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Serum leptin through childhood and adolescence

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Summary

OBJECTIVE Leptin is the protein product of the recently cloned *ob* gene, that has been implicated in the control of body weight and thermogenesis, but also independently stimulates the reproductive axis. As major changes in body composition and gonadal function occur during human adolescence, we have assessed serum leptin concentration through childhood.

SUBJECTS AND MEASUREMENTS Serum leptin was measured in a radioimmunoassay in samples from 235 healthy children from 5 to 18 years of age. Its relationship to body mass index (BMI) (expressed as standard deviation score (SDS)) and the changes in concentration both within and between sexes over the stages of puberty were analysed.

RESULTS Serum leptin was present at similar concentrations in both sexes over the prepubertal years and increased in parallel into early puberty (breast stage (B) 2, genital stage (G) 2). Thereafter serum leptin in the boys declined to a nadir in G5. In contrast in girls, leptin remained constant in mid-puberty rising to a peak at B5. Factors influencing leptin (BMI SDS, age and testicular volume) were assessed therefore in the pre- and peripubertal stages (B1–2, G1–2) compared to the later pubertal stages (B3–5, G3–5). In all groups, leptin was positively correlated to BMI SDS ($r^2 = 38$–41% in girls, $r^2 = 31$–35% in boys). However in B1–2 and G1–2, leptin was also positively related to age, which contributed a further 27% and 20% respectively to the variability. In B3–5, age only accounted for an additional 5% in leptin variability. In contrast in G3–5, leptin was related positively to BMI SDS ($r^2 = 35\%$) and negatively to testicular volume ($r^2 = 24\%$).

CONCLUSIONS The influence of BMI on leptin is a significant factor throughout the prepubertal and pubertal years of both sexes. The additional negative effect of testicular volume in the boys contributes to the sexual dichotomy in leptin concentration at the completion of puberty. The similar rise in leptin over the prepubertal years into early puberty in both sexes, related not only to BMI SDS but also independently to age, would suggest that leptin may have a facilitatory role in human pubertal development.

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The presence of leptin and leptin receptor gene abnormalities in strains of obese mice has firmly established a role for this 16 kD protein and its signalling system in the pathogenesis of murine obesity (Zhang *et al*., 1994; Chen *et al*., 1996; Lee *et al*., 1996). Studies in normal and fasted mice have begun to identify the mechanisms of leptin action: its serum concentration reflects body fat content (Frederich *et al*., 1995), it is reduced in starvation (Ahima *et al*., 1996) and increased after food intake or insulin treatment (Saladin *et al*., 1995), while its administration to ob/ob mice reduces feeding and body weight (Weigle *et al*., 1995). It is proposed that leptin, released from the adipocyte, acts through a long feedback loop to suppress hypothalamic neuropeptide Y expression, and hence reduce appetite (Stephens *et al*., 1995), thereby acting as a regulator of body weight.

The human homologue of the *ob* protein has recently been cloned (Isse *et al*., 1995). Studies in normal weight and obese humans have confirmed that serum leptin concentrations accurately reflect body mass index and percent body fat (Maffei *et al*., 1995; Considine *et al*., 1996; Ma *et al*., 1996), with leptin mRNA being overexpressed in adipocytes from those with obesity (Hamilton *et al*., 1995; Lonnqvist *et al*., 1995). These observations are consistent with the development of a state of leptin resistance in human obesity, where the central mechanisms that reduce food intake are not activated by high levels of circulating leptin.

It has recently been suggested, however, that leptin may have a wider role in the response of the neuroendocrine system to starvation (Ahima *et al*., 1996). The changes in the thyroid, adrenal and gonadal axes, that occur in starved mice, can be partly reversed by restoring leptin to normal levels (Ahima *et al*., 1996). In addition, fertility in the sterile ob/ob female mouse can be achieved by leptin administration (Chehab *et al*., 1996), while leptin given to normal prepubertal mice appears to reduce their time to first oestrus and mating (Chehab *et al*., 1997). As major changes in food

Table 1 Auxological characteristics of subjects: mean (±1 SD)

