Circulating Leptin in Normal Children and During the Dynamic Phase of Juvenile Obesity

Relation to Body Fatness, Energy Metabolism, Caloric Intake, and Sexual Dimorphism

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In 112 obese compared with 42 lean children, we found that serum leptin is elevated early in the evolution of childhood-onset obesity (28.4 ± 1.4 vs. 4.5 ± 0.4 ng/ml in lean children, $P < 0.0001$) and correlates with adiposity. Obese children also had higher serum leptin normalized to fat mass. Despite high serum leptin, obese children ingested 2–3 times more calories than did lean control subjects ($P < 0.0001$) and gained weight rapidly (10.2 ± 0.3 vs. 2.9 ± 0.1 kg/year in control subjects, $P < 0.0001$). Girls had higher leptin levels than did boys, in obese as well as in nonobese children, and showed a closer correlation between adiposity and serum leptin. Elevation of serum leptin was comparable before and after puberty in obese boys, but puberty further increased leptin levels in obese girls (36 ± 3 ng/ml), resulting in a clear sexual dimorphism with pubertal obese boys (22 ± 5 ng/ml, $P < 0.005$). In conclusion, increased serum leptin reflects but does not halt fat deposition in childhood obesity. After normalization to body adiposity, leptin was found to be increased independently by obesity status, female sex, and female sexual maturation. ***Diabetes*** **46:989–993, 1997**

Leptin, a protein coded by the *ob* gene, is released by adipose tissue in the circulation and regulates body weight in mice (1). Mutations in the *ob* gene in *ob/ob* mice (1), as well as mutations in the *ob* receptor gene in *db/db* mice (2–4), result in obesity. In obese mice, except in the *db/db*, injections of recombinant leptin decrease food intake, increase energy expenditure, and cause weight loss (5–7). Leptin is suspected to be a signal allowing the central nervous system, which contains leptin receptors (8), to perceive the amount of adipose tissue stored in the body. If leptin triggers processes through which the brain could control feeding behavior and energy metabolism (1) in humans, one expects leptin to limit the deposition of fat via a feedback regulatory sequence: storage of adipose tissue, increased leptin, decreased food intake, and increased energy expenditure.

Adults with stable obesity have an overexpression of the *ob* gene in their adipose tissue (9–12) and elevated serum leptin (10). At this stage of obesity evolution, elevated leptin could be part of the homeostatic response to limit further fat accumulation. Elevated leptin could also reflect primary resistance to leptin in obese patients, as postulated by Considine et al. (10). According to the available animal models, this resistance could be due to mutations in the *ob* gene (1) or the *ob* receptor gene (2) or to impairment at any level of leptin action.

Our model of dynamic obesity of early onset (13–17) offers an opportunity to study leptin levels at a time of active fat deposition and to distinguish effects of sex and pubertal maturation.

RESEARCH DESIGN AND METHODS

Subjects. We studied 42 lean children (22 boys and 20 girls, mean age ± SE, 11 ± 0.4 years) and 112 obese children and adolescents (50 boys and 62 girls, 11.5 ± 0.3 years). Obesity was defined as a body weight greater than 120% of the ideal value for age and height. Clinical and biological characteristics of the studied children are presented in Tables 1–3.

None of the children was taking any medication or had evidence of endocrine or metabolic disease other than obesity. All were studied while they were rapidly gaining weight, although at various rates (Tables 1 and 2). A blood sample was collected from each subject while fasting, and the serum was frozen at –80°C until analysis. Energy expenditure and glucose and lipid oxidation were measured in 32 obese and 20 lean control children. Food consumption preceding the study was evaluated by a dietitian using a questionnaire designed to quantify food intake over a whole week. Obese children had an alleged caloric consumption of 3,915 ± 207 kcal in girls and 4,170 ± 211 kcal in boys, values comparable to our previous estimations (14). Food intake was 1,780 ± 125 kcal/day ($P < 0.0001$) in lean children.

After the study, all obese children were placed on an hypocaloric diet: their weight decreased at an average rate of 790 ± 220 g/month the first 6 months of follow-up (range –3,600 to +1,400 g per month).

