

# Leptin in human reproduction

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The recent discovery of the obese (*ob*) gene has provided new insight into the mechanism which controls body fat mass. Leptin, a product of the *ob* gene, serves as the link between fat and the brain. This protein, by acting at the level of the hypothalamus, decreases food intake and increases energy expenditure. Animals that lack leptin (*ob/ob* mice) develop profound obesity and become infertile. Treatment of these animals with leptin reduces food intake and restores normal fertility. Although leptin is important for the control of fat stores in certain species, the role of this substance in the development of human obesity remains obscure. However, it has been speculated that, in humans, obesity is related to leptin resistance. The relationship between fat and reproduction has been recognized for >20 years. This article discusses the relationship between leptin and human reproduction. In particular, recent knowledge about the possible role of leptin in various conditions such as puberty, polycystic ovary syndrome and pregnancy is reviewed. Also, the article discusses the possible role of leptin in ovarian function and the relationship of this protein with gonadal steroids. It is expected that future research will clarify the physiological importance of leptin in human reproductive function.

*Key words:* leptin/obesity/ovary/pregnancy/puberty

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initially cloned from the choroid plexus (Tartaglia *et al.*, 1995).

Subsequently, leptin receptors were also identified in other regions of the brain as well as in peripheral tissues, such as the liver and pancreas (Kieffer *et al.*, 1996; Schwartz *et al.*, 1996a; Emilsson *et al.*, 1997). In the brain, leptin receptors are particularly located in the arcuate nucleus and ventromedial hypothalamus, i.e. in places where food intake and energy balance are regulated (Kieffer *et al.*, 1996; Schwartz *et al.*, 1996a). Two forms, a long and a short form, of the receptor have been found (Tartaglia *et al.*, 1995; Chen *et al.*, 1996; Lee *et al.*, 1996). Recent evidence has suggested that leptin receptor is encoded by the diabetes gene (*db*) (Chen *et al.*, 1996).

## Introduction

Leptin is a 16 kDa protein that is secreted almost exclusively by the adipocytes. The gene that produces leptin is called the obese (*ob*) gene and has been recently isolated by positional cloning (Zhang *et al.*, 1994). The word leptin is derived from the Greek word 'leptos' that means thin. It is widely accepted that leptin, by reducing food intake and increasing thermogenesis, controls body fat tissue and hence body weight. Mutations of the *ob* gene that result in massive obesity and diabetes have been described in rodents (Bray and York, 1997, review). Leptin acts by binding to a specific receptor which was

## Actions of leptin

The main action of leptin is to decrease appetite and increase energy expenditure. This effect is exerted on the hypothalamus, but the mechanism is not fully understood. It seems likely that leptin reduces the production of neuropeptide Y (NPY) from the arcuate nucleus of the hypothalamus (Leibowitz, 1994). This peptide under normal conditions increases food intake in animals, and after chronic administration produces obesity (Stanley *et al.*, 1986). In addition, intracerebroventricular infusions of NPY in rats increase the expression of leptin mRNA in adipose tissue and can result in obesity

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(Rohner-Jeanrenaud, 1995). Furthermore, the concentrations of NPY in the hypothalamus of *ob/ob* mice are high and decrease after treatment with leptin (Halaas *et al.*, 1995; Peyllemounter *et al.*, 1995; Stephens *et al.*, 1995). Therefore, NPY could mediate the reducing effect of leptin on food intake in these animals. The fact, however, that in mice deficient in NPY food intake is normal, but decreases after the administration of leptin, contradicts this assumption (Erickson *et al.*, 1996). Other mechanisms, mediating the actions of leptin on food intake, may involve the products of pro-opiomelanocortin gene (Cheung *et al.*, 1997a; Thornton *et al.*, 1997). Apart from the effects on the hypothalamus, leptin may also exert effects on other sites, as leptin receptor variants have been identified in various peripheral tissues including the hepatic cells. When these cells are exposed *in vitro* to high concentrations of leptin, several insulin-induced activities are attenuated (Cohen *et al.*, 1996). Furthermore, leptin is able to inhibit insulin binding in isolated rat adipocytes and this suggests that leptin may play a role in obesity-related insulin resistance (Walder *et al.*, 1997).

### Control of leptin production

The mechanism which controls the secretion of leptin from the adipocytes is not clear. In humans, leptin is secreted in frequent pulses with an average of about 32 pulses in a 24-h period (Licinio *et al.*, 1997). Significant negative correlations between rapid leptin fluctuations and those of adrenocorticotrophin (ACTH) and cortisol have been found, and this suggests that leptin in the central nervous system may suppress the activity of the hypothalamic–pituitary–adrenal axis (Licinio *et al.*, 1997). A diurnal pattern of leptin secretion has been observed recently in humans, with a nocturnal rise that resembles that of prolactin and thyroid-stimulating hormone (Sinha *et al.*, 1996). The reason for the nocturnal increase of leptin is not clear, but it may represent a delayed response to the last meal of the day and may be important for the suppression of appetite during sleeping (Sinha *et al.*, 1996).

The secretion of leptin can be affected by various factors, including food intake. In rats, fasting decreased the expression of mRNA of leptin, while food intake restored it within 4 h to concentrations comparable to those of fed controls and in a way similar to that of insulin administration (Saladin *et al.*, 1995). The latter may suggest that in these animals the effect of food intake on *ob* gene expression is exerted through insulin. In normal or obese human subjects, food intake did not result in any acute changes in serum leptin concentrations during the first 3 h of the experimental period (Korbonits *et al.*, 1997). On the other hand, fasting decreased leptin concentrations only in the last 4 h of a 20 h fasting period (Korbonits *et al.*, 1997). These delayed responses of leptin values to changes in food intake indicate that a feedback-type relationship between the two parameters is rather obscure. With

longer starvation, leptin values are markedly reduced (Maffei *et al.*, 1995; Kolaczynski *et al.*, 1996). It is possible that if leptin is the link between peripheral fat and the brain, it acts as a starvation signal rather than as a signal of increased body fat (Ahima *et al.*, 1996).