	Boys Tanner stage					Girls Tanner Stage				
	G1	G2	G3	G4	G5	B1	B2	B3	B4	B5
n	64	16	10	7	13	42	17	9	14	43
Age (years)	8·3 (2·1)	11·6 (0·9)	13·2 (1·7)	14·6 (1·3)	16·7 (2·1)	7·9 (1·6)	11·2 (0·9)	11·2 (0·9)	13·0 (0·9)	14·9 (1·4)
Height (cm)	131·4 (14)	149·6 (7·8)	159·2 (11·2)	171·2 (2·0)	178·2 (8·6)	128·5 (11·4)	147·5 (6·4)	151·4 (8·4)	158·4 (4·5)	163·5 (5·6)
Weight (kg)	29·8 (10·2)	41·7 (5·3)	52·7 (9·2)	60·1 (8·9)	72·0 (10·5)	27·1 (6·8)	39·1 (6·9)	41·3 (4·0)	48·7 (5·2)	58·7 (9·7)
BMI (kg/m^2)	16·9 (2·6)	18·4 (2·6)	20·7 (2·8)	20·5 (2·9)	22·7 (2·6)	16·2 (2·0)	17·9 (2·4)	18·0 (1·2)	19·5 (1·9)	21·9 (3·1)

intake, body composition and gonadal function occur during the pubertal years, we have measured serum leptin concentrations in a cross-sectional study of normal children and adolescents.

Subjects and methods

Subjects

Schoolchildren ($n = 235$, 110 male, 125 female) aged 4·9 to 18·4 years were recruited with local ethical committee approval. Those with either chronic medical conditions or in receipt of regular medication were excluded. Height (cm) was measured on a portable stadiometer, calibrated with a machined metre rod, and weight (kg) was measured with electronic scales. Genital or breast development was graded according to Tanner stages, with testicular volume (ml) defined by comparison to a Prader orchidometer. Anthropometric data are summarized in Table 1. All assessments were undertaken between 1000 h and 1100 h, 3–4 h after breakfast, during which a blood sample was drawn.

Standard deviation scores ([observed value − mean value] ÷ standard deviation) (SDS) for height and body mass index (weight (kg) ÷ height^2 (m)) (BMI) were calculated from the 1990 age and sex specific normal anthropometric data (Freeman *et al*., 1995; Cole *et al*., 1995). BMI SDS was used in analysis to control for the change in BMI with age. The group were taller than average-mean height SDS in both sexes +0·43 (SD 1), while the mean BMI SDS for girls was +0·2 (1) and for boys +0·4 (1).

Leptin assay

Leptin concentrations were determined using a commercial radioimmunoassay (Linco, St Charles, Mo, USA), which uses human recombinant (hr)-leptin for both the standards and tracer, with antisera raised against hr-leptin. Duplicate serum samples were incubated with ^{125}I-leptin and antisera overnight at 4°C, followed by precipitation of bound and free antibody by incubation with anti-rabbit IgG. Supernatants were decanted after centrifugation, the pellets counted for radioactivity and leptin concentrations determined with reference to a standard curve. The limit of detection of the assay was 0·5 μg/l. The intra- and interassay coefficients of variation ranged from 3·4 to 8·3% and 3·6 to 6·2% respectively over a leptin concentration range of 4·9 to 25·6 μg/l.

Statistical analysis

All analysis was performed using the Statistical Package for Social Sciences (SPSS). Leptin concentrations were log transformed to generate a normal distribution: all data are reported as the geometric mean ± one tolerance factor. Height, height SDS, weight, BMI and BMI SDS are expressed as mean ± 1 SD. Differences between groups were determined using the independent samples *t*-test and one-way analysis of variance (ANOVA) with the Bonferroni post-hoc test. A P value <0·05 was used to indicate statistical significance.

The relationships between variables were assessed by achieving a 'best fit' line with either a first degree (linear), second degree or third degree polynomial, and 95% prediction intervals around this line were calculated. In describing the relationship between leptin concentration and pubertal development (see Fig. 2), each Tanner stage was ranked by age and separated into bins of equal duration. The Chow test was applied to determine whether regression lines were significantly different. To assess the influence of multiple parameters on leptin, stepwise multiple linear regression (MLR) was used.