All protocols were approved by the institutional review board of Cochin Faculty, University René Descartes, and all subjects gave informed consent.

Leptin radioimmunoassay. Leptin was measured in plasma or serum samples by radioimmunoassay by means of reagents supplied by Linco Research (St. Louis, MO). The anti-leptin antiserum was raised in rabbits against highly purified recombinant human leptin. This antiserum did not cross-react with insulin, insulin-like growth factors I and II, glucagon, or interleukin 2 in doses of 10 µg/ml. The tracer was ^{125}I-labeled human leptin. It was stable for at least two months at 4°C, with relative binding at the 0 point decreasing only from 55 to 47%. Recombinant leptin standard or serum sample in duplicate was incubated in phosphate-buffered saline, pH 7.4, containing 0.1% Triton X-100 and 1% bovine serum albumin with anti-leptin antiserum and ^{125}I-labeled leptin (about 15,000 cpm in 100 µl) for 21 h at 4°C. Then the bound fraction was precipitated with a conjugate of polyethylene glycol and anti-rabbit antiserum and centrifuged. The supernatant was decanted and the pellet counted in a γ-counter. The perfor-

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TABLE 1
Clinical characteristics of the studied obese and nonobese children

	Obese children	Nonobese children	*P*
n	112	42	—
Age (years)	11.8 ± 0.3	10.7 ± 0.5	NS
Pubertal stage	12.2 ± 0.2	1.9 ± 0.3	NS
Body weight (kg)	68.6 ± 2.0	32.7 ± 1.7	<0.00001
Percent IBW	164.5 ± 2.1	98.7 ± 1.2	<0.00001
BMI (kg/m^2)	29.2 ± 0.4	16.7 ± 0.3	<0.00001
Fat mass (kg)	29.6 ± 1.0	10.5 ± 0.7	<0.00001
Fat mass (% body wt)	44.3 ± 0.8	31.1 ± 0.8	<0.00001
Weight gain past year (kg)	10.2 ± 0.3	2.9 ± 0.1	<0.00001

Pubertal stage was assessed according to Tanner. IBW, ideal body weight.

mance of the standard curve was as follows: ED_{80}: 1.48 ± 0.09 ng, ED_{50}: 6.07 ± 0.51 ng, ED_{20}: 31.7 ± 3.49 ng. The intra-assay coefficients of variation were 3.6 and 3.3% at the levels 1.4 and 14.2 ng/ml, respectively. The inter-assay coefficients of variation were 4.6 and 3.7 at the levels 1.4 and 14.2 ng/ml, respectively. The detection limit was computed according to Currie (16): the critical limit (95th percentile of the blank value) was 0.1 ng/ml, and the qualitative detection limit (level different from the blank at 95% significance) was 0.3 ng/ml. Recovery of human leptin added to a plasma pool was 105 ± 4% (mean ± SD). Serial dilutions of high-level samples strictly parallel the standard curve. Mean fasting level (± SD) was 3.6 ± 0.7 ng/ml in nonobese men and 8.8 ± 3.9 ng/ml in nonobese menstruating women.

Indirect calorimetry. At 8:30 A.M., after an overnight fast, continuous indirect calorimetry was performed using a Deltatrac Metabolic Monitor (Datex, Finland), and urinary nitrogen was measured, as reported in a previous study of obese children (14). During the 1-h experiment, the children rested comfortably on a bed and watched television or read.

Other analyses. Insulin was measured by radioimmunoassay using reagents provided by CIS Biointernational (Gif-sur-Yvette, France) (17). The standard was human insulin calibrated to the International Preparation 66/304. Serum glucose was measured using glucose oxidase (Yellow Springs Instruments, Yellow Springs, OH). Plasma free fatty acids were measured using a colorimetric method (18).

The percentage of fat-free mass and body fat was determined by bioelectric impedance analysis (Eugedia, France), a method that has recently been validated in children (19). Although variability in within-subject predictive accuracy may compromise delineation of small changes within individuals in a group, bioelectric impedance is considered reliable for comparison between groups with major differences in body composition (20). Oral glucose tolerance tests were performed by giving each subject 40 g/m^2 glucose after overnight fasting. Plasma glucose and insulin levels were measured in the fasting state and 120 min after glucose ingestion.

