" OLIGO/AMENORRHOEA "

Endocrine Profiles, Ovarian Ultrasound, Insulin Resistance and Anthropometric Factors : Relationships Between Insulin Resistance and Ovarian Function

Thesis

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by

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ABSTRACT

There is a lack of longitudinal studies of infertile patients with oligo/amenorrhoea examining changes in hormone concentrations and ovarian morphology. In this prospective longitudinal study, women with oligo/amenorrhoea (n = 42) were studied in detail and compared with a weight-matched group of regularly menstruating females (n = 20). Women with oligo/amenorrhoea exhibited an insignificant increase in body weight (BMI) but truncal-abdominal fat (WHR) was significantly higher than that of the control group. The mean basal FSH concentrations were similar in both the groups; E_2 and SHBG concentrations were lower, and LH, T and FAI were significantly higher in patients than in controls. The comparison of the mean follicular phase endocrine profile during the 5-week window of observations, showed that the ovary was in a dynamic state, even in the absence of follicular growth and ovulation, and that E_2, LH, T and consequently FAI were variable, both between and within patients. The mean follicular phase SHBG concentration was the most stable endocrine variable, and SHBG was also the only endocrine parameter able to distinguish between ovulatory and anovulatory cycles. Significantly higher concentrations of SHBG were found in spontaneously occurring ovulatory cycles of patients in comparison to those in patients who were anovulatory during the period of observation; the former group SHBG levels were compatible with the levels of controls measured in the follicular phase. The dynamic test for measurement of insulin resistance (IR), the SITT, was carried out twice in patients with oligo/amenorrhoea (n = 34) and in controls (n = 20). The SITT yielded similar KITT-values in patients and controls, there was considerable within patient variation and no relationship with anthropometric variables. Fasting I, GLU/INS ratio and FIRI ($\log_{10}$ FIRI) appeared to be better markers of IR than the KITT-value in patients with oligo/amenorrhoea, as they showed the significant expected relationships with anthropometric variables, especially WHR. The differences in IR between the groups appeared to be related to body mass differences as they disappeared after controlling for anthropometric variables. The longitudinal window of observations showed that the occurrence of follicular growth and spontaneous ovulation was common in patients with oligo/amenorrhoea (n = 18). However, inadequate luteal phase function (assessed by PI) was demonstrable in most of cases. It was apparent that LH, androgens and IR had no direct influence on the occurrence/absence of follicular growth and spontaneous ovulation. The follicular IGF-I concentrations were significantly lower in patients than in controls, despite similar follicular GH concentrations. The IGF-I did not correlate with follicular FSH but it did correlate with FAI. The IGF-I was significantly higher in patients who ovulated than in those who did not ovulate. Body fat distribution (WHR), rather than body weight (BMI), had a significant negative

impact on the incidence of spontaneous ovulation. Leptin concentrations were strongly correlated with anthropometric variables, FI and FIRI (log_{10} FIRI). However, there was no significant relationship between leptin and the occurrence of spontaneous ovulation. Longitudinal US observations confirmed that the ovary is not a static organ in such patients and that TOV, the number and distribution of follicles all showed considerable intra- and inter-patient variability. The most sensitive US criterion for the diagnosis of PCOs was the thickened stroma, while TOV was the least sensitive and most variable parameter. Ultrasound diagnosis of unilateral PCO was observed in a number of patients (n = 8). The mean TOV of the study patients was significantly larger than reported normal values. The US indices, TOV, follicle number and distribution and stromal thickness, did not correlate with endocrine, metabolic or anthropometric variables during the period of observation. Asymmetry of the ovarian volume was noticeable in 64.4% of cases and about half of the patients showed more than 100% increase in TOV during monitoring, regardless of the presence of follicular growth. Almost half of the patients showed a mixed picture of peripheral and central follicular distribution and a change from peripheral to central distribution or the reverse, was observed in > 60% of cases. It was found that the larger TOV favoured peripheral distribution of follicles, was associated with significantly higher follicle numbers and the occurrence of bilaterally thickened stroma compared with the smaller ovary. Women with PCOD (n = 11) demonstrated higher WHR, fasting TG and lower HDL-C concentrations than did a group of healthy females. Post-heparin hepatic lipase assays showed that the concentrations, in women with PCOD, were significantly higher than those in normal females and approached the male range.

These studies showed that women with oligo/amenorrhoea, showed dynamic changes in ovarian and biochemical / endocrine parameters when examined serially. These changes were independent of ovulation and did not appear to be directly related to any of the metabolic parameters. The only parameter which was significantly linked to ovulation was SHBG.

The results of these studies improve our insight into oligo/amenorrhoea and the associated PCOD and mean that in future any investigations performed on such patients should involve serial rather than single observations.

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PREFACE

This research work was conducted by DR. HUDA M. AL-NASER AL-ZEKRI in the Department of Obstetrics and Gynaecology, Glasgow University, at Glasgow Royal Infirmary, Glasgow, during the period 1993-1995, under the supervision of DR. JOHN R. T. COUTTS, Reader in Reproductive Endocrinology in the same Department. The patients were patients undergoing investigation/treatment through the Gynaecology Department, Glasgow Royal Infirmary and were under the overall clinical supervision of DR. R. W. S. YATES, the consultant in charge.

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AUTHOR'S DECLARATION

I, HUDA M. I. AL-NASER AL-ZEKRI, do hereby declare that the work presented in this dissertation is original, was carried out by me and has not been presented for an award of a degree in any other university.

Signature : .....

Date : 9th May 1997

ABBREVIATIONS USED IN THE THESIS

A	Androstenedione
ACTH	Adrenocorticotrophic Hormone
AD	Autosomal Dominant mode of inheritance
A-P	Antero-Posterior diameter
AR	Autosomal Recessive mode of inheritance
β-cells	Beta-cells of pancreas.
BMI	Body Mass Index
BLH	Bioreactive Luteinizing Hormone
CAH	Congenital Adrenal Hyperplasia
C19, C21	Hormones containing 19 and/or 21 carbon atoms
CHD	Coronary Heart Disease
CTRL	Controls
C.V.	Coeffient of Variation
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulphate
DHT	5 α-Dihydrotestosterone
DUB	Dysfunctional Uterine Bleeding
DXM	Dexamethazone
E_1	Oestrone
E_2	Oestradiol
E_3	Oestriol
EGF	Epidermal Growth Factor
FAI	Free Androgen Index, a measure of FT (see below)
FBC	Full Blood Count
5 α-R	5 α-Reductase
FI	Fasting Insulin
FIRI	Fasting Insulin Resistance Index
FSH	Follicle Stimulating Hormone
FT	Free Testosterone, measured as FAI (see above)
FT4	Free Thyroxine

GH	Growth Hormone
GLU/INS Ratio	Glucose/Insulin Ratio
Gn	Gonadotrophin(s)
GnRH	Gonadotrophin Releasing Hormone
GnRH-A	Gonadotrophin Releasing Hormone-Agonist
GTT	Glucose Tolerance Test
HDL	High-Density Lipoprotein
HDL-C	High-Density Lipoprotein-Cholesterol
HGH	Human Growth Hormone
HL	Hepatic Lipase
HLA	Human Leucocyte Antigen
11OH deficiency	11-Hydroxylase enzyme deficiency
21OH deficiency	21-Hydroxylase enzyme deficiency
17 α-OHP	17 α-Hydroxyprogesterone
3 β-OHSD	3 β-Hydroxysteroid Dehydrogenase enzyme
17KSR	17-Ketosteroid Reductase enzyme
I	Insulin
IDDM	Insulin Dependent Diabetes Mellitus
IGF	Insulin-like Growth Factors
IGF-I	Insulin-like Growth Factor-I
IGFBP's	Insulin-like Growth Factor Binding Proteins
IGS	Inappropriate Gonadotrophin Secretion
ILH	Immunoactive Luteinizing Hormone
IR	Insulin Resistance
IS	Insulin Sensitivity
ITT	Insulin Tolerance Test
KITT	The Rate Constant for Plasma Glucose Disappearance following intravenous I injection (Bonora *et al* 1989)
LDL	Low-Density Lipoprotein
LDL-I	Low-Density Lipoprotein subfraction I
LDL-II	Low-Density Lipoprotein subfraction II
LDL-III	Low-Density Lipoprotein subfraction III
LDL-C	Low-Density Lipoprotein-Cholesterol
L-Dopa	Dopamine

LH	Luteinizing Hormone
MCR	Metabolic Clearance Rate
M/I	M represents the amount of glucose metabolised estimated from the amount of glucose infused to maintain euglycaemia or hyperglycaemia. I is the mean plasma I concentrations during steady state euglycaemia (60-120 minutes) or hyperglycaemia (20-120 minutes). M/I ratio is used as a measure of tissue sensitivity to I (Bonora *et al* 1989)
MRI	Magnetic Resonance Imaging
NIDDM	Non-Insulin Dependent Diabetes Mellitus
P	Progesterone
PI	Progesterone Index
PCO	Polycystic Ovaries
PCOD	Polycystic Ovarian Disease
PCOS	Polycystic Ovarian Syndrome
PRL	Prolactin
PT	Patients
RI	Roundness Index
SHBG	Sex Hormone Binding Globulin
SHBG-BC	Sex Hormone Binding Globulin Binding Capacity
SITT	Short Insulin Tolerance Test
S-L	Stein-Leventhal
T	Testosterone
TRH	Thyroid Releasing Hormone
T3	Triiodothyronine
T4	Thyroxine
TG	Triglyceride
TT	Total Testosterone
TGF-α	Transforming Growth Factor-α
TGF-β	Transforming Growth Factor -β
US	Ultrasound
VLDL	Very Low Density Lipoprotein
WHR	Waist-Hip Ratio
X-linked R	X-linked Recessive mode of inheritance

CHAPTER ONE - INTRODUCTION

1.1 GENERAL INTRODUCTION

In Britain it is believed that about 14% of couples experience difficulty either achieving pregnancy or having a liveborn child (Hull, 1992), although rates as high as 25% have been reported when shorter durations of infertility are used (Greenhall & Vessey, 1990). At least half of these couples will present with secondary infertility, and the infertility will remain unresolved in a further half of each group (Hull, 1992).

In general, the main categories of infertility appear to be : ovulatory disorder (mainly amenorrhoea or oligomenorrhoea) about 30%; sperm defects and unexplained infertility each about 25%; and pelvic and/or tubal damage about 20%. Much less frequent causes are endometriosis (5%), cervical mucus defect (4%) and coital impairment (6%) (Hull, 1992).

Anovulation is a very common problem which presents itself in a variety of clinical manifestations including amenorrhoea, irregular menses and hirsutism. Serious consequences of chronic anovulation are infertility and a greater risk of developing endometrial carcinoma.

Normal ovulation requires coordination of the menstrual system at all levels, the central hypothalamus - pituitary axis, the feedback signals and local responses within the ovary. The loss of ovulation may be due to any one of a variety of factors operating at each of these levels. The end result is a dysfunctional state : anovulation (Insler & Lunenfeld, 1990).

Anovulation is a key feature of PCOD. Polycystic ovarian disease represents a heterogeneous group of conditions ranging from a mere ultrasonic ovarian finding to the full clinical syndrome described by Stein & Leventhal in 1935 (Shaw, 1991). The symptoms may be the consequence of the loss of ovulation : dysfunctional bleeding, oligo/amenorrhoea, hirsutism and infertility. Each presentation requires a specific diagnostic and therapeutic approach (Insler & Lunenfeld, 1990).

1.2 BACKGROUND

1.2.1 HISTORICAL BACKGROUND

Insler & Lunenfeld (1990) reported that Antonio Vallisneri in 1721 described the classical clinical and anatomical features of what is now called "Polycystic Ovarian Disease". Chereau (1844) was the first to describe the "sclerocystic" disease of the ovaries, which was described as thick, pearly white ovarian capsule. Removal of both ovaries was a popular procedure in Europe thereafter, particularly in cystic degeneration or sclerocystic disease. Pozzi (1894) and Waldo (1895) then advocated a less radical procedure, the wedge resection (Futterweit, 1984).

"Microcystic degeneration" was described by Forgue and Massabuau in 1910 as a condition with many pea-sized follicular cysts scattered over the ovarian cortex. Irving F. Stein and Michael L. Leventhal (1935) were the first to relate polycystic ovaries in some of their patients with amenorrhoea and infertility; prior to that amenorrhoea had been related only to small atrophic ovaries.

Based on diagnostic studies of wedge resection biopsies, Stein and Leventhal (1935) gave their description of a syndrome, named after them, consisting of clinical features of amenorrhoea, infertility, obesity and hirsutism associated with bilaterally enlarged cystic ovaries (Stein & Leventhal, 1935). These were S-L ovaries: enlarged ovaries with thickened tunica. There has been much interest in the condition since then.

1.2.2 DEFINITION

In the presence of a diversity of clinical manifestations and biochemical variabilities, there is no one universally accepted definition of PCOD. It has become clear that the criteria of anovulation, infertility, obesity, hirsutism and the presence of bilaterally enlarged polycystic ovaries are restrictive as with time, an increasing number of patients with PCOD and divergent clinical findings have been observed. The classical picture was also found to be associated with a number of endocrine disorders of diverse aetiology. Therefore, the so called "S-L syndrome" is now only applied to those patients with the criteria described above whereas the term PCOD is now adopted to describe a broad heterogeneous spectrum of abnormalities, characterized by "poly-follicular" rather than "polycystic" ovaries (Futterweit, 1984).

The most clear cut definition of PCOD consists of an association of chronic anovulation, multifollicular ovaries, thick stroma, hyperandrogenism and IGS (an increased plasma concentration of LH relative to that of FSH - increased LH:FSH ratio) (Futterweit, 1984).

Multifollicular ovaries, observed in women with weight-loss related amenorrhoea, are considered to represent a normal ovarian response to a hypothalamic disturbance of Gn control (Adams *et al* 1985). Such ovaries are usually normal in size or slightly enlarged and filled by ≥ 6 cysts 4-10 mm in diameter; in contrast to women with PCOD, stroma is not increased. Such women are not hirsute and serum concentrations of LH and FSH are normal and decreased respectively. Ovarian morphology reverted to normal in ovulatory cycles, whereas in PCOD the polycystic pattern persisted.

PCOD is categorized as WHO Group II ovarian insufficiency with hypothalamic-pituitary dysfunction (Breckwoldt *et al* 1986). The classic SL-PCOD (type 1 PCOD) is described by the association of (a) bilateral polyfollicular ovaries, (b) anovulatory infertility, (c) menstrual dysfunction, and (d) hirsutism.

This disease is commonly associated with obesity, hyperandrogenism, increased peripheral conversion of androgens to oestrogens, and an elevated LH to FSH ratio (Stein & Leventhal, 1935). This may result from an aberrant puberty caused by an abnormal neural development in the brain with a resulting hypothalamic-pituitary-ovarian axis alteration (Mechanick & Futterweit, 1984; Mechanick & Futterweit, 1986).

While type II non classical PCOD includes those without one or two of those classical clinical signs, they do have PCO and an abnormal adrenal androgen secretion (Wu & Mikhail, 1979; Mechanick & Futterweit, 1984).

The term PCOD, or polyfollicular ovarian disease, is applied to a wide clinical array of ovarian, adrenal, and mixed ovarian and adrenal disorders which eventually culminate in polyfollicular ovaries in various stages of growth and atresia (Mechanick & Futterweit, 1986). It is considered as a spectrum of disorders with hyperthecosis at one end and asymptomatic fertile women with polycystic ovaries at the other end, involving complex inter-relationships between hypothalamus-pituitary, adrenals and ovaries; this may result in oligomenorrhoea or anovulation and excess androgenicity (Berger *et al* 1975).

There can be clinical, biochemical, histopathological or ultrasonic definitions of PCOD. Some would consider PCOD to include more than one entity or it could represent different degrees of the same disorder.

1.2.3 PREVALENCE OF PCOD

Polycystic ovarian disease is probably the most common endocrinological disorder resulting in chronic anovulation among women during their reproductive lives including adolescence (Vaitukaitis, 1983; Franks *et al* 1988; Dunaif, 1992b). Little is known of the true incidence of polycystic ovaries and it appears quite variable. This is to be expected since neither clinical manifestations, biochemical changes characterizing the disorder, nor the pathophysiological mechanism(s) underlying the disease have been well defined and generally understood.

In a series of 12,160 unselected gynaecological laparotomies, the prevalence of polycystic ovaries was 1.4% (Insler & Lunenfeld, 1990). Ginsburg & Havard (1976) had reported a much higher incidence of PCOD - about 17% in their experience. Polson *et al.* (1986b; 1988) performed pelvic ultrasonographic examination in 158 normal women of reproductive age. Polycystic ovaries were defined ultrasonically by the presence of $\geq$ 10 cysts 2 - 8 mm in diameter, associated with an increase in the ovarian stroma (Adams *et al* 1986b). They reported that 22% of the ovaries examined were polycystic, although when they reviewed their menstrual histories they found PCO in only 9% of the women with regular cycles (vs. 75% of those with menstrual irregularities). They concluded that the prevalence of polycystic ovary in women with regular cycles was very low. The incidence is quite different if menstrual disorders or hirsutism are present. PCOD, diagnosed by US, was reported in 26% of amenorrhoeic women, 53- 86% of oligomenorrhoeic women (Adams *et al* 1986a; Eden & Place, 1989) and ranging between 75 and 92% of hirsute patients after exclusion of adult onset CAH (Adams *et al* 1986a; Baron, 1991). This shows that PCOD diagnosed by US is very common in anovulatory women (57%) (Adams *et al* 1986a) and this incidence might be increased by the presence of obesity (Eden & Place, 1989). Tulppala and his group (1993) assessed the occurrence of PCO in 50 patients with history of recurrent miscarriage and found 44% of their patients had PCO detectable by vaginal US. This frequency was higher than that in a control group but it was not predictive of the outcome of a subsequent pregnancy. It can therefore be concluded that although more definitive diagnostic criteria are needed, PCOD is more prevalent in women with menstrual irregularities (oligo/amenorrhoea).

1.3 IMPLICATIONS ON GENERAL HEALTH

Insulin is a polypeptide hormone which is secreted by the β-cells of the pancreas and affects carbohydrates, protein and lipid metabolism. Under normal circumstances, I regulates glucose metabolism mainly in liver, muscle and adipose tissues. In the liver, I inhibits the production of glucose by inhibiting gluconeogenesis and glycogenolysis and promoting glycogen storage. In muscle and adipose tissue, I stimulates the uptake, storage, and use of glucose. Insulin also has a major role in the systemic regulation of protein and lipid metabolism. Other actions of I which are less known include stimulation of potassium transport in muscle, cellular differentiation in adipocytes and its effect on ovarian androgen production.

Women with PCOD may have major metabolic complications. Hyperinsulinaemia and IR may be involved in the pathogenesis of PCOD. Insulin resistance is defined as a subnormal biologic response to a given concentration of I. The clinical consequences of IR results from either deficient or excessive I action.

Insulin resistance has a major role in the pathogenesis of many health disorders which include :

1. Type A syndrome of severe IR is an inherited disorder due to defects in I-receptor locus. It is characterized by marked endogenous hyperinsulinaemia with or without glucose intolerance, acanthosis nigricans and ovarian hyperandrogenization. The presentation is usually of amenorrhoea, hirsutism and virilization in a lean patient.

Variants of type A syndrome include leprechaunism and lipodystrophy. Leprechaunism is a rare congenital syndrome characterized by intrauterine growth retardation, dysmorphic features, lipoatrophy, acanthosis nigricans and IR. This disorder affects newborn females who present with cystic ovaries, hirsutism and cliteromegaly. Lipodystrophy, where patients may have no adipose tissue, is characterized by acromegaly, dysmorphic features, genital hypertrophy and cardiomyopathy.

2. Type B syndrome where IR is caused by the presence of autoantibodies to the I receptor. The patients present with uncontrolled diabetes, acanthosis nigricans and ovarian hyperandrogenization.

3. Non-insulin dependent diabetes mellitus caused by a defect in the functional capacity of the pancreatic β-cells to secrete I or by IR. The ability of I to stimulate glucose uptake in muscles and fat is impaired. Obesity alone may be associated with a similar but less severe defect.

4. Severe IR is associated with ovarian thecal hyperplasia, hyperandrogenism and obesity. Hyperinsulinaemia resulting from IR may contribute to the pathogenesis of the PCOD.

5. Hyperinsulinaemia is implicated in the pathogenesis of cardiovascular disease, essential hypertension and hypertriglyceridemia. The clinical association of IR, hypertension, increased VLDL and decreased HDL-C concentrations in plasma is termed " syndrome X ".

The association between hyperinsulinaemia and these significant health disorders requires intensive investigation for a better understanding of the pathogenesis of disorders characterized by IR.

1.4 OBJECTIVES OF STUDY

Polycystic ovarian disease is a fairly common cause of female infertility presented with menstrual dysfunction (mainly oligomenorrhoea). The pathophysiology of PCOD is far from clear and is still the subject of many hypotheses and speculations.

Women with PCOD have been shown to have significant peripheral IR similar to individuals with NIDDM (Dunaif, 1992a). These women are at increased risk of developing NIDDM at a very early age. The effect is exacerbated by the presence of obesity, a common finding in PCOD patients. The association of hyperinsulinaemia and hyperandrogenism has led to a better understanding of PCOD pathogenesis and to the recognition of its subsequent metabolic as well as reproductive complications. It should be appreciated that as a consequence to PCOD, women may have major metabolic complications.

Hyperinsulinaemia may be the trigger mechanism of the pathogenesis of PCOD. Insulin resistance is generated peripherally. Fasting hyperinsulinaemia may be used as a guide to the existence of IR and the SITT might be a suitable method for assessing IR (Akinmokun *et al* 1992; Hirst *et al* 1993). The changed sensitivity of peripheral tissue to I activity may constitute a primary event in the genesis of IR. The literature shows conflicting reports regarding the correlation between hyperinsulinaemia and ovarian hyperandrogenism in PCOD.

Some growth factors may have important roles in modulating ovarian responses to Gn. Among them, IGF-I seems to be an important stimulatory factor of granulosa cell differentiation. The physiological activity of IGF's is modulated by a number of specific binding proteins (IGF-BP's). IGF-I is stimulatory to FSH action (i.e. potentiates ovarian steroidogenesis), whereas I inhibits binding of IGF-I to its

receptors. IGF-I may also potentiate the ability of LH to stimulate androgen production. It is postulated that I, directly or via IGF-I, may modify ovarian steroidogenesis or the action of androgens at target tissue levels. Obese PCOD patients, who also tended to have higher androgen levels, have been reported to have more pronounced IR than non-obese women which may influence IGF-I concentration (Slowinska-Srzednika *et al* 1992).

Among hormones known to cause or predispose to IR are GH, cortisol and PRL. Increased feedback of IGF-I at the level of the pituitary gland could lead to a reduction in GH secretion.

Leptin, a recently discovered hormonal product of the obesity gene expressed in adipocytes, is thought to play a role in the regulation of food intake and metabolism (Barash *et al* 1996; Caro *et al* 1996). In mice, leptin receptors were identified in the ovary and leptin was found to stimulate gonadal function (Chehab *et al* 1997). Little is known about the physiologic action of leptin in humans, although it is clear that circulating leptin concentrations are linked with total adiposity, and it may show abnormal secretions in women with PCOD.

The main objectives of this study were :

1. To study the relationships between I levels and those of glucose, A, T, FT (FAI), DHEA-S, cortisol, SHBG, E_2, PRL and GH.

2. To compare the efficacy of the "SITT" method as a measure of IR, to that of the FI and other estimates of IR or IS - FIRI, GLU/INS ratio.

3. To investigate the role of obesity and hyperandrogenization in influencing I hypersecretion; the following groups of patients were studied :

   a) obese PCOD vs non obese PCOD patients,

   b) obese PCOD vs obese normal controls,

   c) hyperandrogenic PCOD vs PCOD patients with normal androgen levels.

4. To study the impact of anthropometric parameters on the endocrine and metabolic variables.

5. To investigate the discrepancy that exists in the literature with regard to the GH status of PCOD patients. Fasting GH and the change in its levels during the SITT were compared between PCOD patients and normally ovulating volunteers.

6. To study the relationship between the changes in ovarian activity (including ultrasound and E_2) and LH levels, and the values of IR, GH and IGF-I activity.

7. Insulin sensitivity in ovulatory and anovulatory cycles were compared.

8. To compare leptin concentrations in women with oligo/amenorrhoea with those of weight-matched controls, in relation to anthropometric variables and whether leptin may be involved in the blockade of follicular maturation and ovulation often seen in patients with oligo/amenorrhoea.

9. To compare fasting concentrations of plasma free fatty acids and serum lipoprotein lipids in women with PCOD with those of weight-matched controls, and in relation to anthropometric, endocrine and metabolic variables.

CHAPTER TWO - REVIEW OF THE LITERATURE

2.1 CLINICAL MANIFESTATIONS

The existence of bilateral PCOD has been known for a long period of time. However, a broad interest was stimulated in 1935 when S-L described an associated syndrome consisting of menstrual irregularity and presenting mainly as amenorrhoea, a history of sterility, hirsutism and to a lesser degree, presence of retarded breast development and obesity (Stein & Leventhal, 1935). With time, atypical clinical presentations were noticed in a significant number of cases. Analysis of histories of 100 documented PCO cases showed common findings, which may enable PCO syndrome to be distinguished from other causes of chronic anovulation (Yen, 1980) :

1. Mean age at menarche (12.3 years) was close to the mean of 12.9 years found in the normal population. Primary amenorrhoea does occur, but rarely.

2. Post menarcheal menstrual irregularity persists in most cases.

3. Onset of clinically discernible excessive hair growth (hirsutism) either before or around the time of menarche (a reliable history can be obtained from the parents).

4. Obesity is common prior to the onset of the menarche.

Therefore these abnormalities suggest an early onset, occurring before and during puberty, prior to complete cyclic development of the hypothalamic-pituitary-ovarian system. The clinical syndrome has come to be known as PCOD because of the presence of multiple subcapsular follicular cysts (2 - 8 mm in diameter) and a thickened ovarian capsule. PCOD classical group (S-L syndrome) is composed of oligo/amenorrhoea, hirsutism, obesity, infertility and bilaterally enlarged ovaries, while the non-classical PCOD includes those patients without one or two of the classic clinical signs but who do have PCO (Wu & Mikhail, 1979). The clinical expression of endocrinological conditions such as hirsutism, hyperinsulinism, acanthosis are not so important to define the syndrome since they may or may not be present; with wide individual variations in the number and severity of symptoms (Venturoli *et al* 1989). In patients with ultrasonic demonstration of PCOD, it was found that 50% had the classic signs and symptoms associated with S-L syndrome, 25% had variants of the syndrome and in 25% no abnormal clinical signs and symptoms were evident (Swanson *et al* 1981). The major presentations of patients

with PCOD are menstrual disturbance, infertility and hirsutism and they represent the main reasons for such patients seeking medical advice (Futterweit, 1984).

2.1.1 MENSTRUAL DISTURBANCE

Most women have normal onset of menarche, although oligomenorrhoea may be significant and persistent from the beginning of menses (Futterweit, 1984). A considerable number of patients show menstrual abnormalities from the time of menarche or shortly thereafter (Goldzieher & Green, 1962; Ibrahim *et al* 1966; Yen, 1980; Vaitukaitis, 1983; Mechanick & Futterweit, 1984; Mechanick & Futterweit, 1986). Neither the duration nor the severity of the symptoms was correlated with the criteria of the syndrome (Goldzieher & Green, 1962). In a study carried out on Thai women (Rojanasakul *et al* 1989), it was interesting to note that the history of menstrual irregularity, androgenic symptoms and being overweight began about the perimenarcheal period in most patients. This finding may support the hypothesis that the disorder starts around the period of puberty and persists thereafter. Chronic anovulation refers to menstrual arrhythmia that results from repeated ovulatory failure and is manifested clinically as amenorrhoea or menstrual acyclicity (Yen, 1986). A spectrum of amenorrhoea, oligomenorrhoea, polymenorrhoea, obesity, dysmenorrhoea and occasionally even regular cycles is encountered (Coney, 1984). PCOD is a common cause of oligomenorrhoea in women without classical signs of PCOD (Eden *et al* 1988b). An unselected series of 73 women with amenorrhoea and 75 women with oligomenorrhoea were analyzed (Franks *et al* 1988); PCO were found in 26% of women presented with amenorrhoea and in 87% of those who complained of irregular periods. Similar results were presented by Adams *et al.* (1986b). Goldzieher & Axelrod (1963) reviewed a total of 187 references containing 1097 cases. Amenorrhoea was observed in only about half of the patients. It really varied from 15 - 77% and regular cycles were noted in 12% of the patients. PCOD was diagnosed in 22% of women who volunteered for pelvic ultrasonic examination with normal cycles and normal Gn levels, whereas 75% of women with irregular cycles or amenorrhoea had PCO by US (Adams *et al* 1986b). Prolonged acyclic oestrogen stimulation of the endometrium resulted in dysfunctional uterine bleeding (Gindoff & Jewelewicz, 1987). The incidence of anovulatory cycles in eumenorrhoea has been estimated to be 11% while plasma hormones are within normal ranges. This may be a transient phenomenon possibly resulting from mild hypothalamic dysfunction and is reversible to ovulatory cycles (Wu & Mikhail, 1979). Although PCOD in eumenorrhoeic patients is rare it has been reported (Goldzieher & Axelrod, 1963; Adams *et al* 1986b).

2.1.2 INFERTILITY

Infertility is one of the major reasons why these patients consult a physician. The incidence of infertility varied between 35 and 94% (Goldzieher & Green, 1962; Goldzieher & Axelrod, 1963) and in one series was 76% (Goldzieher & Axelrod, 1963). Failure of ovulation or oligo-ovulation are the main causes of infertility (Gindoff & Jewelewicz, 1987) although ovulation was observed in 12-40% of PCOD patients (Goldzieher & Axelrod, 1963).

2.1.3 HIRSUTISM

Hirsutism is an increased hair growth of the masculine pattern in women as a reflection of androgen stimulation of hair follicles. The degree of hirsutism in PCOD does not always correlate with the magnitude of the androgen excess (Yen, 1986). This apparent dissociation between androgen levels and the degree of hirsutism reflects different degrees of sensitivity of the target tissues to androgen. Hirsutism is a manifestation of androgenicity, whether it is due to androgen excess, to an increased sensitivity to androgen, or to both. It may or may not be progressive. The time course of development of the androgenic signs is of great importance; a recent rapid onset of androgenization requires urgent and special diagnostic methods for distinguishing PCOD from other causes of androgen excess. It generally implies excessive hair that is coarser, longer, darker or more intense than in normal females of the same age and race. Principally, the sexual or hormone-dependent hair follicles are involved, such as that of the pubic, axillary, abdominal, chest and facial hair. Hirsutism may develop in women as a result of two mechanisms: (i) elevated androgen levels with abnormal androgen production and/or metabolism (Coney, 1984; Schriock *et al* 1985; Gindoff & Jewelewicz, 1987); (ii) increased 5α-R.

In PCO patients, 30 - 40% are not hirsute despite the presence of elevated androgen levels. The presence of hirsutism is determined not only by a modest increase in circulating androgens but also by the sensitivity of the hair follicles to androgens. It would appear that utilization of androgens by the skin and hair follicles is greater in PCOD patients who develop hirsutism (Yen, 1986). Decreased hair follicle sensitivity to androgens probably explains the failure of hirsutism to occur in some patients. Circulating levels of SHBG play a complex role that contributes to the concentration of FT (McKenna *et al* 1984). It is possible that local oestrogen formation by the hair follicles may play a modulating role in determining the degree of hirsutism. Hair follicle is a sex steroid target but the mechanism of hair growth regulation is poorly understood (Yen, 1980). Hirsutism has been reported to occur in around 50% of PCOD patients

(Stein & Leventhal, 1935; Ibrahim *et al* 1966; Chang *et al* 1983; Adams *et al* 1986a; Wajchenberg *et al* 1986). In one review the incidence was reported to range between 17 - 83%, with an average of 69% (Goldzieher & Axelrod, 1963). The onset of hirsutism was noticed in 4 patients before the age of 15 years giving an incidence of 62% (Goldzieher & Green, 1962). Hirsutism is graded according to the Ferriman and Gallwey scoring system (1961) where a score of 8 is considered the limit for normal feminine hair growth as the 95th centile of normal menstruating women falls in this range. The diagnosis is possible among 75% of all hirsute patients after exclusion of late-onset CAH (Baron, 1991).

2.1.4 OBESITY

Body mass index is calculated as weight in Kg/(height in metres)^2 (Eden *et al* 1989c). Obesity is defined as BMI ≥ 25 Kg/m^2 (Bates & Whitworth, 1982; Conway *et al* 1989; Eden *et al* 1989c) or BMI ≥ 29 kg/m^2 (Kazer *et al* 1990; Slowinska-Srzednika *et al* 1992). Others define obesity as body weight exceeding ideal body weight by 20% or more (Bates & Whitworth, 1982). Women with PCOD tend to be obese (although this may reflect a genetic tendency, (Polson *et al* 1988)). Most of the researchers agreed that changes in body weight in PCOD lead to variations in clinical and biochemical presentations of the syndrome. In patients attending infertility clinics, 15% were found to be obese, hirsute and oligomenorrhoeic (Eden *et al* 1989c). Although obesity has been reported to be infrequent in PCOD in some series (Goldzieher & Green, 1962; Ginsburg & Havard, 1976) in others up to 50% of PCOD patients had some degree of obesity (Bates & Whitworth, 1982; Yen, 1986). Marked obesity and signs of masculinization tended to be correlated (Goldzieher & Green, 1962). Hirsutism and oligomenorrhoea were strongly associated with obesity and increase in FAI in PCOD patients (Gindoff & Jewelewicz, 1987; Conway *et al* 1989; Eden *et al* 1989c). Free androgen index is correlated with BMI. The pathophysiology of this increase in fat disposition, usually apparent at the time of puberty, is unknown. However, recent research has shown that I is a trophic agent. Fat cells are oestrogen targets and the increased adipose tissue in PCOD patients may be causally related to the exposure of fat cells to chronically elevated and unopposed extra-ovarian oestrogen production, probably beginning at the time of puberty (Yen, 1986). Obesity at puberty or later in reproductive years is one mechanism for onset of PCOD by increased production of E_1 (Bates & Whitworth, 1982). Sex hormone binding globulin-binding capacity is also decreased in obesity (Kim *et al* 1979; Laatikainen *et al* 1983). Obesity is often associated with IR (Polson *et al* 1988; Rebuffé-Scrive *et al* 1989). It was found that obese PCOD patients had higher plasma I values than non-obese, but lean body mass, glucose tolerance, plasma TG, blood pressure were not different in spite of almost a two times increase in body fat mass in obese PCO females.

There is a preferential abdominal accumulation of adipose tissue (Ibrahim *et al* 1966) with a high WHR in both PCO groups (Rebuffé-Scrive *et al* 1989). While comparing the non-obese PCOD control women, with equal body fat mass, the PCOD women had higher blood pressure, plasma TG and I, as well as a tendency to increased lean body mass. It might be that hyperandrogenicity plays a part in body composition in this disease but it has not been proved if metabolic abnormalities are a consequence of these endocrine disturbances. Body fat distribution is more closely related to hypertension and metabolic derangement than total fat mass in PCOD. Whether these metabolic abnormalities are due to obesity is not clear.

Simple weight reduction reduced plasma androgen excess and restored cyclic ovulation in 85% of women who lost more that 15% of body weight (Bates & Whitworth, 1982; Schriock *et al* 1985). It was reported that 77% of infertile females conceived spontaneously after weight reduction (Bates & Whitworth, 1982) suggesting that the increased extraglandular aromatization found in association with obesity was reversible and that a reduction of extraglandular E_1 production facilitated restoration of cyclic ovulation in women with chronic anovulation. Androstenedione and T are decreased following weight reduction. Simple weight reduction is recommended as a first method of treatment (Bates & Whitworth, 1982) and other modalities of therapy should be used as adjuvants if the patient fails to respond to weight reduction alone. Obesity is associated with a lower success rate and spontaneous abortion remains a prominent complication even after GnRH-A suppression (Filicori *et al* 1990). It has been reported that obese patients required larger doses of Gn in PCOD for induction of ovulation. The difference between obese and non-obese PCOD patients was independent of the baseline and/or mid-follicular LH concentration either before or during therapy and it was also independent of maternal age (Hamilton-Fairley *et al* 1992). Treatment of obesity in the hope of restoring normal SHBG levels and decreasing androgen production is generally frustrating but it should be encouraged before considering ovulation induction (Schriock *et al* 1985; Hamilton-Fairley *et al* 1992).

2.1.5 ENDOMETRIAL CARCINOMA

Endometrial hyperplasia can develop in PCOD patients at a relatively young age because of prolonged unopposed oestrogen stimulation of the endometrium (Yen, 1980; Gindoff & Jewelewicz, 1987). An excessive rate of oestrogen production compared to that of ovulatory women, (mainly E_1, from A; extraglandular, with little from the ovary directly) would lead to various degrees of endometrial hyperplasia in a number of patients and in some this hyperplasia progresses to carcinoma (Aiman *et al*

1978; Yen, 1980; Yen, 1986; Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987). Reliable estimates of the incidence of endometrial carcinoma in PCOD are not available. Goldzieher & Green (1962) in their review of 466 cases of surgically proven PCO, collected 46 cases of endometrial carcinoma in association with PCOD. Endometrial hyperplasia was diagnosed in 5 patients and endometrial carcinoma in 3 patients out of 17 patients studied in one series (Aiman *et al* 1978). In the great majority of cases, the carcinoma is well differentiated and with high survival rate (Schwartz *et al* 1987). To prevent the endometrial changes, periodic treatment with progestational agents is indicated; medroxyprogesterone acetate is used in a dose of 10 mg for 5 days every 6 weeks (Schriock *et al* 1985). Induction of ovulation is possible if endometrial biopsy indicates improvement. This treatment carries minimal risk to the patient and if no improvement is observed during conservative therapy, hysterectomy can be performed. There is no evidence that the delay is harmful to the survival of the patient (Schriock *et al* 1985). A high coincidence of ovarian tumours has been reported in these patients and this should be taken into account during their evaluation (Schwartz *et al* 1987).

2.1.6 GALACTORRHOEA

Galactorrhoea may be a presenting symptom (Futterweit & Mechanick, 1988). Its exact incidence is as yet not defined, but it has been reported in 13% of patients with PCOD in one series (Futterweit, 1984). Careful examination of breasts should be performed routinely to identify the presence or absence of galactorrhoea.

2.1.7 INSULIN RESISTANCE

Hyperinsulinaemia resulting from IR is common among women with PCOD (Moller & Flier, 1991) and it is similar to that of individuals with NIDDM. Patients with PCOD are among the youngest women at risk for NIDDM. About 20% of obese PCOD patients have impaired glucose tolerance or frank NIDDM by their third or fourth decades (Dunaif, 1992a). The effect is exacerbated by the presence of obesity, a common finding in PCOD patients. Non-obese PCOD patients also have an increased risk of NIDDM, probably at a later stage of life. Hyperinsulinaemia has been associated with hyperandrogenism. This association has been suggested to affect lipid profiles and therefore, places PCOD patients at increased risk of cardiovascular disease.

2.1.8 OTHER PRESENTATIONS

Clitoral enlargement and virilism could be identified in 21% of PCOD patients with ranges between 0 - 28% (Goldzieher & Green, 1962). An adequate degree of oestrogenic activity as determined by breast and vaginal mucosal development is invariably present. In the majority of cases, bilaterally enlarged cystic ovaries can be detected on examination (Goldzieher & Green, 1962) although unilateral enlarged or even normal sized ovaries are also encountered (Ibrahim *et al* 1966). Normal ovaries were diagnosed in 30 - 40% (Futterweit & Mechanick, 1988).

Cystic acne, or diffuse scalp hair loss may be a presenting symptom (Futterweit & Mechanick, 1988). Polycystic ovarian disease may also be associated with acanthosis nigricans (Schriock *et al* 1985). Acanthosis nigricans, described as a brown velvety, sometimes as verrucous, discoloration of the skin frequently noted around the nape of the neck and axillae was reported in 5-10% of PCOD patients and with skin tags in 20% of cases (Schriock *et al* 1985; Futterweit & Mechanick, 1988). Acanthosis nigricans is present in a number of endocrine disturbances including PCOD, hyperthecosis and pituitary tumours. Its significance may be its correlation with IR (Futterweit, 1984). Virilization (cliteromegaly, deep voice and temporal baldness) is uncommon in PCOD, so if present other disorders of androgen abnormality must be excluded (Insler & Lunenfeld, 1990). It was noticed that the incidence of extensive virilization and endometrial carcinoma increased with advanced age (Goldzieher & Green, 1962).

Conway *et al.* (1989) studied 556 PCOD patients. They divided the patients into high and normal LH groups. Infertility was more prevalent in the high LH group, but not hirsutism. Amenorrhoea was less in the high LH group. Infertility was diagnosed in 21% with higher significant mean T and LH and FSH concentrations. Hirsutism was diagnosed in 61% of cases with significantly greater mean BMI, higher T and lower mean FSH. Hyperprolactinaemic patients had significantly lower mean T concentrations and smaller ovarian volumes than normoprolactinaemic patients. There was no difference in infertility, hirsutism, menstrual irregularity, uterine size and endometrial thickness between the hyper and normal prolactinaemic groups. It can be concluded that the range of incidence of each symptom reported by different authors is dramatically large. These clinical variations have been claimed to be simply different stages in the evolution of the disease process. However, this explanation is unsupported by evidence and no detectable association between the duration of the disease and the completeness of the syndrome (Goldzieher & Green, 1962) has been demonstrated. The wide range observed in the incidence of each of the used symptoms probably indicates differences in the diagnostic criteria or in the sources from which the patients were obtained (Goldzieher & Green, 1962; Venturoli *et al* 1989). It is well recognized that the combination of ovulatory failure (and infertility), hirsutism, obesity and bilaterally

enlarged cystic ovaries is not unique to the PCO syndrome. It is also associated with Cushing's syndrome, CAH, virilizing ovarian and adrenal tumours, hyperprolactinaemia and hyper/hypothyroidism (Yen, 1980). Therefore formation of rigid clinical criteria will not fit many patients who subsequently are shown to have PCOD (Goldzieher & Green, 1962; Gindoff & Jewelewicz, 1987). PCOD probably represents a whole array of different disturbances leading to a similar structural change in the ovaries and clinical diagnosis is therefore presumptive in the majority of cases. Despite the high prevalence of PCOD in the normal population (22%) it cannot be considered as a normal ovarian variation but is a definite morphological entity (Polson *et al* 1988). Frequent follow up and laboratory testing are required because clinical features may vary at different times, with episodes of anovulation alternating with ovulatory cycles or oligomenorrhoea (Futterweit & Mechanick, 1988).

The prevalence of PCOD detected by transvaginal ultrasound scan in women with a history of recurrent spontaneous miscarriage was reported to be high (Sagle *et al* 1988; Tulppala *et al* 1993). It ranged between 44-82% while the prevalence of PCOD was 18-20% in the control population. Hyperandrogenism associated with PCOD appeared to be a significant factor (Tulppala *et al* 1993). About $1/5^{th}$ of women with recurrent miscarriage are hyperandrogenic and 83% of them miscarried again. Other researchers tried to relate the weight of the patient to the poor outcome. Miscarriage rate was found more frequent in the obese group (60%) than the lean group (27%) treated with Gn and was independent of mid-follicular LH concentration and the maternal age (Hamilton-Fairley *et al* 1992). Bohrer & Kemmann (1987) found that obesity does contribute to the high miscarriage rate.

2.2 CONCEPTS OF PATHOPHYSIOLOGY

The pathophysiology of PCOD is still uncertain and is the subject of hypotheses and speculations. This is true, in particular, of the possible mechanisms in the initiation and the maintenance of the syndrome.

Excessive FSH stimulation was postulated as the cause of ovarian changes when Ingersoll and McDermott (1950) were the first to suggest that PCOD might be due to excess LH secretion but this was not documented by hormonal assays at that time. McArthur *et al.* (1958) were the first to document increased urinary excretion of LH in PCOD women (Givens, 1982). Some studies have incriminated the ovary as the main site of androgen hypersecretion, but the possible role of the adrenal gland in the aetiology of PCOD is still a question. Pituitary dysfunction does not appear to be the source of the defect. The role of the hypothalamus has been also explored and is still open to more detailed investigations.

It is uncertain whether the clinical abnormalities are due primarily to abnormal steroidogenesis by the ovary or the adrenal cortex which produces elevated levels of circulating androgens with secondary changes in Gn secretion or whether higher centres like the hypothalamus initiate abnormal Gn release which in turn causes polycystic changes in the ovary and abnormal steroidogenesis (Duignan *et al* 1975; Mahesh *et al* 1978).

The clinical and biochemical derangements associated with PCOD result in a classic picture of acyclic Gn secretion, androgen hypersecretion, increased peripheral conversion of androgens to oestrogen, deficient E_2 production compared to that expected at mid-cycle, anovulation and infertility.

2.2.1 INAPPROPRIATE GONADOTROPHIN SECRETION (IGS)

Follicle stimulating hormone and LH must act in harmony to ensure the proper interaction between oestrogens and androgens at the ovarian level to modulate normal follicular function. The optimal conditions for follicular maturation depend on LH:FSH ratio (Heineman *et al* 1984). It appeared that successful maturation of small antral follicles during the early follicular phase was enhanced when there was a relatively high FSH concentration, thus a low LH:FSH ratio. It is well established that this normal sequence of events does not occur in PCOD (Coney, 1984) and as a result of a chronic persistent increase in the LH:FSH ratio, hyperandrogenism and relative hyperoestrogenism result with different ratio of E_1:E_2 (Venturoli *et al* 1989). The presence of numerous follicles in different stages of development and atresia are compatible with inappropriate Gn secretion and inadequate folliculogenesis in PCOD (Yen *et al* 1970b). Abnormal Gn secretion is a characteristic feature of PCOD. Inappropriate gonadotrophic secretion , that is, increased LH:FSH ratio, occurs in approximately 70% of PCOD patients (Hylka & diZerega, 1990). Repeated sampling of serum LH and FSH for several cycles in patients suspected to have the disease may further increase the frequency of IGS which is an important feature of PCOD (Mechanick & Futterweit, 1986). Therefore different LH levels do not necessarily mean different subgroups but rather a reflection of different hormonal statuses from time to time.

Abnormal Gn secretion could be due to the central nervous system, hypothalamic-pituitary, ovarian and adrenal derangements. Combinations of causes have also been described.

PCOD may be represented by two distinct stages : a causative central nervous system generator stage and a resultant effector stage, manifested by a series of events involving the ovaries and the brain (Futterweit, 1984; Mechanick & Futterweit, 1984; Mechanick & Futterweit, 1986). The events which create an

initial state of IGS are referred to as the "generator" stage. Abnormal neural development in the brain decreases the pituitary set-point for negative and positive ovarian hormone feedback. This results in inappropriately elevated LH levels compared to those of FSH thus termed IGS. It is maintained by ovarian hyperandrogenaemia, increased peripheral aromatization of androgens, yielding elevated free E_1 and unbound E_2 levels. Oestrone suppresses release of FSH while E_2 exerts a positive feedback on LH release by increasing pituitary sensitivity to GnRH. Therefore, a vicious cycle is created in this effector stage causing polyfollicular ovaries and increased ovarian androgen production (Mechanick & Futterweit, 1984; Mechanick & Futterweit, 1986).

The origin of the primary defect of PCOD is not settled. There is a general agreement on the pubertal onset of PCOD (Mechanick & Futterweit, 1984; Mechanick & Futterweit, 1986; Lobo, 1988; Venturoli *et al* 1989) that establishes a persistent faulty hypothalamic-pituitary-ovarian axis (Mechanick & Futterweit, 1984). Anovulation is one of the most common features of the developing reproductive system and it is physiological during adolescence (Schaison, 1988; Venturoli *et al* 1989). Children in early puberty have a surge in LH secretion once a day that is approximately synchronous with the nocturnal sleep period. Younger children and girls of late puberty or adulthood have no special surge period; they have episodic LH secretion of an essentially constant magnitude throughout the 24-hour cycle (Zumoff *et al* 1983). It has been reported that the normal mid-cycle surge in LH that initiates ovulation usually begins during sleep and high nocturnal levels do not seem to be necessary for ovulation (Venturoli *et al* 1988; Mauvais-Jarvis & Bricaire, 1989). Desynchronization of the "LH clock" from the sleep period may result in failure of the mid-cycle LH surge to occur and anovulation. This is followed by an endless positive feedback cycle in which excessive androgen production leads to excessive E_1 formation, excessive LH secretion and excessive ovarian androgens (Zumoff *et al* 1983). Thirty percent of anovulatory adolescents showed LH mean values similar to those of PCOD patients, and similar pulsatile patterns with increased pulse amplitudes which were significantly higher than those of normal adolescents but had a similar pulse frequency (Venturoli *et al* 1989). Low pulse frequency in adolescents could be due to neuro-endocrine immaturity. Polycystic ovarian disease patients and adolescents both have similar LH circadian secretion (peak in the afternoon) (Venturoli *et al* 1989). As many of the girls are adolescents, they cannot be called PCOD but rather PCOD-like. Some individuals show improvement of both LH pattern and the ovarian aspect in late adolescence while persistence of PCOD features beyond adolescence, probably indicates an establishment of deranged endocrinological pathways. However, no obvious discriminative marker(s) is yet known.

Kandeel *et al.* (1980) observed that the mean height of their PCOD patients was less than that of controls, which led them to postulate the presence of an early adrenal androgen release resulting in a

premature epiphyseal closure. Onset of menarche is delayed in many PCOD patients and in the majority, menstruation has never been regular (Ibrahim *et al* 1966; Falsetti *et al* 1989).

2.2.2 HYPOTHALAMIC / PITUITARY DYSFUNCTION

Abnormal LH circadian control could upset the follicular maturation and steroidogenesis. This would provide evidence for a primary central nervous system defect in PCOD. This might also support the hypothesis of the presence of a low dopaminergic inhibitory tone responsible for enhanced pulsatile LH secretion in PCOD (Venturoli *et al* 1988). β-endorphins have been proposed as the most important opioids involved in pathophysiology of disturbed Gn secretion in PCOD (Lobo & Goebelsmann, 1982; Laatikainen *et al* 1987; Petraglia *et al* 1990). The central opioid activity does not seem to be increased in PCOD patients despite the fact that circulating endorphins levels are increased (Laatikainen *et al* 1987). Dopamine, serotonin and noradrenaline are neurotransmitters contributing to LH regulation (Segos *et al* 1989; Hylka & diZerega, 1990). It has been suggested that dopamine may play a physiological role in the genesis of LH pulses, therefore an alteration of hypothalamic-dopaminergic regulation of LH secretion has been implicated in abnormal LH secretion in PCOD (Lightman *et al* 1981; Lobo & Goebelsmann, 1982; Murdoch *et al* 1984; Lobo, 1988; McKenna, 1988; Paradisi *et al* 1988; Berga & Yen, 1989; Ferriani *et al* 1989; Segos *et al* 1989; Hylka & diZerega, 1990; Petraglia *et al* 1990; Yoshino *et al* 1990; Lanzone *et al* 1993). However, dopamine receptor blockade was found not to block LH pulses; therefore it does not alter LH response to GnRH, possibly having its effect at the hypothalamic level rather than due to changes in pituitary sensitivity to GnRH. It has been reported that dopamine was a major factor causing a decrease in secretion of immunoactive LH but it did not correlate with the ratio of BLH : ILH (Lobo *et al* 1984). The aetiology of chronically acyclic Gn secretion in PCOD is thought to remain in the domain of inappropriate feedback by ovarian and androgen factors rather than being a primary hypothalamic defect (Berga & Yen, 1989; Lobo, 1988). The role of opioids in modulating reproductive function is diffuse from brain to gonads but it is thought that each works independently (Petraglia *et al* 1990). Hypothalamic opioids act on release of GnRH/LH axis while gonadal opioids modulate the release of steroid hormones. Ovulation is the key event which is characterized by the most relevant changes in hypothalamic, plasma and follicular fluid opioids. At present, it may be suggested that opioids play a major role in regulation of reproductive events. The efficacy of naltrexone (long-acting opioid antagonist) in normalizing LH levels after a GnRH bolus may represent a useful tool for PCOD management (Lanzone *et al* 1993).

It has been suggested that the mechanism of dopaminergic control of PRL is disturbed (Alger *et al* 1980; Milewicz, 1984; Ferrari *et al* 1988; Minakami *et al* 1988b) with hyperprolactinaemia. Hyperprolactinaemia has been reported in PCOD patients with variable prevalence (20-50%). Ferrari *et al.* (1988) in their series found high plasma PRL levels in about 12% of their PCOD patients with great day-to-day variability, that is, persistent physiological circadian rhythmicity. The role of increased PRL in association with PCOD is thought to be through either an abnormal hormone status acting on hypothalamic regulation, directly on Gn secreting cells or indirectly by decreasing dopaminergic tone (McKenna, 1988). Alternatively hyperprolactinaemia may induce disorders of ovarian steroidogenesis leading to morphological ovarian changes. It was suggested that a significant portion of PCOD patients have abnormal PRL secretion and that the oestrogen pool is augmented by increased synthesis from androgen conversion and enhanced sensitivity of lactotroph cells can be assumed despite not all PCOD patients having hyperprolactinaemia (Milewicz, 1984). The data of persistent circadian rhythm of PRL secretion, the normal response to TRH and dopamine antagonist (domperidone) and the lack of effect of dopaminergic drugs such as bromocriptine and L-dopa, on plasma LH in PCOD, do not support the hypothesis of a central dopamine impairment in PCOD at least as far as regulation of PRL secretion (Ferrari *et al* 1988; Minakami *et al* 1988b; Ferriani *et al* 1989; Segos *et al* 1989). However, involvement of central dopaminergic changes in the pathogenesis of IGS in PCOD cannot be excluded. It is thought that as the prevalence of hyperprolactinaemia in PCOD may be low rather than high; the association of hyperprolactinaemia with PCOD may be coincidental rather than a pathogenically related phenomenon (Minakami *et al* 1988b).

The role of PRL in the pathophysiology of PCOD is therefore unknown but its receptors are present in both ovarian and adrenal glands (Vaitukaitis, 1983). The data argued that dopamine is not the sole regulator of release and secretion of LH. Despite the high secretion of LH, an adequate pituitary store may be present in PCOD and LH is being synthesized. This is indicated by an augmented LH response following clomiphene or LHRH (Yen *et al* 1970c; Dnignan *et al* 1975). Hence, the hyperdynamic state of Gn secretion in PCOD could be due to an increased sensitivity of the pituitary gland to GnRH (Bates & Whitworth, 1982; Givens, 1982; Lobo *et al* 1984; Levin *et al* 1991; Dunaif, 1992b). The discrepancy between LH and FSH levels in PCOD suggests an increased responsiveness of the positive feedback mechanism in the high LH group (Givens *et al* 1976). This is supported by increased responsiveness of LH secretion to LHRH. There is considerable variation in response to LHRH in PCOD patients (Givens, 1982). It is postulated that there is an increase in endogenous GnRH (Givens, 1982; Waldstreicher *et al* 1988). Increased LH pulse amplitude may result from a combination of hypothalamic and pituitary effects whereas pulse frequency clearly reflects hypothalamic events - that is an accurate reflection of

GnRH secretion (Waldstreicher *et al* 1988). Whether PCOD is due to a central (neurotransmitter) or to a hypothalamic-pituitary (neurohormone) abnormality remains unsolved. It is rather difficult to resolve because of the lack of non-invasive measures for assessing the neurotransmitters and multiple inputs regulating the neurohormones (central nervous system, positive and negative feedback).

2.2.3 THE ROLE OF ABNORMAL STEROIDOGENESIS IN THE PATHOPHYSIOLOGY

It is assumed that optimal folliculogenesis is dependent upon relative changes in FSH and LH secretion during the first half of the cycle and that the cyclic pattern is lost in PCOD (Yen *et al* 1970b; Chang *et al* 1982; Givens, 1982). In the absence of normal variations in the levels of Gn, increased LH:FSH ratio, there will be arrested follicular development, theca cell hyperplasia, multiple cysts of various sizes, impaired E_2 production and hyperandrogenaemia.

It was once believed that ovulatory failure in PCO patients was due to the mechanical barrier of the thickened ovarian capsule. However, this could not be confirmed by restoration of cyclic ovulatory menses following unilateral oopherectomy or successful induction of ovulation with clomiphene (Mahesh *et al* 1978; Schwartz *et al* 1987; McKenna, 1988). There is accumulating evidence that excess androgen plays a role in the pathogenesis of PCOD (Yen, 1980; Lobo & Goebelsmann, 1982; Francis *et al* 1990; Nakamura, 1990; Pache *et al* 1992a). Hyperandrogenaemia seems to originate from the abnormally high number of cystic atretic follicles seen in PCOD and not from an augmented androgen production per follicle (Pache *et al* 1992a). Hyperandrogenaemia is mainly regulated by 5∝-R activity and SHBG which is an important modulating factor of androgen action (Lobo & Goebelsmann, 1982). Androgens by some mechanism in some cases can distort Gn secretion (Devesa *et al* 1987; Dunaif, 1992b) to produce the typical Gn secretion abnormality of PCOD. There are three mechanisms for their action: (i) androgen can be converted by aromatization both in peripheral tissues (adipose tissue and muscles) and in the hypothalamus into oestrogen, (ii) oestrogens that can be made are E_1 and E_2. Oestrone is primarily derived from A and T can be converted into E_2, (iii) E_2 continues to be secreted by the ovary in PCOD (Dunaif, 1992b). There is no evidence for an androgen effect on the pituitary gland as there were no changes in sensitivity to GnRH while oestrogens appear to act at the pituitary (Dunaif, 1992b) by selectively increasing reserves of LH causing an enhancement of LH release in response to GnRH. Reduction of circulating steroid levels appears to be a more important factor in the resumption of ovulation than is disturbance in hypothalamic regulation (Gambrell *et al* 1973). Studies to determine

ovarian and adrenal contributions to androgen excess yielded conflicting results because of limitations of the methods for assessing glandular sources of androgen production by selective venous catheterization and DXM suppression tests (Dunaif, 1992b). Hyperandrogenism may be adrenal, ovarian or combined in origin. It was demonstrated that purely ovarian or adrenal androgen overproduction is not very frequent (< 39%) (Fulghesu *et al* 1991). Several dynamic tests for suppression or stimulation (DXM suppression test, ACTH stimulation test) to differentiate between ovarian and adrenal contributions have been attempted with controversial results. Dynamic tests can also detect the presence of genetic enzyme defects which affect adrenal steroid production in 9% of hyperandrogenaemic women (Fulghesu *et al* 1991). It is now appreciated that there are three compartments for androgens : ovarian, adrenal and peripheral conversion (Lobo, 1988). Combined suppression of ovarian (with long acting GnRH) and adrenal (DXM) provided evidence that the adrenal contributes to androgen excretion (Devesa *et al* 1987; Lanzone *et al* 1990b; Dunaif, 1992b). The ovary is thought to be the main source of androgen and peripheral T and A excess (Aiman *et al* 1978; Kim *et al* 1979; Chang *et al* 1983; Fleming *et al* 1985; Puzigaca *et al* 1991). The majority of the serum T originated from the ovary and the correlation between T and DHEA-S suggested the possibility that DHEA-S may serve as an important precursor for ovarian steroidogenesis. It is suggested that the primary defect in some forms of PCOD is in the ovary rather than in the hypothalamus or pituitary (Berger *et al* 1975; Ginsburg & Havard, 1976; Vaitukaitis, 1983; Adams *et al* 1986a; Berga & Yen, 1989; Francis *et al* 1990; Rittmaster & Thompson, 1990). It is thought that the decline in sex steroids of ovarian origin would eliminate a persistent positive feedback which allows LH back to normal levels while not affecting FSH, therefore normalizing the ratio and causing ovulation (Katz *et al* 1978). Exogenous oestrogen administration resulted in a slow decline in both Gn in normal patients while in PCOD patients LH decreased but FSH did not (Yen *et al* 1970b) and with an enhanced LH responsiveness to GnRH (Chang *et al* 1982). This suggests an intact oestrogen negative feedback mechanism (Yen *et al* 1970b). The effect could be due to E_1 or mildly increased E_2 levels. The disparity between Gn levels may reflect the preferentially inhibitory feedback action of oestrogen on FSH compared to LH (Chang *et al* 1982). It was reported that the positive and negative feedback mechanisms for LH secretion are qualitatively intact but may differ quantitatively among individuals (Givens, 1982). Synthesis and secretion of FSH are suppressed by excess circulating oestrogens (Vaitukaitis, 1983).

Despite all of this data, tissue culture studies showed the ability of isolated granulosa and theca cells from mid-antral follicles to secrete C21 and C19 steroids in the absence of Gn stimulation and their intact steroidogenic capacity (Wilson *et al* 1979) seems to exclude a primary ovarian steroidogenic dysfunction.

No causative abnormality of adrenal function or ACTH secretion has been established (Kandeel *et al* 1980). The mechanism of increased androgen production by the adrenal cortex is unknown. In one series this was found to occur in 60% of cases (9/15 patients) (Kandeel *et al* 1980). It could be due to high LH stimulating adrenal androgenesis (Yen *et al* 1970b). The contribution from the adrenal gland cannot be excluded (as the source of peripheral excess) (Berger *et al* 1975; Aiman *et al* 1978; Rittmaster & Thompson, 1990). There is actually mild hypersecretion of adrenal androgens associated with PCOD without apparent specific dysfunction (McKenna, 1988). Elevated adrenal androgen levels have remained unexplained (Lobo *et al* 1983). In PCOD, the adrenal related androgen production is positively related to baseline values. The higher the baseline, the more important is the amount of secretion of these androgens. In PCOD 1/3rd of the patients have adrenal-derived excess androgens based upon high DHEA-S levels (Fulghesu *et al* 1991). The adrenal gland may trigger PCOD in some females. Alternatively, it is also possible that ovarian T and A could start it. A certain degree of a chronic state is dictated by the LH stimulating effect, leading to an increase in unbound T and A, an increase in E_2, exaggerated LH secretion with IGS and chronic anovulation and subsequently increased androgen (Lobo & Goebelsmann, 1982).

There is some abnormality in the regulation of adrenal steroidogenesis in many PCOD patients (Devesa *et al* 1987; McKenna, 1988; Fulghesu *et al* 1991; Dunaif, 1992b) characterized by elevated levels of 17α-OHP and DHEA-S and exaggerated 17α-OHP, A and cortisol responses to exogenous ACTH (Devesa *et al* 1987).

Ovarian hyperandrogenism may induce adrenal enzymatic defects (Vermesh *et al* 1988). It was proposed that ovarian 17KSR deficiency, transmitted as AR trait or X-linked R, may result in hirsutism during puberty, along with menstrual dysfunction and PCOD (Pang *et al* 1987; Toscano *et al* 1990). There is no clear cut evidence for a deficiency in 3β-OHSD activity in PCOD patients (Hague *et al* 1989). Congenital adrenal hyperplasia and PCOD have been interrelated. It was found in one series that only 6.4% of PCOD patients had evidence of 21OH deficiency (Hague *et al* 1989), so it is unlikely that PCOD is due to an adrenal defect.

Follicular maturation is FSH dependent, therefore it seems more logical to consider that the central defect is a failure of FSH action which may be exacerbated by high levels of androgens within the follicles. If no ovulation occurs, there is no subsequent P rise and so the normal negative feedback control of Gn secretion is disturbed. The expected inter-cycle rise of FSH fails to occur (Franks *et al*

1988). Although P is essential for a normal Gn surge, it operates by synergizing the obligatory action of E_2 (Liu & Yen, 1983).

Polypeptide growth factors are paracrine modulators of FSH action and the most important of these are: (i) stimulatory : IGF-I and II,TGF-β ; (ii) inhibitory: TGF-α, EGF and inhibin. Other paracrine modulators of FSH are oestrogens and androgens. These paracrine growth factors are of potential importance in controlling the action of FSH (Franks *et al* 1988).

Inhibin is produced exclusively by granulosa cells and has been proposed as a non-steroidal marker of granulosa cell function and follicle viability. The relative FSH decrease to GnRH may primarily reflect pituitary desensitization (Waldstreicher *et al* 1988) and bioactive inhibin was reported to be elevated in PCOD and could play a role in granulosa cell dysfunction (Tanabe *et al* 1990; Pache *et al* 1992a). Inhibin activity in the ovary depends on follicular viability and not on follicular size. Since PCOD follicles contain fewer granulosa cells per follicle in comparison with normal follicles, yet contain comparable levels of inhibin, it is possible that the inhibin secretion per granulosa cell is greater in PCOD than in the normal ovary (Tanabe *et al* 1990). Nevertheless, there was no evidence for a defect in ovarian inhibin production (Waldstreicher *et al* 1988; Pache *et al* 1992a).

In summary, the hyperplastic ovarian theca is thought to be secondary to LH stimulation, immature ovarian follicles are secondary to relative suppression of FSH release and subcapsular follicles are secondary to chronic anovulation due to disordered Gn secretion, while increase in follicular atresia and cortical thickening are due to high androgen levels (Dunaif, 1992b). Possible causes of PCOD include a vicious cycle of increased serum T levels, leading to increased serum LH levels, leading to further increases in serum T levels. This remains a functional disorder while morphological changes in the ovaries are not found, but a second vicious cycle becomes involved (high serum T levels leading to LH/FSH separation, leading to further increases in serum T levels), then morphological changes in the ovaries, i.e. PCO will come about (Takahashi *et al* 1991). In females with high T, the presence of a morphological abnormality on the ovaries has an effect on Gn production and so the incidence of menstrual irregularity and hirsutism. If PCOD is morphologically identified and clinical symptoms appear, the interactions of the hypothalamic-pituitary-ovarian system are disturbed. Even if no morphological indications of PCOD are present but abnormalities of serum androgen and Gn are evident, the possibility of PCOD developing should be considered. If PCOD is found, but androgens and Gn are normal; then the possibility of future hormonal abnormalities should be suspected (Takahashi *et al* 1991). Regular hormonal assays are therefore required for diagnosis.

2.2.4 THE ROLE OF OBESITY IN THE PATHOPHYSIOLOGY

There is little doubt that obesity and anovulation are interrelated phenomena (Friedman & Kim, 1985). Over half of the patients with PCOD have some degree of obesity (Ahlgren *et al* 1976; Yen, 1986; Lobo, 1988; Dale *et al* 1992b). The role of increase adiposity in PCOD was suggested by findings of increased body weight and BMI in PCOD patients when compared with idiopathic hirsutism and control patients (Byrne *et al* 1991). The pathophysiology of this increase in fat disposition, usually apparent at the time of puberty, is unknown. However, it is postulated that oestrogen regulates adipocyte replication and differentiation. Fat cells are oestrogen targets and the increased adipose tissue in PCOD patients may be casually related to the exposure of fat cells to chronically elevated and unopposed extra-ovarian oestrogen production, probably beginning at the time of puberty. Obesity may play a role in the pathogenesis of PCOD (Fisher *et al* 1974; Bates & Whitworth, 1982; Pasquali *et al* 1983; Falsetti *et al* 1989). Obesity at puberty or later in reproductive life could be an initiating factor by increasing E_1 production and consequently altering pituitary Gn secretion. Obesity has been found to cause changes in steroid hormone metabolism (Lobo *et al* 1981; Laatikainen *et al* 1983) through a decrease in SHBG concentrations with resultant increase in unbound, biologically active, steroids (E_2, T), therefore contributing to the increase in Gn secretion. Obesity was associated with high free androgen indices, low LH:FSH, low concentration of LH, A and SHBG (Paradisi *et al* 1986; Anttila *et al* 1991; Invitti *et al* 1991; Anttila *et al* 1992). The mechanism by which body weight could induce lower LH secretion in PCOD patients is not clear. It could be due to sex steroid imbalance. β-endorphins are thought to be more elevated in obese PCOD patients and by an inhibitory mechanism may suppress the LHRH-LH system to a greater extent than in non-obese PCOD patients (Paradisi *et al* 1986). The apparent success of weight reduction in suppressing the hyperandrogenaemia (Friedman & Kim, 1985; Eden *et al* 1989c), causing a decrease in E_1, normalizing Gn secretion and restoring ovulation suggest a common link (McKenna, 1988). Inappropriate Gn secretion and ovarian changes are consequences of obesity which could be due to the presence of hyperoestronaemia and hyperinsulinaemia (Friedman & Kim, 1985; Pasquali *et al* 1986; McKenna, 1988; Invitti *et al* 1991; Lanzone *et al* 1991; Carmina *et al* 1992). The increased aromatase activity and hyperinsulinaemia observed in obese subjects probably plays a major role in causing the hyperandrogenaemia, either by stimulating LH secretion or by directly stimulating the ovary, as in the case of I (Friedman & Kim, 1985). In addition to ovarian hyperandrogenaemia, pituitary hypothalamic dysfunction has been observed in response to obesity. Inadequate central serotonin stimulation, excessive dopamine stimulation, and insensitivity to endorphins may all be involved in the pituitary hypothalamic dysfunction, as well as resistance to weight reduction (Lobo & Goebelsmann, 1982; Friedman & Kim, 1985).

There are several mechanisms by which obesity could be involved in the pathogenesis of PCOD: (i) increased body weight is associated with increased extraglandular aromatization of androgens into oestrogens, primarily E_1 and decreased plasma SHBG levels, (ii) obesity is associated with increased circulating levels of I which may be involved in the pathogenesis of PCOD by directly stimulating ovarian androgen production and/or altering Gn release, (iii) it is possible that obesity in PCOD is either centrally mediated (via changes in hypothalamus, control of food intake or endogenous opiate secretion) or is secondary to an androgen mediated increase in body weight and thus may be associated with a unique form of PCOD (Dunaif *et al* 1988).

2.2.5 INSULIN RESISTANCE

Hyperinsulinaemia may be the trigger mechanism of the pathogenesis of PCOD (Urdl *et al* 1991). Insulin resistance is defined as normal glucose levels in the presence of hyperinsulinaemia (Burghen *et al* 1980). Insulin resistance has been linked to hyperandrogenaemia (Shoupe & Lobo, 1984). Fasting hyperinsulinaemia may be used as a guide to the existence of IR (Shoupe *et al* 1983) and the SITT is another method for assessing IR (Akinmokun *et al* 1992) (Chapter 5-Insulin Sensitivity). In women with PCOD, independent of the degree of obesity, IR was demonstrated in terms of increased basal I levels and I responses to oral GTT (Burghen *et al* 1980; Bates & Whitworth, 1982; Pasquali *et al* 1982; Chang *et al* 1983; Dunaif *et al* 1987; Givens *et al* 1987; Schwartz *et al* 1987; Coiro *et al* 1989; Dunaif *et al* 1989; Dunaif *et al* 1990; Falcone *et al* 1990). Chang and his colleagues (1983) demonstrated raised serum I concentrations in lean women with PCOD compared with lean controls, and observed an association between serum I and androgen concentrations. An apparent association between hyperandrogenaemia and hyperinsulinaemia in the human female has been widely appreciated since 1921 (Adashi, 1990).