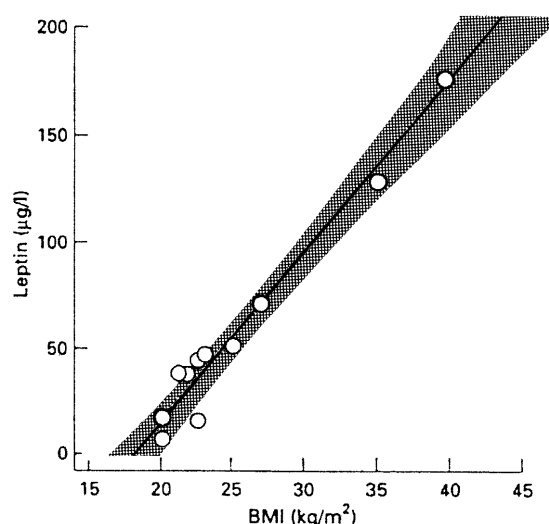
Apart from food, leptin secretion can be also affected by various hormonal factors. Although an inverse relationship between the activity of the hypothalamic–pituitary–adrenal axis and leptin concentrations has been reported (Korbonits *et al.*, 1997; Licinio *et al.*, 1997), results regarding the effects of exogenous glucocorticoids on the secretion of leptin are conflicting, with most studies demonstrating a stimulatory effect (Larsson and Ahren, 1996; Dagogo-Jack *et al.*, 1997; Pappaspyrou-Rao *et al.*, 1997; Tataranni *et al.*, 1997). Insulin is another substance that stimulates the secretion of leptin in humans both *in vitro* (Wabitsch *et al.*, 1996) and *in vivo*. However, the *in vivo* effect is not evident when short-term insulin infusion, i.e. for <6 h, is applied (Kolaczynski *et al.*, 1996; Clapham *et al.*, 1997). In contrast, induction of long-term hyperinsulinaemia in humans stimulates leptin secretion from isolated abdominal adipocytes and increases the concentrations of leptin in the circulation (Kolaczynski *et al.*, 1996).

*In vitro* data have shown that leptin secretion can be reduced after an increase in intracellular cAMP (Slieker *et al.*, 1996), and this possibly mediates the reducing effect of isoproterenol administration on leptin secretion in humans (Donahoo *et al.*, 1997). Somatostatin also reduces leptin secretion in humans *in vivo*, probably via a direct effect on adipocytes (Donahoo *et al.*, 1997), while the effect of growth hormone (GH) on leptin secretion has not been clarified. Nevertheless, GH deficiency in adults with hypopituitarism is accompanied by increased concentrations of leptin, but the degree of increment is greater than that expected from the degree of obesity (Al-Shoumer *et al.*, 1997). Also, in normal subjects a negative correlation between leptin and GH secretion has been found, and the possibility that leptin may provide a link between adiposity and GH secretion cannot be excluded (Gill *et al.*, 1997). Recent data have shown that not only the increased fat mass, but also the decreased lean mass, contributes to the increase in serum leptin values in normal and GH-deficient individuals (Gill *et al.*, 1997). It is possible, therefore, that leptin mediates the reducing effect of body composition on GH secretion in elderly subjects (Gill *et al.*, 1997). Finally, interleukin-1, given to women and men suffering from cancer, induces a temporal increase in leptin concentrations (Janik *et al.*, 1997). A relationship between leptin concentrations and insulin as well as between leptin and the soluble form R55 of the receptor of tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) has been found in normal subjects and in patients with non-insulin-dependent diabetes mellitus, suggesting that the TNF $\alpha$  system may play a role in the control of leptin secretion in humans (Mantzoros *et al.*, 1997a).

## Obesity

Mutations of the *ob* gene (*ob/ob*) in mice that lead to leptin deficiency result in hyperphagia and profound obesity as well as in diabetes, insulin resistance and infertility (Halaas *et al.*, 1995; Pelleymounter *et al.*, 1995; Chehab *et al.*, 1996). Treatment with leptin reduces food intake and body weight and restores fertility in both male and female *ob/ob* animals (Halaas *et al.*, 1995; Pelleymounter *et al.*, 1995; Mounzih *et al.*, 1997). Another form of recessively inherited obesity and diabetes (*db/db*) that occurs in mice (Coleman, 1978) is characterized by abnormalities in leptin receptor, related to an abnormal splice variant, that result in a defect in intracellular signal transduction (Lee *et al.*, 1996). This makes *db/db* mice unresponsive to exogenous administration of leptin, indicative of leptin resistance (Stephens *et al.*, 1995; Lee *et al.*, 1996). In contrast to *db/db* mice, the fatty rats (*fa/fa*) carry a mutation in the extracellular domain of the receptor, but they respond to exogenous leptin given intracerebroventricularly (Cusin *et al.*, 1996). Apart from mutations in leptin receptor, hyperleptinaemia and leptin resistance can be related to defects distal from the leptin receptor, possibly associated with the transport of leptin into the central nervous system, as seems to be the case in the New Zealand obese mouse (Igel *et al.*, 1997).

In humans, measurement of leptin in serum can be easily done with the use of specific immunoassays, and this has provided an opportunity to study leptin concentrations in various conditions. It has been shown that serum leptin values correlate significantly with body mass index (BMI) (Figure 1) and percentage body fat both in women and men (Considine *et al.*, 1996; Hardie *et al.*, 1997; Shimizu *et al.*, 1997). The correlation between serum leptin concentrations and BMI is also significant in patients with anorexia nervosa, bulimia nervosa and non-specific eating disorders (Ferron *et al.*, 1997). In these cases, changes in leptin values only reflect body weight and cannot provide a specific index for the prognosis of the disease (Ferron *et al.*, 1997). In general, significantly higher serum leptin concentrations have been found in obese subjects than in lean controls (Sinha *et al.*, 1996). Loss of weight in obese subjects for 8–12 weeks results in a significant reduction both of leptin concentrations and the expression of *ob* mRNA content of adipocytes, but leptin values increase again during the period of maintenance of the lower weight (Maffei *et al.*, 1995; Considine *et al.*, 1996). A decrease in body weight of only 10% can result in ~53% reduction in serum leptin concentrations, suggesting that, apart from fat, other factors, such as caloric intake and energy expenditure, may regulate the secretion of leptin (Considine *et al.*, 1996). However, changes in leptin values for a longer period, i.e. for ~40 weeks, are more strongly related to changes in body weight and fat mass than to caloric intake (Wadden *et al.*, 1998).