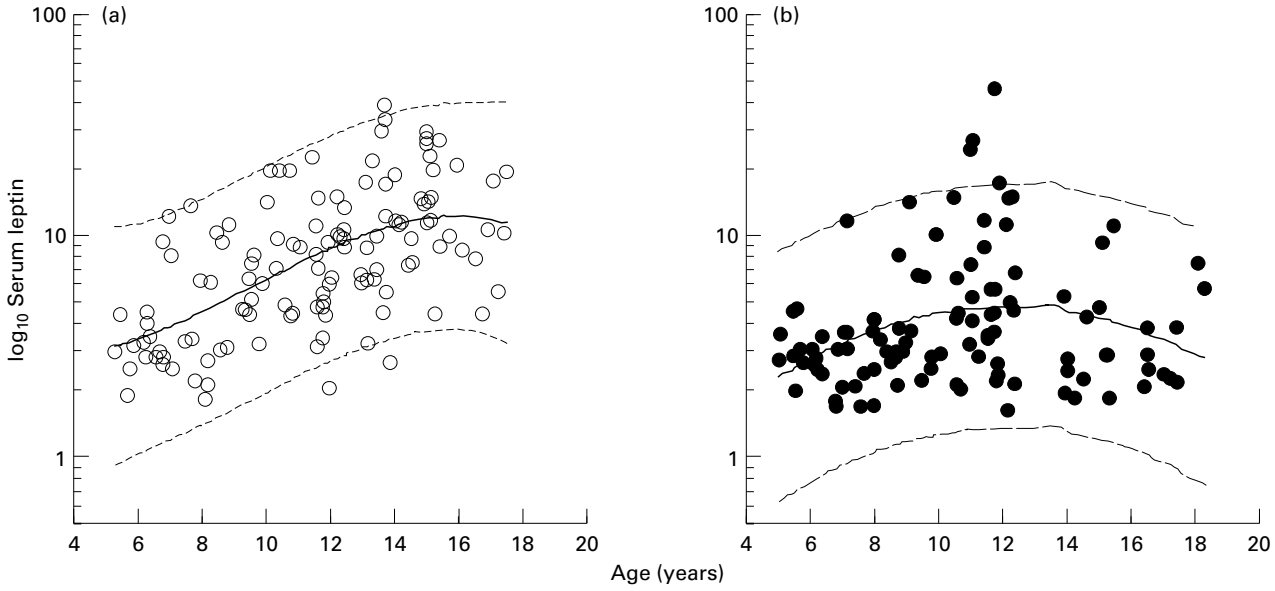

Fig. 1 Serum leptin concentration (ng/ml) (on a $\log_{10}$ scale) *versus* age (years) in (a) the girls (○) and (b) the boys (●). The best fit line (solid line) for the girls was $\log_{10}[\text{leptin}] = -0{\cdot}001x^3 + 0{\cdot}17x^2 - 0{\cdot}095x + 0{\cdot}608$ ($r = 0{\cdot}59$, $P < 0{\cdot}001$), and for the boys was $\log_{10}[\text{leptin}] = -0{\cdot}006x^2 + 0{\cdot}147x - 0{\cdot}215$ ($r = 0{\cdot}31$, $P < 0{\cdot}01$), where x = age (years). The broken lines represent 95% prediction intervals around the best fit line.

Results

All subjects had leptin concentrations within the detection range of the assay. Leptin concentrations were higher in girls than boys (7·4 (3·6, 15) μg/l in girls *versus* 3·9 (2, 7·4) μg/l in boys, $P < 0{\cdot}001$). This difference could be attributed to changes occurring in puberty, in that prepubertal levels were similar in the two sexes (4·3 (2·5, 7·4) μg/l in girls *versus* 3·5 (2, 6·3) μg/l in boys, NS) but were significantly higher in pubertal girls than in pubertal boys (9·7 (5·2, 18·1) μg/l in girls *versus* 4·4 (2·1, 9·1) μg/l in boys, $P < 0{\cdot}001$).

