Hyperleptinaemia is associated with impaired gonadotrophin response to GnRH during late puberty in obese girls, not boys

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Abstract

In ob/ob mice, leptin deficiency results in hypogonadotrophic hypogonadism, impaired sexual maturation and infertility, which are all corrected by leptin administration. In humans, pubertal development and menarche are related to the attainment of a critical amount of body fat. To examine whether changes in circulating concentrations of leptin could be a hormonal signal influencing gonadotrophin secretion, we studied 98 adolescents and young adults of both sexes, aged 13–19 years, whose weight varied from normal to massively obese and whose sexual maturation was between Tanner stages 3 and 5. We measured leptin, sex steroids and circulating gonadotrophin concentrations in the basal state and in response to GnRH. In perimenarchial and young adult girls, we found that the LH and FSH responses to GnRH were negatively correlated with body mass index (BMI; $r = -0.45$ and -0.47 respectively, $P < 0.0025$) and circulating leptin ($r = -0.53$ and -0.49 respectively, $P < 0.002$). Decreased LH and FSH responses to GnRH were associated with increased adiposity and hyperleptinaemia. Our data do not establish, but are consistent with a direct neuroendocrine negative effect of excess leptin on the central reproductive system of obese girls. In boys of comparable adiposity, we found no influence of BMI or leptin on gonadotrophin concentrations, which is another aspect of the sexual dimorphism characterizing human leptin physiology.

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Introduction

Leptin, a protein coded by the ob gene, is secreted by adipose tissue (1), and possibly brain (2), to serve as a component of a regulatory loop linking body fat mass to food intake and energy expenditure (3). In addition, a role for circulating leptin in the regulation of reproduction is suggested by studies both in animal models and humans. Leptin is necessary for the sexual maturation and reproductive life of rodents. Female ob/ob mice, which have a mutated ob gene and leptin deficiency (1), remain prepubertal indefinitely, with no oestrous cycles. Administration of recombinant leptin not only reverses their obesity, but allows ovulation, pregnancy and parturition (4). Ob receptors are expressed in hypothalamus, pituitary gonadotrophs and ovary, supporting the role of leptin in reproductive physiology (5, 6). Leptin accelerates the onset of female puberty in normal mice (7, 8), and appears to have a role in the onset of puberty of female rats (9), consistent with previous observations regarding body fat and sexual maturation in both these species (10, 11). In humans, the influence of nutrition and body composition on puberty and reproductive physiology has long been recognized (10, 12). Menarche typically occurs at a minimal body

weight of 48 kg, independent of the age of sexual maturation (13). Women whose percentage body fat decreases to less than 15% of the ideal value have disturbed reproductive capacity, whereas obese women have dysfunction of the gonadal hormone system and polycystic ovary syndrome more frequently than lean women (14), and morbidly obese women have an increased rate of infertility (15, 16).

Leptin is, at present, the only known marker of adipose tissue the circulating concentration of which increases in linear proportion to the body fat mass (17). A sexual dimorphism of circulating leptin concentrations has been consistently observed in normal and obese adults (18–20) and children (21, 22), leptin values being 20–30% greater in female than in male individuals of comparable body composition. These experimental and clinical observations suggest that serum leptin could serve as a gender-dependent message from the adipose tissue to the reproductive system.

Puberty is characterized by increasing concentrations of gonadal oestradiol in girls and testosterone in boys, driven by increasing concentrations of pituitary gonadotrophins, which are, in turn, regulated by gonadotrophin-releasing hormone (GnRH) released by

Table 1 Main characteristics of the adolescents studied. Values are means $\pm$ s.d.

	Girls (<i>n</i> = 50)		Boys (<i>n</i> = 48)	
	Obese (<i>n</i> = 42)	Lean (<i>n</i> = 8)	Obese (<i>n</i> = 35)	Lean (<i>n</i> = 13)
Age (years)	14.7 $\pm$ 0.2	14.1 $\pm$ 0.8	14 $\pm$ 0.3	14.5 $\pm$ 0.4
Weight (kg)	83.6 $\pm$ 2.1	47.3 $\pm$ 3.2	77.5 $\pm$ 3.2	46.1 $\pm$ 2
Height (cm)	160.9 $\pm$ 0.8	156.5 $\pm$ 1.1	160 $\pm$ 1.8	159.2 $\pm$ 3
BMI (s.d.)	+6.5 $\pm$ 0.6	0.4 $\pm$ 0.9	+5.6 $\pm$ 0.7	-1.2 $\pm$ 0.3
BMI (kg/m ²)	33.4 $\pm$ 0.9	18 $\pm$ 0.8	31.1 $\pm$ 1	16.8 $\pm$ 0.2
Leptin (ng/ml)	36.4 $\pm$ 1.7	14.3 $\pm$ 2.7	23 $\pm$ 1.8	5.6 $\pm$ 1.2

hypothalamic neurones (23, 24). In the present study, we attempted to determine the relationship between leptin and gonadotrophin secretion in lean and obese adolescents during the late stages of puberty.

