Introduction
1. INTRODUCTION

An improved understanding of the control of food intake is a priority nowadays because of the enormous toll on human health taken by obesity and related disorders. The discovery of the fat melting hormone ‘leptin’ as a regulator of food intake is a new weight loss hope.

Leptin is an adipocyte derived novel peptide hormone which regulates body weight by signalling the amount of stored fat (Pinto et al., 2004). It is a 16 kDa protein with 167 amino acid residues transcribed from the Ob (Obese) gene. The human leptin gene is on chromosome 7q31; its DNA has more than 15,000 base pairs and there are three exons, the major coding sites driving protein synthesis (Auwerx and Staels, 1998). Leptin is mainly produced in white adipose tissue and the receptor is expressed mainly in the hypothalamus (Benoit et al., 2004).

1.1 REGULATION OF FOOD INTAKE AND BODY WEIGHT

The circulating leptin informs the brain of body fat. Leptin promotes weight loss by suppressing appetite and stimulating metabolism. Neuropeptide Y (NPY), abundant in the hypothalamus, is responsible for the feeling of hunger. The expression and release of NPY is inhibited by leptin. As weight is gained, the leptin levels are increased. This inhibits the function of NPY creating a response to obesity – food intake is decreased, energy expenditure is increased and the sympathetic activity is increased. On the other hand, as weight is lost, there is a decrease in leptin circulation, which increases the effect of NPY causing a response to starvation (Takahashi and Cone, 2005). The appetite is increased which will reduce unnecessary energy expenditure, including reproductive function. The overall
temperature of the animal is decreased and there is an increase in parasympathetic activity.

Leptin serves as an intracellular messenger as well. The amount of leptin in the body will directly influence the amount of leptin that is produced by adipose tissue. Diabetic and obese mice usually have dysfunctional leptin or no leptin at all in the blood stream. When leptin binds to the leptin receptor which is a class 1 cytokine, the leptin signal is transmitted via janus kinase 2 to the signal transducers and activators of transcription. If the receptor is mutant, then it is impossible to signal for the cell to stop growing and producing leptin. If leptin is not produced the animal can become obese (Friedman and Halaas, 1998).

Leptin is the afferent signal in a negative feedback loop that maintains constancy of adipose tissue mass. Leptin is secreted from adipocytes either as a 16kDa protein or bound to a soluble form of its receptor (Ob-R). The level of leptin is positively correlated with differences in body fat. Increased leptin results in negative energy balance whereas decreased levels lead to positive energy balance. Leptin acts mainly on the hypothalamus. Extensive connections exist between the hypothalamus and other brain regions. Leptin acts centrally to decrease food intake and modulate glucose and fat metabolism. Peripheral effects on T cells, pancreatic islets and other tissues have also been demonstrated (Martin and Myers, 2004).

1.2 CONCENTRATION OF LEPTIN IN PLASMA AND SERUM

In humans, a strong positive correlation is observed between serum leptin levels and the amount of body fat and adipocyte leptin mRNA as in rodents. The plasma concentration of leptin was positively correlated with plasma concentration of growth hormone (GH) and non esterified fatty acids (Bzock et al., 2001). Plasma leptin concentration progressively increased with higher grade of hypertensive
retinopathy. Serum leptin concentrations exhibit a sexual dimorphism with circulating leptin levels higher in women than in men (Martin et al., 2002). Circulating leptin concentrations are positively correlated with measures of obesity including body mass index (BMI) and percent body fat. A strong positive correlation between leptin mRNA levels and BMI in humans has also been confirmed (Vidal et al., 1996).

1.3 LEPTIN AND OBESITY DISORDERS

Obesity is a common medical problem and a risk factor for illness such as hypertension, diabetes, degenerative arthritis and myocardial infarction. The cut-off points proposed by Indian Council of Medical Research (ICMR) expert committee for the classification of overweight in adult based on their BMI is given in Table 1.1, which defines obesity as a body mass index greater than 30. Obesity is caused by a constellation of factors including excessive energy intake, insufficient energy output, genetic predisposition, low fat oxidation rate, low plasma leptin levels and psychologic stressors (Sharma, 2001). A significant correlation between serum leptin and BMI has been found, but there was considerable variation among obese patients (Maffei et al., 1995). While some appeared to be insensitive to leptin, others had low levels for the degree of obesity. Perhaps leptin treatment could help the latter group.