**Figure 1.** Relationship between leptin concentration and body mass index (BMI) in normally cycling non-pregnant women. The shaded area denotes the 95% confidence interval about the linear regression  $y = -140.5 + 7.8x$ ,  $r = 0.982$  ( $P < 0.001$ ). Adapted from Hardie *et al.* (1997) with the permission of Blackwell Science Ltd.

Although in obese subjects leptin concentrations in serum are much higher than in lean counterparts, in cerebrospinal fluid they are only slightly higher (Caro *et al.*, 1996). It is possible that a defect in the transport mechanism of leptin into the cerebrospinal fluid may exist in some cases of human obesity, thus providing a mechanism for leptin resistance (Caro *et al.*, 1996; Schwartz *et al.*, 1996b). Alternatively, leptin resistance in obesity may be related to the abnormal rhythmicity of plasma leptin, with suppressed diurnal amplitudes, which has been reported in obese as compared to lean subjects (Saad *et al.*, 1998). Furthermore, gender may influence the 'leptin–obesity axis', since serum leptin concentrations have been found to be higher in women than in men (Havel *et al.*, 1996; Rosenbaum *et al.*, 1996; Shimizu *et al.*, 1997). Also, in cases with progressive obesity, leptin concentrations increased three times more rapidly in women than in men (Kennedy *et al.*, 1997), while relative diurnal amplitudes of plasma leptin were higher in men than in women (Saad *et al.*, 1998). Testosterone may be responsible for the lower concentrations of leptin in men (Behre *et al.*, 1997), but the higher values in women may be also the result of a kind of leptin resistance (Saad *et al.*, 1997).

Despite the fact that leptin values are higher in obese than in lean subjects and that a relationship between leptin and adipose tissue mass has been demonstrated in several studies, the extend to which leptin regulates body weight through a feedback mechanism has not been clarified. Such a role for leptin could have practical implications in terms of treatment with recombinant leptin to maintain weight loss after dieting, pro-

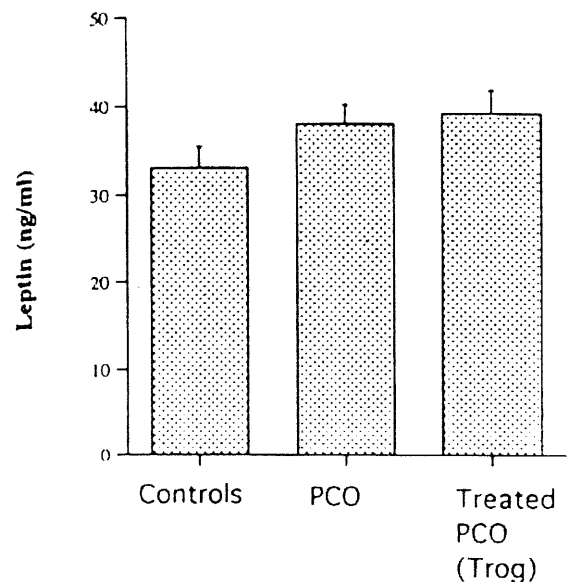
vided that severe leptin resistance does not exist. In animals, mutations of the leptin gene or of the cytoplasmic domain of the leptin receptor result in obesity, but in humans obesity has not been linked to mutations of the leptin gene (Considine *et al.*, 1995), although a mutation involving the deletion of a single nucleotide of the gene for leptin was recently found in two severely obese children with very low serum leptin concentrations (Montague *et al.*, 1997). Nonetheless, leptin resistance may be a characteristic of human obesity. It is evident that further studies are required to clarify the role of leptin in human obesity and the potential use of this substance for therapeutic purposes.

### Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is one of the most common abnormalities in premenopausal women, and is characterized by menstrual irregularities, chronic anovulation, hirsutism and increased luteinizing hormone (LH) and/or androgen secretion (Franks, 1989). Insulin resistance and hyperinsulinaemia are frequently found in women with PCOS, and some of them are obese.

Evidence has been provided since the early 1970s that fat mass may directly affect ovulation and fertility (Frisch, 1990). It has been known for years that the adipose tissue is an extragonadal source of oestrogen and that metabolism of these steroids is affected by fatness. In particular, lean women produce more 2-hydroxylated forms of oestrogen, while obese women produce relatively more 16-hydroxylated forms (Schneider *et al.*, 1983; Snow *et al.*, 1989). Also in obese women, free serum oestradiol is elevated due to decreased capacity for oestrogen to bind to sex hormone-binding globulin (SHBG) (Siiteri, 1981). It is well known that in weight-related amenorrhoea there is a disturbance in the secretion of gonadotrophins. In particular, anorectic women demonstrate a decreased response of LH to gonadotrophin-releasing hormone (GnRH) and, as in prepubertal women, follicle stimulating hormone (FSH) response is greater than that of LH (Nillius and Wide, 1977). However, when weight is rapidly gained, LH responsiveness returns progressively to normal (Nillius and Wide, 1977). Furthermore, a critical body weight is required for an individual girl at menarche (Frisch, 1990). All of these observations, together with the recent isolation of leptin, support the hypothesis that leptin is probably the missing link between body fat and reproduction (Conway and Jacobs, 1997, review).

Some women with PCOS are obese. With the possible involvement of leptin in the development of human obesity, as in animals, several investigators have attempted to examine the role of this protein in the pathophysiology of PCOS. The first study, in which serum leptin concentrations were measured in women with PCOS, was published in 1996 (Brzechffa *et al.*, 1996). In that study, 29% of the women with PCOS had serum leptin values above the 99% prediction interval for their BMI. Those women with insulin resistance had



**Figure 2.** Leptin concentrations in normally cycling women (controls) and in women with polycystic ovary (PCO) syndrome before and after treatment with troglitazone (200 mg or 400 mg for 3 months). Adapted from Mantzoros *et al.* (1997) with the permission of The Endocrine Society.

serum leptin values higher than controls, while a positive correlation was found between leptin concentrations and insulin sensitivity (Brzechffa *et al.*, 1996). Since insulin is known to increase leptin mRNA in adipocytes (Saladin *et al.*, 1995), it is possible that insulin may stimulate the secretion of leptin and, therefore, leptin may participate in certain cases of PCOS (Brzechffa *et al.*, 1996).