Statistical analysis. Linear regression analyses were performed relating measures of body composition, obesity history, and indexes of adiposity to serum leptin concentration. Between-group univariate analyses (obese vs. nonobese, female vs. male) were made by one-way analysis of variance with regard to serum leptin concentrations and variables such as BMI, fat mass (expressed in kg and as percent body weight), age, etc., using analysis of variance for quantitative variables. Correlations between leptin and BMI and fat mass (kg and percent) were calculated using Spearman's correlation coefficient in obese and lean patients. All significant independent variables were then examined for interactions between variables by stepwise multiple regression analysis to leptin as dependent variable and sex, BMI, fat mass, and age. Between-group analyses to determine whether sex or obesity status altered the relationship between leptin and measures of body composition were made by multiple analysis of covariance using the grouping variable as a covariate. For all statistical tests, significance was defined as $P < 0.05$. Computations were made with BMDP statistical software.

RESULTS

Clinical and metabolic characteristics of the studied children are presented in Tables 1–3. The indexes of body fatness used in this study, BMI and fat mass (kg and percent body weight), were closely correlated in obese and nonobese children, with correlation coefficients ranging from 0.64 to 0.84.

The mean serum leptin concentration in the 112 obese children was 28.4 ± 1.4 ng/ml compared with 4.5 ± 0.4 ng/ml in the 42 nonobese children ($P < 0.0001$). Univariate analysis in obese children showed that serum leptin correlated with BMI in obese ($r = 0.63$, $P < 0.001$) and nonobese children ($r = 0.60$, $P < 0.001$) (Fig. 1). Serum leptin also correlated in obese and nonobese children with fat mass expressed in

TABLE 2
Clinical characteristics of the obese children according to gender and pubertal status

	Obese boys			Obese girls		
	All	Before puberty	During puberty	All	Before puberty	During puberty
n	50	23	27	62	27	35
Age (years)	12 ± 0.4	9.6 ± 0.5	14 ± 0.3	10.8 ± 0.4	8.7 ± 0.3	12.6 ± 0.5
Pubertal stage	2.2 ± 0.2	1	2.8 ± 0.2	2.2 ± 0.2	1	3.2 ± 0.2
Duration of obesity (years)	7.5 ± 0.5	6.6 ± 0.6	8.7 ± 0.7	7 ± 0.5	5.3 ± 0.4	8.9 ± 0.7
Body weight (kg)	71 ± 3	57 ± 4	84 ± 3	63 ± 3	49 ± 2	76 ± 5
Percent IBW	160 ± 2	159 ± 3	160 ± 3	167 ± 4	165 ± 4	168 ± 7
BMI (kg/m^2)	29.2 ± 1	27.4 ± 1	31.1 ± 1	29.2 ± 1	27.1 ± 1	31.3 ± 2
Fat mass (kg)	30 ± 1	24 ± 1	37 ± 1	28.5 ± 1	21.5 ± 1	36 ± 2
Fat mass (% body wt)	43 ± 1	42 ± 1	44 ± 1	46 ± 8*	44 ± 1	48 ± 1
Weight gain last year (kg)	10.7 ± 0.7	10.1 ± 0.9	11.5 ± 0.9	9.6 ± 0.4	8.6 ± 0.4	10.8 ± 0.6

Pubertal stage was assessed according to Tanner. *$P < 0.02$ vs. obese boys. IBW, ideal body weight.

TABLE 3
Circulating leptin, glucose, and insulin concentrations in the obese children according to sex and pubertal status