Table 1.1 Cut-off Points proposed by an Indian Council of Medical Research (ICMR) expert committee for the classification of overweight

<table>
<thead>
<tr>
<th>BMI (kg m$^2$)</th>
<th>ICMR classification</th>
<th>Popular description</th>
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</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td>Underweight</td>
<td>Thin (Underweight)</td>
</tr>
<tr>
<td>18.5 - 27.8</td>
<td>-</td>
<td>Healthy, normal</td>
</tr>
<tr>
<td>27.9 - 32.2</td>
<td>Grade 1 overweight</td>
<td>Overweight</td>
</tr>
<tr>
<td>32.2 - 39.9</td>
<td>Grade 2 overweight</td>
<td>Obese</td>
</tr>
<tr>
<td>&gt; 40.0</td>
<td>Grade 3 overweight</td>
<td>Morbidly obese</td>
</tr>
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</table>
1.4 LEPTIN AND DIABETES

There has been a debate as to whether leptin was important in the development of type 2 diabetes (Zimmet and Alberti, 1996; Taylor et al., 1996). As discussed previously, leptin can impair insulin production, and some data indicate that leptin could also play a role in the development of peripheral insulin resistance. Thus, a hypothesis for the interference of leptin in the development of insulin resistance and type 2 diabetes in obese subjects could be that high leptin levels as observed in obesity lead to hyperglycemia through suppression of the glucose induced insulin secretion by the pancreas. Peripheral insulin resistance, possibly also induced by hyperleptinaemia, may then add to further glucose intolerance, overruling this leptin-induced suppression of insulin secretion and eventually induce hyperinsulinaemia. Another hypothesis is that the high serum leptin levels in obesity result in desensitization of the receptor and thus defective leptin receptor signalling in β-cells, which leads to chronic hyperinsulinaemia and may thus, contribute to the pathogenesis of diabetes (Seufert et al., 1999).

1.5 LEPTIN IN HYPERTENSION, DIABETES MELLITUS, AND POLYCYSTIC OVARIAN DISEASE

In the short term, leptin may function as a potassium-sparing diuretic natriuretic factor (Jackson and Li, 1997), in the long term, it increases norepinephrine turnover and sympathetic nerve activity in rodents (Haynes et al., 1997; Dunbar et al., 1997) and humans (Snitker et al., 1997). These results in increased blood pressure in rodents (Dunbar et al., 1997; Shek et al., 1998), but a potential role of leptin in the pathogenesis of hypertension in humans remains to be conclusively demonstrated. Obesity, hypertension, and insulin resistance are closely related in humans (Mantzoros and Flier, 1995). However, although administration of
leptin improves insulin resistance in mice (Sivitz et al., 1997) and insulin resistance has been associated with increased leptin levels in one study in humans (Segal et al., 1996), several independent studies have shown that serum leptin levels are similar in patients with type 2 diabetes mellitus and controls (Mantzoros et al., 1997; Nolan et al., 1996). The role of circulating leptin has also been investigated in a heterogeneous group of patients with the polycystic ovary syndrome, which is associated with insulin resistance (Conway and Jacobs, 1997). In most studies, serum leptin levels in women with the polycystic ovary syndrome did not differ from those of normal women (Mantzoros et al., 1997; Chapman et al., 1997), but in one study (Brzchff, et al., 1996), a group of women with the polycystic ovary syndrome had increased leptin levels. However, because recent data indicate that leptin may directly affect glucose and fat metabolism and because leptin receptors have been identified in the ovaries (Karlsson et al., 1997), it has been proposed that locally acting leptin (Spicer and Francisco, 1997; Zachow and Magoffín, 1997) may be more important than circulating leptin in the pathogenesis of the polycystic ovary syndrome and type 2 diabetes.

1.6 LEPTIN REGULATION IN CARDIAC VASCULAR DISEASE

While acute leptin infusion failed significantly to alter the heart rate over a span of 90 min (Fruhbeck 1999), chronic leptin treatment over one week and cerebral injection of leptin elicited a significant increase in heart rate along with an increase in sympathetic nervous activity (Shék et al. 1998). An astonishing positive correlation between hyperleptinaemia and tachycardia has been confirmed in mildly obese or mildly hypertensive human subjects (Narkiewicz et al. 1999). While an increase in heart rate may enhance cardiac output and provide short-term beneficial effects, sustained tachycardia may cause cardiac hypertropy and ultimately heart failure. The higher heart rate in the hyperleptinemic individuals will impose a
greater myocardial workload and therefore predispose the heart to pathophysiological changes, leading to congestive heart failure and myocardial infarction. This is supported by the clinical and experimental observations of obesity-induced cardiac hypertrophy, and the direct correlation between BMI and the left ventricular size in human and animal models (Sowers 1998, Ren et al. 2000). Such a relationship may be transposed into a direct correlation between left ventricular size and plasma leptin levels. While it seems reasonable that the higher heart rate under hyperleptinaemia may be due to leptin-induced sympathetic activation, an independent association between leptin levels and heart rate was observed in heart transplant recipients with sympathetic denervation (Winnicki et al. 2001). This finding suggests a direct effect of leptin on heart rate conceivably through cardiac leptin receptors, although direct effects of leptin on the cardiac conducting system and cardiac growth still warrant further study.

