between serum concentrations of LH and summed concentrations of insulin in women with PCO.

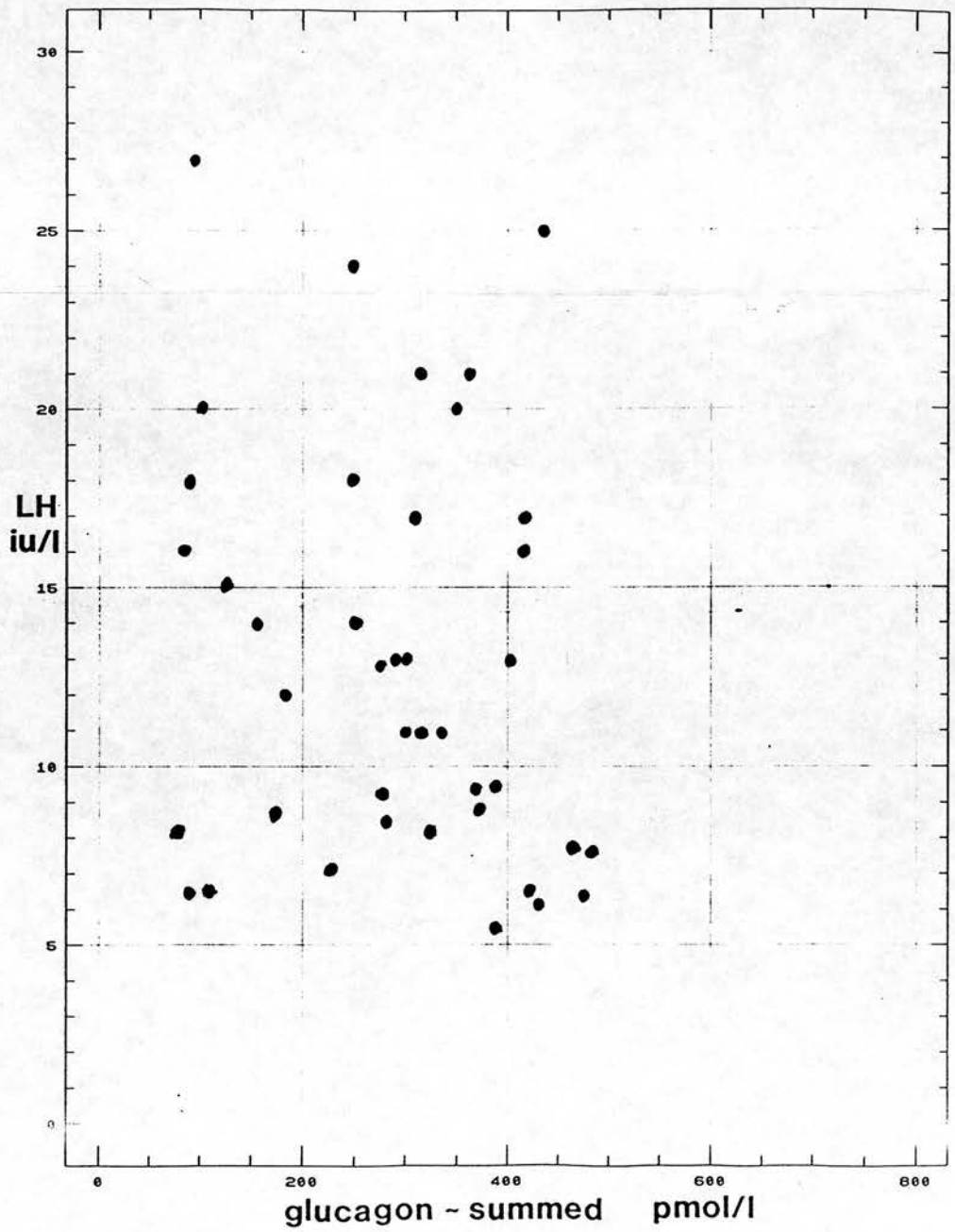


Figure 4.13: Relationship between serum concentrations of LH and summed concentrations of glucagon in women with PCO.

No significant correlations were found between HbA_1 and summed glucose values ($r=0.056$, $p=0.74$). HbA_1 results were markedly skewed, however, by data from one woman with a diabetic glucose tolerance curve (National Diabetes Data Group 1985) and who, inexplicably, had the lowest HbA_1 result of all the women studied. When data from this patient were excluded, a significant positive correlation between HbA_1 and summed glucose levels was apparent ($r=0.389$, $p=0.02$, Figure 4.14).

DISCUSSION

Hyperinsulinaemia, previously demonstrated in women with PCO (Burghen et al 1980), has been confirmed in this study. Summed glucose and insulin values were greater in obese subjects with PCO than in non-obese women with PCO and weight-matched control women, and in 2 (8%) of these women impaired glucose tolerance was demonstrated. Nevertheless, concentrations of glucagon in response to glucose decreased significantly in women with PCO and in the control group of normal weight women, although not in obese normal women, and an inverse relationship between the glucagon response and the concentrations of total T and A was demonstrated in obese women with PCO but not in the other groups.

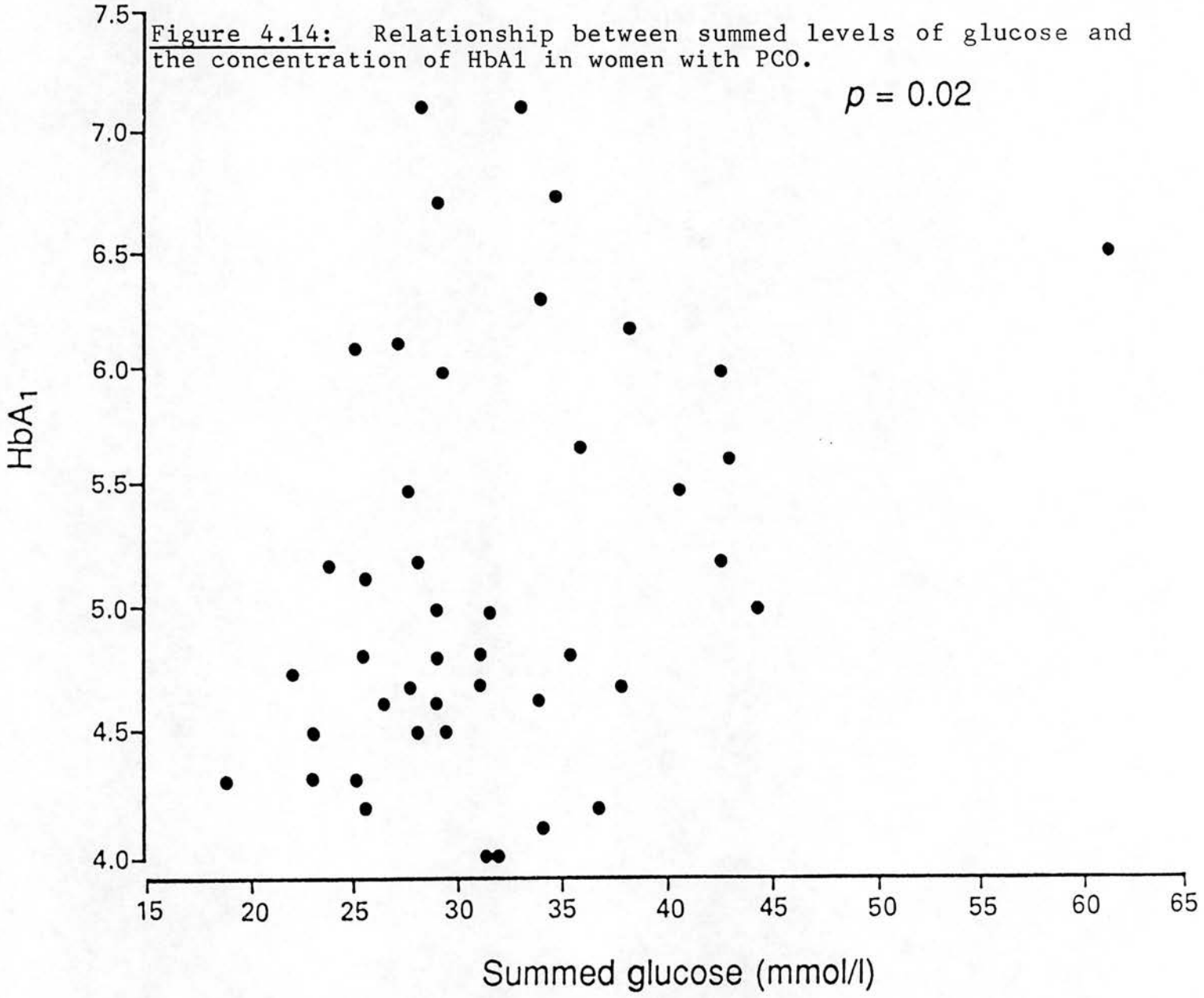


Figure 4.14: Relationship between summed levels of glucose and the concentration of HbA1 in women with PCO.

Elevated androgen concentrations in women with PCO may be responsible for the development and maintainance of hyperinsulinaemia and insulin resistance (Burghen et al 1980, Shoupe et al 1983). However, the higher insulin concentrations and insulin resistance associated with elevated androgen concentrations in women with PCO does not occur in men in whom concentrations of androgens are much greater (Yki-Jarvinen 1984). Furthermore, prolonged administration of testosterone or androstenedione to female Rhesus monkeys does not significantly alter insulin sensitivity (Billiar et al 1986). This suggests that androgens, at least alone, are not the cause of altered insulin homeostasis in women with PCO.

Hyperinsulinaemia induces defects in insulin action (Clark et al 1983) and peripheral insulin resistance in women with androgen excess may result from associated increases in insulin levels. No mechanism for androgen-mediated hyperinsulinaemia has been identified, although Peiris et al (1989) proposed that in hyperandrogenic women with PCO, the anabolic effect of androgens may result in a selective increase in the proportion of insulin-resistant fast twitch muscle fibres. In the rat, however, the actions of androgens upon muscle are independent of those of insulin (Hager and Kalkhoff 1989).