The primary role of I is to facilitate the supply of glucose to cells. If insufficient glucose is made available, because of resistance to the action of I, the pancreas produces more I. In cases of adequate pancreatic reserve, normal circulating glucose levels are maintained at higher serum I concentrations. If the pancreatic reserve is not adequate, impaired glucose tolerance and diabetes mellitus ensue (Shaw, 1991).

There are several processes which are known to result in resistance to the action of I and therefore hyperinsulinaemia : raised concentrations of "anti-I" hormones (acromegaly, Cushing's syndrome,

phaechromocytoma), structural defects of the I receptor (type A syndrome of IR) and the presence of antibodies against the I receptor (type B syndrome of IR). More common than any of these phenomena is obesity. The mechanism by which obesity causes IR is a complex series of events whereby reduced receptor numbers and post-receptor defects cause resistance to the action of I. The resulting hyperinsulinaemia in turn maintains the reduction in the number of receptors "down-regulation" (Shaw, 1991). Interestingly, PCOs are associated with several of the above conditions and obesity is a common finding.

The primary disturbance is apparently IR, which produces a compensatory hyperinsulinaemia to maintain euglycaemia. IR, therefore, may be due to any of the following :

1. Decreased number or abnormal function of I receptors.

2. I receptor antibodies.

3. Post-receptor defects in I expression. (Ahlgren *et al* 1976; Burghen *et al* 1980; Pasquali *et al* 1982; Shoupe *et al* 1983; Givens *et al* 1987; Jialal *et al* 1987; Falcone *et al* 1992).

Levels of I depend on the secretion rate of the β-cells of the pancreas, on the hepatic removal of I and on the type of stimulation (Pasquali *et al* 1982). There is some sort of β-cell parallel to the IR of peripheral tissue. IR is mainly generated peripherally; the changed sensitivity of peripheral tissue to I activity may constitute a primary event in the genesis of IR. This behavior has been demonstrated in obese, PCOD patients with acanthosis nigricans. Therefore, a heterogeneous group of pathogenesis exists in which the common feature is reduced peripheral IS (Randazzo *et al* 1989).

Mechanisms underlying the association of hyperandrogenism and hyperinsulinism are complex and of considerable interest (Chang *et al* 1983; Dunaif *et al* 1987; Laatikainen *et al* 1989). Obese patients were reported to have a significant correlation between I and FT levels while non-obese had significant correlations between I and FSH, DHEA-S and SHBG levels (Dunaif *et al* 1987). Dehydroepiandrosterone sulphate suppression by I is impaired in IR PCOD patients (Falcone *et al* 1990; Farah *et al* 1990). This may contribute to androgen excess. Hyperinsulinism is a heterogeneous feature of PCOD patients where 80% of obese PCOD patients showed hyperinsulinaemic pattern while only 30% of obese controls and of non-obese PCOD patients showed this hyperinsulinaemia (Lanzone *et al* 1989).

Many studies have shown a positive correlation between I and androgen levels (Burghen *et al* 1980; Pasquali *et al* 1982; Chang *et al* 1983; Pasquali *et al* 1983; Poretsky *et al* 1984; Shoupe & Lobo, 1984;

Jialal *et al* 1987; Dunaif & Graf, 1989; Lanzone *et al* 1989; Adashi, 1990; Taketani & Mizuno, 1990; Elkind-Hisch *et al* 1991; Nestler *et al* 1991; Buyalos *et al* 1992) with significant correlation in obese and non-obese patients while other researchers found that hyperinsulinaemia did not play a major role in ovarian hyperandrogenism in PCOD (Geffner *et al* 1986; Dunaif *et al* 1987; Lanzone *et al* 1989; Lanzone *et al* 1990c; AbdelGadir *et al* 1991; Anttila *et al* 1991; Dale *et al* 1992a; Dale *et al* 1992b; Toscano *et al* 1992) since hyperinsulinaemia persisted even after ovarian inhibition with a long acting GnRH-A. Hirsute patients were found not to have higher serum I levels than non hirsute patients (AbdelGadir *et al* 1991). Theoretically hyperandrogenaemia, per se, may promote hyperinsulinaemia but despite the relatively high circulating androgen in men, only a minority of them display IR. Exogenous T has no effect on fasting glucose in normal men. Lowering of circulating androgen levels in hyperandrogenic hyperinsulinaemic patients by medical or surgical means does not appear to exert a measurable effect on the IR state as assessed by GTT, and I requirement (Shoupe & Lobo, 1984; Adashi, 1990). The argument that I may stimulate ovarian androgen biosynthesis has received increasing support from both *in-vitro* and *in-vivo* studies with ovarian theca-interstitial cells the possible site of I receptors and action. The theca-interna cells' surface exhibits specific I receptors (Adashi, 1990; Nagamani & Stuart, 1990; Anttila *et al* 1991). Supraphysiologic as well as physiologic concentration of I, acting alone or with pituitary Gn, enhance ovarian androgen production by theca-interstitial cells (Dunaif & Graf, 1989; Adashi, 1990; Lanzone *et al* 1990c).

Peripheral resistance to I occurs in PCOD and the resultant hypersecretion of I may modulate ovarian/adrenal function and produce abnormal steroid secretion (Pasquali *et al* 1983; Poretsky *et al* 1984; Geffner *et al* 1986; Dunaif & Graf, 1989; Lanzone *et al* 1990c; Nestler *et al* 1991; Sharp *et al* 1991). Patients with PCOD and elevated androgens had higher IR than those with chronic anovulation and normal androgens of similar body weight (Shoupe & Lobo, 1984). The I response to oral glucose correlated positively with serum A and T concentrations, independent of the effect of LH (Shaw, 1991). It is hypothesized that many lean women with PCOD have fasting hyperinsulinaemia and that I stimulates the ovarian production of androgens which contributes to menstrual disturbances. Therefore high I could be involved in the pathogenesis rather than being a result of the primary disorder (Geffner *et al* 1986; Taketani & Mizuno, 1990).

Acute elevation of LH does not appear to cause IR in PCOD (Shoupe & Lobo, 1984; Geffner *et al* 1986). The fact that BLH but not ILH was found related to I raised the possibility that I or altered glucose metabolism in IR subjects may change the bioactivity of BLH. This is supported by the fact that BLH is lower in obese patients and correlated negatively to BMI (Anttila *et al* 1991). Since endogenous opiates are involved in modulating pancreatic islet function and increases in I secretion, hyperinsulinaemia may

be opiate dependent (Givens *et al* 1987; Laatikainen *et al* 1989; Laatikainen *et al* 1990; Lanzone *et al* 1991) but it was found that opioid blockade (by naloxone) did not affect IR response to glucose or IGF-I whereas IGF-binding protein (IGFBP-1) decreased (Laatikainen *et al* 1990). Therefore the role of opiates in the pathogenesis of PCOD is unclear (Givens *et al* 1987) and whether this may result in a reduction of hyperandrogenaemia is not known. Glucagon is not implicated in peripheral IR in PCOD (Golland *et al* 1990).

Insulin is positively correlated with BMI (Pasquali *et al* 1983; Shoupe & Lobo, 1984; Anttila *et al* 1991; Carmina *et al* 1992). Hyperandrogenaemia, obesity and acanthosis nigricans can be independently associated with IR (Jialal *et al* 1987; Lanzone *et al* 1989; Taketani & Mizuno, 1990). Acanthosis nigricans is a common finding in obese hyperandrogenic females (29%) and was found to be associated with significantly higher I levels when it occurred in obese PCOD women. The PCOD women with acanthosis nigricans had sex hormones and Gn levels similar to those in women without acanthosis nigricans, which suggests that they formed a subgroup of PCOD rather than a distinct endocrine disorder (Dunaif *et al* 1987). In acanthosis nigricans anti-receptor antibodies and I antibodies have been demonstrated (Pasquali *et al* 1982; Shoupe *et al* 1983) with normal receptors. It was found that only women with PCOD have hyperinsulinaemia independent of obesity (Dunaif *et al* 1987). The negative impact of PCOD and obesity on I action is additive (Dunaif *et al* 1989; Anttila *et al* 1991).

Insulin growth factors are peptides with structural similarities to pro-I and I, and have been shown to be intra-ovarian modulators of steroid synthesis (Urdl *et al* 1991; Shaw, 1991). The IGFs have growth-promoting and I-like metabolic effects. The liver seems to contribute to most of the circulating IGFs in adults. Granulosa cells contain and secrete IGFs. The IGF-I potentiates the stimulating effect of FSH on ovarian aromatase activity, E_2 and P secretion and expression of the LH receptor. Insulin-like growth factor-I also stimulates accumulation of androgens in the ovary (Shaw, 1991). The serum IGF-I level is regulated by GH. Growth hormone deficiency is associated with low serum IGF-I levels. The IGF-I levels decline during fasting. The IGFs are bound to specific binding proteins in serum. There have now been at least six IGFBPs identified. The circulating level of IGFBP-1 is regulated by I, whereas that of IGFBP-3 is regulated by GH. The IGFBP-1 has been found to inhibit FSH-induced granulosa cell proliferation and its serum concentration is inversely correlated to the I concentration. Obese women with PCOD and hyperinsulinaemia have subnormal serum IGFBP-1 levels. The levels of IGFBP-1 have a positive correlation with those of SHBG, which also are low in PCOD (Shaw, 1991).

The mechanism by which I stimulates androgen production by the ovary may be complex. Fasting I concentrations correlated positively with serum IGF-I and negatively with IGFBP-1 concentrations in

lean women with PCOD. The IGFBP-1 is thought to play a role in regulating the bioavailability of IGF-I and is known to be regulated by I (Shaw, 1991). Micromolar concentrations of I have been found to inhibit IGF-I binding to its receptors in multiple cells and tissues. Insulin, by causing a reduction in the circulating IGFBP-1 levels may increase the potential for IGF-I to stimulate the ovary for androgen production. The demonstration of theca-interna cells IGF receptors and their apparent coupling to the stimulation of androgen production may account for the pharmacological action of I (Adashi, 1990; Nagamani & Stuart, 1990; Anttila *et al* 1991; Buyalos *et al* 1992). Frequently, the onset of hirsutism is accompanied by weight gain in women with PCOD (Shaw, 1991). Obese patients have been found to have low IGFBP-1 levels (Anttila *et al* 1991). It is speculated that elevated BLH in lean PCOD patients stimulates theca androgen production whereas in obese patients high I concentration may act through IGF-I receptors and potentiate Gn-induced androgen production. The effect of obesity is to exacerbate the endocrine abnormalities of PCOD. Weight gain is associated with a rise in serum I and a consequent fall in SHBG concentrations (Shaw, 1991). It is possible that the IR of obesity is not only quantitatively greater than that related to PCOD but also qualitatively distinct, resulting in the need for higher serum I concentrations before significant stimulation of androgen synthesis occurs. It is therefore possible that obesity is both a result of, and a contributing factor to, the endocrine imbalance in PCOD (Shaw, 1991).

It would appear that acute elevation of circulating I levels during the euglycaemic clamp technique may not be sufficient to promote ovarian androgen production (Adashi, 1990). Women with PCOD and hyperthecosis have hyperinsulinaemia and IR. It is possible that I in supraphysiological concentrations exerts its steroidogenic action on ovarian stromal cells through IGF-I receptors. Insulin and IGF-I levels were found positively correlated. Elevated (Damajanovic *et al* 1990; Urdl *et al* 1991) or normal (Kazer *et al* 1990; Slowinska-Srzednika *et al* 1992) serum IGF-I and decreased HGH were found in PCOD. Among hormones known to cause or predispose to IR are GH, cortisol and PRL (Chang *et al* 1983). Concentrations of growth factors were examined in 28 patients with clinical and endocrinological signs of PCOD. The HGH:IGF-I ratio reflects the functional dynamics of these substances. The IGF-I concentrations were increased, therefore, HGH:IGF-I ratios were significantly decreased in PCOD patients than controls (Urdl *et al* 1991). The number of I receptors, receptor affinity and I receptor complexes were significantly higher in PCOD than controls (Urdl & Desoye, 1991; Urdl *et al* 1991). Elevated I and IGF-I levels seem to play a pathogenic role in PCOD by influencing synthesis and/or secretion of this growth factor and its binding protein (IGF-BP1) by liver cells and other IGF-I producing organs. Growth hormone has also been shown to produce relative IR (Burghen *et al* 1980).

Obese PCOD patients showed higher IR with hyperinsulinaemia, which is thought not to be entirely due to obesity because IR levels were lower in patients with simple obesity than in obese PCOD patients.

Hyperinsulinaemia in simple obesity could be due to impaired I removal in the liver (Pasquali *et al* 1982). Therefore, IR is probably the most important factor in determining the hyperinsulinaemia in obese PCOD patients.

Falcone *et al.* (1991) reported an absence of association between the degree of IR and inhibin concentrations. In addition, the same authors found that the women with PCOD had inhibin levels comparable to those of the control group during the early follicular phase but significantly lower than late follicular phase or luteal phase. They postulated that inhibin concentration in women with PCOD most likely reflects ovulatory state with lack of a dominant follicle and subsequent ovulation; therefore, inhibin may reflect impaired follicular maturation and that Inhibin and E_2 are both markers of follicular maturation. Several hormones that are known to stimulate granulosa cell differentiation appear to enhance inhibin production *in-vitro*. During a normal menstrual cycle, inhibin levels change very little from the early to mid-follicular phase. In late follicular phase, inhibin levels rise with the LH surge; peak inhibin levels occur in the mid-lutcal phase. Inhibin secretion is not acutely affected by I secretion in normal or hyperandrogenic females (Falcone *et al* 1991). *In-vitro* experiments with PCOD granulosa cells indicated the following : (i) physiological concentrations of IGF-I are as effective as FSH in stimulating E_2 production; (ii) IGF-I and FSH act synergistically to control the level of E_2 production and (iii) this synergy was not observed with I or IGF-II therapy (Holly *et al* 1990). The mechanism(s) responsible for arrest of folliculogenesis have to be established.

Insulin-like growth factor-I has been shown to potentiate the ability of LH to stimulate interstitial theca cell androgen production and an increase in local IGF-I activity could contribute to the ovarian hyperandrogenism characteristic of PCOD. Also, increased feedback of IGF-I at the level of the pituitary could lead to a reduction in GH secretion (Kazer *et al* 1990). There is considerable evidence that IGF-I plays an important role in the human ovary and that physiological activity of IGF's is modulated by a number of IGFBP's. There are at least 4 discrete types of IGFBP's in ovarian follicular fluid and it is suggested that each may be produced independently within the ovary (Holly *et al* 1990). The possibility that I, directly or via IGF-I may modify the paracrine control of ovarian steroidogenesis or the action of androgens at target tissue levels remains the subject of investigation (Toscano *et al* 1992). Hyperandrogenized patients can be subdivided into (i) those with IR, normal or minimal increase in LH and markedly elevated I levels (obesity is associated with this type); (ii) those with elevated LH levels, no IR and normal I concentration (high BLH is associated with this type) (Anttila *et al* 1991; Dale *et al* 1992b).

Fasting I concentrations are raised in 1/3 of lean and 3/4 of obese women with PCOD (Shaw, 1991). Insulin, and perhaps IGF-I, has a gonadotrophic type action promoting ovarian androgen synthesis and contributing to menstrual disturbance.

In conclusion, although there may be a threshold level of free androgens in women above which IR may be affected, androgens are only one of the multiple factors influencing the development of IR. Peripheral androgen production and LH may not correlate with hyperinsulinaemia in PCOD (Shoupe & Lobo, 1984). Polycystic ovarian disease represents a distinct subphenotype of IR with a different genetic basis from IR associated with typical NIDDM (Ahlgren *et al* 1976).

2.2.6 THE ROLE OF LEPTIN

Leptin, the so called the " fat-melting hormone ", is a newly discovered hormonal product of the obese gene. It is derived from the Greek word " leptos " which means thin (Caro *et al* 1996). Leptin is expressed by adipocytes and thought to play a role in the regulation of food intake and metabolism (Barash *et al* 1996; Caro *et al* 1996). Adipose tissue produces leptin that is then secreted into the systemic circulation (Klein *et al* 1996). Leptin receptors are widely distributed in the brain (including the hypothalamus and the choroid plexus), liver, lungs, heart, kidney, testes, adipose tissue and spleen (Caro *et al* 1996). The reported plasma leptin half life is 25 minutes (Klein *et al* 1996). Plasma leptin concentrations are gender specific, being higher in women than in men (Haffner *et al* 1996; Segal *et al* 1996). There is a circadian rhythm for leptin concentration (Pijl *et al* 1996), with highest levels between midnight and early morning hours and lowest around noon to mid-afternoon. It was postulated that the nocturnal rise in leptin could have an effect in suppressing appetite during the night while sleeping (Haffner *et al* 1996).

There is a strong positive correlation between serum leptin concentration and body fat, therefore, serum leptin concentrations reflect the amount of adipose tissue in the body (Caro *et al* 1996). When energy intake and energy output are in balance, leptin reflects the amount of TG stored in the body as adipose tissue. When a disturbance causes an increase in fat deposition and BMI (positive energy balance), an increase in energy expenditure and a decrease in energy intake due to a reduction in appetite will occur. These compensatory changes are necessary to restore the steady-state energy balance with no addition to the adipose tissue. The reverse would occur if the disturbance causes fat loss (Caro *et al* 1996). The same authors also found a large decrease in serum leptin concentration in the presence of a relatively

small change in body weight suggesting that leptin is regulated by other factors than the size of the adipose tissue depot, such as caloric intake and fasting.

Caro *et al.* (1996) proposed that most obese humans are resistant to their endogenous production of leptin since the majority of human obesity is characterized by hyperleptinaemia. This increase in leptin production may be caused by leptin antibodies, leptin antagonists, or increased production of leptin binding proteins resulting in a decrease of the free leptin reaching the brain. It is not known yet where the site(s) of leptin action is located in the hypothalamic-pituitary-gonadal axis.

It is known that hormones, such as I, glucocorticoid and adrenergic agents, regulate leptin secretion. Acute I does not stimulate leptin secretion in humans, while chronic I plays an important role in stimulating leptin secretion (Caro *et al* 1996).

Studies have revealed an association between nutritional status, adiposity and reproductive maturity in female animals. Chehab *et al.* (1997) reported that normal pre-pubertal female mice injected with leptin reproduced earlier than controls and showed accelerated maturation of the reproductive tract. In addition, leptin-treated female mice were found to have significantly elevated serum levels of LH and increased ovarian and uterine weights (Barash *et al* 1996). Therefore, it was suggested that leptin may be a metabolic signal triggering puberty (Chehab *et al* 1997), and stimulating the reproductive endocrine system of normal female mice (Barash *et al* 1996; Chehab *et al* 1997).

2.3 LABORATORY DIAGNOSIS

Biochemical studies historically were confined to analyses of urinary steroid metabolites. Elevated excretion of urinary 17-ketosteroids had been observed by many investigators. Although it is known that ovarian androgen production as T will produce less elevation of urinary 17-ketosteroids than an equivalent level of androgen production as A or DHEA from the adrenal gland (Goldzieher & Green, 1962) it was evident that there was a spread of values which precluded categorization of these patients into well defined subgroups. In addition, measurement of urinary 17-ketosteroids is of little value since both adrenal and ovarian ketosteroids are metabolized to the same excretory products (androsterone and etiocholanolone) (Goldzieher & Axelrod, 1963). As a consequence, dynamic studies of steroid metabolism were developed in an attempt to distinguish the ovarian contribution from that of the adrenal. More recently the advent of radioimmunoassays opened up new approaches to the understanding of both the disease processes and the diagnosis of PCOD.

Polycystic ovarian disease is characterized by abnormalities in plasma levels of LH, oestrogens, androgens, PRL and glucose metabolism. The clinical features of women with PCOD may vary at different times, with episodes of anovulation alternating with regular menstrual cycles. Therefore frequent laboratory testing of these women is essential (Gindoff & Jewelewicz, 1987; Futterweit & Mechanick, 1988).

2.3.1 GONADOTROPHINS

Luteinizing hormone is a heterogeneous molecule with the possibility of 2 or more molecular forms of LH being secreted with different biological activities (Lobo *et al* 1984). Variation in LH levels in PCOD can be large. There are three groups of investigators with three types of results concerning the levels of LH. The first group believe that LH values are persistently elevated compared to the levels observed in the follicular phase of the normal cycle (Duignan *et al* 1975; Rajaniemi *et al* 1980; Lobo *et al* 1981; Chang *et al* 1983; Laatikainen *et al* 1983; Heineman *et al* 1984; McKenna *et al* 1984; Abdulwahid *et al* 1985; Dunaif, 1986; Messina *et al* 1986; Eden *et al* 1988a; Homburg *et al* 1988; McKenna, 1988; Minakami *et al* 1988a; Venturoli *et al* 1988; Waldstreicher *et al* 1988; Eden *et al* 1989a; Murdoch *et al* 1989; Rojanasakul *et al* 1989), the second group divided the PCOD into two types, those with persistently elevated serum LH levels and those with levels in the normal range (up to 10 - 20% of their patients) (Goldzieher & Green, 1962; Givens *et al* 1976; Yen, 1980; Givens, 1982; Vaitukaitis, 1983; Schriock *et al* 1985; Mason *et al* 1986; Wajchenberg *et al* 1986; Gindoff & Jewelewicz, 1987; Franks *et al* 1988; Conway *et al* 1989), and the third group considered LH levels to be high, normal or low (Gambrell *et al* 1973). Different LH levels do not necessarily mean different subgroups, but may reflect different hormonal statuses of individuals from time to time (Givens, 1982). The daily concentrations of LH in individual patients were highly variable (Gambrell *et al* 1973; Gindoff & Jewelewicz, 1987). The discrepancies between different reports regarding LH levels might result from the conflict between the nature of the LH secretion and the methods used for measuring the levels of this hormone in the blood. Measuring the hormone levels in plasma once daily can lead to confusing results, depending on the timing of blood sampling in relation to fluctuations of the hormone (Yen, 1980; Schwartz *et al* 1987; Dolyan *et al* 1989; Insler & Lunenfeld, 1990).

Follicle stimulating hormone was found to be in the low-normal range by almost all investigators (Duignan *et al* 1975; Katz *et al* 1978; Rajaniemi *et al* 1980; Bates & Whitworth, 1982; Chang *et al* 1983; Lobo *et al* 1983; Heineman *et al* 1984; Abdulwahid *et al* 1985; Messina *et al* 1986; Wajchenberg

et al 1986; Mavroudis *et al* 1988; Minakami *et al* 1988a; Venturoli *et al* 1988; Waldstreicher *et al* 1988; Murdoch *et al* 1989; Rojanasakul *et al* 1989).

Abnormal Gn secretion pattern is expressed by high LH:FSH ratio even if LH is normal (Givens, 1982). This abnormal ratio is of diagnostic value in the disease (Lobo *et al* 1981; Chang *et al* 1983; Lobo *et al* 1983; Heineman *et al* 1984; Schriock *et al* 1985). Abnormal ratios of more that 2.5 can be found in 60 - 70% of PCO patients (Futterweit, 1984; Futterweit & Mechanick, 1988). The ratio varies depending on the assay methods used; this is expected since different criteria are used in the different assays (Lynch *et al* 1989). An abnormally high ratio of LH:FSH is not unique to PCOD patients but is also observed in chronic liver disease, hyperthyroidism and simple obesity (Givens, 1982) and can occur with other hyperandrogenic disorders such as adult onset 21OH deficiency and androgen secreting tumours (Dunaif, 1986). Therefore, it is important that the reference ranges for each hormone be assessed for each method and that the upper normal limits of LH:FSH should not be quoted without stating the methods employed. The LH:FSH ratio may change if follicular development and the occasional ovulation occur (Lynch *et al* 1989) indicating that the best timing for assessment of the Gn status is the early follicular phase. The disparity between FSH and LH may be explained by the negative feedback effect of oestrogen which is greater for FSH than for LH. Follicle stimulating hormone is relatively insensitive to GnRH and the multicystic ovaries may secrete increasing amounts of follicular inhibin which selectively decreases FSH (Yen, 1980). Normally the disappearance rates of FSH like that of LH can be resolved into at least 2 components : the initial (fast) fraction with $t_{1/2}$ of 3.9 hours and the second (slow) component with $t_{1/2}$ of 7.04 hours. The metabolic clearance rate of FSH is about half that of LH (Yen *et al* 1970a). In studying the LH receptors in ovarian follicles from PCOD patients, the number present was lower than in normal pre-ovulatory follicles but was similar to that of early normal follicular phase follicles suggesting that the deranged follicular development may be due to tonic elevation of LH producing down regulation of its own receptors, resulting in a decrease in the number of available receptor sites rather than a lack of LH receptors (Rajaniemi *et al* 1980). Due to wide fluctuations of Gn levels, urinary Gn measurements were suggested (Kulin *et al* 1975; Givens *et al* 1976) and 3 hourly urine samples assessing Gn were well correlated with the levels in 24 hour Gn excretions of urine. Urinary LH was high in most patients. There may be qualitative differences in LH. Methods using monoclonal antibodies are specific for detecting only the intact molecule. Values obtained using such methods were found to be lower than those obtained with methods employing polyclonal antibodies. Monoclonal methods possess good specificity towards the molecule enabling better detection of conditions with qualitative variations in the LH molecule (Venturini *et al* 1989). Patients with PCOD have elevated mean BLH levels and a significantly higher BLH : ILH ratio (Lobo *et al* 1983; Lobo *et al* 1984; Gindoff & Jewelewicz, 1987;

Schwartz *et al* 1987; Mavroudis *et al* 1988) than at any stage of the normal cycle indicating that LH secretion is both qualitatively and quantitatively different from that of the normal female. The ILH may not be increased. The sensitivity of the pituitary gland and the hyperdynamic state of Gn secretion may result in the increased release of BLH (Gindoff & Jewelewicz, 1987). The increased ratio leads to the suggestion that a more biologically active form of LH may be secreted in these patients. Bioactive LH may be an important hormonal marker in the clinical diagnosis of PCOD (Lobo *et al* 1983). Increased ILH has been reported in a few studies (Vaitukaitis, 1983; Lobo *et al* 1984). Elevated ILH and LH:FSH ratio may be contributed to by higher oestrogen circulation, a relative deficiency in central dopamine and elevated levels of GnRH. It is not known if these factors also raise BLH (Lobo *et al* 1984). There was no correlation found between BLH, ILH and androgen levels (Mavroudis *et al* 1988). Treatment with oestrogen and P resulted in a decrease in ILH, BLH and the ratio. The decreased ratio may be due to either a direct effect of oestrogen and/or P on the biological quality of LH or to a direct effect through the negative feedback.

Endocrine glands usually secrete hormones in an intermittent or pulsatile pattern including all hypophyseal hormones. Pulsatile secretion as well as amplitude may transmit information to the target cells thus inducing the activation or inhibition of their activity. Direct sampling for GnRH is not possible in humans; the best way to study neuro-endocrine activity of the hypothalamus on the Gn is to study the Gn secretory pattern. It is important to have a precise and sensitive assay method for pulse detection (Genazzani *et al* 1989). In normal menstruation episodic pulsatile secretion of LH changes throughout the cycle. Luteinizing hormone pulse frequency remained constant during the follicular phase. Changes in pulse frequency and duration are explained by the secretion of gonadal steroids and their feedback effects on the hypothalamic-pituitary axis. Other substances may affect LH pulse release, e.g. opiate receptor antagonist, naloxone, which increases LH pulsatile frequency during the luteal phase (Genazzani *et al* 1989). The detailed Gn pulse patterns are not well defined. Luteinizing hormone is secreted in a pulsatile pattern. In any study of hormonal pulsatility, two problems have to be solved : (1) duration of the study and (2) frequency of sampling. Reduction in sampling frequency and/or the duration of the study will result in a significant reduction in the number of pulses detected. The basal serum LH level is the result of GnRH pulses which are released at relatively regular intervals into the portal vessels. Most researchers describe an LH pulse interval of 1 - 2 hours during the follicular phase of the normal cycle. The LH pulse frequency observed in PCOD is similar to that seen in secondary amenorrhoea. Therefore increased LH pulse frequency is not unique for PCOD while pulse amplitude is higher in patients with PCOD and lower in patients with non-PCO secondary amenorrhoea compared to that of the normal follicular phase (Burger *et al* 1985). There is a marked degree of variability in LH

pulses but not in FSH pulses (Laatikainen *et al* 1983; Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987; Venturoli *et al* 1988). Enhanced pulse amplitude seems the main factor responsible for the high LH levels. Circadian pulsatility together with episodic LH pulsatility could promote a rhythmic ovarian activity. Anovulation would result from a disturbance of this pulsatility (Venturoli *et al* 1988). The frequency of LH pulses can vary from 1 to 5 in a 6-hour period while the amplitude can range from 5.5 - 31.0 mIU/mL (Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987). The increased pulsatile release of LH is probably related to the increased sensitivity of the pituitary gland to endogenous GnRH, released at higher frequency (Genazzani *et al* 1989; Murdoch *et al* 1989). Most studies agree that an increased pulse amplitude occurred in PCOD (Laatikainen *et al* 1983; Burger *et al* 1985; Mason *et al* 1986; Gindoff & Jewelewicz, 1987; Venturoli *et al* 1988; Waldstreicher *et al* 1988; Genazzani *et al* 1989; Murdoch *et al* 1989) while conflicting results on pulse frequency were reported; with no change in some series (Burger *et al* 1985; Mason *et al* 1986; Venturoli *et al* 1988; Genazzani *et al* 1989; Murdoch *et al* 1989) and a significant rise in others (Yen, 1980; Laatikainen *et al* 1983; Gindoff & Jewelewicz, 1987; Waldstreicher *et al* 1988). This discrepancy could result because, even in normal subjects, LH is secreted in a complicated pattern of superimposed pulses of different frequencies. Pulses were detected at frequencies of 1 hour and 2 - 3 minutes (Murdoch *et al* 1989). In addition to pituitary responsiveness, there may be qualitative differences in LH, that is the BLH (Schwartz *et al* 1987). Therefore, there is a heterogeneous pattern of LH pulsatility in PCOD. Frequent measurements are recommended to define patterns of LH. There is no clear correlation between the clinical picture and the LH pulse pattern (Mason *et al* 1986) but in severe cases of obesity and hirsutism, lower pulse amplitude LH peaks were detected than those of women of normal weight (Laatikainen *et al* 1983; Mason *et al* 1986). It is suggested that in obese PCOD patients, a greatly elevated level of biologically active T suppresses the LH secretion. Plasma LH responses to GnRH were significantly higher in PCOD than in normal patients. This is probably related to E_2 concentrations. Gonadotrophin releasing hormone stimulation of FSH levels was normal or low (Mortimer *et al* 1978; Chang *et al* 1983; Vaitukaitis, 1983; McKenna *et al* 1984; Gindoff & Jewelewicz, 1987; Minakami *et al* 1988a; Rossmanith *et al* 1989). Gonadotrophin releasing hormone stimulation was found to result in increased BLH:ILH ratio (Lobo *et al* 1983; Schwartz *et al* 1987). This response may reflect several factors: (i) increased pituitary cell sensitivity to GnRH induced by chronically elevated oestrogen levels, (ii) altered dopaminergic modulation of hypothalamic neurons that secrete GnRH and (iii) other neurotransmitter defects (Vaitukaitis, 1983). Despite these differences a marked degree of overlap existed between PCOD patients and controls.

CONCLUSION

Most authors agreed on the significance of the increased LH pulse amplitudes in PCOD patients over the controls while the increase in LH pulse frequency was not unique for PCOD patients. Therefore, it is important to have precise and sensitive assay methods for pulse detection.

2.3.2 ANDROGENS

The normal ovary, in addition to the adrenal gland, secretes three major C19 steroid androgens: T, A and DHEA synthesized by stroma and thecal internal cells. The relative contributions of the adrenals and the ovaries may vary during the course of the menstrual cycle (Yen, 1986). Androstenedione is secreted by the ovary and the adrenal with a variable ratio exhibiting a diurnal variation. Thus, in the morning, the adrenal contribution may be 80 % of the total A production and decreases by evening in a similar way to that of cortisol. Daily adrenal secretion of A exceeds that of both ovaries in the early follicular phase. The mature follicle secretes increasing amounts of A. Therefore, the time of sampling is important. Testosterone is considered the most potent androgen. Its action is exerted either directly or indirectly depending on the target tissue. The expression of androgenicity in sexual target cells requires the formation of DHT and androstenediol by the enzyme 5∝-R, mainly in hair follicles and external genitalia. Testosterone and A are precursor hormones (prehormones) of plasma DHT. Circulating T is derived from the adrenals (25%), the ovaries (25%) and from peripheral conversion of prehormones (precursors) at sites like liver, fat and skin tissues (50%). At least 2/3rd of plasma DHT is formed by peripheral conversion of A, and to a lesser extent T, while little is derived from direct ovarian secretion. It is suggested, therefore, that the plasma DHT concentration in normal women may reflect the activity in peripheral target tissues for androgen. The metabolic clearance rate of DHT is slow due to its high binding affinity to SHBG. The physiological role of DHEA in humans is unclear (Yen, 1986). Androgens produced in increased amounts from the ovary are often reflected by increased T and A levels in the circulation. Prolonged exposure to elevated LH levels may lead to hyperplasia of theca cells and increased production of the ovarian androgens, A and T. In spite of an increased androgen production, PCOs have a decreased aromatization activity of androgens to oestrogens which is mainly due to the relative deficiency of FSH coupled with the absence of large follicles (Schriock *et al* 1985). Dehydroepiandrosterone and DHEA-S are less potent than other androgens (Yen, 1986). They represent the major androgens secreted by the adrenal gland with less than 10% of the daily DHEA secretion being of ovarian origin. There is neither intraglandular nor peripheral conversion from delta 4 steroids (T and

A) to delta 5 steroids (DHEA and DHEA-S). Normally there is conversion from delta 5 to delta 4 and from delta 5 to E_1 at extraglandular sites but this conversion is insignificant in amount. Dehydroepiandrosterone-sulphate appears to be hydrolyzed continuously to DHEA and secretion of DHEA by the adrenal cortex is ACTH dependent with diurnal variation as for cortisol. Dehydroepiandrosterone-sulphate induces both androgenic and oestrogenic effects in humans. There are no short term variations in DHEA-S levels, therefore its measurement is an easy and reliable method for evaluation of adrenal androgen secretion.

Hyperandrogenaemia is a characteristic feature of PCOD but not all patients display the manifestations of androgen excess. Elevation of almost all androgens has been found to occur in PCOD patients and the principal androgens secreted by the ovary are T and A (Gambrell *et al* 1973; Duignan *et al* 1975; Ginsburg & Havard, 1976; Givens *et al* 1976; Aiman *et al* 1978; Kim *et al* 1979; Quaglarello & Weiss, 1979; Kandeel *et al* 1980; Bates & Whitworth, 1982; Chang *et al* 1983; Laatikainen *et al* 1983; Milewicz *et al* 1983; Pittaway *et al* 1983; Heineman *et al* 1984; McKenna *et al* 1984; Murdoch *et al* 1984; Cunningham *et al* 1985; Fleming *et al* 1985; Haning *et al* 1985; Gross *et al* 1986; Polson *et al* 1986; Wajchenberg *et al* 1986; Carlström *et al* 1987; Schwartz *et al* 1987; Eden *et al* 1988a; Venturoli *et al* 1988; Conway *et al* 1989; Eden *et al* 1989a; Eden *et al* 1989c; Matteri *et al* 1989; Murdoch *et al* 1989; Lynch *et al* 1989; Rossmanith *et al* 1989).

Although there is almost universal agreement regarding the elevations of T and A, other androgens like DHEA and DHEA-S have been reported as elevated in some patients (Cumming *et al* 1982; Chang *et al* 1983; Horrocks *et al* 1983; Pittaway *et al* 1983; Carmina *et al* 1986; Wajchenberg *et al* 1986; Schwartz *et al* 1987; Rojanasakul *et al* 1989).

Dehydroepiandrosterone-sulphate was found raised in 30 - 47% of PCOD patients with mild clinical presentation (Carmina *et al* 1986; Carlström *et al* 1987) and more than 90% was of adrenal original (Carlström *et al* 1987). The explanation for the raised DHEA-S levels is not known. The rise could be explained by increased PRL and E_1 levels (Carmina *et al* 1986; Schwartz *et al* 1987) but DHEA-S was found higher than normal with normal PRL level (Rojanasakul *et al* 1989). It was suggested that the elevation was not due to adrenal enzyme deficiency but to a tonic hyperstimulation of the adrenals. The possibility of an exaggerated secretion of some pituitary hormones with adrenal androgen stimulating activity must be considered. It was suggested that some cases of PCOD with high DHEA-S might be due to a mild form of CAH which only manifested in the adult but this was disproved by an ACTH test. Dehydroepiandrosterone-sulphate is mainly of adrenal origin. Other alternative hypotheses for DHEA-S sources are : (i) due to high E_1 concentration, oestrogen may inhibit the enzyme 3β-OHSD and increase

the DHEA response to ACTH in patients with PCOD, so adrenal abnormality may be a secondary effect arising because of a relative deficiency in 3β-OHSD, (ii) an abnormality of factors which control adrenal androgen secretion, possibly ACTH (Horrocks *et al* 1983). Bioactive LH is found positively correlated with DHEA-S (Lobo *et al* 1983) which might explain adrenal androgenization.

Androgen status was evaluated by measuring plasma TT although T is elevated in ≤ 50% of cases of "benign androgen excess conditions" including PCOD (Pittaway *et al* 1983). About 97 - 99% of T is protein bound mainly to SHBG. The biologically active fraction of T is the free portion (FT) which should be measured because even a small increase in T can reduce its own SHBG resulting in an increase in FT (Quagliarello & Weiss, 1979; Milewicz *et al* 1983; Carlström *et al* 1987; Eden *et al* 1989a). It is reported that FT is found elevated even when TT is normal or decreased (Milewicz *et al* 1983). Total T is suitable for detecting androgen producing tumours. Free androgen index which is defined as T/SHBG x 100 (T:SHBG ratio) (Cunningham *et al* 1985) is a better predictor of androgenic status (Quagliarello & Weiss, 1979; Cunningham *et al* 1985; Carlström *et al* 1987; Eden, 1988; Eden *et al* 1988a; Eden *et al* 1988b; Eden *et al* 1989a; Eden *et al* 1989c) than is TT. In comparison, raised T was found in 53% of patients, raised FT in 53% only while FAI was high in 90% of patients with better prediction (Carlström *et al* 1987) although having a few false positive results in conditions like CAH and androgen secreting tumours which are rare (Eden, 1988). An FAI > 4.5 is suspicious of PCOD (Eden, 1988; Eden *et al* 1988a; Eden *et al* 1989c) and FAI is affected by BMI (Eden, 1988; Eden *et al* 1989a), age and the phase of the menstrual cycle (Eden *et al* 1988a) and should be calculated in the early follicular phase. There are conflicting data regarding the correlation between androgens and Gn, with positive (Conway & Jacobs, 1987), negative (Duignan *et al* 1975) and no correlation (Heineman *et al* 1984; Dunaif, 1986; Messina *et al* 1986) all reported while 17∝-OHP showed a significant inverse correlation. This could be seen as evidence for a negative feedback directly or indirectly exerted by 17∝-OHP on LH and FSH. Therefore the importance of 17∝-OHP was stressed in determining the hormonal status of the syndrome (Messina *et al* 1986).

Although there is general agreement that androgens rise in PCOD the source of excess androgens secretion is questionable. All the previously described tests are helpful but not useful to determine the source of the androgens. Selective simultaneous catheterization of adrenal and ovarian veins is suggested to be the only certain way of determining the source of androgen production and the contribution of each gland (Ginsburg & Havard, 1976; Milewicz *et al* 1983; Wajchenberg *et al* 1986). This showed ovaries to be the source of the excess androgen secretion, but the technique has limitations. Treatment with GnRH-A resulted in a decrease in LH and subsequently E_2, E_1, A and T. Oestrogens were reduced to

low/normal values and androgens although reduced remained elevated. This may indicate that a considerable amount of the androgen is derived from the ovary (Fleming *et al* 1985). It has been established that the adrenal glands contribute to the androgen secretion in patients with PCOD but the significance is as yet unclear (Aiman *et al* 1978; Gross *et al* 1986; Wajchenberg *et al* 1986).

The commonly used adrenal dynamic tests (DXM suppression test, and ACTH stimulation test) do not provide an accurate localization of androgen production (Berger *et al* 1975; Ginsburg & Havard, 1976; Kim *et al* 1979; Milewicz *et al* 1983; Wajchenberg *et al* 1986). The five day DXM test may suppress both ovarian and adrenal secretion and the ACTH test may give heterogeneous results.

2.3.3 OESTROGENS AND EXTRAGLANDULAR HORMONE CONTRIBUTION

Anovulatory cycles in women with PCOD are characterized by a failure of normal follicular maturation. Levels of E_2 in PCOD patients are similar to the levels seen in the early follicular phase of a normal cycle (Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987). In a normal cycle, E_1 production is 100 ug per day, and of this 40 ug arises from peripheral conversion (Yen, 1980; Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987). Peripheral conversion of the elevated A is probably the reason for the high E_1 levels observed in PCOD patients (Aiman *et al* 1978; Yen, 1980; Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987).

Typically E_2 concentrations are within normal ranges while E_1 is usually high (Katz *et al* 1978; Kandeel *et al* 1980; Rajaniemi *et al* 1980; Bates & Whitworth, 1982; Chang *et al* 1983; McKenna *et al* 1984; Cunningham *et al* 1985; Schriock *et al* 1985; Wajchenberg *et al* 1986) with an increased E_1:E_2 ratio. The high E_1 provides inhibitory feedback on the hypothalamic-pituitary system and is thought to be the key factor in maintaining chronic anovulation (Yen, 1980).

Persistent endogenous production of oestrogen is of limited diagnostic value in identifying and classifying patients with PCOD (Insler & Lunenfeld, 1990). It has been found that many women with oligo-ovulation or anovulation show persistent oestrogen production although their gonads do not exhibit the characteristic structural changes associated with PCOD. It is also important to realize that absolute values of oestrogen in plasma do not identify the sources of their secretion.

Oestrone and E_2 may be produced by the ovary, by the adrenal or by peripheral conversion (extraglandular aromatization) of A (Aiman *et al* 1978; Insler & Lunenfeld, 1990). Even in women with

presumably ovulatory cycles, a considerable variability of hormonal levels from cycle to cycle in the same subject has been recorded. The increased LH levels acts upon the ovarian stroma to stimulate A secretion and to a lesser extent T secretion. In females, A is converted to E_1. Women with PCOD are found to have an increased concentration of E_1 relative to E_2 when compared to normal women because of the increased A (Bates & Whitworth, 1982). Administration of GnRH-A resulted in a marked decrease of A, T, E_1 and E_2 to the levels of the oophorectomized female (Chang *et al* 1983; Gindoff & Jewelewicz, 1987; Musacchio, 1988).

It has been suggested that elevated E_1 causes a chronic feedback on Gn secretion leading to increased LH secretion and FSH suppression although it is also possible that elevated LH levels lead to ovarian hyperproduction of E_1. Most of the circulating oestrogen does not originate from direct ovarian secretion but from peripheral conversion of androgens (Heineman *et al* 1984). Plasma E_1 is mainly derived from peripheral conversion of A. It was hypothesized that those patients with the hyperandrogenic state and with lowest aromatase activity demonstrated idiopathic hirsutism, while hyperandrogenic subjects with mean or elevated aromatase activity developed PCOD (McKenna *et al* 1984). Since PCOD patients are frequently obese, there is increased extraglandular tissue residing in adipose tissue capable of converting androgen precursors into oestrogens, mainly E_1. In a study, E_1 was found elevated to three times the normal value but with no demonstrable correlation with obesity (Laatikainen *et al* 1983).

It is thought that oestrogens, rather than androgens contribute to the distortion of Gn release seen in hyperandrogenic females as chronic exposure to androgenic gonadal steroid regulation of hypothalamic-pituitary axis in PCOD is different from that seen in men and acute androgenic administration did not result in distortion of Gn release in androgenized women (Dunaif, 1986).

2.3.4 SEX HORMONE BINDING GLOBULIN (SHBG)

The transport of hormones from their sources to the designated "target" or sites of action requires that the binding affinity of the cellular receptors be greater than that of the carrier protein to permit dissociation from the latter and association with the former.

In comparison to the binding affinity for T, SHBG has three times the affinity for DHT but about 1/3rd that for E_2. The SHBG-bound steroids are not readily available for target-tissue binding and action. The free fraction and the albumin-bound fraction are biologically active. The production of SHBG is promoted by oestrogens and inhibited by androgens. The relationship between SHBG and the balance

between androgens and oestrogens is important in interpreting levels of circulating hormones and their biologic action at target tissues. Alterations in SHBG levels could be due to physiological conditions; decreasing during puberty which is normal and does not depend on increased androgen levels and increasing during pregnancy while pathologically, low SHBG concentrations may result from progestins (except medroxyprogesterone acetate), glucocorticoid excess as in Cushing's syndrome, GH excess as in acromegaly and from thyroid hormone deficiency. Thyroid hormone is the only hormone other than oestrogen that stimulates SHBG production (Yen, 1986). Elevated plasma androgen on the other hand lowers the circulating levels of SHBG. Thus in PCOD patients with increased androgens, the SHBG level is lowered with a subsequent increase in the free biologically active portion of the hormones (Ginsburg & Havard, 1976; Kim *et al* 1979; Carter *et al* 1983; Laatikainen *et al* 1983; Schwartz *et al* 1987; Eden *et al* 1988a; Eden *et al* 1989c; Rojanasakul *et al* 1989). The level of SHBG was found decreased in 60% of PCOD patients (Carlström *et al* 1987). Decreased SHBG could explain the manifestation of hirsutism in PCOD patients with normal TT levels (Ginsburg & Havard, 1976; Cunningham *et al* 1985). Obesity causes a further decrease in SHBG (Kim *et al* 1979; Laatikainen *et al* 1983; Cunningham *et al* 1985). Free T concentrations may contribute to suppression of SHBG in obesity. Reduced SHBG levels in obese subjects may be secondary to increased FT levels. A fall in SHBG levels, in addition to that caused by androgens, will amplify changes in sex steroids by increasing the circulating non-protein-bound fractions, the biologically active moieties (Cunningham *et al* 1985). It would appear that obese PCOD patients have no excessive androgen production but rather higher biologically active free and albumin-bound T (Laatikainen *et al* 1983). Factors other than androgens may be responsible for SHBG suppression in obesity (Cunningham *et al* 1985).

Dehydroepiandrosterone-sulphate is not a significant modulator of plasma SHBG (Cunningham *et al* 1985). Small doses of DXM could result in an increase of SHBG level. This increase is independent of changes in androgen and oestrogen and may be due to a direct effect of glucocorticoid on synthesis and degradation of SHBG. Sex-hormone binding globulin is found lowest in the late follicular phase (Eden *et al* 1988a) and it decreases with advanced age (Cunningham *et al* 1985). Treatment with clomiphene citrate resulted in an increase in SHBG similar to levels seen in spontaneous ovulatory cycles. It is likely that loss of the usual rise in E_2 in both the follicular and luteal phases of an ovulatory cycle is the main reason for the low SHBG concentration found in PCOD (Eden *et al* 1989b).

2.3.5 PROLACTIN

Hyperprolactinaemia is frequently found in association with infertility and hypogonadism, and may occur in 1/3rd of PCOD patients (Vaitukaitis, 1983; Futterweit, 1984). Hyperprolactinaemia in PCOD may result from the effects of the abnormal serum oestrogen concentrations on pituitary lactotrophs (Futterweit, 1984; Schriock *et al* 1985) but the presence of normal PRL function in normoprolactinaemic PCOD patients excludes a specific impairment of lactotroph function. The hyperoestrogenic state in PCOD or the milder elevations of oestrogen, predominantly E_1, is due mainly to peripheral conversion of androgens (mainly A) and decreased SHBG which result in exaggerated oestrogen concentrations (Schriock *et al* 1985). Hyperresponsiveness to TRH is frequently noted probably because of E_1 excess (McKenna *et al* 1984).

It is thought that PRL may exert a direct effect on DHEA-S secretion because the production rate was decreased in PCOD women treated with bromocriptine and was related to a similar decline in serum PRL (Gindoff & Jewelewicz, 1987; Schwartz *et al* 1987). Hyperprolactinaemia may be a reflection of a central abnormality such as altered dopaminergic activity, affecting Gn and PRL secretion (Goldzieher & Green, 1962; Schriock *et al* 1985). All study patients with PCOD in one series who had elevated PRL levels, showed an excessive response to dopamine receptor antagonist (metoclopramide) (Goldzieher & Green, 1962). Serum PRL was found negatively correlated with T, LH and ovarian volume (Conway & Jacobs, 1987). Administration of GnRH has been shown to stimulate PRL release (Shoupe & Lobo, 1985).

2.3.6 INSULIN AND SHORT INSULIN TOLERANCE TEST (SITT)

Since the description by Himsworth in 1936 that human disease could be associated with reduced sensitivity to I (IR), several methods have been developed for detection and quantification of IR (Caro, 1991). Insulin resistance is diagnosed when normal glucose levels exist in the presence of hyperinsulinaemia (Burghen *et al* 1980). The GTT, euglycaemic I-clamp technique, the minimal model technique, and the SITT are all provocation methods which have been used to quantify IS. Other tests include measurement of IS *in-vitro* in cell cultures and tissue samples. However, most of these tests are complicated to perform on a large series of patients and there has been much debate about the relative merits and limitations of each of them.

Total body IS can be assessed via the intravenous GTT, or minimal model. Although the euglycaemic I-clamp technique is the most widely accepted standard method for estimating IR against which other methods are compared, it is expensive, time-consuming, requires specialized equipment and highly trained staffing and the feedback control of glucose levels is complex; all of which make it a laboratory investigative procedure rather than suitable for use in a clinical setting (Caro, 1991). These disadvantages demonstrate the need for an acceptable method to measure IS that could be used in large scale studies or even clinically.

An alternative to the euglycaemic clamp, the SITT is a short useful alternative(Akinmokun *et al* 1992; Hirst *et al* 1993). A close correlation was found between the glucose disappearance rate (KITT) and the M/I ratio derived from the SITT and the euglycaemic hyperinsulinaemic clamp in both normal and diabetic subjects. Results suggest that the SITT is a suitable method of assessing IS and particularly useful for large-scale studies, although the requirement for arterial blood adds a measure of complexity (Matthews *et al* 1985; Akinmokun *et al* 1992; Holte *et al* 1994a). The SITT is simple and rapid to perform. The SITT method uses the ratio of decline in blood glucose after the intravenous administration of I as an index of IR. The method is used increasingly in clinical research because studies have shown that the rate of fall of blood glucose during the SITT, the KITT-value, significantly correlated with the euglycaemic clamp (Akinmokun *et al* 1992). Another advantage of the SITT is that the test is safe, reproducible and could be used to measure IR in large-scale epidemiological studies (Akinmokun *et al* 1992; Hirst *et al* 1993). The degree of IR and deficient β-cell function can be assessed from a patient's FI and glucose concentrations (Matthews *et al* 1985). The fasting plasma glucose concentration depends primarily on the rate of hepatic glucose release, which in turn is regulated by I concentration. Fasting plasma GLU/INS concentration ratio may provide the simplest estimate of IR. It has been observed that the GLU/INS ratio calculated from a single fasting plasma sample correlated with the measurement of IS determined by the euglycaemic clamp, the minimal model technique, and the oral GTT (Caro, 1991). The higher the plasma FI concentration for a given fasting plasma glucose, the more IR an individual is. Fasting insulin and FIRI are simple and reproducible measures of IS. Cleland *et al.* (1996) reported that fasting hyperinsulinaemia may be used as a guide to the existence of IR and that $\log_{10}$ FIRI was highly correlated with the euglycaemic hyperinsulinaemic clamp ($r = 0.67$, $P < 0.001$). Therefore, simpler estimates of IR such as FI, GLU/INS ratio and FIRI may obviate the need for more complex procedures to obtain a useful index of IR in large-scale studies.

It is now well established that hyperinsulinaemia is involved in, and may be a trigger phenomenon in the pathogenesis of PCO (Urdl *et al* 1991). Basal and glucose-stimulated hyperinsulinaemia are recognized features of PCOD (Dunaif & Graf, 1989).

CONCLUSION

Laboratory tests may include estimation of LH, FSH, E_1 and E_2 with elevation of LH:FSH and E_1:E_2 ratios. These are compatible with PCOD but these can be seen in any type of chronic anovulation or other conditions like testicular feminization syndrome (Yen, 1986). It can be concluded that no single biochemical test can diagnose PCOD (Ginsburg & Havard, 1976; Schriock *et al* 1985) because the biochemical spectra include minimally to markedly elevated plasma androgen levels and normal to extremely high LH levels (Givens *et al* 1976). It is important to note that Gn, androgen and oestrogen production depend on the stage of follicular phase which is of great importance for sampling and interpreting results. It is suggested that the initial laboratory evaluation of patients suspected to have PCOD should include the following tests : plasma LH:FSH, T, DHEA-S, PRL, A and 17∝-OHP. Additional studies of: plasma SHBG, FT, % FT, E_2, E_1, DHT, I (IR), ACTH test, GnRH test and TRH test (if hyperprolactinaemic), are not always essential but give a clearer picture of the associated endocrine dysfunction (Futterweit, 1984).

2.4 THE ROLE OF ULTRASONOGRAPHIC SCANNING IN THE DIAGNOSIS OF PCOD

Ultrasonography can aid in the non-invasive diagnosis of gynaecological abnormalities. It is an accurate and reliable method that can help in determining ovarian morphology and size (Campbell *et al* 1982; Orsini *et al* 1985; MacDougall *et al* 1992).

In post-pubertal females, the ovaries enlarge rapidly under hormonal stimulation involving mainly the width and thickness where the enlargement is attributed to an increase in the number of follicular cysts (Sample *et al* 1977).

Ovarian volume is calculated using the formula : [4/3 x π x d1/2 x d2/2 x d3/2] ;

where d1 = widest transverse diameter; d2 = longest diameter orthogonal to this in the A-P axis; d3 = maximum vertex diameter]; (Campbell *et al* 1982). It normally ranges between 1.8 - 5.7 cm^3; while the average ovarian volume of polycystic ovaries is 12.5 cm^3 (range is 6 - 30 cm^3) - 2-5 times normal size (Swanson *et al* 1981). Polycystic ovaries as defined by US scanning are very common in anovulatory women (57%). Polycystic ovaries were found in 26% of patients with amenorrhoea, 87% of patients with

oligomenorrhoea and 92% of those with idiopathic hirsutism (Adams *et al* 1986a). Enlarged polycystic ovaries could be found in ovulating and fertile patients (Ferriman & Purdie, 1965) where a corpus luteum could be detected in 16/23 US-diagnosed PCOD patients (Adams *et al* 1986a). Bilateral enlarged globular shaped ovaries are rare and usually asymmetrical in size (Conway *et al* 1989). A unilaterally enlarged PCO was reported by Rojanasakul *et al.* (1989) who studied 54 Thai women with PCOD using a high resolution real-time US scanner - two patients had unilateral polycystic ovaries.

Measurement of ovarian volume should be more accurate than determination of any one dimension (Sample *et al* 1977) and should be helpful in assessing inappropriate hormonal stimulation. While ovarian volume, expressed as the mean of both ovaries (Hague *et al* 1990), has been suggested as an indication of abnormal ovarian size, this may not be practical (Ginsburg & Havard, 1976; Yeh *et al* 1987; Rojanasakul *et al* 1989) as normal ovarian volume does not exclude the diagnosis of PCOD (Hann *et al* 1984; Orsini *et al* 1985; Taketani, 1990; Puzigaca *et al* 1991). It was found that the average size of ovaries in PCOD patients was much larger than that of healthy females (Hann *et al* 1984; Yeh *et al* 1987) but that 29.7% of ovaries in patients with PCOD were normal in size. Enlarged ovarian volume is therefore not a necessary criterion for the diagnosis of PCOD (Ginsburg & Havard, 1976; Puzigaca *et al* 1991).

Sonographic findings recorded by Adams *et al.* (1986a) and Conway *et al.* (1989) for diagnosing PCOD were as follows : multiple cysts (≥ 10) 2 - 8 mm in diameter, arranged either peripherally around a dense core of stroma or scattered throughout an increased amount of stroma (or both). The ovaries are usually enlarged. This should be distinguished from multicystic ovaries normally seen during puberty and also associated with hypothalamic amenorrhoea due to weight loss. Those ovaries have larger cysts than PCO and normal stroma (Abdulwahid *et al* 1985; Adams *et al* 1985; MacDougall *et al* 1992). Conway *et al.* (1989) studied 556 PCOD patients and tried to correlate trans-abdominal ultrasonographic features to clinical and endocrinological manifestations. They found that subjects in a high LH group had significantly higher mean serum FSH and T concentrations and larger ovaries compared to those in the normal LH group while Givens (1982) argued that the division of US versus raised LH levels concentrations, does not necessarily mean different subgroups but may reflect a different hormonal status of each individual from time to time.

Takai *et al.* (1991) studied ultrasonically and endocrinologically 69 euprolactinaemic patients with anovulation and high LH secretion. They divided PCOD patients into three groups: (i) patients with neither hirsutism nor increased A and/or T concentrations; (ii) patients without hirsutism but with increased A and/or T concentrations; (iii) patients with hirsutism and increased A and/or T

concentrations. Polycystic ovaries were diagnosed ultrasonographically in 88, 84 and 100% of patients in types i, ii and iii respectively. These authors postulated that each type may represent a subset of the whole spectrum of PCOD from SL syndrome to simple anovulation with increased LH secretion, and, that type (i) precedes type (ii) PCOD.

Hyperprolactinaemic PCOD patients had significantly lower mean T concentrations and smaller ovarian volumes than normoprolactinaemic subjects, while no difference in ovarian volume was found between obese and normal weight subjects (Conway *et al* 1989).

Eden & Place (1989) found a high correlation between an elevated FAI and ultrasonic and laparoscopic diagnosis of PCOD. Free androgen index is an index of FT activity (Carter *et al* 1983). Clinical history and ultrasonographic data were analyzed and their possible interrelationships in a group of 72 PCOD patients, were evaluated (Puzigaca *et al* 1991). Twenty three point six percent of PCOD women were found to have an ovarian volume within the normal range, while 72.2% had enlarged ovaries. Serum androgen levels (especially A) were higher in PCOD patients with enlarged ovaries although hirsutism was found in equal amounts in patients with normal and enlarged ovaries. Patients with the most enlarged ovaries, who also have the highest serum A and E_2 concentrations, had the most frequent occurrence of amenorrhoea.

Ovarian morphology was classified according to the presence or absence of echo-free cystic functional structures into (1) predominantly solid if fewer than 4 small ($\leq$ 9.0 mm) cystic structures were detected in the ovary; (2) predominantly cystic if multiple small cystic structures or at least one large ($\geq$ 10.0 mm) cyst was present (Conway *et al* 1989; Rojanasakul *et al* 1989). Orsini *et al.* (1985) could demonstrate the classical ultrasonic picture of PCOD (i.e. symmetrically enlarged ovaries with numerous tiny cysts) in only 36.3% of their cases while Rojanasakul *et al.* (1989) found the typical ultrasound appearance in 2/3 of their patients and 1/4 of them had bilaterally solid ovaries.

In correlating ultrasonic evaluation with clinical and hormonal data (Parisi *et al* 1984) the criteria included ovarian size, shape, margins, structure of the ovary and uterine:ovarian ratio. Uterine:ovarian ratio was calculated as the maximum A-P diameter of the uterine fundus in relation to the longitudinal diameter of the ovary. The degree of ovarian enlargement had a significant correlation with both the age and the duration of symptoms. Furthermore, the ovaries tended to have a more rounded shape in older patients and in those who had had PCOD the longest. This might indicate that both ovarian size and shape are functions of the disease duration and that PCOD, once established, causes progressive enlargement of the ovaries, which tend to become completely round in shape. Identification of large

ovaries with a rounded appearance may therefore be an indication of advanced stages of the disease, which once established, is the cause of progressive enlargement of these organs. Other researchers found a striking correlation between cycle history and ovarian appearance and they considered calculating the ovarian volume (Polson *et al* 1988). Some authors (Parisi *et al* 1984; Futterweit & Mechanick, 1988; Puzigaca *et al* 1991) stressed the value of the uterine:ovarian ratio and it was always < 1 in PCOD cases; Orsini *et al.* (1988) found this in 77.3% of their patients. In contrast, ovarian volume measured by ultrasound, was not considered essential for diagnosis and difficulties were encountered in finding the reference plane for measurement (Rojanasakul *et al* 1989). Ovarian volume did not show a significant correlation with endocrine hormones (Gn, T, E_2, I) or with echogenicity (AbdelGadir *et al* 1991). These authors also reported that bigger ovaries were not produced in response to prolonged duration of symptoms, higher LH levels or to any specific Gn pulse pattern. These ovaries did not produce more T than smaller ones and this confirms that normal sized ovaries may have the same histological and biochemical abnormality as do enlarged ovaries in PCOD patients. AbdelGadir and his colleagues (1992a) concluded that ovarian size did not indicate the severity of the condition and that more elaborate treatment regimens are not indicated on the sole criterion of enlarged ovaries .

Roundness index is defined as the ratio between the width (the second largest ovarian diameter) and the length (the maximum ovarian diameter) (Yeh *et al* 1987). The shape of the ovary in PCOD, expressed as the RI, was found to be not different from that of healthy females and there was no significant correlation between the size and the shape of the ovaries. There was also no correlation between RI and ovarian volume and the ranges and average of the RI of the ovaries in PCOD patients were the same as those of normal healthy ovaries. This indicates that enlargement of the ovaries in PCOD is due to a diffuse process and that the ovaries maintain their shape as they enlarge. The shape of the ovary or RI is therefore of no value in diagnosing PCOD. A considerable number of PCOD patients (25.5 - 40%) have normal sized ovaries so the size of the ovary is neither a specific nor sensitive criterion for ultrasonic diagnosis of PCOD (Yeh *et al* 1987; Puzigaca *et al* 1991). Yeh *et al.* (1987) described ovarian cysts in general and divided them into: (1) developing follicles (0.4 - 1.4 cm in size) which is characteristic of PCOD; (2) maturing follicles (1.5 - 2.9 cm) which were much rarer in PCOD patients than in normal patients (13.5% vs 36%); and (3) follicular cysts (> 3.0 cm) which had the same frequency in PCOD and normal subjects. Ovarian cysts range in diameter from 2 - 8 mm and may be arranged in the periphery of an ovary or through the parenchyma with more variability in size. High-resolution, real-time sector scanning improves the accuracy of ultrasonic texture evaluation of the ovary, differentiating from bowel loops, and enabling the detection of much smaller echo-free cystic functional structures (Swanson *et al*

1981; Campbell *et al* 1982; Parisi *et al* 1984; Orsini *et al* 1985; Yeh *et al* 1987; Rojanasakul *et al* 1989; AbdelGadir *et al* 1991) than with the static B-scanner.

The precise diagnosis of PCOD depends on histologic findings of follicular cysts and increased stroma of the ovaries that are usually but not always enlarged. These are features that can be identified, using high resolution US of the ovaries (Adams *et al* 1986a; Faure *et al* 1989; Rojanasakul *et al* 1989) as numerous small cystic structures surrounding an echogenic area at the middle representing the dense stroma. Various other ultrasonic patterns could be due to patient selection, stages of the disease, different US machines and examiners (Rojanasakul *et al* 1989). The most important morphological feature is an increased number of developing follicles, usually > 5 in each ovary which was found to be 82.4% sensitive and 100% specific with an overall diagnostic accuracy of 85.9% for PCOD (Yeh *et al* 1987). In general, it can be concluded that the presence of multiple small cysts of the ovaries in women with menstrual irregularities should lead to the suspicion of PCOD while the presence of a solid structure does not exclude this condition. Difficulties could be found in identifying the ovaries in extremely obese patients or if there has been previous pelvic surgery.

Methods which have been suggested for better diagnosis of PCOD include (i) scanning ovaries from various angles by placing the transducer on every part of the lower abdomen; (ii) scanning ovaries thoroughly and carefully by sweeping from one edge of ovary to the other edge slowly; (iii) adjusting the gain setting for each patient to optimize follicular visualization; (iv) appropriately distending the bladder and (v) using a small part scanner when ovaries are superficially located (Yeh *et al* 1987). It is well known that the ovarian location, the uterine position, the degree of bladder distension and bowel fullness are more common limiting factors than ovarian size itself in ultrasonic visualization (Orsini *et al* 1985).

Earlier studies of PCOD focused on ovarian morphological findings which were considered to be an important criteria. It was then found that PCOD changes of ovaries were often associated with other well-defined diseases such as Cushing's Syndrome and other endocrine disorders (Ardaens *et al* 1991) or adrenal or ovarian tumours capable of producing androgens (Parisi *et al* 1984; Taketani, 1990). Women with weight-loss and hypothalamic amenorrhoea, usually have a multifollicular ovarian appearance with normal stroma on ultrasonic examination. Ovaries are normal or slightly enlarged filled with ≥ 6, 4 - 10 mm follicles but unlike PCOD patients, they are not hirsute and their Gn are normal (Adams *et al* 1985). It was therefore generally agreed to consider ovarian imaging as complementary to endocrine evaluation (Parisi *et al* 1984; Tucker *et al* 1984; Dolyan *et al* 1989; AbdelGadir *et al* 1992b) and it also helps to predict the response to therapy. About 22% of females with other endocrinopathies would have been mistakenly diagnosed as PCOD if US diagnosis had been used as the sole diagnostic criterion (Insler &

Lunenfeld, 1990; AbdelGadir *et al* 1992a; AbdelGadir *et al* 1992b; Anttila *et al* 1992). Uterine scanning for evidence of endometrial masses was emphasized (Swanson *et al* 1981) to exclude endometrial carcinoma. Some authors suggested MRI as a better contrast-resolution is obtained which enables visualization of organ structures not seen with other techniques. Magnetic resonance imaging showed superiority in detecting PCOD morphology over US (Faure *et al* 1989). As typical findings are often seen in US or MRI, there is still heterogeneity (Faure *et al* 1989; Quartero *et al* 1989). However, recent technological advances in US and specifically the advent of high frequency trans-vaginal US is of particular value in the study of PCOD.

Recently many authors have favoured the vaginal ultrasonographic approach over the transabdominal approach for increased facility in establishing the diagnosis (Gindoff & Jewelewicz, 1987; Ardaens *et al* 1991). The typical appearance of PCO by vaginal US shows the subcapsular follicular cysts in a "pearl necklace" pattern around the periphery of the somewhat enlarged ovaries (Dunaif, 1992b). Vaginal US was found helpful to diagnose PCOD in 44% of a study population with a history of recurrent miscarriage (Tulppala *et al* 1993). Ardaens *et al.* (1991) conducted a study through which they evaluated the superiority of vaginal over abdominal US for PCOD diagnosis. Vaginal US allowed a better analysis of the ovarian stroma as it improved the ultrasonic study of internal PCO criteria. Increased ovarian stroma seems to be the most sensitive and specific sign of PCOD (incidence 64.5% in vaginal US vs. 15.1% abdominal US). While abdominal US allows correct measurement of ovarian sizes, which is less accurate with vaginal US, not all PCOD ovaries are enlarged. Abdominal US is difficult to perform in obese patients especially to visualize the stroma and to detect the microcysts but this is more feasible with vaginal US. The criteria used to diagnose PCO by vaginal ultrasound were : (i) cross section > 10 cm^2 (increased ovarian area); (ii) Uterine volume/ovarian length < 1; (iii) RI > 0.7 (excessive roundness index); (iv) number of follicles > 5 (PCO appearance); and (v) increased amount of hyperechoic stroma. Stroma was considered abnormal when it was mainly central with an area exceeding that of microcysts that were pushed together towards the ovarian periphery and/or when it was dense with enhanced microcysts whose walls appeared thickened. Although vaginal US is superior in detecting the stromal density and especially helpful in obese patients, it has its limitations in measurement of longer ovarian axes and in visualization of superficially located ovaries. Hence the authors emphasized the need for abdominal US before scanning the patients vaginally but they did not recommend a scanning system because the various parameters were not equal in terms of specificity and sensitivity. In conclusion, a wide spectrum of US ovarian findings can be demonstrated in PCOD extending from apparently normal to markedly enlarged cystic ovaries, but only a limited part of this

spectrum is covered by the classic sonographic picture of ovaries, and uterine volume and the uterine volume/ovarian length ratio should be included to improve the diagnostic accuracy of US.

2.5 HISTOPATHOLOGICAL FINDINGS

Diagnosis need not depend on ovarian size or specific clinical presentation but the final analysis can be made histologically following microscopic examination of the tissue. Polycystic ovaries may occur in normal sized or enlarged ovaries with common macro and microscopic findings (Ginsburg & Havard, 1976; Schwartz *et al* 1987; McKenna, 1988; Insler & Lunenfeld, 1990). The pathological spectrum extends from normal sized to massively enlarged ovaries.

The typical PCO has been traditionally described as being grossly enlarged, pearly white and with a thick capsule and numerous subcapsular cysts (Insler & Lunenfeld, 1990).

Stein and Leventhal (1935) described the ovaries of their patients (n = 7) as enlarged with thickened tunica albuginea, where the only consistent pathological findings in all their biopsies were the presence of follicular cysts lined by theca cells and a thickened tunica. Goldzieher & Green (1962) examined in detail the histological features of ovaries from 18 PCOD patients and a thickened tunica albuginea was the most noticeable feature and was almost invariably present. The thickening resulted from an increase in the number of collagen fibres which resulted in a thickened capsule surrounding the ovary (normal ovarian capsule was found to be approximately 100 u wide while in PCO the thickness was 2-6 times that). There were numerous graafian follicles in all stages of development and atresia except for the pre-ovulatory stage. Corpora lutea were seen in only 2 ovaries (11%). There were no consistent stromal changes. A thickened tunica was thought to be unlikely to cause a hormonal disturbance in PCOD but excess androgens in the circulation might cause capsular fibrosis.

There were variations in the histological pictures of 9 wedge resection biopsies examined (Ibrahim *et al* 1966) but the main features included: (i) thickened tunical albuginea (up to 10 times the normal) while increased fibrosis was not prominent; (ii) the presence of numerous small follicles and a thin layer of granulosa cells situated all around and under the ovarian capsule; (iii) other less characteristic features included hyperplasia and luteinization of the theca interna cells.