In the complete cohort, the relationship between leptin concentration and age was best described by a third-degree polynomial in the girls and a second-degree polynomial in the boys (Fig. 1A+B). In prepubertal girls and boys, leptin concentrations increased in parallel with age (Fig. 2A+B). Although concentrations were not different between boys and girls in any prepubertal age band, girls attained a leptin concentration >5 μg/l more than 1 year earlier than boys (mean age 8·6 (1·3) years for the girls *versus* mean age 10·1 (1·4) years in the boys, $P < 0{\cdot}05$).

In both sexes the relationship between leptin and pubertal stage was best described by third-degree polynomials (Fig. 2A+B). During male puberty, there were significant changes in leptin (ANOVA $P = 0{\cdot}006$), with the peak reached early at G2 (Fig. 2B) or at a testicular volume (TV) of 8 ml (Fig. 3). Leptin concentrations at G5 were lower than those in G2 ($P < 0{\cdot}05$). In contrast, leptin increased to B2 in female puberty, then remained constant in mid-puberty with a peak at B5 (Fig. 2A): concentrations at all pubertal stages (B2–B5) were different from the concentration in B1 (all $P < 0{\cdot}05$). In a comparison between the sexes, leptin concentrations were not different in early puberty (B2–3 *versus* G2–3), but diverged in late puberty (B4–5 *versus* G4–5, both P values $< 0{\cdot}01$).

As the sexual dichotomy in leptin concentration appeared only in later puberty, assessment of the influence of factors, such as BMI SDS, age and testicular volume, has been undertaken in girls and boys, grouped according to pre- and early pubertal stages (B1–2, G1–2) compared to later pubertal stages (B3–5, G3–5). There were significant positive linear correlations between leptin and BMI SDS in all groups; the slopes were similar but the intercept increased between B1–2 and B3–5 (Fig. 4A–D). In stepwise MLR (Table 2), leptin was positively related to BMI SDS and age in B1–2, G1–2 and B3–5, but positively related to BMI SDS and negatively to testicular volume in G3–5.

Discussion

Studies of leptin in humans have been carried out in normal weight and obese adults. Serum leptin concentrations are markedly elevated in obesity (Maffei *et al*., 1995; Considine *et al*., 1996; Ma *et al*., 1996), suggesting leptin resistance, with reduced CSF to serum leptin ratios as a putative mechanism for the latter (Schwartz *et al*., 1996; Caro *et al*., 1996). Leptin correlates most significantly with percentage body fat and less