1.7 SALIVARY GLANDS LEPTIN REGULATION

Leptin and its receptor are expressed and distributed in the major salivary glands of humans. Leptin distributed throughout the major salivary glands with obvious intracellular concentrations in granula. In contrast, immunostaining for the leptin receptor was found exclusively in the membranes of the glandular cells. A high density of the leptin receptor was localised in the epithelia of the duct lumen. PCR analysis proved the autonomous expression of leptin by the salivary glands independently from adipocytes.

In the light of recent findings of leptin influencing the growth of rodent salivary glands, the presence and distribution of leptin and its receptor suggests an autocrine role of salivary leptin within the glands (Bohlender et al., 2003).
1.8 ROLE OF LEPTIN IN THE PLACENTA

Placental tissues from humans, rodents and farm animals contain leptin and its receptor. Leptin produced by the human placenta has the same size, charge and immunoreactivity as leptin produced by adipose tissue. However, the expression of human placental leptin appears to be regulated by a placenta-specific upstream enhancer. The occurrence of leptin and its receptor in a range of species and placental types is described, and its significance during pregnancy. Placental leptin contributes to the increase in maternal circulating concentrations of leptin during late pregnancy when it is likely to have an endocrine role in regulating maternal energy balance. Placental leptin may have angiogenic and immunomodulatory activities, which affect the placenta in an autocrine or paracrine manner. It also appears to affect fetal growth and development by binding to leptin receptors present in fetal organs (Ashworth et al., 2000).

1.9 EXPRESSION OF LEPTIN IN GASTRO INTESTINAL MUCOSA

Leptin has been identified in the lower half of the stomach glands both in the pepsinogen granules of chief cells and in the granules of a specific endocrine cell type, suggesting that leptin action is exerted by both exocrine and endocrine pathways. Gastric leptin is sensitive to the nutritional state, being rapidly mobilized in response to food intake following fasting, or after the administration of satiety factors; this suggests a role for this protein in the short-term regulation of feeding, acting in collaboration with satiety peptides such as cholecystokinin (Pico et al., 2003). Leptin, produced by gastric cells and by adipocytes, could act on both acute and chronic regulation of feeding behaviour respectively, giving information to the brain on the availability of external (food) and internal (fat depots) energy resources, thus participating in short- and long-term satiation.
1.10 LEPTIN ROLE IN SEXUAL DIMORPHISM

Leptin is an adipocyte derived peptide hormone known to be involved in the regulation of body weight, energy expenditure and food intake (Zhang et al., 1994). It is a product of human homolog of the ob gene, over expressed in adipose tissue of obese humans and is highly correlated with adiposity. Reports have indicated (Considine et al., 1996) a strong relationship between leptin concentration and body mass index (BMI) (a surrogate for overall adiposity), but relatively few studies have examined the relation between regional fat distribution and leptin levels. It is also not clear whether the upper body adiposity is more strongly correlated with leptin concentration than lower body adiposity, and observed association of body fat distribution is independent of overall adiposity (Haffner et al., 1996). Till date no report is available on the measurement of serum leptin and its relation to adiposity in Indian population and hence our primary concern has been to estimate the levels of leptin in obese and normal weight subjects in south Indian population. The present study examined the influence of BMI, age, gender and hormonal profiles such as serum testosterone, free testosterone, β estradiol and androstenedione on serum leptin concentrations in thin, normal weight, overweight, obese, morbidly obese men and women to further understand the combinatory roles of different determinants on the secretion of leptin in adipocyte. Previous studies have demonstrated that in morbidly obese men and women circulating leptin is positively correlated with BMI and other measures of obesity. Also, differences in serum leptin levels have been previously demonstrated between young and old subjects (Rosenbaum et al., 1996). However, no systemic study had been performed examining the age profile of leptin levels in women and men with respect to BMI (Thin, normal weight vs overweight, obese, morbidly obese) and hormones known to change during the aging process. To address this issue, a study was performed in a
large group of subjects covering a wide range of age and BMI. We confirmed that BMI is the major determinant of leptin levels and provided novel evidence showing that age may affect leptin secretion in non-obese and obese subjects. Through a cross-sectional analysis, treating the data either as groups of age or as continuous variables, we demonstrated that in both sexes there is a rise in leptin levels throughout life that is independent from BMI and other hormonal variables. Also, gender had a major influence on the relationship among leptin, BMI, and aging, with leptin concentrations rising more rapidly as a function of BMI and rising more strongly as a function of age in women than in men. It is well established that aging affects body composition, such as reduced muscle strength and increased fat depots and hypertension in 30 subjects in the age group (41 yrs to 62 yrs) with due credence to those having the habit of smoking and alcoholism. This is the first study demonstrating an influence of sex steroids on serum leptin concentrations in South Indian Population. With our assay system we established a BMI-dependent normal range for men and women. In this assay system, morbidly obese women have 3-fold higher leptin levels than thin and normal women are largely explained by BMI. The BMI is a good indicator of obesity and total amount of adipose tissue; therefore, the information on the percentage of body fat in the morbidly obese men and women, which has been determined in this study. The inclusion of several hormones in our regression model showed that only testosterone in men and estradiol and androstenedione in women were independent contributions to serum leptin levels, possibly accounting for part of the leptin sexual dimorphism in South Indian Population.
Adipocytes have been shown to be a major source of leptin in the body, but leptin synthesis has also been demonstrated in ovarian granulosa cells (Cioffi et al., 1997; Antczak and Van Blerkom, 1997). Although no definitive conclusions can be drawn, it seems unlikely that the leptin increase during IVF treatment would be predominantly ovarian in origin. On the contrary, the relative leptin increase was negatively correlated with the ovarian response as measured by the number of follicles and oocytes. This relationship was further supported by a positive relationship between the percent increases in leptin and FSH concentrations. Further, the percent leptin increase was positively associated with adiposity in leptin responders. In addition that the negative relationship of leptin increase and ovarian response was found when cFSHd was used as a covariate. The number of follicles retrieved was not related to the measures of body composition, adiposity, or basal leptin or insulin concentrations. Therefore, a high leptin response rather than adiposity as such was significantly related to reduce ovarian responsiveness. Taken together, the possibility that increased leptin production during ovarian hyperstimulation is related to adiposity and reduced ovarian responsiveness to FSH administration (Cioffi et al., 1997).

