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# Leptin, polycystic ovaries and polycystic ovary syndrome

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**As soon as leptin was discovered four years ago, its potential as a player in the polycystic ovary syndrome (PCOS) was explored in a primitive way, though little light was shed on the enigma that is PCOS. As a second wave of leptin research is now available, we review how the expanded role of the cytokine in reproduction might yet impact upon our understanding of PCOS**

*Key words:* insulin resistance/leptin/polycystic ovary syndrome/obesity

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## Introduction

Previously in this journal we discussed the role of leptin as a hormone intimately involved in the reproductive process (Conway and Jacobs, 1997). Since then, the literature relating to leptin physiology and pathophysiology has expanded and several papers on serum leptin concentrations in women with polycystic ovary syndrome (PCOS) have been published (see below). We present here our perspective on this important area, and make some recommendations for further research.

It is widely acknowledged that most of the clinical problems that occur in PCOS are worse in overweight women. Indeed, one perception of the pathophysiology of the syndrome is to view obesity and its metabolic problems as the most important environmental challenge that result in its clinical expression (Jacobs, 1987). The notion here is that it is the development of obesity that is responsible for transformation of the asymptomatic woman with polycystic ovaries detected by ultrasound into the symptomatic patient with PCOS (Jacobs, 1987).

The impact of obesity is usually considered to operate through the associated insulin resistance (IR) (Conway and Jacobs, 1993). There are indeed many descriptions of the amelioration of clinical problems by the control of IR through diet (Kiddy *et al*., 1992) and drugs (Nestler *et al*., 1989, 1998; Dunaif *et al*., 1996). In addition to its role as a reporter of fat cell repletion, leptin may also determine a broad range of neuroendocrine vegetative functions through its interaction with the neuropeptide Y axis (NPY) (Stephens *et al*., 1995). Leptin may thereby mediate some of the adverse effects of obesity on ovarian function in women with PCOS.

Leptin enters the central nervous system (CNS) via the choroid plexus, interacts with its receptor in the hypothalamus, and inhibits synthesis and release of NPY (Stephens *et al*., 1995). The identification of leptin receptors in the ovary (Cioffi *et al*., 1997; Karlsson *et al*., 1997) suggests an additional mode of action of leptin, an action that becomes particularly attractive when one considers the high circulating concentrations of leptin that characterize obese people. In this review the possibility will be raised that, while centrally the obese patient with PCOS may be relatively leptin-deficient, in the periphery her ovaries may be overexposed to leptin.

## Polycystic ovaries, polycystic ovary syndrome and insulin resistance

Since the introduction of high-resolution pelvic ultrasound, it is generally accepted that the morphological appearance of polycystic ovaries can be detected in as many as 20% of the normal female population (Franks, 1995). Many of these women are asymptomatic. The development of symptoms, such as menstrual disturbance and those consequent on hyperandrogenism, is usually associated with the development of IR (Conway and Jacobs, 1993). In women with PCOS, the resistance is specifically to insulin-stimulated extra-splanchnic disposal of glucose

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(Dunaif *et al*., 1989). As a consequence of this peripheral IR, euglycaemia can only be maintained through compensatory hypersecretion of insulin. The IR spares the liver (the fasting glucose concentration is normal, while serum sex hormone binding globulin and high-density lipoprotein concentrations are depressed), perhaps the skin (Conway and Jacobs, 1990) and the ovary. Ovarian dysfunction results, in direct proportion to the intensity of compensatory hyperinsulinism (Conway *et al*., 1990). An understanding of the development of symptoms, i.e. of the evolution of PCOS, is therefore closely tied to an understanding of the causes of insulin resistance and their interaction with each other.