Subsequent studies, however, have not confirmed these data. In these studies, serum leptin concentrations did not differ significantly between women with PCOS and normal controls (Figure 2) (Laughlin *et al.*, 1997; Mantzoros *et al.*, 1997b; Rouru *et al.*, 1997). In addition, conflicting data exist concerning the relationships between leptin and insulin in women with PCOS. Most of the studies, including ours (Krasas *et al.*, 1998), have shown that BMI is the most important variable responsible for all the correlations seen between leptin and other parameters in women with PCOS (Chapman *et al.*, 1997; Mantzoros *et al.*, 1997b; Rouru *et al.*, 1997). One cannot exclude, however, the possibility that some variables, such as insulin, may affect leptin concentrations independently of BMI.

Existing data regarding the influence of various hormonal parameters on leptin secretion in women with PCOS are not consistent. For instance, although in one study a significant positive correlation was found between serum leptin and free testosterone concentrations (Rouru *et al.*, 1997), this was not found in other studies (Brzechffa *et al.*, 1996; Chapman *et al.*, 1997; Mantzoros *et al.*, 1997b). Also, no significant correlations were found between leptin and other parameters, such as

SHBG, androgens or oestradiol in the majority of the studies (Chapman *et al.*, 1997; Mantzoros *et al.*, 1997b; Rouru *et al.*, 1997). However, significant positive correlations between leptin values and the ratios of oestradiol/SHBG, oestrone/SHBG and testosterone/SHBG have been reported in women with PCOS (Laughlin *et al.*, 1997). Regarding the concentrations of gonadotrophins, the results are also conflicting. An inverse relationship between leptin and 24-h mean LH concentrations and 24-h mean LH pulse amplitude was found in one study (Laughlin *et al.*, 1997), while another study showed no significant correlations (Rouru *et al.*, 1997).

The fact that leptin concentrations are normal in a large proportion of women with PCOS does not exclude the possibility that this protein is involved in the pathophysiology of this syndrome. Nevertheless, several questions are raised. Although leptin values do not differ significantly between controls and women with PCOS, it is not known if body composition can affect leptin concentrations. On the other hand, leptin is secreted in a pulsatile way, and this should be taken into account for comparison purposes. Furthermore, it is not known if the normal pattern of pulsatile secretion of leptin is altered in PCOS. Finally, it may be possible to approach the question of the role of leptin in PCOS by ways other than the simple measurement of circulating leptin values.

Leptin may act not only on the hypothalamus, but also on other tissues, such as at the level of the ovary. A possible direct intra-ovarian effect of leptin was recently noted in a study in rats in which the synergistic action of insulin-like growth factor (IGF)-1 and FSH on oestradiol production by granulosa cells in culture was attenuated by the addition of leptin (Zachow and Magoffin, 1997). Also, leptin inhibited insulin-induced oestradiol and progesterone production by bovine granulosa cells (Spicer and Francisco, 1997). However, it is not known whether leptin can exert similar effects in human ovary and particularly in women with PCOS, although it was recently shown that leptin inhibited LH-stimulated oestradiol production by human granulosa cells in culture (Karls-son *et al.*, 1997). Furthermore, recent studies have demonstrated the presence of leptin receptors in human granulosa cells and, therefore, a direct action of leptin at the ovarian level is possible (Cioffi *et al.*, 1997). It is evident that further studies are required to clarify the effects of leptin in the ovary and its possible physiological role, as well as the role of this protein in the pathophysiology of PCOS.

## Human menstrual cycle

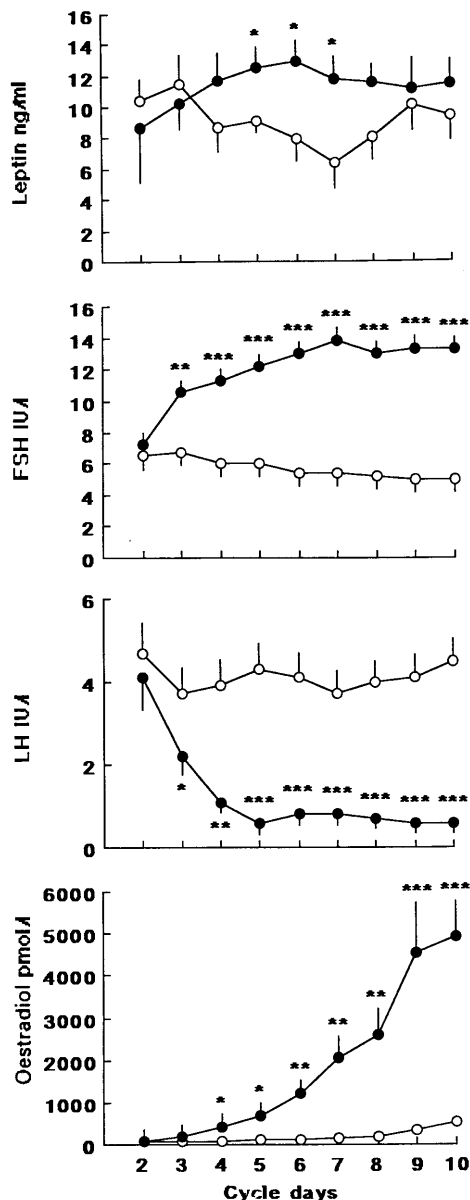
### Natural cycle

The importance of leptin for normal fertility has been demonstrated in *ob/ob* mice lacking leptin. In these animals, administration of leptin induces an increase both in LH concentrations and in ovarian and uterine weights (Barash *et al.*, 1996). A question, however, has been raised as to

whether leptin could play a physiological role during the normal menstrual cycle. The fact that serum leptin values are lower in men than in women and lower in postmenopausal than in premenopausal women indicates that leptin production may be affected by gonadal steroids (Rosenbaum *et al.*, 1996; Shimizu *et al.*, 1997). It is possible that gender differences are related to testosterone, which in men shows an inverse relationship with leptin values (Behre *et al.*, 1997). However, the possibility that oestrogen may also participate is not excluded.