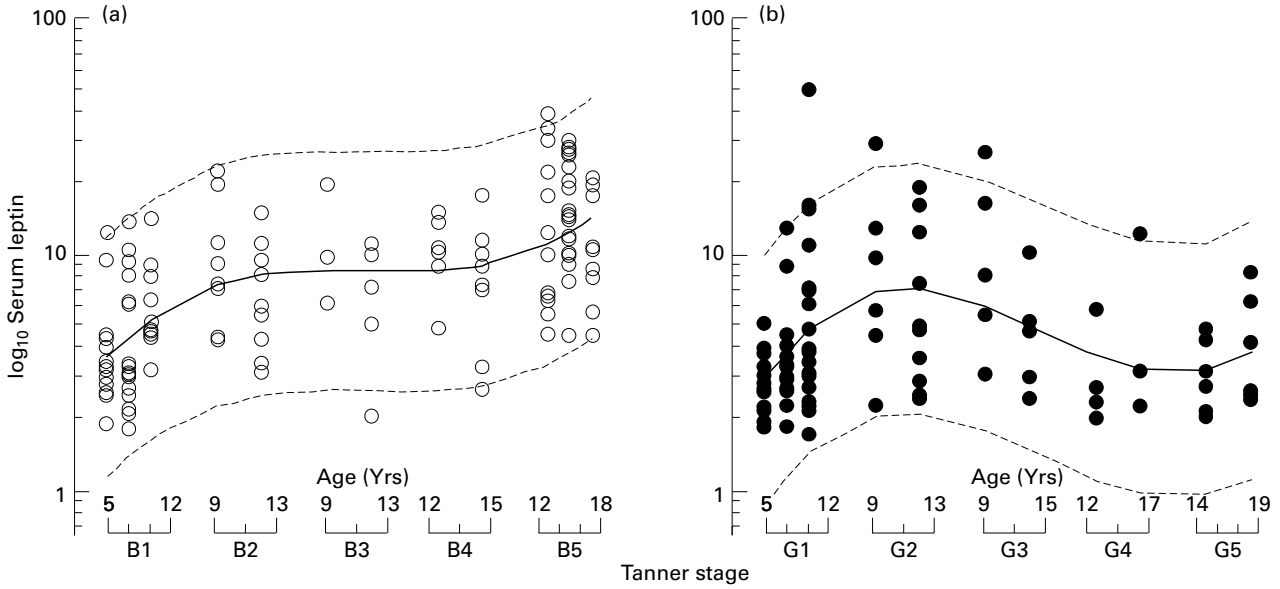

Fig. 2 Serum leptin concentration (ng/ml) (on a $\log_{10}$ scale) versus pubertal stage, ranked by age, in (a) the girls (○) and (b) the boys (●). The best fit line (solid line) for the girls was $\log_{10}[\text{leptin}] = 0{\cdot}029x^3 - 0{\cdot}275x^2 + 0{\cdot}858x + 0{\cdot}036$ ($r = 0{\cdot}6$, $P < 0{\cdot}001$), and for the boys was $\log_{10}[\text{leptin}] = 0{\cdot}047x^3 - 0{\cdot}47x^2 + 1{\cdot}337x - 0{\cdot}364$ ($r = 0{\cdot}44$, $P < 0{\cdot}001$), where x = pubertal stage, ranked by age. The broken lines represent 95% prediction intervals around the best fit line.

so with BMI (Maffei *et al*., 1995; Considine *et al*., 1996), indicating that the latter is a relatively poor index of fat mass. Nevertheless the relationship between leptin and BMI SDS reported here is similar to that found between leptin and BMI in adults. There is a wide range of leptin concentrations for any value of BMI in adults (Maffei *et al*., 1995; Considine *et al*., 1996; Ma *et al*., 1996), and this also applies to BMI SDS in children. Mechanisms controlling leptin production therefore may be predetermined for that individual, providing some evidence for a spectrum of leptin sensitivity.

Limited data on leptin through childhood is available: Hassink *et al*. (1996) reported that in obese and normal-weight children over puberty leptin levels, as expected, were

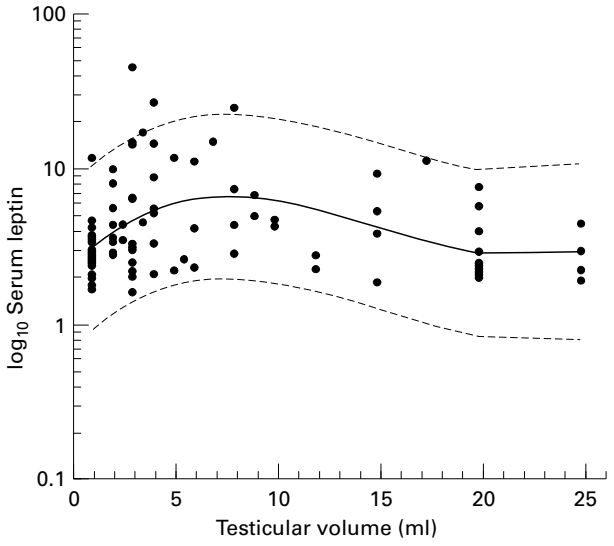

Fig. 3 Serum leptin concentration (ng/ml) (on a $\log_{10}$ scale) versus testicular volume (ml) in the boys. The best fit line (shown in bold) was $\log_{10}[\text{leptin}] = -0{\cdot}37x^3 + 0{\cdot}13x^2 - 0{\cdot}01x + 0{\cdot}002$ ($r = 0{\cdot}43$, $P < 0{\cdot}001$), where $x =$ testicular volume (ml). The broken lines represent 95% prediction intervals around the best fit line.