	Obese boys			Obese girls		
	All	Before puberty	During puberty	All	Before puberty	During puberty
n	50	23	27	62	27	35
Fasting plasma glucose (mmol/l)	4.1 ± 0.1	4 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	4.4 ± 0.1
Fasting plasma insulin (μU/ml)	14 ± 1	10 ± 1	18 ± 2	18 ± 2	15 ± 4	20 ± 3
Glucose at 120 min OGTT (mmol/l)	5.2 ± 0.4	4.9 ± 0.2	5.7 ± 0.2	5.6 ± 0.2	5.4 ± 0.3	5.8 ± 0.3
Insulin at 120 min OGTT (mU/ml)	69 ± 8	54 ± 7	85 ± 10	83 ± 9	67 ± 11	96 ± 10
Free fatty acid (mmol/l)	0.58 ± 0.04	0.62 ± 0.08	0.54 ± 0.04	0.64 ± 0.04	0.69 ± 0.06	0.62 ± 0.04
Basal energy expenditure (kcal/min)	1.16 ± 0.04	1.02 ± 0.04	1.31 ± 0.03	1 ± 0.03	0.91 ± 0.02	1.12 ± 0.04
Lipid oxidation ($mg \cdot m^{-2} \cdot min^{-1}$)	33 ± 2	31 ± 4	35 ± 2	28 ± 2	29 ± 3	26 ± 2
Glucose oxidation ($mg \cdot m^{-2} \cdot min^{-1}$)	70 ± 4	78 ± 5	61 ± 4	76 ± 4	80 ± 4	71 ± 5
Serum leptin (ng/ml)	25 ± 3	24 ± 3	22 ± 5	32 ± 4*	28 ± 6	36 ± 3†‡
Serum leptin per unit fat mass ($ng \cdot ml^{-1} \cdot kg^{-1}$ fat mass)	0.83 ± 0.06	0.96 ± 0.09	0.68 ± 0.13	1.11 ± 0.07*	1.14 ± 0.08	1.08 ± 0.09†

*$P < 0.005$ vs. boys; †$P < 0.005$ vs. pubertal boys; ‡$P < 0.01$ vs. prepubertal girls.

kilograms ($P < 0.001$) or percent body weight ($P < 0.001$). Correlations in obese children were comparable with that described in adults with stable obesity (10). When expressed per unit fat mass, serum leptin was found to be higher in obese than in lean children (1.0 ± 0.05 vs. 0.46 ± 0.03 ng/ml per kg fat mass, $P < 0.001$) and in girls than in boys, both in obese (Table 3) and nonobese children (0.52 ± 0.04 vs. 0.38 ± 0.04 ng/ml per kg fat mass, $P < 0.02$).

Since absolute fat mass was closely dependent on age and obesity duration ($r = 0.85$ and 0.87, respectively, $P < 0.001$), serum leptin correlated with these parameters. Percent fat mass did not correlate with obesity duration and was weakly related to age ($r = 0.38$, $P < 0.002$). Serum leptin correlated weakly, but positively, with the rate of weight gain the year preceding the study ($r = 0.38$, $P < 0.002$), suggesting that fat deposition was not restricted by high leptin concentrations. Neither was leptin predictive of future weight loss during the first 6 months following the prescription of a standard hypocaloric diet.

After adjustment for age, pubertal status, and adiposity indexes, serum leptin was higher in obese girls (32 ± 4 ng/ml) than in obese boys (25 ± 3 ng/ml, $P < 0.0001$). This difference was not related to the slightly greater fat content in obese girls than in obese boys, and the difference before and after puberty was not due to changes in fat content (Table 2). Serum leptin was comparable in boys before and after puberty, despite a slightly higher fat content in the latter group (Tables 2 and 3). In contrast, obese girls had a 30% rise in serum leptin concentrations during puberty.

Stepwise multiple regression analysis found that serum leptin was significantly correlated with BMI and sex in obese and nonobese children (Table 4).

When adjusted for BMI using covariance analysis, serum leptin was also significantly higher in lean girls than in lean boys ($P < 0.0001$). Similar relationships were observed with the other indexes of adiposity. Adjusted for sex using mul-