Insulin resistance in PCOS has been reviewed extensively (Dunaif, 1997; Nestler, 1997). Dunaif and her colleagues have described a specific defect in transduction of the insulin signal (autophosphorylation of the serine rather than tyrosine residues of the intracellular component of the insulin receptor) which is considered to be a constitutive feature of fibroblasts of women with PCOS (Dunaif *et al*., 1995). This defect is thought to be an inherited feature of women with PCOS. In addition, as children enter puberty, IR develops in response to the increase of growth hormone secretion that underlies the acceleration in growth at this age (Amiel *et al*., 1991). To give an idea of the dimensions of this effect, insulin requirements typically double in children with diabetes during the adolescent growth spurt. In the girl with polycystic ovaries, a combination of these two forms of IR create the background to the development of obesity and of the symptoms of PCOS. Obesity itself, present in some 40% of women with PCOS (Balen *et al*., 1995), worsens IR and so causes further deterioration of ovarian function. Should the patient come from a family with diabetes mellitus, there is the added risk of developing the IR of non-insulin-dependent diabetes mellitus. Finally, as will be discussed, serum leptin concentrations rise with increasing obesity, and there is evidence that leptin can itself impair insulin action in hepatocytes (Cohen *et al*., 1996). These several processes, which are at the least additive, are summarized in Table I. Whether they combine synergistically to provoke ovarian dysfunction is at present a matter for conjecture.

Two questions now arise: first, could a disturbance in the leptin–NPY pathway contribute to the processes just described; and second, is there any evidence for such a disturbance?

**Table I.** Causes of insulin resistance in polycystic ovary syndrome (PCOS)

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|---|---|
| 1. | PCOS-specific impaired transduction of insulin signal (Dunaif *et al*., 1995) |
| 2. | Adolescent growth spurt (Amiel *et al*., 1991) |
| 3. | Obesity |
| 4. | Non-insulin-dependent diabetes mellitus (in patients with familial susceptibility) |
| 5. | Leptin inhibition of insulin action on hepatocytes (Cohen *et al*., 1996) |

## Leptin–NPY axis: implications for reproductive disturbances

As we reviewed last year, leptin is a helical cytokine of the tumour necrosis factor group, which was originally identified as the product of the *ob* gene in mice. The obese (ob) mouse and diabetes (db) mouse have provided models for the study of obesity for many years (Bray and York, 1997). The *ob* gene, isolated by positional cloning, encodes a 167 amino acid protein (Zhang *et al*., 1994). The mutation identified in the ob mouse results in a premature stop codon (Arg105STOP). The leptin receptor is a single transmembrane-domain receptor of the cytokine receptor family (Tartaglia *et al*., 1995). The db mouse, indistinguishable phenotypically from the *ob* mouse, expresses a mutant leptin receptor and is therefore the product of leptin resistance (Chua *et al*., 1996).

In the ob (but not the db) mouse, administration of leptin results in weight loss through both decreased appetite and increased energy expenditure (Pelleymounter *et al*., 1995). In wild-type mice, leptin administration at pharmacological doses has only a minor effect on weight loss, making a physiological role for leptin in weight reduction uncertain (Pelleymounter *et al*., 1995; Halaas and Friedman, 1997). The leptin-deficient mouse is infertile and has subnormal gonadotrophin concentrations with an impaired response to castration (Swerdloff *et al*., 1976), i.e. it has hypogonadotrophic hypogonadism. Weight loss alone, when forced by dietary restriction upon the *ob*/*ob* mouse, does not reverse infertility. Leptin administration, however, results in a prompt return of fertility in the female *ob*/*ob* mouse, presumably through stimulation of gonadotrophin releasing hormone (GnRH) (Chehab *et al*., 1996). Indeed, leptin-treated *ob*/*ob* mice have higher serum concentrations of luteinizing hormone (LH) (particularly in females) and follicle stimulating hormone (FSH) (particularly in males) compared with pair-fed, saline-treated *ob*/*ob* mice (Barash *et al*., 1996).

Human counterparts of the ob and db mice have now been reported. A missense mutation, identical to the C→T transition in the first base of codon 15 that leads to the appearance of the premature stop codon in the *ob*/*ob* mouse, has been described in a Turkish kindred (Strobel *et al*., 1998). The mutation impaired normal processing of leptin through the secretory pathway, rather than inducing protein breakdown. The phenotype of homozygous adults (low serum leptin concentrations associated with hyperphagic obesity and hypothalamic hypogonadotrophic hypogonadism) suggests that in humans too, leptin not only controls body mass but is also necessary for the initiation of puberty. More widespread pituitary dysfunction was observed in patients homozygous for a mutation in the gene encoding the leptin receptor (Clement *et al*., 1998). In this consanguineous family, a G→A base substitution in the splice donor site of exon 16 resulted in a truncated leptin receptor that lacked both transmembrane and intracellular domains. Affected individuals had very raised serum leptin concentrations, together with a history of early-onset morbid obesity and lack of pubertal development. There was also reduced secretion of growth hormone (causing a mild but significant delay in growth) and thyrotrophin (causing mild hypothalamic hypothyroidism).