Conversely, insulin may be responsible for or contribute to increased androgen production in women with PCO (Taylor et al 1982). Barbieri et al (1986, 1988) and Dunaif and Graf (1989) suggested that insulin may act directly upon ovarian insulin receptors (Jarrett et al 1985) or upon those of insulin-like growth factor 1 (IGF-1) (Poretsky et al 1985, Bayer et al 1986) to stimulate production of androgens. In support of this hypothesis, prolonged hyperinsulinaemia has been shown to increase binding to and the numbers of IGF-1 receptors in rat ovarian stroma (Poretsky et al 1988), whilst in vitro studies have demonstrated the production of androgens by ovarian stroma from hyperandrogenic women in a dose-dependent manner (Barbieri et al 1986, Poretsky and Kalin 1987). However, sustained hyperinsulinaemia in hyperandrogenic women does not stimulate production of testosterone from the ovary in vivo (Nestler et al 1987, Stuart et al 1987, Dunaif and Graf 1989).

The relationship between insulin, and ovarian production and serum concentrations of androgens, is complex (Dunaif et al 1988). Although fasting and summed insulin were related to free T ratios in the study population as a whole, this did not apply to any individual group of PCO or control subjects except for the correlation between the summed concentration of insulin

and free T in the obese women with PCO, and even this applied only to "overweight" but not more obese women with PCO. Correlations between concentrations of insulin and testosterone or androstenedione have been demonstrated (Burghen et al 1980, Chang et al 1983b, Shoupe et al 1983, Pasquali et al 1983, Bruno et al 1985, Jailal et al 1987, Dunaif et al 1987) but not confirmed by others (Geffner et al 1986, Alper and Garner 1987, Sathanandan et al 1988). Most studies that have demonstrated a relationship between testosterone and insulin have been smaller than this and have, therefore, been subject to possible statistical error, whilst larger studies are inconsistent in their conclusions (Dunaif et al 1987, Pasquali et al 1983). The differences may also reflect differing criteria for selection of subjects.

Maintained elevation of circulating concentrations of insulin using hyperinsulinaemic euglycaemic clamping methods (DeFronzo et al 1979) has been shown to cause an acute increase in the concentrations of A but not of T in women with PCO (Stuart et al 1987, Nestler et al 1987, Dunaif and Graf 1989). The disparity between the responses of A and T has not been explained, but attributed to a shortage of substrate, a preferential secretion of A, or to time-dependent changes in the effect of insulin upon the ovary (Dunaif and Graf 1989). A more

prolonged hyperinsulinaemic stimulus did not increase ovarian secretion of androgen by the ovary in rats, although insulin and IGF-1 receptor numbers were increased (Poretsky et al 1988). In contrast, administration of diazoxide, which inhibits endogenous insulin release, to obese women with PCO, also resulted in decreased concentrations of free and total T, but no change in A, SHBG or DHEAS levels (Nestler et al 1989). The decreased concentration of T was attributed to a diazoxide-induced reduction in insulin stimulation of the ovary.

In contrast to the findings of Schriock et al (1988), there was no confounding close relationship between insulin and testosterone to influence that existing between insulin and DHEAS in the PCO groups. This study is not able to determine any influence of DHEAS upon tissue sensitivity to insulin, and no relationship was demonstrated between DHEAS and insulin in any group.

Pasquali et al (1987) proposed that as concentrations of LH were lower in obese than non-obese women, the action of insulin on the ovary in non-obese women with PCO may be additive to that of LH whereas in obese women, by contrast, insulin alone may be sufficient to sustain the increased ovarian secretion of androgens. However, in

this study, LH values in obese and non-obese women with PCO were similar and there was no relationship between BMI and serum LH concentrations. Indeed, the concentrations of T, but not of A, was lower in obese than in non-obese women with PCO, in contrast to that associated with simple obesity, whereas had LH and insulin effects been additive, obese subjects with PCO would have been expected to have higher concentrations of androgens.

Only two of the subjects with PCO had a minor increase in prolactin concentration, confirming that prolactin is not implicated in the insulin resistance of the majority of women with PCO.

GLUCAGON AND INSULIN RESISTANCE IN PCO

In this study fasting and glucose-induced concentrations of glucagon were similar in obese and non-obese women with PCO and in non-obese control women, despite significant differences in concentrations of insulin and insulin resistance. Thus, the hypothesis of elevated serum concentrations of glucagon in women with PCO is not supported.

The greater response of glucagon in the obese than in non-obese normal women confirms the effect of obesity upon glucagon concentrations (Borghi et al 1984). Glucagon values did not decrease in response to glucose in this group, as they did in women with PCO and non-obese control subjects. Thus, the mechanism responsible for insulin resistance and hyperinsulinaemia in women with PCO differs from that responsible for insulin resistance associated with obesity (Borghi et al 1984), NIDDM (Reaven et al 1987), gonadal dysgenesis (Costin and Kogut 1985) and glucagonoma (Boden et al 1978), in all of which conditions hyperglucagonaemia co-exists. Indeed, Dunaif et al (1989) considered that PCO is associated with a unique disorder of insulin action.

Although resistance of pancreatic alpha-cells to insulin may explain the failure of glucose to suppress glucagon secretion in obese subjects (Borghi et al 1984) the data from this study indicate normal reactivity of pancreatic alpha-cells to insulin in women with PCO despite associated insulin resistance.

The normal response of glucagon to glucose in the hyperandrogenic women in this study is consistent with a lack of hepatic insulin resistance, resistance to insulin only occurring within the peripheral tissues. Sustained

hyperglucagonaemia, in contrast, is a feature of syndromes associated with both hepatic and peripheral insulin resistance, such as non-insulin dependent diabetes mellitus (Reaven et al 1987) and gonadal dysgenesis (Costin and Kogut 1985), and with administration of drugs such as danazol (Williams et al 1985) and synthetic androgens (Williams et al 1986) which induce insulin resistance and have prominent hepatic metabolism. Peiris et al (1989a) showed insulin resistance in hyperandrogenic women to be the result of peripheral but not hepatic resistance to the action of insulin. This would be consistent with the findings of this study. However, Dunaif et al (1989) suggested that peripheral and hepatic insulin resistance coexist in PCO. Subtle abnormalities of glucagon in women with PCO may not have been detected by this study, particularly as assessment of hepatic extraction or secretion rates of glucagon could not be assessed.

A relationship between concentrations of total testosterone and androstenedione and those of glucagon in the basal state and in response to oral glucose was shown for the obese women with PCO, and in particular the group with a BMI in excess of 30 kg/m^2. The number of women in these groups was sufficient to permit statistical comparison with only small risk of chance error.

Nevertheless, interpretation of these relationships is complicated by demonstrating mean concentrations of total testosterone, androstenedione and glucagon similar to those in non-obese women with PCO although the latter group did not exhibit similar relationships. The results suggest that as concentrations of total testosterone and androstenedione increase, those of glucagon decrease, a pattern of response opposite to that proposed or which has been identified in other hyperglucagonaemic states. These data do not establish whether testosterone and androstenedione directly influence concentrations of glucagon in plasma, therefore serum concentrations of androgens were altered therapeutically and the influence upon concentrations of glucagon re-examined (Chapters 5 and 6).

GLYCOSYLATED HAEMOGLOBIN IN PCO

This study has demonstrated that obese women with PCO have a significantly greater blood glucose response to oral glucose loading in comparison with that in non-obese women with PCO and weight-matched normal women. Dunaif et al (1987) found a 20% incidence of impaired glucose tolerance in an obese group of women with PCO (mean body weight 154%), whilst this study found an incidence of 2 of

24 women (8%)(mean BMI, 32.5kgm^{-2}). These women were not previously recognised as having any abnormality of glucose tolerance.

HbA_1 and fasting glucose concentrations did not differ significantly between the PCO and normal women. A significant positive correlation between HbA_1 and summed glucose values was demonstrated, but only after exclusion of data from one patient with extreme values.

Measurement of HbA_1 is less sensitive than the OGTT for the full assessment of glycaemic control. However, an OGTT is a time-consuming investigation, which would not be performed in the normal investigation of women presenting with symptoms suggestive of PCO except in the research environment, and which assesses acute glycaemic responses only. HbA_1 concentrations, however, reflect levels of blood glucose over a more prolonged interval (Bunn et al 1976). It was hoped to be able to demonstrate that assessment of the concentration of HbA_1 would be of value in the screening of women with PCO to easily select those who would be at an increased risk for abnormal or impaired glucose tolerance, and who could then undergo further investigation of glycaemic control.

These data have demonstrated, however, that the measurement of HbA_1 is not sufficiently discriminatory to enable identification of impaired glucose tolerance in PCO. However, the overall incidence of impaired glucose tolerance in the subjects was low. If subjects with high fasting or 1 hour blood glucose values were selected for further study, the incidence of elevated HbA_1 values may be higher. Nevertheless, the HbA_1 values suggest that in the physiological state, despite the presence of hyperinsulinaemia and insulin resistance, abnormal mean 24 hour glucose levels are not a significant feature of most obese women with PCO. This suggests that hyperinsulinaemia is a secondary response to insulin resistance, rather than a response to impaired glycaemic levels.

CHAPTER 5: SERUM GONADOTROPHINS AND SEX STEROIDS IN RESPONSE TO TREATMENT WITH ANTIGONADOTROPHINS, ANDROGENS OR ANTIANDROGENS IN NORMAL WOMEN AND WOMEN WITH PCO

INTRODUCTION

Many studies have demonstrated that alteration in the sex steroids and gonadotrophins in women, by treatment with antigonadotrophins, sex steroids or antiandrogens, influences glucose tolerance and insulin levels (Yen and Vella 1965, Spellacy et al 1972, Beck et al 1975, Cole and Kitabchi 1978, Gossain et al 1983, Shoupe et al 1983, Wynn and Godsland 1986).