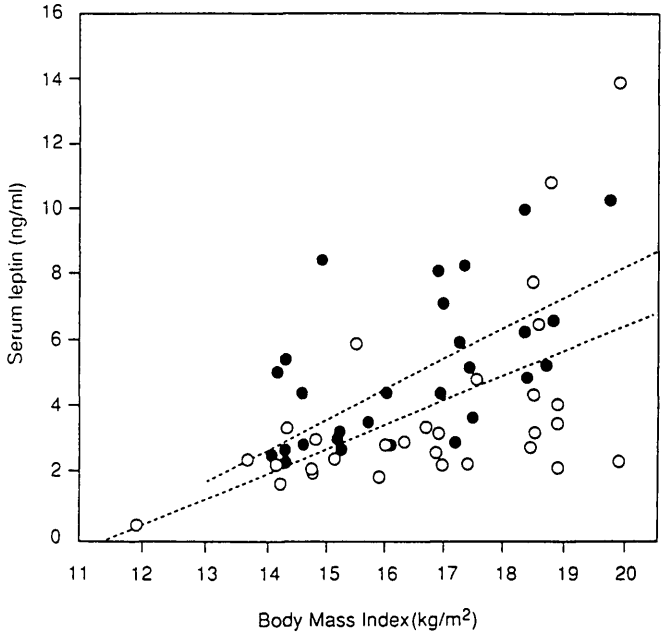

FIG. 1. Relationship between BMI and serum leptin concentration in nonobese children. The equations describing the regressions are: $y = 1.0x \pm 11.5$ ($r = 0.74$, $P < 0.001$) in girls (●) and $y = 0.99 - 13$ ($r = 0.54$, $P < 0.001$) in boys (○).

TABLE 4
Summary table for multiple stepwise regression analyses in the studied children

	Step	Variable entered	Number of variables included	Multiple r
Obese	1	BMI	1	0.63
	2	Sex	2	0.67
Nonobese	1	BMI	1	0.60
	2	Sex	2	0.66

tiple analysis of covariance, the relationship between leptin and BMI was different in obese and nonobese children (Figs. 1 and 2).

In the obese children, serum leptin correlated with fasting insulin ($r = 0.45$, $P < 0.002$) but not with fasting glucose or with insulin, glucose, or glucose-to-insulin ratio at 120 min of the oral glucose tolerance test. Leptin did not correlate with plasma free fatty acid or with basal rates of energy expenditure or carbohydrate or lipid oxidation (Table 2). Basal energy expenditure was closely related to lean body mass in the obese children ($y = 17x + 890$, $r = 0.77$, $P < 0.0001$).

A subset of three obese patients (one girl, two boys), all of whom were prepubertal, had surprisingly low leptin concentrations with respect to their body fatness: they were consistently lying clearly outside of the 95% confidence interval of the regression of serum leptin with BMI, and fat mass in kilograms or percent body weight (Fig. 2). This observation of hypoleptinemic obese children is comparable with that of the 5% of obese adults who have decreased leptin production relative to the amount of body fat (10). In contrast, another group of four patients (three girls, one boy) had remarkably high leptin concentrations with respect to the leptin-BMI relationship (Fig. 2). No relevant phenotypic differences were detected in these children compared with the rest of the obese cohort.

DISCUSSION

The present study shows that increased leptin levels are an early feature of juvenile obesity. The relationship between serum leptin and body fat content in our obese children resembles that observed in adults with long-standing, stable obesity (10). Increased leptin levels in obese adults were interpreted as indicating leptin resistance. Our data, obtained at a stage when obesity is constituting rapidly, support this interpretation because leptin did not appear to act as a satiety regulator in the obese children, who kept an exaggerated appetite and a remarkably high caloric intake despite hyperleptinemia.

During the dynamic phase of fat deposition, our obese children have a slight increase of their basal energy expenditure, as observed previously (14). The lack of relationship between increased serum leptin and increased energy expenditure, however, does not support an active signaling role for leptin to regulate energy metabolism in the obese children. In fact, the slightly higher basal energy expenditure in the obese children appears to simply reflect their increased lean body mass.

Not only was leptin elevation unable to inhibit rapid fat deposition in young obese patients, it correlated positively with weight gain. For example, the obese children with leptin levels above 40 ng/ml were gaining weight at a rate of 15.3 ± 2 kg/year, 3–4 times the normal rate.

Pharmacological doses of leptin decrease food intake, increase energy expenditure, and cause weight loss in mice that have mutations of the *ob* gene (5), diet-induced obesity (6), or normal weight (7). Our data in obese juvenile patients suggest that the use of leptin as a treatment aiming to decrease body weight would certainly require much higher circulating leptin levels than those observed here or, more logically, a drug able to improve leptin action.