Leptin receptors have been identified in various peripheral tissues (e.g. ovary) as well as in the hypothalamus and choroid plexus. A novel isoform of the leptin receptor has been identified in human haemopoietic cells, prostate and ovary (Cioffi *et al*., 1996). Within the hypothalamus, leptin interacts with its receptor to inhibit synthesis and release of NPY (Stephens *et al*., 1995). NPY is the most abundant neuropeptide in brain. It has various functions and is involved in regulation of circadian rhythm, the response to anxiety and stress, peripheral vascular resistance and cardiac contractility. It is the only peptide known to induce obesity through prolonged central administration (Stephens, 1998). The link between leptin and the GnRH neurone is mediated through NPY, although there is some redundancy in the hypothalamic signalling pathway, since the NPY knock-out mouse not only maintains normal body weight but is also fertile (Erickson *et al*., 1997). NPY is thought to be an important mediator of the body's response to starvation, while additional factors (melanocyte stimulating hormone and the melanocortin-4 receptor) are involved in the hypothalamic response to obesity (Friedman, 1998).

In humans, expression of the *ob* gene in white fat cells is stimulated by insulin, glucocorticoids, noradrenaline and nutrients (Saladin *et al*., 1995; Rohner-Jeanrenaud and Jeanrenaud, 1996). In normal and obese subjects, between 5% and 20% of leptin circulates in a high-molecular weight complex (Houseknecht *et al*., 1996). Circulating serum leptin concentrations and *ob* gene expression in adipose tissue are increased in obese humans (Considine *et al*., 1996), so human obesity does not seem to be a state of leptin deficiency. Evidence that obesity is a state of leptin resistance is limited. The raised circulating leptin concentrations of obese individuals are thought appropriate for the amount of fat tissue (although see later for the situation in PCOS). There is a diurnal variation in serum leptin concentrations (Sinha *et al*., 1996), these being three times higher in women than in men, and the difference persisting after correction for fat mass. Oestrogen is not responsible, because neither menopausal status nor oestrogen administration has much effect on serum leptin concentrations (Havel *et al*., 1996; Rosenbaum *et al*., 1996). Part of the sexual dimorphism in leptin concentrations may be explained by the effect of androgens (Wabitsch *et al*., 1997). On the other hand, since expression of leptin messenger RNA is higher in subcutaneous than visceral fat (Hube *et al*., 1996), the difference may be more related to the higher ratio of subcutaneous to visceral adipose mass in women compared with men (Kotani *et al*., 1994).

## Leptin secretion in women with PCOS

Serum leptin concentrations in women with PCOS have been reported to be higher than (Brzechffa *et al*., 1996) or similar to (Chapman *et al*., 1997; Laughlin *et al*., 1997; Mantzoros *et al*., 1997; Rouru *et al*., 1997) those in weight-matched controls. In a challenging editorial, Caro (1997) considered that, for a given body mass index, the literature indicated that leptin was not different in controls compared with women with PCOS. Caro also commented, however, that investigations in this area had not yet addressed the broad spectrum of leptin pathophysiology, in the sense that body composition, androgen levels, the distribution of leptin between its bound and free forms and the pulsatile and circadian rythmicity of its secretion had not been incorporated into these studies. To which might be added, bias in the selection of patients for study (related to the definition of PCOS) and in the selection of controls (some 20% of which, unless they were excluded after ultrasound examination, might be expected to have polycystic ovaries) were also not accounted for.