Cyproterone acetate (CPA) is a derivative of hydroxyprogesterone used in the treatment of hirsutism and acne. It possesses anti-androgenic, progestational, anti-oestrogenic and anti-gonadotrophic properties and reduces synthesis and secretion of glucocorticoids when used in high doses (Neumann 1987). Alone, or in combination with ethinyl oestradiol (35-50 micrograms daily), CPA induces a rapid fall in the serum concentrations of total T, free T, A, DHEAS, LH, FSH, oestrone and oestradiol and an increase in the concentration of SHBG (Falsetti et al 1987, Couzinet et al 1986).

Spironolactone has complex anti-androgenic activity, including inhibition of 17-hydroxylase and 17,20-lyase activity in the adrenal and ovary. It also increases aromatase activity, thereby decreasing concentrations of total T and A of ovarian and adrenal origin (Young et al 1987). It is effective in the treatment of hirsutism in women with PCO (Givens 1985) although it has adverse oncogenic effects associated with long term use in animals (Committee of Safety of Medicines, 1988).

Treatment with analogues of gonadotrophin releasing hormone (GnRH) results in a state of hypogonadotrophic hypogonadism in normal women and in those with PCO, after an initial stimulation interval lasting for up to 14 days (Calogero et al 1987). The decrease in the secretion and peripheral concentrations of T and A may be less than that of oestradiol in the same woman (Rittmaster 1988). Analogues of GnRH induce a similar degree of suppression of endometriosis as does treatment with danazol (Tummon et al 1989). The GnRH agonist analogues are effective in the treatment of menorrhagia due to uterine leiomyomata and in suppression of gonadotrophin and ovarian activity in women with PCO (Chang et al 1983b, Fleming et al 1985, Lanzone et al 1987, Steingold et al 1987, Couzinet et al 1986, Calogero et al 1987).

Danazol, widely used for treatment of menorrhagia and endometriosis, is a synthetic non-steroidal testosterone derivative structurally related to oxymetholone and possesses complex androgenic activity (Bevan et al 1984). By high-affinity binding to androgen receptors on sex hormone-binding globulin (SHBG), the androgen binding capacity of SHBG is markedly reduced.

This chapter examines the influence of therapeutic modulation of gonadotrophins and androgens by antigonadotrophins, androgens or antiandrogens in normal women and in those with PCO.

METHODS

Women in the PCO groups were allocated non-randomly to one of three treatment groups, in accordance with their therapeutic requirements (Table 5.1). Twenty-six women received cyproterone acetate 2 mg and ethinyl oestradiol 35 micrograms ['CPA/EE'](Dianette, Schering Health Care, Burgess Hill, Sussex) daily for 21 days, recommencing after a 7 day drug-free interval. Six women received spironolactone, 100mg twice daily, whilst 9 women received buserelin ([D-ser (But)^6] LHRH-(1-9)-ethylamide, Suprefact, Hoescht, Hounslow, Middlesex), 1200 micrograms

intranasally in divided doses daily, and 3 women received no treatment.

Women in the control groups were allocated to one of two treatment groups, selected by computer-randomised programme (Table 5.1). Six women received danazol (Sterling Winthrop, Guildford, Surrey) 400mg daily, and 10 women received goserelin depot (C-ser(But)^{6}, azgly^{10}-GnRH, Zoladex, ICI Pharmaceuticals, Alderley Edge, Cheshire), 3.6 mg subcutaneously, every 28 days. Seven women with leiomyomata received no treatment.

After 42 days, all the women receiving treatment were studied, with blood samples taken in an identical manner to pre-treatment studies. Samples from each subject were measured in the same batch to eliminate interassay variation.

TABLE 5.1: OPTIONS FOR TREATMENT FOR STUDY SUBJECTS.

TREATMENT GROUP	SUBJECT NUMBER	TOTAL (n)
PCO SUBJECTS		
Cyproterone acetate/ ethinyl oestradiol	3, 4, 7, 12, 15, 16, 17, 18, 20, 21, 23, 25, 26, 27, 28, 31, 32, 33, 35, 36, 37, 38, 39, 41, 42, 43	26
Spironolactone	10, 19, 22, 30, 34, 40	6
Buserelin	2, 5, 8, 9, 11, 13, 14, 24, 44	9
CONTROL SUBJECTS		
Danazol	45, 50, 54, 58, 61, 62	6
Goserelin	46, 47, 48, 52, 53, 56, 60, 63, 65, 66	10

RESULTS

CONTROL SUBJECTS

The mean body mass index before treatment did not differ between the two treatment groups (Table 5.2). During the treatment period, the median BMI in the danazol-treated group increased by 2.1%, equivalent to an increase in the BMI of $0.7 kgm^{-2}$ but there was no change in the BMI in the goserelin-treated group (Table 5.2).

Concentrations of LH, FSH, total T, A, free T, DHEAS and SHBG in serum before and during treatment with danazol or goserelin are shown in Table 5.3. Before treatment, there was no difference in values between the two groups. The concentration of LH in the goserelin-treated group decreased to 4.6 (2.6) iu/l, lower than in the danazol-treated group (7.9 (1.9) iu/l, $p<0.01$). Concentrations of total T and A remained unchanged in the goserelin group but decreased in the danazol-treated subjects (total T, 1.9 (0.7) to 1.3 (0.5) nmol/l; A, 5.8 (1.7) to 4.5 (1.8) nmol/l respectively, $p<0.05$). The mean concentration of SHBG was markedly reduced in danazol-treated subjects from 65 (33) to 9 (10) nmol/l ($p<0.01$) and there was a corresponding increase in the calculated free testosterone index (3.3 (1.6) to 13.3 (4.2) units; $p<0.01$). The SHBG

TABLE 5.2: BODY MASS INDEX (BMI) AND CHANGE IN BMI (%) IN CONTROL SUBJECTS TREATED WITH GOSERELIN OR DANAZOL

	DANAZOL			GOSERELIN		
n	6			10		
NUMBER	BEFORE	DURING	% CHANGE	NUMBER	BEFORE	DURING	% CHANGE
45	22.8	23.6	+3.5	46	24.8	24.0	-3.2
50	18.9	19.2	+1.0	47	21.6	21.2	-1.9
54	23.8	24.1	+1.3	48	21.4	21.5	+0.5
58	24.0	25.6	+6.7	52	18.5	18.2	-1.6
61	18.6	19.6	+5.4	53	23.8	24.1	+1.3
62	19.2	19.6	+2.2	56	28.9	29.4	+1.7
				60	21.5	21.4	-0.5
				63	28.0	28.9	+3.6
				65	18.2	18.1	-0.5
				66	31.8	30.2	-5.0
Median	21.0	21.6	+2.9	Median	22.7	22.8	0.0
Range	(18.6-24.0)	(19.2-25.6)	(+1.0-+6.7)	Range	(18.2-31.8)	(18.1-30.2)	(-5.0-+3.6)

TABLE 5.3: MEAN (SD) CONCENTRATIONS OF GONADOTROPHINS, ANDROGENS AND SHBG IN NORMAL WOMEN BEFORE, AND DURING TREATMENT WITH DANAZOL OR GOSERELIN.

	DANAZOL		GOSERELIN	
n	6		10	
	BEFORE	DURING	BEFORE	DURING
LH (iu/l)	8.3 (2.5)	7.9 (1.9)	7.6 (2.6)	4.6 (2.6)##
FSH (iu/l)	6.1 (4.2)	5.7 (3.9)	5.7 (2.9)	4.0 (5.1)
T (nmol/l)	1.9 (0.7)	1.3 (0.5)#	2.0 (0.6)	1.6 (0.6)
A (nmol/l)	5.8 (1.7)	4.5 (1.8)#	7.5 (2.8)	5.7 (2.6)
DHEAS (umol/l)	3.8 (1.5)	3.0 (1.3)	4.9 (4.4)	4.6 (1.2)
SHBG (nmol/l)	65 (33)	9 (10)##	51 (17)	45 (12)
Free T (units)	3.3 (1.6)	13.3 (4.2)##	4.3 (1.5)	3.8 (1.7)

#$p<0.05$ ##$p<0.01$, vs pre-treatment

concentration did not change in the goserelin-treated group. No change was apparent in DHEAS values in either group.

PCO SUBJECTS.

Clinical data for the 3 groups of women with PCO, treated with CPA/EE, spironolactone or buserelin, are shown in Table 5.4. No significant change in BMI was observed in any group during treatment.

Concentrations of LH, FSH, total T, A, free T, DHEAS and SHBG in the serum in the three groups before and during treatment are shown in Table 5.5. LH and FSH values decreased in the women treated with CPA/EE and buserelin but did not change in those treated with spironolactone. Treatment with CPA/EE markedly reduced values of total T from 3.6 (1.3) to 2.5 (1.2) nmol/l, A from 13.9 (5.3) to 7.2 (4.9) nmol/l, DHEAS from 8.0 (2.9) to 5.6 (1.5) micromol/l and free T from 12.2 (6.1) to 1.7 (5.9) units, and increased those of SHBG from 34 (14) to 162 (41) nmol/l ($p<0.001$ for all values). In buserelin-treated subjects, values of total T were reduced from 2.9 (1.4) to 2.0 (1.0) nmol/l and those of A from 12.5 (4.1) to 9.5 (3.1) nmol/l ($p<0.05$), but those of DHEAS, SHBG and free T were unchanged. Spironolactone reduced values of

TABLE 5.4: BODY MASS INDEX (BMI) AND CHANGE IN BMI (%) IN SUBJECTS WITH PCO TREATED WITH CYPROTERONE ACETATE AND ETHINYL OESTRADIOL (CPA/EE), BUSERELIN OR SPIRONOLACTONE.