Berger *et al.* (1975) divided the patients into two groups according to ovarian pathology : (I) typical - "Type I" markedly enlarged ovaries (2-4 times the normal size) with multiple follicular cysts visualized

underneath the fibrotic ovarian cortex with thickened capsule and usually luteinized follicular cells with stromal hyperplasia; (II) atypical - "Type II" small to slightly enlarged ovaries with sub-cortical cysts and thickened capsules. Histologically, stromal hyperplasia and thecal luteinization were occasionally observed in type II PCO. Morphologically, the ovaries may be small and sclerocystic or enlarged. Capsular thickening is variable but was not always present. Subcapsular fibrosis and cyst formation (4 - 10 mm in diameter) were always present and were characteristic of the syndrome, in both large or small ovaries (Ginsburg & Havard, 1976; Parker *et al* 1980; Schwartz *et al* 1987; McKenna, 1988; Insler & Lunenfeld, 1990); even a unilaterally enlarged ovary was reported (Parker *et al* 1980). Typically, no corpora lutea and/or corpora albicantia were seen (Schwartz *et al* 1987), although corpora lutea were reported in 11% of the series of Goldzieher & Green (1962).

The steroidogenic capacities of isolated granulosa and theca cells from mid-antral follicles (4 - 7 mm in diameter) of PCOs were studied and compared to those of normal ovaries (Wilson *et al* 1979). The findings strongly suggested that the mid-antral follicles of PCOs were normal and the authors concluded that chronic anovulation in PCOD patients may not be causally connected with an inherent endocrine abnormality in the theca and granulosa cells of the developing follicles. Chronic anovulation is not due to deficiency in the steroidogenic potential of the follicles or to a deficiency of Gn receptors on PCO follicular cells but tonic elevation of serum LH may cause down regulation of its own receptors leading to atresia of granulosa cells, luteinization of thecal cells and accumulation of small cystic follicles in the ovaries (Rajaniemi *et al* 1980). The pathogenesis of development of enlarged ovaries in PCOs is unknown and little information is available to correlate biochemical and histological parameters. This task has gained the special interest of some investigators. Goldzieher & Green (1962) failed to find a correlation between the thickness of the capsule and the clinical findings, severity of symptoms and the advancement of the disease. Ovarian hyperandrogenism can be associated with histologically normal ovaries (Kim *et al* 1979). Some investigators have proposed that the initiation of ovarian enlargement (involving theca cell hyperplasia and theca luteinization) in PCOD is caused by excessive Gn, especially excess LH, (Gambrell *et al* 1973; Givens *et al* 1976; Kim *et al* 1979; Rajaniemi *et al* 1980; Yen, 1980; Conway *et al* 1989) and the associated excessive androgen production, where the amount of ovarian androgen production may parallel the degree of histologic change (Kim *et al* 1979). Histologic changes under the effect of long-term androgen (T) treatment were studied in 10 patients who underwent sex reassignment surgery. The findings were compared to those among PCOD patients and a normal control group (Amirikia *et al* 1986). It was concluded that exogenous androgen can thicken the tunica albuginea and basal membrane giving similar histological changes to those seen in PCOD ovaries under excess endogenous androgen production.

A direct action of excessive androgen on the ovaries may account for capsular fibrosis (Yen *et al* 1970b). As enlargement occurs, the increased steroid production may lower LH secretion to normal. There may be also a concomitant increase in sensitivity of the ovary to LH as hyperplasia progresses (Givens *et al* 1976). Other investigators (Schwartz *et al* 1987) found no correlation between LH and ovarian size and suggested that the gross enlargement of the ovaries was a manifestation of a prolonged period of anovulation and was of no diagnostic value in classifying patients into subgroups of the syndrome. Some authors considered hyperthecosis as one of the histological manifestations of PCOs (Insler & Lunenfeld, 1990). Polycystic ovaries classified as Group 1 hyperthecosis or called thecosis interna consisted of bilateral multiple follicular cysts and superficial collagenization with persistence and predominance of normal or altered theca interna but with no stromal theca cells (Fienberg, 1981), i.e. theca interna hyperplasia of the ovary (Futterweit, 1984). On the other hand other authors questioned whether hyperthecosis is one of the inherent morphological features of PCOD (Insler & Lunenfeld, 1990; Speroff *et al* 1983) or represents a completely separate entity (Insler & Lunenfeld, 1990) with diffuse hyperplastic ovarian stroma rather than limited to theca interna as in PCOD (Aiman *et al* 1978). Some or all morphological characteristics of PCO can appear in individuals who certainly do not have PCOD.

Polycystic changes of the ovaries are also found in association with Cushing's Syndrome, CAH, adrenal and ovarian tumours (Insler & Lunenfeld, 1990) and in women with morbid obesity except for the lack of cystic appearance (Fisher *et al* 1974). It should be emphasized that histological changes in the ovary are not present in all PCOD patients. Variations do occur and may be related to the variable clinical and laboratory manifestations of the syndrome (Yen, 1980).

2.6 GENETIC INHERITANCE

Polycystic ovarian disease is thought to be due to genetic rather than environmental causes. It has been shown in cytogenic studies that: (i) androgen binding protein is present in the cyst fluid of patients with PCOD; (ii) defects in aromatization mechanisms that interfere with conversion of C-19 oxygenated steroids into oestrogens occur , (iii) chromosomal abnormalities exist , and (iv) there are occurrence of familial cases (Parker *et al* 1980).

Most women with PCOD have a normal 46, XX karyotype (Yen, 1980). It is possible that in some cases an autosomal anomaly can lead to PCOD (Schwartz *et al* 1987) although a typical and consistent chromosomal abnormality is not associated with PCOD. Parker *et al.* (1980) carried out cytogenetic

studies in 15 PCOD patients; 5 of them showed trisomy 14 in 2 - 4% of their cells, which was considered to be significant as such an occurrence is rare in the general population.

The relationship to HLA has aided in understanding the genetic transmission of an inherited disorder. Congenital adrenal hyperplasia (21OH deficiency) has close genetic linkage with HLA on chromosome no. 6 (Hague *et al* 1989). Several authors have described the association of CAH and PCOD. Broster *et al.* (1938) was the first to suggest the association of CAH and PCOD. Hague *et al.* (1989) reported a 6.4% incidence of PCOD patients with evidence of 21OH deficiency. The same author (Hague *et al* 1990) showed a similar association in post-pubertal females (76%). Cystic ovarian changes were diagnosed in the majority of CAH patients (Eden *et al* 1989c; Levin *et al* 1991; AbdelGadir *et al* 1992b) and the last authors also found that the PCO appearance persisted even after normalization of androgens with corticosteroids. Yen (1980) proposed that PCOD could be inherited as an x-linked disorder and the incidence was higher if inherited from the father (Ginsburg & Havard, 1976). As 17 ketosteroid deficiency could be transmitted as AR and could be an x-linked R, the discovery of its deficiency in some families of PCOD patients might explain the occurrence of menstrual dysfunction and PCOD (Pang *et al* 1987; Toscano *et al* 1990).

In studying the prevalence in families, Mahesh *et al.* (1978) diagnosed PCOD in 3 sisters, and it has also been diagnosed in identical twins (Goldzieher & Green, 1962). Mandel *et al.* (1983) studied 4 families in whom at least 2 siblings had clinical evidence of PCOD. Futterweit (1984) had recommended investigating all women with a family history of menstrual irregularity for PCOD. In addition, Polson *et al.* (1988) found that 44% of women with irregular periods, diagnosed to have PCO by ultrasound, had a sister or a mother with irregular periods (compared to 4% in those with regular periods). As Hague *et al.* (1990) studied relatives of their PCOD patients, they found that 67% of pre-menopausal mothers and 86% of sisters of PCOD patients were found to have PCO as well. Although the sample of families studied was small (4), Mandel *et al.* (1983) found that PCOD does not exhibit linkage to the HLA system. A study of the prevalence of ultrasonically detected PCO in family female members of 59 patients suggested a dominant inheritance of PCOD with variable phenotypic expression, but the prevalence was higher than expected for either an x-linked or AD inheritance (Hague *et al* 1986).

The occurrence of hypertension, hyperlipidaemia, hyperuricaemia, IDDM, obesity and acanthosis nigricans in families with PCOD is of special interest (Yen, 1980). Although there is some evidence of a genetic factor of inheritance, larger studies are needed to ascertain the mode of inheritance.

2.7 DIFFERENTIAL DIAGNOSIS

The so-called "PCO-like Syndrome" (Yen, 1980)

Any condition causing anovulation with LH-dependent androgen excess secretion and increased extraglandular production of oestrogen may result in a gross appearance of the ovaries similar to that seen in PCOD (Yen, 1980; Futterweit, 1984). Appropriate oestrogen secretion occurs only when FSH-dependent orderly follicular maturation takes place. Thus, when the LH:FSH ratio is increased, often due to acyclical peripheral oestrogen feedback, chronic anovulation with increased ovarian androgen production ensues resulting in a gross appearance of the ovary similar to that seen in the PCO syndrome.

Cushing's Syndrome

Oligo/amenorrhoea or menstrual irregularity, obesity and hyperandrogenism occur in the majority of pre-menopausal women with Cushing's Syndrome (Yen, 1980; Yen, 1986) simulating PCOD. Chronic anovulation and menstrual irregularity may be due to disturbed cyclical Gn release secondary to central nervous system defence and/or increased peripheral production of oestrogens (Yen, 1980). Although cortisol release is in excess with loss of normal circadian rhythm, some degree of sex steroid increase from the adrenal gland may also be present (Yen, 1980; Yen, 1986). An overproduction of ovarian androgens due to chronic anovulation may occur leading to the formation of PCO-like ovaries.

Congenital Adrenal Hyperplasia (CAH)

Congenital adrenal hyperplasia is a variety of disorders of adrenal steroidogenesis which result from an inherited deficiency of one of several enzymes necessary for normal adrenal steroid synthesis (Futterweit, 1984). The most common defect is that of adrenal 21OH or 3 β-OHSD isomerase enzymes. Severe hirsutism, menstrual dysfunction and infertility with varying degrees of androgen excess, mainly reflecting adrenal androgens, are present. The differential feature from PCOD is the marked elevation of 17 α-OHP and P both basally and in response to ACTH stimulation (Yen, 1980; Futterweit, 1984; Yen, 1986; Levin *et al* 1991). The elevated androgens are readily suppressed by DXM and stimulated by

ACTH. The deficiency of 3 β-OHSD can be manifested with elevated LH:FSH ratio, decreased SHBG and androgen excess (mainly delta 5 androgens - DHEA, DHEA-S and androstenediol) (Yen, 1986). The diagnosis is made by elevation of plasma pregnenolone, 17 α-hydroxypregnenolone, DHEA, DHEA-S with relatively low levels of delta 4 compounds such as P, 17 α-OHP and A (Futterweit, 1984).

Androgen-producing Tumours

"Virilizing ovarian and adrenal tumours". Several types of ovarian and adrenal tumours produce androgens and cause chronic anovulation and often virilization. These tumours include "hilar cell tumours", "arrhenoblastoma" (Sertoli-Leydig cell tumours), "benign cystic teratomas", "luteinized thecoma", "adrenal nest tumours of the ovary", and "adrenal adenoma" and "carcinoma" (Yen, 1980; Yen, 1986). Any rapidly progressing hirsutism in association with amenorrhoea and cliteromegaly is highly suggestive of a virilizing tumour. Markedly elevated plasma T values may be diagnostic (Futterweit, 1984). Therefore adrenal and ovarian ultrasonography and adrenal CT scanning will be of great help in localizing the tumour.

Oestrogen-producing Tumours

Granulosa-theca cell tumours are the most common hormone-producing neoplasms of the ovary, accounting for 15 - 20% of all solid ovarian tumours (Yen, 1986). These usually produce mainly oestrogen but frequently also produce androgens.

Ovarian hyperthecosis

The term "hyperthecosis" is a non-neoplastic pathologic lesion of the human ovary, characterized by a diffuse hyperplastic process in the human ovary resulting in diffusely distributed islands of luteinized thecal cells throughout the ovarian stroma (Yen, 1980; Fienberg, 1981; Yen, 1986). The clinical picture is usually composed of severe hirsutism, mild cliteromegaly, obesity, temporal balding, oligomenorrhoea and resistance to clomiphene therapy (Futterweit, 1984). They may respond to ovarian wedge resection. Obesity, diabetes mellitus and hypertension occur with greater frequency in hyperthecosis.

Hyperthecosis and PCOD may differ from one another histologically. Islets of luteinized theca cells in the ovarian stroma are not present in ovaries of PCOD patients (Yen, 1980; Futterweit, 1984; Yen, 1986). Therefore, in the absence of a specific clinical or biochemical difference between the two, hyperthecosis should be included within the spectrum of PCOD (Futterweit, 1984).

Hyperprolactinaemia

Although the clinical picture (hirsutism, seborrhoea, with or without amenorrhoea and galactorrhoea) may resemble PCOD, the presence of hyperprolactinaemia, normal or low Gn levels, and selective increase of DHEA and DHEA-S distinguishes those patients from those with PCOD (Yen, 1980; Yen, 1986).

PCOD-like syndrome due to hypersecretion of LH and PRL is relatively rare (Yen, 1980). Clinically, the patients present with hirsutism, infrequent menses and galactorrhoea. Laboratory studies showed hyperprolactinaemia, with inappropriate elevation of LH and low FSH levels. The aetiology is unknown. Luteinizing hormone and PRL levels showed a marked decline following dopamine infusion, a response similar to that found in hyperprolactinaemic and PCOD patients. Treatment with bromocriptine results in normalization of PRL but not LH (Yen, 1980).

Hyper/Hypothyroidism

Marked changes in SHBG and sex steroid metabolism and secretion could result from an excess or deficiency of thyroid hormones resembling PCOD. Plasma T concentrations are elevated in hyperthyroidism and a significant increase in the conversion of T to A occurs (Yen, 1980). There is an increase in circulating oestrogen levels in hyperthyroidism resulting from peripheral conversion rather than from direct glandular secretion. Chronic oestrogen elevation results in anovulatory cycles through inappropriate feedback and elevated LH.

In hypothyroidism, the MCR for T is increased due to the reduced SHBG while the MCR for A is normal. The conversion of A to T is increased and, consequently, an increase in the conversion of T to E_2 occurs. However, the metabolism of E_2 appears to be altered as well, since preferential 16-hydroxylation takes place with the formation of E_3 instead of E_2 (Yen, 1980). Oestriol is less potent than

E_2 in feedback regulation of Gn secretion resulting in chronic anovulation and/or menstrual disturbance (Yen, 1980). Exclusion of thyroid dysfunction in suspected cases is relatively apparent.

Idiopathic hirsutism

This is defined as hirsutism in women with relatively normal T levels and regular menstrual cycles (Yen, 1986). Hirsute women with normal serum T levels may have hypersensitivity of the target cell to androgens, due to increased 5α-reduction of T to DHT or may be due to a primary increase in the concentration of androgen receptors. The androgen receptor is not regulated by androgens in the skin in contrast to the 5α-R activity which increases androgen action in androgen stimulated areas such as pubic skin (Futterweit, 1984; Yen, 1986). The degree of hirsutism correlates with the relative rate of conversion of T to DHT (5α-R activity). The mechanism, which accounts for the increased 5α-R activity remains to be clarified. Although the majority of PCOD patients have elevated circulating androgen levels, up to 30% are not hirsute (Futterweit, 1984). This sensitivity may be determined by the target tissue.

CHAPTER THREE - CLINICAL AND LABORATORY PROCEDURES

3.1 PATIENTS

3.1.1 INCLUSION CRITERIA

Patients were selected according to the following criteria :

1. Age less than or equal to 38 years
2. Menstrual cycle > 41 days (oligomenorrhoeic)
3. Infertile (primary or secondary infertility)
4. Laparoscopy or hystrosalpingogram, carried out within the last three years, indicating normal pelvic organs and patent fallopian tubes

3.1.2 SOURCES OF RECRUITMENT

The patients in this study were recruited from the specialized Infertility and Reproductive Endocrinology clinics at :

Glasgow Royal Infirmary (GRI), Glasgow Western Infirmary, and Royal Alexandra Hospital, Paisley. All cases were managed at GRI.

A number of obese patients was included to allow for the analysis of the role of obesity in the pathophysiology of PCOD.

3.1.3 PATIENTS CLINICAL DATA

For all patients selected, the following data were collected and recorded in the "Patient Infertility Sheet" (Appendix I) :

- Name of the patient and her partner, residency and telephone number
- Age at menarche
- Menstrual and obstetric history
- Duration of infertility
- History of medication and response
- Onset of symptoms in relation to menarche, marriage, childbirth, use of contraceptives ... etc.
- Progression of symptoms
- Detailed family history.

All of the patients had a general examination with special emphasis on :

- BMI
- Thyroid enlargement
- Galactorrhoea
- Abdominal masses
- Hair distribution
- Signs of other endocrinopathies
- Pelvic examination.

Androgenization was assessed using the Ferriman & Gallwey scoring system and hirsutism was diagnosed when the score was > 7. Virilism was diagnosed if one or more of the following clinical features was present :

- Clitoral hypertrophy
- Breast atrophy
- Male-type baldness

- Deepening of the voice.

3.1.4 PATIENT INFORMATION SHEET

Once a suitable patient was found, a "Patient Information Sheet" was given to her. This information sheet contained simple descriptions of the procedures and the tests to be carried out (Appendix II).

3.1.5 THE CONSENT FORM

The study protocol was approved by the Ethical Committee of the Greater Glasgow Health Board. An informed written consent was obtained in each case before proceeding to the investigations. It was clearly stated in the consent that the patient could withdraw from the study at any time without jeopardizing her future follow up or treatment (Appendix III).

3.1.6 CATEGORIES OF PATIENTS

Patients recruited according to the above mentioned criteria were defined to have polycystic ovarian disease by the presence of at least 2 of the following diagnostic criteria :

1. Clinical diagnosis.

The patient showed some or all of the symptoms and signs of PCOD. These include :

i- menstrual irregularity

ii- hyperandrogenization

iii- primary or secondary infertility

iv- obesity.

2. Ultrasound diagnosis.

Ultrasonographic examination of ovaries showed at least two of the following :

i- ovarian volume > 9.0 cm^3

ii-≥ 10 follicles of 2-8 mm, peripherally or centrally distributed in the ovarian stroma, at any cut section, in one ovary.

iii- thickened ovarian stroma.

3. Biochemical diagnosis.

The patient showed some or all of the following biochemical criteria :

i- LH : FSH ratio > 2:1 during the early follicular phase (between day 5-8) of the menstrual cycle

ii- T or A levels above the upper limit of normal values of the biochemistry laboratory at GRI

iii- FAI > 4.5.

3.1.7 PATIENTS RECRUITED TO THE STUDY

The aim of the study was to recruit about 150 patients in order to achieve hopefully significant answers to the objectives of the study (Section 1.4). During the study workshop time scale, 1993-1995, about 150 patients were interviewed in the 3 hospitals allocated for recruitment of patients through their specialized " Infertility and Reproductive Endocrinology Clinics ". In spite of huge effort and time spent and persistence, and due to problems of recruitment, only 42 patients who agreed to participate in the study were found to satisfy the inclusion criteria.

All patients gave blood samples and underwent US scans (Section 3.3.3 and 3.3.5). However, only 34 patients had 2 SITTs performed (Section 3.3.8.1). The rest of the patients did not undergo the test because of either patient's refusal to undergo this invasive test or for logistic reasons as the patient presented late, in the last few months of the study time-scale.

All data represent all of the patients for all hormones except IGF-I which was carried out for only 18 patients. This was due to laboratory through-put problems within the time scale of the study for IGF-I assay. The remainder will be assayed and analysed for future publication purposes.

One patient had failed to continue the 5-week longitudinal observations, therefore, she was excluded from the relevant analyses (Chapter Six).

Eleven out of 34 patients, who were chosen randomly, underwent the post-heparin HL test (Chapter Seven). The sample was small due to logistic reasons.

3.2 CONTROLS

3.2.1 INCLUSION CRITERIA

The "volunteers control group" were recruited according to the following inclusion criteria :

1. Age matched to the patients
2. Menstrual cycles regular (i.e. between 24 and 35 days)
3. Preferably parous (i.e. no difficulty in getting pregnant)
4. No contraceptive pills for at least the last 2 months
5. Physically and mentally normal
6. Included a group of lean and a group of obese (BMI $\geq$ 29) volunteers.

All "control group" subjects had regular menstrual cycles and were studied during the midfollicular and midluteal phases of their menstrual cycle. None was hirsute, or had diabetes mellitus. None had received any hormonal medication within the last 2 months before being studied.

3.2.2 SOURCES OF RECRUITMENT

The control group consisted of regularly menstruating volunteers who were students, hospital staff or were recruited from local Health Centres.

3.2.3 CONFIRMATION OF OVULATION

The mean plasma concentrations of E_2, LH, FSH, T, SHBG and FAIs of the control group in samples collected during the early follicular phase are shown below. Ovulation was documented in the same

group by a typical rise in E_2 and a rise in plasma P values during the midluteal phase. One of the controls (n = 20) was excluded because she did not ovulate during the monitoring period as indicated by the midluteal plasma P (1.6 ng/mL) (Table 3-I).

Table 3-I : Follicular & Luteal Phase Endocrine Data in the Control group

	Follicular Phase	Luteal Phase
E_2 (pg/mL)	65.00 ± 34.2 (25 - 145)	91.15 ± 39.35 (47.5 - 215)
LH (IU/L)	5.253 ± 4.390 (1.60 - 19.8)	
FSH (IU/L)	5.150 ± 1.730 (2.95 - 9.85)	
LH / FSH ratio	1.070 ± 0.799 (0.281 -3.47)	
T (nmol/L)	1.529 ± 0.928 (0.30 - 3.20)	
SHBG (nmol/L)	62.53 ± 25.77 (28 - 120)	
FAI (TT/SHBG x 100)	2.904 ± 2.125 (0.361 - 8.00)	
P (ng / mL)		12.20 ± 7.16 (4 - 26.0)

3.2.4 CONTROLS RECRUITED TO THE STUDY

There was difficulty in recruiting weight-matched controls with normal and regular menstrual cycles. Only 20 controls agreed to participate in the study. However, one control was excluded after investigation; retrospectively she was found to have irregular menstrual rhythm rather than normal cycles.

All the controls agreed to undergo the blood tests and to have 2 SITTs but unfortunately, were reluctant to have US scans. Therefore, the literature data for normal US observations were used for reference for this study.

3.3 PROCEDURE OF INVESTIGATIONS

3.3.1 ANTHROPOMETRIC MEASURES

Body mass index was calculated as weight (kg) divided by height (m) squared (BMI = wt/(height)^2). Obesity was defined as BMI ≥ 29 Kg / m^2.

Waist/hip ratio was calculated from the circumferences measured in duplicate in the supine position (waist : midway between the lower rib margin and the iliac crest; hip : widest circumference over the great trochanters).

3.3.2 ENDOCRINE INVESTIGATIONS

Blood for the endocrine measurements was obtained in a standardized manner between 08:00 - 10:30 h. Oestradiol was measured using a competitive fluoroimmunoassay (Delfia Estradiol; Wallac Ltd, Turku, Finland). Luteinizing hormone, FSH, SHBG and PRL were assayed using noncompetitive sandwich fluoroimmunoassays (Delfia hLH, Delfia hFSH, Delfia SHBG, Delfia PRL; Wallac Ltd, Turku, Finland). Testosterone, A, P, cortisol, 17α-OHP, I, GH and C-peptide were measured using competitive radioimmunoassay (Coat-A-Count TT Kit, Coat-A-Count A, Coat-A-Count P, Coat-A-Count cortisol, Coat-A-Count 17α-OHP, Immulite I, Double Antibody Human Growth Hormone, Double Antibody C-Peptide Kits; Diagnostic Products Corporation, Los Angeles, CA, USA). The FAI or the FTI was calculated as T concentration (nmol/L) x 100, divided by SHBG concentration (nmol/L). Dehydroepiandrosterone sulphate (DHEA-S) was measured using competitive radioimmunoassay (ImmuChem™ DHEA-S Coated Tube Kit; ICN Biomedicals, Inc., Costa Mesa, CA, USA). The IGF-I was assayed using competitive radioimmunoassay (IGF-I/Somatomedin-C Coated Tube RIA Kit; Euro-Diagnostica B.V., Health Care Biotechnology). Plasma glucose was measured using the glucose oxidase method (Glucose Reagent Kit - Olympus AU5200, Olympus Optical Co Ltd). Triidothyronine, T4 and FT4 were measured using chemiluminescent enzyme immunoassays (Immulite Total T3 and Immulite Total T4 Kits; Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma leptin concentrations were measured in duplicate using solid-phase ^{125}I-radioimmunoassays (Linco Research, St. Charles, Mo).

Oestradiol values were expressed in pg/mL. The standard values for LH and FSH were expressed in IU/L calibrated against the WHO 2nd International Standard (IS) for pituitary LH for immunoassay (80/552), and the 2nd International Reference Preparation (IRP) of pituitary FSH/LH (ICSH), human, for bioassay (78/549), respectively. The values for PRL (mIU/L) and hGH (ng/mL) used the 3rd IS for PRL (84/500) and 1st IRP of human GH for immunoassay (66/217 and 80/505), respectively.

Hepatic lipase was assayed in post-heparin plasma. In this assay, gum arabic stabilized TG emulsion containing glycerol tri [I-^{14}C] oleate at a specific activity of 30 uCi/mmol TG fatty acid was use as substrate. The fatty acid products of lipolysis were captured by bovine serum albumin, extracted from the

glycerides and counted by liquid scintillation. The activity of HL was assayed in the presence of 1.0 M NaCl to inactivate lipoprotein lipase. Enzyme activities are expressed in umol of fatty acids released per hour per mL of plasma (umolFA/mL/h).

The between- and within-assay coefficients of variation for the hormones are listed in Appendix IV.

3.3.3 TIMING OF INVESTIGATIONS

- The investigations were started at least 14 days after the last menstrual period in oligomenorrhoeic cases
- For those cases who had prolonged periods of amenorrhoea, a blood estimation of E_2 and a pelvic ultrasound scan were carried out randomly. The investigations usually started once E_2 concentrations were found to be < 100 pg/mL and the ultrasound scan showed no follicles > 10.0 mm in diameter.
- Controls with regular menstrual cycles were started in the follicular phase (i.e. between days 5-8).

3.3.4 FIRST VISIT TO THE CLINIC

During the first visit the following were carried out :

1. Recruitment of cases according to inclusion criteria
2. Simple introduction to the study
3. The " Patient Infertility Sheet " was completed
4. Blood investigations and ultrasound scans were arranged
5. Semen analysis was checked
6. The " Patient Information Sheet " was handed over
7. The patient was consented when appropriate (i.e. if inclusion criteria were fulfilled).

3.3.5 LONGITUDINAL INVESTIGATIONS

The basic duration of the investigations was 5 weeks for oligo/amenorrhoeic cases.

3.3.5.1 Day One

An endocrine profile was performed to establish the circulating levels of :

1. FSH, LH, E_2, TT, SHBG, FAI, A, 17α-OHP, DHEA-S, cortisol, PRL and thyroid function tests
2. First SITT was performed (as described in 3.3.8.1)
3. A basic pelvic ultrasound examination (transvaginal ultrasound) was performed, identifying specifically the ovarian volume, echogenicity, stromal thickness and the presence of microcysts.

3.3.5.2 Weeks (1-5)

All patients were monitored for any ovarian activity twice weekly by measuring plasma E_2 levels and once a week by an ultrasound examination. The monitoring was carried out for 5 weeks.

If E_2 concentrations and/or the ultrasound scan indicated no ovarian activity, the E_2 sampling was terminated and the SITT was repeated during the 5th week.

If E_2 concentrations and/or ultrasound scans indicated any ovarian activity at any stage during the 5 week period, then E_2 was shifted to daily estimations and the ultrasound examination for follicular maturation and ovulation was performed as required over the following 2 weeks. At the midluteal phase serum P concentrations were estimated and the SITT was repeated.

3.3.6 ASSESSMENT OF LUTEAL FUNCTION

Ovarian activity was monitored twice weekly by measuring plasma E_2 levels. The monitoring was carried out for a maximum of 5 weeks.

If E_2 levels indicated ovarian activity at any stage during the 5-week period, E_2 was shifted to daily measurements. Ovulation was defined by the occurrence of an "LH surge" followed by a significant rise in P (≥ 4.0 ng/mL). The "LH surge" was diagnosed by the presence of at least doubling of the preceding LH values from one sample to the next to a concentration > 10.0 IU/L. Evidence of adequate luteinization was confirmed if a single P concentration of ≥ 4.0 ng/mL was detected, 48 hours after the "LH surge". At midluteal phase serum P concentration was estimated to assess luteal function. This required a sample to be taken between days LH peak +4 and LH peak +9 inclusive. The mean of the samples at the follicular phase represent the mean of all the samples of the observation period for those who did not ovulate, and the mean of all samples of the follicular phase calculated at least 2 days before the LH surge for those who ovulated.

3.3.7 ULTRASOUND SCANNING

3.3.7.1 Ultrasound Scanner

Ovarian ultrasonography was performed using a Siemens P/C, Sonoline SL-1 machine, Serial No. NM 80460 using sector real-time transvaginal transducers of frequencies of 5.0 and 7.5 MHz. The machine was equipped with a Polaroid Camera, 35 mm SLR - Camera adapter. All ultrasound examinations were performed by one operator (HA).

Vaginal US allows a much more precise analysis of internal ovarian morphology to be performed. As the probe can be brought closer to the ovaries, than by abdominal ultrasound, higher ultrasound frequencies (5.0 to 7.5 MHz) can be used, yielding high resolution ovarian pictures.

3.3.7.2 Technique of Ovarian Ultrasonography

Pelvic scans were carried out vaginally. This technique does not require the full bladder which is necessary using the abdominal ultrasound technique to displace the gas-containing bowel out of the pelvis. A special vaginal probe was used, covered by a clean condom, changed after each patient, and smeared with a jelly at its apex.

The uterus was identified, and the more transonic structures, the ovaries, were identified on either side of the uterus. The right and the left ovary were both examined. The following sonographic findings were recorded :

1. Ovarian volume = length(d_1)/2 x width(d_2)/2 x thickness(d_3)/2
2. The greatest number of echo free, round, or ovoid translucent structures known as follicles, < 10 mm in size, in one US plane was recorded, regardless of their distribution
3. Presence or absence of hyperechogenic ovarian stroma.

Because the ovary is mobile and not spherical, the measurements of the three diameters vary with position. However, the ovarian volume remains constant. In order to calculate the ovarian volume, the following procedure was performed :

When the largest available section of the ovary in the transverse plane had been displayed, the widest transverse diameter (d_1) and the longest diameter orthogonal to this in the A-P axis (d_2) were measured. The transducer was then rotated through 90° and when the ovary was identified in the longitudinal plane the maximum vertical diameter (d_3) was measured. The procedure took from 10-15 minutes per patient.

In order to increase the reproducibility of scanning, the basal US measurements of ovarian volume were repeated on 2 occasions for each patient. The details of the patient's mean and coefficient of variation are listed in Appendix V.

High-resolution, real-time US, enables the detection of small cysts (2 to 8 mm) within an ovary. These microcysts looked translucent on the US screen and may be arranged in the periphery of the ovary or throughout the parenchyma.

Follicle size was determined from two dimensions, longitudinal and A-P. The mean of these two measurements was taken to be the follicle diameter.

As the evaluation of the ovarian stroma is purely visual, inter-observer bias was prevented since all scans were done by one operator. The stroma was considered abnormal when it was mainly central with an area exceeding that of the microcysts that were pushed together toward the ovarian periphery, and/or when it was hyperechogenic and dense with enhanced contrast between the microcysts and the stroma, and/or when it infiltrated the microcysts whose walls appeared thickened.

3.3.7.3 Ultrasound Information Collected

All patients had a basal pelvic US examination performed on the first day of investigations. Ovarian volume, echogenicity, stromal thickness and the presence of microcysts were assessed. All cases were monitored for any ovarian activity once a week by an US examination. The monitoring was carried out for 5 weeks. Whenever E_2 concentrations and/or US scans indicated any ovarian activity during the 5 weeks' period, US examination for follicular maturation and ovulation was performed as required over the following 2 weeks.

3.3.8 DYNAMIC PROCEDURES

3.3.8.1 Short Insulin Tolerance Test (SITT)

All subjects were instructed to remain on their usual diet prior to study. None was known to have diabetes or impaired glucose tolerance, and no subject was on any medication affecting glucose tolerance. Each subject was studied on two separate occasions with an interval of at least 2 weeks for controls and 3 weeks for patients. Human actrapid I (Novo Laboratories, Nordisk, UK) was used.

The test was conducted after an overnight fast, between 08:30 - 11:00 hours. The fasting periods ranged from 8-12 hours, and the duration of each test was 1 hour and 15 minute. The weight of the patient was recorded in kilograms. The patient was then asked to lie on a couch, and a teflon cannula (Biovalve venflon, G:22, Ecouen France, made in Belgium) was inserted into a large antecubital vein. The following, pre-I injection, blood samples were collected :

1. The basal, "-15" time blood sample, 15 mL, for glucose, I, and E_2
2. "-5" time sample, 2 mL, for glucose
3. "0" time sample, 17mL, for glucose, GH, FSH, LH, E_2, TT, A, cortisol, 17α-OHP, DHEA-S, PRL and thyroid function tests.

- To arterialize the venous blood, the hand was placed in a water bath at a constant temperature of 43°C, for 10 minute prior to the start of I infusion and kept there until the end of the test. To ensure that only blood from the hand was sampled, the vein was retrogradely cannulated with a butterfly needle. Total I dose was calculated as 0.1 IU/Kg body weight and diluted in a bag of 100 mL normal

saline. The total calculated dose of I was then withdrawn from the dilution bag. Insulin was administered, by constant infusion, intravenously into the teflon cannula. Timing was calculated in relation to I infusion time. Blood was sampled as follows :

- For glucose at 1, 3, 5, 7, 9, 11, 13 and 15 minute (2 mL each), as a standard procedure. Sampling for glucose was continued at 20, 25 and 30 minute if no symptomatic hypoglycaemia developed.
- For I at 9 and 15 minute.
- For GH at 15, 30 and 60 minute (5 mL for each sample).
- Samples at 30 and 60 minute for Gn, full androgen profiles, PRL and thyroid function tests. Ten mL was required for each of these samples.

Blood for glucose estimation was sampled through the butterfly needle while that for the hormones was sampled through the venflon. Needles were flushed between sampling with normal saline. The fast was terminated at +15 minute if the patient felt hypoglycaemic; otherwise the test was continued until +30 minute. A 10-20 mL sample of 50% glucose was injected intravenously to terminate the effect of the I injection and the patient was given a snack. All glucose blood samples were placed immediately on ice until analyses.

3.3.8.2 Post-Heparin Hepatic Lipase Activities and LDL Subfraction Distribution

The aim of the heparin test was to correlate the LDL subfraction distribution and concentration with anthropometric indices, plasma lipid and lipoprotein concentrations, the activities of post-heparin lipoprotein and hepatic lipase status in PCOD patients.

Eleven patients from the whole study group who presented with oligomenorhoea and with biochemical evidence of hyperandrogenism agreed to undergo the plasma lipids study.

Inclusion Criteria :

1. Normal FBC
2. Patients who have given their informed consent to participate in this study.

Exclusion Criteria :

As described earlier in Chapter Three plus in addition :

1. Contraindications to intravenous heparin such as : sensitivity to heparin, history of any bleeding disorder (e.g. haemophilia), recent injury or trauma, Rheumatic fever or stroke.
2. Patients taking any medication known to interfere with lipoprotein concentrations such as non-steroidal anti-inflammatory drugs.

The selected oligomenorrhoeic patients were advised to fast overnight and requested to report to the clinic on the following morning during their 5-week monitoring period, bringing with them a 24-hour collection of urine. Fasting blood (total of 30 mL in EDTA) was collected for :

- Plasma concentrations of cholesterol, TG and HDL
- Determination of VLDL, LDL and HDL subfraction distributions
- Cholesterylester transfer protein activity.

The patients were then heparinized with 70 U/Kg body weight and a blood sample (10 mL in heparin) was collected for lipoprotein and HL. All blood samples were placed immediately on ice. The patients blood pressure was also recorded on the day of sampling.

3.3.9 STATISTICAL ANALYSES

Results were analyzed using Student's two-tailed *t*-test (for paired and unpaired data as indicated) and for comparison of means between two groups; Mann-Whitney *U*-test was used for variables not normally distributed. Group differences in category variables were tested with Fisher's exact test. Pearson correlation coefficients were used for simple linear correlations : $P < 0.05$ was taken as significant. Where such correlation coefficients are reported in any results section they were performed using the Pearson method. For evaluating the relationship between anthropometric, endocrine or metabolic variables, i. e. if there was a significant impact of BMI or WHR on the variables, the material was subgrouped according to BMI into < 29 and ≥ 29 Kg/m^2 and/or WHR into < 0.8 and ≥ 0.8. A number of analyses of covariance were used as an alternative method whenever appropriate. Results of all determinations shown in this thesis are expressed as mean values of the appropriate group of patients or

controls ± SD. In all tables where figures are shown as " x ± y "; y represents the standard deviation of the mean x.

All statistical analyses were performed using " Prism ", version 2, GraphPad Software, Inc.

CHAPTER FOUR - BASELINE CHARACTERISTICS OF PATIENTS & CONTROLS

4.1 INTRODUCTION

This chapter comprises three main subsections of investigations : clinical characteristics and endocrine characteristics in both patient and control groups and ultrasonographic observations in the patient group only. Clinical characteristics include age, clinical presentation of patients and anthropometric observations as well as the inter-relationships between anthropometric and clinical observations. The subsection of endocrine characteristics includes data from study group patients and controls, the relationships between endocrine characteristics and clinical presentations. Ultrasonographic observations include basal ovarian scan findings and their relationships with clinical presentations including anthropometric variables and endocrine variables.

4.2 CLINICAL CHARACTERISTICS

4.2.1 AGE

The characteristics of the study and control populations with respect to age and smoking habits are presented in Table 4-I . It shows that the mean age for the patients with oligo/amenorrhoea (mean = 29.1 years) was lower than that of the control group (mean = 34.0 years). This reflected the difficulties in recruiting weight-matched control subjects. There was no difference in the incidence or degree of smoking between the 2 groups.

Table 4-I : Characteristics of the Patients and the Controls

	Patients	Controls	P value
n	42	19	
Age	29.07 ± 4.23 (21 - 37)	34.00 ± 6.57 (18 - 41)	0.0008
Smoking	7.14 ± 10.77 (5 - 40)	3.68 ± 6.63 (5 - 20)	0.2021

4.2.2 CLINICAL PRESENTATION

Table 4-II summarizes the clinical features of the study group, and shows that 35 (83.3%) of the women had oligomenorrhoea, and seven (16.7%) had amenorrhoea. The patients with oligomenorrhoea, showed different clinical histories in that 18 patients (51.4%) had had the problem since the menarche, seven patients (20.0%) dated the problem following the use of oral contraceptive pills while 3 of the whole group (8.6%) started to notice the change in their menstrual pattern after delivery. The rest of the patients (n = 7, 20.0%) were unable to date when their menstrual disturbance started. Primary infertility was a complaint in 61.9% of the women with oligo/amenorrhoea; the mean duration of the infertility was 4.31 years (range 1-14 years).

Dysfunctional uterine bleeding was a complaint in only 5 (11.9%) of those patients who presented with oligo/amenorrhoea (Table 4-II).

Hirsutism was diagnosed in 26 patients (61.9%) with Ferriman and Gallwey scores of ≥ 7 points, and 14 (33.3%) of them had more than mild hirsutism and virilization. Almost all of the patients had noticed progression of the symptoms - oligomenorrhoea and hirsutism in particular - with advance in their age, commonly coincident with significant weight gain. None of the patients showed acanthosis nigricans. Galactorrhoea was an infrequent observation (4 patients, 9.5%).

None of the patients or the controls had diabetes mellitus or cardiovascular problems. A positive family history of menstrual irregularity was reported in 9 cases (21.4%).

Table 4-II : Clinical Features of the Patients

	Presenting features (n)	Total (%)
Oligomenorrhoea	35	83.3
Amenorrhoea (Primary/Secondary)	7	16.7
Primary infertility	26	61.9
Secondary infertility	16	38.1
Dysfunctional bleeding	5	11.9
Androgenization	26	61.9
Virilization	14	33.3
Galactorrhoea	4	9.5
Overweight (BMI ≥ 25 Kg/m^2)	24	57.1
Obesity (BMI ≥ 29 Kg/m^2)	17	40.5

4.2.3 ANTHROPOMETRIC OBSERVATIONS

The BMI for the patients was not significantly higher than that of the control group. However, the mean value of the patients' WHR was significantly higher than that of the control group, indicating different weight (fat) distribution between the patients and the controls (Table 4-III).

Table 4-III : Anthropometric Observations

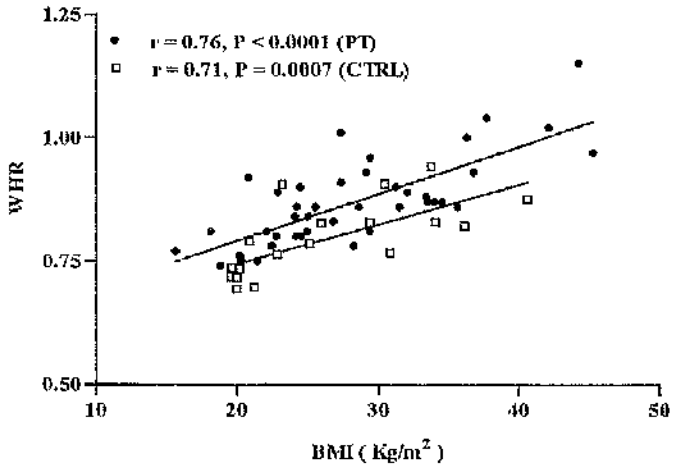
	Patients	Controls	P
n	42	19	
BMI	28.09 ± 7.06 (15.63 - 45.31)	25.98 ± 6.64 (19.6 - 40.65)	0.2756
WHR	0.87 ± 0.09 (0.74 - 1.15)	0.79 ± 0.076 (0.69 - 0.94)	0.0017

Seventeen patients (40.5%) and 7 controls (36.8%) were obese (BMI ≥ 29 Kg/m^2). Twenty seven (64.3%) patients admitted a significant increase in their weight coinciding with the start or worsening of their symptomatology, with an average increase of 18.3% in their weight (range 8.8% - 39.5%).

A highly significant correlation was found between BMI and WHR in the patients as well as in the control group, irrespective of the difference in weight distribution which was indicated by the difference in WHR (Figure 4.1).

Figure 4.1 :

Correlation : WHR and BMI in Patients and Controls



4.2.4 RELATIONSHIP BETWEEN CLINICAL PRESENTATION AND ANTHROPOMETRIC VARIABLES

There was no significant relationship between BMI or WHR and menstrual pattern, androgenization or virilization.

4.3 ENDOCRINE CHARACTERISTICS

4.3.1 COMPARISON OF ENDOCRINE DATA - PATIENTS AND CONTROLS

Women with oligo/amenorrhoea were found to have higher plasma concentrations of LH, and T, and lower levels of FSH and SHBG than their weight-matched controls (Table 4-IV) who were sampled between days 5 and 8 of their menstrual cycle. This resulted in increases in LH:FSH ratios and in FAI respectively. Basal plasma concentrations of 17∝-OHP, DHEA-S, E_2, P, PRL and thyroid function tests were similar in the patients and the controls (Table 4-IV).

In the patient group a high LH (>10.0 IU/L; mean ± 1SD of control group) was found in 47.6%, LH:FSH ratio was >2 in 48.8%, T was >2.5 nmol/L (mean ± 1SD of control group) in only 24.4 %, while 53.7% had a high FAI (> 4.5; mean ± 1SD of control group). In comparison, 2 controls (10.5%) had a high LH and LH:FSH ratio while 15.8% (3) had T >2.5 nmol/L and 26.3 % (5) had FAI > 4.5.

The results suggested that the patients had lower FSH levels than those obtained in the early follicular phase of normally ovulating women (Table 4-IV), but the difference was not statistically significant.

Although 4 patients had clinically demonstrated galactorrhoea, only one had a high PRL level (1645mIU/L).

Table 4-IV : Endocrine Results for the Patients and the Controls

	Patients	Control	P
E_2 (pg/mL)	59.09 ± 31.54	65.00 ± 34.20	NS
LH (IU/L)	10.02 ± 5.30	5.25 ± 4.39	0.0012
FSH (IU/L)	4.95 ± 1.29	5.15 ± 1.73	NS
LH : FSH ratio	2.05 ± 1.05	1.07 ± 0.80	0.0007
T (nmol/L)	2.27 ± 1.10	1.53 ± 0.93	0.0132
SHBG (nmol/L)	47.57 ± 24.28	62.53 ± 25.77	0.0336
FAI*	5.57 ± 4.16	2.62 ± 1.78	0.0061
P (ng/mL) Follicular.	0.719 ± 0.48	1.14 ± 1.67	NS
P (ng/mL) Luteal	6.89 ± 6.23	12.20 ± 7.16	0.0067
A (nmol/L)*	8.09 ± 4.36	6.13 ± 2.85	0.0795
17∝-OHP (nmol/L)	1.44 ± 0.76	1.48 ± 1.14	NS
DHEA-S (umol/L)	1971.2 ± 1306.6	2126.0 ± 1788.0	NS
PRL (mU/L)	291.12 ± 240.03	424.30 ± 519.60	NS
T4 (nmol/L)	114.92 ± 25.035	109.70 ± 19.29	NS
T3 (nmol/L)	144.15 ± 26.976	140.10 ± 29.59	NS
FT4	14.36 ± 3.55	14.42 ± 2.32	NS

Groups were compared using Student's t-test, except for values with (*) where Mann-Whitney nonparametric test was used for comparison. Progesterone was estimated only for those patients (n=17) and controls (n=19) who ovulated at follicular and luteal phases. NS means P ≥ 0.05.

The correlations of the basal endocrine profiles in all subjects are shown in Figures 4.2 - 4.4. In the patients, the LH was not correlated to T or FAI, but there was a weak correlation with FSH (Figure 4.3). There was a tendency for FSH to rise with increased severity of high LH. There was also a weak negative correlation between the patients' T concentrations and FSH levels in both study groups (Figure 4.4), which could be related to some follicular activity contributed by those individuals who ovulated.

Figure 4.2 :

Correlation : E_2 and Testosterone in Patients and Controls

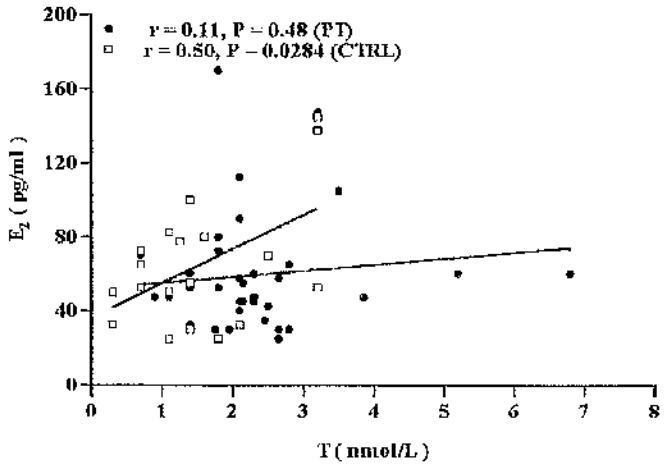


Figure 4.3 :

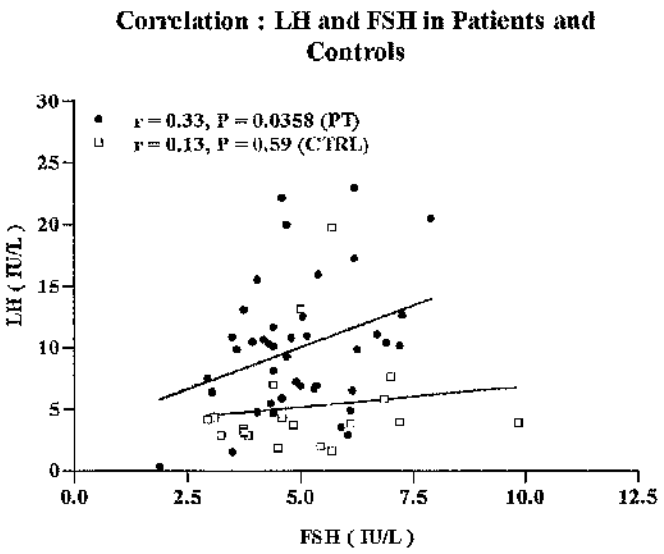
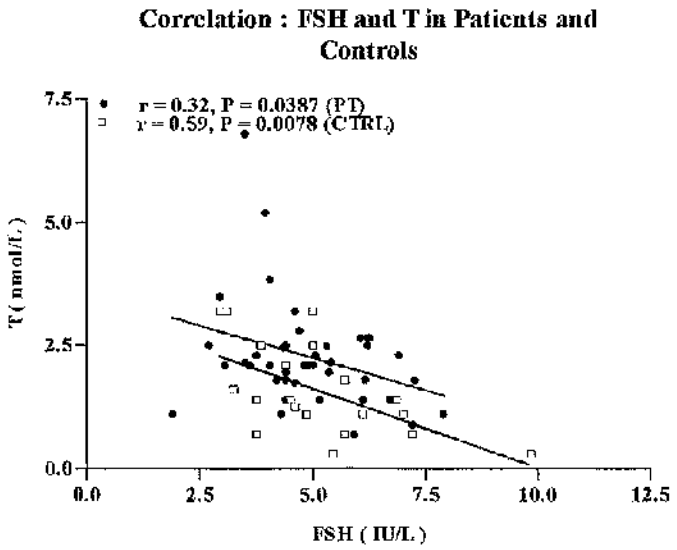


Figure 4.4 :



There were no differences in the mean basal values of T and FAI between the group of patients who had high LH (> 10.0 IU/L) and those with normal basal LH concentrations (≤ 10.0 IU/L) (Table 4-V).

Table 4-V : Mean Basal T and FAI in Patients with Normal/High Basal LH Concentrations

	Normal LH		High LH		P
T (nmol/L)	(n = 20)	2.08 ± 0.64	(n = 22)	2.46 ± 1.36	NS
FAI	(n = 21)	5.71 ± 4.76	(n = 21)	5.42 ± 3.45	NS

4.3.2 RELATIONSHIP WITH CLINICAL PRESENTATION

The relationship between the presenting clinical symptoms and the endocrine profiles are shown in Tables 4-VIa and 4-VIb. Patients who presented with prolonged history of menstrual disturbance, oligomenorrhoea since menarche, were found to have lower levels of FSH than those whose menstrual irregularity started later (P = 0.0325). Women with DUB were found to have lower basal levels of E_2 than other study subjects (P = 0.0228).

Table 4-VIa shows that patients presenting with amenorrhoea had significantly lower plasma E_2 concentrations than those with oligomenorrhoea (P = 0.0458) but LH, FSH, LH:FSH, T, SHBG and FAI were not statistically different.

Androgenized and virilized patients showed higher mean concentrations of LH, FSH, T, LH:FSH and FAIs, and lower levels of E_2 and SHBG compared with the rest of the study group, but these differences were not statistically significant.

Patients with primary infertility had significantly higher SHBG (P = 0.0313), and significantly lower FAIs (P = 0.0267), than those with secondary infertility. This finding could be due to weight changes although their BMI and WHR were similar.

Table 4-VIa : The Relationship between the Presenting Symptoms and the Endocrine Profiles

Presenting symptom	Prevalence	Hormone concentrations in Patients			
	n & (%)	E_2 (pg/mL)	LH (IU/L)	FSH (IU/L)	LH : FSH
Oligo.since menarche	18 (42.9)	55.29±26.13	9.73±3.42	4.60±0.86*	2.17±0.82
Other Oligo/amenorrh.	24 (57.1)	63.80±35.52	10.65±6.15	5.35±1.33*	2.04±1.17
Hirsutism	27 (64.3)	57.87±31.36	10.13±5.11	4.99±1.29	2.06±0.95
No hirsutism	15 (35.7)	62.14±33.81	9.79±5.84	4.89±1.32	2.02±1.27
Virilized	13 (31.0)	57.69±35.68	10.96±4.17	5.43±1.32	2.07±0.80
No virilization	29 (69.1)	60.09±30.59	9.57±5.76	4.73±1.23	2.04±1.17
DUB	5 (11.9)	45.00±38.66*	9.20±2.95	5.07±1.23	1.85±0.62
No DUB	37 (88.1)	61.32±33.44*	10.13±5.57	4.94±1.31	2.07±1.10
Oligomenorrhoea	35 (83.3)	62.72±32.57*	10.54±5.32	4.90±1.16	2.19±1.05
Amenorrhoea	7 (16.7)	42.86±23.47*	7.44±4.75	5.21±1.88	1.33±0.76
1° infertility	28 (66.7)	61.20±31.10	9.94±5.05	5.05±1.34	2.01±1.04
2nd infertility	14 (33.3)	55.71±34.17	10.15±5.94	4.77±1.21	2.12±1.12

The groups were compared using Student's t-test, where (*) indicates significant P< 0.05.

Table 4-VIb : The Relationship between the Presenting Symptoms and the Endocrine Profiles

Presenting symptom	Prevalence	Hormone concentrations in Patients		
	n & (%)	T (nmol/L)	SHBG (nmol/L)	FAI
Oligo.since menarche	18 (42.9)	2.30±0.97	42.47±21.89	5.94±2.70
Other Oligo.	24 (57.1)	2.30±1.20	51.41±26.23	5.43±5.05
Hirsutism	27 (64.3)	2.44±1.23	44.57±23.62	5.66±3.43
No hirsutism	15 (35.7)	1.95±0.73	53.36±25.39	5.40±5.45
Virilized	13 (31.0)	2.32±1.11	47.69±24.57	5.69±3.29
No virilization	29 (69.1)	2.25±1.11	47.52±24.60	5.51±4.56
DUB	5 (11.9)	1.53±0.58	45.80±33.93	4.43±2.61
No DUB	37 (88.1)	2.38±1.12	47.82±23.28	5.73±4.33
Oligomenorrhoea	35 (83.3)	2.36±1.15	49.23±23.33	5.70±4.38
Amenorrhoea	7 (16.7)	1.83±0.67	39.50±29.09	4.95±3.04
1° infertility	28 (66.7)	2.22±0.70	52.61±26.47*	4.56±2.99*
2nd infertility	14 (33.3)	2.56±1.61	37.86±16.09*	7.52±5.39*

The groups were compared using Student's t-test, where (*) indicates significant P< 0.05.

4.3.3 RELATIONSHIP BETWEEN ENDOCRINE AND ANTHROPOMETRIC VARIABLES

4.3.3.1. BMI

The subgrouping for BMI is arbitrary, therefore the results may be highly dependent on the limits applied. Accordingly, to examine the effect of excess weight, results were analyzed with a cut-off point of BMI equals 25 Kg/m^2 indicating overweight (subgroups < 25, ≥ 25 Kg/m^2), and the same analyses were repeated with BMI equals 29 Kg/m^2 to indicate the obese patients and controls (subgroups < 29, ≥ 29 Kg/m^2).

The major endocrine impact of obesity is shown in Tables 4-VIIa and 4-VIIb.

Patients :

Plasma levels of E_2, LH, LH:FSH ratio, the levels of 17∝-OHP, and DHEA-S were similar in lean and obese patients (Table 4-VIIa). Plasma concentrations of FSH, SHBG, and PRL were significantly lower, and those of T and the FAI were both significantly higher in obese than in lean patients. The differences were less significant when the higher BMI cut-off point was applied.

Table 4-VIIa : The Impact of BMI on the Endocrine Profiles of the Patients

	<25	≥25	P	<29	≥29	P
n	18	24		25	17	
E_2 (pg/mL)	58.89	59.67	NS	60.10	58.13	NS
LH (IU/L)	10.24	9.84	NS	9.94	10.13	NS
FSH (IU/L)	5.64	4.42	0.003	5.28	4.45	0.024
LH:FSH ratio	1.78	2.26	NS	1.86	2.34	NS
T (nmol/L)	1.86	2.60	0.0189	1.97	2.75	0.049
A (nmol/L)				7.38	9.15	NS
SHBG (nmol/L)	59.78	38.02	0.0028	54.82	36.25	0.013
FAI	3.57	7.13	0.0026	3.60	8.65	0.0004
17∝-OHP (nmol/L)	1.42	1.46	NS	1.44	1.45	NS
DHEA-S (umol/L)	836.14	2297.83	0.004	1886.80	2103.1	NS
PRL (mU/L)	381.94	220.04	0.059	343.76	208.88	0.034

The groups were compared using Student's t-test. $P < 0.05$ is significant

Controls :

BMI showed a significantly negative impact on SHBG (Table 4-VIIb), and consequently a higher FAI in obese compared to lean controls. Other hormones showed no significant association with obesity.

Table 4-VIIb : The Impact of BMI on the Endocrine Profiles of the Controls

	<25	≥25	P	<29	≥29	P
n	10	9		12	7	
E_2 (pg/mL)	62.25	65.00	NS	67.50	57.81	NS
LH (IU/L)	4.60	6.04	NS	4.83	6.04	NS
FSH (IU/L)	4.78	5.51	NS	4.82	5.63	NS
LH:FSH ratio	0.95	1.22	NS	1.00	1.20	NS
T (nmol/L)	1.24	1.92	0.099	1.39	1.86	NS
A (nmol/L)				6.24	5.87	NS
SHBG (nmol/L)	75.60	45.20	0.0072	73.58	40.63	0.0037
FAI	2.06	4.71	0.0483	2.26	5.07	0.0765
17∝-OHP (nmol/L)	1.54	1.34	NS	1.59	1.21	NS
DHEA-S (umol/L)	1620.0	2570.0	NS	1841.67	2475.0	NS
PRL (mU/L)	462.30	362.40	NS	414.42	409.25	NS

The groups were compared using Student's t-test. $P < 0.05$ is significant

4.3.3.2 WHR

Similarly, the impact of WHR on the endocrine profiles is shown in Tables 4-VIIIa and 4-VIIIb.

Patients :

Basal E_2, LH, FSH, LH:FSH ratio, T, A, 17∝-OHP and PRL levels were similar for patients with WHR < 0.8 and those with WHR ≥ 0.8 (Table 4-VIIIa). The levels of SHBG, were related to WHR. The difference between the mean values of SHBG for patients with WHR < 0.8 compared to those with WHR ≥ 0.8 was highly significant (P = 0.0014). Consequently, similar findings were observed for FAI (P = 0.0006) and the levels of DHEA-S (P = 0.0212).

Table 4-VIIIa : The Impact of WHR on the Endocrine Profiles of the Patients

	< 0.8	≥ 0.8	P
n	8	34	
E_2 (pg/mL)	60.94 ± 27.74	58.94 ± 33.16	NS
LH (IU/L)	7.96± 5.74	10.51± 5.15	NS
FSH (IU/L)	5.51± 1.41	4.82± 1.24	NS
LH:FSH ratio	1.48± 0.87	2.19± 1.06	0.0876
T (nmol/L)	1.78± 0.67	2.39± 1.15	NS
A (nmol/L)	7.90±5.48	8.13±4.14	NS
SHBG (nmol/L)	71.0± 25.98	41.89± 20.47	0.0014
FAI	2.72± 1.61	6.26± 4.30	0.0006
17∝-OHP (nmol/L)	1.05± 0.31	1.54± 0.81	NS
DHEA-S (umol/L)	1293.8 ± 689.95	2135.5 ± 1373.77	0.0212
PRL (mU/L)	453.75 ± 493.74	251.69 ± 102.37	NS

The groups were compared using Student's t-test. P < 0.05 is significant

Controls :

There was no statistically significant difference in E_2, LH, FSH, LH:FSH ratio, 17∝-OHP, DHEA-S and PRL between controls with WHR < 0.8 and those with WHR ≥ 0.8 (Table 4-VIIIb). Testosterone levels and FAI were significantly higher, and SHBG levels were significantly lower in controls with larger WHR than in those with smaller WHR.

Table 4-VIIIb : The Impact of WHR on the Endocrine Profiles of the Controls

	< 0.8	≥ 0.8	P
n	11	8	
E_2 (pg/mL)	57.73± 23.60	70.83± 43.80	NS
LH (IU/L)	4.97± 5.18	5.74± 3.12	NS
FSH (IU/L)	5.52± 1.93	4.68± 1.29	NS
LH:FSH ratio	0.93± 0.89	1.27± 0.62	NS
T(nmol/L)	1.02± 0.51	2.26± 0.88	0.001
A (nmol/L)	5.40±2.34	7.07±3.18	NS
SHBG (nmol/L)	72.73± 27.54	45.33± 17.23	0.0184
FAI	1.62± 1.17	5.54± 3.14	0.0061
17∝-OHP (nmol/L)	1.26± 1.24	1.66± 0.99	NS
DHEA-S (umol/L)	1718.2± 1098.9	2555.6± 2301.7	NS
PRL (mU/L)	427.46± 649.9	393.89± 292.5	NS

The groups were compared using Student's t-test. P < 0.05 is significant

4.3.3.3 Comparison of Patients and Controls with Respect to the Influence of Obesity

BMI :

Basal plasma levels of E_2, FSH, 17∝-OHP, DHEA-S and PRL did not show any statistically significant difference when patients were compared to the control group of similar BMI (Tables 4-IXa and 4-IXb). However, the levels of LH and the LH:FSH ratio were significantly higher when comparing the patients to the controls for all BMI subgroupings. Testosterone and FAI mean values were increased, and SHBG values were decreased for both the overweight (≥ 25 Kg/m^2) and the obese (≥ 29 Kg/m^2) individuals compared to the lean patients and controls. However, the differences in T and SHBG concentrations reached statistical significance only when comparing the lean patients to the lean controls for both the cut-off points of BMI < 25, as well as < 29 Kg/m^2 (data in table). The differences did not reach statistical significance comparing the patients to the controls for the higher BMI of ≥ 25 and ≥ 29 Kg/m^2, (Tables 4-IXa and 4-IXb).

Luteinizing hormone levels were found to be equivalent in obese and lean patients as well as in the control group. However, when comparing patients to controls of matched BMI, the differences were significant for all BMI ranges (Tables 4-IXa and 4-IXb).

Table 4-IXa : The Impact of Overweight on the Endocrine Profiles of the Patients in Comparison to the Controls

	PT(< 25)	CTRL (< 25)	P	PT (≥ 25)	CTRL (≥ 25)	P
n	18	10		24	9	
E_2 (pg/mL)	58.89	62.25	NS	59.67	65.00	NS
LH (IU/L)	10.24	4.60	0.022	9.84	6.04	0.024
FSH (IU/L)	5.64	4.78	NS	4.42	5.51	NS
LH:FSH ratio	1.78	0.95	0.044	2.26	1.22	0.006
T (nmol/L)	1.86	1.24	0.021	2.60	1.92	NS
SHBG (nmol/L)	59.78	75.60	NS	38.02	45.20	NS
FAI	3.57	2.06	0.094	7.13	4.71	NS
17∝-OHP (nmol/L)	1.42	1.54	NS	1.46	1.34	NS
DHEA-S (umol/L)	836.14	1620.0	NS	2297.83	2570.0	NS
PRL (mU/L)	381.94	462.30	NS	220.04	362.40	NS

The groups were compared using Student's t-test. P < 0.05 is significant

Table 4-IXb : The Impact of Obesity on the Endocrine Profiles of the Patients in Comparison to the Controls

	PT (< 29)	CTRL (< 29)	P	PT (≥ 29)	CTRL (≥ 29)	P
n	25	12		17	7	
E_2 (pg/mL)	60.10	67.50	NS	58.13	57.81	NS
LH (IU/L)	9.94	4.83	0.017	10.13	6.04	0.014
FSH (IU/L)	5.28	4.82	NS	4.45	5.63	NS
LH:FSH ratio	1.86	1.00	0.026	2.34	1.20	0.004
T (nmol/L)	1.97	1.39	0.033	2.75	1.86	NS
A (nmol/L)	7.38	6.24	NS	9.15	5.87	NS
SHBG (nmol/L)	54.82	73.58	0.036	36.25	40.63	NS
FAI	3.60	2.26	0.082	8.65	5.07	0.068
17∝-OHP (nmol/L)	1.44	1.59	NS	1.45	1.21	NS
DHEA-S (nmol/L)	1886.8	1841.7	NS	2103.13	2475.0	NS
PRL (mU/L)	343.76	414.41	NS	208.88	409.25	NS

The groups were compared using Student's t-test. $P < 0.05$ is significant

The correlations between BMI and T, SHBG and FAI in both groups of patients and controls are demonstrated in Figures 4.5 - 4.7. There was no significant correlation between BMI and T in either patients or controls. A significant negative correlation was observed between BMI and SHBG in both groups which was reflected in the FAI.

The two regression lines for patients and controls comparing T and BMI (Figure 4.5), were not statistically different (95 % confidence interval for the slope of patients was -0.0155 to 0.1192 and for the controls -0.003931 to 0.09174). Therefore, for a unit increase in BMI, SHBG and T responded similarly in the patients and in the controls.

Figure 4.5 :

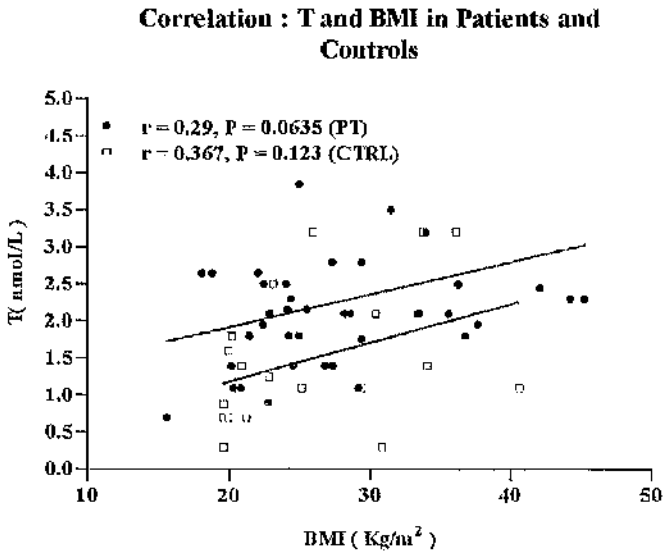


Figure 4.6 :

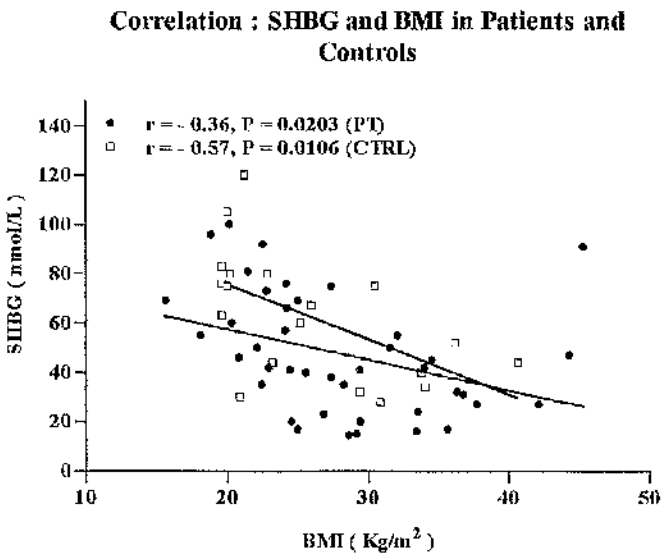
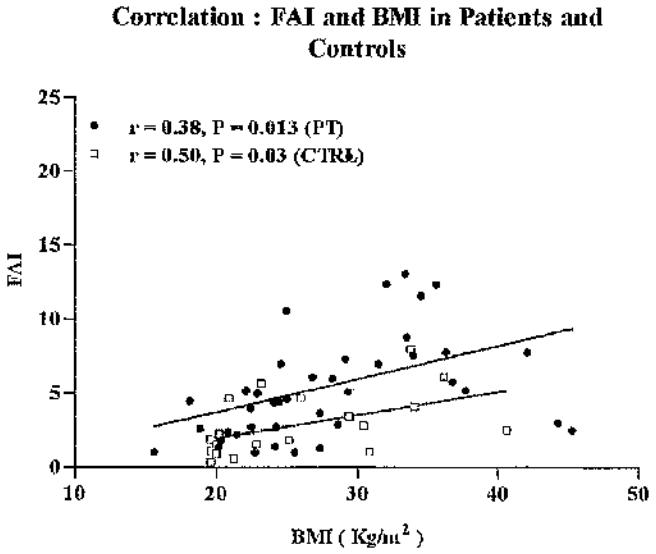


Figure 4.7 :



WHR :

Plasma concentrations of E_2, FSH, , SHBG, FAI, A, 17∝-OHP, DHEA-S and PRL were similar in both patient and control subgroups of similar WHR (Table 4-X).

The levels of LH and FAI were higher in patients than controls with WHR < 0.8 as well as WHR ≥ 0.8. But these levels reached statistical significance only with the higher WHR (Table 4-X). The LH levels and LH:FSH ratio were significantly higher in the patients than the controls at WHR ≥ 0.8 (Table 4-X).

Testosterone levels were not significantly higher in patients than controls with higher WHR (P = 0.15), but were significantly higher in controls with larger WHR than in controls with smaller WHR (P = 0.001). When comparing the mean value of the patients to that of the controls with similar WHR, the difference in T levels reached statistical significance with smaller WHR women only (P = 0.0132 for WHR < 0.8 and 0.7403 for WHR ≥ 0.8).

The SHBG levels were influenced by the WHR. The mean values of SHBG for both patients and controls with WHR< 0.8 were significantly higher than those with WHR ≥ 0.8 (P = 0.0014 and 0.0184, respectively). The findings were the same for FAI (P = 0.0006, and 0.0061); very significantly higher indices being found for both patients and controls with WHR ≥ 0.8.