The importance of body weight and composition and their relation to diagnostic criteria is exemplified by the report of Arroyo *et al*. (1997). These authors investigated the influence of adiposity on gonadotrophin secretion in women with PCOS. They found that the amplitude, although not the frequency, of LH pulses fell as the body mass index rose in women with PCOS, but not in women with normal menstrual cycles. Since many investigators (particularly in North America) require a raised serum LH concentration to establish a diagnosis of PCOS, the complexity of the interaction of pituitary function with obesity (and therefore with leptin secretion) is evident. Moreover, obesity in PCOS is characterized by an increase in visceral fat (Bjorntorp, 1996) (which increases the ratio of the circumference of the waist to that of the hip), i.e. an increase in the type of fat that relatively undersecretes leptin compared with subcutaneous fat.

That leptin levels in women with PCOS are, to an important extent, maintained by the associated compensatory hyperinsulinaemia was recently shown by Krassas *et al*. (1998), who found that treatment with diazoxide reduced serum leptin concentrations, *pari passu* with the fall of insulin secretion. The relationship of leptin secretion to IR was investigated in a very detailed study by Laughlin *et al*. (1997). Among several factors studied, they found that, independently of body mass index and percent body fat, only the 24-hour mean insulin concentration contributed significantly to leptin levels. Despite this relationship and the 2-fold higher mean insulin concentration in patients with PCOS compared with controls, the expected increase of serum leptin concentrations was not in fact observed. The authors considered their results were most readily explained by the presence of a PCOS-specific form of IR in adipocytes, which impairs the stimulatory effect of insulin on leptin secretion (Ciaraldi *et al*., 1997). This conclusion is consistent with the negative correlation of serum leptin concentrations with insulin sensitivity in both slim and obese women with PCOS reported by Micic *et al*. (1997).

The above studies raise the possibility that in women with PCOS, leptin secretion is less than expected because of IR and

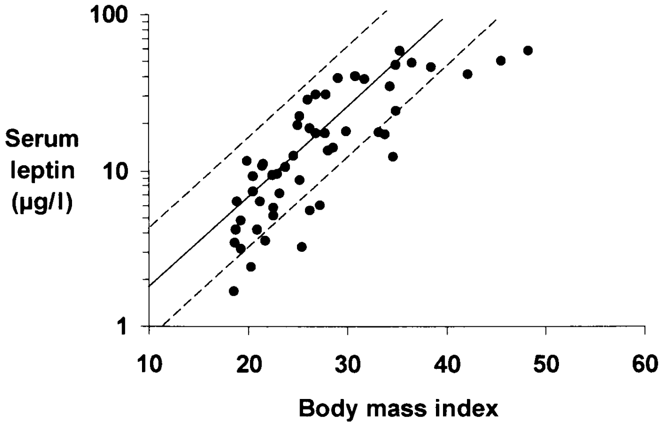


**Figure 1.** Fasting serum leptin measurements in women with polycystic ovary syndrome plotted against Body mass index. Lines indicate the median and 95th centiles for a large, normal population cohort.

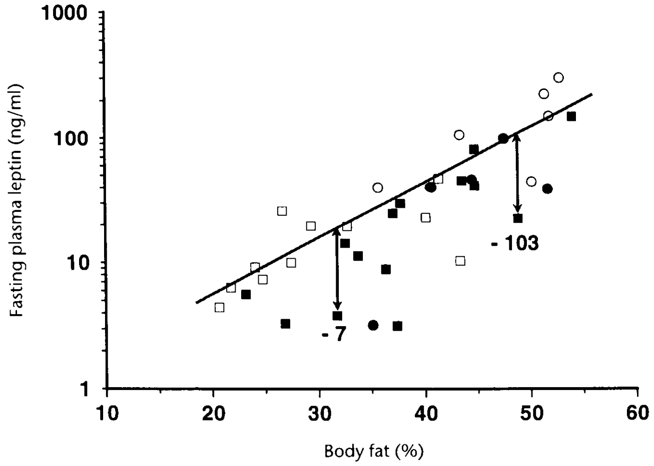


**Figure 2.** Relationship between fasting leptin concentrations and percent body fat. Females are represented by circles, males by squares. Open symbols show individuals with stable weight; closed symbols show those who gained weight during a three-year follow-up. The numerical labels illustrate the effect of the logarithmic scale on the degree to which individuals with a subnormal leptin concentration deviate from the mean (Ravussin *et al.*, 1997).