	CPA/EE		
n	26		
NUMBER	BEFORE	DURING	% CHANGE
3	25.7	25.4	-1.2
4	23.2	23.9	+3.0
7	28.1	28.1	0
12	19.3	20.3	+5.2
15	24.6	24.4	-0.8
16	20.5	19.4	-5.4
17	29.1	26.5	-8.9
18	31.1	31.3	+0.6
20	24.7	24.4	-0.7
21	32.8	32.0	-2.4
23	23.9	23.0	-3.8
25	24.1	24.3	+0.8
26	26.1	27.9	+6.9
27	21.5	21.7	+0.9

CPA/EE (Cont.)			
NUMBER	BEFORE	DURING	% CHANGE
28	33.0	31.2	-5.5
31	24.4	23.2	-4.9
32	33.5	33.0	-1.5
33	22.9	22.4	-2.2
35	37.8	37.9	+0.3
36	38.8	38.5	-0.8
37	19.6	20.6	+5.1
38	19.1	18.8	-1.6
39	24.5	24.1	-1.6
41	22.4	21.9	-2.2
42	28.8	29.3	+1.7
43	24.9	24.5	-1.6
Median	24.8	24.5	-1.0
Range	(19.1-38.8)	(18.8-38.5)	(-8.9-+6.9)

TABLE 5.4: (Cont.)

BUSERELIN

n 9

NUMBER	BEFORE	DURING	% CHANGE
2	31.7	31.9	+0.6
5	26.8	26.8	0
8	26.7	27.0	+1.0
9	20.7	21.1	+1.1
11	35.4	35.4	0
13	30.6	29.3	-4.2
14	34.0	34.2	+0.6
24	34.2	34.2	0
44	43.9	47.4	+8.0
Median	31.7	31.9	+0.6
Range	(20.7-43.9)	(21.1-47.4)	(-4.2-+8.0)

SPIRONOLACTONE

6

NUMBER	BEFORE	DURING	% CHANGE
10	23.2	23.0	-0.9
19	41.5	41.0	-1.2
22	19.5	18.4	-5.6
30	29.9	29.8	-0.3
34	33.4	34.6	+3.6
40	22.1	21.3	-3.6
Median	27.1	26.4	-1.1
Range	(19.5-41.5)	(18.4 41.0)	(-5.6 +3.6)

TABLE 5.5: MEAN (SD) CONCENTRATIONS OF GONADOTROPHINS, ANDROGENS AND SHBG IN WOMEN WITH PCO BEFORE AND DURING TREATMENT WITH CYPROTERONE ACETATE AND ETHINYLOESTRADIOL (CPA/EE), BUSERELIN OR SPIRONOLACTONE.

	CPA/EE		BUSERELIN		SPIRONOLACTONE	
n	24		9		6	
	BEFORE	DURING	BEFORE	DURING	BEFORE	DURING
LH (nmol/l)	13.3 (6.5)	5.7 (5.1)$^{###}$	12.8 (5.3)	6.9 (6.3)$^{\#}$	11.9 (4.7)	11.6 (7.0)
FSH (nmol/l)	4.1 (1.8)	2.1 (1.4)$^{###}$	5.6 (2.1)	4.2 (1.6)$^{\#}$	5.3 (1.1)	4.2 (3.1)
T (nmol/l)	3.6 (1.3)	2.5 (1.2)$^{###}$	2.9 (1.4)	2.0 (1.0)$^{\#}$	2.8 (0.8)	2.1 (0.4)$^{\#\#}$
A (nmol/l)	13.9 (5.3)	7.2 (4.9)$^{###}$	12.5 (4.1)	9.5 (3.1)$^{\#}$	12.0 (2.7)	9.0 (3.3)
DHEAS (umol/l)	8.0 (2.9)	5.6 (1.5)$^{###}$	5.8 (3.7)	5.9 (2.5)	8.5 (4.6)	7.1 (2.7)
SHBG (nmol/l)	34 (14)	162 (41)$^{###}$	25 (12)	25 (7)	20 (7)	18 (4)
Free T(units)	12.2 (6.1)	1.7 (5.9)$^{###}$	13.4 (7.8)	9.2 (6.7)	15.6 (5.9)	12.2 (4.0)

$^{\#}p<0.05$ $^{\#\#}p<0.01$ $^{###}p<0.001$

total T from 2.8 (0.8) to 2.1 (0.4) nmol/l ($p<0.01$), but did not affect those of free T, A, DHEAS or SHBG.

DISCUSSION

Significant changes in gonadotrophin and androgen values occurred in response to administration of danazol or goserelin to normal women, and cyproterone acetate and ethinyl oestradiol, buserelin or spironolactone to women with PCO. The changes are in accordance with other published reports of the use of danazol (Bevan et al 1984, Tummon et al 1989), goserelin or other analogues of GnRH in normal women (Tummon et al 1989), and cyproterone acetate and ethinyl oestradiol (Couzinet et al 1986, Falsetti et al 1987), buserelin or other analogues of GnRH (Chang et al 1983a, Steingold et al 1987) and spironolactone (Givens 1985, Young et al 1987) in women with PCO.

Fasting concentrations of glucose, insulin, C-peptide and glucagon and their responses to glucose were reassessed in the changed environment during treatment to determine the role of androgens in the development of insulin resistance and in modification of plasma

concentrations of glucagon. The details of these studies are presented in Chapter 6.

CHAPTER 6: CHANGE IN GLUCOSE, INSULIN, C-PEPTIDE AND GLUCAGON RESPONSES TO GLUCOSE LOADING IN NORMAL WOMEN AND IN WOMEN WITH POLYCYSTIC OVARY SYNDROME DURING MODULATION OF SERUM ANDROGEN AND/OR GONADOTROPHIN CONCENTRATIONS.

INTRODUCTION

A number of published studies have investigated the change in glucose and insulin values associated with alteration of concentrations of androgens in women with PCO, by treatment with spironolactone (Shoupe and Lobo 1984), an analogue of GnRH (Geffner et al 1986), ketoconazole (Pepper et al 1987), a combined oral contraceptive (Cole and Kitabchi 1978) and cyproterone acetate with ethinyl oestradiol and diet (Pasquali et al 1986).

Other studies have investigated the effects of experimental hyperinsulinaemia on androgen values in women with PCO, acutely during an oGTT (Smith et al 1987), by hyperinsulinaemic euglycaemic clamp studies (Stuart et al 1987, Nestler et al 1987, Dunaif and Graf 1989) and by inhibition of pancreatic insulin secretion with diazoxide (Nestler et al 1989).

The role of androgens in the development of insulin resistance in women with PCO has yet to be elucidated. As discussed in Chapter 4, androgens may be responsible for alteration in peripheral insulin sensitivity (Peiris et al 1989), but insulin may increase secretion of androgens from the ovary (Barbieri et al 1988). Suppression of circulating androgens by administration of spironolactone (Shoupe et al 1983) was associated with a decrease in fasting and glucose stimulated concentrations of insulin although there was no correlation found between changes in concentrations of insulin and total testosterone. Treatment with analogues of GnRH, which markedly reduces circulatory concentrations of sex steroids, has no effect upon the insulin response to glucose (Geffner et al 1986), and cyproterone acetate does not influence concentrations of insulin independently from the effects of weight loss (Pasquali et al 1986).

Combined oestrogen and gestagen contraceptive preparations have a deleterious effect upon glucose tolerance which may be related to the progestagen (Wynn et al 1979) as ethinyl oestradiol alone does not alter glucose tolerance (Spellacy et al 1972). Even in those women with normal glucose tolerance, serum concentrations of insulin are increased (Yen and Vella 1968, Gossain et al 1983, Wynn and Godsland 1986). Progesterone, unlike

ethinyl oestradiol, augments glucose-induced release of insulin from murine pancreatic cells in vitro, possibly by an alteration of islet cell sensitivity to glucose (Nielsen 1984).

Binding of insulin to receptors on monocytes or erythrocytes obtained from non-obese normal women during the follicular phase of the menstrual cycle is similar to that during administration of low-dose combined oral contraception (Tsibris et al 1980). However, administration of a combination of ethinyl oestradiol and levonorgestrel induces a temporary state of peripheral insulin resistance, although without any change in hepatic sensitivity to insulin or correlation between peripheral insulin sensitivity and the insulin response to oral glucose (Skouby et al 1987, Kasdorf and Kalkhoff 1988).

In a woman with PCO and acanthosis nigricans, Cole and Kitabchi (1978) reported reversal of hyperinsulinaemia with a combination of mestranol and norethisterone, although this finding has not been substantiated by further studies.

Combined oral contraceptives do not affect plasma glucagon responses to oral or intravenous glucose administration in normal women (Gossain et al 1983),

although the response of glucagon to arginine stimulation is variable (Beck et al 1975, Gossain et al 1983). In animal studies, administration of gestagen results in increased portal vein concentrations of glucagon, whilst administration of oestrogens has the opposite effect (Mandour et al 1977).

Cyproterone acetate used alone does not appear to affect serum concentrations of insulin, nor is there a direct effect upon body weight (Breckwold 1980). In women with PCO, the combination of CPA (50 mg), ethinyl oestradiol (30 micrograms) and weight loss resulted in a fall in serum androgen values, although no correlation was demonstrated between the change in concentrations of androgens and either insulin secretion or insulin resistance, and women with similar weight loss without other treatment showed an equivalent reduction in fasting insulin values (Pasquali et al 1986).

In hyperinsulinaemic women with PCO, treatment with spironolactone resulted in a significant fall in fasting and glucose-stimulated concentrations of insulin, although without correlation between changes in insulin values and those of total T (Shoupe and Lobo 1984). Spironolactone may accelerate glucose utilisation (Evans, personal communication in Peiris et al 1987). The effects of

spironolactone upon plasma glucagon values have not been reported.