Table 2 Stepwise multiple linear regression: $\log_{10}$[Leptin] was entered as the dependent variable; (A) BMI SDS and age were entered as explanatory variables in girls; (B) BMI SDS, age and testicular volume (ml) were entered as explanatory variables in boys

Group	Variables in equation	Coefficient	*P* value	Model R^2
Females				
B1/2	1) BMI SDS	0·176	0·0000	(0·38)
	2) Age	0·066	0·0000	0·65
B3-5	1) BMI SDS	0·197	0·0000	(0·41)
	2) Age	0·031	0·0314	0·46
Males				
G1/G2	1) BMI SDS	0·159	0·0000	(0·31)
	2) Age	0·055	0·0000	0·51
G3-5	1) BMI SDS	0·195	0·0001	(0·35)
	2) TV	−0·025	0·0005	0·59

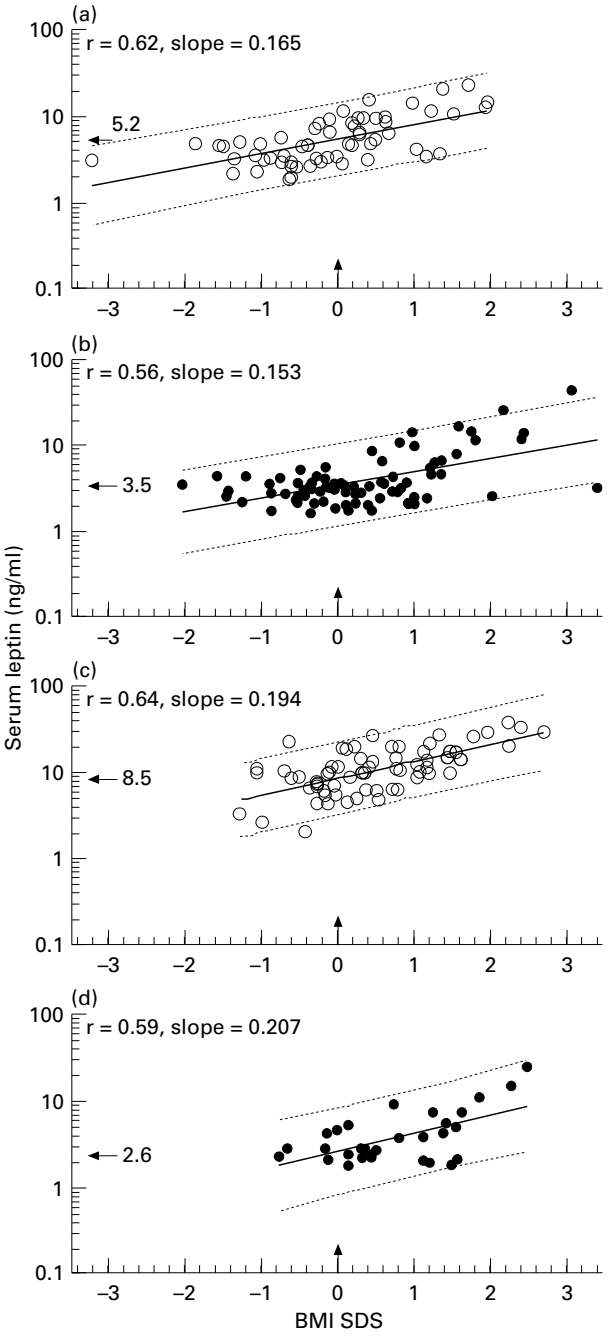

Fig. 4 Serum leptin concentration (ng/ml) (on a $\log_{10}$ scale) versus BMI SDS in (a) girls in B1–2 ($r = 0{\cdot}62$, slope $= 0{\cdot}165$). (b) boys in G1–2 ($r = 0{\cdot}56$, slope $= 0{\cdot}153$). (c) girls in B3–5 ($r = 0{\cdot}64$, slope $= 0{\cdot}194$) and (d) boys in G–5 ($r = 0{\cdot}59$, slope $= 0{\cdot}207$). (○ girls, ● boys). The r value for each linear regression is given above (all $P < 0{\cdot}001$). Chow tests indicated no difference in slope in all groups, but the intercept when BMI SDS $= 0$ (arrowed) was significantly higher in pubertal girls than prepubertal girls and pubertal boys.