1.12 LEPTIN REGULATION IN OVARY

Leptin action in the ovary is conceivable, since the mRNAs for leptin and its receptors, both long and short isoforms (Glasow et al., 1998; Kutoh et al., 1998; Kitawaki et al., 2000), have been detected in granulosa and cumulus cells of pre-ovulatory follicles from women undergoing IVF (Cioffi et al., 1997). Furthermore, leptin immunofluorescence has been located has been in inner
granulosa cells, and in non-fertilized and fertilized oocytes as well as in preimplantation stage embryos (Antczak and Van Blerkom, 1997). Leptin suppresses steroidogenesis in granulosa cell cultures when co-stimulated by FSH and dexamethasone (Barkan et al., 1999). Leptin also weakly inhibits gonadotrophin- and /or insulin-like growth factor (IGF)-I-induced steroidogenesis in bovine thecal and granulosa cells cultured for 2 days in serum-free medium (Spicer et al., 2000). However, other findings have demonstrated leptin’s stimulatory effect on aromatase activity and estrogen production in luteinized granulosa cells from human pre-ovulatory follicles (Kitawaki et al., 1999). With regards to the intact ovary, leptin receptor mRNA has been found in porcine (Lin et al., 2000) and in human ovaries (Karlsson et al., 1997), although the long and short receptor isoforms were not distinguished. Leptin positive cells change in distribution and density in the intact human ovary as evidenced by RT-PCR studies (Loffler et al., 2001). In polycystic ovaries, most follicular cysts displayed a moderate to strong leptin response (Loffler et al., 2001).

1.13 LEPTIN AND ENDOCRINAL DEFECTS: REPRODUCTIVE DISORDERS

Leptin has been suggested to serve as a permissive signal to reproductive functions (Barash et al., 1996). A leptin deficient ob/ob mouse is known to have central hypogonadism, which is reversed after chronic leptin treatment, and leptin is found to reverse the hypogonadotropic, hypogonadism that is induced by starvation. Also, it has been reported that leptin treatment in normal female mice accelerates puberty. Leptin also seems to play a role in fertility. In obese female mice whose failure to make leptin was attributed to a gene mutation, infertility due to hormonal abnormalities prevented them from ovulating. Also shown was that injections of leptin induce ovulation, which allowed those female to become pregnant and bear
healthy offspring (Mounzih et al., 1997). Leptin receptors found on ovaries suggest that leptin may act directly to control ovulation. An alternative hypothesis may be that leptin modulates estrogen expression and may serve as a signal from fat to the brain about the adequacy of fat stores for ovulation and menstruation (Shimizu et al., 1997). The regulation of reproductive function seems to be to the ability of leptin to enhance the secretion of gonadotropin releasing hormone (GnRH) and thus luteinising hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary (Gonzalez et al., 2000).

1.14 POLYCYSTIC OVARY SYNDROME

PCOS, also known as hyperandrogenism chronic anovulation or as Stein-Leventhal syndrome is a benign disorder that commonly results in infertility. PCOS is characterized acne and hirsutism (excessive hair growth). Most women with PCOS also have ovaries filled with multiple benign cysts (Franks, 1995). PCOS is relatively common and seen in approximately 6-10% of all females. (pcos.freeservers.com). This is one of the most common hormonal abnormalities in women of reproductive age and is a leading cause of infertility. The abnormal action seen in PCOS can be understood by glimpsing at the normal hormonal patterns required for ovulation (Table 1.2).