The insulin response to oral glucose is not affected by treatment with GnRH agonist analogues (I.C.I. in-house data on file) although their effect upon plasma glucagon values are not known. Geffner et al (1986) reported that a long-acting analogue of GnRH did not affect fasting or glucose-stimulated concentrations of insulin in three women with PCO, although serum values of T, A and oestradiol were suppressed to castrate levels. Values of LH were not reported. They suggested that hyperandrogenism is not responsible for maintainance of hyperinsulinaemia in women with PCO, although their findings also suggest that hyperinsulinaemia does not maintain increased ovarian secretion of androgens in the presence of low serum concentrations of LH.

Other studies have investigated the effects of experimental hyperinsulinaemia on androgen values in women with PCO, acutely during an oGTT (Smith et al 1987), by hyperinsulinaemic euglycaemic clamp studies (Stuart et al 1987, Nestler et al 1987, Dunaif and Graf 1989), and by inhibition of pancreatic insulin secretion with diazoxide (Nestler et al 1989).

Sex differences in insulin action are modest. Diabetes mellitus is more common in post-pubescent males than females matched for weight and age (Kannel et al 1979). Insulin-mediated glucose disposal is 45% lower in men (Yki-Jarvinen 1984), and men have higher plasma concentrations of insulin but equivalent concentrations of C-peptide compared with age- and weight-matched women (Hale et al 1985), illustrating that androgens may mediate an inhibition of hepatic insulin clearance. In mice, male animals treated with streptozotocin or by subtotal pancreatectomy develop diabetes more frequently than females, and administration of androgens to the females increases the subsequent frequency of diabetes (Paik et al 1982).

Testosterone therapy has no effect upon fasting glucose concentrations in normal subjects (Taalat et al 1957) and implants of testosterone or androstenedione administered to female Rhesus monkeys do not significantly alter basal or glucose-stimulated insulin or C-peptide values (Billiar et al 1987). In normal men, DHEAS does not alter peripheral insulin sensitivity (Nestler et al 1988).

Several synthetic androgens induce hyperinsulinaemia and impair glucose tolerance, in contrast to natural

androgens. Methyltestosterone (McCullagh and Jones 1941, McCullagh and Lewis 1942), oxymetholone (Woodward et al 1981) and methandienone (Wynn et al 1962) impair glucose tolerance and induce insulin resistance, whilst oxymetholone also induces severe hyperglucagonaemia (Williams et al 1986). These effects are evident in men, as seen from the development of insulin resistance associated with use of anabolic steroids in powerlifters (Cohen and Hickman 1987).

During treatment with danazol, hyperinsulinaemia, insulin resistance and hyperglucagonaemia develop independently of any associated weight gain although basal insulin concentrations are not increased (Wynn 1977, Gottenberg et al 1982, Williams et al 1985, Vaughan Williams et al 1989). It has been suggested that hyperglucagonaemia during danazol treatment contributes to the development of insulin resistance (Williams et al 1985) via a direct effect upon the pancreas (Gottenberg et al 1982) although alternatively the "androgenic nature" of danazol may be responsible (Williams et al 1985, Vaughan Williams et al 1989).

The data presented in Chapter 5 detailed the changes in concentrations of gonadotrophins and androgens in normal women and in women with PCO during treatment aimed

at modifying the hormonal milieu. Fasting concentrations of glucose, insulin, C-peptide and glucagon and their responses to glucose were reassessed in this changed endocrine environment to determine the role of androgens in the development of insulin resistance and alterations of plasma concentrations of glucagon.

METHODS

After completion of the oGTT (Chapter 2), women in the PCO groups were allocated non-randomly to one of three treatment groups, in accordance with their therapeutic requirements, and women in the control groups were allocated to one of two treatment groups, selected by computer-randomised programme, as described in Chapter 5 (Table 5.1). After 42 days, all the women receiving treatment underwent a second oGTT, performed in a manner identical to that described above. Medications were taken as normal on the day of the repeat oGTT.

Samples from each subject were measured in the same batch to eliminate interassay variation.

RESULTS

CONTROL SUBJECTS.

Fasting concentrations of insulin and C-peptide were unchanged during treatment in both obese and non-obese normal women, but the fasting concentration of glucagon had increased from 63 (16) to 215 (84) pmol/l ($p<0.01$) and that of glucose decreased from 4.4 (0.4) to 3.8 (0.4) mmol/l ($p<0.05$) in danazol-treated subjects. There was no change in fasting concentrations of insulin or glucagon in goserelin-treated subjects (Table 6.1).

The summed concentrations of glucose after glucose loading did not differ from the respective pre-treatment values in either group (Table 6.2). However, in the danazol-treated group, glucose values between 30 and 90 minutes after glucose loading were greater than corresponding values before treatment ($p<0.05$), but had returned to fasting levels by 150 minutes. There was a marked increase in the insulin response in this group, values between 30 and 150 minutes after glucose loading and the summed insulin value being greater than the respective values before treatment (summed values 150.2 (30.4) iu/l before treatment v. 413.3 (215.0) iu/l during

TABLE 6.1: FASTING CONCENTRATIONS OF PLASMA GLUCOSE, INSULIN, C-PEPTIDE, AND GLUCAGON IN RESPONSE TO ORAL GLUCOSE BEFORE AND DURING TREATMENT IN NORMAL WOMEN TREATED WITH DANAZOL OR GOSERELIN

	BEFORE TREATMENT		DURING TREATMENT	
	DANAZOL	GOSERELIN	DANAZOL	GOSERELIN
n	6	10	6	10
Glucose (mmol/l)	4.4(0.4)	4.3(0.5)	3.8(0.4)	4.3(0.5)
Insulin (umol/l)	4.1(0.9)	5.3(2.0)	4.3(3.4)	6.7(3.3)
Glucagon (pmol/l)	63(16)	64(27)	215(84)##	73(27)
n	4	4	4	4
C-peptide (pmol/l)	0.4(0.1)	0.3(0.3)	0.8(0.3)	0.4(0.3)

#p<0.05 ##p<0.01 v. pre-treatment values.

TABLE 6.2: SUMMED VALUES OF PLASMA GLUCOSE, INSULIN, C-PEPTIDE, AND GLUCAGON IN RESPONSE TO ORAL GLUCOSE BEFORE AND DURING TREATMENT IN NORMAL WOMEN TREATED WITH DANAZOL OR GOSERELIN

	BEFORE TREATMENT		DURING TREATMENT	
	DANAZOL	GOSERELIN	DANAZOL	GOSERELIN
n	6	10	6	10
Glucose (mmol/l)	29.0(4.2)	29.6(5.4)	33.6(5.4)	25.5(4.7)
Insulin (iu/l)	150.2(30.4)	178.6(77.3)	413.3(215.0)#	171.4(70.4)
Glucagon (pmol/l)	337(79)	291(146)	839(465)#	323(133)
n	4	4	4	4
C-peptide (pmol/l)	11.4(1.4)	6.8(2.9)	20.1(8.5)#	6.1(4.6)

#$p<0.05$ ##$p<0.01$ v. pre-treatment values.

treatment, $p<0.05$). The insulin response was unchanged in goserelin-treated women.

C-peptide data were only available in 4 subjects in each group. The cumulative C-peptide response to oral glucose was greater during treatment with danazol, from 11.4 (1.4) to 20.1 (8.5) pmol/l) than during treatment with goserelin (Table 6.2).

The cumulative glucagon response to oral glucose increased from 337 (79) pmol/l to 839 (465) pmol/l in the danazol-treated group ($p<0.05$, Table 6.2) but was unchanged in the goserelin-treated women.

No relationship was demonstrated between the changes in the insulin or glucagon response to glucose loading as a result of danazol or goserelin treatment and the changes in androgen or LH concentrations in either group.

PCO SUBJECTS

Fasting concentrations of glucose, insulin, glucagon and C-peptide did not differ from pre-treatment values in any of the treatment groups (Table 6.3).

TABLE 6.3: FASTING CONCENTRATIONS OF PLASMA GLUCOSE, INSULIN, C-PEPTIDE AND GLUCAGON IN RESPONSE TO ORAL GLUCOSE BEFORE AND DURING TREATMENT IN WOMEN WITH PCO TREATED WITH CYPROTERONE ACETATE AND ETHINYL OESTRADIOL, BUSERELIN OR SPIRONOLACTONE.

	BEFORE TREATMENT			DURING TREATMENT		
	CPA/EE	BUSERELIN	S'LACTONE	CPA/EE	BUSERELIN	S'LACTONE
n	26	9	6	26	9	6
Glucose mmol/l	4.2(0.5)	4.8(1.4)	4.0(0.5)	4.2(0.5)	4.9(1.2)	4.4(0.3)
Insulin iu/l	7.9(5.5)	15.0(11.9)	10.5(4.2)	8.4(4.6)	15.7(11.5)	12.1(4.1)
Glucagon pmol/l	66(23)	71(13)	84(13)	80(41)	68(19)	95(26)
n	9	4	3	9	4	3
C-peptide pmol/l	0.9(0.6)	1.2(0.7)	0.9(0.1)	0.9(0.7)	1.4(0.9)	1.1(0.1)

TABLE 6.4: SUMMED VALUES OF PLASMA GLUCOSE, INSULIN, C-PEPTIDE AND GLUCAGON IN RESPONSE TO ORAL GLUCOSE BEFORE AND DURING TREATMENT IN WOMEN WITH PCO TREATED WITH CYPROTERONE ACETATE AND ETHINYL OESTRADIOL, BUSERELIN OR SPIRONOLACTONE.