Study participants and methods

Study participants

The main characteristics of the study participants are presented in Tables 1 and 2. We recruited 98 pubertal adolescents aged 13–19 years: 50 girls (eight non-obese and 42 obese) and 48 boys (13 non-obese and 35 obese). All of them had completed or were completing their pubertal development. Forty-two of the girls had apparently normal menstrual functioning; eight had not had their first menses. None had hirsutism or hyperandrogenism, or ultrasound or hormonal signs of polycystic ovary syndrome (PCOS). Body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in metres. BMI is considered to be a reliable index of adiposity in pubertal adolescents (22, 25). Obesity was defined as a BMI or body weight greater than 120% of the ideal value for age and height (26). Pubertal staging was performed according to Tanner.

None of the participants was taking any medication or had evidence of metabolic or endocrine disease other than obesity.

Protocols were approved by the institutional review board of University René Descartes-Faculté Cochin Saint Vincent de Paul. All the study participants and their families gave their informed consent to their inclusion in this clinical research study.

Hormone assays

Gonadal function was assessed by measurement of serum concentrations of oestradiol, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in basal conditions and in response to GnRH stimulation, during the follicular phase. LH and FSH were measured 15, 30, 45, 60, 90 and 120 min after i.v. injection of GnRH 100 μ g/m² (27). Testosterone was measured by a direct radioimmunoassay (Orion, Turku, Finland). The intra- and interassay coefficients of variation were 8% and 8.5% respectively for a concentration of 0.6 ng/ml and <8% at greater concentrations, and the limit of detection was 0.05 ng/ml. Oestradiol was measured by a double-antibody radioimmunoassay (Clinical Assays, Stillwater, Minnesota, USA). The intra-assay coefficient of variation was 5.8% at 25 pg/ml and 3.2% at 90 pg/ml, the interassay coefficient of variation was 2.4% at 26 pg/ml and 3.8% at 83 pg/ml, and the limit of detection was 2.5 pg/ml. Leptin was measured in plasma or serum samples by radioimmunoassay by means of reagents supplied by Linco Research Inc. (Saint Louis, Missouri, USA), as reported elsewhere (22).

Table 2 Sexual development and hormones in the adolescents studied. Values are means $\pm$ s.d.

	Girls (<i>n</i> = 50)		Boys (<i>n</i> = 48)	
	Obese (<i>n</i> = 42)	Lean (<i>n</i> = 8)	Obese (<i>n</i> = 35)	Lean (<i>n</i> = 13)
Uterine length (cm)	62.8 $\pm$ 1.4	62 $\pm$ 1.6	—	—
Testes volume (ml)	—	—	10.1 $\pm$ 1.5	10.7 $\pm$ 2.4
Basal LH (IU/ml)	3 $\pm$ 0.2	3.4 $\pm$ 0.7	1.4 $\pm$ 0.2	1.8 $\pm$ 0.2
LH peak* (IU/ml)	13.4 $\pm$ 0.9	18.6 $\pm$ 1.2	10.4 $\pm$ 1	13.1 $\pm$ 1.7
Basal FSH (IU/ml)	3.1 $\pm$ 0.2	3.9 $\pm$ 0.1	1.9 $\pm$ 0.2	2 $\pm$ 0.3
FSH peak* (IU/ml)	6.8 $\pm$ 2.4	9.5 $\pm$ 2.8	3.9 $\pm$ 0.4	3.9 $\pm$ 0.8
Oestradiol (pg/ml)	46.3 $\pm$ 4.1	45.2 $\pm$ 2.4	—	—
Testosterone (ng/ml)	—	—	4.3 $\pm$ 0.5	4.6 $\pm$ 1.1

* After GnRH.