Table 4-X : The impact of WHR on the Endocrine Profiles of the Patients in Comparison to Controls

	PT (< 0.8)	CTRL (< 0.8)	P	PT (≥ 0.8)	CTRL (≥ 0.8)	P
n	8	11		34	8	
E_2 (pg/mL)	60.94±27.74	57.73±23.60	NS	58.94±33.16	70.83±43.80	NS
LH (IU/L)	7.96±5.74	4.97±5.74	NS	10.51±5.15	5.74±3.12	0.0118
FSH (IU/L)	5.51±1.41	5.52±1.93	NS	4.82±1.24	4.68±1.29	NS
LH:FSH ratio	1.48±0.87	0.93±0.89	NS	2.19±1.06	1.27±0.62	0.0177
T (nmol/L)	1.78±0.67	1.02±0.51	0.0132	2.39±1.15	2.26±0.88	NS
A (nmol/L)	7.90±5.48	5.40±2.34	NS	8.13±4.14	7.07±3.18	NS
SHBG (nmol/L)	71.0±25.98	72.73±27.54	NS	41.89±20.47	45.33±17.23	NS
FAI	2.72±1.61	1.62±1.17	NS	6.26±4.30	5.54±3.14	NS
17∝-OHP (nmol/L)	1.05±0.31	1.26±1.24	NS	1.54±0.81	1.66±0.99	NS
DHEA-S (umol/L)	1293.8±690.0	718.2±1098.9	NS	2135.5±1373.8	555.6±2301.69	NS
PRL (mU/L)	453.75±493.7	427.46±649.9	NS	251.697±102.4	393.89±292.5	NS

The groups were compared using Student's t-test. P < 0.05 is significant

Weak but significant correlations were observed between SHBG, and PRL and WHR of the patients (r = -0.36, P = 0.0179; r = -0.31, P = 0.0484, respectively) (Figures 4.9 and 4.11). The significant correlation between PRL and WHR was due to one very high PRL value in a patient with a low WHR, which was lost when this patient was excluded (P = NS).

For the control group, the negative correlation between WHR and SHBG was tighter and more significant (r = -0.61, P = 0.0052) as shown in Figure 4.9, and significant positive correlations between

T_f and FAI and WHR (r = 0.64, P = 0.003; r = 0.78, P < 0.0001, respectively; Figures 4.8 and 4.10) were also observed.

Figure 4.8 :

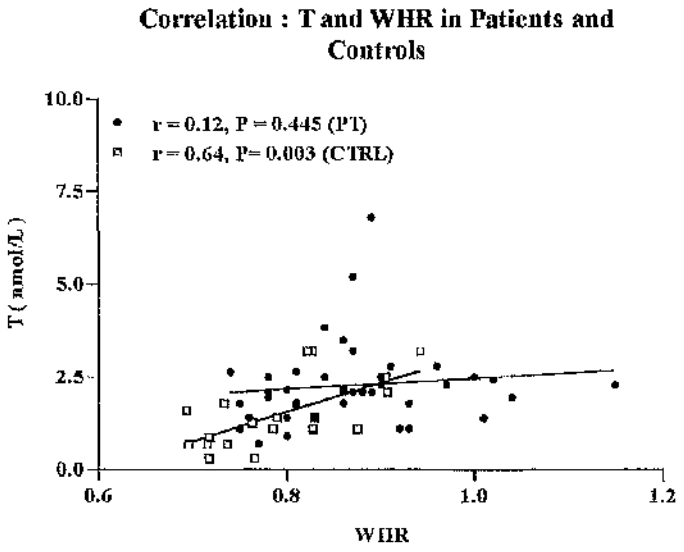


Figure 4.9 :

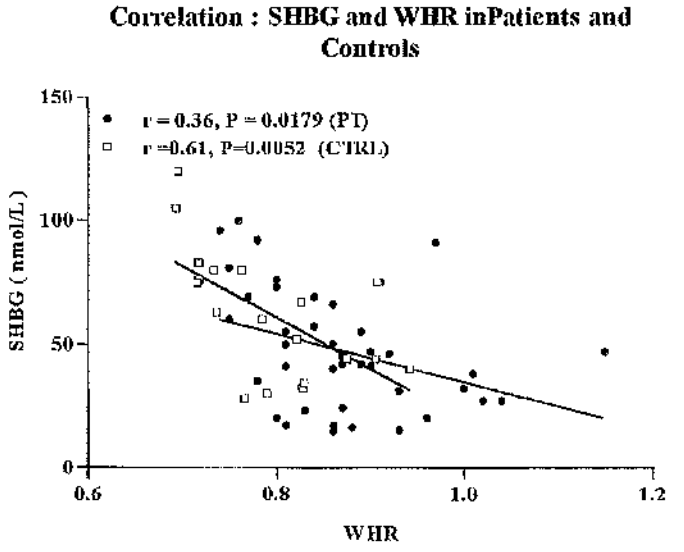


Figure 4.10 :

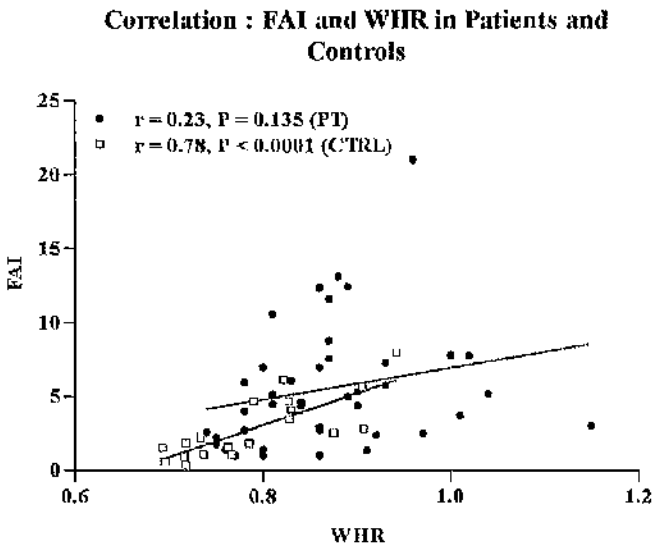
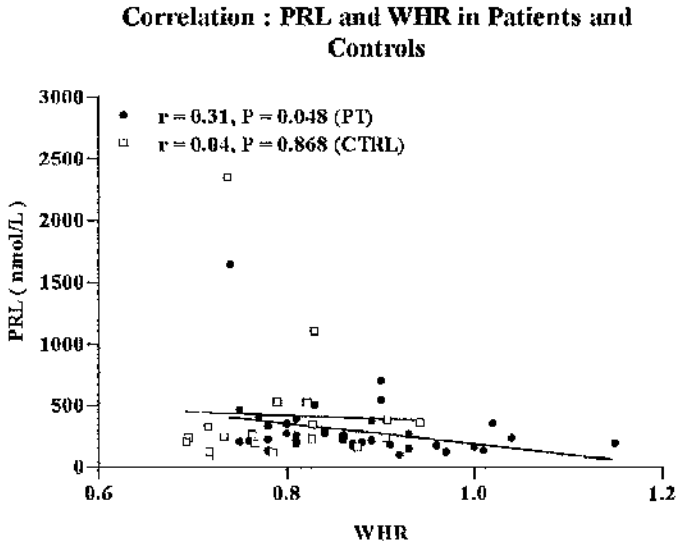


Figure 4.11 :



4.4 ULTRASONOGRAPHIC OBSERVATIONS

4.4.1 BASAL OVARIAN SCAN OBSERVATIONS

Control Data :

Unfortunately, there was no US examination for the control group because of their reluctance to volunteer for the examination. The mean (± SD) ovarian volume for normal subjects was taken from published reference ranges as 5.2 ± 1.9 cm^3 (Yeh *et al* 1987) and 5.4 ± 1.6 cm^3 (Polson *et al* 1988; Franks, 1989). The cut-off point of normal ovarian volume, in this study, was considered to be 2 SD above the mean normal ovarian volume, that is 9.0 cm^3. Therefore, the ovary was considered enlarged in patients when the volume was greater than 9.0 cm^3.

Patient Data :

The US scanning was always effected > 14 days after the first day of the last menstrual period. The mean ovarian volume for oligo/amenorrhoeic patients was 9.36 cm^3, while the mean of TOV was 18.73 cm^3 (Table 4-XI).

Table 4-XI : The Ovarian Volume in Patients

	mean ± SD (cm^3)	range (cm^3)
No. of Patients	42	
Rt. ovary	9.58 ± 6.33	1.09 - 31.54
Lt. ovary	9.13 ± 5.55	1.83 - 27.30
TOV	18.73 ± 8.88	2.92 - 40.50
Ovarian Volume (mean Rt./Lt.)	9.36 ± 4.44	1.09 - 29.42

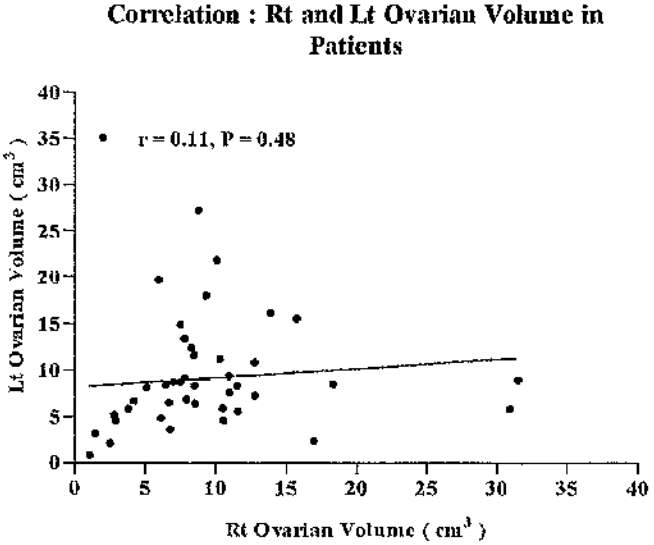
Two out of 42 patients showed normal US ovarian picture, while the rest (n = 40) satisfied the criteria of "PCO" US (Chapter Three). Therefore, the following analyses included those patients with "PCO" US diagnosis only (40 patients).

Table 4-XII : The Ovarian Volume in "PCO" Patients

	mean ± SD (cm^3)	range (cm^3)
No. of Patients	40	
Rt. ovary	9.79 ± 6.26	1.48 - 31.54
Lt. ovary	9.34 ± 5.46	2.13 - 27.30
TOV	19.15 ± 8.57	4.60 - 40.50
Ovarian volume (mean Rt./Lt.)	9.57 ± 4.29	2.32 - 20.24

There was heterogeneity within subjects, as 10 patients (24.4%) showed one ovary less than 50% of the size of the other, and this general finding resulted in there being no relationship between Rt. and Lt. ovarian volumes (Figure 4.12). This is a remarkable observation showing the dynamic nature of the organ, even in the relatively stable conditions of oligo and amenorrhoea.

Figure 4.12 :



Twenty two (55%) of patients had at least one ovary of a volume > 9.0 cm^3, and only 40% had both ovaries > 9.0 cm^3. The basal US ovarian characteristics of the patients are shown in Table 4-XIII. The mean number of ovarian follicles (2 - 8 mm) was 11.68 ± 2.55 (range 5 - 20 follicles) on the right ovary,

and 12.61 ± 3.81 (range 6 - 30 follicles) on the left ovary (Table 4-XIII). Ovarian follicles were peripherally distributed in only 46 ovaries (56.1 %), while the rest showed central distribution.

Table 4-XIII : Ultrasonographic Ovarian Characteristics

	Rt. Ovary	Lt. Ovary	P
No. of Patients	40	40	
Ovarian Volume (cm^3)	9.58 ± 6.33 (1.09-31.54)	9.13 ± 5.55 (0.83-27.30)	NS
No. of Follicles (2-8 mm)	11.68± 2.55 (5 - 20)	12.61± 3.81 (6 - 30)	NS

The groups were compared using Student's t-test. P ≥ 0.05 is NS

All 40 PCO patients (95.2 %) had at least 10 follicles (2 - 8 mm in diameter) in one section on one ovary, and bilaterally in 36 patients (85.7%). Also 40 patients (95.2 %) showed thick (dense) stroma in at least one ovary, while 32 patients (80.0 %) had an increased amount of stroma in both ovaries.

The US ovarian characteristics and a comparison between "unilateral PCO" with "bilateral PCO" are shown in Table 4-XIVa and 4-XIVb. The mean ovarian volume and TOV was significantly larger in the patients with bilateral PCO than those with unilateral PCO diagnosis. The number of follicles was higher with bilateral PCO than unilateral PCO (P = 0.0127) but the distribution of the follicles was similar (Table 4-XIVb). However, this observation could be masked by the small size of the unilateral PCO sample.

Table 4-XIVa : Ultrasonographic Ovarian Characteristics in Patients

	One Ovary	Both Ovaries
	n (%)	n (%)
PCO Diagnosis	8 (20)	32 (80.0)
Follicles ≥ 10	40 (95.2)	36 (85.7)
Thick Stroma	40 (95.2)	32 (80.0)

Table 4-XIVb : Comparison between"Unilateral PCO"and"Bilateral PCO"

	Unilateral PCO	Bilateral PCO	P
No. of Patients	8	32	
Rt. Ovarian Volume	8.09 ± 9.74	9.94 ± 5.35	NS
Lt. Ovarian Volume	5.06 ± 2.41	10.12 ± 5.67	0.019
Mean Ovarian Volume	6.58 ± 5.38	10.03 ± 3.99	0.048
TOV	13.17 ± 10.75	20.07 ± 7.98	0.048
Mean No. of Follicles	9.25 ± 2.38	12.67 ± 2.61	0.0127
Peripheral Follicular Distribution	2	15	
Central Follicular Distribution	3	8	
Mixed Follicular Distribution	3	9	

4.4.2 CORRELATION OF OVARIAN VOLUME WITH CLINICAL PRESENTATION

Women with large ovarian volumes (>9.0 cm^3) were more commonly presented with oligomenorrhoea than with amenorrhoea but the difference was not statistically significant (P = 0.0931). The duration of menstrual irregularity, the presence of DUB, androgenization and/or virilization were not associated with ovarian volume.

There was no significant correlation between TOV, the presence of a thick ovarian stroma and the duration of infertility, androgenization or virilization. There was no correlation between the clinical presentation and the presence of unilateral or bilateral polycystic ovaries diagnosed by US scanning. However, patients with unilateral thick stroma were found to have longer periods of amenorrhoea than those with bilateral changes (ranges 14-18 vs 5-12 weeks, respectively; P = 0.0395).

4.4.3 CORRELATION OF OVARIAN VOLUME WITH ANTHROPOMETRIC VARIABLES

For the purpose of comparisons between patients with different BMI and WHR, the mean ovarian volumes were used. There was no relationship between the mean ovarian volume, and the BMI and WHR as shown in Table 4-XV and Figures 4.13 and 4.14.

Table 4-XV : The Correlation between BMI, WHR & Ovarian Volumes in the Patients

	OV.VOLUME ≤ 9.0 cm^3		OV.VOLUME > 9.0 cm^3		Total
	n (%)	mean ± SD	n (%)	mean ± SD	
BMI < 25	10 (23.8)	5.58 ± 2.58	7 (16.7)	12.64± 3.79	17
BMI ≥ 25	12 (28.6)	6.69 ± 2.09	13(30.9)	12.94± 3.21	25
BMI < 29	13 (31.0)	6.04 ± 2.43	9 (21.4)	11.46± 3.38	22
BMI ≥ 29	8 (19.0)	6.05 ± 2.20	12(28.6)	13.10± 3.33	20
WHR < 0.8	6 (14.3)	6.10 ± 2.42	2 (4.8)	9.78 ± 0.17	8
WHR ≥ 0.8	15 (35.7)	6.02 ± 2.32	19(45.2)	13.18± 0.79	34

Figure 4.13 :

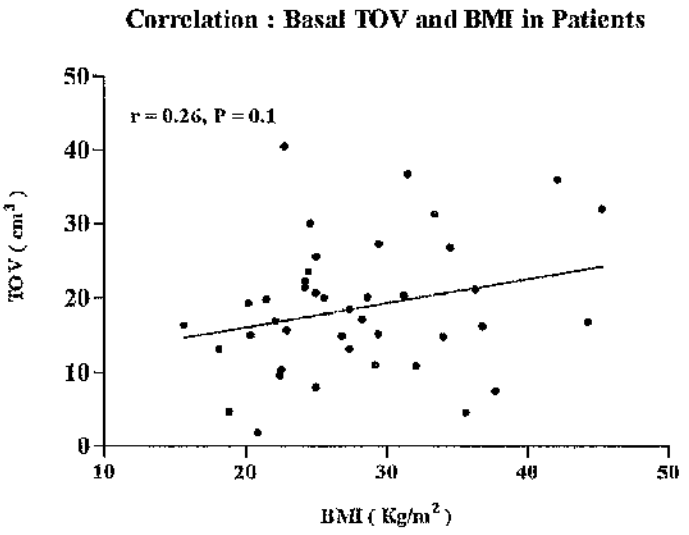
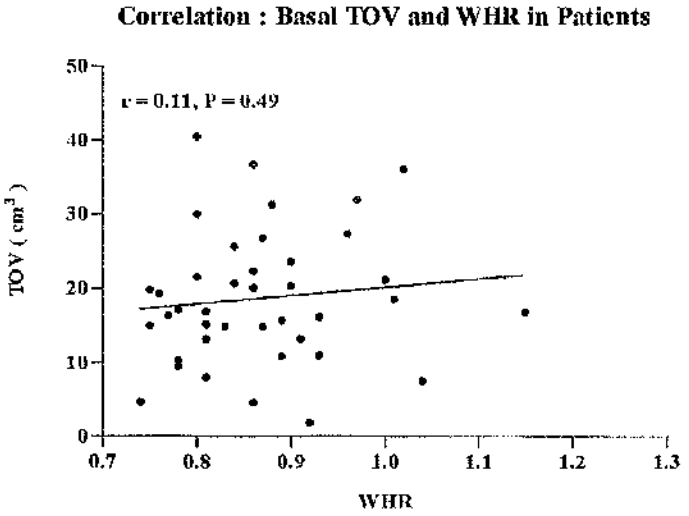


Figure 4.14 :



4.4.4 CORRELATION OF OVARIAN VOLUME WITH ENDOCRINE RESULTS

The endocrine variables were also correlated with the TOV. There was no correlation between the TOV and the endocrine profiles (Table 4-XVI). Although the basal levels of E_2, LH, LH:FSH, T, and SHBG

were higher in patients with larger ovarian volumes (> 9.0 cm^3), the differences were not significant. Patients with larger ovarian volume (> 9.0 cm^3) showed lower levels of FSH, FAI, 17∝-OHP, PRL and DHEA-S but the differences were not statistically significant.

Peripheral distribution of the ovarian follicles was shown to be associated with higher ovarian volume, BMI, WHR, E_2, LH, FSH, LH:FSH, T, FAI, 17∝-OHP, DHEA-S, and lower SHBG and PRL concentrations but no difference was significant.

Patients with unilateral PCO had higher levels of basal E_2, LH, FSH,T, SHBG and FAI than those with bilateral PCO but none of these differences was significant.

The correlations between the endocrine, anthropometric parameters and the TOV are also shown in Table 4-XVI.

Table 4-XVI : Correlations between Endocrine and Anthropometric Parameters and TOV

	r	SLOPE	P
TOV / BMI	0.257	-0.05460 to 0.5237	NS
TOV / WHR	0.112	-0.2030 to 0.4055	NS
TOV / E2	0.125	-0.2076 to 0.4315	NS
TOV / LH	0.161	-0.1717 to 0.4614	NS
TOV / FSH	0.083	-0.2478 to 0.3964	NS
TOV / LH:FSH	0.179	-0.1536 to 0.4758	NS
TOV / T	0.075	-0.2935 to 0.4237	NS
TOV / SHBG	0.030	-0.3620 to 0.4123	NS
TOV / FAI	0.095	-0.3032 to 0.4657	NS
TOV / P (F)	0.309	-0.0171 to 0.5752	NS
TOV / 17∝-OHP	0.264	-0.1374 to 0.5911	NS
TOV / PRL	0.267	-0.5634 to 0.09005	NS
TOV / DHEA-S	0.097	-0.4981 to 0.3388	NS

4.5 DISCUSSION

Polycystic ovarian disease is characterized by heterogeneous clinical and endocrine features. The controversy and uncertainty about its aetiology, has attracted numerous publications. It has been shown that most women diagnosed as PCOD had menstrual disorders and/or hirsutism, but there were patients who were non-hirsute and others who showed evidence of ovulation (Goldzieher & Green, 1962; Goldzieher & Axelrod, 1963; Yen, 1980).

Clinical Findings

None of the study patients had evidence of an androgen-secreting tumour, Cushing's syndrome, CAH or thyroid disease. All the women of the control group considered themselves to be normal with regular menstrual rhythm, and had not found it necessary to consult a doctor for menstrual disturbance, infertility or hirsutism.

Menstrual Disorders and Endocrinology

In this study, only 42.9 % of women with oligo/amenorrhoea (PCOD) had all the classical signs of the disorder as described by Stein & Leventhal (1935), namely oligomenorrhoea, hirsutism and obesity. Hirsutism prevalence was comparable to the findings reported in the literature for patients with PCO (Franks, 1989; Holte *et al* 1994c). However, the presence of oligomenorrhoea and DUB were more, and amenorrhoea was less prevalent in the patient group probably due to the inclusion criteria.

In the present study, the mean basal plasma E_2 concentration was similar in the patients and the controls, but significantly lower in patients with DUB than those without DUB ($P = 0.0228$). The effects of "unopposed" oestrogen (that is, without the "protective" effect of progesterone) on the uterus are thought to account for endometrial hyperplasia and DUB in anovulatory women with PCO. However, the uterus is larger and the endometrium is thicker than normal even in ovulating women with PCO (Adams *et al* 1988). Therefore, it is difficult to explain these findings on the basis of unopposed oestrogen (Franks, 1989). It has been reported that the concentrations of free, non-bound E_2 is elevated in women with PCO despite normal total E_2 concentrations (Lobo *et al* 1981; Lobo, 1988) because of either the androgen-induced decrease in SHBG and/or the inhibition of binding by the excess androgens (Lobo & Goebelsmann, 1982). However, Polson *et al.* (1987) were unable to find any significant increase in the absolute concentration of free E_2 in serum.

Hirsutism and Endocrinology

There were no differences in endocrine profiles between women with hirsutism and those without, coinciding with findings of other observers (Franks, 1989). Both groups had raised serum concentrations

of T and the mean concentrations were slightly higher in the hirsute than in the non-hirsute women; however, these differences did not reach statistical significance (hirsute : 2.44 ± 1.23 nmol/L; non-hirsute : 1.95 ± 0.73 nmol/L). In hirsute patients the correlation of the serum T with the degree of hirsutism is poor, a number of hirsute patients having normal levels of serum T (Franks, 1989). Some hirsute women with a normal serum T concentration have an increased T production rate and therefore an increased MCR. The higher incidence of hirsutism in the obese women may be explained by reduced SHBG causing increased free androgen in peripheral tissues in obese subjects (Franks, 1989).

Galactorrhoea and Hyperprolactinaemia

Although galactorrhoea was observed in 9.5% of the patients, hyperprolactinaemia was an infrequent observation (2.4%), compared to the 7% reported by Franks (1989). An interrelationship between hyperprolactinaemia and PCOD is complex. Some patients with PRL-secreting pituitary tumours have hirsutism as well as amenorrhoea (Franks, 1989). Women with hyperprolactinaemic amenorrhoea are known to be associated with the findings of PCOD (Yen, 1980). Elevation of PRL could be due to increased serum concentrations of E_1 or the unopposed action of oestrogen on the pituitary (McKenna, 1988) as well as increased free oestrogen (i.e. normal oestrogen with decreased SHBG). The insignificant observation of hyperprolactinaemia in this study could be due to the selection criteria but whatever the association between elevated PRL and PCO, PRL is not a major contributing factor in the patients investigated in this study.

Endocrine Findings

When radioimmunoassay became widely available in the early 1970's, a great reliance was put on the use of biochemical markers of "the syndrome" rather than the histological appearance. The major problem is that there is no universal agreement on the biochemical definition of PCOD (Franks, 1989).

LH

In this study, significantly higher LH concentrations were observed in the patients with oligo/amenorrhoea than in the control group ($P = 0.0012$). Twenty patients (47.6%) were found to have high basal LH levels greater than 1 SD above the mean of the normal control group (> 10.0 IU/L). Yen

(1980) reported that about 10% of women with PCOD had normal LH concentrations. An LH level of > 10.0 IU/L was acceptably specific to diagnose PCOD (Yen, 1980; Eden, 1988; Eden *et al* 1989c) with a sensitivity of 53% (Eden & Place, 1989). However, a higher cut-off point has been described in the literature - > 11.1 IU/L (Franks, 1989). If this was applied here, only 29.3% would satisfy this criterion which might question the exclusion of PCOD diagnosis based on a single LH concentration. The cut-off normal value applied depends very much on the assay used. Modern assays (monoclonal antibody assays) are more specific and give lower levels than those obtained with previous (polyclonal antibody assays) assays.

It has been reported that some women with all the other clinical and biochemical features of PCOD had normal serum LH concentrations (Givens *et al* 1976) at the time of sampling. It is therefore, important to sample patients in the early follicular phase to avoid the confusion of the LH surge (Eden *et al* 1989c). Furthermore, a high concentrations of LH during the follicular phase was found to have a dramatic effect on the success rate of induction of ovulation and the conception outcome and it may cause early pregnancy loss (Homburg *et al* 1988).

A weak but significant correlation ($r = 0.33$, $P = 0.036$) was found between LH and FSH. A similar observation was reported by Eden *et al.* (1988; 1989c). The LH:FSH ratio > 2 was found in less than half of the patients. This supports the statement that the LH:FSH ratio has a relatively poor ability to diagnose the PCOD.

FSH

The results in this study, showed that basal FSH concentrations were similar in patients and controls. This observation does not agree with those reported by Yen *et al.* (1970b) which showed higher values in normally ovulating women than in anovulatory women with PCOD during the first eight days of the follicular phase. Although it was suggested that subnormal FSH levels might contribute to anovulation in the PCOD, given the high effectiveness of exogenous FSH in inducing ovulation in these women (Yen, 1980; Franks, 1989), most studies have failed to confirm lower levels of FSH in women with PCOD than in controls (Duignan *et al* 1975; Dunaif *et al* 1988; Conway *et al* 1989; Franks, 1989). The presence of similar E_2 and FSH concentrations in patients and controls could mean that there is no impairment of FSH release and therefore FSH probably is not the main cause of anovulation in PCOD as suggested by Chang *et al.* (1982). The relationship between FSH concentration and the occurrence of spontaneous

ovulation in the group of patients with oligo/amenorrhoea, will be discussed in Chapter Six. Patients with oligomenorrhoea since menarche had lower FSH concentrations (P = 0.0451) than other oligomenorrhoea patients which could be related to the degree of chronicity of the disorder. However, the results in some studies may be affected by small samples or because the timing of the blood sampling in controls was not restricted to the early follicular phase.

Androgens and SHBG

The mean concentrations of T were significantly higher in the patients than the controls (P = 0.0132). Although the T concentration was higher in hirsute patients than those without hirsutism, this difference was not significant. This may emphasize the importance of measuring FT, expressed by FAI.

Testosterone concentrations of > 2.5 nmol/L (mean + 1SD of the study control subjects) were considered abnormal which agreed with other observers (Eden, 1988; Eden *et al* 1989c). Despite the raised mean serum T concentrations in women with PCO, 75.6% had values which were in the normal range (< 2.5 nmol/L) in comparison to half the patients reported by Franks (1989). If the patients were subdivided according to whether serum T concentrations were raised or normal, LH concentrations were higher in the hyperandrogenic group, although the difference was not statistically significant. On the contrary, slightly lower LH values in the hyperandrogenic group were reported (Franks, 1989), but these were still nearly twice as high as the mean LH levels in the controls. On the other hand, basal T concentrations were similar in the patients with high LH to those patients with normal LH concentrations (≤ 10.0 IU/L). In addition, serum T concentrations in the subgroup with normal LH levels were still significantly greater (P = 0.047) than in normal subjects. It seems that patients with PCOD show a disorder of androgen which is independent of elevated LH concentrations (Fleming *et al* 1985).

As is the case with LH measurements, a random sample of T is not necessarily an accurate reflection of androgen secretion but, in contrast to LH, this has less to do with episodic secretion of androgen than the fact that a random serum T measurement is an inadequate index of either androgen production by the ovary (or adrenal) or of the bioavailability of circulating androgen to target tissues (Franks, 1989).

In this study no significant correlation was found between T and SHBG; although the reverse has been reported (r = - 0.43) (Franks, 1989). Basal concentrations of SHBG were significantly lower in the patients than the controls (P = 0.0336) which agrees with other reports (Yen, 1980). However, in the population of women with PCO reported by Franks in 1989, the mean and median concentrations of

SHBG were not significantly different from those in normal women in the early to mid-follicular phase of the cycle. Sex hormone binding globulin is made by the liver and serum levels fall in the presence of excess androgen, especially T (Anderson, 1974; Carter *et al* 1983; Cunningham *et al* 1985). If levels of SHBG fall, T will be released and FT will increase. It has been suggested that low SHBG concentrations in the serum are a reflection of increased transport of the "biologically active" SHBG-androgen complex into the tissues but as yet there is no evidence to support this view.

The basal SHBG concentrations showed the most significant and consistent relationships with the anthropometric parameters in patients and controls, compared with other endocrine variables. Similar observations were found with the mean follicular SHBG concentrations (Chapter Six).

The activity of androgen in target tissues is determined by two main factors. The first is the androgen binding capacity in serum of SHBG (Anderson, 1974) and albumin. The second is further metabolism of T or A to more potent 5α-reduced products.

In this study, FAI was the most sensitive biochemical criterion for the diagnosis of PCOD. An abnormal FAI was considered if FAI was > 4.5 (mean + 1SD of the control data). A high follicular level of FAI > 4.5 was observed in 53.7% of the patients. The FAI is useful for detecting the PCOD in oligomenorrhoeic women as there appear to be few false positives. A follicular phase FAI > 4.5 should be considered as highly suspicious of PCOD (Eden, 1988; Eden *et al* 1988a; Eden *et al* 1988b). Although an early follicular phase estimation of T and SHBG, to allow the calculation of FAI, has been recommended in the biochemical investigation of the oligomenorrhoeic woman (Eden, 1988) this is difficult to apply in clinical practice for patients with oligo/amenorrhoea. Adding SHBG and FAI to LH, FSH and T gives a better chance of detecting PCOD.

Here, similar basal DHEA-S concentrations were observed in the patients and the controls. There has been a debate over the years as to whether the source of excess androgen secretion in women with PCOD is the ovary or the adrenal. A proportion of subjects with PCOD was found to have elevated concentrations of DHEA-S (Yen, 1980), and the high prevalence of PCOs in subjects with CAH due to 21OH deficiency (Hague *et al* 1990) together with the occurrence of "PCO-like" features in women with Cushing's syndrome suggests that there is a link between hypersecretion of adrenal androgens and PCOD. However, studies of the family data suggested that the adrenal production defect could be unrelated to the causes producing PCO changes in the majority of cases (Hague *et al* 1989; Hague *et al* 1990).

Ultrasonographic Findings

The availability of the high-resolution US scanning, especially with a transvaginal transducer, offered a non-invasive method of defining the typical polycystic morphology of ovaries in women with reproductive disturbances (Swanson *et al* 1981; Parisi *et al* 1984; Adams *et al* 1985; Adams *et al* 1986b).

In this study, PCOs were identified in all the patients with oligomenorrhoea and 71% of patients with amenorrhoea. Adams *et al.* (1986b) were able to identify PCO in 85% of women with oligomenorrhoea and in 26% of the women with amenorrhoea.

The mean ovarian volume of patients diagnosed to have PCO was 9.57 cm^3; small compared to the 11.6 cm^3 reported by Franks (1989) and the 15.1 cm^3 reported by Holte *et al.* (1994c). This could be explained by the difficulty in identifying the follicular phase, hence, variations in timing of the scanning in oligo/amenorrhoeic patients. The ovaries can be normal in volume and still polycystic (Givens *et al* 1976).

There were no differences in US and biochemical indices between women with hirsutism (androgenized) and those without. Women with oligomenorrhoea had a larger mean ovarian volume than those with amenorrhoea, however, this difference was not significant. AbdelGadir *et al.* (1992b) concluded that ovarian size did not indicate the severity of the condition. On the contrary, patients with the most enlarged ovaries were reported to have the most frequent occurrence of amenorrhoea (Puzigaca *et al* 1991).

Although US demonstration of bilateral, symmetrically enlarged, globular shaped ovaries has been described as a criterion for diagnosis of PCOD (Parisi *et al* 1984), such evidence was, practically, not possible to obtain in this study. A wide spectrum of ovarian sizes was seen in patients with PCOD. The TOV showed a wide variation within study patients (1.9 - 40.5 cm^3). Yeh *et al.* (1987) found normal sized ovaries present in PCOD patients. In a considerable number of patients in the present study (82.9%), at least one ovary was normal in size, and both ovaries were normal in size in 36.6% . This observation was higher than that reported by Hann *et al.* (1984) and by Yeh *et al.* (1987) - 29% . The upper limit of normal ovarian volume was reported to be 7.5 cm^3, with a mean of 5.2 ± 1.9 cm^3 , and gross asymmetry in size, that is one smaller than 50% of the other, was found in 20.9% in one series (Yeh *et al* 1987). A higher incidence was found in the present study, (24.4%). This observation shows the dynamic nature of the ovaries, even in relatively stable conditions such as oligo/amenorrhoea.

However, the differences in observations may be due to differences in criteria for selection of patients and/or in the ultrasound technology. These observations will be discussed further in Chapter Six.

Enlargement of the ovaries in PCOD is due to a diffuse process, and the ovaries maintain their original shape when they enlarge, indicating that the shape or the RI of the ovaries is of no value in diagnosing PCOD (Yeh *et al* 1987). Because of the considerable variations in the ovarian sizes, the large sized ovary is neither a sensitive nor a specific criterion for the US diagnosis of PCOD. However, the majority of ovaries (95.9%) can be seen by means of a real - time scanner (Yeh *et al* 1987).

The frequency of visualization of follicles (2 - 8 mm) was high in PCOD patients using a real - time scanner with high frequency and a vaginal probe. In the present study, follicles (≥ 10) were seen in 95.2% of PCOs, with almost equal distribution in the stroma of the ovary (peripheral or central). Yeh *et al.* (1987) reported that the presence of multiple small cysts (≤ 8 mm in size) is the most important US criterion for the diagnosis of PCOD - similar to the developing follicles of healthy individuals but increased in number. Transvaginal US is preferred for visualization of the ovarian details, especially in obese patients. The study of Adams *et al.* (1986b) utilizing US had shown that PCOs are common in oligomenorrhoeic women without hirsutism or obesity.

Thick stroma was identified in all the patients with oligomenorrhoea and in 71.4% of the patients with amenorrhoea. Therefore, thick stroma was the most sensitive single US criterion to diagnose PCOs. An increase in the ovarian stroma was added to the US criteria. Increased ovarian stroma has been reported in the literature as the most sensitive and specific US sign of PCOD (Ardaens *et al* 1991).

The mean basal ovarian volume in the "high T" group was similar to that in the "normal T"group. This finding is in contrast to previous observations that androgen production in women with PCO can be related to ovarian volume (Puzigaca *et al* 1991).

The prevalence of a unilateral PCO was higher (20%) than reported in the literature (3.7%, (Rojanasakul *et al* 1989)). The group of patients who had a unilateral PCO were observed to have a significantly smaller mean basal ovarian volume, TOV and a lesser number of small follicles but follicular distribution was not different from those with bilateral PCO ovaries. The clinical presentation, age, BMI, WHR and the basal endocrine profiles were similar in both groups. It may be argued that bilateral US PCO change is not necessary to have the typical clinical and endocrine criteria of PCOD. These findings are confirmed with the longitudinal observations in Chapter Six. However, whether patients with a unilateral PCO would have a mild degree of the disease, have more prevalence of spontaneous ovulation and contribute to the present confusion of understanding PCOD remain to be studied. Long-term follow-

up may be necessary to determine whether a patient with a unilateral PCO will develop bilateral disease in the future.

Role of Obesity

Obesity is common in the study patients (40.5%). A higher mean BMI was recorded for both the patients and controls (28.09, 26.64 Kg/m^2 ; respectively) compared to the means reported by Franks (1989) (24.2 Kg/m^2 for patients and 22.0 Kg/m^2 for controls) and by Holte *et al.* (1994c) (25.7 Kg/m^2 for patients and 25.1 Kg/m^2 for controls). Body mass index had no significant influence on hirsutism, menstrual pattern or ovarian volume, similar to findings reported in the literature (Holte *et al* 1994c). Although there was no statistically significant difference in BMI between patients and controls, the difference in WHR was highly significant (P = 0.0023); this is in contrast to the observations of Holte *et al.* (1994c).

Patients with LH > 10.0 IU/L were found to be less obese, with slightly larger ovarian volume but these differences did not reach statistical significance, similar to the findings reported by Franks (1989). The differences in LH concentrations were significant when comparing patients to controls for all the BMI range (see Table 4 - IXa and IXb), although more noticeable if the cut-off point of BMI was taken as ≥ 29. Therefore, LH levels may be more related to PCOD than to BMI. Similar observations have been reported by others (Laatikainen *et al* 1983; Paradisi *et al* 1986; Anttila *et al* 1991; Dale *et al* 1992b). Holte *et al.* (1994c) showed a negative association between LH and obesity in both patients and normal control groups; an effect of obesity, independent of PCOD. Follicle stimulating hormone was significantly lower in obese patients than in the non-obese (P = 0.024) but the correlation between FSH and BMI was weak (r = 0.27, P = NS). It is reported in the literature that although the mean serum FSH in obese women was not significantly lower than that in non-obese subjects there was a weak but significant inverse correlation between FSH and BMI in the obese but not in non-obese women (Franks, 1989). This may explain the higher incidence of anovulation in the obese subjects. A clinical implication of these findings is that the diagnostic value of LH levels or the LH:FSH ratio for PCOD could actually be higher than has been recently proposed if obese women with PCOD are carefully compared with BMI-matched controls rather than with normal non-obese women.

Obesity is associated with a rise in T and a fall in SHBG (Bates & Whitworth, 1982; Cunningham *et al* 1985), therefore; the presence of gross obesity may affect the interpretation of a high FAI (Eden, 1988; Eden *et al* 1988a; Eden *et al* 1988b). There was no significant correlation of T with SHBG; although the

reverse has been reported (r = - 0.43) (Franks, 1989), but the inverse correlation between SHBG and BMI was clear (r = -0.36) in the present study. Obese normal women had low levels of SHBG compared with lean individuals whereas the PCO women of normal BMI had normal SHBG concentrations despite significantly elevated serum T levels. Therefore, BMI is the most important factor affecting SHBG concentrations. These results confirmed the previously observed negative effects of obesity and PCOD on SHBG levels.

Obese women were reported to be more hirsute than those of normal weight (73% vs 56%), and more likely to have menstrual disturbances than lean subjects with PCOD (Goldzieher & Green, 1962; Goldzieher & Axelrod, 1963; Dunaif *et al* 1988). Similar observations of hirsutism, with BMI ≥ 25, were found in this study (21% vs 43 %), as well as menstrual irregularity. However; these observations were absent when BMI ≥ 29 is used. Despite the tendency for the obese women to be more hirsute and have more cycle disturbance than non-obese PCO patients, there was no significant difference between the groups in Gn, T or A concentrations. These observations confirmed previous reports (Dunaif *et al* 1988; Franks, 1989).

CONCLUSION

All patients recruited had menstrual irregularity (oligo/amenorrhoea with or without DUB) and infertility. Patients' presentation was biased since their referral to the outpatient clinic was due to either a menstrual problem or infertility or both. About half of the patients were either obese or hyperandrogenized.

Women with PCOD had higher serum concentrations of LH (double the control value), LH:FSH, T and FAI, lower SHBG, and similar FSH, E_2, 17∝-OHP, DHEA-S, PRL and thyroid function tests to those of the controls. The same findings were reported by Holte *et al.* (1994c), except for the lower FSH levels and the latter finding higher values for DHEA-S.

Regarding the biochemical diagnosis - LH:FSH ratio > 2:1 was diagnosed in only 20 patients (48.8%). The presence of high LH concentration (> 10.0 IU/L) was found in < 50% of the study patients, and the presence of a correlation between LH and FSH weaken the sensitivity of LH:FSH as a diagnostic criterion of PCOD. Elevated T concentrations (> 2.5 nmol/l) was a finding in less than 1/3rd of the patients (24.4%) while FAI of > 4.5 was found in 53.7% of the patients. The accuracy of the diagnosis is

increased when more than one criterion is applied. However, 26.2% of the whole group of patients were found to satisfy all of the biochemical diagnostic criteria.

Sex hormone binding globulin showed the most significant and consistent relationships with the anthropometric parameters in patients and controls, compared with the other endocrine variables.

On US examination, a unilateral PCO was diagnosed in eight patients (20.0%). Thick stroma, the most sensitive single criterion, was found in at least one ovary in 95.2% of the patients. However, ovarian volume > 9.0 cm^3, of either ovary, was found in only 55.0%, the least sensitive ultrasonographic parameter. Both ovaries showed more than 10 follicles in one plane in 85.7% of patients. However, combination of more than one US criterion increases its diagnostic value. The TOV showed a wide variation with patients and that the ovary is not static, even in the relatively stable conditions of oligo/amenorrhoea.

The intra-individual variation in biochemical markers (Franks, 1989) questions the selection of controls based entirely on the absence of PCO-like symptoms and biochemical markers. This is particularly the case in obese women, as obesity is common in PCOD (40.5%) and the prevalence of PCOD in obese women can be expected to be high, given the high prevalence of PCO in the general female population (Polson *et al* 1988).

In the present study, subjects with PCOD, diagnosed on US scan, had significantly higher BMI, LH concentrations, LH:FSH ratio, T concentrations and FAI and lower SHBG levels than did the controls. However, it has been reported that oligo/amenorrhoeic subjects with normal ovaries had significantly lower BMI, T and FAI than did those with regular cycles (Eden, 1988; Eden *et al* 1988a; Eden *et al* 1988b).

CHAPTER FIVE : INSULIN SENSITIVITY

5.1 INTRODUCTION

Insulin resistance is a metabolic state in which physiological concentrations of I produce a less than normal biological response. The fasting plasma glucose concentration depends primarily on the rate of hepatic glucose release, which in turn is regulated by the I concentration (Caro, 1991; Dunaif, 1992a). The initial step in I action is binding of the hormone to cell surface receptors which causes activation of tyrosine-specific protein kinase activity. The receptor itself is phosphorylated. Subsequent events include generation of intracellular signals, modulation of enzyme activities, and importantly, stimulation of the ability of cells to take up and use glucose. Disturbance at any of these sites could result in IR (Harrison *et al* 1976; Ciaraldi *et al* 1992; Dunaif, 1992a). Therefore, IR may be due to : decreased number or defective function of I receptors (type-A), I receptor antibody (type-B), or post-receptor defects in I expression (type-C) (Moller & Flier, 1991; Dunaif, 1992a). Normal glucose concentration can be maintained if I-resistant persons are able to maintain a state of chronic hyperinsulinaemia (Dunaif *et al* 1990; Moller & Flier, 1991). Insulin resistance will result in decreased I mediated glucose uptake into peripheral tissue and increased glucose release from liver, defects that are partially compensated by increased I secretion. Since a partial compensatory response to IR is hyperinsulinaemia and because IR may not be equally manifested in all I sensitive tissues, there may be a relative increase in I action in some tissues (Caro, 1991). The evidence has appeared to suggest that although hyperinsulinaemia may prevent NIDDM from developing, the cost of this is substantial. Specifically, it has been proposed that resistance to I-stimulated glucose uptake and hyperinsulinaemia may play a central role in the cause and clinical course of several diseases, including high blood pressure, abnormal lipoprotein metabolism and CHD.

Elevated I and/or C-peptide concentrations have been associated with increased risk of development of NIDDM in most populations. An increased fasting proinsulin/I ratio, which may be a marker for early β-cell dysfunction, has been associated with increased development of NIDDM (Haffner, 1996). The plasma FI concentration is largely determined by the glucose concentration and the basal hyperglycaemia in diabetes appears to arise from the feedback loop between the liver and β-cells, thereby maintaining an effective I action in the liver and at the periphery. The degree of basal hyperglycaemia is therefore determined by a combination of β-cell deficiency and IR (Matthews *et al* 1985).

Hyperinsulinaemia, secondary to a poorly characterized disorder of I action, is a feature of PCOD (Chang *et al* 1983; Pasquali *et al* 1983; Dunaif *et al* 1989; Dunaif *et al* 1990; Rajkhowa *et al* 1994; Harrington & Balen, 1996) and patients with PCOD are at risk to develop NIDDM (Dunaif *et al* 1990). In the normal person there is a 14-minute cycle of plasma I secretion known to occur. Although many factors have an influence on the basal concentrations of FI and glucose, there is a major role for the simple interaction of plasma glucose and I between the liver, β-cells and periphery (Matthews *et al* 1985). The cellular mechanisms that account for target tissue IR in PCOD are unknown. Insulin resistance in PCOD involves a marked defect in glucose transport sensitivity without significant alterations in receptor dynamics. This unique feature distinguishes it from IR in obesity and NIDDM where both receptor and post-receptor defects are involved (Ciaraldi *et al* 1992). Obesity can also have an impact on I action (Ciaraldi *et al* 1992). It has also been suggested that androgens modulate IS in PCOD (Bruno *et al* 1985). Studies have shown that chronic hyperinsulinaemia may be responsible for the hyperandrogenism in PCO (Shoupe *et al* 1983; Buyalos *et al* 1993; Elkind-Hisch *et al* 1993; Rajkhowa *et al* 1994), although the mechanism(s) is unclear. Endogenous opiates are at least partially responsible for the hyperinsulinaemia and IR in PCOD (Givens *et al* 1987; Laatikainen *et al* 1990); although their role in the pathogenesis of PCOD is unclear.

This chapter sets out to explore the relationships between IS and disturbances of ovarian function seen in patients with oligo/amenorrhoea. After consultation, the method selected to estimate IS was the " Short Insulin Tolerance Test " (SITT), in which the sensitivity to I administration is estimated over a 15 minute period by calculating the rate of decline in glucose concentrations. Fasting I (and glucose) measurements were taken as the initial part of the test, so simpler estimates (FI and FIRI) may also be used (Cleland *et al* 1996).

There are well established relationships between anthropometric variables and IS, so these can be used to compare the results of laboratory estimates of IS.

Basal metabolic variables of the study groups were recorded as were the changes which occurred during the SITT, i.e. over the 60 minutes following the administration of I. The mean of 3 samples at 15 minutes, 5 minutes and 0 minute prior to the I injection was calculated and used as the basal value for glucose (Chapter Three).

The SITT was carried out on two occasions in each subject. In the patients, the first was at least 15 days after the last menstrual period and in the controls the first was between cycle days 5-8. The test was repeated in the luteal phase in the controls and in those patients who ovulated, whilst in patients who did

not ovulate the test was repeated five weeks after the first test. The possible influence of the luteal phase was studied and the variations in the tests of IS were examined. Correlations between the SITT values (mean of 2 tests) and the anthropometric variables were effected. Failure to confirm the well-established relationships between IS and the anthropometric variables as well as the practical problems of the SITT are discussed under 5.4.3. The alternative methods of estimating IS : - FI and FIRI- were examined, and the relationships between these and the anthropometric variables were compared. These estimates were used to examine the relationships between IS and ovarian activity, including follicular growth and luteinization, and hyperandrogenism.

5.2 BASAL METABOLIC VARIABLES (PATIENTS AND CONTROLS)

The fasting basal serum levels of the metabolic variables measured before the first SITT test in the patients in comparison to the control group, are shown in Table 5-I.

Table 5-I : Basal Metabolic Variables observed at the first SITT for the Patients and the Weight and Age-matched Control group

	Patients	Controls	P
n	34	19	
Fasting glucose (mmol/L)	5.13 ± 0.49	5.09 ± 0.58	NS
FI (mIU/L)	12.47 ± 9.93	7.53 ± 5.62	0.057
C-peptide (ng/mL)	1.21 ± 0.66	1.04 ± 0.69	NS
IGF-I (nmol/L)	13.24 + 5.51	33.08 ± 14.24	< 0.0001
GH (ng/mL)*	2.26 ± 4.45	2.48 ± 3.17	NS
Cortisol (ug/dL)	14.29 ± 6.56	15.50 ± 4.53	NS
PRL (IU/L) *	291.1 ± 240.0	436.2 ± 532.0	NS

Groups were compared using Student's t-test, except for values with (*) where Mann-Whitney nonparametric test was used for comparison. NS means P ≥ 0.05. Number of patients was 34, except IGF-I was 18.

The fasting glucose and I estimations were taken at 08:00 hour after an overnight fast. The glucose concentration represented the mean of 3 values for each individual prior to the administration of I.

The cut-off accepted value for normal FI in this study, derived from the control group data, was 13 mIU/L (mean ± 1SD of own control group), which agrees with the findings of other observers (Dunaif *et al* 1989; Buyalos *et al* 1991; Nestler *et al* 1991; Akinmokun *et al* 1992; Dale *et al* 1992b).

All individuals had normal fasting blood glucose concentrations, with the highest values of 6.6 mmol/L for patients and 6.8 mmol/L for controls and the mean values were similar in both groups. Fourteen of the 34 patients (41.2%), and 5 out of 19 controls (26.3%) had FI concentrations significantly higher than the normal cut-off value. This distribution would explain the borderline significance seen when comparing the two groups (P = 0.057) (Table 5-I).

There was a significant difference in the fasting level of IGF-I between the two groups (Table 5-I). This agrees with observations from Barreca *et al.* (1996) who found lower IGF-I levels in follicular fluid of patients with PCOD than in normally ovulating women. Slowinska-Srzednicka *et al.* (1992) reported a similar observation in serum, but the difference between their patients and controls was not significant.

The mean basal GH appeared to be slightly lower, and the fasting C-peptide was slightly higher in the patients than the control group but these differences were not significant. However, the actual GH levels were unclear in both groups, since many (33.3%) of the samples were below the lower limit of assay sensitivity (0.2 ng/mL). Fasting serum cortisol and PRL levels were similar in patients and controls.

Correlations of the Basal Concentrations of the Metabolic Variables and the Reproductive Hormones

1. Fasting Insulin

There was no significant correlation between the basal FI and E_2, LH, FSH, LH:FSH, T, 17α-OHP, A or DHEA-S in either patients or the control group. However, basal FI was negatively and significantly correlated with SHBG in both patients and controls, ($r = -0.45$, $P < 0.0001$; $r = -0.65$, $P = 0.0025$; respectively) (Figure 5.1). Although there was no correlation with T, basal FI levels were positively correlated with FAI in both patients and controls, ($r = 0.50$, $P < 0.0001$; $r = 0.47$, $P = 0.041$; respectively) (Figure 5.2).

Figure 5.1 :

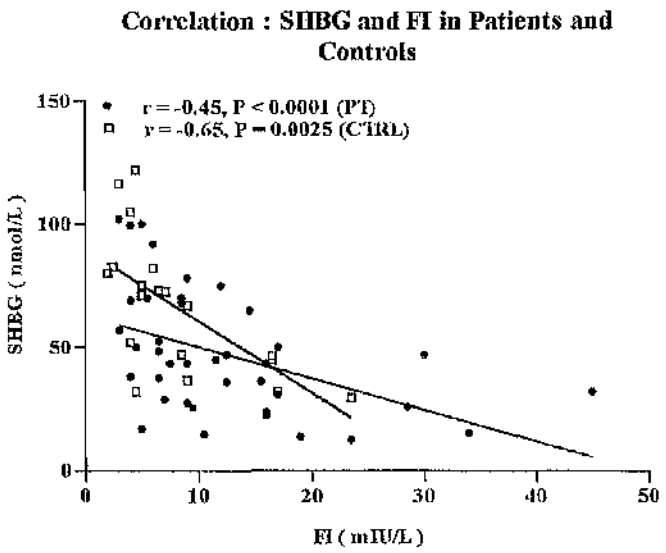
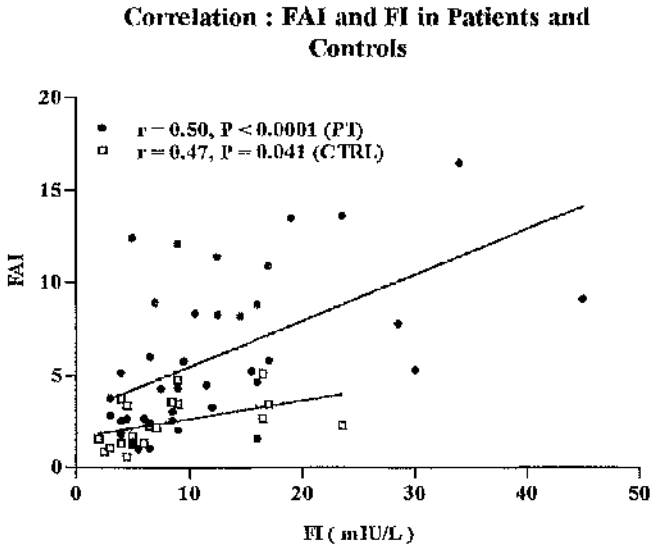
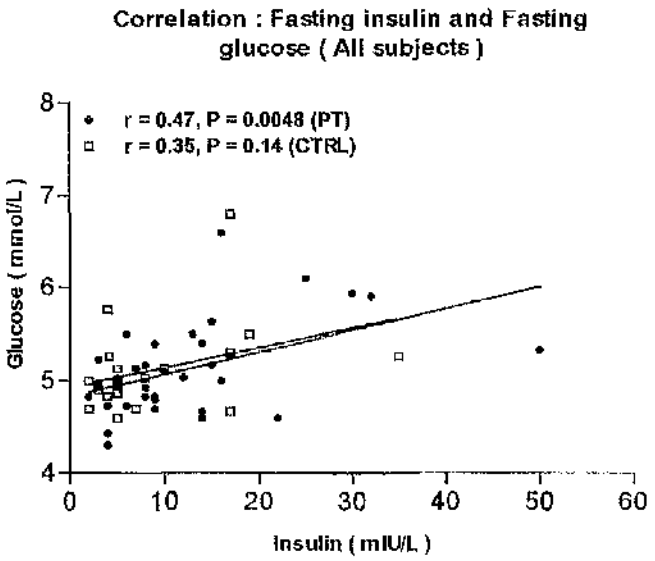


Figure 5.2 :



In the patient group, basal FI was significantly correlated with the basal fasting glucose concentration (r = 0.47, P = 0.0048), whereas for the controls there was no correlation (r = 0.35, P = 0.14) (Figure 5.3). There was no correlation between FI and IGF-I, GH, cortisol or PRL in either group of women.

Figure 5.3 :



2. Fasting Glucose

Basal fasting glucose was not correlated with basal E_2, LH, FSH, LH:FSH, T, SHBG, FAI, 17α-OHP, DHEA-S, A, PRL, cortisol, GH, IGF-I or C-peptide in either group of the study.

3. Growth Hormone and IGF-I

No significant relationship was found between basal GH levels and these of any of the basal E_2, LH, FSH, LH:FSH, T, SHBG, FAI, A, 17α-OHP, cortisol, fasting glucose, FI, IGF-I or C-peptide in either the patients or the controls.

Basal IGF-I values in the patients were not correlated with E_2, LH, FSH, LH:FSH, T, SHBG, A, 17α-OHP, cortisol, fasting glucose or C-peptide. However, FAI demonstrated a positive, although weak, correlation with IGF-I values ($r = 0.46$, $P = 0.0465$). In the controls, there was no correlation between IGF-I values and any of the other variables measured.

5.3 INSULIN SENSITIVITY : METHODS USED TO ASSESS INSULIN SENSITIVITY IN THIS STUDY

Since the description by Himsworth in 1936 that human disease could be associated with reduced sensitivity to I (IR), several methods have been developed for detection and quantification of IR (Caro, 1991). Insulin resistance is diagnosed when normal glucose levels exist in the presence of hyperinsulinaemia (Burghen *et al* 1980), and generally derives from abnormal metabolic activity in peripheral tissues, while hyperinsulinaemia results not only from increased secretion of I by β-cells, but also from impairment of receptor-mediated clearance of I by resistant peripheral target cells. The clinical consequences of IR result from either deficient (due to reduced uptake) or excessive (due to high circulating levels) I action. An elevated FI is a common feature of IR. The GTT, euglycaemic I-clamp technique, the minimal model technique and SITT are all provocation methods which have been used to quantify IS. Other tests include measurement of IS *in-vitro* in cell cultures and tissue samples. However, most of these tests are complicated to perform on a large series of patients and there has been much debate about the relative merits and limitations of each of them.

METHODS FOR ESTIMATING INSULIN SENSITIVITY

Total body IS can be assessed via the intravenous GTT, or minimal model, where an intravenous glucose injection is followed by frequent determinations of plasma glucose and I, and the results of this correlate well with the euglycaemic clamp technique (Dunaif, 1992a). The test requires less staffing and could be used in a highly specialized clinical setting (Caro, 1991). Although the euglycaemic insulin-clamp technique is the most widely accepted standard method for estimating IR against which other methods are compared, it is expensive, time-consuming, requires specialized equipment and highly trained staffing, and the feedback control of glucose levels, all of which make it a laboratory investigative procedure rather than suitable for use in a clinical setting (Caro, 1991). These disadvantages demonstrate the need for an acceptable method to measure IS that could be used in large scale studies or even clinically.

One possible alternative is the glucose/I infusion test which correlates well with the euglycaemic clamp but is costly in time (3 hours).

Another alternative to the euglycaemic clamp, the SITT is a short useful alternative (Akinmokun *et al* 1992; Hirst *et al* 1993). Results suggest that the SITT is a suitable method of assessing IS and particularly useful for large-scale studies, although the requirement for arterial blood adds a measure of complexity (Matthews *et al* 1985; Akinmokun *et al* 1992; Holte *et al* 1994a). The SITT is simple and rapid to perform. The method is used increasingly in clinical research because studies have shown that the rate of fall of blood glucose during the SITT, the KITT-value, significantly correlated with the euglycaemic clamp (Akinmokun *et al* 1992). Another advantage of the SITT is that the test is safe, reproducible and could be used to measure IR in large-scale epidemiological studies (Akinmokun *et al* 1992; Hirst *et al* 1993).

The degree of IR and deficient β-cell function can be assessed from a patient's FI and glucose concentrations (Matthews *et al* 1985). Fasting plasma GLU/INS ratio may provide the simplest estimate of IR. The higher the plasma FI concentration for a given fasting plasma glucose, the more IR an individual is. It is technically simple and inexpensive, and the availability of more accurate I assay methods has helped to overcome its limitations. Fasting I and FIRI are simple and reproducible measures of IS (Cleland *et al* 1996).

The literature shows conflicting reports about the correlation between hyperinsulinaemia and ovarian hyperandrogenism in PCOD. Some growth factors, such as IGF-I, may have an important role in modulating the ovarian response. Among hormones known to cause or predispose to IR are GH, cortisol and PRL. The SITT test was the method used in this study to investigate such correlations.

The aim was to use the SITT to investigate IR in patients with oligo/amenorrhoea (PCOD) and controls by reference to anthropometric criteria and also aspects of ovarian activity. Simpler methods of IR assessment were also available and used to compare with the results from the SITT.

5.4 THE SHORT INSULIN TOLERANCE TEST (SITT)

5.4.1 CORRELATION OF " SITT " VALUES WITHIN SUBJECTS

The mean fasting hormonal and metabolic variables at the first and the repeat SITT, of the patients and the controls are shown in Table 5-II.

Mean fasting blood glucose concentrations were similar in the first and second SITT, and FI concentrations were almost identical on the two occasions of testing for both patients and the controls.

There was no significant difference in the GH, IGF-I or C-peptide in the t_0 sample in the first and second SITTs in either group. It should be emphasized that IGF-I values, on both occasions were significantly higher in the control group than in the patients ($P < 0.0001$). Fasting GH values were almost half the levels of the controls ($P < 0.0001$), when the repeat SITT was effected few weeks later.

Table 5-II : Metabolic Variables in the Basal State during SITT's for the Patients and the Control group

	Patients			Controls			
	1st SITT	2nd SITT	P	1st SITT	2nd SITT	P	P**
FI (mIU/L)	12.47± 9.93	12.09± 9.17	NS	7.53±5.62*	8.65±6.69*	NS	NS
Fasting glucose (mmol/L)	5.13± 0.49	4.98± 0.52	NS	5.09± 0.58	5.11± 0.44	NS	NS
C-peptide(ng/mL)	1.21±0.66	1.21± 0.88	NS	1.04± 0.69	1.10± 0.59	NS	NS
GH (ug/mL)	2.26±4.45*	1.06±2.77*	NS	2.48±3.17*	2.19± 2.86*	NS	<0.0001
IGF -1 (nmol/L)	13.24±5.51	11.88±5.58	NS	33.08±14.24	29.83±11.17	NS	<0.0001
Cortisol (ug/dL)	14.29± 6.56	12.67±6.53	NS	15.50± 4.53	12.18± 4.48	0.0365	NS
PRL (IU/L)	291.1± 240*	294.6± 277*	NS	436.2± 532*	237.5±119.5	NS	NS
KITT-value (%/min)	4.14 ± 2.15	4.24 ± 2.36	NS	4.91± 3.07	4.18± 1.46	NS	NS

Groups were compared using Student's t-test, except for values with (*) where Mann-Whitney nonparametric test was used for comparison. (P**), comparing patients with controls, was calculated using analysis of variance where $P \geq 0.05$ is NS.

There was a close correlation between FI level at t_0 before the first and the repeat SITT in both the patients and the control group ($r = 0.86$, $P < 0.0001$; $r = 0.78$, $P = 0.0001$; respectively) (Figure 5.4). Similarly, a significant correlation was found between the t_0 GLU/INS ratio during the first and the repeat SITT in the patients, ($r = 0.80$; $P < 0.0001$). It was not significant, however, for the controls ($r = 0.33$; $P = 0.19$) (Figure 5.5).

Figure 5.4 :

Correlation : FI during SITTs in Patients and Controls

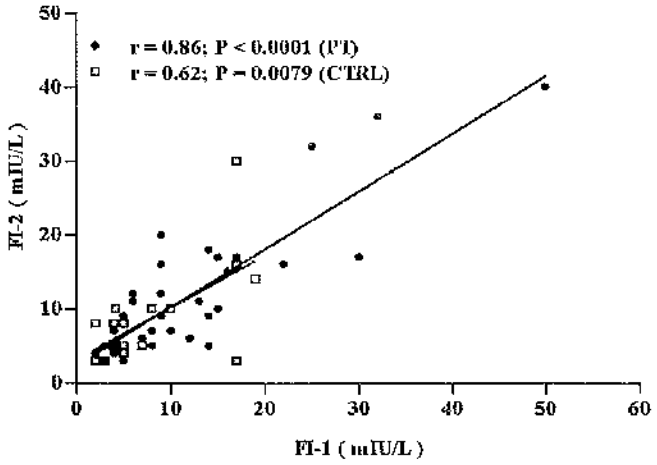
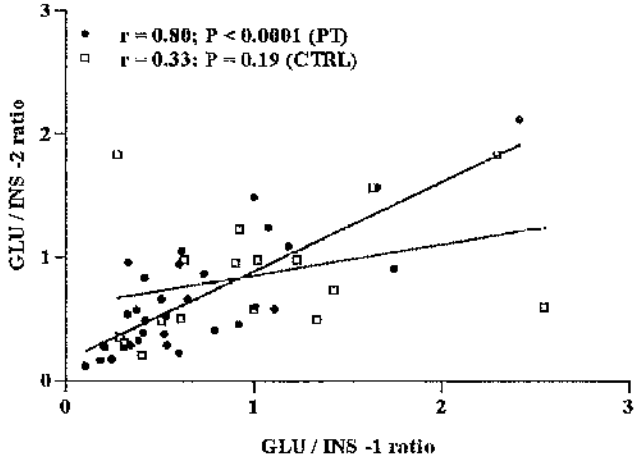


Figure 5.5 :

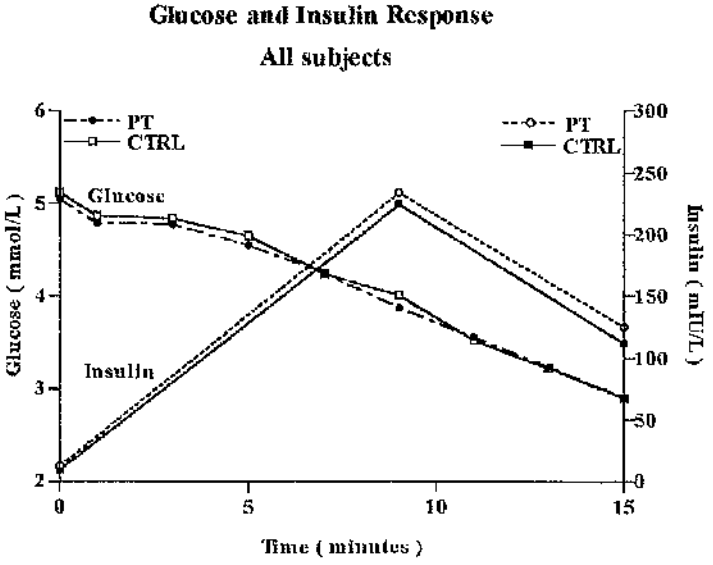
Correlation : GLU/INS ratio during SITTs in Patients and Controls



The glucose and I responses during the SITTs are depicted in Figure 5.6. For the purposes of the graph, I concentration should start at t_0, i.e. assume $t_0 = t - 15$. Following the intravenous injection of I, blood glucose levels started to fall from the third minute in both groups.

An index of IS, assessed using the rate constant KITT, was derived from a semilogarithmic plot of the decay in blood glucose values between t_3 and t_{15} (least squares analysis of concentration gradient) after the injection of I (dose = 0.1 IU/Kg). The KITT-value, was therefore calculated from the slope of glucose disappearance between 3 - 15 min, using arterialised blood samples. The mean KITT-value of 4.55 ± 2.27 in the control group appeared to be similar to those published for control populations by Hirst *et al.* (1993) and by Akinmokun *et al.* (1992). However, the high standard deviation (50%) may indicate an inherent weakness in the reliability of the test.

Figure 5.6 :



Following intravenous injection of I, the mean blood glucose concentration, fell from 5.08 (t_0 - sample) to 2.91 mmol/L (t_{15} - sample) for patients, and from 5.14 to 2.90 mmol/L for controls, while the mean plasma I concentrations rose to supraphysiological levels at 9-minutes and declined by 15-minutes (Table 5-III). Circulating I levels at 9-minutes were similar in the two groups but showed wide variations (SD) (Table 5-III).

Table 5-III : Mean glucose and I responses of patients and controls to injected I during the SITT

	Glucose (mmol/L)			I (mIU/L)		
Time	Patients	Controls	P	Patients	Controls	P
0 - samples	5.08 ± 0.45	5.09 ± 0.46	NS	12.34± 9.149	8.09 ± 5.53	0.071
1 min-samples	4.79 ± 0.42	4.87 ± 0.42	NS			
3 min-samples	4.78 ± 0.41	4.84 ± 0.44	NS			
9 min-samples	3.87 ± 0.53	4.01 ± 0.45	NS	234.1±125.2	205.8±80.18	NS
15min-samples	2.91 ± 0.72	2.90 ± 0.68	NS	125.3±180.0	112.3± 93.40	NS

The KITT-values (KITT-1 and KITT-2), for the individual patients as well as for the controls in both SITT tests are listed in Table 5-IV.

Table 5-IV : The KITT-Values in the duplicate tests in Patients and Controls

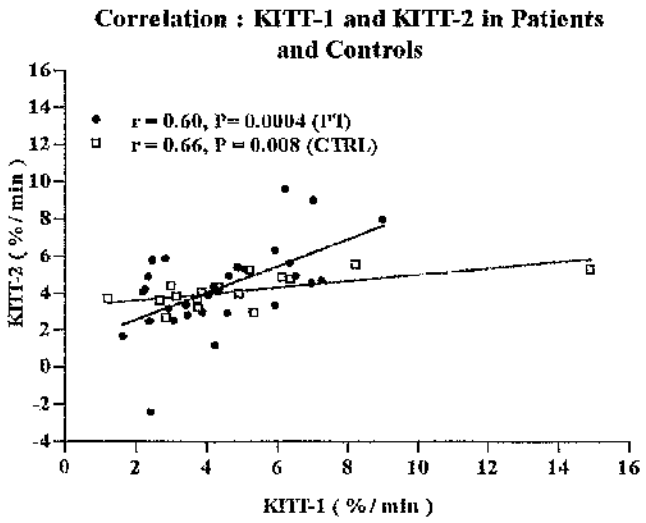
Patients				Controls	
KITT-1	KITT-2	KITT-1	KITT-2	KITT-1	KITT-2
2.36	4.89	2.93	3.15	3.14	3.85
2.43	2.42	1.58	1.58	4.33	4.33
4.24	1.18	8.99	7.97	3.00	4.39
5.07	5.30	4.82	1.63	1.21	3.71
3.42	3.34	2.39	2.48	2.85	2.65
4.21	4.36	4.07	3.92	5.21	5.28
4.91	5.41	6.35	5.64	14.89	5.33
2.27	4.20	6.52	4.92	3.86	4.05
7.02	8.99	5.93	3.35	2.68	3.60
3.89	2.94	4.64	4.96	6.52	6.25
6.24	9.60	4.58	2.90	5.34	2.94
2.84	5.09	6.23	6.23	3.99	3.99
2.20	4.04	6.96	4.53	3.22	3.76
2.76	2.76	7.26	4.67	3.74	3.66
3.07	2.51	2.47	5.79	6.37	4.74
4.87	5.43			3.66	3.66
0.40	0.40			6.13	4.86
5.93	6.34			3.98	4.91
3.46	2.79			5.56	8.22

In the patients, the mean KITT-value during the first SITT (KITT-1), and of the repeat SITT were 4.14 % / min, and 4.24 % / min, respectively.

There was a significant correlation between the KITT-1 and the KITT-2 values in both the patient group and the controls, (r = 0.60, P = 0.0004; r = 0.66, P = 0.008; respectively) (Figure 5.7). This shows that the SITT yielded some level of reproducibility within patients when the analyses were effected few weeks

apart in the patients and after 2 weeks in the controls. However, the correlations indicated that the test was less reproducible within patients or controls than the FI values shown above (KITT: r = 0.60 and 0.66, FI: r = 0.86 and 0.78, in patients and controls; respectively). This low level of correlation between KITT-1 and KITT-2 within patients is also lower than that reported for SITT when compared to the euglycaemic hyperinsulinaemic clamp (r = 0.86 in normal and 0.81 in diabetic subjects) (Akinmokun *et al* 1992).