In PCOS, the normal menstrual cycle is disrupted. First, women with PCOS usually have an increase in LH secretion from the brain. An elevated LH promotes secretion of androgens from the ovaries. In turn, the increased androgen production causes wasting of the developing ovarian follicles and interferes with the production of a dominant follicle. This results in disruption of normal estrogen production by the ovaries and the absence of a mid-cycle LH surge. Normally an egg
is released from the dominant follicle, but in PCOS, the follicles do not mature properly and instead, develop into ovarian cysts (Abbott et al., 2002).

Table 1.2 A Typical menstrual Cycle

<table>
<thead>
<tr>
<th>Menstrual Phases</th>
<th>Typical No. of Days</th>
<th>Hormonal Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular (Proliferative)</td>
<td>Days 1 through 6:</td>
<td>Estrogen and progesterone start out at their lowest levels.</td>
</tr>
<tr>
<td></td>
<td>Beginning of</td>
<td>FSH levels rise to stimulate maturity of follicles. Ovaries</td>
</tr>
<tr>
<td></td>
<td>menstruation to end of</td>
<td>start producing estrogen and levels rise, while progesterone</td>
</tr>
<tr>
<td></td>
<td>blood flow.</td>
<td>remains low.</td>
</tr>
<tr>
<td></td>
<td>Days 7 - 13:</td>
<td>The endometrium (the inner portion or lining of the uterus)</td>
</tr>
<tr>
<td></td>
<td>The endometrium</td>
<td>thickens to prepare for the egg implantation.</td>
</tr>
<tr>
<td></td>
<td>(the inner portion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>or lining of the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>uterus) thickens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>to prepare for the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>egg implantation.</td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>Day 14:</td>
<td>Surge in L.H. Largest follicle bursts and releases egg into</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fallopian tube.</td>
</tr>
<tr>
<td>Luteal (Secretary)</td>
<td>Days 15 - 28:</td>
<td>Ruptured follicle develops into corpus luteum, which produces</td>
</tr>
<tr>
<td>Phase, also known as the</td>
<td></td>
<td>progesterone.</td>
</tr>
<tr>
<td>Premenstrual Phase</td>
<td></td>
<td>Progesterone and estrogen stimulate blanket of blood vessels to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>prepare for egg implantation.</td>
</tr>
<tr>
<td></td>
<td>.. If fertilization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>occurs:</td>
<td>Fertilized egg attaches to blanket of blood vessels which supplies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nutrients for the developing pregnancy. Corpus luteum continues to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>produce estrogen and progesterone.</td>
</tr>
<tr>
<td></td>
<td>... If fertilization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>does not occur.</td>
<td>Corpus luteum deteriorates. Estrogen and progesterone levels drop.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The blood vessel lining sloughs off and menstruation begins.</td>
</tr>
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</table>
The biochemical and metabolic features of PCOS are as follows:

- Compared with the follicular phase of the normal menstrual cycle, women with PCOS exhibit an elevated LH/FSH ratio of 2-3:1. The underlying cause of this pattern of gonadotropin secretion is linked to an accelerated GnRH activity and heightened pituitary response to GnRH (Abbott et al., 2002).

- About 20% of obese women with PCOS have impaired glucose tolerance or type II Diabetes mellitus. PCOS is considered a more important risk factor for glucose intolerance than ethnicity or race (Ehrmann et al., 1999).

- Insulin resistance accompanied by compensatory hyperinsulinemia (elevated fasting blood insulin levels) is another common feature in PCOS. There is increasing data that hyperinsulinemia produces the hyperandrogenism of polycystic ovary syndrome by increasing ovarian androgen production particularly testosterone and androstenedione and by decreasing the serum sex hormone binding globulin concentration (Pagotto et al., 2002). The high levels of androgenic hormones interfere with the pituitary ovarian axis, leading to increased LH levels, anovulation, amenorrhea, and infertility.

1.15 LEPTIN IN PCOS

Despite the evidence that leptin may regulate gonadotropin secretion, a definitive role for leptin in the pathophysiology of PCOS has not been established, as serum leptin levels in PCOS patients do not differ from those in age-matched and
weight-matched controls (Rouru et al., 1997). In addition, treatment with antiandrogens, estrogens and insulin sensitizers have generally not been shown to affect serum leptin levels in humans (Panidis et al., 2000), although one study showed a modest effect of insulin sensitizers after short-term treatment (Morin-Papunen et al., 1998).