the type of fat that accumulates. Indeed, in a cohort of women with PCOS, we found leptin concentrations that were about 20% lower than in controls across a wide range of body weights (Figure 1). The results suggest that, in neuroendocrine terms, obesity goes 'under-reported' in patients with PCOS. The results are reminiscent of the low leptin concentrations reported to precede weight gain in non-diabetic Pima Indians (Ravussin *et al.*, 1997). A group of these subjects, extensively studied because of their proneness to obesity and non-insulin-dependent diabetes mellitus, had been followed for approximately three years. After adjustment for body fat, the mean plasma leptin concentration was lower in the 19 who subsequently gained weight compared with that in the 17 whose weight remained stable (Figure 2). Perhaps reduced leptin secretion forms part of the so-called 'thrifty' genotype (Neel, 1962), the set of genes postulated to account for the high prevalence of obesity (Balen *et al.*, 1995) and diabetes (Dahlgren *et al.*, 1992; Pierpoint *et al.*, 1998) that occurs in certain populations exposed to modern nutrition.

It remains to consider the possibility of an impact of leptin directly on the ovary. Karlsson and colleagues (1997) found transcripts in human granulosa and theca cells which encoded both the short and long isoforms of the leptin receptor (it is the latter which activates the signalling pathway). They found that leptin inhibited LH-stimulated oestradiol production by granulosa cells, but had no effect on cells incubated in the absence of LH. This finding, together with the impairment by leptin of the augmentation by insulin-like growth factor-I (IGF-I) of FSH-stimulated oestradiol production by rat granulosa cells (Zachow and Magoffin, 1997), indicates inhibition by leptin of the ovarian response to gonadotrophins. Perhaps the effect on the ovary of high circulating concentrations of leptin in obese patients with PCOS explains their otherwise surprisingly impaired response to gonadotrophin stimulation (White *et al.*, 1996). It seems likely that the response of the ovaries of such patients represents a balance between the stimulatory effects of insulin and the inhibitory effects of leptin.

An additional role for leptin in reproduction is suggested by its presence within the preimplantation embryo, where the polarity of its distribution might imply a regulatory role in development (Antczak and Van Blerkdom, 1997). Leptin protein, but not its mRNA, are found with the oocyte, presumably supplied by cells of the cumulus oophorus. Within the embryo, both leptin and its signal transducer STAT3, become unevenly distributed in the blastomere. These polarized domains of potential regulatory proteins suggest a role in determining the animal pole and establishment of the trophoblast. Yet another twist to the leptin story comes from a recent report of its role as an angiogenic factor (Sierra-Honigmann *et al.*, 1998). Within the ovarian follicle the angiogenic effect of leptin may effect dominant follicle selection and lead to the formation of a corpus luteum. Could leptin deficiency in PCOS result in the failure of dominant follicle selection?

To summarize, leptin secretion increases with obesity and is stimulated, inter alia, by insulin. In women with PCOS, insulin-stimulated leptin secretion is limited by IR in adipocytes. An important component of the obesity of women with PCOS is accumulation of visceral fat, which secretes less leptin than subcutaneous fat. We postulate that lower than appropriate satiety signals permit further development of obesity. Increasing obesity results in progressively severe IR with eventual decompensation of reproductive and metabolic function. At the ovarian level, high leptin concentrations may impair ovarian function by reducing the response to gonadotrophin stimulation.

Further studies in this area should, in our opinion, focus on details of leptin secretion, particularly around the time of puberty, since there is already evidence at this time of disturbed hypothalamic–pituitary–ovarian and metabolic function in girls with PCOS (Apter *et al*., 1995; Porcu *et al*., 1997). The role of androgens may be readily studied, particularly because there are now several effective anti-androgens with different modes of action (Conn and Jacobs, 1997). The development of obesity with the evolution of PCOS in women treated with sodium valproate offers an intriguing opportunity for clinical investigators (Sharma and Jacobs, 1997), as do the abnormalities of eating and nutrition that are so commonly seen in women with PCOS (McCluskey *et al*., 1991; Jahanfar *et al*., 1995). Perhaps the most important exhortation to future researchers in this area is Caro's plea for them to design studies based on leptin physiology, rather than simply pull samples out of the freezer that were collected during other experiments from women with PCOS (Caro, 1997).

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