Figure 5.7 :



Follicular " SITT " vs Luteal " SITT "

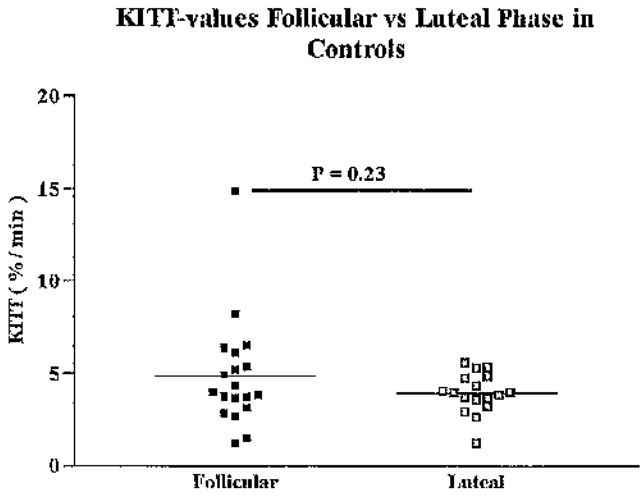
When controls were assessed in follicular and luteal phases, no significant difference was observed in the metabolic variables, except for cortisol which was significantly higher in the follicular than the luteal phase (Table 5-V). The follicular phase mean KITT-1 was similar to the luteal phase mean KITT-2, (Figure 5.8).

Table 5-V : Metabolic Variables in Follicular vs Luteal SITT in Controls

	1st SITT	2nd SITT	P
FI (mIU/L)	7.53 ± 5.62*	8.65 ± 6.69*	NS
Fasting glucose (mmol/L)	5.09 ± 0.58	5.11± 0.44	NS
C-peptide (ng/mL)	1.04 + 0.69	1.10 ± 0.59	NS
GH (ng/mL)	2.48 ± 3.17*	2.19 ± 2.86*	NS
IGF-I (nmol/L)	33.08 ± 14.24	29.83 ± 11.17	NS
Cortisol (ug/dL)	15.50 ± 4.53	12.18 ± 4.48	0.0365
PRL (IU/L)	436.2 = 532.0*	237.5 ± 119.5	NS
KITT-value (%/min)	4.91 ± 3.07	4.18 ± 1.46	NS

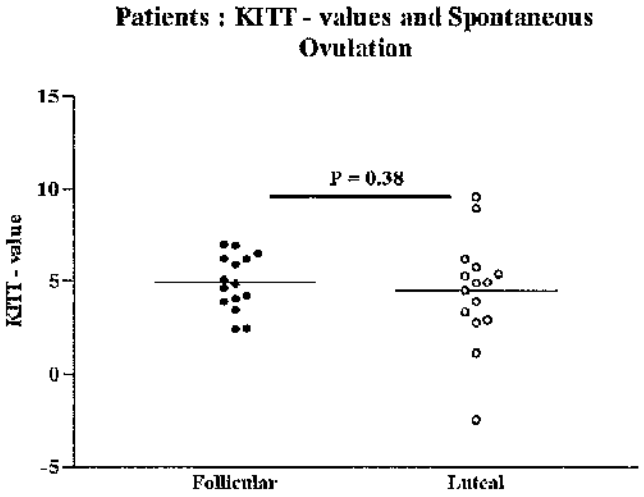
Groups were compared using Student's t-test, except for values with (*) where Mann-Whitney nonparametric test was used for comparison. P > 0.05 is NS.

Figure 5.8 :



The difference in IS between ovulatory and anovulatory cycles in the patient group, measured by the mean KITT-values is demonstrated below, and it shows similar results in the two subgroups, (Figure 5.9). This means that the mean KITT-value is valid for both patients and controls, to be used in any further analysis.

Figure 5.9 :



5.4.2 RELATIONSHIP BETWEEN "SITT" AND ANTHROPOMETRIC VARIABLES

Insulin sensitivity and anthropometric criteria enjoy well established relationships (Sharp *et al* 1991; Rajkhowa *et al* 1994), such that any test for IR may be assessed relative to those criteria to confirm reliability. There is already some level of doubt with respect to the reliability of the SITT as assessed in these experiments because it showed poorer correlations than FI levels within individuals, and the mean KITT values in the patients and controls showed no difference. The SITT was therefore examined with respect to anthropometric criteria and compared to the other methods of assessing IR which were available.

5.4.2.1 Relationship with BMI

Correlations between BMI and the basal metabolic variables are demonstrated in Table 5-VI. The mean KITT-values failed to show any relationship with BMI in either patients or controls (Figure 5.10), while the FI and fasting glucose (in the patient group) showed the expected relationship between obesity and IR.

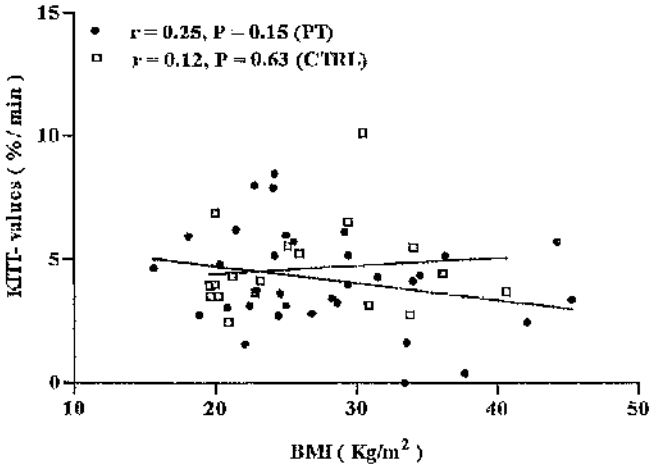
Table 5-VI : Correlations between BMI and the Basal Metabolic Variables

	Patients		Controls	
	r	P	r	P
FI (mIU/L)	0.55	0.0003	0.75	0.0001
Fasting glucose (mmol/L)	0.54	0.0011	0.27	0.271
C-peptide (ng/mL)	0.39	0.022	0.46	0.0381
GH (ng/mL)	-0.22	0.2116	0.49	0.0296
IGF-1 (nmol/L)	0.39	0.0975	0.33	0.194
mean KITT - values	0.25	0.15	0.06	0.80

P ≥ 0.05 is not significant.

Figure 5.10 :

Correlation : Mean KITT-values and BMI



5.4.2.2 Relationship with WHR and Waist

Correlations between WHR and the basal metabolic variables are shown in Table 5-VII.

Table 5-VII : Correlations between WHR and the Basal Metabolic Variables

	Patients		Controls	
	r	P	r	P
FI (mIU/L)	0.40	0.0178	0.49	0.029
Fasting glucose (mmol/L)	0.55	0.0009	0.12	0.630
C-peptide (ng/mL)	0.16	0.368	0.38	0.1109
GH (ng/mL)	-0.33	0.0573	0.46	0.0412
IGF-I (nmol/L)	0.27	0.2697	0.36	0.1609
mean KITT-values	0.15	0.39	0.18	0.45

P ≥ 0.05 is not significant.

As with BMI, mean KITT-values did not show any relationship with waist measurements or WHR (Figures 5.11 and 5.12), while the FI and fasting glucose (in the patient group) showed significant correlations with truncal obesity.

Figure 5.11 :

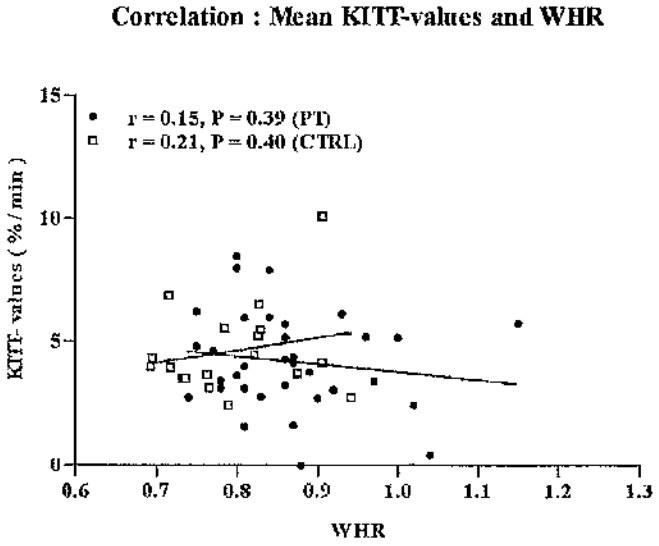
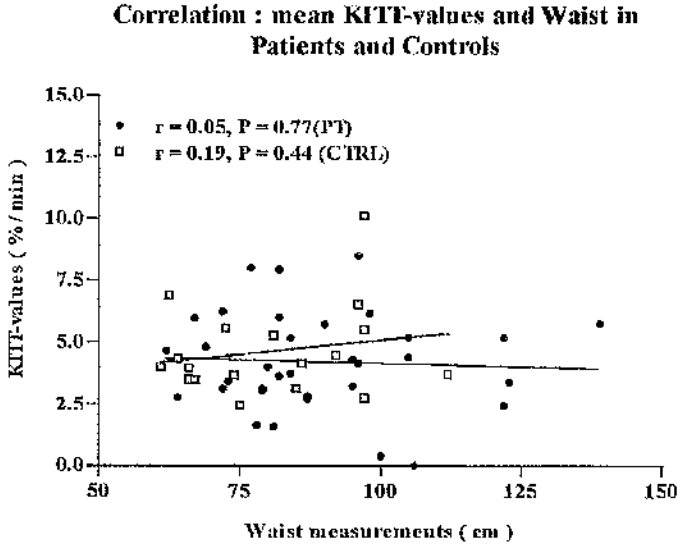


Figure 5.12 :



5.4.3 CRITICISM OF THE " SITT "

Although the SITT has been evaluated and found to be a safe, simple and "reliable" test to assess IS (Akinmokun *et al* 1992; Hirst *et al* 1993), in this study the KITT failed to comply with the well established relationships between IR and the anthropometric variables BMI and WHR. This may be due to inherent problems with the endocrinology of the test, or to simple practical problems. Furthermore, the SITT gave disappointing poor consistency within individual patients or controls.

5.4.3.1 Practical Problems

There were some practical problems. First, I-induced hypoglycaemia is unpleasant for the subject and potentially dangerous. Two of the patients (5.9%) (Figure 5.13), and one of the controls (5.0%), developed symptomatic hypoglycaemia and had a blood glucose level of $\leq$ 2.0 mmol/L by 15 minutes. Their I concentrations at 15 minutes were 20 and 80 mIU/L for the patients and 32 mIU/L for the control subject. Second, difficulties were encountered in maintenance of the intravenous access used to collect an arterialised blood sample for glucose estimation over the period of test. This problem was more pronounced in obese individuals and those who smoked.

5.4.3.2 Variations of Insulin Profiles

In spite of fairly consistent glucose responses (Figure 5.13), I concentrations showed variable patterns and inconsistent profiles following I injection during the SITT. These variable profiles were observed in both patients and control groups and occurred despite dosage adjustment according to BMI (Figures 5.14 and 5.15).

Figure 5.13 :

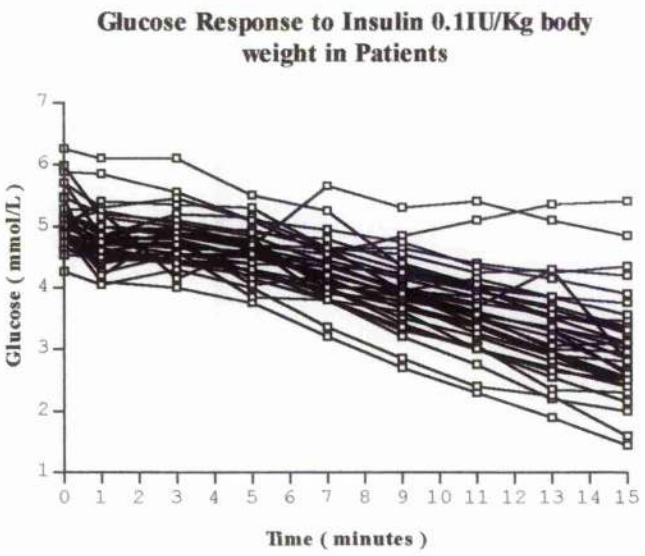


Figure 5.14 :

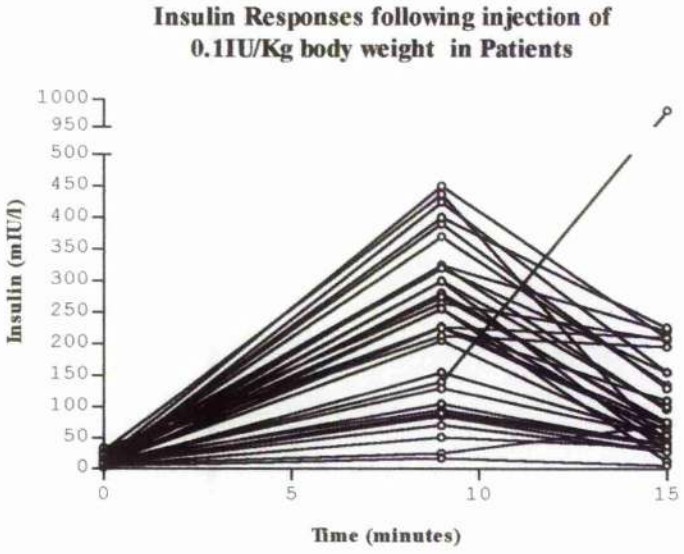
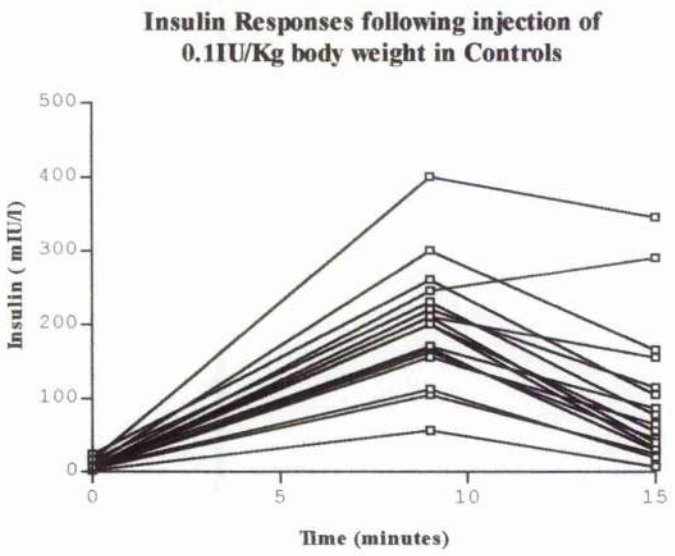


Figure 5.15 :



5.5 ALTERNATIVE ESTIMATES OF INSULIN SENSITIVITY

5.5.1 FI and FIRI

It has been reported that FIRI is a reproducible measure of IS (Matthews *et al* 1985; Cleland *et al* 1996). The FIRI is calculated from the formula : fasting glucose × FI / 25 (Cleland *et al* 1996), such that individuals who show both raised glucose and I levels could be IR, in contrast to the GLU/INS ratio which would tend to normalise the assessment. It may be a reasonable measure of IR in both non-diabetic and diabetic individuals. Its use may obviate the need for more complex procedures to obtain a useful index of IR in large-scale studies. Cleland *et al.* (1996) reported a mean FIRI of 2.77 for normal non-diabetic individuals. A significant correlation was reported between $\log_{10}$ FIRI and IS during euglycaemic hyperinsulinaemic clamp ($r = 0.67$, $P < 0.001$) (Cleland *et al* 1996).

Weak, but significant correlations were found between the mean KITT-values and FI and mean KITT-values and FIRI (Figures 5.16 and 5.17).

Figure 5.16 :

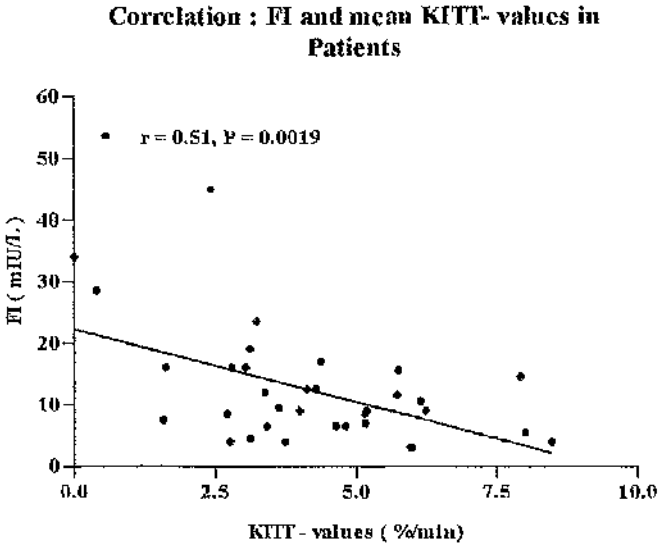
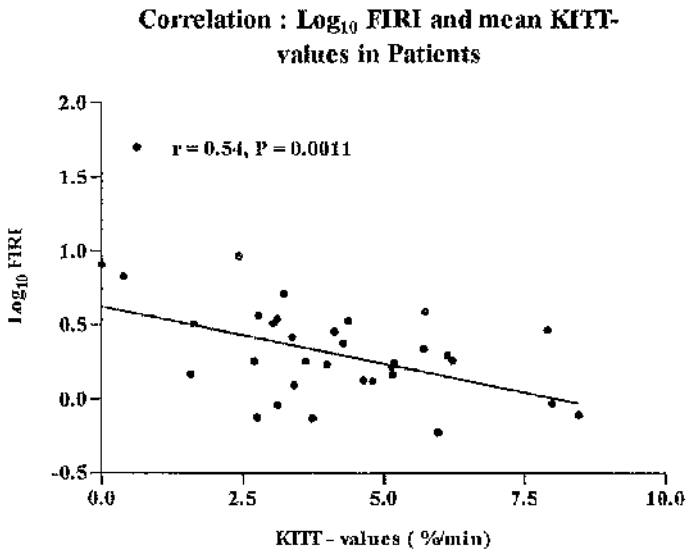


Figure 5.17 :



Fasting I, FIRI and $\log_{10}$ FIRI were significantly reproducible, in patients and in controls, during the first and repeat SITTs. These correlations are shown in Table 5-VIII and Figures (5.18 - 5.20). The mean values of FIRI for patients and controls were lower than those reported for normal non-diabetic

individuals (2.77) (Cleland *et al* 1996). The FIRI is dependent on sampling in the fasted state, and on the reproducibility of the I assay used.

Table 5-VIII : Mean Values of FI and FIRI within Patients and Controls

	Patients			Controls			
	1st SITT	2nd SITT	P	1st SITT	2nd SITT	P	P*
KITT (%/min)	4.14±2.15	4.24±2.36	NS	4.91±3.07	4.18±1.46	NS	NS
FI (mIU/L)	12.47±9.93	12.09±9.17	NS	7.53±5.62	8.65±6.69	NS	NS
GLU/INS ratio	0.67±0.50	0.64±0.40	NS	0.97±0.66	0.81±0.53	NS	NS
FIRI	2.52±2.31	2.44±1.98	NS	1.94±1.89	2.11±2.02	NS	NS

Groups were compared using Student's t-test. (P*), comparing patients with controls, was calculated using analysis of variance where $P \geq 0.05$ is NS.

Figure 5.18 :

Correlation : FI during SITTs in Patients and Controls

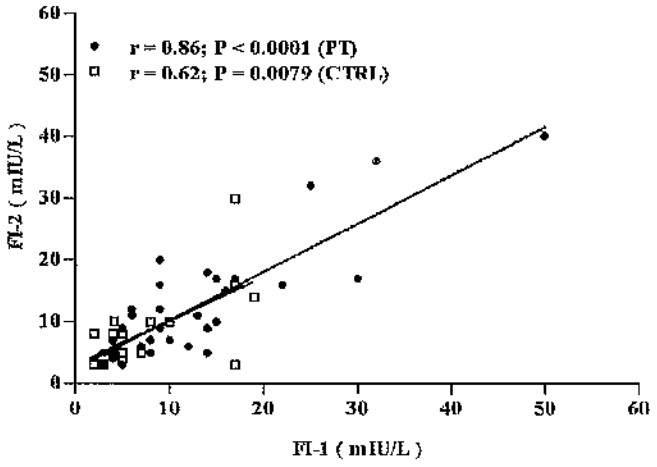


Table 5-IX shows the correlations between indices for measurement of IS between SITT-1 and SITT-2 in patients and controls. The KITT-value was the least consistent within patients and controls, while FI and FIRI showed equally good correlations. There was a surprisingly poor correlation between the two tests in the weight-matched control group, probably due to 2 exceptional values observed. Although the SITT-2 was carried out in the luteal phase, cycle phase showed no effect (Table 5-V; Figure 5.8).

Table 5-IX : Correlations between SITT-1 and SITT-2 within Patients and Controls

	Patients		Controls	
	r	P	r	P
KITT (%/min)	0.60	0.0004	0.66	0.008
FI (mIU/L)	0.86	< 0.0001	0.78	0.0001
GLU/INS ratio	0.80	< 0.0001	0.33	0.19
FIRI	0.83	< 0.0001	0.85	< 0.0001

P ≥ 0.05 is not significant.

Figure 5.19 :

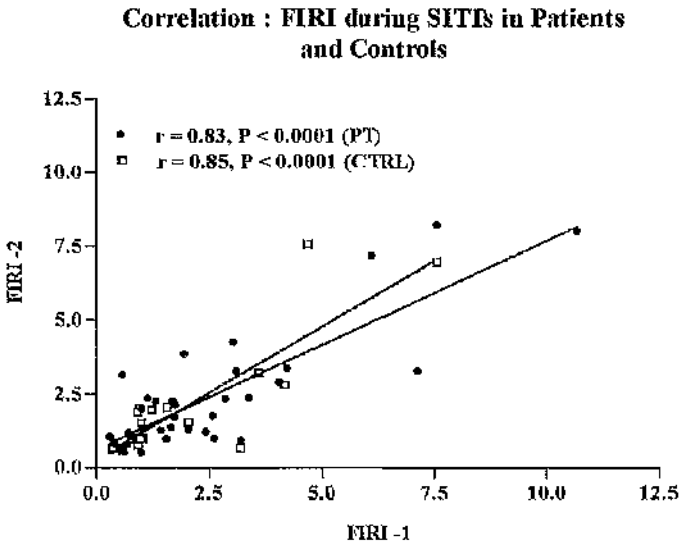
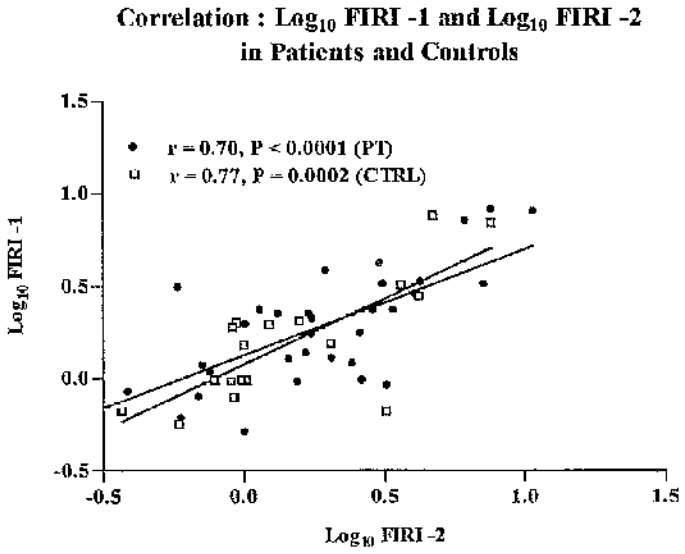


Figure 5.20 :



5.5.2 CORRELATION OF FI AND FIRI WITH ANTHROPOMETRIC VARIABLES

As stated above, the expected correlations of anthropometric variables with KITT-values was poor, so investigations were carried out to determine if the relationships of FI or FIRI with these variables were more reliable criteria reflecting IR, and showing the expected relationships.

5.5.2.1 Relationship between FI, GLU/INS ratio and FIRI and BMI

The relationships between BMI and FI, GLU/INS ratio and $\log_{10}$ FIRI were all significant in both patients and controls (Figures 5.21 - 5.23).

Figure 5.21 :

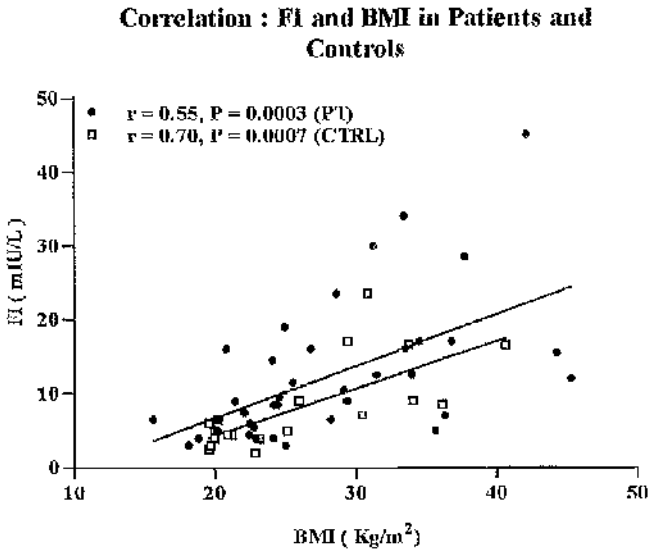


Figure 5.22 :

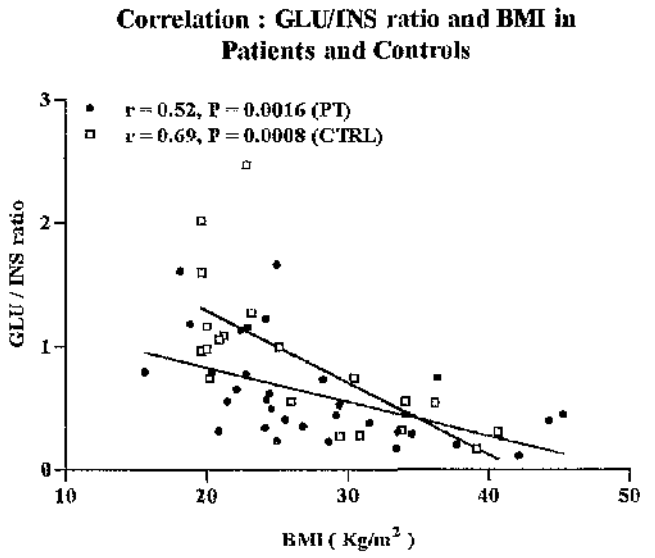
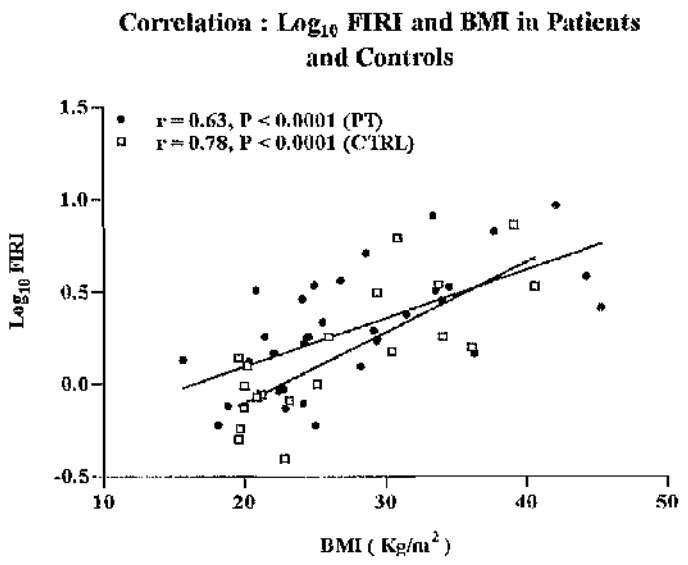


Figure 5.23 :



While the KITT-values did not show any difference between lean and obese patients and controls, other indices of measuring IR were significantly higher in obese than lean individuals (patients and controls) (Table 5-X). Lean patients showed significantly higher FI, GLU/INS ratio and FIRI than their weight-matched controls but obese individuals (patients and controls) were similar.

Table 5-X : The Relationships between BMI and Indices of IR in Patients and Controls

	Patients			Controls				
	< 29	≥ 29	P	< 29	≥ 29	P	P*	P**
KITT (%/min)	4.54±2.17	3.49±2.04	NS	4.22±1.93	5.59±4.04	NS	NS	NS
FI (mIU/L)	8.79±5.52	17.53±11.1	0.0024	4.67±1.90	16.33±8.57	0.0002	0.0178	NS
GLU/INS ratio	0.76±0.42	0.39±0.17	0.0058	1.24±0.54	0.40±0.19	0.0006	0.0074	NS
FIRI	1.83±1.21	3.79±2.57	0.0049	0.93±0.40	3.55±2.15	0.0006	0.0194	NS

Groups were compared using Student's t-test. (P*) compares patients and controls of BMI < 29, while (P**) compares patients and controls of BMI ≥ 29. P ≥ 0.05 is NS.

5.5.2.2 Relationships between FI, GLU/INS ratio and FIRI and WHR and Waist Measurements

Similar relationships, although less significant, were found between WHR and FI, GLU/INS ratio and $\log_{10}$ FIRI. The WHR and waist measurements (cm) showed almost identical relationships with FI and $\log_{10}$ FIRI in the patients. In the control group, stronger correlations were observed between FI and $\log_{10}$ FIRI and waist measurement than that of WHR (Figures 5.24 - 5.29) .

Figure 5.24 :

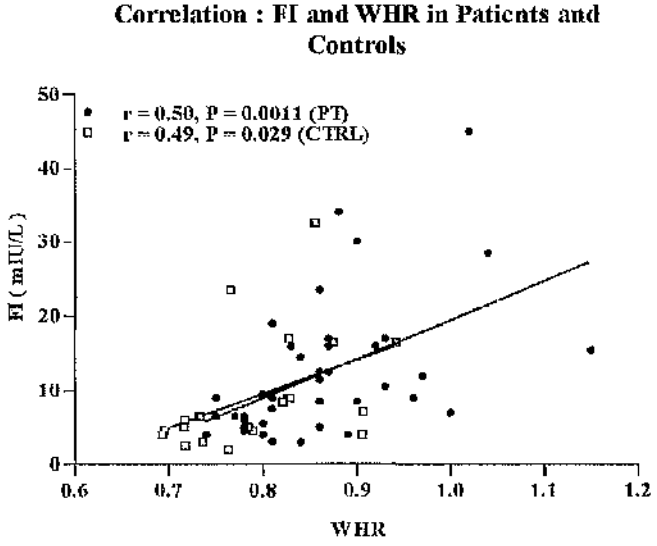


Figure 5.25 :

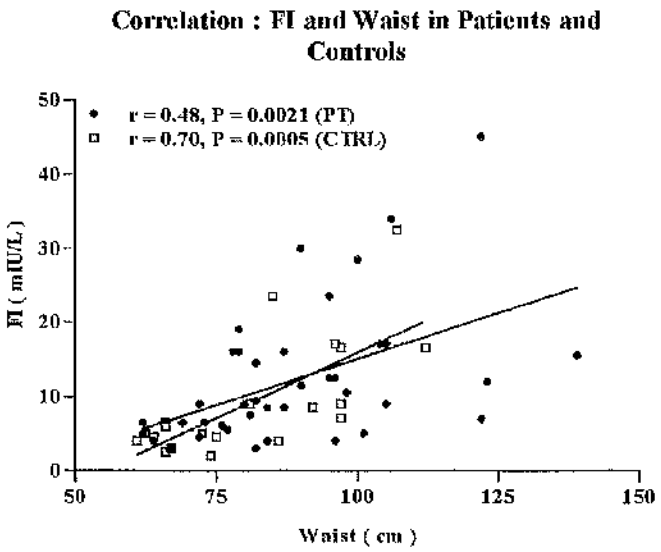


Figure 5.26 :

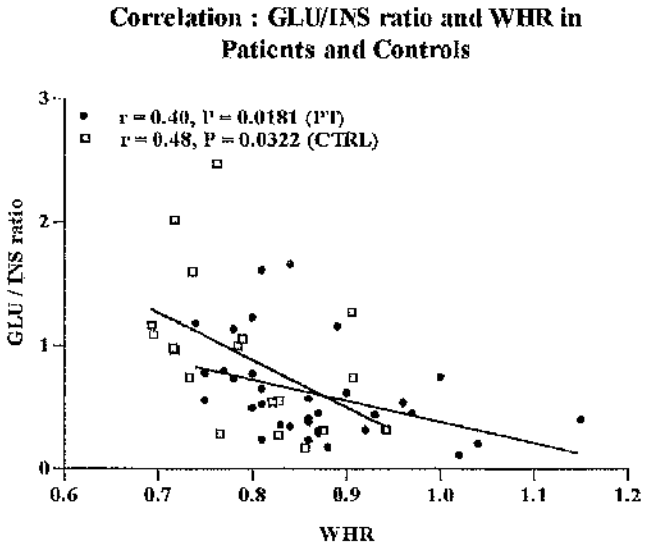


Figure 5.27 :

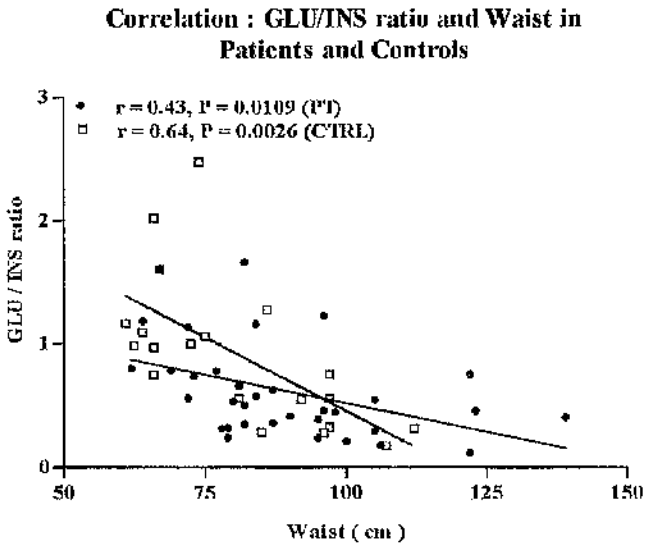


Figure 5.28 :

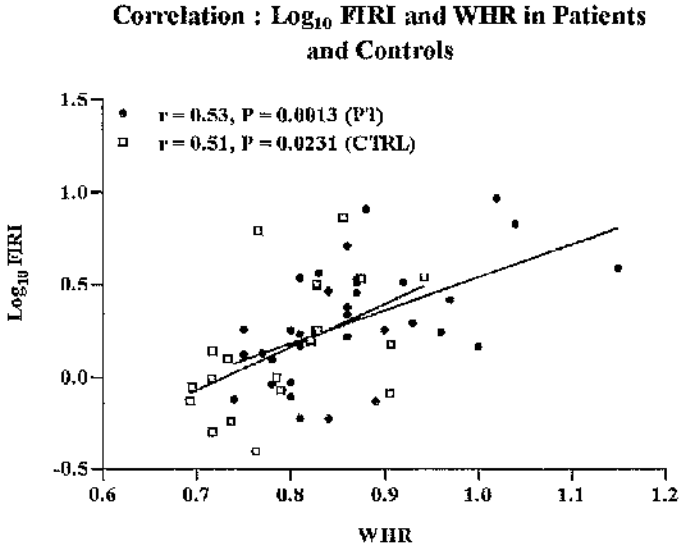
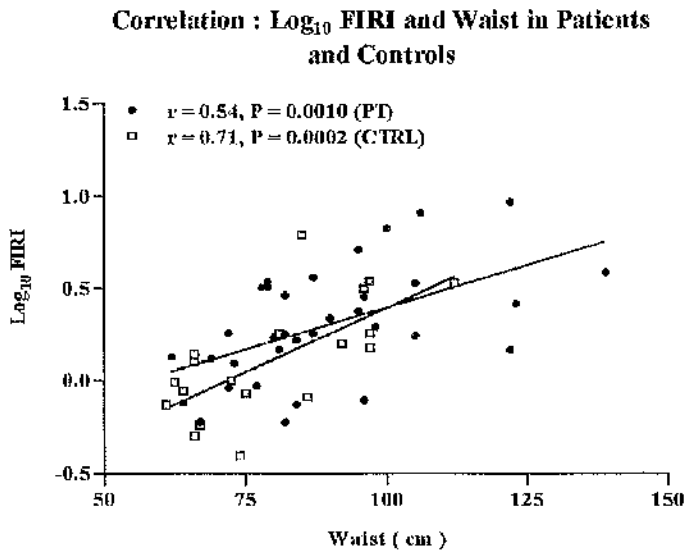


Figure 5.29 :



KITT-values were similar in individuals with WHR < 0.8 and those with WHR ≥ 0.8. However, the differences were significant when FI, GLU/INS ratio and FIRI were compared between subgroups of WHR (Table 5-XI). No significant difference was noticed between patients and their WHR-matched controls.

Table 5-XI : The relationships between WHR and Indices of IR in Patients and Controls

	Patients			Controls			
	< 0.8	≥ 0.8	P	< 0.8	≥ 0.8	P	P* P**
KITT (%/min)	4.15±1.99	4.14±2.22	NS	3.89±1.99	5.84±3.63	NS	NS NS
FI (mIU/L)	6.0±1.56	14.02±9.69	0.0266	6.05±5.95	13.34±8.57	0.0376	NS NS
GLU/INS ratio	0.87±0.24	0.56±0.40	0.0843	1.22±0.61	0.53±0.33	0.0071	NS NS
FIRI	1.23±0.37	2.87±2.16	0.0775	1.35±1.64	2.76±1.94	0.0945	NS NS

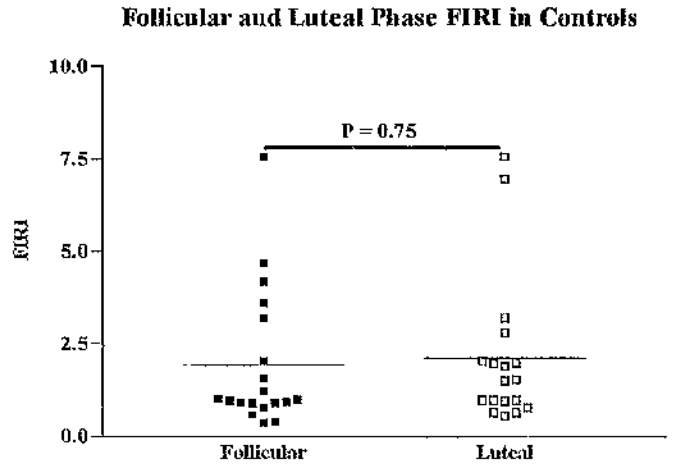
Groups were compared using Student's t-test. (P*) compares patients and controls of WHR < 0.8, while (P**) compares patients and controls of WHR ≥ 0.8. P ≥ 0.05 is NS.

5.5.3 INFLUENCE OF CYCLE PHASE (CONTROLS)

Values of FIRI determined in the follicular phase are compared with those in the luteal phase of the controls (Figure 5.30). Cycle phase showed no significant influence on FIRI. The mean follicular phase

FIRI (1.92 ± 1.89, range 0.37 - 7.56) was not different from the luteal phase FIRI (2.11 + 2.02, range 0.56 - 7.56).

Figure 5.30 :



5.5.4 HYPERANDROGENISM AND INSULIN SENSITIVITY

The relationships between the circulating androgen concentrations and the various estimates of IS were studied (Table 5-XII). There was no significant correlation between FI and T, A, or DHEA-S. Similar observations were noticed with $\log_{10}$ FIRI and GLU/INS ratio.

Table 5-XII : Correlations between Hormonal Indices of Hyperandrogenism and Estimates of IS

	FI				$\log_{10}$ FIRI			
	PT		CTRL		PT		CTRL	
	r	P	r	P	r	P	r	P
T (nmol/L)	0.13	0.47	0.05	0.85	0.22	0.22	0.15	0.53
A (nmol/L)	0.14	0.42	0.26	0.29	0.10	0.56	0.11	0.64
SHBG (nmol/L)	-0.45	< 0.0001	-0.65	0.0025	-0.54	< 0.0001	-0.57	0.0004
FAI	0.50	< 0.0001	0.47	0.041	0.55	< 0.0001	0.57	0.0002
DHEA-S (umol/L)	-0.16	0.36	0.05	0.84	0.18	0.31	0.10	0.58

On the other hand, there were powerful correlations between the estimates of IR and SHBG (Figures 5.31 -5.32) and FAI (Figures 5.33 - 5.34) in both patients and controls. Fasting I and $\log_{10}$ FIRI showed almost identical patterns.

Figure 5.31 :

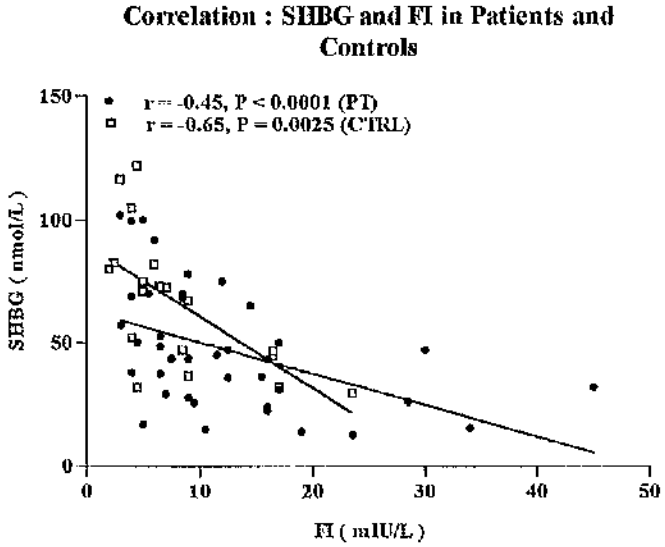


Figure 5.32 :

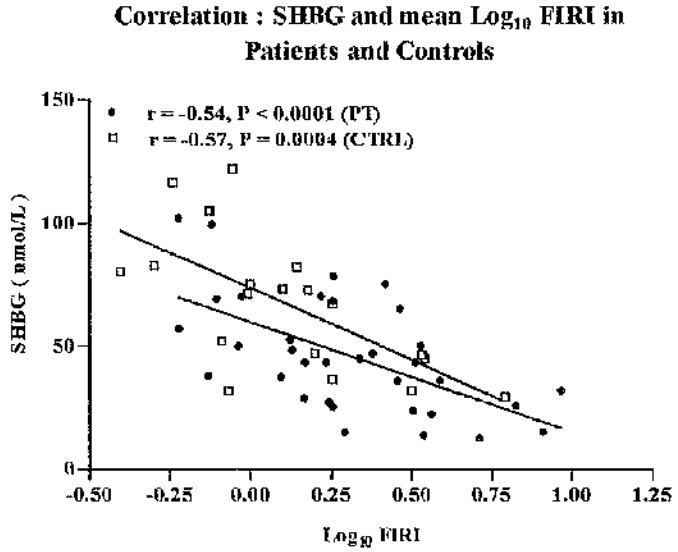


Figure 5.33 :

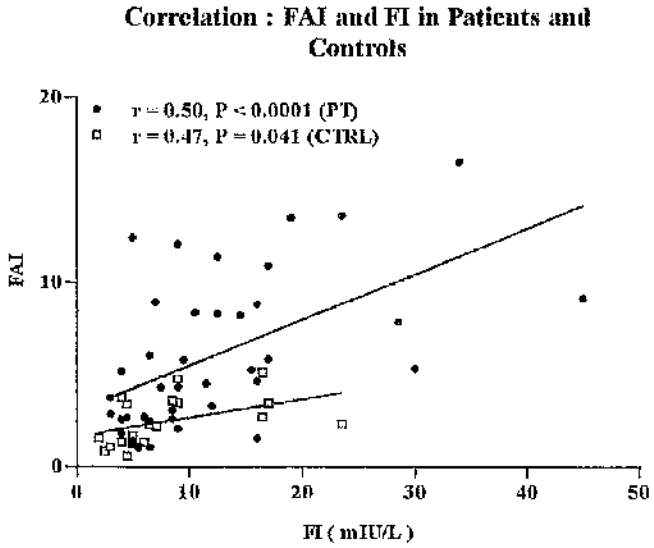
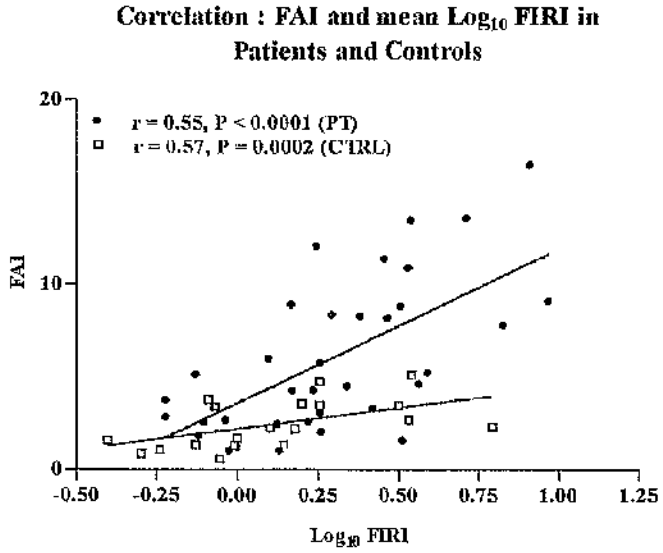


Figure 5.34 :



Androgenized patients, with low SHBG (mean ± SD of that of the control group), as expressed by FAI ≥ 4.5 (mean ± 1SD of study controls), showed significantly higher values of FI, $\log_{10}$ FIRI and lower GLU/INS ratios than did those with normal FAIs (Table 5-XIII), (Figures 5.35 - 5.39).

Table 5-XIII : Relationship between Hyperandrogenism and IS (Patients)

	FAI < 4.5	FAI ≥ 4.5	P
No. of Patients	16	18	
FI (mIU/L)	7.23 ± 3.44	16.79 ± 10.38	0.0006
GLU/INS ratio	0.83 ± 0.41	0.42 ± 0.25	0.0014
FIRI	1.49 ± 0.75	3.55 ± 2.37	0.0021

Figure 5.35 :

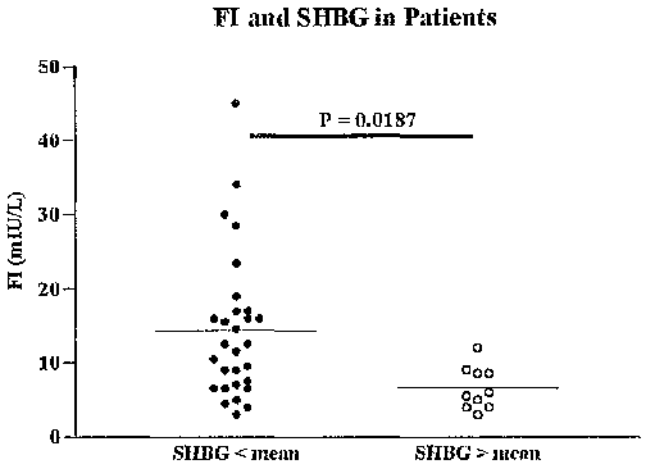


Figure 5.36 :

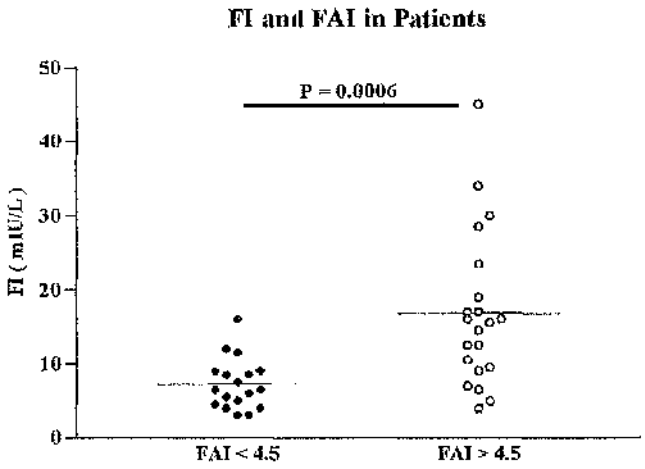


Figure 5.37 :

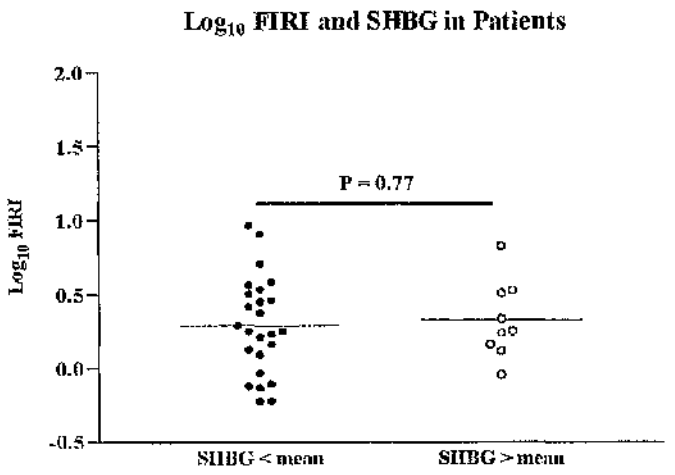


Figure 5.38 :

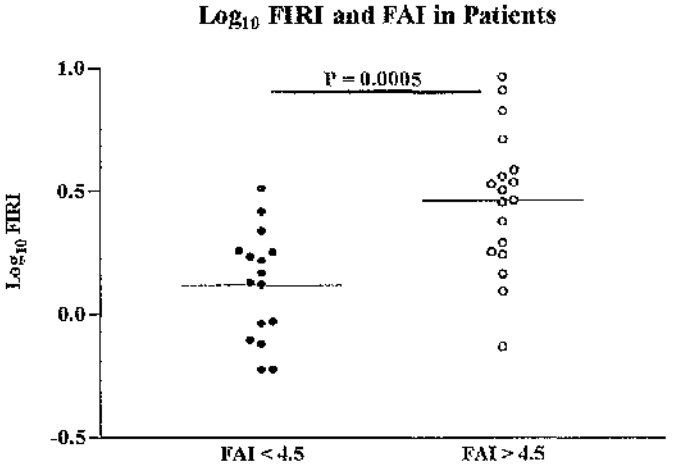
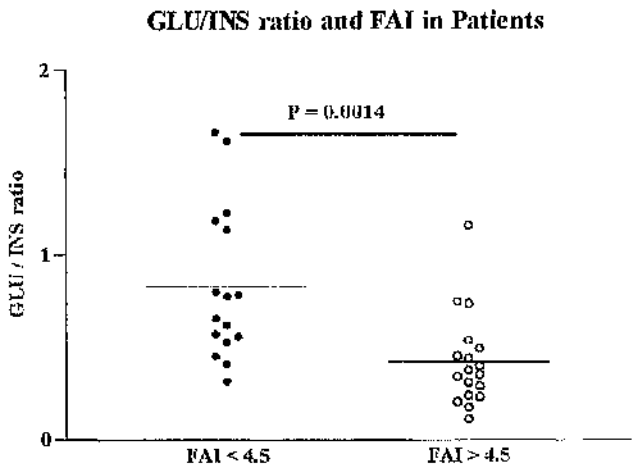


Figure 5.39 :



5.6 RESPONSES OF OTHER VARIABLES TO INSULIN ADMINISTRATION (PATIENTS AND CONTROLS)

The central, adrenal and ovarian endocrine responses to I administration, in patients and controls, are demonstrated in the following section.

5.6.1 CENTRAL RESPONSE

Patients

Gonadotrophins, GH and PRL responses to I injection in patients are shown in Table 5-XIVa. Growth hormone, one of the regulatory hormones for I, showed a significant increase at 30-minutes which was sustained until 60-minutes. Gonadotrophins and PRL showed no change.

Table 5-XIVa : Mean Central Responses to I administration during SITTs in Patients

	0-minute	30-minute	P	60-minute	P*	P**
LH (IU/L)	8.75 ± 5.59	8.45 ± 4.66	NS	8.45 ± 6.12	NS	NS
FSH (IU/L)	4.97 ± 1.32	5.09 ± 1.34	NS	4.99 ± 1.47	NS	NS
GH (ng/mL)	1.69 ± 3.77	3.81 ± 6.74	0.0018	2.69 ± 4.06	0.05	NS
PRL (IU/L)	303 ± 278	313 ± 433	NS	292 ± 294	NS	NS

Groups were compared using Student's t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

Controls

Similarly, in controls there were no significant changes in Gn and PRL responses to I injection (Table 5-XIVb). Growth hormone showed, however, a different pattern with a rise at 30-minutes which was insignificant (due to wide variations between subjects), and then decreased significantly at 60-minutes.

Table 5-XIVb : Mean Central Responses to I administration during SITTs in Controls

	0-minute	30-minute	P	60-minute	P*	P**
LH (IU/L)	4.13 ± 3.90	3.84 ± 3.83	NS	3.66 ± 3.42	NS	0.058
FSH (IU/L)	4.05 ± 1.90	4.06 ± 1.93	NS	3.99 ± 1.96	NS	NS
GH (ng/mL)	2.34 ± 2.98	4.07 ± 5.43	NS	2.11 ± 2.04	0.039	NS
PRL (IU/L)	340 ± 398	361 ± 412	NS	320 ± 326	NS	NS

Groups were compared using Student's t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

5.6.2 ADRENAL RESPONSE

Cortisol and DHEA-S responses to I injection in patients at 0-minute, 30-minutes and 60-minutes, are demonstrated in Table 5-XV. Cortisol, another regulatory hormone to the action of I, showed a late, though significant response at 60-minutes. Dehydroepiandrosterone-sulphate was measured at 60-minutes and was not significantly different from the basal value. The controls showed similar patterns in cortisol and DHEA-S responses.

Table 5-XV : Mean Adrenal Responses to I administration during SITTs in Patients

	0-minute	30-minute	P	60-minute	P*	P**
Cortisol (ug/mL)	13.3±6.4	13.4±6.4	NS	15.89±6.54	0.021	0.005
DHEA-S (umol/L)	1805±1187			1732±1158		NS

Groups were compared using Student's t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

5.6.3 OVARIAN RESPONSE : ALL TESTS

Patients

The patients' main ovarian endocrine responses following exogenous I injection are shown in Table 5-XVIa. Oestradiol showed a dramatic decrease at 30-minutes following the I injection and continued to decline at 60-minutes, accounting for about a 17% decrease from the basal (t_0 = 60.28 pg/mL) values. Testosterone followed the same pattern, although to a lesser degree, and was significantly decreased at 60-minutes compared to 0-minute (11% decline at 60-minutes).

Table 5-XVIa : Mean Ovarian Responses to I administration during SITTs in Patients

	0-minute	30-minute	P	60-minute	P*	P**
E_2 (pg/mL)	60.28 ± 23.19	54.85 ± 21.25	0.0002	49.85 ± 17.99	<0.0001	<0.0001
T (nmol/L)	2.19 ± 1.36	2.05 ± 1.34	NS	1.93 + 1.18	NS	0.006
SHBG(nmol/L)	46.55 ± 25.24	54.32 ± 26.9	NS	47.99 ± 26.54	NS	NS
FAI	6.10 ± 4.67	3.94 ± 2.84	NS	5.68 ± 4.14	NS	NS
IGF-I (nmol/L)	12.58 ± 5.5			12.97 ± 5.43		NS

Groups were compared using Student's paired data t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

Controls

Similar observations were noted in the control group as in the patients (Table 5-XVIb). No significant change in SHBG or IGF-I levels was observed but E_2 and T concentrations and FAI were significantly decreased across the test.

Table 5-XVIb : Mean Ovarian Responses to I administration during SITTs in Controls

	0-minute	30-minute	P	60-minute	P*	P**
E_2 (pg/mL)	77.27 ± 39.09	68.14 ± 34.60	<0.0001	63.29 ± 30.20	0.0124	0.0011
T (nmol/L)	1.37 ± 0.74	1.29 ± 0.71	NS	1.15 ± 0.56	0.057	0.061
SHBG(nmol/L)	66.11 ± 30.54			67.50 ± 31.85		NS
FAI	2.48 ± 1.69			2.01 ± 1.29		0.0386
IGF-I (nmol/L)	31.64 ± 12.83			31.59 ± 12.15		NS

Groups were compared using Student's paired data t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

Role of BMI

Growth hormone response after I administration was more pronounced in lean patients than obese patients (Table 5-XVII), although the responses in the control group were less remarkable.

Table 5-XVII : Role of BMI in the Metabolic Responses to I Administration

	Patients (P-value)						Controls (P-value)					
	< 29			≥ 29			< 29			≥ 29		
	0-30	30-60	0-60	0-30	30-60	0-60	0-30	30-60	0-60	0-30	30-60	0-60
GH (ng/mL)	0.027	0.0011	NS	NS	0.05	0.025	NS	0.071	NS	NS	NS	NS
Cortisol (ug/mL)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IGF-I (nmol/L)			NS			NS			NS			NS

Responses to I administration in Ovulatory Cycles

Follicular Phase

The responses to I were analyzed, separating follicular and luteal phase tests. These are illustrated in Table 5-XVIIIa. They show a similar trend to those described for the whole group but the decrease in T is not significant (P = 0.083).

Table 5-XVIIIa : Mean Ovarian Responses to I administration in the Follicular Phase of Ovulatory Cycles in Patients

	0- minute	30-minute	P	60- minute	P*	P**
E_2 (pg/mL)	59.60 ± 34.28	53.40 ± 31.16	0.0072	48.80 ± 27.84	0.0009	< 0.0001
T (nmol/L)	2.19 ± 0.88	1.98 ± 1.03	0.083	1.95 ± 1.01	NS	0.0924
SHBG(nmol/L)	45.22 ± 22.40			44.02 ± 25.85		NS
FAI	6.02 ± 4.25			6.31 ± 4.27		NS
P (pg/mL)	0.75 ± 0.52	0.63 ± 0.38	NS	0.76 ± 0.52	NS	NS
IGF-I (nmol/L)	13.24 ± 5.51			12.56 ± 3.69		NS

Groups were compared using Student's paired data t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

Luteal Phase :

Ovarian T and P but not E_2 responses after 1-hr were more pronounced when the luteal phase of ovulatory cycles in the patients were analyzed (Table 5-XVIIIb). In the luteal phase SHBG

concentrations rose significantly during the test and as a consequence FAI values fell significantly. In the tests performed in the luteal phase the P values decreased significantly during the test.

Table 5-XVIIIb : Mean Ovarian Responses to I administration in the Luteal Phase of Ovulatory Cycles in Patients

	0-minute	30-minute	P	60-minute	P*	P**
E_2 (pg/mL)	79.44 ± 42.58	70.29 ± 36.68	0.0169	64.38 ± 32.29	0.0032	0.0009
T (nmol/L)	2.38 ± 2.05	1.63 ± 1.61	0.033	1.78 ± 1.46	NS	0.0225
SHBG(nmol/L)	50.41 ± 22.22			64.06 ± 26.89		0.0101
FAI	5.93 ± 5.57			3.55 ± 3.21		0.0279
P (pg/mL)	6.89 ± 6.22	6.56 ± 6.40	NS	5.83 ± 5.12	0.077	0.0064
IGF-I (nmol/L)	16.33 ± 5.32			13.33 ± 4.93		NS

Groups were compared using Student's t-test. (P*) compares 30-min with 60-min, while (P**) compares 0-min with 60-min. P ≥ 0.05 is NS.

5.7 DISCUSSION

The SITT and FI and FIRI

In this study, the SITT test was the dynamic test chosen to measure IR. The fall in plasma glucose concentration in the test depends on both the inhibition of endogenous glucose production (principally from the liver), and the stimulation of glucose uptake by tissues (principally the muscles); the faster the decline in plasma glucose concentration, the more sensitive the individual is to I. The dose of I used, (0.1 IU/Kg), is supraphysiological, causing suppression of hepatic glucose production/release. Hence, the rate of glucose disappearance from the circulation is a measure of glucose uptake by the tissues. However, hypoglycaemia often results when blood glucose reaches its nadir, by about 20-minutes, and this will trigger a pronounced counter-regulatory response which in turn reverses the decline in plasma glucose concentration. The glucose disappearance rate in the first 15 - 20 minutes should therefore measure only the action of the injected I (Akinmokun *et al* 1992). The rate of fall of blood glucose (KITT-value) was calculated from values in samples collected at 2-minute intervals between 3 and 15 minutes. The rate of fall in percent per minute was calculated using the least squares analysis and this was taken as an index of IS (Rajkhowa *et al* 1994) (Appendix V).

The KITT-values found in this study are similar to those reported by other observers (Harrison *et al* 1976; Akinmokun *et al* 1992; Hirst *et al* 1993). The mean KITT-value in normal weight, non-diabetic adults reported was 3.0 %/min (range 0.59-4.84) (Harrison *et al* 1976), higher than that expected in diabetic individuals. In this study, the mean KITT-value for patients was 4.20 ± 1.99 %/min (range 0.005 - 8.48). However, the mean KITT-values showed no difference between patients and their weight-matched controls. Rajkhowa *et al.* (1994) showed that the KITT-value, used as an index of IS, correlated inversely with BMI, and obese patients with PCOD had significantly lower KITT-values than did obese controls, but lean PCO women were similar to lean controls. The data reported in this thesis do not agree with these observations either within the obese or lean groups.

The correlations between KITT-1 and KITT-2 in both patients and controls (r = 0.60, 0.63, respectively), indicated the poor reproducibility of the test within the subjects. These correlations were poorer than the correlations of FI within subjects of this study (r = 0.86, 0.78; respectively), and the correlations reported for SITT and the euglycaemic clamp (r = 0. 86 for normal and 0.81 for diabetic subjects) (Akinmokun *et al* 1992). Analysis of the profiles of I concentrations during the SITT showed considerable inconsistency. This may be related to the poor correlation between the 2 SITTs in each subject. Furthermore, the KITT-values failed to confirm the well-established relationships with anthropometric variables. The reliability of the SITT must therefore be doubted; the FI or FIRI appeared to be more reliable tests for estimating IR with regard to intra-subject consistency and also relationship with anthropometric criteria.

Although a weak correlation was found in this study between the mean FI and the mean KITT-values and between the mean FIRI and the mean KITT-values in patients; the FI and FIRI were found to be more reliable within subjects and also showed the expected relationship with anthropometric criteria. Evidence from Elkind-Hirsch *et al.* (1993) showed that in patients with PCOD, FI correlated well with IR measured by intravenous GTT (r = 0.66, P < 0.02), suggesting that FI levels are predictive of IR. The mean FIRI recorded by Cleland *et al.* (1996) suggested that it may be an alternative method with the benefit of including patients with raised glucose and I.

INSULIN and GLUCOSE

All the individuals in the study had normal fasting glucose concentrations and the mean value of glucose for the patients was similar to that of the control group. This agreed with the findings of other observers (Buyalos *et al* 1993; Holte *et al* 1994a). Basal fasting glucose did not correlate with any of the endocrine

or metabolic variables and the glucose response to the injected I (0.1 IU/Kg) was similar in both groups of women.

The cut-off accepted value for normal FI concentration in this study, derived from the control group data (mean ± SD), was 13.0 mIU/L. Similar values have been employed in other studies (Dunaif *et al* 1989; Buyalos *et al* 1991; Nestler *et al* 1991; Akinmokun *et al* 1992; Dale *et al* 1992b). Significantly higher mean FI concentrations were observed in patients with PCOD irrespective of BMI (Shoupe *et al* 1983; Chang *et al* 1983; Bruno *et al* 1985; Lanzone *et al* 1990a; Buyalos *et al* 1993; Conway *et al* 1993; Filicori *et al* 1996). In this study 41.2% of the patients and 26.3% of the control group were found to have a higher FI than the cut-off normal value, this resulting in only a borderline significant increase in FI when comparing the patients to the controls (P = 0.057). A significant linear correlation between FI and fasting glucose values was found in the patients only (r = 0.47, P = 0.0048), suggesting a resistance to I in the patient group, but not in the controls.

C - PEPTIDE

In this study, fasting C-peptide levels were found to be similar in the patients and the control group. These data contrast with the observation of Filicori *et al.* (1996) who reported lower levels in patients with PCOD, while Holte *et al.* (1994a) showed higher levels in controls; these levels were increased in patients with PCOD over the whole range of BMI. The increased C-peptide concentrations in these other studies were presumably a byproduct of the increased I production. It is clear that the data concerning C-peptide are confused, and the results from this study with weight-matched cases of oligo/amenorrhoea and normal menstrual rhythm suggest that it has no pathophysiological role in the disturbances of ovarian function measured under these circumstances.

INSULIN RESISTANCE and ANDROGENS

None of the parameters used in this study to estimate IS showed any significant relationships with total circulating T or A in the study group. Similar findings have been reported in the literature (Ciaraldi *et al* 1992; Buyalos *et al* 1993; Rajkhowa *et al* 1994), with no significant correlation of hyperinsulinaemia with raised T and A concentrations in obese and non-obese PCO women. These authors suggested that hyperandrogenaemia and IR were independent features of PCO and that obesity exacerbates the clinical

expression of hyperandrogenaemia. Others (Chang *et al* 1983; Bruno *et al* 1985; Geffner *et al* 1986) have concluded that T concentrations, in patients with PCOD and their controls, were highly correlated to fasting hyperinsulinaemia and IR which may be partially responsible for the observed imbalance in glucose/insulin homeostasis. The data presented in this study support the former hypothesis that IR and hyperandrogenism are independent features.

In this study, FI was significantly correlated with SHBG (negatively) and FAI in both patients and the control group. The correlation between IR and FAI in the oligo/amenorrhoea group appears to be secondary to the inverse correlation of SHBG to serum I. Although I has been shown to have direct effects on ovarian theca cells, *in vitro*, the effects of I on androgen metabolism are also likely to be by modification of synthesis and secretion of SHBG, which in turn, may lead to profound changes in androgen clearance and production (Sharp *et al* 1991). Insulin, rather than sex steroids, appears to be the primary regulator of circulating SHBG concentrations (Dunaif, 1992a). Insulin is thought to lower serum SHBG levels by acting directly to reduce hepatic SHBG synthesis (Buyalos *et al* 1993; Rajkhowa *et al* 1994) such an effect of I was not seen in healthy non-obese women in the present study. There were significant inverse correlations of SHBG with BMI in both patients and controls indicating that hyperinsulinaemia in obese patients lowered the SHBG levels. This was subsequently reflected in the FAI in both the patients and in the control group, and hence enhances the manifestation of hyperandrogenaemia. Hyperandrogenized patients, with high FAI, showed IR as expressed by significant hyperinsulinaemia ($P = 0.0006$) and higher FIRI values ($P = 0.0021$) than patients with normal FAIs. Similar observations have shown weak but significant positive correlations between FI and FAI (Lanzone *et al* 1990a; Dale *et al* 1992b).

The cause and effect relationship between I and androgens in PCOD is still controversial. Insulin has been implicated in the increased ovarian production of androgens (Nagamani & Stuart, 1990; Buyalos *et al* 1993; Elkind-Hirsch *et al* 1993; Rajkhowa *et al* 1994), while it has also been suggested that androgens modulate IS in PCOD (Bruno *et al* 1985). Studies have indicated that chronic hyperinsulinaemia may be responsible for hyperandrogenism in PCO by acting directly or indirectly to enhance LH-mediated androgen synthesis by the ovary (Shoupe *et al* 1983; Buyalos *et al* 1993; Elkind-Hirsch *et al* 1993; Rajkhowa *et al* 1994). By testing women known to be hyperandrogenic due to increased BMI, it was possible to show that hyperandrogenism may be the trigger to the cycle of androgen-insulin excess. Whether this is a direct effect of androgen or is mediated by secondary factors, such as elements of the GH-IGF axis, is unclear (Speiser *et al* 1992). The bulk of the evidence in the literature indicate that the role of androgens in IR is questionable. Men are not more IR than women and oophorectomy or suppression of circulating androgens have failed to improve IS in women (Dunaif, 1992a). Reversal of

hyperandrogenism did not normalise IS (Geffner *et al* 1986; Dunaif *et al* 1990; Lanzone *et al* 1990a; Speiser *et al* 1992). The data presented here confirm that endogenous androgens do not play a role in sustaining IR. Therefore, there is an association of elevated androgens and changes in IS but the mechanisms by which hyperinsulinaemia induces hyperandrogenaemia are still unresolved.

Hyperinsulinaemia may exert its steroidogenic action on the ovary through IGF-I and its receptors. Insulin and IGF-I are closely related peptides and both have been shown to potentiate LH-induced ovarian androgen synthesis. The human ovary contains I and IGF-I receptors, and both I and IGF-I stimulate human ovarian androgen production (Dunaif, 1992a; Elkind-Hisch *et al* 1993). Insulin suppression of locally produced IGF-I binding proteins might be one of the factors in the pathogenesis of I-induced hyperandrogenaemia by increasing IGF-I receptor binding in the ovary and enhancing androgen production by theca-interstitial and stromal cells (Elkind-Hisch *et al* 1993). It has been suggested that if I is to produce ovarian hyperandrogenism, I concentrations must be increased into the range that can activate the IGF-I receptors, and polycystic ovarian changes must be present that predispose the ovaries to secrete excess androgens (Dunaif, 1992a). Androgens may produce irreversible changes in target tissue sensitivity to I action. Therefore, suppression of hyperandrogenaemia would not alter such effects and may not be a clinically effective modality to improve I action and subsequently decrease the risk of NIDDM in patients with PCOD (Dunaif *et al* 1990; Dunaif, 1992a).

ADRENAL ANDROGEN

In the present study, no significant relationship was found between the concentrations of DHEA-S and IR. Conflicting information concerning the acute effect of hyperinsulinaemia on circulating adrenal androgens, specifically DHEA-S has been reported. In women with PCOD, Pasquali *et al.* (1983) demonstrated a negative correlation between DHEA-S and fasting GLU/INS ratio, and Falcone *et al.* (1990) showed that FI concentrations were significantly associated with the basal DHEA levels. Suppression of DHEA concentrations normally caused by I is impaired in women with PCO and IR and this impairment may be another manifestation of a peripheral reduction in I action. This disturbance may contribute to adrenal androgen excess in some women with PCOD and may reflect changes in adrenal responsiveness or peripheral clearance (Falcone *et al* 1990). However, this mechanism was not apparent in the groups of women observed in this study.

GROWTH HORMONE / REGULATORY HORMONES

Baseline GH concentrations were low in both patients and the control group. While PCO patients in the study by Kazer *et al.* (1990) showed lower GH levels, normal GH levels have been reported by others in patients described as PCO (Chang *et al* 1983; Geffner *et al* 1986; Slowinska-Srzednika *et al* 1992). Low GH levels could be due to decreased secretion from the anterior pituitary, increased clearance from the circulation, or an abnormality of the GH. Kazer *et al.* (1990) suggested that GH levels may be low due to impaired GH secretion, since I can reduce GH production. In the present study, no correlation was established between GH and FI, supporting the concept that there was no abnormality in GH secretion related to hyperinsulinaemia.