Despite similar follicular fluid and plasma leptin levels, relatively higher leptin-binding activity in the preovulatory follicle may result in lower follicular fluid free leptin levels. Importantly, normal women and women with PCOS who succeeded in becoming pregnant within three cycles of in vitro fertilization had significantly lower follicular fluid leptin levels compared to those who failed to become pregnant (Federesak et al., 2000). Polycystic ovary syndrome (PCOS), one of the most common disorders of premenopausal women, involves menstrual disturbances, chronic anovulation and hyperandrogenism (Rouru et al., 1997). Hypersecretion of androgens is a typical biochemical feature of PCOS and they frequently have increased secretion of luteinizing hormone (LH) and insulin resistance (Laughlin et al., 1997). Therefore, PCOS patients are good model for studying body fat distribution and measurement of obesity and ob gene expression in relation endocrinal parameters. The interaction of leptin with gonadotropins and sex steroids is of particular interest in thin PCOS subjects. In this study, we examined the correlation between serum leptin levels and body mass index (BMI) in comparison with various measures of fat distribution, including waist and hip circumference and their ratio, triceps and abdominal skin-fold thickness, association of insulin resistance/hyperinsulinemia, overall and central obesity, glucose concentration, dyslipidemia (higher triglycerides and lower high density lipoprotein [HDL]-cholesterol]), hypertension and percentage of body fat in women with PCOS and non-PCOS subjects. Serum levels of sex hormone-binding globulin (SHBG),
dehydroepiandrosterone sulfate (DHEAS), androsterodione, LH, follicle-stimulating hormone (FSH), prolactin, beta-estradiol, LH : FSH ratio, testosterone and free testosterone were analyzed in PCOS and non-PCOS subjects, and compared with that of normal weight control women. Serum leptin levels are significantly correlated to bodyweight, BMI, percentage of body fat and especially to body fat distribution (abdominal skin-fold thickness, triceps skin-fold thickness, waist circumference, hip circumference, waist to hip ratio) in overweight, obese and morbidly obese PCOS and non-PCOS subjects, compared with that of normal weight control, thin PCOS and non-PCOS subjects. The most significant finding/observation was the increase in leptin levels in thin PCOS, irrespective of BMI, and this is indicative of the influence of ovarian hormonal profile, which is characteristic of PCOS subjects that might determine the estimate of leptin. In addition to these effects of leptin on the reproductive axis, there are several additional reasons to assess the state of leptin in PCOS. Interestingly, there was an increase in leptin levels in the 18–44 year age group subjects in thin, obese and morbidly obese PCOS subjects. There was an inverse correlation between serum leptin and serum SHBG in thin, obese, overweight and morbidly obese PCOS subjects, which is largely explained by BMI values. It was significant to note that upper body fat distribution was found to be elevated, thus contributing to the increased adiposity in overweight, obese, morbidly obese PCOS and non-PCOS subjects, showing a two- to threefold increase when compared to normal weight control women, thin PCOS and non-PCOS subjects (Ravishankar Ram et al., 2005).

1.16 ROLE OF CYTOKINES IN PCOS

In PCOS subject’s serum leptin levels correlate well to body weight, body mass index (BMI), percentage of body fat, serum levels of TNF- alpha, IL-6 and IL-8. Obese subjects generally have higher levels of serum leptin, suggesting that
obese humans are not defective in leptin expression, but rather manifest leptin resistance (Vidal et al., 1996). Several studies have noted increased ob mRNA expression in obese humans (Considine et al., 1996) and consistently the elevated serum leptin levels are directly related to adipose tissue ob gene expression. Another important product of the adipocyte is TNF-alpha, which is elevated in the adipose tissue of obese rodents and humans (Ranganathan et al., 1998), and which may play an important role in obesity-related insulin resistance (Hotamisligil et al., 1995). Although adipocyte TNF-alpha concentration is related to obesity, there is much interindividual variation in humans, suggesting that other factors control TNF-alpha expression. Both insulin resistance and TNF-alpha over expression in adipose tissue and skeletal muscle are important features of human obesity, related to each other, as TNF-alpha induces insulin resistance by acting via autocrine-paracrine pathway (Hotamisligil et al., 1993). It is well known that insulin resistance plays a role in the pathogenesis of obesity-related complications. Cytokine IL-8 is produced mainly in macrophages and monocytes and plays a role in modulating inflammatory response. Oxidized low-density lipoprotein (LDL) particles are able to stimulate production and secretion of IL-8 by macrophages from human atherosclerotic plaques and high levels of IL-8 is in macrophage-derived human foam cells (Straczkowski et al., 2002). IL-8 is a potent chemoattractant and is responsible for the recruitment of neutrophils and T lymphocytes into the subendothelial space. It also induces adhesion of monocytes to endothelium and migration of vascular smooth muscle cells (Urakaze et al., 1996). Another adipocyte secretory product that may be involved in insulin resistance is interleukin (IL-6, IL-8), which is a cytokine secreted by many cells, including adipocyte and adipose stromal cells. Like TNF-alpha, IL-6 and IL-8 inhibit the expression of LPL, but, unlike TNF-alpha, IL-6 and IL-8 do not stimulate lipolysis (Mohamed-Ali et al., 1997). IL-6 and IL-8 secretion is increased in the adipocytes of obese subjects and may be important either as a circulating
hormone or as a local regulator of insulin action. Although many studies have examined the role of TNF-alpha in insulin resistance, relatively few of these have been in humans, and none has examined cytokine expression in detail along with the measurement of insulin resistance in obese subjects (Kern et al., 2001). In this study, we examined the serum levels of leptin, TNF-alpha, IL-6 and IL-8 in thin, overweight, obese and morbidly obese women with PCOS subjects and were compared with normal weight subjects (Ravishankar Ram et al., 2005). We found that circulating leptin; TNF-alpha, IL-6 and IL-8 levels are highly correlated with obesity – related PCOS and thin PCOS than in regularly menstruating normal weight subjects, which ultimately leads to defects in leptin action in female infertility.