The intra-assay coefficient of variation was 3.6% at 1.4 ng/ml and 3.3% at 14.2 ng/ml, the interassay coefficient of variation was 4.6% at 1.4 ng/ml and 3.7% at 14.2 ng/ml. Recovery of human leptin added to a plasma pool was $105 \pm 4\%$ (mean $\pm$ s.d.). Serial dilutions of high-concentration samples strictly paralleled the standard curve. The mean fasting concentration ($\pm$ s.d.) was 3.6 ± 0.7 ng/ml in non-obese men and 8.8 ± 3.9 ng/ml in non-obese menstruating women.

Statistical analysis

Linear regression analyses were performed relating indices of adiposity and serum leptin concentration. Correlations between gonadotrophin concentrations (basal and peak) and leptin, BMI and age, were calculated. For all statistical tests, significance was defined as $P < 0.05$. Computations were made with BMDP statistical software.

Results

Clinical and biological characteristics of the children studied are presented according to sex in Tables 1 and 2. BMI correlated positively with age ($r = 0.81$, $P < 0.0001$). The mean serum leptin concentration was 26.9 ± 1.8 ng/ml in the 98 adolescents and 5.5 ± 1.0 ng/ml in the lean subgroup (21/98) ($P < 0.001$). Univariate analysis showed that serum leptin correlated strongly with BMI both in girls

($r = 0.85$, $P < 0.0001$) and in boys ($r = 0.72$, $P < 0.0001$), but did not correlate with age. In both sexes, in agreement with the findings of others (28), mean basal LH and FSH concentrations showed no correlation with age within our narrow range of pubertal stages.

In girls, basal FSH, but not LH, correlated with BMI ($r = 0.45$, $P < 0.005$). The peak LH response in girls correlated with the basal LH value ($r = 0.61$, $P < 0.0001$), and peak FSH correlated with basal FSH ($r = 0.84$, $P < 0.0001$). Peak LH and FSH responses of girls to GnRH test were interrelated ($r = 0.35$, $P < 0.04$), and both correlated negatively with BMI ($r = -0.45$, $P < 0.005$ and -0.47 , $P < 0.0025$), but not with age. Serum leptin in girls correlated negatively with peak LH ($r = -0.53$, $P < 0.0001$; Fig. 1), basal FSH and peak FSH ($r = -0.49$, $P < 0.002$; Fig. 2). Basal and peak LH/FSH ratios were closely correlated ($r = 0.79$, $P < 0.0001$), but were not influenced by leptin concentrations. Oestradiol concentration did not correlate with basal or peak LH, age, BMI or leptin.

In boys, basal LH and post-GnRH peak were correlated ($r = 0.58$, $P < 0.0001$), in addition to basal and peak FSH concentrations ($r = 0.91$, $P < 0.0001$). Basal and post-GnRH LH and FSH values were interrelated ($r = 0.59$, $P < 0.0001$ for each). Correlations of gonadotrophin concentrations with BMI or serum leptin were not found in boys (Figs 1, 2). Testosterone correlated with both basal ($r = 0.46$, $P < 0.002$) and peak LH ($r = 0.44$, $P < 0.01$), and to a lesser degree with leptin ($r = 0.37$, $P < 0.05$), but not with age and BMI.

Figure 1 Relationship between serum leptin concentration and maximal LH value after GnRH injection in girls (●) and boys (◆). The equation describing the regression of LH peak to leptin in girls is: $y = -0.19x + 20$ ($r = -0.53$, $P < 0.01$). There was no significant relationship between these parameters in boys.

Figure 2 Relationship between serum leptin concentration and maximal FSH value after GnRH injection in girls (●) and boys (◆). The regression equation in girls is: $y = -0.12x + 11.4$ ($r = -0.49$, $P < 0.02$). There was no significant relationship in boys.

Discussion

Serum concentrations of leptin in the 98 adolescents (21 lean and 77 obese) were correlated closely with adiposity, as reported previously (21, 22). We found that puberty was associated with an increase in leptin values in girls, but that boys retained lower values for leptin than did the girls, and showed no increase after sexual maturation (22); these data confirmed the observations of others (28–30). The greater androgen concentrations in obese boys are responsible for their lower leptin serum concentrations compared with those in obese girls (21). This sexual dimorphism of pubertal changes in leptin concentrations is observed in obese and in normal adolescents (22).

Per kilogram of body fat, boys produce less leptin after than before puberty (21, 22). A longitudinal study in eight lean boys suggested that serum leptin increases just before the onset of puberty, possibly because of changes in fat mass, then returns to baseline after the initiation of puberty (31).