significantly related to adiposity, but also to Tanner stage independent of adiposity. Advancement into puberty in the whole group was associated with a decline in leptin, suggested to be an index of peripubertal leptin resistance. However we have now demonstrated that, although pre- and post-pubertal boys have similar low concentrations of leptin, a significant elevation in leptin occurs over the prepubertal years into early puberty. In girls there is also a progressive increment in leptin through prepuberty into early puberty, a plateau in the mid stages but a late rise at the end of puberty. A significant difference between the sexes therefore only emerges in B4–5 compared to G4–5. A similar pattern of leptin concentrations over puberty has been reported by Blum *et al.* (1996) in a cross-sectional study of healthy children and adolescents, with concentrations in males rising with age and BMI only to Tanner stage 2, but continuing to rise throughout puberty in females.

In our study, over the prepubertal and pubertal years in both sexes, the correlations between leptin and BMI SDS were similar: a given change in BMI SDS induced a similar change in leptin (Fig. 4). Pubertal girls did have a higher leptin concentration for a given BMI SDS, but this may reflect the larger contribution that fat mass will make to their BMI. Although BMI SDS was the principal factor related to leptin throughout childhood and adolescence, an independent effect of age, accounting for up to 27% of leptin variability, was found in peripubertal children. BMI SDS alone was therefore insufficient to account for the variability in leptin.

In contrast in pubertal females, BMI SDS accounted for 41% of the variability in leptin, age only contributing a further 5%. However, in pubertal boys, testicular volume became an additional significant factor. The inverse relationship between testicular volume and leptin presumably reflects the increasing levels of testosterone over puberty. As testosterone is the main determinant of muscle mass, then the significant decrement in serum leptin concentration could suggest that not only adipose but also lean tissue may determine leptin, positively influenced by fat mass and negatively by skeletal muscle mass. It is noteworthy that the Ob receptor is expressed at low level in both murine and human muscle (Tartaglia *et al.*, 1995; Cioffi *et al.*, 1996).

The secretion of GH (Albertsson-Wikland *et al.*, 1994), its effector peptide IGF-I (Juul *et al.*, 1995), the GH dependent IGF-I carrier protein IGFBP3 (Juul *et al.*, 1995) and sex steroids increase dramatically through the pubertal years. However all tend to reach maximal levels in mid to late puberty. The significant early increments in leptin have already been achieved. However the gradual increment in gonadotrophin secretion from late childhood through to puberty (Bridges *et al.*, 1994) would parallel these early changes in leptin. There may therefore be an interaction between leptin and gonadotrophin secretion in the late childhood years. This may account for the

significant positive influence of age in Tanner stages 1 to 2. Evidence from murine experiments would support such an interaction: leptin administration can reverse the sterility in ob/ob mice (Chehab *et al*., 196), lead to the early induction of reproductive function in normal female mice (Chehab *et al*., 1997), increase LH secretion in female and FSH secretion in male ob/ob mice (Baresh *et al*., 1996) and partially restore LH and testosterone secretion in fasted male mice (Ahima *et al*., 1996). Leptin in the mouse therefore has potent central effects on the reproductive axis, which may also be relevant to humans. In addition expression of human B219/Ob receptor mRNA (Cioffi *et al*., 1996) in ovary, prostate and testis, albeit at low level in the latter tissue, would suggest that leptin could have a direct effect on gonadal tissue.

We therefore hypothesize that the progressive increment in leptin through childhood could facilitate progress into puberty. Clinical evidence to support this hypothesis would include the relatively early maturation of obese children, the suppression of puberty in female gymnasts and in anorexia (DeSouza & Metzeger, 1991) and in our study the attainment of higher leptin levels in girls earlier in childhood than boys, possibly contributing to the earlier onset of puberty in girls. In addition, it was proposed 22 years ago by Frisch & McArthur (1974) that fat mass must reach a threshold to achieve menstruation.

It will require longitudinal studies through childhood in parallel with studies in disordered puberty with or without therapeutic intervention to define exactly the role of leptin. Nevertheless it would appear that this newly identified satiety factor could play a part in the interaction between nutrition and sexual development in children.

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