1.17 OB GENE EXPRESSION STUDY IN PCOS

Leptin, the ob gene is produced by the adipose tissue (Considine et al., 1996), regulates food intake and energy expenditure (Trayhurn et al., 1999) and also plays an influential role in reproduction.

Injecting leptin into ob/ob mice that are infertile and with low levels of gonadotropin, increases the weight of the uterus, ovaries and the number of follicles (Friedman, 1997). Administering leptin treatment to normal female mice accelerates puberty (Chehab et al., 1996), and in humans higher leptin levels have been shown to relate to the earlier onset of menarche. Fertility can be restored by treatment with human recombinant leptin (Chehab et al., 1996). Women undergoing IVF therapy who tend to be obese with reduced ovarian response, display increased serum leptin concentrations, but unchanged leptin concentration in follicular fluid (Cioffi et al., 1997). For women with PCOS, high leptin levels participate in this disturbed gynecological event is still a matter of debate (Loffler et al., 2001). We have previously, confirmed that the hypersecretion of androgen is a typical biochemical
feature of PCOS with the frequently increased secretion of LH and insulin resistance occurs (Ravishankar Ram et al., 2005). Therefore, PCOS patients are good model for studying serum leptin levels, body fat distribution, measurement of obesity and biochemical analysis in relation to endocrinal parameters (Rouru et al., 1997). In this study, we investigated the regulation and correlation between ob mRNA and serum leptin levels, BMI, body fat distribution in relation to endocrinal parameters in thin and degree of obesity PCOS and non-PCOS subjects and were compared with normal weight controls. However, there is still little information on leptin localization in the intact human ovary, endometrium and adipose tissue. For this reason, our objective was to analyse serum leptin and its ob mRNA expression at the PCOS and non-PCOS ovary, endometrium and adipose tissue. Our data confirms the significant correlation between leptin mRNA levels and the BMI, as supported from other reports (Loffler et al., 2001). Interestingly, there was an expression of ob mRNA levels in the 18-40 y age group subjects in thin, obese and morbidly obese PCOS ovary, endometrium and adipose tissue and were compared with normal weight control adipose tissue. Inverse correlation between ob mRNA and serum levels of SHBG, androstenedione in thin, obese and morbidly obese PCOS subjects, which was largely, explained by serum leptin and BMI values. A significant note that, upper body fat distribution was found to be elevated, thus contributing to the increased adiposity in obese and morbidly obese PCOS and non-PCOS subjects, showing a 8 fold increase when compared to normal weight control women, thin PCOS and non-PCOS subjects.

1.18 CYTOKINES EXPRESSION STUDY IN PCOS

PCOS is a common reproductive endocrine abnormality characterized by hyperandrogenism and infertility due to both chronic anovulation and recurrent miscarriage. PCOS subjects generally have higher levels of serum leptin, suggesting
that PCOS are not defective in leptin expression, but rather manifest leptin resistance. Reports have suggested increased ob mRNA expression in PCOS (Loffler et al., 2001) and due to the consistent finding of elevated serum leptin, there is a tendency to assume that serum leptin levels are directly related to endometrium, ovary and adipose tissue ob gene expression. Another important product of the adipocyte is TNF-alpha, IL -6 and IL -8, which are elevated in the adipose tissue of obese humans (Subramanian et al., 1998; Kern et al., 2001; Straczkowski et al., 2002), and which may play an important role in obesity-related insulin resistance (Kern et al., 2003). Although adipose tissue TNF-alpha, IL -6 and IL -8 expression is related to obesity, there is much interindividual variation in humans (Kern et al., 2001; Bruun et al., 2001), suggesting that other factors control TNF-alpha, IL -6 and IL-8 expression levels. For the first time to ascertain the role of leptin and inflammatory markers such as TNF-alpha, IL -6 and IL -8 at autocrine, paracrine and endocrine levels in PCOS and the thus obtained results on over expression of leptin is compared with non-PCOS and normal weight control subjects. This approach is necessitated by the fact that in the case of thin PCOS, over expression of leptin and cytokines is vitnes where that over expression of leptin and cytokines is found to be not the function of BMI.