	BEFORE TREATMENT			DURING TREATMENT		
	CPA/EE	BUSERELIN	S'LACTONE	CPA/EE	BUSERELIN	S'LACTONE
n	26	9	6	26	9	6
Glucose mmol/l	29.2(5.9)	41.4(19.4)	31.7(6.4)	31.4(4.3)	39.8(15.1)	32.3(5.4)
Insulin iu/l	298.2(242.0)	467.7(405.3)	325.3(88.9)	282.7(166.7)	430.0(353.2)	321.4(151.4)
Glucagon pmol/l	255(156)	363(45)	377(72)	333(219)	350(89)	441(369)
n	9	4	3	9	4	3
C-peptide	14.0(8.7)	22.0(14.5)	16.6(3.6)	12.6(7.0)	24.9(12.2)	16.5(2.7)

Treatment with CPA/EE did not alter the summed concentrations of glucose after glucose loading, but there was a delay in the return of blood glucose concentrations to the fasting level, values at 120 and 150 minutes being greater than respective pre-treatment values. Treatment with buserelin or spironolactone did not alter fasting concentrations of glucose (Table 6.3).

None of the treatments influenced the summed values of insulin, C-peptide or glucagon nor were the responses to oral glucose changed (Table 6.4).

No relationship between the changes in concentration of androgen or SHBG and those of insulin or glucagon was demonstrated in any of the groups.

DISCUSSION

In this study, women were investigated to determine whether changes in concentrations of androgens influenced insulin resistance, hyperinsulinaemia and glucagon responses to glucose stimulation in women with PCO. Subjects were not randomised to each treatment and, therefore, although the final composition of the groups

was similar, no comparative between-group data can be undertaken with validity.

Reduction of circulating androgens by administration of an analogue of GnRH had no effect on insulin concentrations or insulin resistance in women with PCO despite a marked decrease in total T and A concentrations. This is in agreement with the findings of Geffner et al (1986). SHBG values and the calculated free T value were unchanged during treatment. Conway et al (1989) have shown that concentrations of total T are greater in women with infertility and PCO than other subjects with PCO diagnosed by ultrasonography. In the current study, the subjects treated with buserelin all complained of infertility, but had total T values similar to those of the whole group of PCO subjects.

In addition, as in the control group, there was no alteration in glucagon values in women with PCO treated with an analogue of GnRH.

Although obesity, hyperinsulinaemia and LH may act independently on ovarian androgen secretion (Dunaif et al 1987), the reduction in the concentration of LH during treatment with GnRH-analogue resulted in a marked diminution of ovarian androgen production in both normal

and PCO subjects without change in insulin values or the BMI. This suggests that, in vivo, insulin alone does not sustain the excess ovarian secretion of T or A in women with PCO, in contrast to the findings of in vitro studies (Barbieri et al 1986).

In spite of decreased circulating concentrations of T and A, those of insulin, C-peptide and glucagon values were unchanged. This is consistent with an absence of influence of T, A or LH on pancreatic, hepatic or peripheral sensitivity to glucose or insulin. However, the methods used may not be sufficiently sensitive to detect small alterations in insulin and glucagon sensitivity, which may have been exposed using hyperinsulinaemic-euglycaemic clamp methods (Skouby et al 1987).

The use of an identical analogue of GnRH for subjects with PCO and control subjects in this study would have been desirable, to enable a direct comparison between these groups to be made, but the treatment requirements and study protocols did not allow this. However, highly significant reductions in the circulating concentrations of gonadotrophins were seen in both groups.

Treatment with spironolactone did not influence insulin resistance, despite a small decrease in serum concentrations of total T. This is at variance with the results of Shoupe and Lobo (1984). The dose of spironolactone used in this study was twice that used by Shoupe and Lobo (1984) and was used for six rather than for two weeks, but the decrease in the concentrations of total T was similar in both studies. The concentration of free T was unchanged in response to treatment with spironolactone in this study, and this may be more important than total T in tissue sensitivity (Peiris et al 1989a).

Peiris et al (1987) suggested that a decrease in insulin values in response to administration of spironolactone may result in an increase in tissue sensitivity to insulin and thus an acceleration of glucose utilisation, although no clinical details or methods used are given to support their conclusions. Improvement in glucose tolerance in response to both spironolactone and bromocriptine therapy has been reported (Blum et al 1981) but in only 2 women with PCO. Prolactin, however, is known to influence insulin resistance, and bromocriptine may alter glucose tolerance independently from the effects of spironolactone.

Spironolactone also affects calcium and magnesium concentrations by its diuretic action and both of these electrolytes may influence tissue sensitivity to insulin (Chapter 7).

The decreases in concentrations of testosterone and androstenedione in the women with PCO during treatment were not associated with changes in those of glucagon. Pre-treatment data from women with PCO in the obese subgroup (BMI >30kg/m^2) showed an inverse relationship between fasting and summed concentrations of glucagon and those of testosterone and androstenedione (Chapters 3 and 4). However, similar analysis of this subgroup during treatment is confounded by inclusion of subjects in three treatment protocols, each potentially influencing concentrations of glucagon by different action, and none of the treatment groups alone included sufficient numbers of subjects for analysis.

The increased insulin response to an oral glucose load in normal women during treatment with danazol (Wynn 1977, Gottenberg et al 1982, Williams et al 1985, Vaughan Williams et al 1989) was confirmed although without change in glucose values. Women treated with goserelin, however, showed no change in fasting glucose or insulin values, nor in glucose or insulin responses to glucose

loading, indicating that goserelin is without significant effect upon insulin action. This may influence the choice of drug for the treatment of endometriosis in those women known to have impaired glucose tolerance (Vaughan Williams et al 1989).

No correlation has been shown between changes in the concentrations of testosterone, androstenedione, or free testosterone and that of insulin associated with the administration of either danazol or goserelin. The hypothesis that the danazol-induced increase in the serum concentration of testosterone is responsible for the development of insulin resistance and hyperglucagonaemia cannot be confirmed (Williams et al 1985, Vaughan Williams et al 1989).

Plasma concentration of glucagon in the fasting state and in response to an oral glucose load significantly increased in non-obese normal women during treatment with danazol. As each subject was used as her own internal control to exclude effects of varied expression of immunoreactive glucagon in the plasma (Jaspan et al 1981), the finding of Williams et al (1985) of severe hyperglucagonaemia in women taking danazol has been confirmed and extended. However, women treated with the GnRH-analogue goserelin, showed no change in the plasma

concentration of glucagon, demonstrating that treatment of endometriosis is not, in itself, responsible for hyperglucagonaemic response to danazol therapy.

Although hyperglucagonaemia was well established within six weeks of starting treatment with danazol, it is not possible to say how rapidly this change developed, nor whether such a change was sustained for the duration of the treatment period. Although a significant increase in weight was seen for the danazol-treated group, the concentration of glucagon was substantially greater than that in the obese control subjects, suggesting that it was not the weight itself that was responsible for the rise in insulin and glucagon levels.

In conjunction with hyperinsulinaemia, an increase in the concentration of glucagon results in a significant shunting of infused glucose towards lipid synthesis and away from glycogen synthesis (DelPrato et al 1987). Danazol is known to increase plasma concentrations of low density lipoproteins and decrease those of high density lipoproteins (Fahraeus et al 1984), possibly as a consequence of the increased insulin and/or glucagon values. In contrast, Farrish et al (1989) have shown that the GnRH-analogue, buserelin, did not alter plasma lipoprotein concentrations when administered for one year.

This is consistent with lack of significant effect of GnRH-analogues and, therefore, endogenous androgens, upon insulin and glucagon action.

One proposed mechanism for the hyperinsulinaemic response to danazol is by a direct stimulation of pancreatic beta-cells (Gottenberg et al 1982). Insulin, however, is a potent inhibitor of glucagon secretion and is the principal regulator of glucagon production in the basal state (Maruyama et al 1984). The development of insulin resistance in response to administration of danazol suggests that insulin becomes less effective in controlling pancreatic glucagon secretion and thereby enables the development of hyperglucagonaemia. It is possible, therefore, that danazol-induced insulin resistance leads to a defect in regulation of the pancreatic alpha-cell, and thus to the development of hyperglucagonaemia.

As confirmed in this study, administration of danazol is associated with a decrease in the serum concentration of SHBG (Bevan et al 1984), probably due to an inhibition of hepatic synthesis (Gershagen et al 1984), and thus a modest rise in calculated free testosterone concentration.

In vitro, testosterone stimulates (Lee et al 1987) whereas insulin inhibits (Plymate et al 1988) synthesis of SHBG. The data in this study do not allow further elucidation of whether changes in concentrations of SHBG in vivo results from a direct action of danazol or an inhibitory effect of insulin.

The changes in concentrations of insulin and glucagon when fasting or in response to an oral glucose load following treatment with either danazol or ethinyl oestradiol and cyproterone acetate are complex. One cannot easily distinguish the direct effects of these drugs on the secretion of insulin and glucagon, and any change in insulin and glucagon secretion which may be secondary to alteration in the secretion of ovarian and adrenal steroids.

CHAPTER 7: CONCLUSIONS AND SUMMARY

The results of these studies suggest that the mechanisms responsible for insulin resistance differs in androgenic conditions of differing aetiology. The lack of a hyperglucagonaemic response to glucose in women androgenised as a result of PCO suggests a different mechanism for insulin resistance in those women from that associated with androgen administration, non-insulin dependent diabetes mellitus and obesity, conditions which are associated with hepatic and peripheral insulin resistance. The increase in concentrations of insulin and glucagon which resulted from treatment with danazol do not appear to be caused directly by increased androgen concentrations.

However, the mechanism for insulin resistance in women with PCO has not been clarified. No consistent relationships between fasting or glucose-stimulated concentrations of insulin, glucagon or glucose and concentrations of androgens were demonstrated, confirming the lack of a simple relationship between concentrations of insulin and androgens. Changes in serum concentrations of oestrogens, which may modify those of androgens, insulin or glucagon, were not measured in this study,

although no relationship to concentrations of androgens or insulin in hyperandrogenic women with PCO was demonstrated by Dunaif et al (1987).