Obese children appear to produce twice as much leptin per unit fat mass than do lean control children, and obese and nonobese girls produce 37% more than boys through mechanisms that have yet to be elucidated: besides the postulated resistance to leptin, differences in fat mass composition, regulation of leptin production (sex hormones, glucocorticoids), or leptin clearance could play a role.

Our observation that obese girls had higher leptin levels than boys is consistent with the sex difference reported for obesity mRNA values in adipocytes from obese adults (11). A comparable sex-related difference was also found in nonobese children. Such sexual dimorphism is not explained

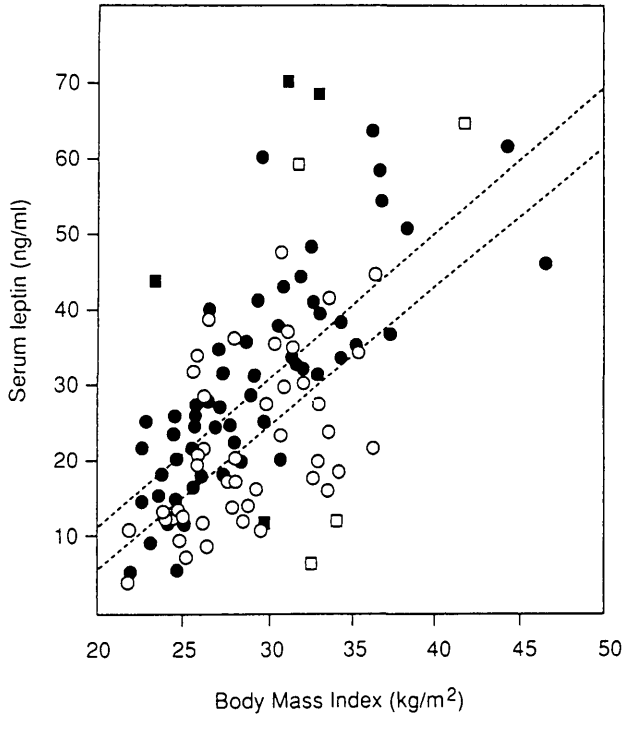

FIG. 2. Relationship between BMI and serum leptin concentration in obese children. The equations describing the regressions are: $y = 1.9x \pm 27$ ($r = 0.80$, $P < 0.001$) in girls (●) and $y = 1.7x - 27$ ($r = 0.54$, $P < 0.001$) in boys (○). Squares indicate obese patients lying outside of the 95% confidence interval of the regression.

by quantitative differences in overall body fatness and became apparent only at time of sexual maturation, suggesting that it could be associated with the hormonal changes taking place at puberty in females. Higher leptin in female adolescents was not associated with higher insulin levels nor with differences in other parameters of energy metabolism. The sexual dimorphism of circulating leptin levels could reflect a physiological role in the regulation of reproduction in humans. In mice, while the *ob/ob* males are normally fertile, administration of leptin to infertile female *ob/ob* mice is needed to restore fertility (21). It is therefore possible that leptin plays a role in the female gonadostat as a signaling hormone reflecting the amount of fat stores at the hypothalamic-pituitary level or directly at the ovarian level (22). The ability to mobilize lipid fuels from fat stores is known to be of major importance for energy metabolism during pregnancy, particularly in fasting periods. Our hypothesis is that quantitative and possibly functional changes in adipose tissue of girls entering puberty may physiologically increase leptin to levels that allow the hypothalamic-pituitary-gonadal axis to complete sexual maturation and prepare for pregnancy. The sexual dimorphism for circulating leptin levels observed in lean girls and boys is consistent with these metabolic considerations. If this hypothesis is true, the lack of premature puberty despite hyperleptinemia in massively obese girls could be taken as an additional index of central leptin resistance.

In summary, our findings are that *1*) serum leptin is largely increased in the dynamic phase of childhood-onset obesity and does not appear to halt fat deposition and *2*) female sex and female puberty are both associated with increased serum leptin, producing a sexual dimorphism of leptinemia in obese as well as in lean children.

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