1.19 IMMUNOHISTOCHEMICAL STUDIES IN PCOS

In recent years, the study of expression of leptin in human endometrium has been of great interest. Accumulating evidence suggest that the endometrium requires leptin to become receptive to the implantation of a blastocyst in the midluteal phase. The leptin mRNA and protein are substantially expressed in human placenta. Leptin has been found to have physiological effects on the placenta, including angiogenesis, growth and immunomodulation. Changes in leptin activity in the fetus and the maternal fetal interface as well as in the endometrium are clearly
important areas of future research. Till date, there have been very few (Loffler et al., 2001; Gonzalez et al., 2000) reports demonstrating expression of leptin system in the reproductive tissues in pathological conditions. Further research is needed to examine the mechanism through which leptin modulates reproductive function. Leptin can be autocrine, paracrine and endocrine mediator that assists in the successful functioning of the reproductive system. The present study is designed to extrapolate the expression of leptin in pathological tissues of reproductive system.

1.20 LEPTIN STRUCTURE AND SEQUENCE ANALYSIS

There has been very little advancement in the field of deciphering the structure of leptin. Leptin has 67% sequence similarity among such diverse species as human, gorilla, chimpanzee, orangutan, dog, cow, pig, rat and mouse. There is a great sequence similarity in the leptin gene sequence between human and gorilla. The frequency of leucine is higher in all the species followed by serine. The amino acid which shows least frequency is tryptophan. Leptin has no apparent sequence similarity with any other protein, making modeling of the structure difficult. A mutant Ob protein leptin E-100 has been crystallized (Zhang et al. 1994). Gene sequences of leptin from 11 species is compared to analyze the codon usage of the gene sequences. The species included are Sheep, simnthropis, mouse, cow, chimpanzee, dog, rat, porcine, gorilla, orangutan and human are taken from data bases. The standard codon table is used and the amino acid sequences are determined. The individual amino acid frequencies of leptin of twenty species were estimated using the PERL program and sequence of leptin of 16 species were downloaded and the sequence homology was studied using the BLAST tool. Hydropathy plot for the leptin of all the species was determined using Kyte-Doolittle software. The secondary structure leptin in all the species were determined using the Network Protein Sequence Analysis tool.
OBJECTIVE OF THE PRESENT STUDY

Chapter I

To estimate serum leptin concentration in thin, normal weight, overweight, obese, morbidly obese and study its relationship to adiposity, BMI, percentage of body fat, body fat distribution, serum biochemical parameters such as the serum levels of glucose, insulin, lipid profile and hormone levels as that of β-estradiol, thyroid stimulating hormone (TSH), androstenedione, testosterone and free-testosterone performed by radio immunoassay.

Chapter II

To analyse the serum leptin concentration in thin, overweight, obese and morbidly obese PCOS and non-PCOS subjects were compared to normal weight subjects and its relationship to adiposity, BMI, percentage of body fat and body fat distribution (abdominal skin fold thickness, triceps skin fold thickness, waist circumference, hip circumference, waist to hip ratio), plasma cytokine analysis such as TNF-alpha, IL-6 and IL-8. Biochemical parameters such as serum levels of glucose, insulin, lipid profile and hormonal profiles such as testosterone, free-testosterone, androstenedione, DHEAS, SHBG, triiodothyronine (T3), thyroxin (T4), thyroid stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxin (fT4), LH, FSH, β-estradiol, and prolactin were obtained using radio immunoassay.

Chapter III

The leptin (ob gene) mRNA quantification was performed from adipose, ovary and endometrium tissue using RT- cPCR and classical PCR. The subjects included in this study were thin, overweight, obese, morbidly obese PCOS and non-PCOS subjects and they were compared with normal weight subjects. Further
mRNA levels of cytokines such as TNF alpha, Interleukins 6 and 8 from these tissues were determined using RT-PCR.

Chapter IV

Immunofluorescent staining of human adipose, ovary, placenta and endometrium tissues was performed in obesity related PCOS and non-PCOS subjects to analyse the expression of leptin in this study were thin, overweight, obese, morbidly obese PCOS and non-PCOS subjects and they were compared with normal weight subjects.

Chapter V

The amino acid sequences of leptin from various species were aligned to study the conservation in sequence and structure. Gene sequence analysis was compared using multiple sequence alignment and optical alignment methods. Percentage of amino acid homology, the frequency of individual amino acid, hydropathy plot and secondary structure was computed using bio-informatics' tools. Gene sequence codon bias was also computed for different sequences of leptin.