The study patients were weight-matched with their control counterparts, and no correlations were found between body mass and either GH or IGF-I in the study groups. However, lean individuals had higher (though not significant perhaps due to the limited sensitivity of the assay) mean fasting serum GH concentrations than obese subjects. This may support a relationship between GH and body mass in the form of obesity, although the timing of blood sampling might have masked this relationship as GH exhibits a diurnal variation (Hatasaka *et al* 1994). It is also known that the nutritional status alters GH, as acute fasting or chronic malnutrition raises GH and obesity lowers it (Oerter *et al* 1992).

No significant difference in fasting serum cortisol and/or PRL levels were found between the patients and the controls, similar to previous reports (Chang *et al* 1983; Geffner *et al* 1986). The patients and controls showed similar rates of fall of glucose during the SITT, and the control group's GH responses showed a similar pattern to that of the patients. However; these responses were not significantly different in lean or obese individuals compared to the patients. The regulatory responses of GH, cortisol, glucagon and catecholamines vary from individual to individual and according to the rate of fall of glucose, hence interpretation of the results is difficult (Pochner *et al* 1984).

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) and IGFBPs

Basal serum IGF-I concentrations were significantly lower in oligo/amenorrhoeic patients than in the controls ($P < 0.0001$) in spite of normal levels of GH; this observation was confirmed at the repeat SITT effected 5 weeks later. These findings support the observations of Barreca *et al.* (1996) who found lower IGF-I levels in follicular fluids from patients with PCOD than in follicular fluid from normally ovulating women. However, normal (Kazer *et al* 1990; Sharp *et al* 1991; Slowinska-Srzednika *et al* 1992; Holte *et*

al 1994a) and elevated (Laatikainen *et al* 1990) IGF-I concentrations have also been reported by others. These marked differences could be due to the methods used for IGF-I assays, which depend upon extraction of IGF-I from its binding proteins, although simple explanation of the conflicting observations are not obvious.

Growth factors are polypeptides that regulate the replication and differentiated function of cells. Growth factors are present in the systemic circulation and are synthesized by a variety of cell systems and tissues; therefore, they may act as systemic or local regulators of cell metabolism. In the circulation, growth factors are present, free or bound to specific binding proteins as they may also act at a local level. The synthesis and effects of the locally produced factor can be modified by systemic hormones in tissues expressing the hormonal receptor(s). Whereas GH is one of the most important regulators of IGF-I synthesis, other hormones such as oestrogens, parathormone and glucocorticoids affect the synthesis of IGF-I (Canalis, 1992). Cells that secrete IGF-I also secrete one or more IGFBPs. So far, at least six structurally related IGFBPs have been identified and are termed IGFBP-1 through 6. The IGFBPs like IGFs are hormonally controlled. The IGFBP-3, the predominant form of IGFBP present in human serum, is GH dependent, whereas serum concentrations of IGFBP-1 are suppressed by I. The function of the various IGFBPs is not exactly known; they could be important in increasing the half-life of IGFs, presenting IGFs to their receptors, or modulating the activity of IGFs by binding the biologically active free IGF. IGFs have biochemical and functional properties resembling those of I. Insulin-like growth factor-I induces hypoglycaemia when infused to rats or humans because, like I, it increases intracellular glucose transport. Furthermore, IGF-I decreases I degradation. It is important to note that IGFs are expressed by a variety of tissues, and changes in local production are not likely to be detected by serum measurements. IGF-I tends to be low and IGFBP-I is elevated in untreated DM patients because its production is inhibited by I. Insulin-like growth factor-I production is regulated through different intracellular mechanisms in liver and peripheral tissues (Tiitinen *et al* 1990).

The synthesis and secretion of IGFBPs may play a major role in the regulation of IGF hormonal action at the target cell. The role of IGFBPs in ovarian physiology remains unknown. FSH decreases the release of IGFBPs, by an uncertain mechanism, and this may enhance the cellular action of endogenously generated and also circulating IGFs at the granulosa cells (Adashi *et al* 1988; Adashi *et al* 1991; San Roman & Magoffin, 1992). The ability of FSH to inhibit the release of IGFBPs from granulosa cells is dose- and time-dependent. Low-dose exposure for a relatively short period of time may be compatible with the needs of the granulosa cell in the earlier phases of follicular development. Therefore, the diminished IGFs would be compatible with the relatively slow early follicular development. More prolonged exposure to high doses of FSH and the consequent increase in IGFs may be sufficient to

maintain and enhance the terminal phases of follicular development. FSH-insensitive follicles undergoing atresia may be compromised by decreased IGFs (Adashi *et al* 1991).

The changes in tissue IGF-I concentrations and thereby activities may be influenced by changes in specific IGF-I binding proteins which were not evaluated in the present study. It has been reported that IGFBP levels are inversely correlated with I, resulting in more bioactive unbound IGF-I activity in hyperinsulinaemia which may contribute to increased androgen production (Franks, 1989) even in the presence of normal or reduced total IGF-I concentrations. In this study, basal IGF-I demonstrated no relationship with T or SHBG but a weak positive correlation with FAI ($r = 0.46$, $P < 0.05$) was observed in the patient group only.

Circulating levels of serum IGF-I, which do not vary during the day and are not influenced by meals (Kazer *et al* 1990), did not correlate with basal GH concentrations. No correlation was found between IGF-I and obesity. However; lean controls showed significantly higher IGF-I levels than obese controls, and both lean and obese patients. Nutritional status can alter GH and IGF-I in normal subjects in opposite directions and IGF-I levels change significantly with age and sex. In children, acute fasting or chronic malnutrition lowers IGF-I and overnutrition raises IGF-I (Oerter *et al* 1992). It seems that the relationship between IGF-I and obesity may not be a simple linear correlation. Thus it is important to weight match controls in any study of PCO and/or disturbed ovarian activity.

The human ovary has both I and IGF-I receptors. Receptors for IGF-I have been identified in rat interstitial and granulosa cells, however; specific binding sites for I (Poretsky *et al* 1984; Nagamani & Stuart, 1990) and for IGF-I (Nagamani & Stuart, 1990) have been identified in human ovarian stroma. In the normal ovary under physiological conditions, I may exert its effect on ovarian steroidogenesis mainly through its own receptors. However, in PCO, when I is present in higher concentrations, I might mediate its action through IGF-I receptors (Nagamani & Stuart, 1990), particularly when IGF-I concentrations are reduced.

In vitro studies with granulosa cells of polycystic ovaries indicate that physiological concentrations of IGF-I are as effective as FSH in stimulating E_2 synthesis and it has been shown that follicular fluid contains IGF-I. The *in vitro* studies with human granulosa cells showed that IGF-I and FSH may act synergistically to control the level of E_2 production (Erickson *et al* 1990). Whether a dysfunction in IGF-I activity is involved in the failure of follicular maturation and reduction in E_2 secretion, observed in PCOs may be questioned or follicular fluid of PCOs may contain inhibitors of IGF-I action such as binding proteins. Insulin and IGF-I are closely related peptides and both I and IGF-I receptors have been

found in human ovarian tissue (Elkind-Hirsch *et al* 1993). Ciraldi and coworkers (1992) showed functionally intact I receptor binding in adipocytes taken from women with PCOD and a post-receptor defect may be involved. There is the possibility of hyperinsulinaemia as a consequence of IR and an increase in circulating bioavailable IGF-I collectively exerting their actions on the ovary through the IGF-I receptors (Elkind-Hirsch *et al* 1993).

Measurements of IGF-II have been less useful than IGF-I as markers of endocrine disorders (Canalis, 1992). The granulosa cell IGF-I receptor may constitute one of several variables responsible for follicular selection (Adashi *et al* 1988).

Follicles of women with PCOs were reported to contain high concentrations of A but reduced E_2 and significant amounts of aromatase activity. However, FSH is unable to stimulate normal amounts of E_2 production and release *in vivo* due to a reduction in the number of the granulosa cells and there may also be a defect in the granulosa cells due to the presence of one or more substances in these follicles which modify the action of FSH. Recently, it has been shown that in the presence of physiological concentrations of IGF-I the same amount of FSH is capable of stimulating maximal E_2 biosynthesis suggesting that IGF-I may be important for sensitizing the granulosa cells to FSH (Adashi *et al* 1988; Mason *et al* 1993; Barreca *et al* 1996). Decreased IGF-I concentrations or activity in oligo/amenorrhoeic patients may be related to ovulation (Chapter Six). Growth hormone administration, by increasing IGF-I levels in follicular fluid, may correct the IGF imbalance and overcome the defect in granulosa cells in PCO patients and thus improve ovarian follicular maturation (Barreca *et al* 1996).

RESPONSES TO EXOGENOUS INSULIN ADMINISTRATION

Growth hormone, one of the central regulatory hormones for I, showed a significant increase at 30-minutes after I administration, probably the expected response to hypoglycaemia induced by the exogenous I injection. This increase was statistically significant in the patients but not so in the control group. Lean patients showed a more significant rise in GH at 30-minutes than in obese patients and in lean and in obese controls. This difference cannot be attributed to obesity as obese controls showed similar responses to their lean counterparts. Other central hormones, such as Gn and PRL, did not show a change during the SITT in either patients or controls. Although I stimulated Gn secretion in isolated rat pituitary cells, human studies indicated that I infusion failed to alter Gn release in normal or in

PCOD patients (Dunaif & Graf, 1989; Dunaif, 1992a). Cortisol, another regulatory hormone for insulin, showed a delayed rise at 60-minutes after I administration..

Dehydroepiandrosterone-sulphate was measured 60-minutes following the I injection and was not significantly different from its basal levels. The effect of physiological increases in circulating I concentrations on DHEA-S is not well established. Circulating DHEA-S levels during endogenous I release in response to OGTT did not change either in patients with PCOD or in controls (Buyalos *et al* 1991). This is not surprising because of the extensive volume of distribution, the prolonged half life, and the slow MCR of DHEA-S which would make acute changes in circulating levels of this steroid unlikely. Minimal diurnal variability is observed in circulating DHEA-S levels, despite the well established pulsatile and circadian fluctuations of cortisol and DHEA. It is unlikely that an acute increase in I within the physiological range would be important in the regulation of circulating DHEA-S levels in either PCO or euandrogenic women (Buyalos *et al* 1991). However, rapid changes in circulating DHEA-S in response to supraphysiological I levels have been reported (Nestler *et al*, 1987).

A dramatic change was noted in circulating E_2 and T concentrations following I administration. Supraphysiological hyperinsulinaemia resulted in a significant decline in the levels of E_2 at 30-minutes and this decrease continued at 60-minutes and accounted for a drop of about 17% from the basal levels in both the patients and the control group. Testosterone showed a similar pattern, although to a lesser extent (11% decrease from the basal values). Sex hormone binding globulin, FAI and IGF-I did not show any significant changes during the period of monitoring (60-minutes). However, when the luteal phases of the ovulatory cycles in the patients were analyzed separately, E_2, T, FAI and P showed significant decreases; while SHBG showed a significant increase during the period of monitoring. Significant reductions in E_2 and FAI were shown in both the follicular and the luteal phases of the control group, and other hormonal changes were not significant. A reduction in circulating androgen concentrations has been recorded after administration of exogenous I to women with PCO (Dunaif & Graf, 1989; Speiser *et al* 1992). The change in androgen levels following I administration cannot be explained on the basis of the physiological diurnal variation because the control group did not show a significant decrease although the diurnal variation of androgens was reported to be similar in healthy women and in PCO patients (Baird *et al* 1977). The significant reduction in ovarian steroid concentrations was sustained at 60-minutes and a possible effect of acute I administration on ovarian steroid secretion must be proposed. This suppression of ovarian androgens in oligo/amenorrhoeic women argues against a simple, direct relationship between hyperinsulinaemia and hyperandrogenaemia

(Dunaif & Graf, 1989). The decrease in E_2 concentrations was more than the decrease in androgen, suggesting that the effect on E_2 is probably not due to inhibition of supply of androgen precursor alone.

Dunaif & Graf (1989) reported that I infusion to achieve supraphysiological levels decreased the levels of potent androgens (T, DHT and FAI) and acutely increased A and E_2 levels in patients with PCOD. In normal women, the only significant change in steroid levels during I infusion was an increase in E_2 levels but no change in androgen levels were noted. The relationship between I and androgens is complex. While chronic hyperinsulinaemia is associated with hyperandrogenaemia, acute supraphysiological I administration was found to decrease the circulating androgen concentrations. Insulin-like growth factor-I and/or I appears to play a physiological role in the regulation of gonadal steroidogenesis, but supraphysiological I concentrations can act through the IGF-I receptor. Hyperinsulinaemia could act synergistically with other hormonal disturbance(s) such as LH hypersecretion, to enhance abnormal gonadal steroidogenesis by increasing ovarian sensitivity to IGF-I via regulation of the latter's receptors (Dunaif & Graf, 1989). However, the same authors claimed that in extreme IR, profound long-standing hyperinsulinaemia alone may be sufficient to produce hyperandrogenaemia. The study of the kinetics of these responses to I may reveal changes in ovarian steroid output which relate to the problems of failure of follicular maturation seen in PCO patients.

OBESITY

Significantly lower mean GH levels were found in obese individuals compared with lean controls, indicating that the difference is attributable to obesity. However, BMI was correlated negatively with mean serum concentrations of GH in the control group, but not the patients. Low GH levels could be due to decreased secretion from the anterior pituitary, increased clearance from the circulation, or an abnormality of the GH molecule resulting in misleading immunoassay results. Obesity and IR contribute to an increased GH clearance. Lean patients and controls showed a more rapid increase in GH concentrations in response to I administration (30-minutes) than obese individuals, who showed a rather delayed increase of GH (significant at 60-minutes).

Insulin sensitivity and anthropometric variables have a well-recognized relationship (Sharp *et al* 1991; Dale *et al* 1992b; Rajkhowa *et al* 1994). In this study, the mean KITT- values failed to show any relationship with BMI or WHR in either patients or controls. On the other hand; FI, GLU/INS ratio and FIRI proved to be more reliable than the KITT values with the expected relationships being shown with

anthropometric variables, including BMI, WHR and waist measurements. Body mass index, WHR and waist measurements showed almost identical relationships with FI and FIRI in the patients. In the control group, stronger correlations were observed between FI and FIRI with waist measurement than with WHR. Furthermore, comparison of lean and obese individuals showed a more prominent influence of obesity. Fasting I and FIRI were significantly higher in obese than in lean women. In addition obese patients showed levels of FI and FIRI similar to obese controls which strengthens the apparent role of obesity on IR.

Although no single entity is diagnostic of PCOD, obesity is encountered in 35-80% of cases (Dale *et al* 1992b). It has been suggested that obesity is a pathogenic factor in PCOD by contributing to increased extraglandular aromatization of androgens into oestrogens. Obesity is also associated with IR and hyperinsulinaemia (Sharp *et al* 1991; Dale *et al* 1992b; Rajkhowa *et al* 1994).

It is generally accepted that the increased I secretion in obesity serves as a mechanism to compensate for IR. However, the processes that trigger hyperinsulinaemia in human obesity and the possible pathophysiological consequences of hyperinsulinaemia are not fully identified. Insulin resistance has been observed in lean patients with PCO, so the combination of PCOD and obesity may have a synergistic impact on glucose tolerance (Dunaif, 1992a). Current understanding of the IR and obesity is fragmented and inconclusive. Furthermore, much less is known about the IR in non-obese subjects. It has been postulated that those individuals with IR and normal BMI will have increased body fat mass in comparison to individuals matched for age and BMI. Therefore, individuals with an identical BMI may have differing amounts of fat that correlate with the degree of IR. The implication is that normal-weight, I-resistant subjects must have a complementary decrease in lean body mass. In fact, the normal-weight, I-resistant subjects may show increased body fat when compared with I-sensitive weight-matched subjects (Caro, 1991). Women with PCOD may have significant IR that is independent of obesity (Chang *et al* 1983).

Although IR and other obesity-related metabolic abnormalities are frequently associated with overall accumulation of fat in the body, there is growing evidence that the distribution of fat may have an important additional role. Little is known about the relationships between generalized and regional deposition of fat in obesity. Fat deposition in the subcutaneous region of the trunk was found to be significantly and independently associated with peripheral and hepatic IR (Abate, 1996). WHR measurements and waist girth were significantly related to IR (Ferrannini *et al* 1996).

Young I-sensitive lean women may have enhanced likelihood of developing IR associated with both age and weight gain (Ferrannini *et al* 1996). The ability of I to stimulate glucose uptake can vary substantially in non-obese persons with no apparent disease. The degree of obesity and level of physical activity can also modulate I action. The compensatory response to a decrease in I-stimulated glucose uptake is an increase in plasma I concentration, and a significant direct relationship between magnitude of IR and the degree of associated hyperinsulinaemia in non-diabetic individuals has been documented. Insulin resistance was much more clearly associated with upper body fat than with androgens (Holte *et al* 1994a), but hyperinsulinaemia and IR may not be due entirely to obesity, and may be related to the degree of hyperandrogenaemia, mainly of adrenal sources (Pasquali *et al* 1983). However, no significant role of adrenal androgens has been observed in the present study.

OVULATION

When assessment of the controls of the present study, were compared in the follicular and the luteal phase, no significant differences were observed in FI, fasting glucose, C-peptide, GH, IGF-I or PRL concentrations. The follicular phase mean KITT-value was similar to that of the luteal phase in both the control group and in patients who ovulated (n = 17). The cycle phase had no significant influence on FIRI ($P \geq 0.05$). However, significant differences in IS were observed between patients with oligo/amenorrhoea and their weight-matched control group. Anovulatory patients were more IR, with significantly higher FI and lower GLU/INS ratio, than the controls (P = 0.022, 0.039; respectively); these differences were not significant when compared with patients with oligo/amenorrhoea who ovulated spontaneously (P = 0.31, 0.08; respectively). This effect was diluted when FIRI was applied (Chapter Six).

Blood glucose levels have been reported to be increased in anovulatory patients with PCOD and to be greater than those in ovulatory patients (Filicori *et al* 1996). The groups reported in this study showed no difference. The GLU/INS ratio was reduced in both ovulatory and anovulatory patients compared to the control group.

Filicori *et al.* (1996) have suggested that obesity and impaired GTT are negative prognostic factors when ovulation is induced with pulsatile GnRH in PCOD patients. Higher I concentrations in anovulatory compared with ovulatory women with hyperandrogenaemia may indicate that IR in the ovary contributes to the mechanism of anovulation in PCOD, but it is important to use weight-matched controls in such studies. The fact that I levels were not significantly raised in the groups as a whole but were elevated in

anovulatory compared with ovulatory women, despite similar androgen levels, suggests that I is not directly involved in the increased androgen production in these women (Sharp *et al* 1991). The observations of the present study support this statement.

CONCLUSION

Insulin secretion is regulated by numerous metabolic, hormonal, and neural signals, and this hormone also feeds back on the pancreatic β-cell to regulate its own receptors. Total body IS can be assessed by different techniques, although the simpler methods should be implemented for routine clinical practice.

This study showed that the SITT was unreliable for the measurement of IR in oligo/amenorrhoeic patients because the KITT-values were similar in patients and their weight-matched control group, and more importantly; the test failed to show any correlation with anthropometric variables. In addition, the correlation within patients between the 2 SITTs, expressed as KITT-values, was poor and disappointing compared to other parameters such as FI and $\log_{10}$ FIRI. Simpler methods, FI and FIRI, were more convenient, easy to measure and showed the expected relationships with the anthropometric variables - comparable with the standard methods of IR measurement.

The baseline IGF-I concentrations were significantly lower in the patients than the controls on both test occasions despite normal circulating GH concentrations.

Fasting glucose and FI were not significantly different between the patients and the controls. However, significantly higher FI and FIRI were observed when obese and lean subgroups were compared. Thus the importance of weight-matched controls in any study of PCOD and/or disturbed ovarian activity must be emphasized.

The cause and effect relationship between I and androgens is controversial. None of the methods used to estimate IS showed a significant relationship with androgens in this study. The inverse correlation between IS and FAI in the oligo/amenorrhoeic group appeared to be secondary to the inverse correlation of SHBG to serum I. These powerful relationships between SHBG and FI and $\log_{10}$ FIRI were demonstrable in both patient and control groups. Insulin is thought to regulate the circulating SHBG concentrations (Franks, 1989) by acting directly on hepatic synthesis of SHBG (Buyalos *et al* 1993; Rajkhowa *et al* 1994). Hyperandrogenized patients, with high FAI, showed significant hyperinsulinaemia and higher FIRI values than patients with normal FAI.

The prevalence of impaired glucose tolerance or frank diabetes in obese women with PCOD was shown to be 20 % (Dunaif, 1992a), therefore; a measurement of GTT is important in these women (Harrington & Balen, 1996).

Although IR was independent of obesity, the combination of PCOD and obesity had a synergistic impact on glucose tolerance. The presence of hyperinsulinaemia may be important since long-term follow-up of patients with PCOD indicates that they are at increased risk of developing DM and CHD. Research on the mechanisms of anovulation in PCOD, the role of obesity and the clinical significance of the relationship between IR and hyperandrogenism are on-going.

CHAPTER SIX : LONGITUDINAL OBSERVATIONS IN INFERTILE WOMEN WITH OLIGO/AMENORRHOEA

6.1 INTRODUCTION

It has been shown that infertile women with oligomenorrhoea are often capable of undergoing spontaneous episodes of follicular development, maturation and ovulation. This phenomenon was investigated in depth in order to study the endocrine aspects, ovarian morphological development, and anthropometric criteria associated with these events.

All patients were investigated longitudinally for a period of 5 weeks. They were monitored for ovarian activity twice weekly by measuring plasma E_2 levels and once a week by a pelvic US examination. These observations were started a minimum of 14 days following a menstrual bleed. Ovarian volume, the presence of microcysts and stromal thickness were assessed during the US scan. If E_2 concentrations and/or US scan indicated signs of follicular maturation at any stage during the 5-week period, then blood sampling was changed to daily and the frequency of US examinations, was increased. Follicular maturation and ovulation, were assessed over the following two weeks. Cases with evidence of follicular maturation and ovulation were assayed for P in the luteal phase. If concentrations of E_2 and/or the US scan indicated no ovarian activity, the E_2 sampling was terminated after the 5^{th} week.

Results are presented as mean values for each individual patient during each week of observation. By design this was a minimum of 2 samples/week for endocrine estimations and 1 observation per week for US scans. Where follicular growth was noted the number of observations was increased. In sections 6.2 and 6.3 "mean values during the 5-weeks period" the mean represents individual means of all samples analyzed during that week.

There were 3 main sections to the observations : - 1) Changes in the reproductive hormone concentrations during the 5-week window will be discussed in section 6.2. The occurrence of spontaneous ovulation in oligo/amenorrhoeic patients and assessment of the luteal phases are recorded. Relationship of anthropometric variables, LH and hyperandrogenization with the incidence of spontaneous ovulation are examined and discussed, as is the role of IR. 2) Changes in the ovarian US picture during the 5-weeks of observation are recorded in section 6.3. The dynamic changes in TOV,

follicular distribution and the presence of stromal density are examined. 3) Section 6.4 covers the variations in the endocrine parameters and in ovarian volume within patients and correlates this with the clinical presentation of the patients.

Unfortunately there were no ultrasonographic examinations for the control group because of their reluctance to undergo the examination. The upper limit of ovarian volume for normal subjects was taken as 9.0 cm^3 (Chapter Four) and the ovary was considered enlarged in patients when the volume was > 9.0 cm^3.

6.2 CHANGES IN ENDOCRINE VARIABLES DURING THE WINDOW (5-WEEKS) OF OBSERVATIONS

6.2.1 VARIATIONS IN ENDOCRINE OBSERVATIONS

There was a wide range of variations in E_2 concentrations within the whole group of patients and also within individual patients. There was no biological point of reference in such analyses, as not all subjects showed a LH surge, and the occurrence of spontaneous follicular maturation and ovulation at any time rendered comparison of different weeks inappropriate. However, Figure 6.1 shows the considerable range of E_2 values seen during the period of observations varying from menopausal levels (< 30 pg/mL) to normal mid-cycle peak values (approximately 300 pg/mL). Figure 6.2 shows the variations seen within patients during the same time (Table 6-I), indicating that ovarian follicular function is not static in these patients.

Figure 6.1 :

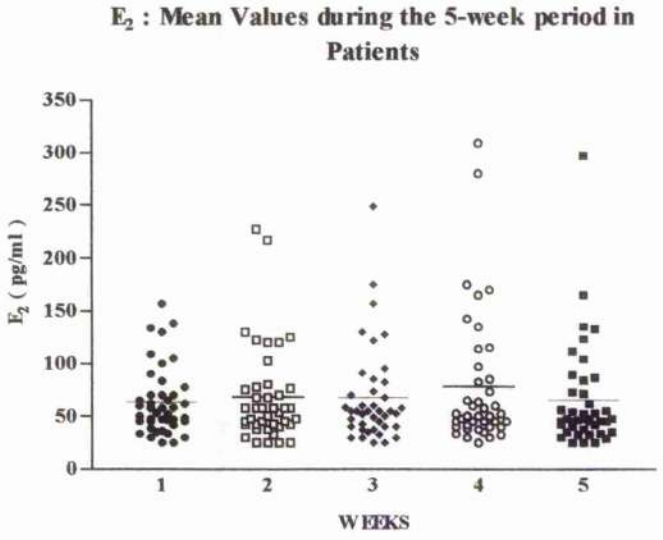
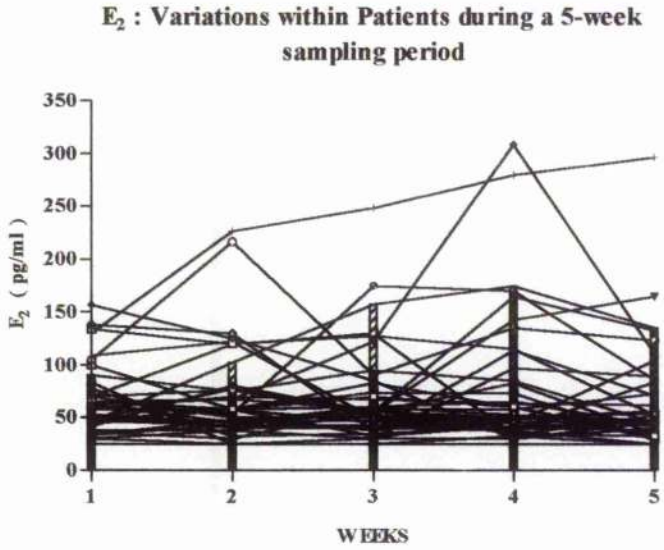


Figure 6.2 :



Similar variations were also observed for the other reproductive hormones monitored during the window of observation - LH, T, SHBG and consequently FAI. Figures 6.3 to 6.10 demonstrate the wide variations confirming that these hormones are not static and serve to indicate how unrepresentative a single hormone value can be.

The profiles of LH showed large variations within the group during the 5-week window (Figure 6.3) (range 1.5 - 39.5 IU/L), and Figure 6.4 shows that the variations within patients were also large. If the upper limit of normal is taken as 10.0 IU/L then 24 patients (57.1%) showed both normal and supranormal values, while 18 patients (42.9%) showed all samples within the normal or supranormal ranges.

Figure 6.3 :

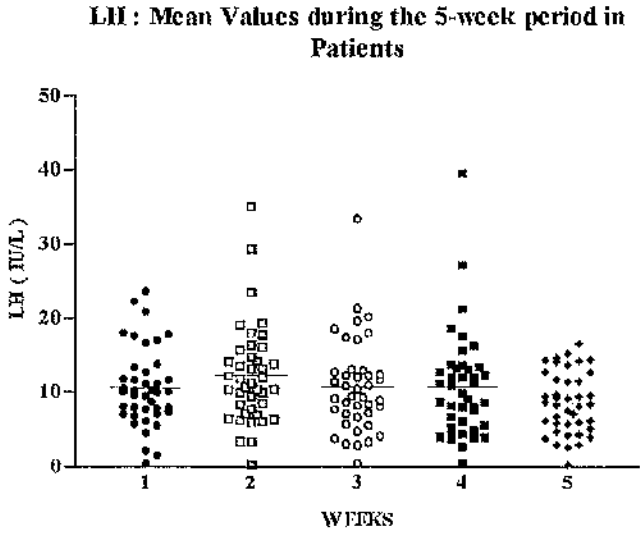
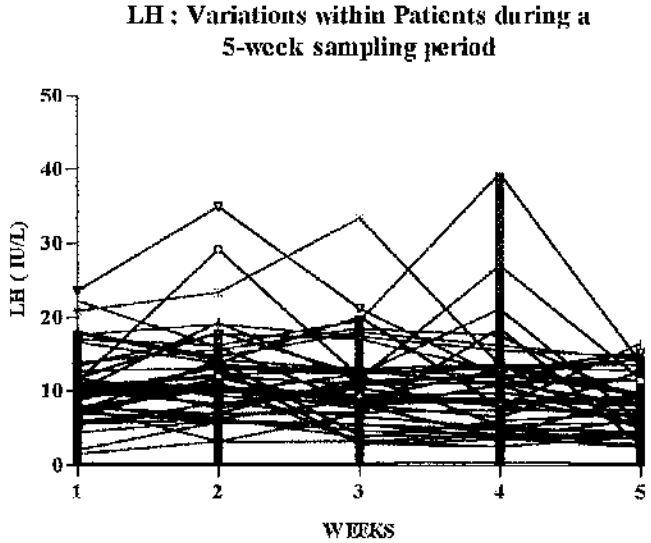


Figure 6.4 :



Similarly, T concentrations showed wide variations within the group (Figure 6.5), and also within patients (Figure 6.6) during the 5-week period of monitoring. Testosterone concentrations were consistently normal (≤ 2.5 nmol/L) in 12 patients while always elevated in only 6 patients. Figure 6.6 and Table 6-I demonstrate the wide variation within patients.

Figure 6.5 :

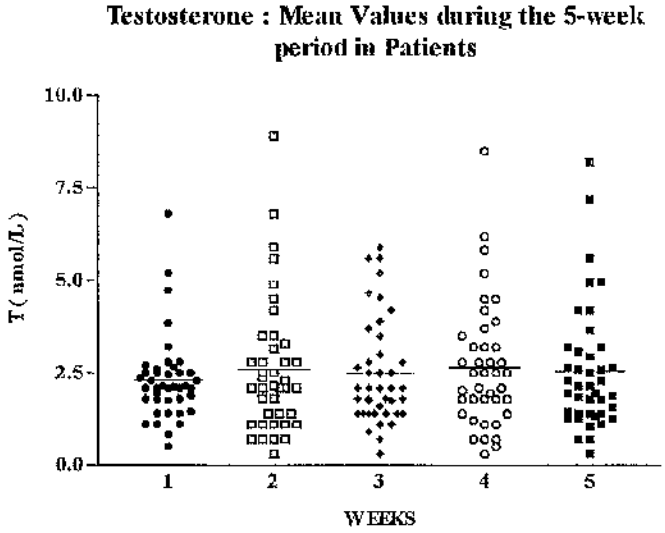
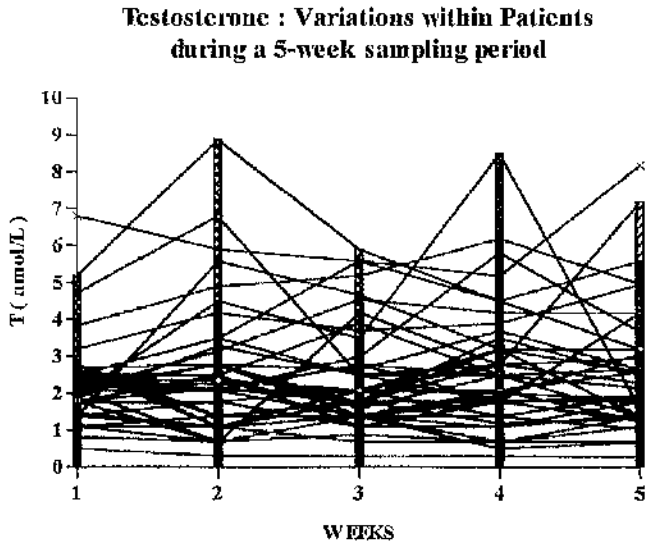


Figure 6.6 :



As with E_2, LH and T, the profiles of SHBG showed large variations within the group, ranging between 11 and 295 nmol/L (Figure 6.7). However, Figure 6.8 shows that SHBG concentrations were relatively stable within individuals during the 5-week window of observation, and showed the most consistent values within patients of all of the parameters measured (Table 6-I).

Figure 6.7 :

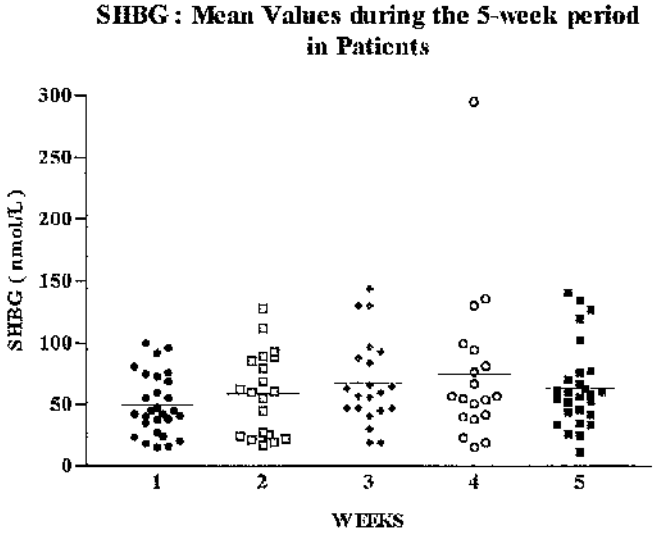
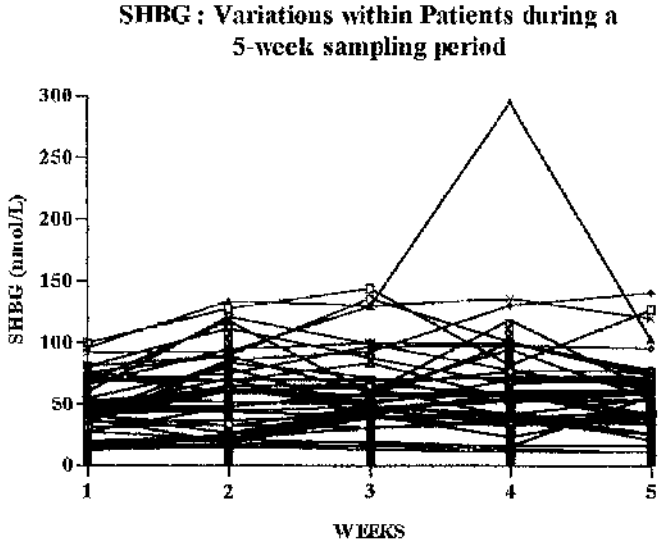


Figure 6.8 :



Consequent to the relatively stable SHBG concentrations, FAI variations within the group and within the patients (Figures 6.9 and 6.10) showed similar patterns to those of T.

Figure 6.9 :

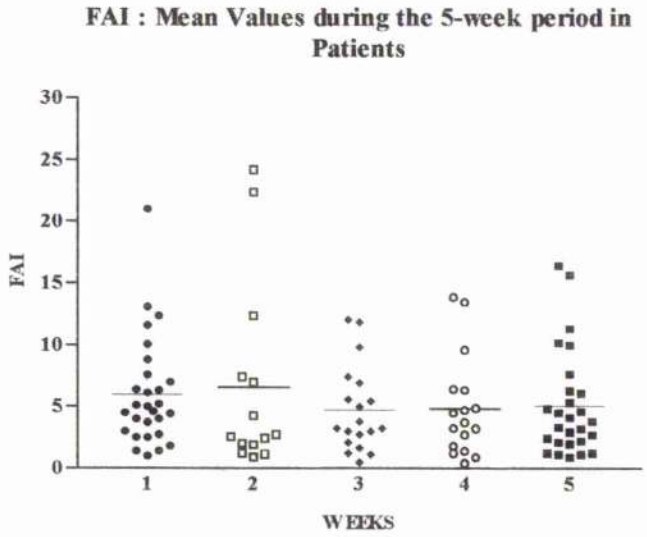


Figure 6.10 :

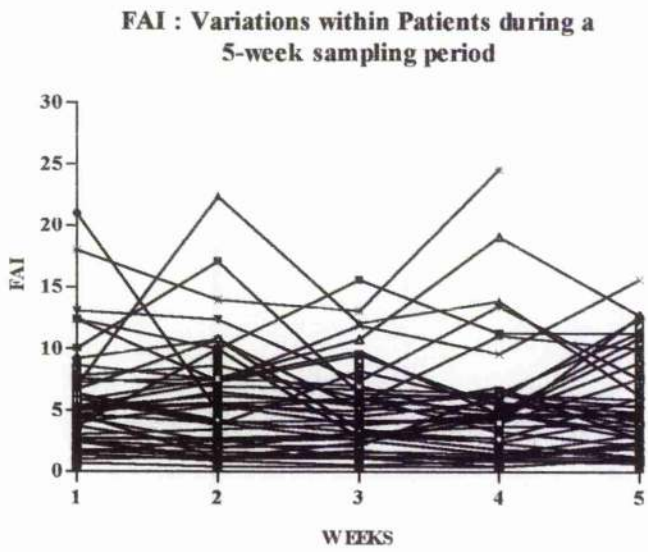


Table 6-I : Variations in the Endocrine Hormones within Patients during the 5-week Period of Observations

Hormone	Average Coefficient of Variation	range (%)
E_2 (pg/mL)	30.1 %	4.0 - 78.7
LH (IU/L)	26.0 %	10.2 - 75.4
T (nmol/L)	31.5 %	8.7 - 64.5
SHBG (nmol/L)	25.4 %	5.0 - 72.6
FAI	36.4 %	6.0 - 110.0

These wide variations in the reproductive hormone concentrations during the 5-week window of observations indicate that ovarian function is not static in oligo/amenorrhoeic patients. This emphasizes the limited value of single sample hormone assays when used as diagnostic criteria or as means to investigate the pathophysiology of the disorder.

6.2.2 RELATIONSHIP BETWEEN ANTHROPOMETRIC VARIABLES AND FOLLICULAR PHASE ENDOCRINE PROFILES

The relationships between anthropometric variables and follicular endocrine profiles in the patients were explored with reference to spontaneous ovulation.

The correlations between mean follicular endocrine profiles and BMI and WHR are demonstrated in Table 6-II. Significant relationships were only observed between the mean follicular hormones representing the androgenic state in the patients, and anthropometric variables (Figures 6.11 - 6.14).

The clearest link between endocrine and anthropometric variables was seen in the SHBG data which was also the most consistent element.

Table 6-II : Correlations between Mean Follicular Endocrine Profiles and Anthropometric Variables

	BMI		WHR	
	r	P	r	P
E_2 (pg/mL)	0.01	NS	0.09	NS
LH (IU/L)	0.03	NS	0.08	NS
FSH (IU/L)	0.05	NS	0.01	NS
LH/FSH Ratio	0.06	NS	0.002	NS
T (nmol/L)	0.38	0.013	0.14	NS
SHBG (nmol/L)	- 0.39	0.0097	- 0.43	0.0042
FAI	0.42	0.0057	0.23	NS
A (nmol/L)	0.06	NS	0.15	NS
P• (ng/mL)	0.32	NS	0.39	NS
17α-OHP (ng/mL)	0.18	NS	0.11	NS
DHEA-S (nmol/L)	0.18	NS	0.06	NS
PRL (IU/L)	0.23	NS	0.29	0.062

P• represents the mean values of luteal phases of ovulatory cycles only. P≥ 0.05 is NS

Figure 6.11 :

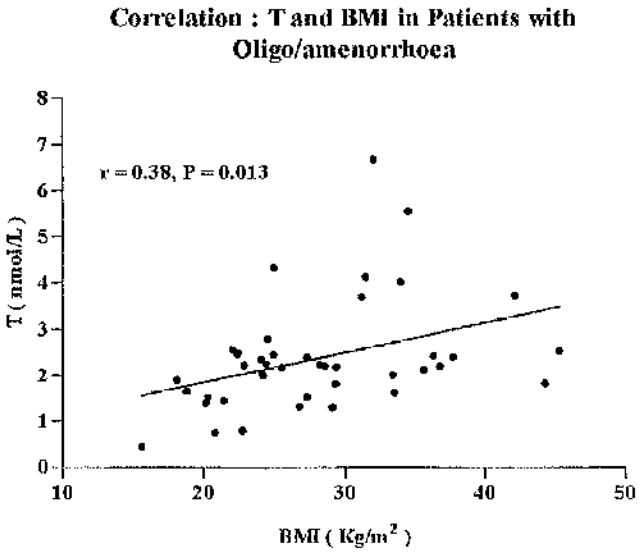


Figure 6.12 :

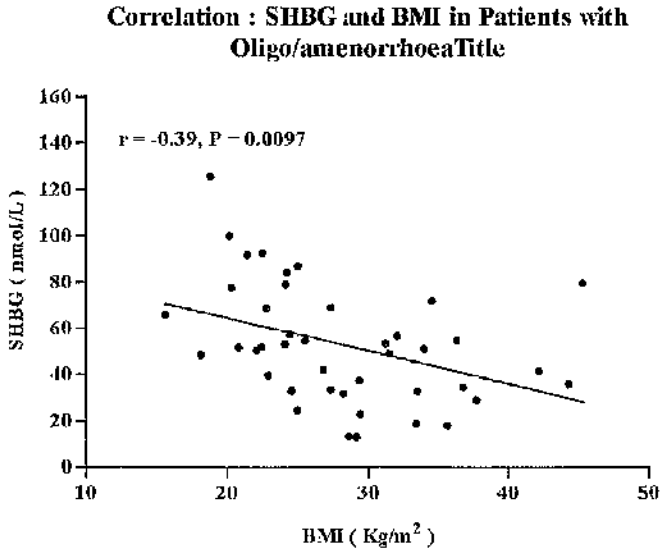


Figure 6.13 :

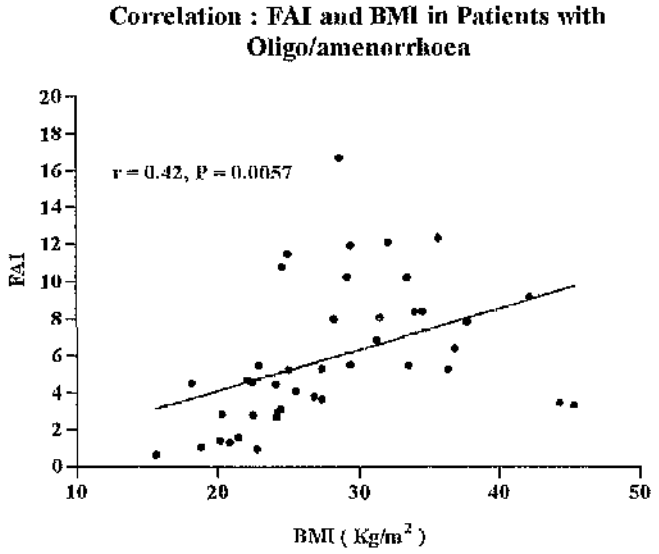
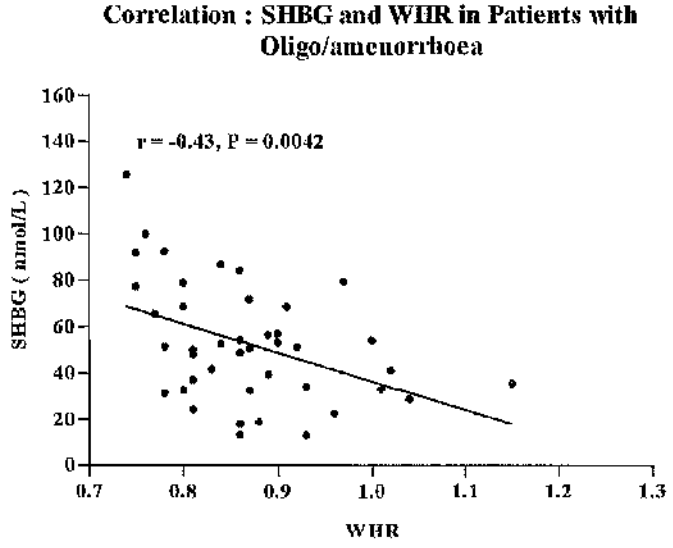


Figure 6.14 :



The mean follicular phase T, SHBG and FAI concentrations showed more powerful relationships with anthropometric variables than did the basal levels of the same hormones (Chapter Four). While the basal levels of T showed no significant relationship with BMI or WHR, the mean follicular phase T correlated significantly with the body mass. On the other hand, the relationships between anthropometric variables

and the basal as well as the mean follicular phase SHBG concentration, and subsequently FAI were consistent (Chapter Four). These relationships were even stronger with the mean follicular phase SHBG and FAI than the single basal concentrations. These observations emphasize the importance of repeated sampling of such hormones, and that a single assay is not an accurate representation of the hormone concentrations. Furthermore, the consistent and powerful relationships between SHBG and anthropometric variables (BMI and WHR) suggest that SHBG may be a better marker of the disorder than E_2, LH or T.

6.2.3 SPONTANEOUS OVULATION

Spontaneous ovulation was defined by the occurrence of a LH surge followed by a significant rise in P above 1.5 ng/mL. The LH surge was diagnosed by the presence of at least doubling of the preceding LH values from one sample to the next to a concentration > 10.0 IU/L. Evidence of adequate luteinization was confirmed if a single P concentration of ≥ 4 ng/mL was detected, at least 48 hours after the LH surge. The mean luteal phase serum P concentration was estimated to assess luteal function. This required a sample to be taken between days LH peak + 4 and LH peak + 9 inclusive.

All samples taken outside the luteal phase were designated as "follicular" phase. The mean of the samples at the follicular phase represent the mean of all the samples of the observation period for those who did not ovulate, and the mean of all the samples of the follicular phase taken at least 2 days before the day of the LH peak for those who ovulated.

Follicular development and ovulation was documented in 18 patients (43.9%) during the period of observation. All the patients who ovulated exhibited a demonstrable LH surge and luteinization with circulating P concentrations ≥ 4 ng/mL. The mean concentration of the mid-luteal phase P secretion (day LH peak + 7) for the patients who ovulated spontaneously was 13.2 ± 4.58 ng/mL (range 5 - 25.5 ng/mL).

6.2.4 ASSESSMENT OF LUTEAL FUNCTION - PROGESTERONE INDEX (PI)

The mid-luteal phase (day LH peak + 7) P secretion for the patients who ovulated spontaneously, was similar to the mean P value of the mid-luteal phase in the control group of this study (13.2 ± 4.58 ng/mL and 12.20 ± 7.16 ng/mL; respectively).

The mean luteal P value (LH peak + 4 to + 9 inclusive) was 11.60 ± 2.30 ng/mL (range 7.11 - 13.30 ng/mL). The luteal phase profile of P was quantified using a PI which describes the area under the curve of the patient's plasma P concentrations on days LH peak + 4 to + 9 inclusive, relative to that described by the mean of the laboratory normal range. The formula used was :

sum [P] (LH + 4 to LH + 9) (PT) × 100 / sum [P] (LH + 4 to LH + 9) (Laboratory normal mean) (Fleming *et al* 1995).

The laboratory normal mean data was derived from daily samples taken from 18 fertile volunteers with normal menstrual rhythm, and from 12 spontaneous conception cycles. The laboratory normal mean AUC value of P (LH +4 to +9 inclusive) was 73.4 ng/mL. A normal profile yielded a PI of 100, and the lower limit of the normal range was 70 (Fleming *et al* 1995). The mean PI of the study patients was subnormal (57.75 ± 29.56) with a range of 13.6 - 112.4. Almost half of the group had a PIs of < 70 (10 out of 18 patients); although normal P concentrations were established in all patients by mid-luteal phase (LH peak + 7 days).

Body Mass and Ovulation

There was no significant difference in BMI between patients who showed spontaneous follicular maturation and ovulation, and those who failed to ovulate, or the control group (Figure 6.15). However, WHR was higher in patients who did not ovulate compared to those patients with spontaneous ovulation and also the controls (P = 0.075, 0.0004; respectively) (Figure 6.16). When the waist measurements were compared between the groups, these relationships were less significant than when WHR measurements were used (Figure 6.17). These important observations suggest that the ratio of body fat distribution, rather than total body mass, may be related to the capacity to undergo follicular maturation and ovulation.

Figure 6.15 :

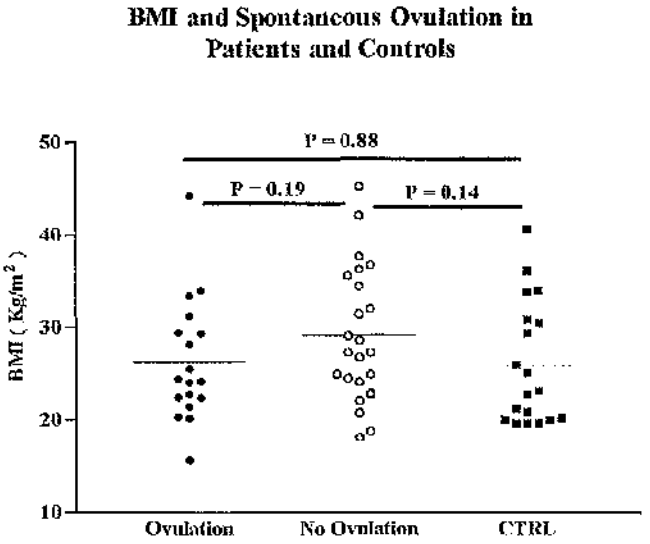


Figure 6.16 :

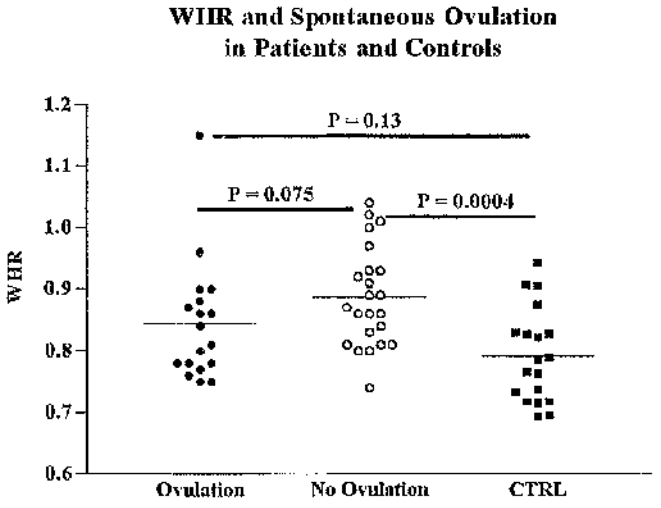
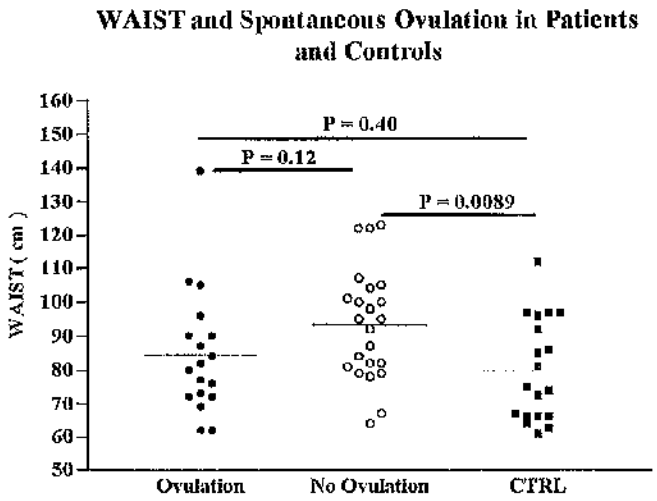


Figure 6.17 :



6.2.5 RELATIONSHIPS BETWEEN ENDOCRINE & METABOLIC VARIABLES AND SPONTANEOUS OVULATION

6.2.5.1 Reproductive Endocrinology and Ovulation

Analyses of the mean follicular phase concentrations of the reproductive hormones in patients who ovulated spontaneously (n = 18) were compared with those who did not ovulate and also with the control group (Table 6-III).

Patients who ovulated and those who did not ovulate showed similar follicular phase values of LH, FSH, LH:FSH ratio, A and 17α-OHP. Significantly lower concentrations of E_2, SHBG, FAI and PRL were determined in the anovulatory patients compared with the ovulators, while differences in T and DHEA-S were not significant.

Compared with the control group, LH, LH:FSH ratio and FAI were raised in both groups of patients; those who ovulated and those who did not.

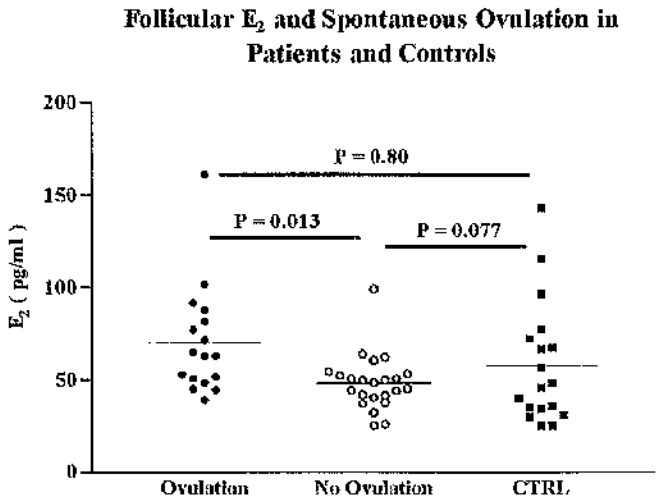
Table 6-III : Comparison of the Mean Follicular Phase Endocrine Variables in relation to Spontaneous Ovulation in Patients and Controls

	Patients			Controls		
	Ovulation	No Ovulation	P		P*	P**
n	18	23		19		
E_2 (pg/mL)	67.35 ± 19.9	48.11 ± 25.98	0.013	65.00 ± 34.20	NS	0.0765
LH (IU/L)	9.67 ± 2.76	9.98 ± 0.74	NS	5.25 ± 4.39	0.0009	<0.0001
FSH (IU/L)	4.41 ± 1.51	5.09 ± 0.45	NS	5.15 ± 1.73	0.018	NS
LH:FSH Ratio	2.11 ± 0.66	2.04 ± 1.43	NS	1.07 ± 0.80	0.0001	0.0001
T (nmol/L)	1.97 ± 0.78	2.59 ± 0.31	0.09	1.53 ± 0.93	0.40	<0.0001
SHBG (nmol/L)	56.66 ± 11.52	48.17 ± 9.13	0.012	62.53 ± 25.77	NS	0.017
FAI	4.52 ± 3.29	6.81 ± 0.48	0.047	2.62 ± 1.78	0.036	<0.0001
A (nmol/L)	7.89 ± 4.15	6.31 ± 3.33	NS	6.13 ± 2.85	NS	NS
P (ng/mL)	13.20 ± 4.58			12.20 ± 7.16	NS	
17α-OHP (ng/mL)	1.56 ± 0.32	1.70 ± 0.47	NS	1.48 ± 1.14	NS	NS
DHEA-S (nmol/L)	2762 ± 1750	2097 ± 272.0	0.079	2126 ± 1788	NS	NS
PRL (IU/L)	226.0 ± 138.6	349.9 ± 29.18	0.0002	424.3 ± 519.6	NS	NS

Groups were compared using Student's t-test. The differences between patients who ovulated and controls were expressed as (P*); whereas the differences between patient's who did not ovulate and controls were expressed as (P**). P ≥ 0.05 is NS.

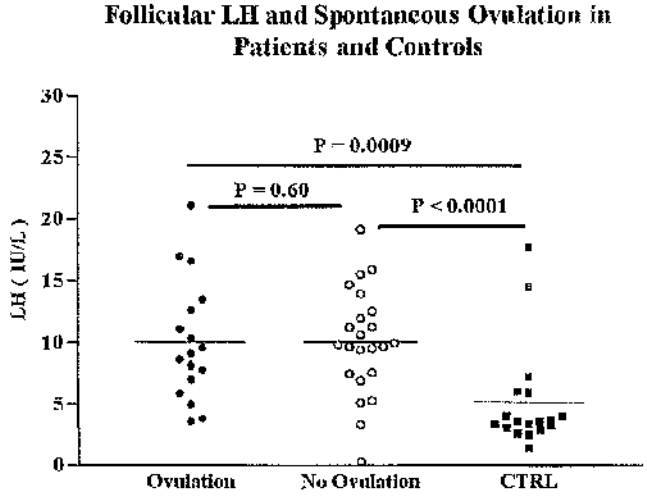
Figure 6.18 shows the distributions of the mean follicular phase E_2 concentrations amongst the patients who failed to ovulate, those who ovulated and the controls.

Figure 6.18 :



The mean follicular phase LH concentration in those patients who ovulated was similar to those who did not ovulate (9.67 ± 2.76 vs 9.98 ± 0.74 IU/L, respectively) (Figure 6.19). The basal LH concentration showed a similar observation.

Figure 6.19 :



High LH vs Normal LH

Consistently elevated follicular LH concentration was observed in 11 oligo/amenorrhoeic patients (26.8%), and 4 of these showed spontaneous ovulation with a mean PI of 33.3 ± 20.82. Six of 14 (42.9%) women with normal levels of LH were observed to ovulate. When they did ovulate, Figure 6.20 shows that the range of PI values seen in the two groups was similar, indicating that elevated LH was not directly related to the incidence of follicular maturation, luteinization, occurrence of spontaneous ovulation or the deficient luteal function observed in these patients.

Figure 6.20 :

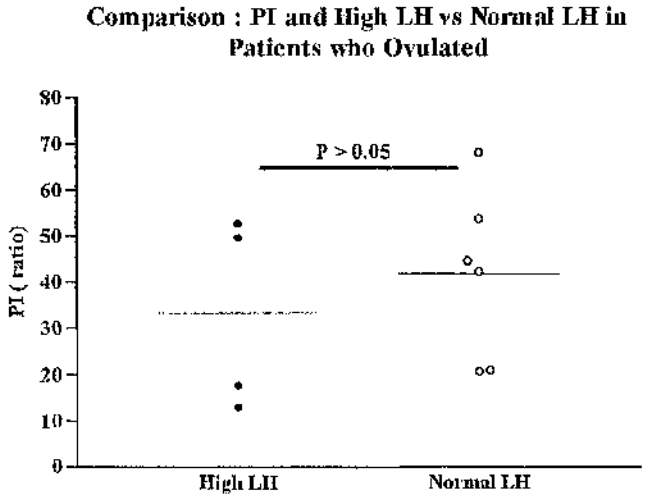


Figure 6.21 shows the distributions of the mean follicular phase T concentrations amongst the patients who failed to ovulate, those who ovulated and the controls. The data show the raised values of T in the no ovulation group compared with the controls, but the difference between them and the patients who ovulated was not significant. The differences in the distribution of SHBG in patients who ovulated, failed to ovulate and the controls are illustrated in Figure 6.22, and show broad overlaps in the data despite the statistical significance. Similarly, Figure 6.23 illustrates the significant differences in FAI among the subgroups. These observations strongly emphasize the influence of follicular SHBG concentrations, rather than LH or TT, on the occurrence of follicular maturation and spontaneous ovulation in patients with oligo/amenorrhoea.

Figure 6.21 :

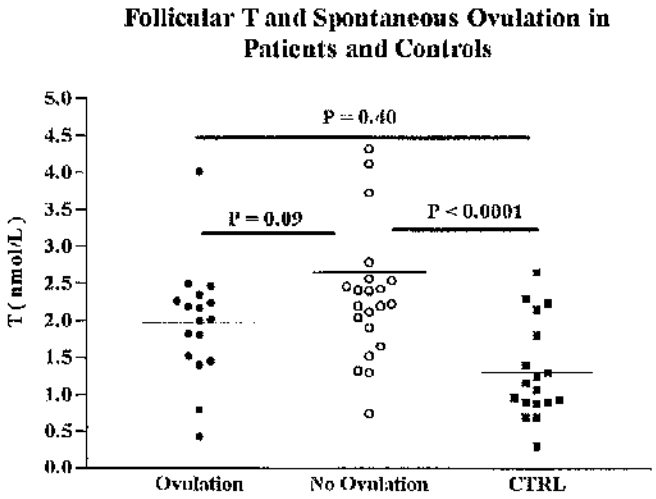


Figure 6.22 :

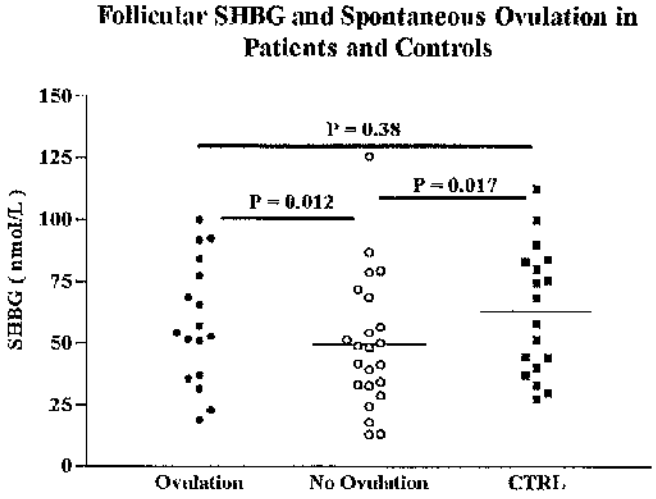
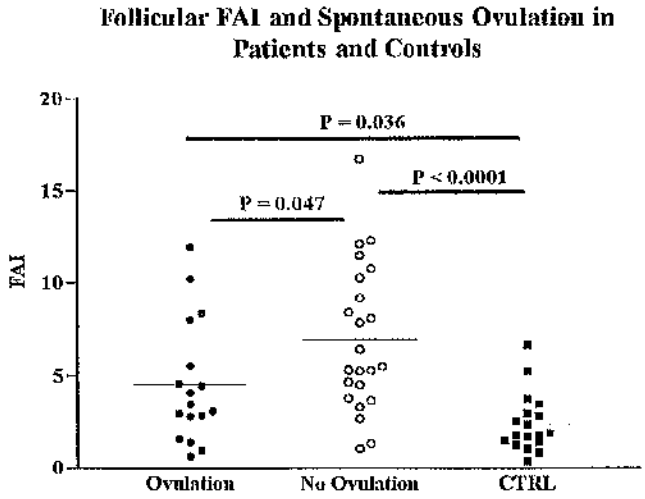


Figure 6.23 :



High T and FAI vs Normal T and FAI

There was no significant difference in the mean follicular phase T concentrations seen in patients who ovulated or those who did not (Table 6-IV). In addition, there was no significant difference in the proportion of patients with elevated T concentrations (mean ± SD of controls = 2.5 nmol/L) who showed spontaneous ovulation (4 out of 10), and those with normal T concentrations (14 out of 30, $P \geq 0.05$). However, the mean follicular T concentrations were significantly higher in the patients with elevated LH values (1.78 ± 0.74 (normal LH), 2.57 ± 1.16 (high LH); P = 0.0105) and the correlation between follicular phase LH and T concentrations was r = 0.29 (P = 0.061).

Examination of the incidence of spontaneous ovulation among patients, with respect to FAI, showed that the proportion of patients with elevated mean follicular phase FAI (mean ± SD of controls ≥ 4.5) who had spontaneous ovulation (7 out of 24) was lower than for those with normal FAI (11 out of 16, P = 0.053). This observation suggests that the more hyperandrogenized (as an effect of SHBG rather than TT) the patient is, the less likely she is to have spontaneous ovulation. Similar relationships were observed when basal concentrations of T and FAI were applied.

Table 6-IV : Relationship between T and FAI and Spontaneous Ovulation in Patients

	Spontaneous Ovulation		No Ovulation		P
	Normal (n)	High (n)	Normal (n)	High (n)	
T (nmol/L)	1.72 ± 0.59 (14)	2.78 ± 0.83 (4)	2.18 ± 0.85 (16)	4.04 ± 1.74 (6)	NS
FAI	2.38 ± 1.15 (11)	7.58 ± 2.89 (7)	2.64 ± 1.19 (5)	8.48 ± 3.44 (17)	NS

6.2.5.2 Metabolic Variables and Ovulation

Mean basal cortisol, GH, FI, fasting glucose, C-peptide and KITT- values were similar in patients who had spontaneous ovulation or patients who did not ovulate and in the control group (Table 6-V). A significantly higher mean basal IGF-I concentration was found in the controls than in both subgroups of patients, and in addition the values of IGF-I were also significantly higher in patients who ovulated than in those who did not (Table 6-V) (Figure 6.24).

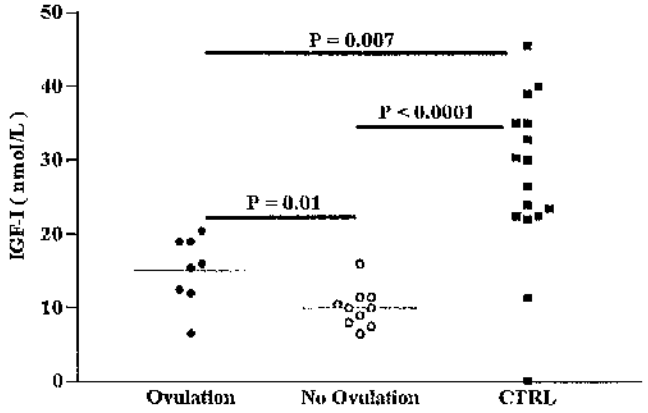
Table 6-V : Comparison of the Mean Follicular Phase Metabolic Variables in relation to Spontaneous Ovulation in Patients and Controls

	Patients			Controls		
	Ovulation	No Ovulation	P		P*	P**
n	18	22		19		
Cortisol (ug/dL)	13.03 ± 5.94	15.31 ± 7.21	NS	13.89 ± 3.99	NS	NS
GH (ng/mL)	3.09 ± 5.85	1.68 ± 3.02	NS	2.39 ± 2.38	NS	NS
IGF-I (nmol/L)	16.29 ± 4.35	11.1 ± 5.38	0.010	29.07 ± 12.48	0.007	< 0.0001
FI (mIU/L)	10.67 ± 6.98	13.78 ± 12.08	NS	8.09 ± 5.53	NS	0.066
Fasting glucose (mmol/L)	5.16 ± 0.57	5.11 ± 0.45	NS	5.09 ± 0.46	NS	NS
C - Peptide (ng/mL)	1.03 ± 0.64	1.32 ± 0.66	NS	1.05 ± 0.59	NS	NS
KITT - value (% / min)	4.94 ± 1.52	3.61 ± 2.42	NS	4.73 ± 1.92	NS	NS

Groups were compared using Student's t-test.The differences between patients who ovulated and controls were expressed as (P*); whereas the differences between patient's who did not ovulate and controls were expressed as (P**).P ≥ 0.05 is NS.

Figure 6.24 :

Relationship between IGF-I and Spontaneous Ovulation in Patients and Controls



There was no relationship between IGF-I and E2, LH, FSH, T or SHBG. However, IGF-I was significantly related to mean follicular FAI ($r = 0.48$, $P = 0.041$). It may be that IGF-I stimulates the accumulation of free circulating androgens rather than the production of androgens.

6.2.5.3 Insulin Sensitivity and Spontaneous Ovulation

Fasting insulin concentrations, GLU/INS ratio and FIRI, as simple methods to estimate insulin sensitivity, showed variable relationships with the incidence of spontaneous follicular growth and ovulation in the patient group (Table 6-VI). There was a tendency towards increasing FI and FIRI with the degree of disturbance of ovarian function, but the differences were not significant except when patients who failed to ovulate were compared with controls. The difference in FI between patients who ovulated and those who did not may be masked by a single high reading in the ovulatory group (FI = 34 mIU/L) (Figure 6.25). However, this indicates that I may not have a direct influence on the occurrence of spontaneous ovulation in these patients.

Table 6 - VI : Relationships between Spontaneous Ovulation and Insulin Resistance

	Patients			Controls		
	Ovulation	No Ovulation	P		P*	P**
n	17	17		19		
FI (mIU/L)	10.26 ± 6.97	14.15 ± 9.01	NS	8.09± 5.53	NS	0.022
Glucose/Insulin Ratio	0.61 ± 0.26	0.62 ± 0.49	NS	0.95± 0.49	0.08	0.039
FIRI	2.21 ± 1.73	2.95 ± 2.34	NS	1.70± 1.44	NS	0.054

Groups were compared using Student's t-test. The differences between patients who ovulated and controls were expressed as (P*); whereas the differences between patients who did not ovulate and controls were expressed as (P**). $P \geq 0.05$ is NS.

Although FI was significantly higher in patients who did not ovulate than in the control group; this effect was diluted when $\log_{10}$ FIRI was applied (Figures 6.25 and 6.26).

Figure 6.25 :

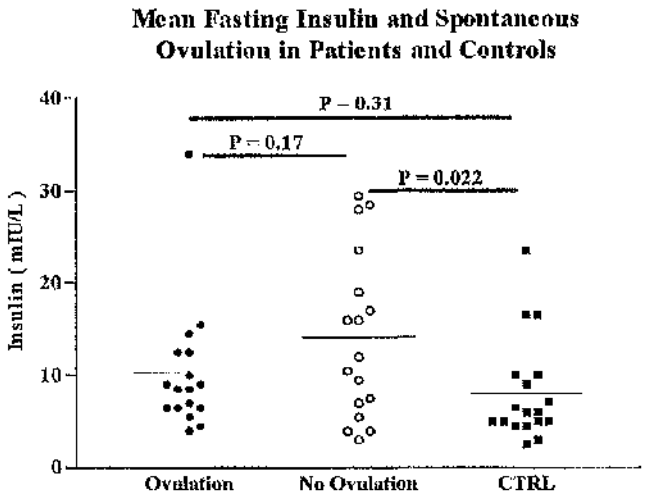
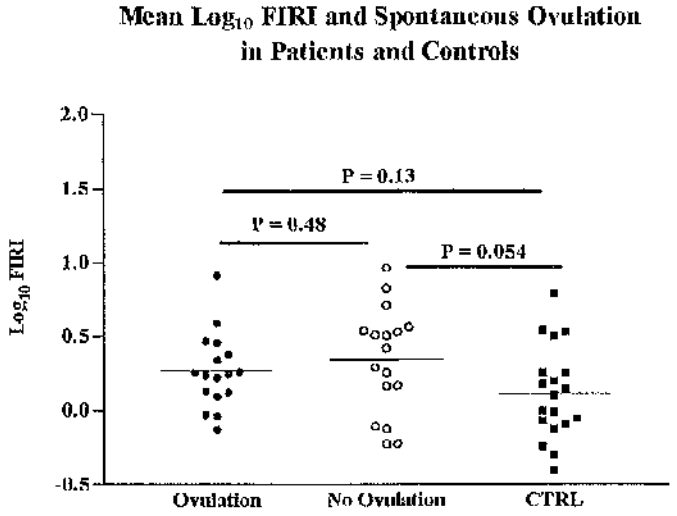


Figure 6.26 :



6.2.5.4 Leptin and Spontaneous Ovulation

The mean plasma concentrations of leptin were similar in patients and controls (Table 6-VII). The mean leptin concentration in patients who ovulated was lower than patients who did not ovulate but the

difference did not reach statistical significance (18.75 ± 13.28 vs 27.54 ± 16.66 ng/mL, P = 0.075). No correlation was found between leptin and E_2, LH, FSH, T, SHBG or FAI.