An inverse relationship between concentrations of glucagon and those of testosterone and androstenedione was demonstrated in the obese women with PCO, but not in non-obese or "overweight" women with PCO nor in the control groups. This pattern of response is the opposite to that proposed or to that seen in other states of insulin resistance and hyperglucagonaemia, but it is not possible to establish the possible mechanisms for this unexpected finding from these data. Nevertheless, a decrease in serum concentrations of androgens by therapeutic manipulation did not appear to influence circulating concentrations of glucagon in hyperandrogenic women.

Serum concentrations of beta-endorphin are increased in obese, hirsute women with PCO (Givens et al 1980, Aleem and McIntosh 1984). In obese normal subjects, beta-endorphin infusion results in increased secretion of insulin and glucagon, whereas in non-obese subjects, an increase in glucagon concentrations only is seen (Giugliano et al 1987). Although the glucagon status in obese women with PCO was normal despite elevated concentrations of beta-endorphin, the responsiveness of

the pancreas to beta-endorphin appeared to be diminished compared with that in obese normal women (Givens et al 1980, Aleem and McIntosh 1984). Hyperinsulinaemia in obese women with PCO is, therefore, probably not secondary to beta-endorphin stimulated increased pancreatic secretion of insulin. In addition, the increased incidence of glucose intolerance in obese women with PCO (Dunaif et al 1987) does not appear to be related to beta-endorphin, as apparently is the case in obese normal women (Guigliano et al 1988). Nevertheless, opiate blockade by naloxone or nalmefene reduces insulin levels by approximately 25% without influence upon those of glucose in women with PCO (Givens et al 1987). The action of opiate antagonists on the pancreas in these women may also be influenced by alterations in the hypothalamo-pituitary axis (Berga and Yen 1989).

Hyperglucagonaemia contributes to the development of abnormalities of insulin action (Unger 1978) and is associated with impairment of both hepatic and peripheral disposal of glucose (DelPrato et al 1987). In the presence of hyperinsulinaemia, increased concentrations of glucagon promote lipid formation rather than glycogen storage (DelPrato et al 1987), and plasma concentrations of lipoproteins and triglycerides tend to increase. Women with PCO have increased plasma concentrations of free

fatty acids (FFA) and very low density lipoproteins (VLDL), although these do not correlate with serum concentrations of androgens (Mattsson et al 1984, Wild et al 1985). With normal peripheral concentrations of glucagon and normal suppression of glucagon secretion by glucose, the data presented here suggest that increased concentrations of FFA and VLDL in women with PCO are not related to abnormalities of glucagon secretion. Golay et al (1986) have shown that glucagon is not responsible for increased FFA concentrations of obese individuals when compared with non-obese subjects.

Despite hyperinsulinaemia and insulin resistance, HbA_1 values did not indicate significant hyperglycaemia in most women with PCO, and this index is not of value for monitoring subtle abnormalities of carbohydrate metabolism in these subjects.

In most insulin resistant states, changes in the number and function of insulin receptors appear to have a regulatory function (Clark et al 1983, Kahn and White 1988), since they are improved by treatment of the disease. Increases in the number and sensitivity of receptors as a result of decreased concentrations of insulin has been proposed as an explanation for reduced ovarian secretion of androgens in response to treatment

with diazoxide (Nester et al 1989) or weight loss (Pasquali et al 1986). However, other treatments for symptoms of hyperandrogenism in women with PCO do not appear to be mediated through alteration in ovarian insulin receptor number or function. In particular, administration of spironolactone is not accompanied by significant changes in peripheral concentrations of insulin or glucose.

The insulin receptor is an insulin-regulated tyrosine kinase allosteric enzyme, phosphorylating itself and other substrates on tyrosine residues (Kahn and White 1988). Alteration in receptor signal transduction could enhance sensitivity to insulin and has been proposed as a site for future therapeutic manipulation (Kahn and White 1988). Insulin effects within the cell may be mediated or influenced by intracellular calcium (Kissebah et al 1975), high levels of which are associated with resistance to insulin action (Draznin et al 1988), although the influence of changes in concentrations of free calcium on receptor and post-receptor steps has not been elucidated. Release of insulin from the pancreas is also a calcium-dependent process (Hellman 1975), but treatment with nifedipine, a calcium-channel blocking agent, does not appear to alter insulin levels or glucose tolerance in non-insulin dependent diabetics (Collins et al 1987,

Beable et al 1989). Further studies are needed to explore the influence of calcium, and also magnesium, on insulin resistance in women with PCO. Preliminary data from studies of treatment of women with PCO with nifedipine show no alteration in concentrations of androgens or insulin (I.M. Golland, unpublished).

Few prospective longitudinal studies to assess the consequences of hyperinsulinaemia and insulin resistance in women with PCO have been undertaken. It is not known whether there is an increased risk of cardiovascular disease in obese women with PCO compared with that in either non-obese women with PCO or obese eumenorrhoeic women. One study has demonstrated, however, that post-menopausal women with abnormal coronary angiography are more likely to have experienced irregular menses and to be hirsute than those with normal angiography (Wild et al 1988). Decreased insulin sensitivity associated with a degree of impaired glucose tolerance in obese women with PCO, particularly when associated with abnormal concentrations of circulating lipids (Mattsson et al 1984) indicates a possible increased risk of atheromatous vascular disease. These women, however, also have increased circulating concentrations of oestrogens resulting from extraglandular aromatisation of androgens (Yen 1980) or increased secretion from the polycystic

ovary itself (Wajchenberg et al 1988), and which may reduce the incidence of vascular disease. Further prospective studies examining the contributions of hyperinsulinaemia, hypertension and sex steroids to the risk of development of atheromatous vascular disease in women with PCO are required, particularly to consider whether obesity is an independent risk factor.

The risk of development of hyperplasia and carcinoma of the endometrium is increased by prolonged exposure to circulating oestrogens unopposed by progesterone, and the incidence of endometrial carcinoma has been shown to be increased in anovulatory women with PCO (Jackson and Dockerty 1957). It is of interest that in women with endometrial carcinoma concentrations of insulin are increased and the women exhibit insulin resistance in association with stromal luteinisation of the ovaries (Nagamani et al 1988).

Insulin resistance may be an inherited trait (Reaven 1988). A three-fold variation in insulin sensitivity between non-obese individuals with normal glucose tolerance has been demonstrated, with values of peripheral glucose uptake in the most insulin resistant quartile of a degree comparable to subjects with impaired glucose tolerance or non insulin-dependent diabetes mellitus

(Reaven 1988). Inheritance of an insulin resistance trait may explain the development of long-term hyperinsulinaemia and subsequent reduction in insulin receptor number and diminished post-receptor events (Clark et al 1983). An increase in concentrations of insulin may act upon the ovary to increase androgen and IGF-1 production (Barbieri et al 1988) and/or synergistically with an inherited abnormality of ovarian control mechanisms or LH secretion expressed at puberty (Hague et al 1988), thereby contributing to the induction of the polycystic ovary syndrome.

The absence of significantly increased concentrations of glucagon in the peripheral plasma in women with PCO suggests that the mechanism responsible for insulin resistance in these women differs from that associated with administration of androgenic drugs such as danazol, obesity or non-insulin dependent diabetes mellitus. The hypothesis, that glucagon counters the influence of insulin on peripheral tissues but not that on the ovary, thereby permitting an unopposed gonadotrophic action of insulin on androgen secretion (Barbieri et al 1988), is not supported by these studies.

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Lack of predictive value of HbA_1 for impaired glucose tolerance in polycystic ovary syndrome

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Abstract

Twenty-seven women with polycystic ovary syndrome (PCO) and 17 control women had a 75 g oral glucose tolerance test (oGTT) performed. Although glucose tolerance was impaired in the obese (body mass index > 25 kg/m^2) women with PCO, glycosylated hemoglobin (HbA_1) concentrations did not exceed the normal upper limit (7.2%). In all 44 women, there was no correlation between HbA_1 and fasting glucose ($r = 0.082$, $p = 0.63$) but there was a significant correlation between HbA_1 and summed glucose levels through the oGTT ($r = 0.389$, $p = 0.02$). HbA_1 measurement does not predict the presence of impaired glucose tolerance in women with PCO.

Introduction

Women with polycystic ovary syndrome (PCO), irrespective of whether they are obese or not, are hyperinsulinemic both in the fasting state and in response to oral glucose loading[1–4]. Obese women with PCO have impaired glucose tolerance when compared with obese and non-obese eumenorrheic women with PCO[1,4,5], as is shown by significantly higher plasma concentrations of glucose during oral glucose tolerance testing (oGTT). Obese women with PCO do not, however, have elevated fasting glucose concentrations, and there is no constant relationship between the hyperinsulinemic response and the degree of impairment of glucose tolerance.

The long-term consequences of impaired glucose tolerance and hyperinsulinemia in PCO are unknown. Insulin might be partly responsible for the maintenance of excessive ovarian androgen secretion in women with PCO[6,7], but there are no detailed longitudinal studies of the risk of these subjects

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developing diabetes mellitus (DM), vascular disease or hypertension when compared with women with PCO without abnormal glycemic control. At present, assessment of glucose tolerance in these subjects requires that an oGTT be performed, because measurement of fasting concentrations of glucose and insulin are not sufficiently discriminatory.

Measurement of glycosylated hemoglobin (HbA_1) has been used to monitor glycemic control[8] and assist in the diagnosis of DM[9]. Elevated levels of HbA_1 in DM are correlated with the development of diabetic complications[10]. We undertook a study, as part of our investigations into the causes of hyperinsulinemia in obese women with PCO, to establish whether the measurement of HbA_1 would be of value in the prediction of abnormalities in glucose tolerance in women with PCO, and, therefore, be of potential value as a screening test for a group at potentially increased risk for the development of DM or complications of vascular disease.