A recent study measured leptin concentrations in blood samples taken every 3 days during the follicular and the luteal phases of the normal menstrual cycle and every day from cycle days 11–17 (Hardie *et al.*, 1997). No clear pattern of changes in leptin values was found; however, when the values were grouped according to the functional stage of the cycle, it was found that leptin concentrations increased significantly from the follicular to the preovulatory stage, reaching peak values during the luteal phase. At that stage, leptin concentrations were significantly higher than in the follicular phase (Hardie *et al.*, 1997). During the transition from the luteal to the follicular phase, serum leptin concentrations declined to values similar to those in the follicular phase. In that study (Hardie *et al.*, 1997), significant positive correlations were found between leptin and progesterone concentrations, particularly during the luteal phase. That leptin values are higher during the luteal than the follicular phase of the cycle was also found in another recent study (Shimizu *et al.*, 1997) in which, however, no further measurements of leptin during the rest of the cycle were reported.

The reason for the higher values of leptin during the luteal than the follicular phase of the normal menstrual cycle is not known. It is possible that progesterone stimulates the secretion of leptin from the adipocytes which have already been primed by oestradiol (Hardie *et al.*, 1997). This assumption is supported by our recent study in normally cycling women who were investigated during a spontaneous menstrual cycle and a cycle superovulated with purified FSH (Messinis *et al.*, 1998). In that study, leptin was measured in blood samples taken every day during the follicular phase and in a single blood sample in the luteal phase (Figure 3). During the natural menstrual cycle, leptin values showed great variations. However, leptin concentrations declined gradually but significantly from the early to midfollicular phase and then increased gradually up to midcycle to values similar to those in the early follicular phase (Messinis *et al.*, 1998). The declining pattern of leptin during the first half of the follicular phase is difficult to explain. Although a negative correlation between leptin and serum oestradiol values was found at that stage, oestradiol may exert a stimulating rather than a suppressing effect on leptin production (Shimizu *et al.*, 1997). In the same study (Messinis *et al.*, 1998), a significant positive correlation was found between serum leptin and oestradiol values during the



**Figure 3.** Serum leptin, follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol (E2) values (mean  $\pm$  SEM) during the follicular phase of (O) spontaneous and (I) FSH-treated cycles in nine normally ovulating women. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (compared with the spontaneous cycles). Adapted from Messinis *et al.* (1998).

late follicular phase, indicating that oestradiol may stimulate the production of leptin from the adipocytes.

### Cycles superovulated with FSH

In cycles in which women were treated with purified FSH, multiple follicles were developed and the decline in leptin values that occurred during the early follicular phase of the spontaneous cycles was not seen (Messinis *et al.*, 1998). On the contrary, a gradual increase in leptin values from the early

to midfollicular phase (Figure 3) and a significant positive correlation between leptin and oestradiol concentrations were found. In these cycles, leptin values from the midfollicular phase to midcycle did not increase further despite the continuing rise in oestradiol values (Messinis *et al.*, 1998). This suggests that serum concentrations of leptin do not reflect the degree of ovarian hyperstimulation induced by FSH. In the luteal phase of the FSH-treated cycles, serum leptin values were also significantly higher than in the follicular phase, but similar to those in the spontaneous control cycles (Messinis *et al.*, 1998).

It is somewhat possible from these results to infer that either oestradiol stimulates the production of leptin from the adipocytes or that leptin is produced by the ovary. The former is supported by findings in rats demonstrating that ovariectomy leads to a significant reduction both in the expression of the *ob* gene in the white adipose tissue and in serum leptin concentrations, but these effects can be prevented if the animals are pretreated with oestradiol (Shimizu *et al.*, 1997). On the other hand,  $17\beta$ -oestradiol directly stimulates *ob* mRNA after 10 h of in-vitro incubation (Murakami *et al.*, 1995). Also, in normal women, synchronous ultradian fluctuations in leptin and oestradiol concentrations have been found during the follicular phase (Licinio *et al.*, 1998). The possibility that leptin is produced by the ovary in women is supported by recent data demonstrating that leptin mRNA is expressed by granulosa and cumulus cells (Antczack *et al.*, 1997; Cioffi *et al.*, 1997), although this has not been confirmed by others (Karlsson *et al.*, 1997). Despite these conflicting results, our preliminary data (unpublished) demonstrate a significant reduction in serum leptin concentrations in normal women following bilateral ovariectomy.

### Physiological role of leptin

Given these results, the question arises as to whether leptin plays a physiological role during the normal menstrual cycle. The fact that leptin receptors have been detected in human ovary (Karlsson *et al.*, 1997) indicates that leptin may act within the ovary to affect steroidogenesis. In cattle, leptin is able to attenuate insulin-induced steroidogenesis from granulosa cells *in vitro*, without affecting proliferation of the cells (Spicer and Francisco, 1997). The same is true for insulin-stimulated progesterone and androstenedione production by bovine thecal cells, although leptin increases insulin-induced proliferation of these cells (Spicer and Francisco, 1998). In rats, leptin attenuates the synergistic action of FSH and IGF-1 on the production of oestradiol from granulosa cells in culture (Zachow and Magoffin, 1997). If, therefore, leptin is produced by the ovary, it may act as a paracrine factor in the ovary. Alternatively, leptin produced by the adipocytes may be transferred to exert endocrine effects within the ovary. In any case, leptin may play a role in ovarian physiology, and this should be further elucidated.