The strong correlations between leptin concentrations and anthropometric variables in patients and controls, are demonstrated in Table 6-VII and Figures 6.27 - 6.30. The serum leptin levels were higher in obese individuals, patients and controls. Leptin concentrations were significantly higher in lean patients than lean controls ($P < 0.05$), but there was no such difference between obese patients and their counterpart obese controls.

Table 6-VII : Mean Leptin Concentrations and BMI in Patients and Controls

	Patients	Controls	P
n	40	19	
All	23.69 ± 15.53 (3.85 - 66.01)	21.77 ± 16.79 (3.68 - 56.14)	NS
BMI < 29 kg/m^2	15.97 ± 9.90 (3.85-40.28)	9.46 ± 4.28 (3.68-18.17)	0.0448
BMI ≥ 29 kg/m^2	35.03 ± 15.52 (10.0-66.01)	37.89 ± 12.2 (25.37-56.14)	NS
WHR < 0.8	9.25 ± 4.87 (3.85-17.77)	11.05 ± 7.64 (3.68-29.33)	NS
WHR ≥ 0.8	27.08 ± 15.23 (5.52-66.01)	33.70 ± 16.28 (8.34-56.14)	NS

Although the relationship between serum leptin concentrations and fat distribution, expressed as WHR, was more pronounced in patients than the controls, it is apparent that the association of leptin with obesity was similar in the two groups, there being no difference between the correlation criteria (slope, r, and intercept) (Figure 6.27).

Waist measurements were correlated with leptin concentrations (Figure 6.29) and confirmed the strong correlations shown with the other anthropometric variables. This emphasizes that leptin is influenced by body fat, especially truncal obesity.

Figure 6.27 :

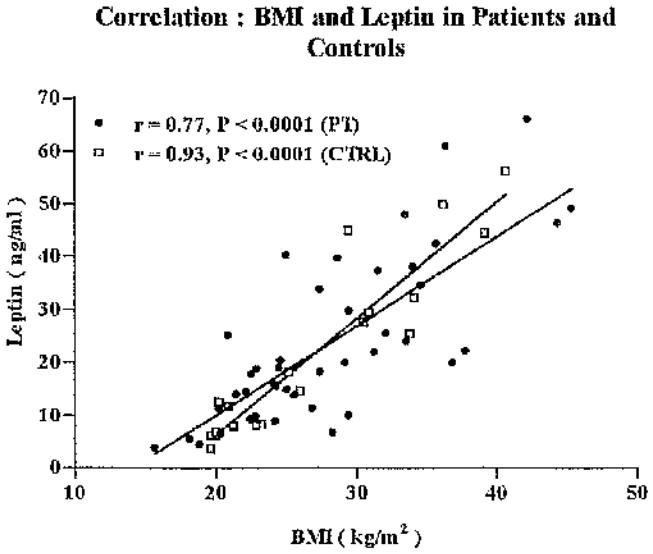


Figure 6.28 :

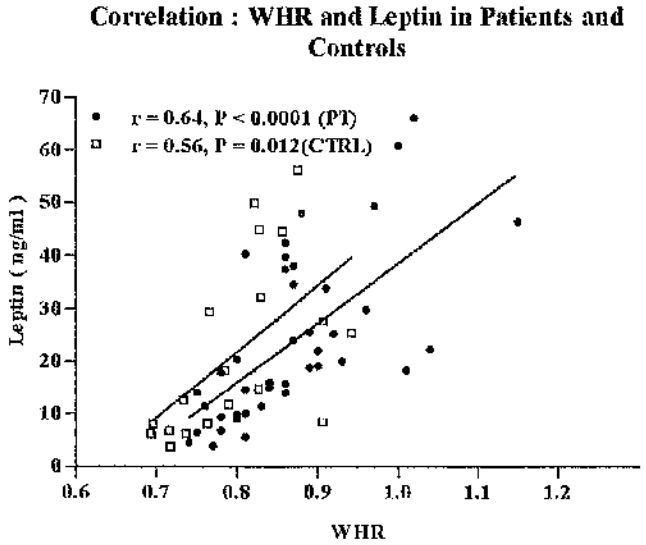
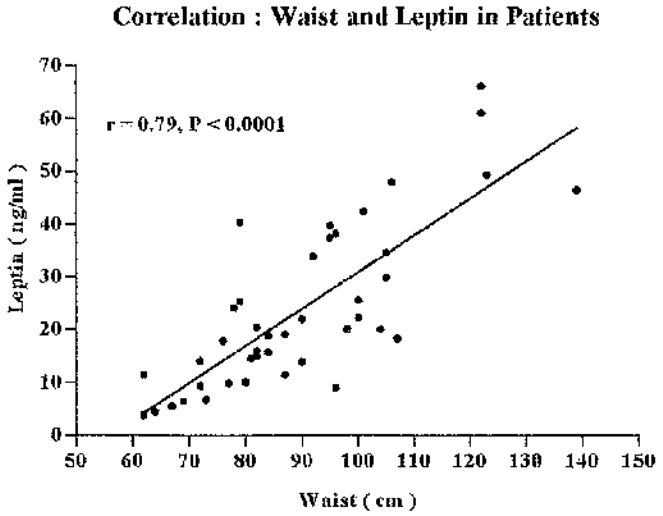


Figure 6.29 :



The relationship between Leptin, BMI and the occurrence of spontaneous ovulation in patients are shown in Table 6-VIII and Figures 6.30 - 6.31. Leptin levels were significantly higher in obese patients whether they ovulated or not. However, in lean anovulatory patients the range of leptin concentration was markedly higher than the range in ovulatory patients. Therefore, the difference in the mean leptin concentration between the lean subgroups could be masked by the wide variation between patients as demonstrated by a high SD.

Table 6-VIII : Relationship between Leptin and Spontaneous Ovulation in BMI-matched Patients

	Spontaneous Ovulation			No Ovulation		
Leptin (ng/mL)	Lean (n=12)	Obese (n=6)	P	Lean (n=11)	Obese (n=11)	P
mean ± SD	12.92 ± 4.81	32.32 ± 14.73	0.0006	17.00 ± 11.22	34.92 ± 18.48	0.0098
range	3.85 - 19.01	10.00 - 47.92		4.44 - 39.71	6.46 - 66.01	

Groups were compared using Student's t-test. The differences were expressed as P-value with $P \geq 0.05$ is NS.

The effect of leptin on the occurrence of spontaneous ovulation was masked in the obese individuals. However, when lean individuals were examined, a borderline significance was found between lean patients and lean controls (Table 6-IX).

Table 6-IX : Relationship between Leptin and Spontaneous Ovulation in Lean patients Compared to Lean Controls

	Lean Patients			Lean Controls		
Leptin (ug/mL)	Ovulatory	Anovulatory	P		P*	P**
n	12	11		11		
mean ± SD	12.92 ± 4.81	17.00 ± 11.22	0.075	9.46 ± 4.28	0.0837	0.050
range	3.85 - 19.01	4.44 - 39.71		3.68 - 18.17		

Groups were compared using Student's t-test. The differences between patients who ovulated and controls were expressed as (P*); whereas the differences between patients who did not ovulate and controls were expressed as (P**). P ≥ 0.05 is NS.

Figure 6.30 :

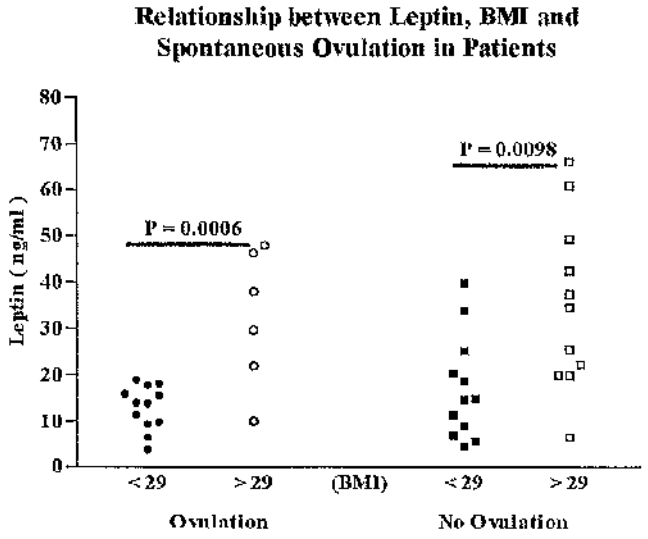
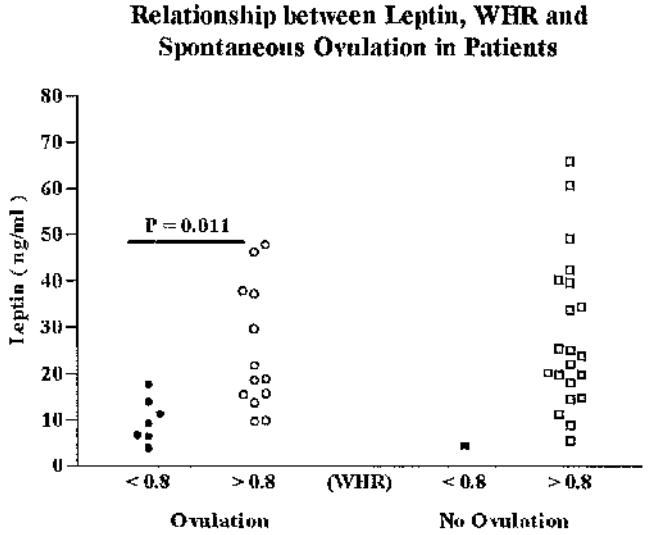


Figure 6.31 :



It is apparent that the occurrence of ovulation was not directly related to leptin concentrations when compared in BMI-matched or WHR-matched patients (Figure 6.32 and 6.33). Similar observations were found when patients were compared to their weight-matched controls (Figure 6.34).

Figure 6.32 :

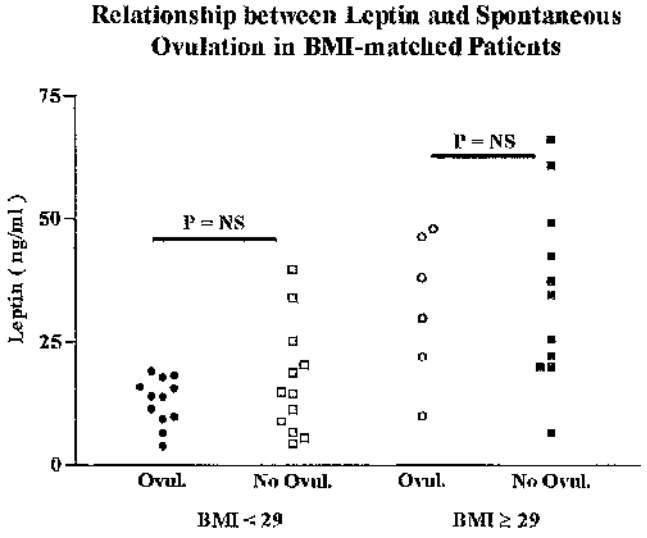


Figure 6. 33 :

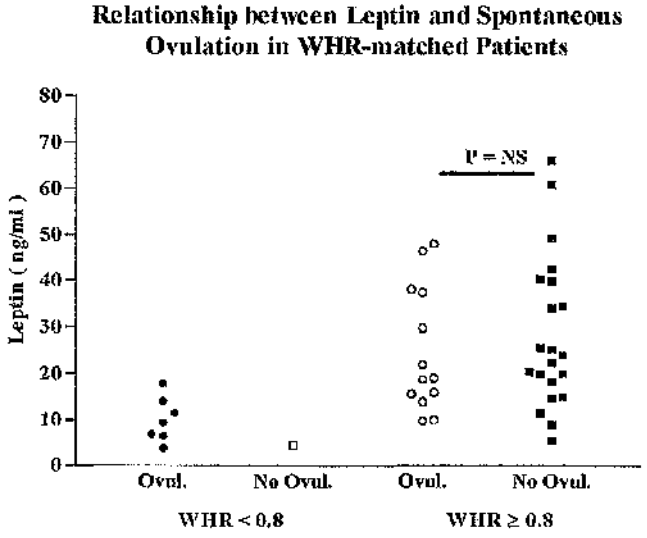
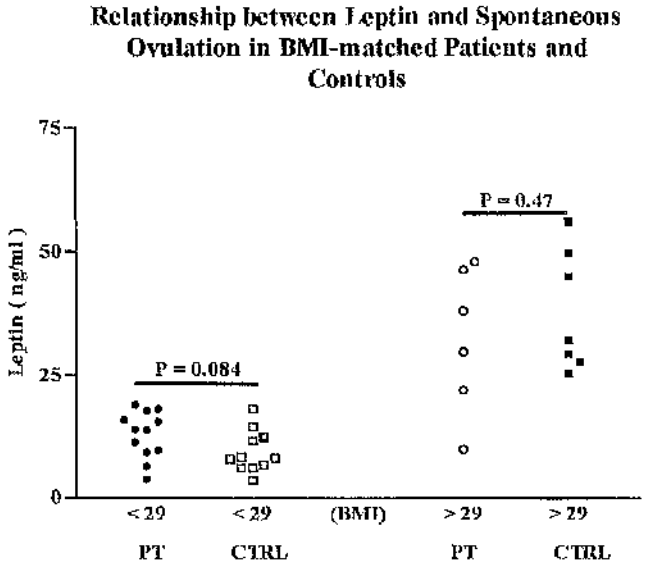


Figure 6.34 :



Figures 6.35 - 6.37 show that there were similar relationships between serum leptin concentrations and authropometric variables in the two groups of patients : ovulators and non-ovulators. Figure 6.35 shows that the relationship between leptin and BMI was similar in the ovulatory patients and the controls, and that the patients showed parallel but slightly offset relationship (i. e. for a given BMI, leptin was higher in the anovulatory patients but the difference was not significant). It is unlikely that the occurrence of spontaneous ovulation in oligo/amenorrhoeic patients is directly influenced by leptin levels.

Figure 6.35 :

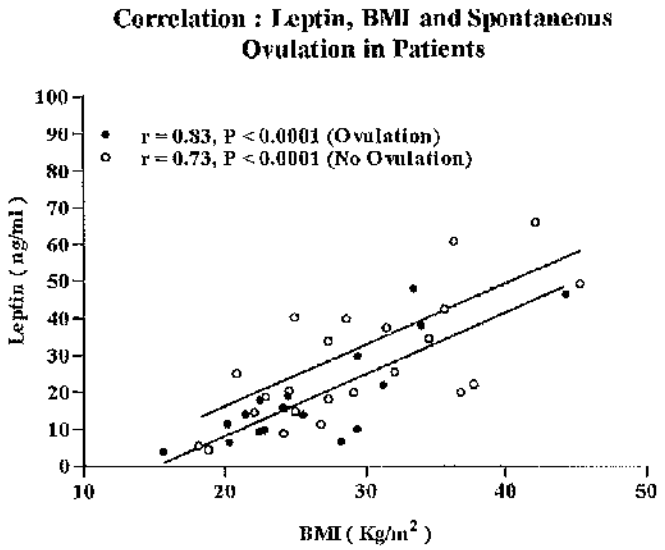


Figure 6.36 :

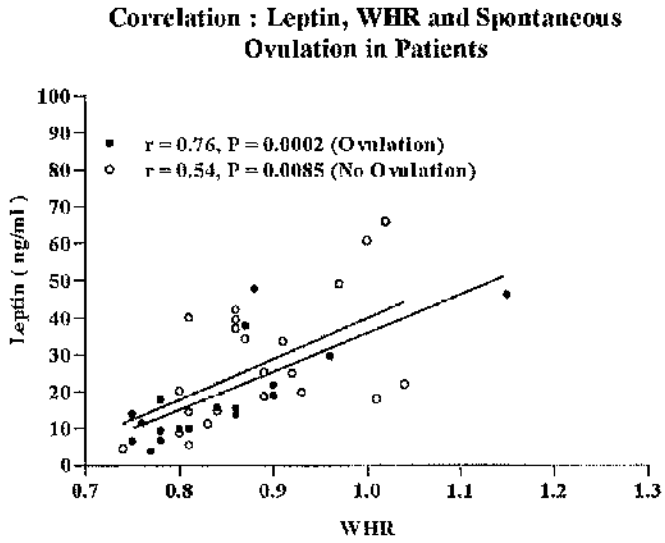
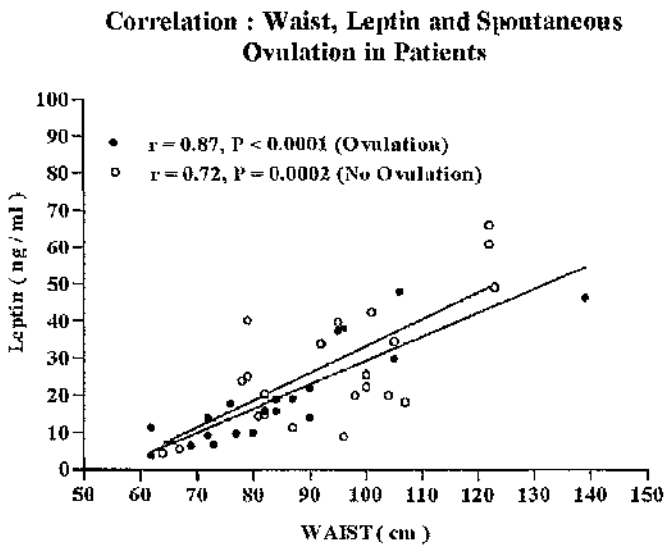


Figure 6.37 :



6.2.6 CORRELATION BETWEEN LEPTIN AND INSULIN RESISTANCE

Highly significant relationships were observed between serum leptin concentrations and the indices of IR, expressed as FI and $\log_{10}$ FIRI, and fasting glucose (Figures 6.38 - 6.40). More significant relationships were observed between FI and leptin and $\log_{10}$ FIRI and leptin concentrations in ovulatory than anovulatory patients (Figure 6.41 and 6.42). Patients with IR (high FI and FIRI) showed significantly higher leptin concentrations. This may indicate that spontaneous ovulation is indirectly influenced by the strong relationship between leptin and IR. There were no relationship between leptin and IGF-I concentrations.

Figure 6.38 :

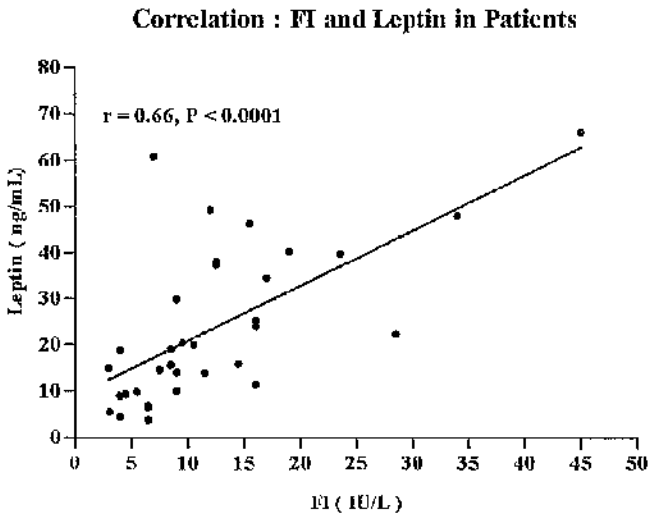


Figure 6.39 :

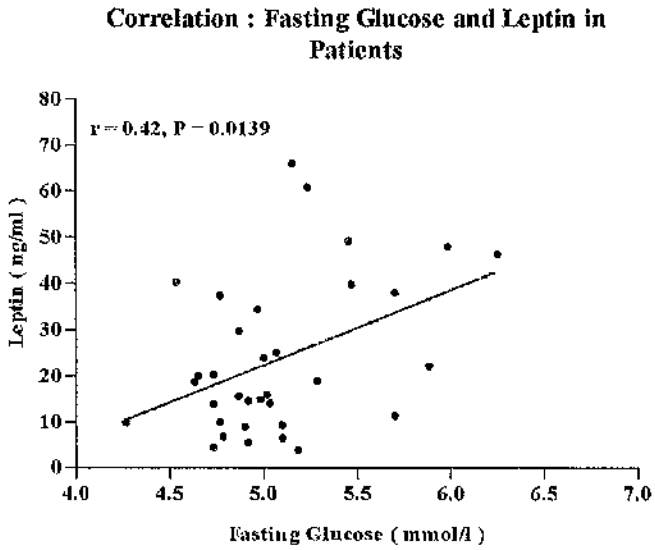


Figure 6.40 :

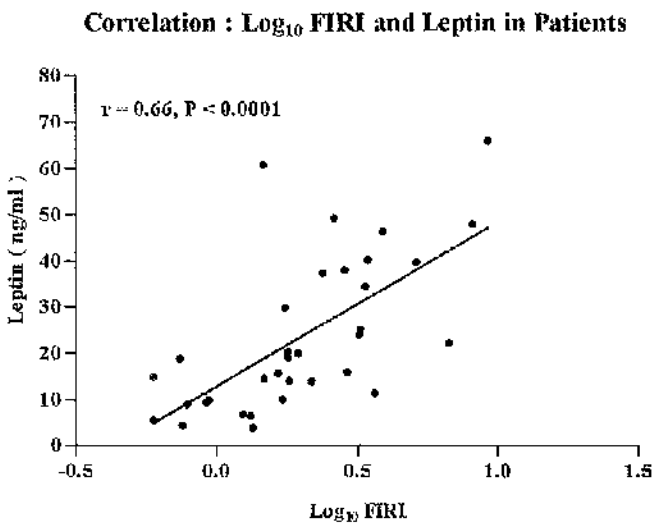


Figure 6.41 :

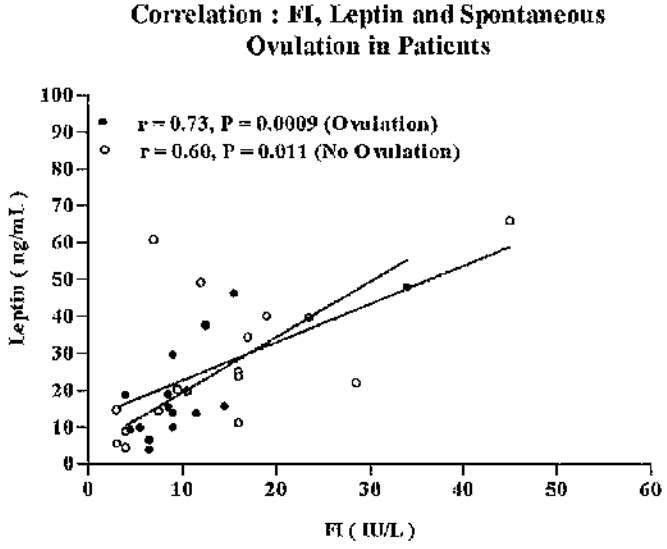
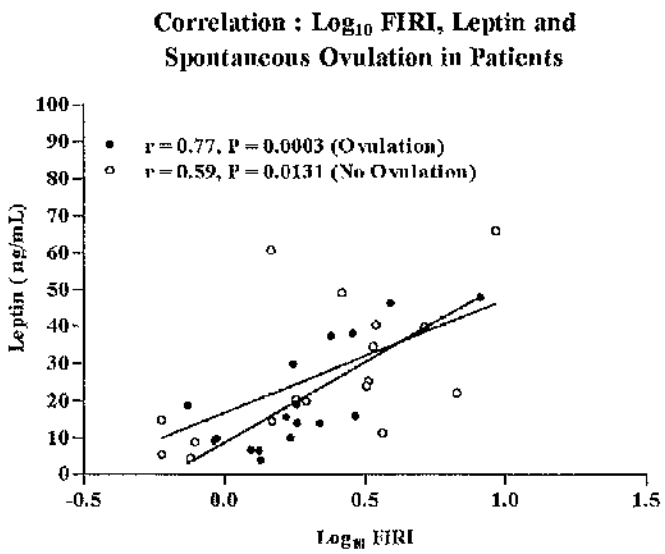


Figure 6.42 :

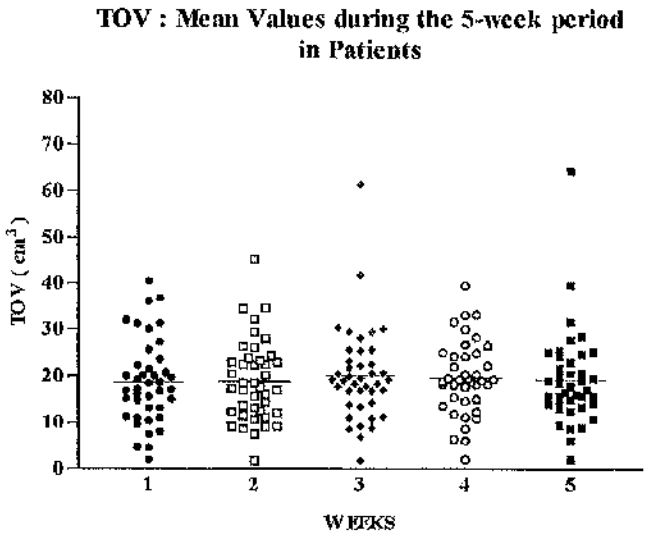


6.3 CHANGES IN OVARIAN ULTRASOUND VARIABLES DURING THE WINDOW OF OBSERVATIONS

6.3.1 VARIATIONS OF TOTAL OVARIAN VOLUME (TOV)

There was a wide range of variations in TOV within the whole group of patients during the 5-week window of observation (Figure 6.43). These variations were even more pronounced within individual patients (Figure 6.44). The range of TOV was wide, varying from < 5 cm^3 to 65 cm^3, (the average coefficient of variation was 23%, range 2.16 - 56.8%).

Figure 6.43 :



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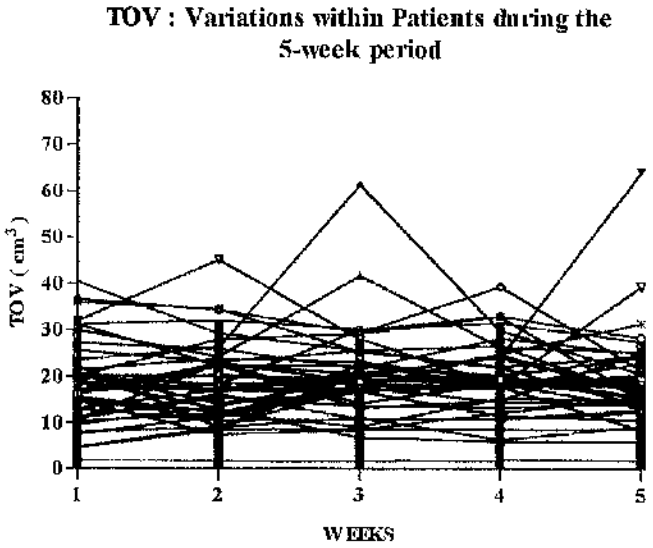


6.3.2 DYNAMIC CHANGES IN TOV

The dynamic changes in ovarian volume were correlated to the changes in the reproductive hormones, the changes in the metabolic variables and the occurrence of spontaneous ovulation during the 5-week window of observations.

6.3.2.1 Relationship with Reproductive Hormones

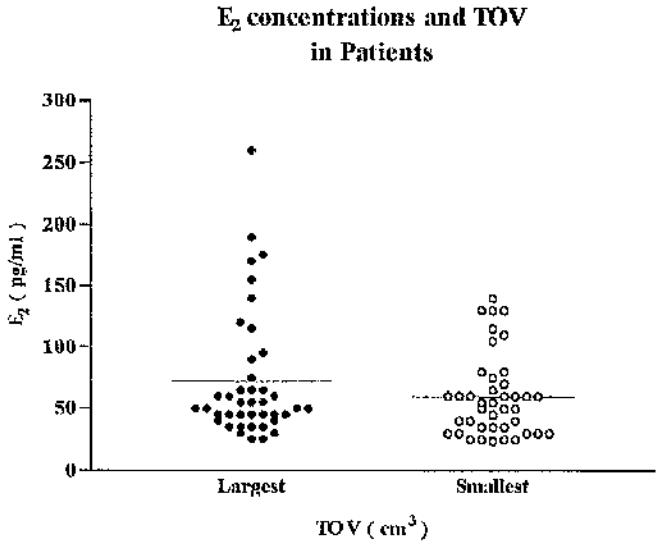
There was no relationship between the TOV and any of the reproductive hormones. In addition, the levels of E_2, LH, FSH, T, SHBG and FAI assayed on the day of the ultrasound scan at the smallest and the largest TOV are demonstrated in Table 6-X and Figures 6.45 - 6.47. It is apparent that there was no influence of ovarian volume on the concentrations of the reproductive hormones.

Table 6-X : The Concentrations of Reproductive Hormones at the Smallest and the Largest TOV during the Window of Observations

	Smallest TOV	Largest TOV	P
E2 (pg/mL)	59.9 ± 33.2	72.4 ± 52.4	NS
LH (IU/L)	9.6 ± 4.9	10.6 ± 5.0	NS
FSH (IU/L)	5.3 ± 2.7	5.6 ± 5.8	NS
T (nmol/L	2.7 ± 1.7	2.6 ± 1.5	NS
SHBG (nmol/L)	55.9 ± 33.3	63.9 ± 32.9	NS
FAI	5.8 ± 4.5	5.1 ± 3.8	NS

The data was compared using paired t-test. $P \geq 0.05$ is NS

Figure 6.45 :



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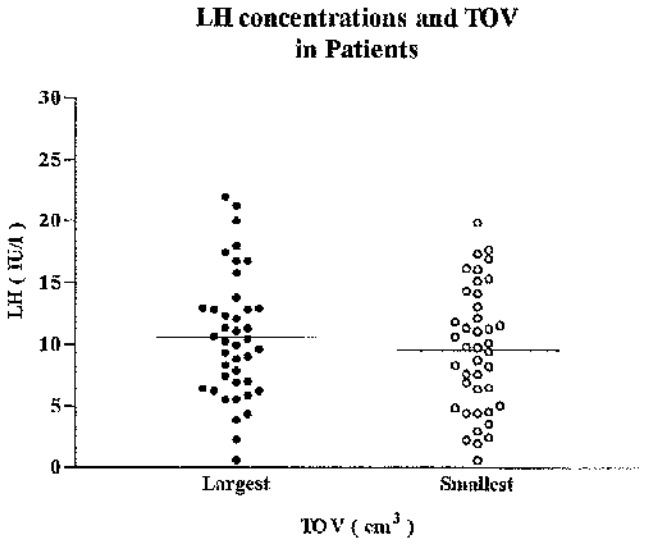

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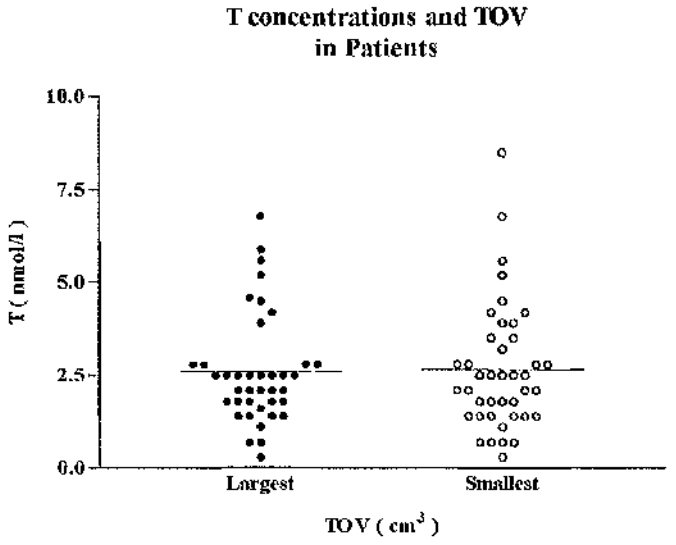


The patients' 5-weeks TOV mean (mean of each patient's 5-week mean TOV) was 19.33 ± 7.45 cm^3.

There were wide dynamic changes in TOV during the period of observation, with 68.3% of patients showing > 60% change in their TOV and 41.5% had ≥ 100% change in TOV during the same period. However, there was no difference in endocrine profiles or anthropometric variables between patients who showed these changes in TOV and those who did not.

The mean difference between maximum and minimum TOV for the patients was 11.23 ± 9.62 cm^3. About half of the patients showed a variation in their TOV more than the group mean difference between minimum and maximum volume.

6.3.2.2 Relationship with Anthropometric Variables

The mean values of TOV of the patients did not correlate with BMI or WHR during the 5-week window of observations. In addition, when the patients were subgrouped to BMI < or ≥ 29 Kg/m^2, TOV measurements were similar in obese and lean patients (Figure 6.48). Similarly, TOV was not significantly different in patients with WHR ≥ 0.8 and those with WHR < 0.8 (Figure 6.49).

Figure 6. 48 :

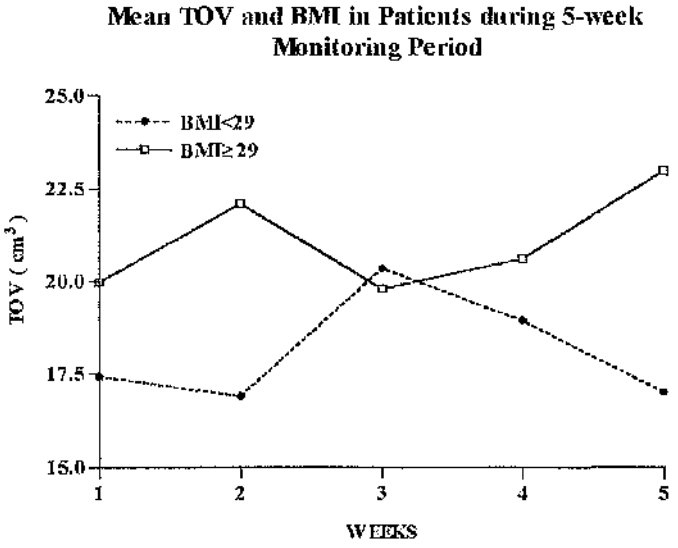
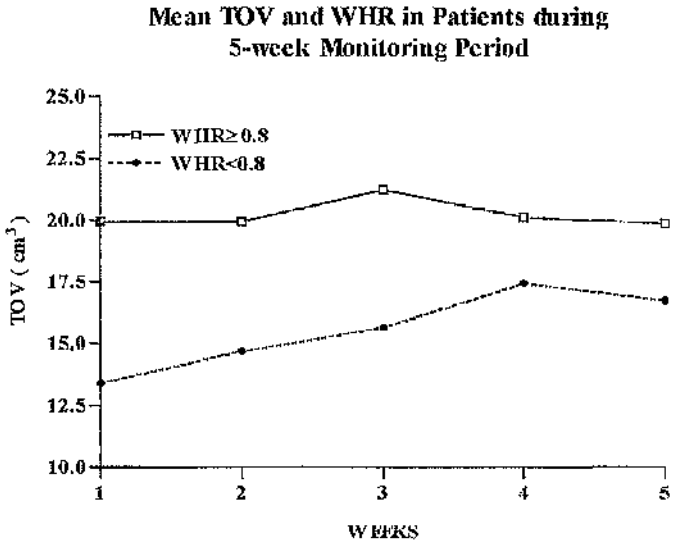


Figure 6.49 :



There was no relationship between the anthropometric variables and the number of follicles or the presence of thick stroma.

6.3.2.3 Relationship with Insulin Resistance and Metabolic Variables

There was no relationship between mean TOV and FI, $\log_{10}$ FIRI, GH or cortisol. Also none of the metabolic variables was correlated with the number of follicles or follicular distribution.

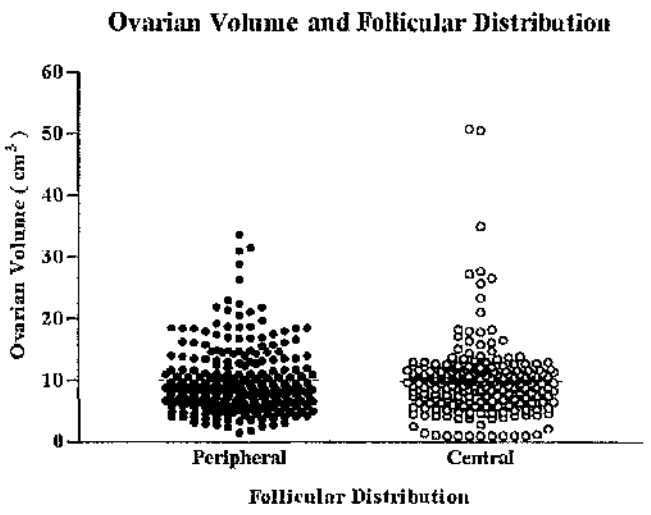
6.3.3 FOLLICULAR DISTRIBUTION (PERIPHERAL / CENTRAL)

There were 407 ultrasonographic ovarian observations for follicular distributions. On 234 occasions (57.5%) a peripheral follicular distribution was observed. Only 6 patients showed a central follicular distribution throughout the period of monitoring, 12 patients always showed a peripheral follicular distribution and the rest of the patients had a mixed picture.

Interestingly, a change from peripheral to central follicular distribution or the reverse was noticed in one or both ovaries in 63.4% of patients, with a total number of 72 changes, seen equally in the right and left ovaries.

The mean ovarian volume was similar when follicles were peripherally (10.3 ± 7.0 cm^3) or centrally (9.8 ± 6.4 cm^3) distributed (Figure 6.50).

Figure 6.50 :

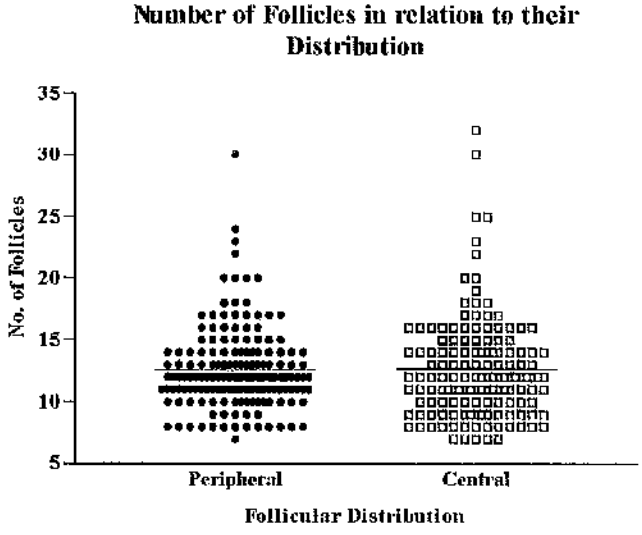


However, when patients with only centrally distributed follicles (6 patients) were compared with those patients with only peripheral (12 patients) or with mixed (peripheral and central) pictures (24 patients), the following was observed : the mean TOV for centrally distributed follicles (13.47 ± 1.54 cm^3) was significantly smaller than the mean TOV for peripherally distributed follicles (18.40 ± 1.05 cm^3) and that of the mixed picture (21.0 ± 0.94 cm^3) (P = 0.0004, < 0.0001; respectively). In addition, patients with centrally distributed follicles had significantly lower mean follicular phase T concentrations and FAI than patients with peripheral or mixed follicular distribution. The mean follicular phase SHBG concentrations were similar in patients who had centrally or peripherally distributed follicles but significantly higher than patients with a mixed picture. The mean follicular phase E_2, LH and FSH were similar in the three groups.

The mean number of follicles per ovary during the 5-week window of observations was 12.5 ± 2.9 (range 5-24). There was no difference in the number of ovarian follicles between patients or within patients. The mean number of follicles was similar when peripherally or centrally distributed. The number of follicles was significantly correlated with TOV (r = 0.53, P = 0.0051) during the 5-week period of observation.

It is apparent from the data, that the larger the ovarian volume the greater the number of follicles pushed peripherally by the thickened ovarian stroma due to hyperandrogenization. However, the number of follicles does not influence their distribution (Figure 6.51).

Figure 6.51 :



6.3.4 STROMAL DENSITY

Thick stroma was demonstrable in both ovaries by US scanning in 31 patients (75.6%), while only 8 patients (19.5%) had a unilateral thick stroma. The TOV measurements were significantly larger in patients who showed bilaterally thickened stroma compared with a unilateral observation during the period of monitoring (Table 6-XI). However, there was no difference in BMI, WHR, E_2, LH, FSH, T, SHBG, FAI and A between these two group of patients.

Table 6-XI : The TOV in Bilateral versus Unilateral Ovarian Thickened Stroma in Patients

	Bilateral	Unilateral	P
n	31	8	
Week -1	20.20 ± 8.10	12.53 ± 10.68	0.0238
Week-2	20.42 ± 7.90	12.78 ± 9.71	0.0188
Week-3	21.67 ± 9.90	13.84 ± 8.43	0.0365
Week-4	20.66 ± 6.40	15.26 ± 11.84	0.073
Week-5	20.78 ± 10.58	13.15 ± 5.75	0.045

6.3.5 ULTRASOUND OBSERVATIONS IN PATIENTS WITH SPONTANEOUS OVULATION

The total number of patients who showed spontaneous ovulation was 18. Surprisingly, during the 5-week window of observations, the mean TOV of patients who ovulated was similar to that of patients who failed to ovulate. The variability in the TOV (difference between minimum and maximum TOV) was no different in this group compared with those who did not ovulate. These observations indicate that in the event of or absence of spontaneous ovulation there is still a large variation in ovarian volume. These changes may also weaken the significance of ovarian volume as a criterion to diagnose PCO as 48.8% of patients showed both " normal " and " supranormal " values.

An equal proportion of patients with a unilaterally (3 out of 8) or bilaterally thickened stroma (15 out of 31) showed spontaneous ovulation.

6.3.6 CORRELATION BETWEEN ULTRASONOGRAPHIC OBSERVATIONS AND CLINICAL PRESENTATION

The TOV or follicular distribution were not related to the length of oligo/amenorrhoea or years of infertility. However, a negative relationship was found between the number of ovarian follicles and the duration of oligo/amenorrhoea ($r = -0.35$, $P = 0.0478$). This means less E_2 and T, indicating less ovarian activity, therefore, fewer oestrogen withdrawal or ovulatory bleeds in these patients.

Patients who had a unilaterally thickened stroma had a significantly longer duration of oligo/amenorrhoea (18.0 ± 14.2 weeks) than those with bilateral (11.73 ± 5.13 weeks) ($P = 0.0395$) changes. No relationship was found between the presence of a unilateral or bilateral thickened stroma and the occurrence of androgenization or virilization.

6.4 DISCUSSION

This study presents data from longitudinal endocrine assessment in patients with oligo/amenorrhoea, and relates ultrasonic evaluation and anthropometric variables to each other.

ENDOCRINE VARIABLES

In this study, the wide range of variations in the profiles of E_2, LH, FSH and T concentrations within the whole group of patients as well as within individual patients during the 5-week window of observations, clearly confirm that ovarian function is dynamic in these patients in spite of oligo/amenorrhoea and the rarity in the occurrence of follicular growth and spontaneous ovulation. This emphasizes the importance of sequential studies, and reveals the limitation of single assessments.

Yen *et al.* (1970b) found that the variation in the fluctuation of daily serum LH concentrations was rather high between patients as well as within patients, although the daily serum FSH concentrations were constant. In patients with PCOs, the cyclic pattern of LH and FSH is typically absent and there is a disproportionately high secretion of LH with constant low FSH secretion resulting in anovulation (Yen *et al* 1970b). It was reported that the high LH concentrations found in PCO patients appeared to be the result of greater amplitude or increased frequency of pulsatile LH release or both (Yen, 1980), and that in

about 10% of PCO patients, basal levels and the pulsatile pattern of LH release are indistinguishable from those of normal cycles in women, which may simply reflect day-to-day variation in LH release or the time at which the sample is taken. More than half (57.1%) of the patients in the present study showed both normal and supra-normal LH concentrations.

Amongst the study patients, SHBG showed the least variation during the period of observation in comparison to the other endocrine variables measured. This observation indicates that SHBG (and subsequently FAI) may be the most suitable endocrine variable for diagnosing or investigating the pathophysiology of the disorder in patients with oligo/amenorrhoea.

SPONTANEOUS OVULATION

Documentation of Spontaneous Ovulation

The elevated LH levels, seen in patients with PCOD, are not caused by an inability of the hypothalamic-pituitary system to respond to the negative or positive feedback effects of oestrogen (Yen, 1980) because following an increase in endogenous E_2 from an induced or spontaneous follicular maturation, an LH surge with subsequent ovulation does occur (Yen, 1980).

About 42% of the study patients showed follicular development and ovulation was documented in all these cases. All the study patients who ovulated spontaneously exhibited a demonstrable LH surge and luteinization with circulating P concentrations $\geq$ 4 ng/mL. These findings confirms Yen's observation.

Fleming *et al.* (1995) reported a similar occurrence of follicular development in their patients with oligomenorrhoea and that a LH surge and luteinization were confirmed in most of their cases.

A lower incidence (25%) of spontaneous ovulation in patients with PCOD was reported by Baird *et al.* (1977) who used 6-weeks period of observation and urinary products to assess ovarian function. This observation was interpreted as that occasional ovulatory cycles may occur spontaneously in patients with PCOD presenting with oligo/amenorrhoea (Baird *et al* 1977). The finding of a corpus luteum as an evidence of ovulation was demonstrable in 22% of the cases with PCOD (Pache *et al* 1990).

In normal menstrual cycles, the LH peak was observed between cycle day 13 and 18, reflecting a considerable variation in ovulation time (Pache *et al* 1990), which is exaggerated in patients with menstrual disturbances, especially oligo/amenorrhoea. Although the window of observation was

extended to 5-weeks, and also blood sampling started > 2 weeks after last menstrual period, it is probable that the incidence of spontaneous ovulation was underestimated.

The discrepancy in the incidence of spontaneous ovulation in patients with PCOD in the literature could be due to the diagnostic criteria of the patients, the wide variation in the presentation, the selection of patients and period of observation, and when the observations were started. Most methods lead to underestimation of the phenomenon because of the variability seen.

The mean concentrations of the mid-luteal phase P secretion for patients of this study who had spontaneous ovulation was 13.2 ± 4.58 ng/mL (range 5 - 25.5 ng/mL) and was similar to the mean P value of the mid-luteal phase in the control group. In addition, the mean of the mid-luteal phase P secretion was almost equal to the mean P (LH peak + 4 to + 9 inclusive) value. These findings indicate that the study patients generally showed adequate luteal function.

The key feature of anovulation in PCOD is failure of normal follicular maturation. Poor follicular maturation is associated with subnormal or absent P secretion and therefore, unopposed oestrogen production by the ovary or by peripheral tissues. The normal negative feedback control of the pituitary is disturbed and the rise of FSH, which occurs in the late luteal phase of normal cycles and is responsible for normal development of the next crop of follicles, fails to occur (Franks *et al* 1985). Perhaps the low FSH levels during the follicular phase induce abnormal follicular maturation or require longer periods of stimulation to initiate follicular maturation (Wu & Mikhail, 1979). Elevated LH and/or androgen levels, hyperinsulinaemia and IR, are believed to be crucial factors involved in maturation arrest of follicles in PCO (Anderson, 1974). However, there is no suggestion from the present study that LH or T have any relationship with the incidence of ovulation. These factors are discussed separately below.

Progesterone Index

In this study, in order to assess the luteal function more accurately, the luteal phase P profile was quantified by the PI. Although normal P concentrations were established in all of the study patients by mid-luteal phase assessment, the mean PI was sub-normal with almost half of the group having a PI of < 70 (lower limit of normal). This indicates that a single measurement of mid-luteal serum P concentration may be inadequate for evaluation of luteal function and ovulation.

A sub-normal PI (< 70) was demonstrated in the majority of the ovulatory cycles, and the mean values of PI for the patients with normal and with elevated LH were similar. This confirms that the occurrence of spontaneous ovulation and the inadequacy of the luteal phase P profiles were independent of follicular phase LH concentrations.

Sherman and Korenman (1974) were first to describe the subnormal P concentration profiles in infertile oligomenorrhoeic patients, indicating an inadequate luteal phase due to their long cycles. Wu & Mikhail (1979) in a small series showed that in oligomenorrhoeic patients ovulation may fail to occur, despite an adequate LH surge, and if ovulation occurs, it tends to be abnormal leading to a deficient luteal phase.

Sub-normal mean plasma P profiles in the early luteal phase in 53% of their oligomenorrhoeic patients who demonstrated LH surges, were reported by Fleming *et al.* (1995) although normal P concentrations were established by the mid-luteal phase. The same authors also found that the proportion of patients with elevated LH was no different from that of the whole group with oligomenorrhoea and that the mean follicular phase LH concentration of those patients showing a normal PI was similar to the mean LH concentration of patients demonstrating follicular growth and a sub-normal PI.

Follicular LH

A significantly higher mean follicular phase LH concentration was found in the study patients in comparison to the control group as has been reported by others (Eden *et al* 1989a; Fleming *et al* 1995). Persistently high follicular LH concentrations were observed in 26.8% of the oligo/amenorrhoeic patients of this study, while 34.1% of the patients showed normal follicular LH concentrations. The mean follicular phase LH concentrations of patients with oligomenorrhoea was reported to be mildly elevated but with a considerable range of values (Fleming *et al* 1995), and about 60% of patients showed consistently elevated LH concentrations during the period of observation.

In the present study, almost similar proportion of patients with normal or high follicular phase LH showed spontaneous ovulation. However, the PI range was not different, indicating that high LH was not a major determinant of the incidence of follicular maturation or the quality of the luteal function in such patients. This agrees with the findings reported by other investigators (Fleming *et al* 1995), where there was no relationship between LH and PI, and the distribution of PIs was similar in the normal LH and elevated LH groups.

In contrast, Sagle *et al.* (1988) reported that LH concentrations are usually normal in women with PCOs who ovulate and Baird *et al.* (1977) have shown that urinary LH concentrations were higher in oligomenorrhoeic patients who did not ovulate than in those who ovulated. Although the mean basal LH value was found to be nearly double in patients than in controls during the early follicular phase, the concentration was variable from patient to patient and also within the same patient from day to day (Baird *et al* 1977). The elevated LH levels have been linked with abnormal ovarian function, although the mechanism is not fully understood. Yen (1980) suggested that the elevated LH concentrations, observed in women with oligomenorrhoea, play a major role in the aetiology of PCOD and can result in failure of follicular maturation and ovulation. However, the results of the present study clearly disagree with such conclusions.

Minakami *et al.* (1988a) showed that the basal LH level, as well as the LH response to LHRH, were dependent on the time since the last menstrual cycle and that LH elevation above normal in PCO patients may be related to their long-standing follicular phases. They also noted that the elevated LH concentrations declined after ovulation to normal or near-normal range at the end of the luteal phase and in the early follicular phase. The declined LH level gradually increased again with the increasing days from the beginning of menstrual flow and remained at a high level until the next ovulation. In the present study, most patients with oligomenorrhoea were sampled at similar time in relation to their menstrual cycle and continued for a period of 5-weeks; a similar proportion of ovulatory cycles was observed in both the high LH group and the normal LH group. This is in addition to wide variations seen within patients at any stage. If LH is to be used as a diagnostic criterion, then the timing of the blood sampling is important, since concentrations in the luteal phase and the early follicular phase may be lower than at other times.

Abnormal secretion of LH and androgens were claimed to contribute to the subfertility of women with PCO (Eden *et al* 1989a), and high follicular phase levels of LH may also impair fertilization (Stanger & Yovich, 1985). A raised serum LH concentrations during the follicular phase of unstimulated (Regan *et al* 1990) or stimulated (Homburg *et al* 1988) ovulatory cycles resulted in a substantial risk of infertility and pregnancy loss (up to 65%).

Homburg *et al.* (1988) reported that exposure of the ovaries to high concentrations of LH during the follicular phase in women with PCOs have a harmful effect on rates of conception and may cause early pregnancy loss. The mean basal serum LH concentrations of oligomenorrhoeic patients who ovulated after pulsatile LHRH therapy was found (by the same authors) not to differ from those patients who failed to ovulate, and there was no difference in LH concentrations between those who did not ovulate and those

who ovulated but failed to conceive. However, the LH concentrations were significantly lower in those who ovulated and did conceive and in those whose pregnancy progressed beyond the first 4 weeks compared with those who suffered early pregnancy loss (Homburg *et al* 1988). In addition, oocytes obtained from the pre-ovulatory follicles during stimulated cycles of women with elevated LH levels were found to have impaired *in-vitro* fertilization ability and the embryos which formed were less likely to implant successfully (Stanger & Yovich, 1985). It would be of interest to study the current group of patients during stimulated cycles.

A study of ovarian tissue taken by wedge resection from patients with PCOD showed that the binding of LH to the membrane receptor, did not basically deviate from normal in PCOD. However, when compared with normally developing follicles, the number of follicle receptors for LH was decreased. It was suggested that abnormal follicular development and function in PCOD may be due to tonic excessive LH stimulation of the follicles too early in development, leading to a decreased number of receptors for LH (Rajaniemi *et al* 1980). However, the observation of apparently normal follicular development in some patients in the present study does not support this hypothesis.

Androgens

Mean follicular T concentrations were not different in patients who did not ovulate compared to those patients who ovulated, but both were higher than the levels in the control group. A similar observation was found by Eden *et al.* (1989a) who examined single samples. Similar proportions of patients in the current study, who had normal mean follicular phase T concentrations and those who had elevated T concentrations showed spontaneous ovulation. The mean follicular T concentrations were significantly higher in patients with elevated LH values ($P = 0.0105$), and there was a weak correlation between LH and T ($r = 0.29$, $P = 0.061$). This indicates that TT was not directly implicated in the incidence or inhibition of spontaneous follicular growth supporting the observations in the literature (Fleming *et al* 1995). A higher (3.7 nmol/L) upper limit of the normal range for TT was reported by Fleming *et al.* (1995) than in the present study, however, there was no difference in the mean follicular phase T concentrations seen in their patients who showed follicular growth and those who did not. Testosterone concentrations were found by the same authors to be positively correlated with LH ($r = 0.47$, $P = 0.008$) but there was no link with the incidence of spontaneous follicular growth. There was no significant difference in the incidence of spontaneous ovulation in patients with elevated or with normal mean

follicular phase T concentrations. However, the T concentrations were significantly higher in patients with elevated LH values ($P < 0.001$). The data of the present study supports these findings.

Eden *et al.* (1989a) suggested that abnormal secretion of TT concentrations and subsequently high FT may contribute to the subfertility of women with PCO, and it was proposed that high follicular phase levels of androgens could impair intrafollicular control mechanisms (Yen, 1980). In this study, a relationship between hyperandrogenism and spontaneous ovulation was only detected through differences in SHBG and consequently FAI, rather than total androgen levels. The differences in the mean follicular FAI values were significant between patients who failed to ovulate and patients who ovulated and the controls, mainly through the effect of SHBG. More patients with normal FAI experienced a spontaneous ovulation than did those with high FAI.

Other Reproductive Hormones and Spontaneous Ovulation

The study patients who ovulated showed similar mean follicular phase FSH, LH:FSH ratio, A and 17α-OHP concentrations, higher E_2 and SHBG concentrations, and significantly lower FAI and PRL values compared to those patients who failed to ovulate.

Although the study patients who ovulated spontaneously showed significantly higher LH concentrations than the control group, the mean follicular E_2 concentrations were similar, indicating the similarity in E_2 secretion during follicular maturation. The concentrations of FSH were significantly lower in patients who ovulated than in the controls. Similar observations of normal E_2 and a low FSH have been reported by others (Sherman & Korenman, 1974).

The E_2 profiles in patients showing follicular growth were reported to be normal when compared with normal controls and there was no difference in the E_2 profiles of patients with elevated LH or normal LH values (Fleming *et al* 1995). Surprisingly, significantly lower follicular phase E_2 concentrations were estimated in urine of patients with PCO who had spontaneous ovulation than in patients with anovulation (Baird *et al* 1977).

It may be suggested that low FSH concentrations, observed in the study patients during the follicular phase, induce abnormal follicular maturation or require longer periods of stimulation to effect follicular maturation as proposed by Wu & Michail (1979). In contrast, ovulatory women with PCO were reported to have larger follicles and higher levels of FSH despite similar concentrations of E_2 , when compared

with the controls (Eden *et al* 1989a). However, modern more specific immunoassays may more reliably detect real differences, and if the observations recorded here represent biological activity then low FSH cannot be the cause of blocked follicular growth. The old hypothesis that low FSH in patients with oligomenorrhoea and PCOD leads to anovulation is wrong as lower FSH concentrations were observed in ovulators than in non-ovulators. Therefore, a new explanation for anovulation in such patients is needed.

Metabolic Variables and IR

Despite similar GH concentrations between the groups of this study, a significantly lower mean follicular phase IGF-I concentration was observed in patients who failed to ovulate spontaneously in comparison to those patients who ovulated and the controls. However, in spite of significantly low IGF-I levels in comparison to the control group, spontaneous ovulation was still observed in patients. This indicates that IGF-I may influence the adequacy of luteal function rather than the occurrence of ovulation.

Barreca *et al.* (1996) found significantly lower IGF-I in follicular fluid from patients with PCOD than in follicular fluid from normal subjects. The human ovary has both I and IGF-I receptors. Specific binding sites for I (Poretsky *et al* 1984; Nagamani & Stuart, 1990) and for IGF-I (Nagamani & Stuart, 1990) have been identified in human ovarian stroma. In presence of hyperinsulinaemia, I might mediate its action through IGF-I receptors (Nagamani & Stuart, 1990), particularly when IGF-I concentrations are reduced. Follicle stimulating hormone inhibits the release of IGFBP's from granulosa cells. Therefore the decreased IGFs would be compatible with the relatively slow early follicular development. FSH-insensitive follicles undergoing atresia may be compromised by decreased IGFs (Adashi *et al* 1991). Increasing IGF-I levels in follicular fluid may correct the IGF-I imbalance and overcome the defect in granulosa cells in PCO patients and improve ovarian follicular maturation (Barreca *et al* 1996).

There was a tendency towards an increase in the indices of IR, assessed by FI, GLU/INS ratio and FIRI, with the degree of disturbance of ovarian function which was more pronounced between the study patients who did not ovulate and the control group. This indicates that IR may have an influence on ovarian function and the occurrence of spontaneous ovulation, possibly through effects of IGF-I.

Anthropometric Variables

In this study, significant relationships were observed between anthropometric variables and the mean follicular hormones representing the androgenic state of the patients; the clearest links were seen in the SHBG data.

It was apparent in this study, that the ratio of body fat distribution (WHR) rather than BMI influences the capacity to undergo spontaneous follicular maturation and ovulation. Weight loss seems an effective form of therapy, resulting in improvement in menstrual cyclicity, return of ovulation (Harlass *et al* 1984; Holte *et al* 1995)and restoration of IS in such patients (Holte *et al* 1995).

Leptin

In this study, the mean values and ranges of plasma leptin concentrations were similar in patients and controls. Leptin concentrations were strongly correlated to BMI, WHR and waist in both study groups. Similar relationships have been found by others (Considine *et al* 1996; Klein *et al* 1996). Obese patients and controls had significantly higher mean leptin concentrations than their lean counterparts ($P < 0.0001$). The concentrations of leptin in obese and lean individuals fell in the same ranges reported by other authors (Considine *et al* 1996; Haffner *et al* 1996). However, when the study patients were compared with weight-matched controls, lean patients had higher leptin concentrations than lean controls but the difference was lost between obese patients and controls. The finding of increased serum leptin concentrations in obese subjects may suggest increased resistance to leptin, although the detection of leptin by immunologic methods does not prove that it is biologically active (Considine *et al* 1996). Considine *et al.* (1996)reported that the fluctuation of serum leptin concentrations was large in the presence of relatively small changes in body weight. These observations may indicate that although the rate of leptin production is directly related to obesity and it is increased per unit body fat (Considine *et al* 1996; Klein *et al* 1996), there are other factors which may regulate its production such as chronically elevated levels of I (Bonora *et al* 1989; Considine *et al* 1996) and IR, sympathetic nervous system activity and corticosteroids (Caro *et al* 1996; Klein *et al* 1996). In this study, a highly significant relationship was found between mean serum leptin concentrations and indices of IR (FI, GLU/INS ratio, $\log_{10}$ FIRI).

No relationship was observed between leptin concentrations and E_2, LH, FSH, T, SHBG or FAI. The relationship between leptin concentrations and TOV was weak ($r = 0.28$, $P = 0.075$). Mean leptin levels

were lower in patients who ovulated than those who did not ovulate but the difference did not reach statistical significance (P = 0.075). The relationship between leptin and the occurrence of spontaneous ovulation was masked in the obese individuals. However, leptin concentrations in lean controls were similar to their levels in ovulatory patients but were lower than in lean anovulatory patients (P = 0.05). However, for a given BMI, leptin concentrations were not significantly different in patients who failed to ovulate, compared with those who did. It is unlikely that the occurrence of spontaneous ovulation in oligo/amenorrhoeic patients is influenced by leptin levels. The relationships between leptin and indices of IR were stronger in patients who ovulated than anovulatory patients. This may indicate that the occurrence of spontaneous ovulation was indirectly influenced by the strong positive correlation between leptin and I.

It has been reported that animals with mutations in the obese gene are obese and lose weight when given leptin, but little is known about the physiologic actions of leptin in humans (Considine *et al* 1996). In mice, leptin receptors were identified in the ovary and leptin administration was found to stimulate gonadal function, causing a significant increase in LH, sex steroid production and increased ovarian and uterine weights, indicating greater amounts of follicular development which were confirmed histologically. In normal animals leptin serves as a metabolic signal to the reproductive system (Barash *et al* 1996). However, similar studies on humans have not yet been carried out.

ULTRASONOGRAPHIC OBSERVATIONS

The accurate assessment of follicular growth and development is important in the investigation and treatment of the infertile woman. Assessment of follicular growth by US scan is important in evaluating and treating all infertile women and in particular oligo/amenorrhoeic patients. Ultrasonic assessment of follicular growth is a useful addition to hormonal measurements (Hackelöer *et al* 1979).

In the present study ovarian structural changes were recorded in women suffering from cycle disturbances and infertility. The observed changes were correlated with clinical and biochemical features. Repeated sonography examinations were performed to assess possible dynamic changes in the ovaries.

TOV

Significant variations in the TOV were observed between and within the patients, regardless of the occurrence of spontaneous ovulation. However, there was no influence of ovarian volume on the mean follicular phase concentrations of LH, E_2 and T. Pache *et al.* (1991), however, showed that there was no statistically significant difference between serial examinations of the same ovary.

In addition comparison between the hormonal concentrations at the lowest and at the largest TOV during the period of observation showed no influence of ovarian volume on the concentrations of any of the reproductive hormones. However, significant correlations have been reported in the literature between ovarian volume and LH and T (Pache *et al* 1993).

In this study, no significant difference in the mean ovarian volume was observed between the right and left ovary. Similar observation have been reported by others (Pache *et al* 1991; Takahashi *et al* 1994b).

The patients' 5-weeks TOV mean was 19.33 ± 7.45 cm^3, and almost half of the patients had mean TOV greater than the group mean. More than half of the patients showed > 60% change in their TOV while 41.5% had doubled their ovarian volume during the period of monitoring. This significant change in TOV was not accompanied by any significant difference in the endocrine hormones, IR or other metabolic variables. Furthermore, the wide variations in TOV were not influenced by BMI or WHR. The mean ovarian volume has been reported to be significantly larger in patients than in controls (Pache *et al* 1991; Pache *et al* 1992b), with 9.8 mL in patients and 5.9 mL in controls (Pache *et al* 1992b) but there was a wide overlap in size, suggesting that the discriminative power of the ovarian volume as a diagnostic US parameter of PCOs is poor.

Marked asymmetry between the two ovaries, i. e., one is smaller than 75% of the other, has been reported in 28.5 - 64.4% in some series (Yeh *et al* 1987; Takahashi *et al* 1994b), however, there was not such a marked asymmetry between the ovaries of controls.

In the present study, no relationship was found between TOV and metabolic variables, including IR. In contrast, I and IR were significantly correlated with ovarian volume in the study of Pache *et al.* (1993).

Follicular Number and Distribution

In this study, the mean number of follicles during the 5-week period of observation was higher than reported in the literature for patients with PCOD and almost twice the number of follicles found in controls (Pache *et al* 1991; Pache *et al* 1992b; Takahashi *et al* 1994b). A significantly higher number of follicles than in the current study was reported (Takahashi *et al* 1994a). However, a considerable overlap existed between control subjects and patients with PCOD in follicular number and size (Pache *et al* 1992b). In their study, a maximum number of 11 follicles could be detected in normal ovaries. On the other hand, a considerable number of ovaries in patients with PCOD contained fewer than 11 follicles. Therefore, the specificity of a cut-off value of 12 or more follicles per ovary may be high, whereas the sensitivity of this level is too low to rule out the diagnosis of PCO. This indicates that follicular number as well as ovarian volume may not be used separately as a single reference to discriminate between normal and PCOs and the question of what should be the threshold follicle number for the ovary to be considered as polycystic should be addressed further.

In the present study, there was no significant difference in the number of small follicles between the right and left ovary which was similar to reports in the literature (Takahashi *et al* 1994b) and the mean number of follicles showed a variation during the period of monitoring which was significantly correlated to TOV. However, others (Pache *et al* 1990; Pache *et al* 1991) showed a slight variation in the follicular number throughout the menstrual cycle, although a considerable inter-individual variation existed.

More than half of the study patients showed a mixed picture of peripheral and central follicular distribution in relation to the stroma in their ovaries. Interestingly, the distribution of follicles in one ovary was not static and a change from peripheral to central or the reverse was observed in > 60% of ovaries. The mean TOV tended to be smaller if the follicles were consistently centrally distributed than if they were peripherally distributed. Larger ovarian volume favours the peripheral distribution of the follicles and was associated with a significantly greater number of follicles than the small ovary. Therefore, the larger the ovarian volume the greater the number of follicles pushed peripherally by the thickened ovarian stroma caused by hyperandrogenization. However, the number of follicles does not influence their distribution. A strong relationship between ovarian volume and follicle number has also been reported in the literature (Pache *et al* 1991).

In this study, no relationship was demonstrated between follicular number and endocrine, metabolic or anthropometric variables. No correlation between follicular number and BMI was found by others (Pache *et al* 1993). However, a correlation has been reported between the follicle number and LH:FSH

and FAI (Pache *et al* 1991), between follicle number and androgens (Pache *et al* 1993; Takahashi *et al* 1994a), and between follicle number and IR (Pache *et al* 1993).

Mean follicle size was not related to mean follicle number, indicating that the extent to which individual follicles grow is not related to the number of maturing or atretic follicles present (Pache *et al* 1991). It has been reported that there was a significant correlation between the number of small cysts on transvaginal US and the number of atretic follicles with hypertrophied and luteinized inner theca cells, and thickened ovarian capsules demonstrated histologically in ovarian tissues from patients with PCOD (Takahashi *et al* 1994a). All stages of folliculogenesis were increased in the PCO and atretic follicles formed about 76% of small cysts on histological examination. When > 10 small cysts in each ovary are observed by transvaginal US, the patient can be histopathologically diagnosed as a PCOD patient with numerous atretic follicles (Takahashi *et al* 1994a). Therefore, the assessment of ovarian morphology by transvaginal US in these patients provides an insight to the pathological state and possibly the degree of progression of the disease (Takahashi *et al* 1994a). Interestingly, significantly more small follicles were associated with a lack of response to ovulation induction with clomiphene citrate, therefore, the US features could be clinically useful for distinguishing the non-responders from the responders to clomiphene citrate (Takahashi *et al* 1994b).

Stromal Density

In this study, thick stroma was the most sensitive US criterion to diagnose PCOs. The majority of cases had bilateral thick stroma while only 21.4% had unilateral thick stroma. Bilateral rather than unilateral thick stroma was associated significantly with larger TOV. A significant correlation has also been reported by others (Robert *et al* 1995). This may be explained by the clarity of visualization when TOV is large. However, there was no influence of LH, androgen concentrations or anthropometric variables on the presence of unilateral or bilateral thick stroma. However, significant relationships have been reported between thickened stroma and LH, T and IR (Pache *et al* 1993).

Total follicle number in both ovaries was found to be well correlated with ovarian stroma echogenicity (Pache *et al* 1991). The echogenicity of ovarian stroma was reported to be increased in 10 - 16% of controls and was normal in 6 - 26% of PCOD patients (Pache *et al* 1992b; Robert *et al* 1995). Increased echogenicity was reported to be the most sensitive and specific sonographic sign of PCOs (Pache *et al* 1992b; Robert *et al* 1995). Increased stroma is considered to be a classical morphological criterion for the diagnosis of PCOs (Pache *et al* 1991). However, assessment of the echogenicity of ovarian stroma is

subjective, with the risk of overestimating the hypertrophy. The inter-observer variation was minimized when all US assessments were carried out by a single operator.

Ovarian capsular thickness was observed in 63% of PCOD patients but no correlation has been found between the thickness of the capsule and the morphology of ovarian structures (Takahashi *et al* 1994a). The same authors found that androgen concentrations and that ovarian capsular thickness were significantly correlated with the number of atretic follicles, with the latter found to be significantly higher in PCOD patients with thickened ovarian stroma than in those without. Therefore, the ovarian capsular thickness might be related not only to hyperandrogenism, but also to the morphology of ovarian structures (Takahashi *et al* 1994a).

Stromal hypertrophy is a frequent and specific US finding in hyperandrogenic and/or oligomenorrhoeic women. Robert *et al.* (1995) demonstrated similar accuracy between visual and computerized analyses for stromal assessment, with the main advantage being avoiding false-positive results in normal patients using the computerized analyses, especially in doubtful cases.

Spontaneous Ovulation

In this study, there was no influence of ovarian volume or the presence of unilateral or bilateral thickened stroma on the occurrence of spontaneous ovulation. The changes in the mean TOV were not restricted to those patients who ovulated spontaneously but significant enlargement of the TOV was attained despite the absence of ovulation. This may even weaken the significance of ovarian volume as a criterion to diagnose PCO.

All patients (43.9%) who had spontaneous ovulation showed a mature follicle by US scan (≥18 mm). However, maturing follicles on US scans (1.5 - 2.9 cm) have been reported to be much lower in PCOD patients (13.5%) than in healthy women (36%) (Yeh *et al* 1987). When ovulation occurs in patients with PCOD, it does not usually exhibit regular intervals which makes the detection of a mature follicle difficult.