Materials and methods

Twenty-seven women, aged 20–34 years (mean 26.7 years), with a diagnosis of PCO based upon the presence of hirsutism, oligomenorrhea, elevated plasma LH and LH/FSH ratio, ultrasound evidence of polycystic ovaries[11], and elevated serum concentrations of one or more of the following androgens, testosterone, androstenedione and dehydroepiandrosterone sulfate, were entered into the study. Fourteen of these women were defined as obese (OB-PCO), with a body mass index (BMI, body weight in kg/height in m^2) greater than 25. The remaining 13 women were of normal weight (NOB-PCO, BMI < 25 kg/m^2). None of the women showed evidence of acanthosis nigricans.

Seventeen healthy women, aged 19–45 years (mean 30.7 years), with regular menstrual cycles and without signs of androgen excess clinically or biochemically, served as controls. Nine of these women were obese (OB-control) and 8 were non-obese (NOB-control).

None of the women studied was known to have impaired glucose tolerance, although 1 woman in the NOB-PCO group had a family history of DM, and none had taken any medication known to affect glucose tolerance during the previous 3 months. The control women were studied during the early or mid-follicular phase of the menstrual cycle, whilst the PCO women were studied without regard to the menstrual dates. Irrespective of the day of the cycle, progesterone measurement at the time of the study confirmed the absence of recent ovulation.

After a 12-hour overnight fast, a standard 75 g oGTT was performed, with venous blood samples taken at 0, 30, 60, 90, 120 and 150 minutes for glucose estimation. HbA_1 was measured in the fasting sample. Plasma glucose was measured by a glucose oxidase method, and HbA_1 was measured by a cation exchange analysis at 415 nm[12]. The summed glucose value was obtained from the sum of all glucose values through the oGTT.

The study was approved by the district ethical committee, and all women gave their informed consent.

Statistical analysis

Changes in glucose concentration over the test period were evaluated using a 2-factor analysis of variance (ANOVA) with repeated measures on 1 factor[13], the factors being study group and time. Differences in the HbA_1 level between the study groups were examined using a single factor ANOVA. The relationship between HbA_1 and glucose levels (fasting and summed) was assessed for each study group separately using Pearson's correlation coefficient. Statistical significance was set at the conventional 5% level throughout.

Results

Mean oGTT levels over the test period are presented for each of the study groups in Figure 1. Fasting glucose concentrations did not differ significantly. In the OB-PCO women, glucose values were significantly higher at 60 and 90 minutes relative to the non-obese groups and also at 120 minutes relative to the NOB-PCO women. There was no significant difference between OB-PCO and OB-control women at any interval during the oGTT. One OB-PCO woman was found to be frankly diabetic, as defined by the criteria of the National Diabetes Data Group[14].

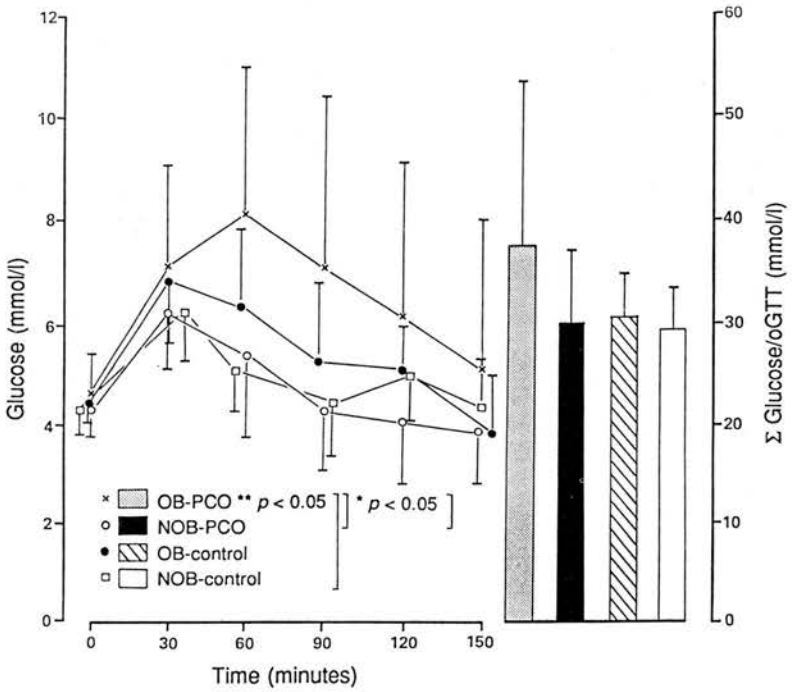

Figure 1 Mean (SD) glucose levels in response to 75 g glucose in obese and non-obese women with PCO and in obese and non-obese control women

Table 1 Age, body mass index and mean (± SD) HbA_1 concentrations in plasma in obese (OB-PCO) and non-obese (NOB-PCO) women with polycystic ovaries, and in obese (OB-normal) and non-obese (NOB-normal) control women

	OB-PCO	*NOB-PCO*	*OB-normal*	*NOB-normal*
Age (range, years)	27.5 (22.6–34.2)	25.5 (20.9–33.1)	32.6 (21.0–45.4)	28.2 (21.7–38.8)
BMI (kg/m^2)	31.8 ± 4.5	22.6 ± 1.8	30.2 ± 3.0	20.8± 2.6
HbA_1 (%)	5.2 ± 1.0★	5.0 ± 0.9★	5.5 ± 1.0★	5.0 ± 0.9★

★$f = 0.38$, $p = 0.69$

Table 1 shows the age, BMI and HbA_1 concentration for each of the 4 groups of women studied. In no case was the HbA_1 concentration above the upper limit for our laboratory (7.2%). HbA_1 concentrations did not differ significantly between the groups. Non-significant correlations were found between HbA_1 and both fasting ($r = -0.179$, $p = 0.28$) and summed ($r = 0.056$, $p = 0.74$) glucose values. The results were markedly skewed, however, by the patient with diabetes who, inexplicably, had the lowest HbA_1 result of all the women studied. Excluding this patient again produced a non-significant correlation between HbA_1 and fasting glucose, but a significant positive correlation between HbA_1 and summed glucose levels ($r = 0.389$, $p = 0.02$; Figure 2).

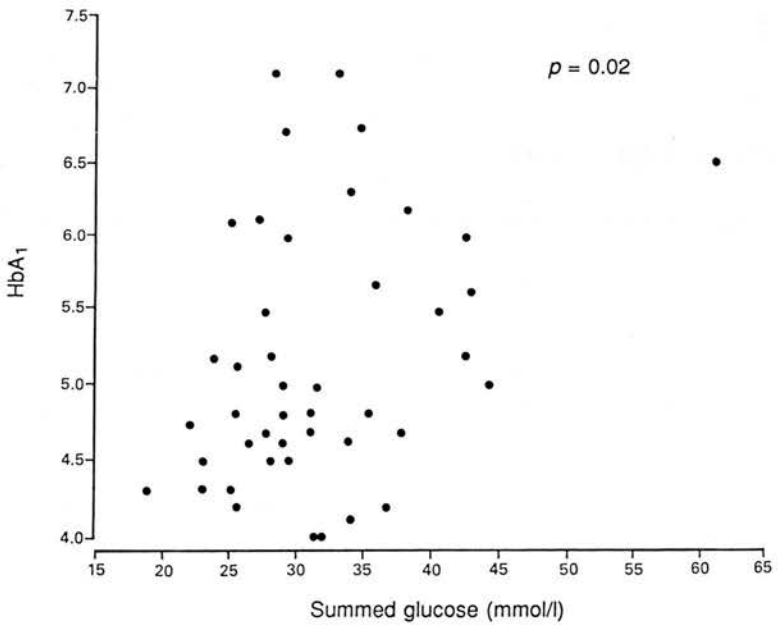

Figure 2 Relationship between the concentration of HbA_1 and the cumulative concentration of glucose after 75 g glucose

Discussion

We have shown in this study that obese women with PCO have a significantly greater blood glucose response to oral glucose loading in comparison with that in non-obese women with PCO and non-obese normal women, although not in comparison with obese normal women. No significant elevation of the fasting blood glucose above normal limits was seen. These results are in agreement with previous studies of obese women with PCO[1,4].

HbA_1 and fasting glucose concentrations did not differ significantly between the OB-PCO, NOB-PCO and obese and non-obese normal women. A significant positive correlation between HbA_1 and summed glucose values was demonstrated, but only after exclusion of data from 1 patient with extreme values.

Measurement of HbA_1 is less sensitive than the oGTT for the full assessment of glycemic control. However, an oGTT is a time-consuming investigation, and one which would not be performed in the normal investigation of women presenting with symptoms suggestive of PCO, except in the research environment. We hoped to be able to demonstrate that assessment of the concentration of HbA_1 would be of value in the screening of women with PCO, to easily select those who would be at an increased risk for abnormal or impaired glucose tolerance, and who could then undergo further investigation of glycemic control.

We have demonstrated, however, that the measurement of HbA_1 is not sufficiently discriminatory to enable identification of impaired glucose tolerance in PCO. In addition, this would suggest that, in the physiological state, despite the presence of insulin resistance, abnormal mean 24-hour glucose levels are not a significant feature of most obese women with PCO.

Women with PCO are hyperinsulinemic compared with weight-matched controls[1,4], which implies a variable degree of insulin resistance, although the underlying biochemical defect is unclear. It has been suggested[6,7] that hyperinsulinemia might be partly responsible for the maintenance of increased androgen concentrations in PCO as a result of direct stimulation of ovarian stroma or augmentation of LH-dependent androgen secretion. However, the greater magnitude of insulin concentrations in obese women is not associated with similarly increased androgen concentrations[17].

Few prospective longitudinal studies assessing the consequences of hyperinsulinemia and insulin resistance in these women have been undertaken. It is not known whether they are at more risk of cardiovascular disease than non-obese women with PCO or obese eumenorrheic women, although the male pattern lipoprotein profile seen in androgenized women with PCO[16,17] suggests that this might be the case.

In conclusion, the absence of a constant relationship between HbA_1 and glucose tolerance in PCO suggests that the measurement of HbA_1 is not of value for the prediction of impaired glucose tolerance in these women.

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