Leptin has also been detected in follicular fluid of women undergoing in-vitro fertilization treatment for infertility, and the values were similar to those in serum (Karlsson *et al.*, 1997). Although no association between leptin values in the follicular fluid and embryo development has been found, increasing concentrations in serum following ovulation have been associated with implantation potential (Cioffi *et al.*, 1997). Nevertheless, in-vitro experiments have recently shown a variable distribution of leptin along the surface of mouse and human embryos, with the inner cells in blastomeres found to contain little if any leptin as compared with the outer cell mass, suggesting thus that leptin may have a critical role in early development of mammalian embryos before implantation (Antczak and Van Blerkom, 1997).

In-vitro data have shown the ability of leptin to stimulate the secretion of GnRH from hypothalamic explants and the release of LH and FSH from the anterior pituitary, effects which are mediated by nitric oxide (Yu *et al.*, 1997a,b). During the follicular phase of the natural cycle, no clear relationship between basal leptin and gonadotrophin concentrations has been found (Messinis *et al.*, 1998). Despite these limitations, the possibility that leptin may play a physiological role in gonadotrophin secretion cannot be excluded, since synchronous pulses of leptin and LH have been found at night during the mid- to late follicular phase of the cycle in healthy women (Licinio *et al.*, 1998).

A recent study has investigated the importance of low leptin concentrations, a condition called hypoleptinaemia, in women athletes (Laughlin and Yen, 1997). A normal pattern of diurnal secretion of leptin was found in the control women, while in the athletes who had amenorrhoea the nocturnal secretion of leptin was abolished. The reason for this is not clear; however, the significant correlation of leptin changes with hypoinsulinaemia and hypercortisolaemia indicates a link between fat, nutritional status and integrity of the reproductive function in women (Laughlin and Yen, 1997). Alternatively, an increase in corticotrophin-releasing hormone (CRH) in the central nervous system of athletes could, by stimulating central adrenergic activity and specific  $\beta_3$  adrenergic receptors in adipocytes, inhibit leptin gene expression and secretion (Gettys *et al.*, 1996; Mantzoros *et al.*, 1996; Sliker *et al.*, 1996). In contrast to athletes, leptin concentrations and the diurnal pattern in women with functional hypothalamic amenorrhoea were similar to those in normal controls (Laughlin *et al.*, 1998).

## Puberty

It has already been mentioned that a critical body weight is required for menarche to occur (Frisch, 1990). On the other hand, leptin is a candidate for the missing link between fat tissue and the brain and, therefore, a possible role for this protein in the onset of puberty has been examined. So far, data are conflicting both in animals and humans. A study in mice

demonstrated that female animals injected with leptin showed an earlier onset of the classic pubertal signs compared with controls (Ahima *et al.*, 1997). That study suggested that leptin is the signal which transmits the information to the brain that there is adequate fat in stores to cover the energy requirements of reproduction. Leptin, therefore, may participate in the timing of puberty. Another recent study in female rats showed that leptin is able to reverse the delayed onset of puberty in animals receiving less than normal quantities of food (Cheung *et al.*, 1997b). This suggested that leptin is not the signal that triggers the onset of puberty, but rather it plays a permissive role, acting as a metabolic gate for puberty to progress (Cheung *et al.*, 1997b). The latter results are in agreement with recent data in male monkeys demonstrating that leptin values did not change before the onset of puberty, i.e. before the increase in serum testosterone values, an indicator of puberty initiation (Plant and Durrant, 1997).

In humans, there are only a few studies which have addressed this issue. In one of them (Clayton *et al.*, 1997), leptin concentrations were found to be similar in boys and girls before the onset of puberty and to increase significantly at stage 2 of puberty. Then, in boys leptin concentrations declined to a nadir in late puberty, while in girls leptin values, after remaining stable in mid puberty, increased significantly during late puberty. At all stages, leptin concentrations correlated significantly with BMI, expressed as standard deviation score. Although these results do not support the notion that leptin acts as a signal for the onset of puberty, they demonstrate that leptin may facilitate human pubertal development. Another study (Mantzoros *et al.*, 1997c) investigated changes in leptin values in eight boys during puberty. The study started when puberty was in stage 1 or early stage 2 and the boys were followed for 2.5–5.1 years. Serum leptin values increased by 50% before the initiation of puberty, i.e. before testosterone started to increase, while after the onset of puberty leptin values decreased to prepubertal levels. The authors of that study (Mantzoros *et al.*, 1997c) concluded that in boys as in mice, leptin acts as a signal for the onset of puberty. Although these data are interesting, the number of boys investigated was small and therefore further studies are required.

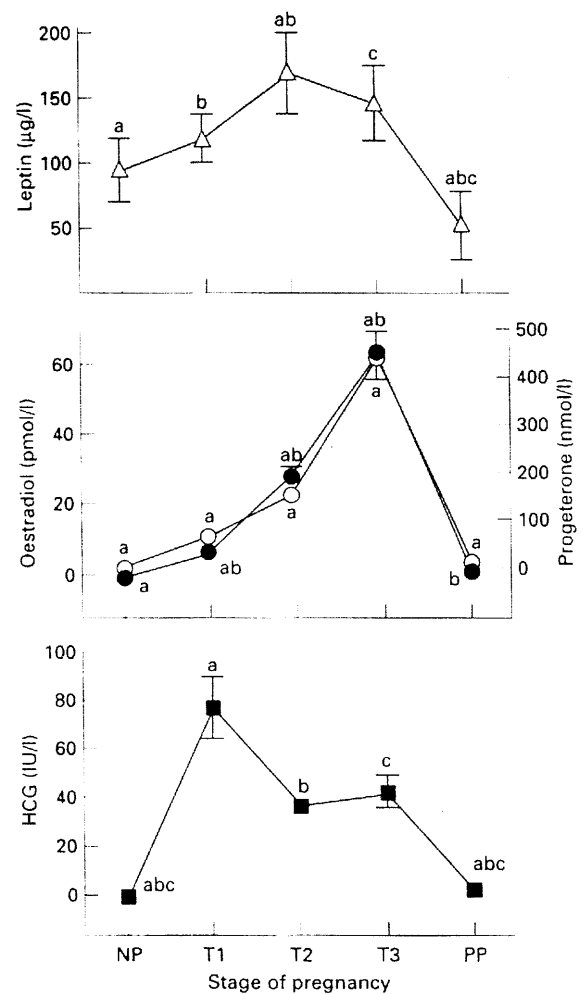
In females, a recent study investigated the relationship between fat mass and the age at menarche by measuring changes in leptin concentrations during puberty (Matkovic *et al.*, 1997). The study included 343 prepubertal girls who were followed over a period of 4 years. It was found that rising leptin values during puberty were associated with a decline of the age at menarche up to a leptin value of 12 ng/ml. In particular, an increase in serum leptin value by 1 ng/ml decreased the age at menarche by 1 month. Also, an increase in body fat by 1 kg lowered the time of menarche by 13 days. These results suggest that there is a critical concentration of leptin that acts as a threshold for the timing of menarche. Although these studies confirm that fat is an important contributor to normal reproductive function, it still remains to investigate

further whether leptin plays a primary role in the onset of puberty in humans or whether it has only a permissive role, like other metabolic factors.