We are not aware of longitudinal studies that have been undertaken in girls in normal puberty. Our previous data indicate that, when normalized for body adiposity, circulating leptin is increased independently by obesity status, female sex and sexual maturation. Greater concentrations of leptin in sexually maturing girls, whether lean or obese, are associated with the pubertal increase in fat content (22). Per kilogram body fat, pubertal girls produce approximately twice more leptin than pubertal boys (22, 29, 30).

De Ridder *et al.* (32) studied the rate at which 68 schoolgirls progressed through the stages of puberty in relation to body fat mass. They found no evidence that body fat mass triggers the onset of puberty. Body fat mass, however, was negatively related with the rate of pubertal development toward menarche. According to these observations, onset of puberty and menarche are not parallel events. These findings do not conflict with population data, which suggest that increased fat content accelerates menarche, not puberty onset (32, 33).

Given these results, we focused our observations on the perimenarchial or early post-menarchial period. We found that BMI and circulating leptin were statistical negative determinants of gonadotrophin secretion. At or near the end of female puberty, increased adiposity and excess circulating leptin are associated with decreased gonadotrophin secretion. The more obese the pubertal girl, the more leptin secreted and the lower the FSH, LH and FSH response to GnRH. LH and FSH concentrations both decrease in proportion with increasing leptin concentrations, so that LH/FSH ratio is conserved. These clinical data are consistent with, but do not establish, leptin as a direct causative signal linking adipose tissue excess to the reproductive function of hypothalamus or pituitary gonadotrophs.

Our results are in apparent contradiction with the effects of leptin observed in animal models of obesity, in which leptin appears to be a physiological signal linking adiposity and central neural networks (34, 35). Ob/ob females have a functional defect of the hypothalamic–pituitary axis (36–39), and reconstitution of reproductive function requires either delivery of hypothalamic

extracts to the third ventricle or administration of pituitary extracts or gonadotropic hormones (40). Repeated administration of recombinant human leptin to ob/ob female mice restores hypothalamic–pituitary function and sexual maturity (4), leptin reverses the hypogonadotrophic hypogonadism induced by starvation (40), and leptin treatment of normal female mice accelerates puberty (7, 8). Two hypotheses could reconcile these experimental observations with the present results. One is that the central nervous system is resistant to circulating leptin in obese patients (17). If pituitary gonadotroph cells are resistant to leptin in obesity, a signal necessary to sexual maturation and function, this could alter gonadotrophin secretion. The resistance could also act at the blood–brain leptin transport level (41). An alternative hypothesis is that physiological leptin concentrations are necessary for proper female gonadotrophin function during puberty and in the post-pubertal period, but that massively increased leptin concentrations or modifications of secretory rhythms (42) as a result of obesity deregulate the gonadotrophin system. The possibility remains also that serum leptin concentrations are only a reflection of the massive increase of body fat, whereas other circulating or central signals are directly involved in the regulation of gonadotrophin secretion.

Adult women with long-term obesity overexpress the ob gene in their adipose tissue (43), and have increased serum leptin (17). They are at increased risk of developing PCOS, a group of clinical and biological manifestations involving hyperandrogenism, menstrual disturbances, and chronic anovulation. Although the aetiology of PCOS has not been elucidated (44, 45), the condition is reminiscent of that in rodents with leptin deficiency or leptin resistance (46). Studies of leptin concentrations in obese women with PCOS at about the age of 30 years of age failed to reveal differences from normally cyclic obese controls (32, 47–51). Although these studies did not find any relationship between increased leptin and PCOS at the time of diagnosis, they do not rule out the possibility that, in subsets of predisposed obese women, increased leptin concentrations may favour the development of PCOS (51). Obese adolescents afford the opportunity to study leptin–gonadotrophin relationships long before the age at which diagnosis of PCOS is usually made – namely at an age 15 years younger than that of the women with PCOS who were studied (47, 48). The marked decrease in gonadotrophin secretion observed in the more obese adolescents could impair ovulation. An inverse correlation of circulating leptin concentration with LH pulse amplitude has been reported in normal women and those with PCOS. Whether chronically decreased gonadotrophin secretion in adolescence and early sexual maturity could contribute to PCOS pathogenesis remains entirely speculative. None of the adolescent or young women studied had ultrasound or hormonal evidence of PCOS.

In boys, no influence of adiposity or leptin on gonadotrophin concentrations was observed. This could be an additional aspect of the sexual dimorphism of leptin concentrations observed in humans.

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