Clinical Presentation

In the study patients, TOV or follicular distribution were not related to the period of infertility and the presence of oligomenorrhoea or amenorrhoea. However, the longer the period of menstrual disturbance, the lesser the number of ovarian follicles observed. This may indicate less ovarian activity, therefore, lower E_2 concentrations and subsequently less withdrawal or ovulatory bleeds. Therefore, a solid variant of US PCO picture reported by Orsini *et al.* (1985) may indicate more severe disease.

The presence of bilateral thick ovarian stroma does not indicate that the disease is more severe than the presence of unilateral thick stroma. On the contrary, the presence of unilaterally thickened stroma was significantly associated with prolonged durations of menstrual disturbance but not with the presence/absence of hyperandrogenism.

There was no significant difference in median follicular number, follicular size, ovarian volume, or ovarian stroma estimates between patients with oligomenorrhoea and patients with amenorrhoea (Pache *et al* 1992b). Hirsutism was positively correlated in previous studies with the number of follicles, ovarian volume, and stroma echogenicity (Pache *et al* 1993; Takahashi *et al* 1994a).

CONCLUSION

There is a tendency to consider oligo/amenorrhoea and PCOD as static conditions, and diagnosis with a single blood sample for hormone assay and/or US assessment has been the practice. However, it is evident from the longitudinal studies that more biological variability exists than strict criteria would allow and a cut-off level for any of the quantitative parameters which would have provided both a satisfactory sensitivity and specificity for diagnosis was not found.

The levels of SHBG showed the least variation within and between patients and was the most consistent of the endocrine variables. In addition, the mean basal as well as mean follicular phase SHBG concentrations showed the most significant relationships with the anthropometric variables, the occurrence of spontaneous follicular growth and ovulation in comparison to the other reproductive hormones. Therefore, SHBG is a better predictor of ovarian function than LH and T.

A significantly lower mean IGF-I concentration was found in the patients who failed to ovulate than in the patients who had spontaneous ovulation and in the controls. Furthermore, the IGF-I concentrations

were significantly lower in the group of patients who ovulated than in the control group. Insulin-like growth factor-I may influence the adequacy of luteal function rather than the occurrence of ovulation.

Insulin and IR did not demonstrate any direct effect on the occurrence of spontaneous ovulation or on the ovarian gross morphology monitored by US. However, there was a tendency towards an increase in FI and FIRI with the degree of disturbance of ovarian function but these differences were not significant. The observed cycles might not represent the entire menstrual history of such patients. It is expected that if IR plays a major role in the pathogenesis of PCOD, it should also correlate with ovulation and polycystic changes of the ovaries. Insulin may influence ovarian activity indirectly through its relationships with IGF-I and leptin. These interactions need further exploration.

It was apparent that WHR, rather than BMI, influences the occurrence of spontaneous follicular growth and ovulation.

The mean values and ranges of plasma leptin concentrations were similar in patients and controls and were strongly related to BMI, WHR and waist in both study groups. However, in the absence of obesity, leptin concentrations were higher in lean patients than lean controls. Although the rate of leptin production is directly related to obesity, there are other factors which may regulate its production such as chronically elevated levels of IR. A highly significant relationship was found between mean serum leptin concentrations and indices of IR. No relationship was observed between leptin concentrations and any of the endocrine variables or TOV. The mean leptin levels were lower in patients who ovulated than those who did not ovulate but this difference was not significant. It is unlikely that the occurrence of spontaneous ovulation is directly influenced by leptin levels. However, studies on the physiologic actions of leptin and its relationship to the reproductive system in humans are needed.

The most sensitive and specific US scan criteria, for the diagnosis of PCOD, shown in this study was the combination of thick stroma and follicular number.

The mean TOV of the study patients was significantly larger than the TOV of the controls. No relationship was found between any of the US indices and endocrine, metabolic or anthropometric variables. Asymmetry of ovaries was quite a common finding and about half of the patients at least doubled their ovarian volume, even in the absence of ovulation. The dynamic state of the ovaries was demonstrable by changes in TOV and the number and distribution of follicles, regardless of the occurrence of spontaneous ovulation, and these changes were not related to hormone concentration changes. Interestingly, a change from a peripheral to a central distribution of ovarian follicles or the

reverse, was observed in > 60% of US occasions. Larger TOV favoured a greater number of follicles with a tendency towards peripheral distribution as well as the presence of a bilateral thick stroma.

Assessment of ovarian morphology by means of US is currently employed as a substitute for histologic examination in the diagnosis of PCOs. However, because of the heterogeneity of the PCOD, US cannot be used alone for diagnosis (Anderson, 1974). One-third of patients with biochemically documented PCOD will have normal-appearing ovaries by US (Takahashi *et al* 1994a). Transvaginal US enables reliable diagnosis of PCOs and characterization of limits for normal number and size of follicles will allow more accurate differentiation between normal and abnormal ovarian function.

Since PCOD represents a wide variation of presentation, ovarian enlargement and follicle formation may vary according to the duration and degree of hormonal disturbance (Haun *et al* 1984). Differences in methods applied (equipment used for US examination, assays applied for hormone measurements, timing of investigations, accuracy in measuring ovarian diameters, etc.), and in the criteria for selection of subjects might account for the conflicting data in the literature regarding this disorder.

CHAPTER SEVEN : LIPOPROTEINS, POST-HEPARIN HEPATIC LIPASE ACTIVITY AND THE RISK OF CORONARY HEART DISEASE IN WOMEN WITH PCOD

7.1 INTRODUCTION

The gender difference in CHD rates in younger adults (20-50 years) and the increased risk following the menopause in women help in understanding the basis of the CHD pathogenesis.

The LDL is associated with a high risk of CHD (Laing *et al* 1993). The main influences on the LDL subfraction profiles are the TG, the activities of hepatic and lipoprotein lipases and the presence of IR (Knopp *et al* 1994; Tan *et al* 1995).

The small, dense LDL (LDL-III) is widely considered to be the most atherogenic form of LDL. When it is present at a concentration in excess of 100 mg/dL there is a 7-fold increased risk of CHD regardless of the plasma cholesterol level. In men with increased waist size, LDL-III was shown to be significantly raised, with a plasma TG in excess of 1.5 mmol/L, higher FI and an elevated content of the large, TG-rich VLDL subfraction.

Males on average demonstrated twice the HL activity of females (Tan *et al* 1995). This enzyme is thought to be responsible for the conversion of LDL-II to LDL-III and it is considered to be under sex hormone control. It is possible that the lipoprotein associated risk in women is due in part to an altered androgen/oestrogen balance which stimulates HL, and in so doing promotes the redistribution of circulating LDL into LDL-III. Women with central obesity, indicated by increased BMI and WHR, and high FI levels were reported to have raised plasma TG and VLDL but accumulation of LDL-II (a more benign LDL subfraction) rather than the LDL-III (Tan *et al* 1995). This was attributed to the 50% reduction in HL in women compared to men. However, the precise effects of changes in anthropometric indices on plasma lipoprotein subfraction distributions are unknown (Tan *et al* 1995).

Early menopause has been shown to be a feature of women who get early CHD. In addition, studies of hormone replacement therapy with unopposed oestrogen have shown about 50% reduction in the risk of CHD. Therefore, it is clear that the hormonal status in females is a potentially important determinant of

risk. This includes oestrogen/androgen status and also the presence of IR, as diabetic women lose their gender associated protection from CHD. Insulin and sex hormones influence a large number of metabolic pathways including those involved in lipid transport.

A review of the known risk factors in women with CHD reveals that the same risk factors are operative as in men but the importance of elevated plasma TG, low HDL-C and an increased WHR has been emphasized (Talbott *et al* 1995).

It is presumed that lipid-associated risk in women is due to the combination of : i) central obesity/IR leading to increased levels of large VLDL and total LDL and ii) an increased androgen/oestrogen ratio which stimulates HL and redistributes circulating LDL into a small, dense, atherogenic LDL-III.

The purpose of this study was to investigate whether HL activity was similar to normal women or men in women with oligomenorrhoea. The majority of such cases demonstrate hyperandrogenism. In the study, HL activity was assayed in post-heparin plasma in 11 women with oligomenorrhoea and PCO.

The objectives were to correlate the LDL subfraction distribution and concentrations with anthropometric indices, plasma lipid and lipoprotein concentrations, the activity of post-heparin HL, the degree of IR and androgen/oestrogen status in women with PCOD. The data were compared with published laboratory normal figures.

7.2 RESULTS

The patients with PCOD (n = 11) had significantly larger BMIs than those of normal females (n = 67) and normal males (n = 71), established from previous studies (Tan *et al* 1995) (Table 7-I). The WHR was significantly higher in patients than in normal female volunteers ($P = 0.01$) but was similar to that of male volunteers ($P \geq 0.05$), indicating the difference in body fat distribution of these patients.

Table 7-I shows that the patients had significantly higher plasma FI and TG, and a significantly lower total HDL-C than the normal volunteers (females and males).

The mean post-heparin HL activity was twice as high in the patients compared to normal females (22.38 ± 8.85 vs 11.4 ± 5.40 umolFA/mL/h; $P < 0.0001$), and indeed fell within the male range (19.5 ± 8.1 umolFA/mL/h; $P \geq 0.05$) (Figure 7.1).

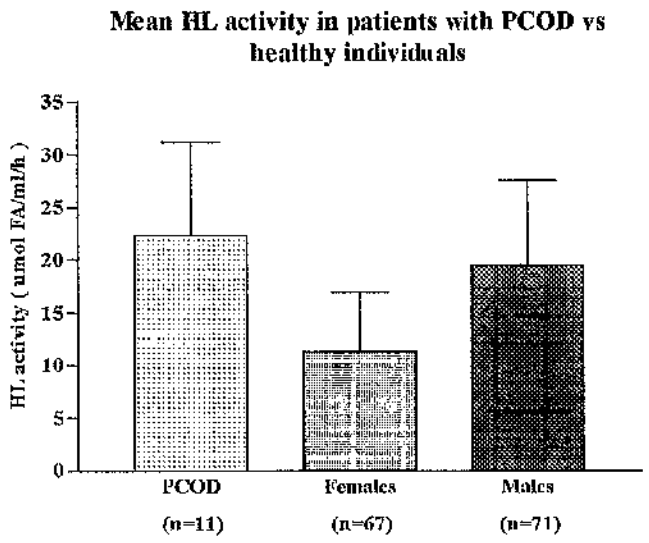
Only one patient had an LDL-III > 100 mg lipoprotein/dL plasma (the level above which significant CHD risk occurs) (Tan *et al* 1995), and showed the highest TG (9.7 mmol/L) concentrations and the lowest LDL% (7.3%). However, this patient exhibited the lowest HL activity (16.61 umolFA/mL/h) of the patient group.

Table 7-I : Anthropometric indices, Plasma Lipids, HL and Lipoproteins in the study groups

	Patients	Female Controls	Male Controls	P*	P**
n.	11	67	71		
Age	28.4 ± 3.6	37.0 ± 11.0	35.6 ± 11.2	0.0113	0.0349
BMI	31.18 ± 6.6	24.6 ± 3.9	24.9 ± 3.0	< 0.0001	< 0.0001
WHR	0.88 ± 0.08	0.80 ± 0.1	0.90 ± 0.1	0.0101	NS
FI (mIU/L)	12.82 ± 7.87	7.65 ± 4.17	8.55 ± 4.3	0.0007	0.0063
F.Glucose(mmol/L)	4.75± 0.37	4.63 ± 0.51	4.93 ± 0.51	NS	NS
Cholesterol (mmol/L)	4.51 ± 0.69	5.20 ± 1.0	5.30 ± 1.1	0.0570	0.0477
TG (mmol/L)	1.56 ± 0.78	1.0 ± 0.5	1.2 ± 0.6	0.0066	NS
HDL-C (mmol/L)	1.15 ± 0.22	1.50 ± 0.3	1.20 ± 0.3	< 0.005	0.0061
LDL-C (mmol/L)	2.84 ± 0.64	3.20 ± 0.9	3.50 ± 1.0	NS	0.0670
VLDL-C (mmol/L)	0.53 ± 0.32	0.50 ± 0.3	0.60 ± 0.3	NS	NS
Chol/HDL ratio	4.11 ± 1.23	3.70 ± 1.2	4.60 ± 1.4	NS	NS
HL (umolFA/mL/h)	22.38 ± 8.85	11.40 ± 5.4	19.50 ± 8.1	< 0.0001	NS
LDL (mg/dL)	181.0 ± 63.6	304.0 ± 97.0	294.0 ± 89.0	0.0007	0.0007
LDL-I (mg/dL)	40.71± 31.5	81.0 ± 41.0	59.0 ± 35.0	< 0.0001	NS
LDL-II (mg/dL)	94.7 ± 41.47	175.0 ± 73.0	172.0 ± 67.0	0.0024	0.0016
LDL-III (mg/dL)	45.3 ± 40.01	44.0 ± 37.0	88.0 ± 81.0	NS	NS

The mean ± SD of the groups were compared using Student's t-test. (P*) compares patients with normal females, while (P**) compares patients with normal males. P ≥ 0.05 is NS.

Figure 7.1 :



Variations in plasma TG levels in the patients correlated positively with LDL-III (r = 0.80, P = 0.0168) (Figures 7.2), VLDL (r = 0.96, P = 0.0001) (Figure 7.3), total cholesterol concentration (r = 0.68, P = 0.0626), and negatively with HDL (r = -0.72, P = 0.0421).

The LDL-C was positively correlated with total serum cholesterol (r = 0.92, P = 0.0013), T (r = 0.73, P = 0.04) and negatively correlated with HDL-2 (r = -0.74, P = 0.0344). Similar observations have been reported by Holte *et al.* (1994b). There was no correlation with SHBG.

On the other hand, LDL-I showed significant positive relationships with SHBG (r = 0.79, P = 0.0208), LDL-mass (r = 0.64, P = 0.0848) and total cholesterol (r = 0.86, P = 0.0056). There was also a negative relationship with $\log_{10}$FIRI (r = - 0.70, P = 0.0515)

Significant correlations were established between LDL-II and E_2 (r = 0.67, P = 0.067), LDL-mass (r = 0.71, P = 0.0498), and cholesterol (r = 0.79, P = 0.0185). The LDL-III was significantly correlated with VLDL-C (r = 0.87, P = 0.0046) (Figure 7.4).

Figure 7.2 :

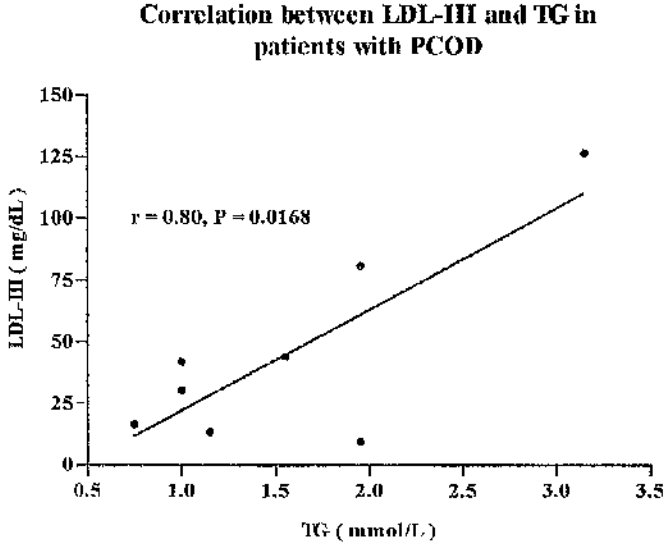


Figure 7.3 :

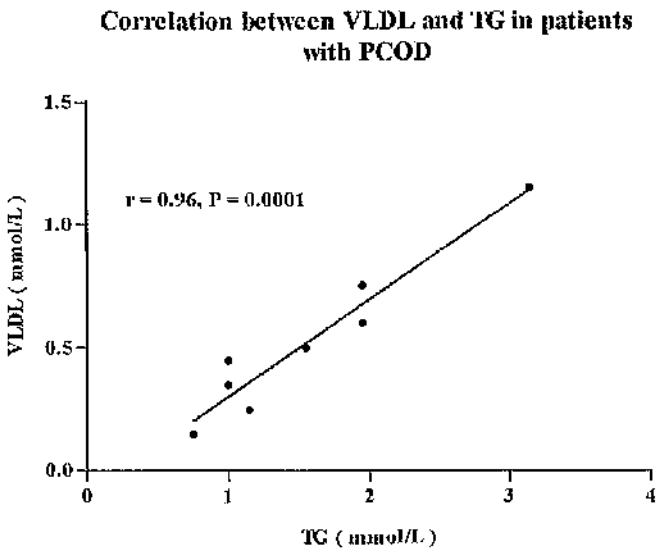
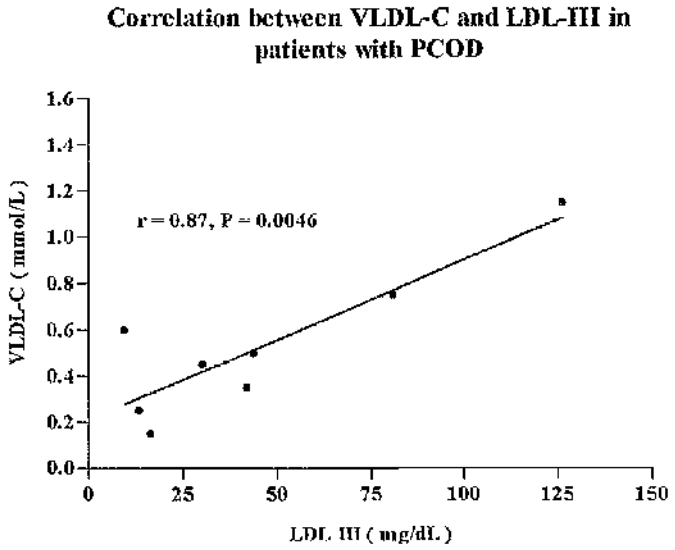


Figure 7.4 :



Total HDL-C levels were negatively correlated with FAI (r = -0.68, P = 0.064), and also with total LDL-C (r = -0.65, P = 0.0815). These relationships showed borderline significance.

Post-heparin HL activity had significant associations with total LDL mass (r = 0.77, P = 0.0256) (Figure 7.5), cholesterol (r = 0.71, P = 0.047), LDL-II (r = 0.91, P = 0.0018) (Figure 7.6), and LDL/HDL ratio (r = 0.74, P = 0.0361).

Figure 7.5 :

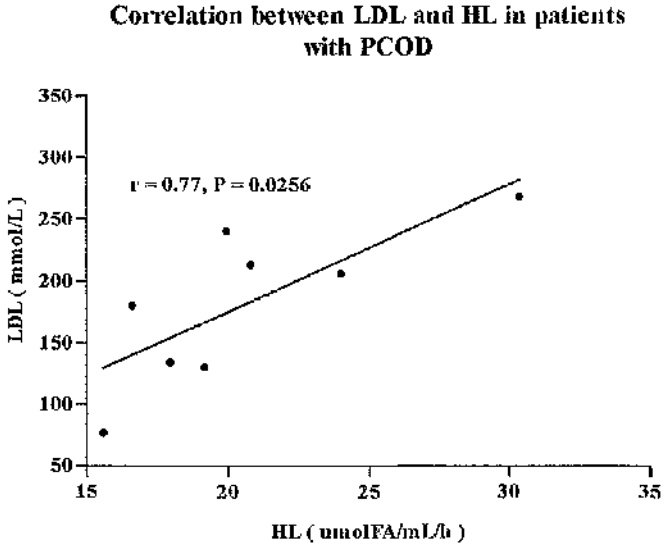
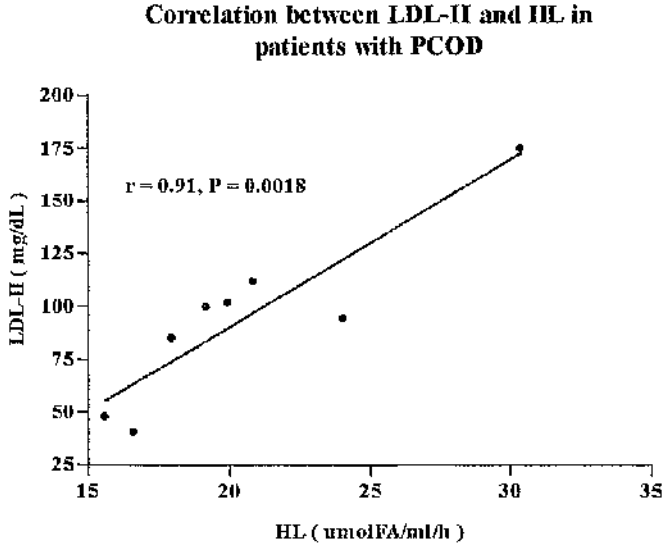


Figure 7.6 :



7.3 DISCUSSION

Due to the recruitment process, the patients with PCOD were younger than the normal volunteers. It was notable that while the BMI was significantly higher in the patients than in the normal volunteers (females and male) the WHRs of the patients and the normal male volunteers were identical, indicating a similarity in the central or truncal fat distribution in the patients with PCOD compared with that of normal males.

In this study, the patients with oligomenorrhoea showed a male CHD risk-factor profile : they showed significantly increased risk factors compared with laboratory normal control women. These included increases in BMI, WHR, FI and TG levels and decreased total HDL. These findings are supported by the results of Talbott *et al* (1995); significantly lower HDL-C was observed in patients who developed CHD ($P < 0.05$) (Hargreaves *et al* 1992), and LDL-C correlated highly with total serum cholesterol (Holte *et al* 1994b), indicating that women with PCOD should be monitored for early detection of these risk factors. It remains to be determined whether the mere existence of oligomenorrhoea may be a marker for increased risk factors irrespective of PCOs.

The IR has been linked to the raised plasma TG, lower HDL-C and the presence of small LDL. Insulin resistance and the presence of central obesity can lead to changes in androgen/oestrogen balance (Tan *et al* 1995). However, in this study, no correlation was demonstrated between FI and cholesterol or TG. Similar observations were reported by Laing *et al.* (1993). In addition, FI did not correlate with HDL or LDL subfraction concentrations, failing to identify I as a direct risk factor for CHD. Similar observations have been reported by others (Hargreaves *et al* 1992). This is in contrast to the findings of Laing *et al.* (1993).

However, when $\log_{10}$ FIRI was applied as a measure of IR, a significant negative correlation was found with the LDL-I. Therefore the more I resistant the patient is, the lower the concentration of LDL-I (the least dense species of the lipoprotein) is. This finding may suggest that $\log_{10}$ FIRI is a more relevant marker of IR than FI, with respect to lipid metabolism.

The LDL-C showed a highly significant relationship with T, while the subfraction LDL-I showed a significant positive correlation with SHBG. The protective lipoprotein HDL-C was negatively correlated with FAI, probably through the effect on SHBG. These data suggest that hyperandrogenaemia may be a stronger determinant of serum lipids in PCOD than IR.

One of the most important observations reported by Talbott *et al.* (1995) was that the differences in risk factors between patients with PCOD and control subjects were generally stronger at earlier ages. This was probably due to an early onset of hormonal changes and obesity and possibly to the distribution of body fat among patients with PCOD. The risk factors in the PCOD women are probably elevated at an earlier age than in non-PCOD women. If the central obesity, hyperinsulinaemia, and low HDL-C and high TG levels noted in the PCOD cases are really a unique profile of risk factors of atherosclerosis and subsequent CHD, then women with PCOD should have a higher incidence of atherosclerosis than control subjects, especially at younger ages. If the risk of atherosclerosis is primarily related to elevated LDL-C, then there is an alternative reason for a higher prevalence of atherosclerosis in PCOD patients. The patients with PCOD present risk-factor characteristics found in younger men and older obese postmenopausal women (Talbott *et al* 1995).

Hepatic lipase plays an important role in determining the relative concentrations of LDL-II and LDL-III in plasma. The HL acts on LDL-II to hydrolyze the TG enriched particles and generate LDL-III. If the enzyme activity is low then LDL-II remains the major species in plasma and is relatively TG enriched. The finding of significantly higher mean HL activity in patients with PCOD than in the normal female volunteers, ($P < 0.0001$), and activity identical to that of normal male subjects, adds weight to the increasing body of evidence that the sex hormone status of PCOD women plays a role in disturbance of the lipoprotein subfractions.

Hepatic lipase is decreased by oestrogen treatment, which is also thought to play a role in the removal of the remnant lipoprotein (Knopp *et al* 1994). The lipoprotein effects of sex hormones are of practical importance. The lipoprotein effects of progestins and androgens on lipoprotein metabolism oppose those of oestrogens. Oestrogens raise plasma TG concentrations but lower LDL and raise HDL levels while progestins and androgens lower TG concentrations. Oestrogen appears to explain the lower LDL levels experienced by women premenopausally. The physiologic effects of oestrogens and progestins are the basis of the male/female difference in lipoprotein levels and cardiovascular disease risk. Reductions in HDL-cholesterol with androgen or androgenic progestin administration are well described. Reductions in HDL are associated with increases in HL activity presumably via increased HDL phospholipid hydrolysis and hepatic uptake. Hyperinsulinaemia and obesity can produce the same lipoprotein picture. These effects raises the risk of CHD in the postmenopausal women and they provide an approach to its prevention. Understanding sex steroid effects on lipoprotein physiology is essential because CHD accounts for 50% of the mortality in women; actually exceeding that in men. While the greater liability of cardiovascular disease in women compared with men may have a number of explanations, whatever

the cause, prevention of cardiovascular disease in women as well as in men is now a very high priority (Knopp *et al* 1994).

IR, hyperinsulinaemia, hypertension, hypertriglyceridemia, and low plasma HDL-C concentrations are closely related variables and tend to occur in the same persons (Hargreaves *et al* 1992). This cluster of metabolic abnormalities might usefully be referred to as syndrome X. In addition to being closely related and present in the same person, the abnormalities associated with resistance to I action, namely, glucose intolerance, hyperinsulinaemia, and a low plasma HDL-C concentration, have been identified as increasing the risk of CHD.

In general, consideration of metabolic risk factors for CHD has focused on the role of LDL-C. However, it is becoming increasingly apparent that CHD can occur in the absence of elevated LDL. Insulin resistance, and the degree to which the endocrine pancreas responds to this defect play important roles in the pathogenesis and clinical course of high blood pressure, dyslipidemia, and CHD (Hargreaves *et al* 1992).

7.4 CONCLUSION

In keeping with other studies, patients with PCOD demonstrated higher WHR and fasting plasma TG concentrations and lower HDL-C concentrations. A significantly higher post-heparin HL activity was demonstrated in patients with PCOD. This enzyme is stimulated by androgens, and it is presumed that the hyperandrogenaemia in this group of women is responsible for stimulating HL activity into the male range. These findings add weight to the increasing body of evidence that the sex hormone status of women plays a major role in the disturbance of lipoproteins in an atherogenic direction.

Women with PCOD demonstrate male type HL activity. Further studies are needed to establish the differential roles of obesity, IR, and sex hormones.

CHAPTER EIGHT : GENERAL CONCLUSIONS, RECOMMENDATIONS & PROPOSALS FOR FUTURE WORK

8.1 INTRODUCTION

The studies described in this thesis were performed to attempt to address the objectives described in Chapter One (Section 1.4). The section below summarises the conclusions which can be drawn from these studies with regard to the various headings.

8.2 DIAGNOSIS OF PCOD

During the literature survey performed as a prelude to carrying out the research described in this study it was obvious that the disease reported in the literature as PCO, PCOD and PCOS was not a homogeneous entity but consisted of a heterogeneous mixture of related conditions. Polycystic ovarian disease is a common cause of female infertility in women with menstrual dysfunction. In one series (Franks *et al* 1988) PCO was diagnosed in 87% of women with oligomenorrhoea and in 26% of those who complained of amenorrhoea. Very few of the patients reported fulfilled the original criteria described by S-L. Furthermore in more recent times the diagnosis of PCOD has become more complicated with the advent of ovarian US scanning. Whilst US has been shown to be of extreme value in assessing the structure and function of ovaries its use as the sole criterion for diagnosing PCOD is obviously suspect particularly in light of the published data (Adams *et al* 1986b) that up to 22% of normally cycling women show PCOs on US examination. It was therefore decided that as part of the investigations performed in this study the usefulness of the various criteria used in PCOD diagnosis would be addressed and an attempt made to decide what would constitute the ideal diagnosis.

All patients recruited had menstrual disturbance (oligo/amenorrhoea with or without DUB) and infertility. About half of the patients were hyperandrogenized.

Women with oligo/amenorrhoea and PCOD showed significantly higher serum basal concentrations of LH, LH:FSH ratio, T and FAI and lower SHBG than did the control group whilst basal E_2, FSH, DHEA-S and PRL concentrations were similar in the study patients and the controls.

The mean follicular phase endocrine profile in patients who ovulated showed significantly higher LH, LH:FSH ratio and FAI, and a significantly lower FSH than the controls while E_2 concentrations were similar. In comparison to patients who failed to ovulate, higher E_2, and lower SHBG and FAI were found in patients who ovulated. Luteinizing hormone and FSH were similar.

In evaluating the sensitivity and the specificity of the biochemical diagnosis of PCOD : high LH levels, raised LH:FSH ratio or elevated TT concentration were not diagnostic of the disorder because less than half of the study patients satisfied any of these biochemical criteria. However, FAI of > 4.5 was the most sensitive endocrine index. The accuracy of the diagnosis was increased when more than one criterion was applied. Only one quarter of the whole group of patients satisfied all of the classical biochemical diagnostic criteria. It is important to remember that these differences may be due to a number of factors : the wide variation in the presentation of patients and their selection criteria; the number and timing of blood sampling in relation to the patient's last menstrual period; hormonal assay methodology as well as the period of observation.

There is a tendency to consider oligo/amenorrhoea and PCOD as static conditions, and diagnosis with a single blood sample for hormone assay and/or US assessment is a common practice. However, it is evident from the longitudinal observations that more biological variability exists than strict criteria would allow and a cut-off level for any of the quantitative parameters which would have provided both satisfactory sensitivity and specificity was not available.

The PI was a better predictor of luteal phase function than a single mid-luteal phase serum P concentration - showing deficient luteal phases in many.

The mean TOV of the study patients was significantly larger than the TOV of controls (using published data). No relationship was found between any of the US indices and endocrine, metabolic or anthropometric variables.

Asymmetry of ovaries was a common finding and about half of the patients at least doubled their ovarian volume, even in the absence of ovulation. The dynamic state of the ovarian structure was demonstrable by changes in TOV and the number and distribution of follicles during the period of observation. Interestingly, a change from a peripheral to a central distribution of ovarian follicles or the reverse, was

observed in > 60% of ovaries. Larger TOV favoured a greater number of follicles with a tendency towards peripheral distribution as well as the presence of bilaterally thickened stroma compared to the smaller ovaries.

Assessment of ovarian morphology by means of US is currently employed as a substitute for histological examination in the diagnosis of PCOs. However, because of the heterogeneity of the PCOD, US alone cannot be used alone for diagnosis. Repeated assessment will give more information than a single scan. A unilateral PCO was diagnosed in 20% of patients. There was no indication that unilateral PCO, diagnosed by US scan, was less severe than bilateral PCOs. However, it would be interesting to follow these patients with unilateral PCO for any progression of the disease.

Thick stroma, the most sensitive single US criterion, was found in at least one ovary in 95.2% of the patients. Enlarged ovarian volume, the least sensitive US parameter, was observed in 55% of patients. Increased Follicle number (> 10) was found in 85.7% of patients. However, combination of more than one US criterion increased the diagnostic accuracy. The most sensitive and specific US scan criteria for the diagnosis of PCOD, shown in this study, was the combination of thick stroma and the number of follicles.

Transvaginal US enables reliable diagnosis of PCOs and characterization of limits for normal numbers and sizes of follicles will allow more accurate differentiation between normal and abnormal ovarian function. Results may also serve as a reference for US monitoring of ovulation induction by exogenous Gn in PCOD patients.

Since the disease represents a wide spectrum of presentation, ovarian enlargement and follicle formation may vary according to the duration and degree of hormonal disturbance (Hann *et al* 1984). Differences in methods applied (equipment used for US examination, assays applied for hormone measurements, accuracy in measuring ovarian diameters, sampling regimens, etc.), and in the criteria for selection of subjects with PCO might account for much of the conflicting data in the literature.

It can be concluded from the data collected in this study, that the combination of at least two endocrine (including SHBG) and two US (including thick stroma) indices, assessed repeatedly, is the most sensitive criteria for the diagnosis of PCOD.

8.3 ASSESSMENT OF IR - COMPARISON OF SITT WITH FI, FIRI & GLU/INS RATIO

Despite the potential advantages of and the reproducibility of the SITT in measuring IR which have been reported in the literature, this study showed that the SITT was unreliable in oligo/amenorrhoeic patients because the KITT-values were similar in patients and their weight-matched control group, and more importantly; the test failed to show any correlation with anthropometric variables. In addition, the correlation within patients between the 2 SITTs, expressed as KITT-value, was poor and disappointing in comparison to FI and $\log_{10}$ FIRI.

Other methods for assessment of IR were also assessed. The FI, FIRI and GLU/INS ratio, were more convenient, easy to measure and showed the expected relationships with the anthropometric variables - comparable with the standard method of IR measurement (euglycaemic clamp). For the purposes of assessing IR in this study the simplest and most reliable assessment was the FI.

8.4 EFFECTS OF OBESITY & HYPERANDROGENAEMIA ON I LEVELS

Obesity was a common feature in the study patients (40.5%). This may indicate that obesity is common in patients with menstrual disturbance and PCOD and that the prevalence of PCOD in obese women can be expected to be high, given that PCO is common in the general female population (Polson *et al* 1988). The combination of PCOD and obesity may have an additive adverse effect on glucose tolerance. The prevalence of impaired glucose tolerance or frank diabetes in obese women with PCOD has been shown to be significant (20%) in other studies (Franks, 1989). The presence of hyperinsulinaemia may be important as well, since long-term follow-up of patients with PCOD indicates that they are at increased risk of developing DM and CHD. Therefore, measurement of GTT in such patients is important.

The cause and effect relationship between I and androgens is controversial. None of the methods used to estimate IR showed a significant relationship with androgen concentrations in this study. The correlation between IR and FAI in the oligo/amenorrhoeic group appeared to be secondary to the inverse correlation of SHBG to serum I. These powerful relationships between SHBG and FI and $\log_{10}$ FIRI were demonstrable in both patient and control groups.

Supraphysiological hyperinsulinaemia following I administration (during the SITTs), resulted in a significant decline in E_2 and T concentrations at 30- and 60 minutes, demonstrating a possible effect of

acute I administration on ovarian steroid secretion. This suppression of ovarian androgens argues against a simple, direct relationship between hyperandrogenaemia and hyperinsulinaemia.

Research on the mechanisms of anovulation in PCOD, the role of obesity and the clinical significance of the relationship between IR and hyperandrogenaemia are on-going.

8.5 EFFECTS OF ANTHROPOMETRIC PARAMETERS ON ENDOCRINE & METABOLIC VARIABLES

Sex hormone binding globulin showed the most significant and consistent relationships with the anthropometric parameters in patients and controls, compared with the other endocrine variables. Fasting glucose and FI were not significantly different between the patients and the controls. However, IR increased in parallel with increasing BMI in both groups. Thus the importance of weight-matched controls in any study of PCOD and/or disturbed ovarian activity must be emphasized.

8.6 THE GH STATUS OF PCOD PATIENTS VS NORMAL

Growth hormone concentrations were similar and normal in patient and control groups despite significant differences in IGF-I levels. Results could be affected by timing of blood sampling and/or limitations of the assay sensitivity. There was no relationship between GH and FI.

8.7 OVARIAN ACTIVITY - EFFECTS OF LH, SHBG, IR, GH & IGF-I : OVULATION VS ANOVULATION

Follicular growth and spontaneous ovulation occurred in 47% of patients with oligo/amenorrhoea during the observation period. Luteinizing hormone, androgens and I levels had no direct influence on the occurrence of spontaneous ovulation.

The levels of SHBG showed the least variation within and between patients and was the most consistent of all of the endocrine variables. In addition, the mean basal as well as mean follicular phase SHBG

concentrations showed the most significant relationships with the anthropometric variables, and the occurrence of spontaneous follicular growth and ovulation when compared to the other reproductive hormones. Therefore, SHBG was a better predictor of ovarian function than LH and/or T.

Waist : hip ratio rather than BMI, influenced the occurrence of spontaneous follicular growth and ovulation.

Insulin and IR did not demonstrate any direct effect on the occurrence of spontaneous ovulation or on the ovarian gross morphology as monitored by the US scan. There was a tendency towards an increase in FI and FIRI with increased degree of disturbance of ovarian function but the differences were not significant. The observed cycles might not represent the entire menstrual history of the patients. It may be expected that if IR plays a major role in the pathogenesis of PCOD, it should also correlate with ovulation and polycystic changes in the ovaries. Insulin may influence ovarian activity indirectly through its relationships with IGF-I and leptin. These interactions need further exploration.

Significantly lower mean basal IGF-I concentrations were found in patients with oligo/amenorrhoea than in the control group, despite similar mean GH concentrations. Furthermore, similar mean IGF-I concentrations were found in patients who failed to ovulate as in patients who had spontaneous ovulation and in controls. The concentrations of IGF-I were also significantly lower in the group of patients who ovulated than in the control group. Insulin-like growth factor-I may influence the adequacy of luteal function rather than the occurrence of ovulation.

Insulin and/or IGF-I appear to play a physiological role in the regulation of gonadal steroidogenesis. Insulin is thought to regulate the circulating SHBG concentration (Franks, 1989) by acting directly on the hepatic synthesis of SHBG (Buyalos *et al* 1993; Rajkhowa *et al* 1994). Hyperandrogenized patients with high FAI showed significant hyperinsulinaemia, higher FIRI values and less occurrence of spontaneous ovulation than patients with normal FAI. Insulin can act through the IGF-I receptors especially when the IGF-I levels are low.

It is apparent from the findings of this study that anovulation in patients with PCOD was not caused by high LH, high androgens or low FSH concentrations. Therefore, a new hypothesis to explain anovulation in such patients is proposed.

The finding of significantly low circulating IGF-I concentrations may be a clue. An IGF-I threshold at the level of the ovary may be critical for the occurrence of ovulation, especially since low IGF-I levels have been reported in follicular fluid of patients with PCOD than in normal women. Low circulating

IGF-I concentrations may indicate dysfunctionally low IGF-I concentrations at the ovarian level. It is known that I can act through the IGF-I receptor and the presence of low IGF-I concentrations favours this action especially in the group of patients with high I.

Significantly low SHBG concentrations were found in PCOD patients and I exaggerates this effect by a direct action on SHBG synthesis in the liver. This results in high circulating levels of free androgens (high FAI).

Although a direct relationship between I and androgens could not be established in this study, it can still be postulated that an interaction between I and free androgens will result in anovulation which is exacerbated by the presence of low IGF-I and low SHBG concentrations and in turn result in increased I activity and hyperandrogenaemia.

The observation in these studies in preliminary data that IGF-I levels were significantly lower in the patient group are extremely interesting but need to be confirmed when all of the results are available. This finding may be of extreme importance since it is well established that by some, as yet unclear, mechanism IGF-I effects ovarian function. Previous reports have described contradictory results with IGF-I levels the same as those in controls (Kazer *et al* 1990; Sharp *et al* 1991; Slowinska-Srzednika *et al* 1992; Holte *et al* 1994a) or greater than those in controls (Laatikainen *et al* 1990). It is possible that the differences in IGF-I levels could be a function of different assay methodology being used in the different studies.

If the findings of low IGF-I concentrations in the PCOD patients is confirmed this may be an important clue with respect to these patients " ovarian dysfunction ".

8.8 LIPID METABOLISM IN PCOD - EFFECTS OF ANTHROPOMETRIC, ENDOCRINE AND METABOLIC VARIABLES

The mean values and ranges of plasma leptin concentrations were similar in patients and controls and were strongly related to BMI, WHR and waist in both groups. However, in the absence of obesity, leptin concentrations were higher in lean patients than lean controls. Although the rate of leptin production is directly related to obesity, other factors may regulate its production - such as a chronic state of IR. A highly significant relationship was found between mean serum leptin concentrations and indices of IR. No relationship was observed between leptin concentrations and any of the endocrine variables or TOV.

Leptin concentrations were not significantly different in patients who failed to ovulate, compared with those who did. It is unlikely that the occurrence of spontaneous ovulation in patients with oligo/amenorrhoea is directly influenced by leptin levels.

Patients with PCOD demonstrated higher WHR and fasting plasma TG concentrations and lower HDL-C concentrations. Significantly higher post-heparin HL activity was demonstrated in patients with PCOD than in normal female volunteers. Women with PCOD demonstrated male type HL activity. Hepatic lipase is stimulated by androgens, and it is presumed that the hyperandrogenaemia in this group of women was responsible for stimulating HL activity into the male range. These findings add weight to the increasing body of evidence that the sex hormone status of women plays a major role in the disturbance of lipoproteins in an atherogenic direction.

8.9 FUTURE WORK

The importance of weight-matched controls in any study of PCOD and/or disturbed ovarian activity must be emphasized.

Total body IS can be assessed by different techniques, although simpler methods should be implemented if possible for routine clinical practice. The results presented in this thesis suggest that FI alone might be as good as any technique.

Further research on the mechanisms of anovulation in PCOD needs to be performed in order to establish the differential roles of obesity, IR, hyperandrogenaemia, IGF-I and leptin on the reproductive system. Particularly the roles of the significant differences in IGF-I and SHBG need further investigation to establish any functional effects.

Because of the heterogeneity of the PCOD and the dynamic state of the ovary, repeated endocrine and US assessments are recommended in any future studies.

The US features could be clinically useful to distinguish between responders and non-responders to the treatment of ovulation induction.

It will be of interest to follow-up the patients with unilateral PCO to observe any progression of the disease.

APPENDICES

APPENDIX I : PATIENT INFERTILITY SHEET

Date of Consultation :

Patient's Name :

Date of Birth : Contact Phone Number (Home) :

(Work) :

Referred by : Occupation :

Husband's Name :

Date of Birth : Occupation :

G.P.

Years since marriage : Parity :

Period of Infertility :

Frequency of Intercourse : Dyspareunia (Y/N) : Type :

Other Sexual Dysfunction (Y/N) :

- Impotence - Premature Ejaculation - Vaginal Soreness - Others

Previous Marriage(s) / Relationship(s) :

Husband : Outcome :

Wife : Outcome :

Gynaecological History :

Age at Menarche :		LMP :	Cycle (K) :	
Regular / Irregular :		Duration of Irregularity :		
Description of Irregularity :				
Blood Loss :	DUB :	Dysmenorrhoea (Y/N) :	Type :	Duration :
IMB (Y/N) :	PCB (Y/N) :	Vaginal Discharge (Y/N) :		

Change in Weight :

Excessive Hair Growth (Y/N) : Duration : Location : Frequency of Removal :

Acne (Y/N) : Greasy Skin : Change of Voice : Breast Discharge :

Current or Previous illness :

Wife	Husband
.....	.....
.....	.....
.....	.....
.....	.....

Operation :

Wife	Husband
.....	.....
.....	.....
.....	.....

History of Medications / Drugs (including smoking and alcohol) :

Wife	Husband
.....	.....
.....	.....
.....	.....

Family History :

General Examination : Appearance :

Height : Weight : BMI : WHR :

Pulse : Blood Pressure :

Thyroid : Breast Examination : -Tanner's Stage (I - V)
- Galactorrhoea :
- Lumps :

Chest Examination : Heart Examination :

Abdominal Examination :

Pelvic Examination :

Hair Distribution (Normal / Abnormal) : Description of Hair Distribution :

APPENDIX II : PATIENT INFORMATION SHEETS

AN EXPLANATION & INSTRUCTIONS FOR PATIENTS UNDERGOING POLYCYSTIC OVARY STUDY

INTRODUCTION

In women with normal ovarian function, an egg is released from the ovary each month; it either meets a sperm and fertilization takes place resulting in a pregnancy, or bleeding will occur (the period). A prolonged menstrual cycle of more than 41 days, called " Oligomenorrhoea ".

"Oligomenorrhoea " can be caused by many endocrine disorders" which might prevent the ovaries from producing an egg every month; this will result in delaying the period and reducing the chances of pregnancy as well.

" Oligo/amenorrhoea " study will involve studying the hormone changes involved in the irregularity of your cycles and this should help in design of best treatment as well.

The blood tests and the scans, which will be carried out as explained below, are named " Oligo screen ".

INSTRUCTIONS FOR PATIENTS

To commence an " Oligo screen ", please contact the nursing staff at the Assisted Conception Services "ACS " Unit within the first 2 weeks of your period starting, with a note of first day of its start.

Make sure that you are not on any treatment that stimulates the ovaries, or hormonal contraceptive for a period of at least 2 months before you start the investigations. A date will be given to you then to start your investigations, which may be between day 15 and 20 after a period, or as convenient if your period was too long ago.

Investigation of your hormone levels involves having approximately 10 mL blood sample taken twice every week for a maximum of 5 weeks. The hormones will be monitored and if ovarian activity is

detected you will need to give a blood sample every day for a maximum of 2 weeks or till your next menstrual period whichever occurs first.

On the day of your first blood samples, you should come fasting overnight, and be at the ACS Unit at 8:30 am. Your weight will be recorded and 10 mLs of blood sample will be taken for basal levels of your hormones, then a specific test called Short Insulin Tolerance Test,"SITT", will be carried out. This is explained simply as follows :

[After a 12-h overnight fast, the first group of blood samples will be collected from you after 5 and 15 minute of the start. This is followed by a single insulin injection into a vein in your arm and a cannula will be kept in your vein order to collect blood samples during the test easily. The second group of blood samples will be collected 5,10,15,20,30 and 60 minutes after the injection. At 15-20 minutes following the insulin injection, you will be given a sweaty drink,(a cup of tea or coffee with sugar for example). Although it rarely happens, you might feel dizzy and sweaty because of the drop in your blood sugar level. This can be easily treated, if necessary, with a drip of glucose connected to your vein through the same cannula previously mentioned].

The first ultrasound scan (us scan) will be performed during the day of your first blood tests, further scans will be assigned as needed.

If the analysis from the laboratory indicates that ovulation may occur, you will be asked to change to daily blood tests for a maximum of two weeks to assess the quality of the ovulation.

A repeat of the "SITT" test will be carried out once more during week 5 after the start of sampling, or 7-10 days after ovulation.

1. Blood Samples

These may be taken either at the hospital or at your G.P.'s Surgery.

Hospital : blood samples are taken at the "ACS" Unit. Times for blood tests are between 9:00am and 9:30am every day, including Saturday and Sunday.

G.P. : If your G.P. is willing to take blood samples for you, bottles, labels, envelopes and containers will be supplied by the hospital. Please label the bottles clearly with your Name and Date of sampling and

ensure their delivery or postage (First Class) to the University Laboratory in the Royal Infirmary Hospital. 10 mLs of blood are required for each sample. You can keep the bottles in the fridge till time of postage.

N.B. The "SITT" test will be done at the hospital and while your are fasted.

2. Ultrasound Scans

Scans are necessary to assess ovarian function: they can detect growing follicles and/or polycystic ovaries. These scans are carried out in the ultrasound room, at the ACS Unit. Vaginal scanning is the method used which does NOT require a full bladder. It is similar to an "internal examination". This is not painful and it gives more accurate information about ovarian activity than other methods. You will need a number of scans during the "Oligo screen" and will be informed after scanning of the date of your next scan.

3. Results :

A clinic appointment will be made after the last blood sample where the results will be discussed and treatment will be assigned for you. Please make sure that you have this appointment on completion of your "Oligo screen" scan.

EXPLANATION & INSTRUCTIONS FOR PATIENTS UNDERGOING THE STUDY OF THE REGULATION OF BLOOD FAT LEVELS BY HORMONES

I understand that a team of researchers at the GRI are investigating the key hormonal factors which control blood fat levels in women.

The studies involve the following :

Taking an initial 5 mL blood sample for a full blood count,

Filling in a questionnaire which will allow the doctor to assess your suitability to receive heparin. The reason for asking you these questions is that heparin is used to thin the blood and in rare circumstances can cause you to bleed. This is much more likely, however, if individuals have had certain medical problems in the past.

Taking a 40 mL fasted blood sample.

On the same occasion as (3) above, the administration of heparin followed by a 10 mL blood sample, after you have been assessed by a doctor as to your suitability for receiving heparin.

Giving a blood sample may be associated with minor discomfort.

I understand that my involvement in this study is entirely voluntary and that I may withdraw at any time.

APPENDIX III : CONSENT FORMS

Consent Form (1) :

Full Name of Patient : .....

Address of Patient : .....

.....

.....

.....

I consent to participate in the " Study of Oligo/amenorrhoea " and agree to take the tests involved in the study including the " Short Insulin Tolerance Test - SITT ". The procedure has been explained to me in detail by the staff and an information sheet given by :

.....

and I am aware that I am free to withdraw from the study without prejudice to my future treatment.

Patient's Signature ..... Date .....

Doctor's Signature ..... Date .....

Consent Form (2) :

I, (Name) : .....

of (Address) : .....

.....

.....

agree to take part in the Study of " Regulation of blood fat by hormones " described in " Patient's Summary ".

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

Patient's Signature Date

....

Doctor's Signature Date

....

Heparin Administration Medical Questionnaire :

Patient's Name : .....

Date of Birth : .....

Dear Patient :

Thank you for participating in our study. You will be given an injection of heparin to enable us to measure the activity of key factors involved in fat metabolism. In order for us to be certain that it is safe to give you the heparin, would you please answer the questions and sign the sheet when you have finished. If you have any further questions or something is unclear, please ask.

1. Are you quite fit at the moment ? YES / NO
2. Are you receiving any medical treatment ? YES / NO
3. If so, please specify : .....
4. Have you taken aspirin recently ? YES / NO
5. As far as you know, are you sensitive to heparin ? YES / NO
6. Do you have haemophilia or any other diagnosed bleeding disease ? YES / NO
7. Have you ever suffered from a stroke ? YES / NO
8. Have you had rheumatic fever ? YES / NO
9. Have you had a recent surgery ? YES / NO
10. Have you had a recent head injury or trauma requiring hospital admission ? YES / NO

Height : ..... Weight :..... Blood Pressure :.....

Signature : ..... Date : .....

APPENDIX IV : THE BETWEEN- AND WITHIN-ASSAY COEFFICIENTS OF VARIATION OF THE ENDOCRINE METHODS

Hormones	Between-assay C. V. (%)	Within-assay C.V. (%)
E_2 (pg/mL)	4.8	2.5
LH (IU/L)	6.4	5.3
FSH (IU/L)	5.9	1.7
T (nmol/L)	7.9	1.4
SHBG (nmol/L)	3.5	3.2
P (ng/mL)	5.6	3.8
A (nmol/L)	6.9	3.4
17∝-OHP (nmol/L)	9.6	3.1
DHEA-S (umol/L)	8.0	5.7
PRL (mU/L)	5.7	8.9
T4 (nmol/L)	6.3	1.3
T3 (nmol/L)	6.4	3.8
FT4	8.3	6.2
FI (mIU/L)	5.4	3.6
Glucose (mmol/L)	3.4	2.2
C-peptide (ng/mL)	7.9	3.6
GH (ng/mL)	6.5	3.7
IGF-I (nmol/L)	10.5	4.0
Cortisol (ug/dL)	8.3	4.9
Leptin (ng/mL)	<10	0.7

APPENDIX V : US SCAN DATA

The Mean and Coefficients of Variation of TOV in Patients

Patients	Mean TOV (cm^3)	Within-Patient C.V. (%)
Patient 1	8.7	1.8
Patient 2	31.2	5.1
Patient 3	21.6	5.7
Patient 4	24.9	8.0
Patient 5	26.7	3.3
Patient 6	19.3	1.7
Patient 7	10.7	1.9
Patient 8	19.2	7.3
Patient 9	40.5	4.7
Patient 10	20.1	7.4
Patient 11	11.6	9.0
Patient 12	20.2	0.2
Patient 13	16.7	7.3
Patient 14	26.9	6.5
Patient 15	18.0	4.0
Patient 16	11.3	6.8
Patient 17	27.7	1.2
Patient 18	9.7	7.5
Patient 19	14.5	1.9
Patient 20	15.8	8.7
Patient 21	18.7	2.4
Patient 22	17.6	5.9
Patient 23	6.5	3.8
Patient 24	13.8	4.0
Patient 25	15.7	4.0
Patient 26	18.2	1.1
Patient 27	19.2	4.8
Patient 28	11.2	10.1
Patient 29	17.2	3.5
Patient 30	16.3	9.1
Patient 31	8.1	9.5
Patient 32	10.4	10.2
Patient 33	2.9	6.7
Patient 34	17.7	6.5
Patient 35	23.5	2.3
Patient 36	35.3	3.0
Patient 37	14.1	10.4
Patient 38	24.5	6.8
Patient 39	19.5	4.1
Patient 40	10.6	4.5
Patient 41	15.1	5.5
Patient 42	8.5	4.3

APPENDIX IV : US SCAN DATA

TRANSVAGINAL US IMAGES OF THE OVARY

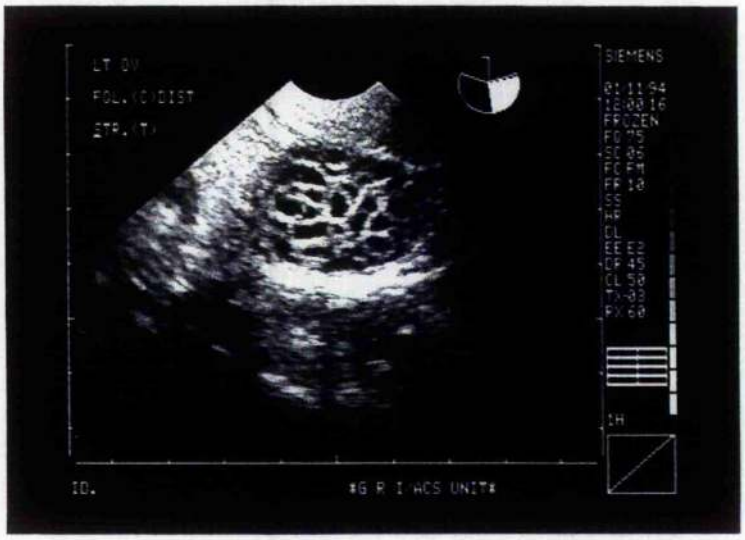


Figure (1) : A picture of an ovary in one of the study patient with oligo/amenorrhoea, showing a " PCO " picture with a large number of follicles centrally distributed with minimal, thick stroma left behind.

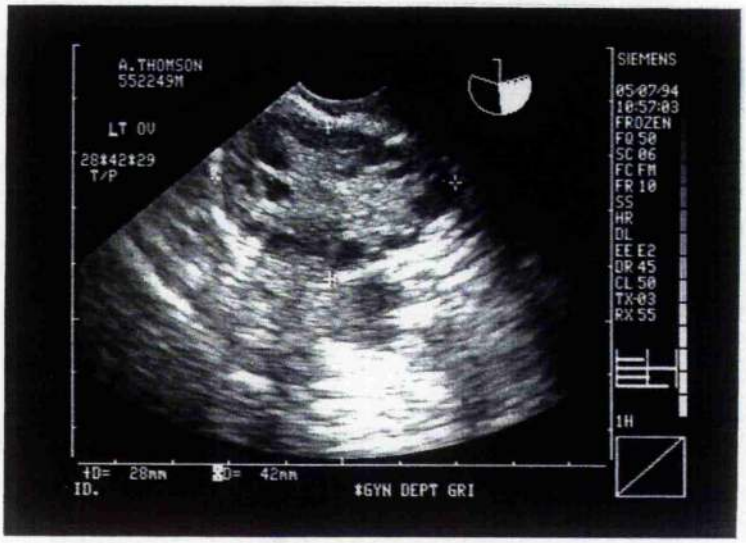


Figure (2) : Another oligo/amenorrhoeic patient with an enlarged " PCO " picture, where the small follicles are multiple and distributed peripherally.

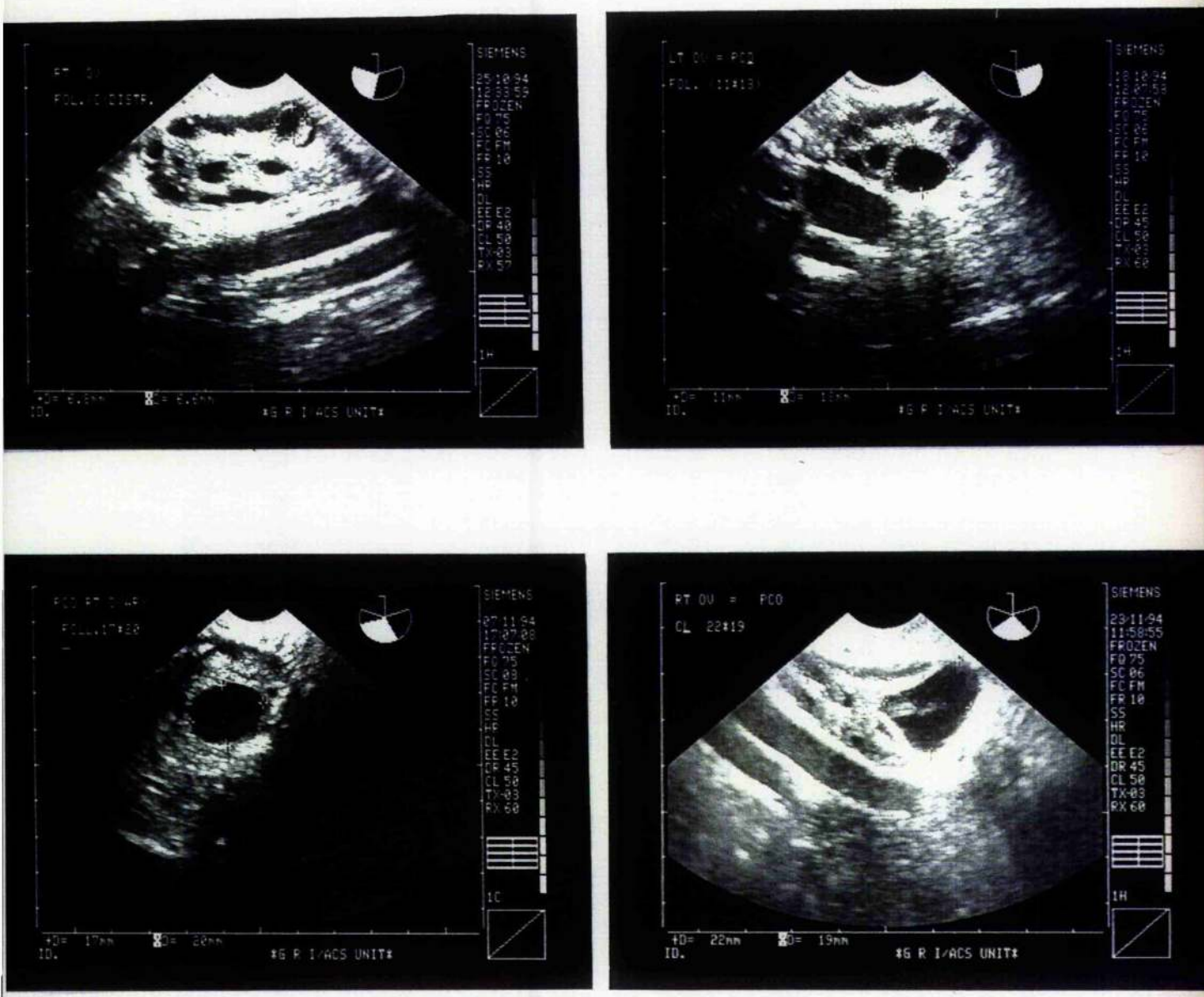


Figure (3a - 3d) : Ultrasound images of an ovary from an oligo/amenorrhoeic patient demonstrating the different stages of follicular growth in a spontaneous ovulatory cycle, (3a) a small follicle of 6.8 × 6.6mm, (3b) a pre-ovulatory follicle of 11× 13mm, (3c) a mature follicle of 17 × 20mm, and (3d) a corpus luteum of 22 × 19mm.

ULTRASOUND SCAN DATA SHEET :

ULTRASOUND DATA

NAME:.....

DOB: ...../...../..... Ht (Ms):..... Wt (Kgs): .....

PARITY: ..... Date of last delivery: .....

Ovarian Surgery: .....

LMP: ...../...../.....

NOTE

	RIGHT						LEFT				
DATE	FD<10mm (N)	DIST P/C	FD>10mm (N)	VOLUME	STROMA N / T(hick)		FD<10mm (N)	DIST P/C	FD>10mm (N)	VOLUME	STROMA N / T(hick)

MP AT END OF CYCLE: .....

APPENDIX VI : METHOD OF CALCULATION OF THE KITT-VALUES IN THE " SITT "

PROGRAM FOR KITT VALUATION

**using data in hudasitt to calculate KITT from glucose decay

```
USE HUDASITT
set filt to KITT = 0 .AND.G3>0.AND.G5>0 .AND. G7>0 .AND. G9>0 □
.AND. G11>0 .AND. G13>0 .AND. G15>0
GO TOP
DO WHILE .NOT. EOF()
SX=63

 SY=LOG(G3)+LOG(G5)+LOG(G7)+LOG(G9)+LOG(G11)+LOG(G13)+LOG(G15)
SXY=(LOG(G3)*3)+(LOG(G5)*5)+(LOG(G7)*7)+ (LOG(G9)*9)+ □
(LOG(G11)*11)+ (LOG(G13)*13)+ (LOG(G15)*15)
SSXY=SXY-((SX*SY)/7)

SXS= 679
SXX=679-((SX*SX)/7)
MKITT=(SSXY/SXX)*-100
REPL KITT WITH MKITT
REPL THALF WITH 69.3/MKITT
SKIP
LOOP
enddo

set filt to KITT30 = 0 .AND.G3>0.AND.G5>0 .AND. G7>0 .AND. G9>0 □
.AND. G11>0 .AND. G13>0 .AND. G15>0 .AND. G20>0 .AND. G25>0 .AND. G30>0
GO TOP
```

```
DO WHILE .NOT. EOF()
SX=138

SY=LOG(G3)+LOG(G5)+LOG(G7)+LOG(G9)+LOG(G11)+LOG(G13)+LOG(G15)+LOG(G20)+LOG(
G25)+LOG(G30)
SXY=(LOG(G3)*3)+(LOG(G5)*5)+(LOG(G7)*7)+ (LOG(G9)*9)+ (LOG(G11)*11) + (LOG(G13)*13)+
(LOG(G15)*15)+ (LOG(G20)*20)+ (LOG(G25)*25) + (LOG(G30)*30)
SSXY=SXY-((SX*SY)/10)
SXS= 2597
SXX=2597-((SX*SX)/10)
MKITT=(SSXY/SXX)*-100
REPL KITT30 WITH MKITT
REPL THALF30 WITH 69.3/MKITT
SKIP
LOOP
ENDDO
USE
CANCEL
```

APPENDIX VII : ABSTRACTS SENT FOR PUBLICATION

ABSTRACT - 1 : Variations in Parameters of Ovarian Function and Insulin Tolerance in Infertile Women with Oligo/amenorrheoa

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Introduction : Infertile women (n = 23) with oligo/amenorrhoea (K > 41 days) are frequently observed to have polycystic ovaries (PCO) and demonstrate evidence of hyperandrogenism and elevated serum LH concentrations, and also insulin resistance. They may also ovulate spontaneously. The aim of this study was use the the short insulin tolerance test (SITT) to estimate the insulin resistance in such patients, to determine its within-patient variability, and to explore its relationship with other parameters in those patients showing evidence of follicular maturation and ovulation, compared with those with no follicular development.

Methods : Blood sampling was initiated > 14 days after a menstrual period at a frequency of 2 samples per week for 5 weeks. When the plasma oestradiol (E_2) indicated that follicular growth was established daily samples were taken to estimate follicular maturation and the luteal phase progesterone profile. The initial SITT was effected at the start of the blood sampling and a second was effected either at the end of sampling or in the luteal phase of those who ovulated.

Results : Forty five SITT tests were effected in 23 patients. The initial results indicate that there was considerable variation in the KITT, both within and between patients. The KITT-value was unrelated to the circulating E_2 concentration or the ovarian volume, which also showed high intra-patient variability. The KITT was also unrelated to the body mass index. Eight of the 23 patients showed evidence of follicular maturation and ovulation during the period of observation. The KITT was no different in those who ovulated compared with those who did not. Patients demonstrating supranormal LH at the SITT (n = 12) showed higher circulating testosterone and lower E_2 concentrations, and also higher KITT-values than those with normal LH.

Discussion : The data indicate that the SITT yielded reliable within patient results. However, there was no association of the SITT data with any of the physiological or endocrine measurements studied. This implies that IR is only loosely associated with specific parameters of PCOD.

The abstract was published in *Journal of Endocrinology* (1995), **144 (3)**, Suppl., P339.

ABSTRACT - 2 : Female Hyperandrogenism Stimulates Hepatic Lipase Activity into the Male range

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Abstract : Recent epidemiological studies have highlighted the importance of low density lipoprotein (LDL) subtype as a risk marker for coronary heart disease. LDL which is small and dense is associated with high risk, independent of total LDL concentration. In a previous population study we demonstrated that in women, the development of a high concentration of small, dense LDL is dependent upon both plasma triglyceride and hepatic lipase levels. This enzyme is regulated strongly by sex hormones levels. On the basis of these findings we formulated the hypothesis that lipoprotein associated risk in women is due in part to an altered androgen/oestrogen balance which stimulated hepatic lipase and in so doing promotes the redistribution of circulating LDL into small, dense, atherogenic LDL.

Methods : To test the relationship between sex hormone status and hepatic lipase activity we measured the activity of this enzyme in eight women with oligomenorrhoea and polycystic ovaries (demonstrated by ultrasound). The majority of such cases demonstrate hyperandrogenism.

Results : The mean hepatic lipase activity for this group of women was significantly greater than the mean activity for normal females (n = 67), established from previous studies (20.55(4.74) vs 11.30(5.68) uM/FFA/mL/hr, P = 0.0007), and indeed fell into the male range.

Discussion : This finding adds weight to the increasing body of evidence that sex hormone status of women plays a major role in perturbing lipoprotein in an atherogenic direction.

The abstract was published in *Scottish Medical Journal* (1995), **40**, p.p. 126.

ABSTRACT - 3 : Oligo/Amenorrhoea - Relationship between Leptin and Spontaneous Ovulation

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Introduction : It is known that the main cause of infertility in women with oligo/amenorrhoea is anovulation. The key feature of anovulation in such patients is failure of normal follicular maturation, and obesity is commonly associated with this condition. Leptin, a recently discovered hormonal product of the obesity gene, is expressed by adipocytes and thought to play a role in the regulation of food intake and metabolism. There is a strong positive correlation between serum leptin concentration and body fat. Leptin receptors are widely distributed in the body, however, it is not known yet where the site(s) of leptin action are located; even in the hypothalamic-pituitary-gonadal axis. In mice, leptin receptors were identified in the ovary and leptin administration was found to stimulate gonadal function, causing significant increases in LH, sex steroid production and increased ovarian and uterine weights, indicating greater amounts of follicular development which were confirmed histologically. Little is known about the physiologic action of leptin in humans. The aim of this study was to determine a relationship between circulating leptin concentrations and the occurrence of spontaneous ovulation in oligo/amenorrhoeic human females.

Methods : Forty one infertile women with oligo/amenorrhoea and PCOD (menstrual cycle > 41 days) and age of ≤ 38 years were recruited for the study. The group of patients were compared with normal and regularly menstruating, age and weight-matched volunteers (n = 19). The investigations were started at least 14 days after the last menstrual period in oligomenorrhoeic cases and between days 508 for the controls.

Results : During the period of observation, 18 patients ovulated spontaneously. The mean leptin concentrations were similar in patients and controls. However, lean patients had higher leptin concentrations than lean controls (P = 0.0449) but there was no such difference between obese patients and controls. No relationship was found between leptin concentrations and E_2, LH, FSH, T or SHBG. The relationship between leptin and the occurrence of spontaneous ovulation was masked in the obese

individuals. However, leptin concentrations in lean controls were similar to its levels in ovulatory patients but were lower than in lean anovulatory patients ($P = 0.05$). However, for a given BMI, leptin concentrations were not significantly different in patients who failed to ovulate, compared with those who did.

Discussion : It is unlikely that the occurrence of spontaneous ovulation in oligo/amenorrhoeic patients is influenced by leptin levels.

The abstract was accepted for the "European Society of Human Reproduction & Embryology Congress" Meeting, (ESHRE), 22-25 June, 1997.

ABSTRACT - 4 : Dynamic Changes Of Ovarian Volume in Patients with Oligo/Amenorrhoea - Absence of Relationship with Hormone Parameters

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Introduction : There is a tendency to consider oligo/amenorrhoea and PCOD as static conditions, and diagnosis with a single ultrasound assessment has been a common practice. However, it is evident from the longitudinal studies that much biological variability exists.

Methods : A group of patients (n = 41) with infertility and oligo/amenorrhoea and PCOD were recruited. The assessment of ovarian morphology, by means of transvaginal ultrasound scanning, was carried out at least twice weekly for a period of 5 weeks. The investigations were started > 14 days after the last menstrual period. Blood samples were collected for E_2, LH, FSH, T and SHBG assays at the time of each scan.

Results : Follicular growth and spontaneous ovulation was observed in 18 patients. Significant variations in the total ovarian volume (TOV) were observed both between and within patients during the 5-week observation period, regardless of the occurrence of spontaneous ovulation. The TOV was not related to the length of oligo/amenorrhoea or years of infertility. In addition, there was no influence of ovarian volume on the mean follicular phase concentrations of the reproductive hormones. Marked asymmetry between the two ovaries on the basal scan, i. e., one being less than 75% of the contralateral was observed in 39% of patients. The patients' 5-weeks TOV was 19.33 ± 7.45 cm^3, and more than half of the patients showed > 60% change in their TOV, while 41.5% had doubled their ovarian volume during the period of monitoring. These significant changes in TOV were not accompanied by any significant difference in the endocrine hormones. Furthermore, the wide variations in TOV were not related to BMI or WHR. Surprisingly, during the 5-week window of observations, the mean TOV of patients who ovulated was similar to that of patients who failed to ovulate. The variability in the TOV (difference between minimum and maximum TOV) was no different in patients who ovulated compared with those who did not ovulate.

Discussion : This study clearly showed that assessment of ovarian morphology by means of ultrasound demonstrated the dynamic state of the ovaries as marked changes in TOV, regardless of the occurrence of spontaneous ovulation, and the changes were not related to hormone concentration changes.

The abstract was accepted for the "European Society of Human Reproduction & Embryology Congress" Meeting, (ESHRE), 22-25 June, 1997.

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