## Pregnancy

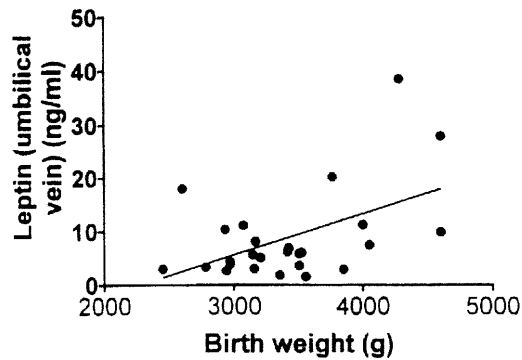
During pregnancy, various metabolic changes resulting in increased body weight and fat mass as well as changes in the secretion of various hormones take place. Therefore, pregnancy can be considered to be a state of relative obesity where leptin might play a role. Several studies have measured leptin values during pregnancy. In one of them (Butte *et al.*, 1997), serum leptin was measured in 65 women at 36 weeks of pregnancy as well as 3 and 6 months after delivery. Leptin concentrations per unit fat mass were significantly higher at 36 weeks than at 3 and 6 months postpartum and the values were higher in women who gained more weight during pregnancy. Significant positive correlations were found between leptin and the following parameters: body weight, BMI, fat mass, percentage fat mass and fasting insulin. After delivery, changes in leptin values correlated significantly with changes in body weight and fat mass and the women who failed to lose weight postpartum had higher leptin values. In the same study (Butte *et al.*, 1997), no differences in leptin values were found between lactating and nonlactating women, although a negative effect of leptin on 24-h milk production was seen, which could be explained by a suppressing effect of this hormone on prolactin secretion. Overall, the results of that study supported the lipostatic role of leptin; however, since a slight decrease in fat mass postpartum was associated with a great decrease in leptin, it is possible that factors other than fat mass may also regulate leptin in pregnant women. Leptin has been also detected in breast milk of lactating mothers (Casabiell *et al.*, 1997). Experiments in rats have shown that  $^{125}\text{I}$ -labelled leptin injected into mothers was detected in the stomach and the circulation of suckling neonates (Casabiell *et al.*, 1997). Although similar experiments have not been performed in humans, it is possible that leptin contained in the milk is absorbed by the gastrointestinal tract of the suckling infant and thus may play a role in the regulation of infant's appetite and adipose tissue.

Changes in serum leptin concentrations throughout the whole pregnancy have been reported so far in only one study, which included five women with singleton pregnancy (Figure 4). Leptin values increased significantly from the first to the second trimester, decreased slightly in the third trimester and declined markedly 4–6 weeks after delivery (Hardie *et al.*, 1997). Apart from a significant correlation with BMI, leptin concentrations correlated significantly with oestradiol concentrations, particularly during the first trimester and postpartum. A significant correlation was also found between leptin and human chorionic gonadotrophin values. The factors that cause the increase in leptin values during pregnancy have not been determined. Despite the significant correlation with



**Figure 4.** Changes in circulating oestradiol (O), progesterone (O), human chorionic gonadotrophin (HCG; n) and leptin ( $\Delta$ ) in normally cycling women prior to, during and in the month after a normal singleton pregnancy ( $n = 5$ ). The data is shown grouped into pre-partum (NP), by trimester (T1, T2, T3) and post-partum (PP, a single blood sample collected 4–6 weeks after parturition) stages of the pregnancy. Values sharing common superscripts (a,b,c) were found to be significantly different to each other (analysis of variance,  $P < 0.05$ ), while values which do not share common superscripts were not significantly different from each other. Adapted from Hardie *et al.* (1997) with the permission of Blackwell Science Ltd.

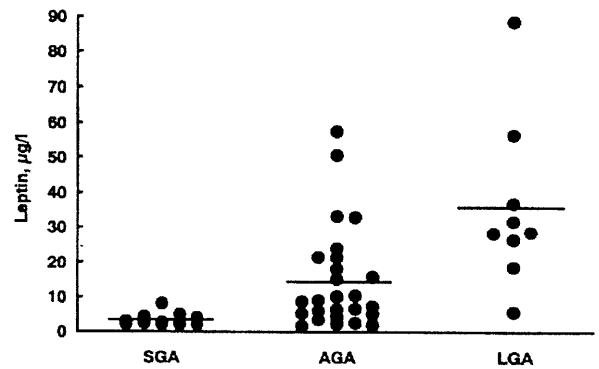
oestradiol, which could stimulate leptin production from the adipocytes (Shimizu *et al.*, 1997), leptin concentrations in the third trimester showed a plateau while oestradiol continued to rise (Hardie *et al.*, 1997). Cortisol and insulin concentrations, which increase during pregnancy (Kuhl, 1991; Chan *et al.*, 1993), may also stimulate the production of leptin from the adipocytes. The possibility, however, exists that leptin is produced by the placenta, since there is evidence that human placenta expresses *ob* gene (Luoh *et al.*, 1997; Masuzaki *et al.*, 1997; Senaris *et al.*, 1997).



**Figure 5.** Relationship between serum leptin concentration in umbilical vein and birth weight in 27 healthy newborns at delivery ( $r = 0.57$ ,  $P < 0.01$ ,  $y = 0.008x - 1.7$ ). Adapted from Schubring *et al.* (1997) with the permission of The Endocrine Society.

The purpose of the increase in leptin concentrations during pregnancy is not clear. Certainly, the correlation of leptin with the increase in body fat indicates that leptin may play a role in the control of maternal weight and metabolism. However, the gradual increase in leptin concentrations during pregnancy and the decrease postpartum indicates that a situation of leptin resistance may exist in pregnant women. A recent study (Schubring *et al.*, 1997) has shown that leptin values in maternal serum at delivery correlate positively with leptin values in amniotic fluid and negatively with placental weight. The values of leptin in maternal serum in that study were significantly higher than in arterial or vein cord blood and did not correlate with them. A close positive correlation between leptin values in umbilical vein and artery was found with no marked difference between the two vessels. Also, leptin values in cord vessels correlated significantly with birth weight and placental weight (Figure 5). Several possibilities are likely (Schubring *et al.*, 1997), such as that leptin in amniotic fluid is probably derived from the maternal circulation, while leptin in cord blood comes from the fetus and/or the placenta. The positive correlation between fetal leptin and birth weight indicates the importance of this protein for the regulation of energy in the fetus and that leptin in the fetus may act as a signal between fat stores and the brain.

When babies are small or large for gestational age, leptin concentrations in cord blood are lower or higher respectively than in neonates of normal weight (Figure 6) (Harigaya *et al.*, 1997; Koistinen *et al.*, 1997). However, within the first 24 h after delivery, leptin values in the blood of large and average-for-gestational-age neonates decline to levels similar to those in small-for-gestational-age babies (Harigaya *et al.*, 1997; Koistinen *et al.*, 1997). The rapid decrease in leptin values in these infants regardless of birth weight suggests that the production of this protein in the fetus may be also controlled by mechanisms other than changes in body fat mass. However, following the first 4 weeks of life, an increase in serum leptin values occurs which is probably related to the increase in body



**Figure 6.** Leptin concentrations in small for gestational age (SGA), average for gestational age (AGA) ( $P < 0.001$  compared with SGA) and large for gestational age (LGA) ( $P < 0.005$  compared with AGA) newborn infants at delivery. Adapted from Koistinen *et al.* (1997) with the permission of The Endocrine Society.

weight (Helland *et al.*, 1998). It is also possible that, at least in part, leptin in the fetus is derived from the placenta. One cannot, however, exclude the possibility that leptin values in the newborn infant reflect the nutritional status.

Regarding leptin values in umbilical cord blood, no sex difference was found in one study (Schubring *et al.*, 1997), suggesting that hormonal control of leptin production in the fetus may differ from that in adults. However, other recent studies have found lower concentrations of leptin in umbilical cord blood of male than of female babies (Matsuda *et al.*, 1997; Tome *et al.*, 1997; Helland *et al.*, 1998). These conflicting data are difficult to explain, however, since both oestradiol and testosterone values were similar in male and female newborns, genetic differences may play a role (Matsuda *et al.*, 1997).

Preterm newborns seem to have similar concentrations of leptin in cord blood to those of full-term infants, but preterm babies with smoking mothers have lower leptin values (Mantzoros *et al.*, 1997d). Leptin values in both preterm and full-term babies delivered from smoking mothers are lower than in those delivered from non-smoking mothers (Mantzoros *et al.*, 1997d). Also, a significant negative correlation between leptin values in cord blood and the number of cigarettes smoked per day by mothers has been found. The mechanism through which smoking can reduce leptin is not known. Smoking per se may adversely affect uteroplacental and fetal blood flow, which may reduce fat stores in adipose tissue. Another explanation could be through an increase in catecholamines induced by smoking which, by increasing lipolysis, decrease leptin concentrations (Gettys *et al.*, 1996).

The ability of human placenta to produce leptin was shown recently (Senaris *et al.*, 1997). Cytotrophoblast cells but not the core of villi are able to synthesize leptin *in vitro*. The extent to which placental leptin contributes to circulating leptin in the fetus is difficult to estimate. The fact that no marked arteriovenous difference has been found in cord blood vessels at term

(Schubring *et al.*, 1997) suggests that placenta is not a primary source of leptin for the fetus. However, the possibility that placental leptin may affect fetal weight at an earlier stage of fetal development cannot be excluded. The presence of leptin receptors in the placenta (Luoh *et al.*, 1997) indicates that leptin may exert paracrine or autocrine effects in this organ. Certainly, further experiments are required to assess if leptin is important for placental physiology and to clarify the role of this protein during pregnancy.

## Conclusions

Leptin is a protein that is produced by the adipocytes and may serve as a link between fat tissue and the brain. The role of leptin in obesity has been clarified in rodents bearing mutations of the *ob* gene or of leptin receptor. Animals that lack leptin or demonstrate resistance to this protein develop profound obesity. Human obesity, however, has not yet been linked to any gene defects and it is more possible that it is related to a kind of leptin resistance. The association between leptin and reproduction has been studied in women with PCOS, but the data so far are conflicting. No clear evidence has been provided that leptin may participate in the pathophysiology of this syndrome and further research is required. On the other hand, gonadal steroids and particularly oestradiol may affect the production of leptin. Oestradiol may exert a stimulatory effect on the adipocytes and testosterone a suppressing effect. Data regarding the expression of leptin gene in the human ovary are conflicting; however, there is some evidence that leptin is produced by the ovary. The fact that leptin receptors have been identified in the ovary and leptin can affect steroidogenesis *in vitro* indicates that this protein may exert endocrine or local effects in the ovary. During the normal menstrual cycle, leptin values are higher in the luteal than in the follicular phase, but the mechanism of this difference and its possible role in implantation need further investigation. Whether leptin is a signal for the onset of puberty in humans or plays a permissive role requires clarification. Finally, during pregnancy leptin values increase gradually in maternal serum, probably reflecting changes in the metabolism, while in the fetus leptin may act as a signal between fat tissue and the brain. It is evident that the data so far are indicative, but not conclusive, that leptin acts as a link between fat tissue and reproduction in humans, and further research is required to establish a physiological role for this protein in the reproductive process.

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