Plasma Leptin in Children: Relationship to Puberty, Gender, Body Composition, Insulin Sensitivity, and Energy Expenditure

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Leptin has been demonstrated to reflect body fat mass (FM) in humans, but the regulation of leptin levels during childhood growth and development is poorly understood. We studied the relation between plasma leptin, fasting insulin, insulin sensitivity, and resting energy expenditure in 22 healthy prepubertal children and 27 adolescents. Body composition was assessed by the $H_2{}^{18}O$-dilution principle, insulin sensitivity by a hyperinsulinemic (40 mU/m^2/min)-euglycemic clamp, and energy expenditure by indirect calorimetry. Plasma leptin in prepubertal children (9.3 ± 2.0 ng/mL) was not different from that in pubertal adolescents (10.9 ± 2.2 ng/mL). Plasma leptin correlated with FM (r = .77, P < .001). There were no gender differences in leptin after controlling for FM differences. In prepubertal and pubertal subjects, plasma leptin correlated with fasting insulin independently of FM (r = .60, P < .001), but did not correlate with insulin sensitivity independently of body fat content. Leptin showed no relationship to resting energy expenditure after adjusting for body composition. The present cross-sectional evaluation of normal children shows that (1) plasma leptin reflects body fat content, (2) leptin concentrations are similar between prepubertal children and pubertal adolescents, (3) there are no gender differences in leptin independent of adiposity, and (4) leptin correlates with fasting insulin but not with insulin sensitivity. Contrary to animal data, our cross-sectional results in healthy children do not suggest a role for leptin in puberty or a female-related leptin resistance as reported in adults. It remains to be determined at which stage of human development the sexual dimorphism in leptin becomes evident.

SINCE THE RECENT CLONING of the gene responsible for hyperphagia and obesity in genetically obese (ob/ob) mice,[1] the scientific literature has overflowed with information regarding leptin and its regulation both in animals and in humans.[2] The ob gene is expressed in adipose tissue, and its protein product, leptin, has been shown repeatedly to be an index of the amount of triglyceride stored in adipose tissue depots.[3-8] It has also been proposed that under non–steady-state energy balance conditions, leptin is acutely regulated. In states of overfeeding, it is upregulated to decrease food intake through central nervous system mechanisms and to increase energy expenditure to maintain original steady-state energy balance and fat-store balance.[2] Additional observations include the permissive role of leptin in the reproductive system[9-12] and its positive relationship with insulinemia.[13] However, little is known about the physiological relationships of leptin in children during growth and development,[6] a state of dynamic change in body composition, energy balance, and insulin sensitivity.[14,15] We therefore aimed to test the relationships of leptin to body composition, puberty, and insulin sensitivity in healthy prepubertal children and pubertal adolescents.

SUBJECTS AND METHODS

Overnight-fasting plasma leptin levels were analyzed in 22 healthy prepubertal children and 27 pubertal adolescents studied in the General Clinical Research Center at Children's Hospital of Pittsburgh (Table 1). Some of these subjects have been reported previously.[16] All studies were approved by the Human Rights Committee of the Hospital, and informed written assent and consent were obtained from the participants and their guardians. All children were in good health as assessed by medical history, physical examination, and routine hematological and biochemical tests. Pubertal development was assessed by physical examination according to the criteria of Tanner,[14] and was confirmed by measurement of plasma testosterone in males and estradiol in females. There were 17 subjects in Tanner stage III puberty, nine females and eight males, and 10 in Tanner stage IV, five females and five males.

Experiments were performed in the postabsorptive state after a 12-hour overnight fast. Body composition was assessed with the use of $H_2{}^{18}O$ as described by our group previously.[16,17] In vivo insulin sensitivity was evaluated by a 40-mU/m^2/min hyperinsulinemic-euglycemic clamp.[16] Resting energy expenditure and postabsorptive glucose and fat oxidation were evaluated by 30-minute continuous indirect calorimetry using a ventilated-hood system (Deltatrac Metabolic Monitor; Sensormedics, Anaheim, CA) with measurement of CO_2 production and O_2 consumption.[15] Plasma samples used for leptin determination were stored at −20°C and were not thawed until the leptin assay was performed. Leptin was determined in duplicate by radioimmunoassay (RIA) using a human leptin RIA kit (Linco, St Louis, MO).[18] The within-assay coefficient of variation was 3.4% to 8.3%, and between-assay variation was 3.6% to 6.2%. Plasma insulin was determined by RIA.[15] The insulin-like growth factor-I (IGF-I) level was measured by RIA after acid-ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA), and testosterone by double-antibody RIA (ICN Biomedical, Costa Mesa, CA).

Data are presented as the mean ± SE. The Student t test was used to compare group means. Univariate relationships were evaluated with least-squares regression analysis. Multiple regression analysis was used to assess multivariate relationships. The goodness-of-fit of the model was measured by R^2, the coefficient of determination, which is the square of the multiple correlation coefficient between the dependent and independent variables.[19,20] Statistical significance is implied by P less than .05.

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Table 1. Physical and Biochemical Characteristics of the Study Subjects

Characteristic	Prepubertal (n = 22)	Pubertal (n = 27)	P
Age (yr)	10.5 ± 0.2	13.5 ± 0.2	<.0001
Sex (male/female)	13/9	13/14	NS
BMI (kg/m²)	18.7 ± 0.7	20.8 ± 0.6	.02
FFM (kg)	32.8 ± 1.5	45.8 ± 1.6	<.0001
FM (kg)	5.4 ± 1.0	8.4 ± 1.1	.06
Body fat (%)	12.3 ± 1.9	15.1 ± 1.8	NS
Leptin (ng/mL)	9.3 ± 2.0	10.9 ± 2.2	NS
IGF-I (ng/mL)	266 ± 23	522 ± 30	<.0001
Testosterone (ng/dL)	27 ± 4	401 ± 20	<.0001
Estradiol (pg/mL)	7 ± 1	25 ± 3	.03

NOTE. Results are the mean ± SE.
Abbreviations: FFM, fat-free mass; NS, nonsignificant.

RESULTS

Leptin Concentrations in Prepubertal Versus Pubertal Subjects

Plasma leptin in prepubertal children (9.3 ± 2.0 ng/mL) was not different from the value in pubertal adolescents (10.9 ± 2.2 ng/mL). Leptin correlated with fat mass ([FM] total group, $r = .77$, $P < .001$; prepubertal, $r = .81$, $P = <.001$; pubertal, $r = .76$, $P = <.001$) and percent body fat (total group, $r = .66$, $P = <.001$; prepubertal, $r = .71$, $P = <.001$; pubertal, $r = .63$, $P = <.001$). The regression equation for leptin and FM in prepubertal children was leptin = 1.5396 (FM) + 1.0235, and in pubertal adolescents, leptin = 1.4795 (FM) −1.5372. The slopes and intercepts between the two regression lines were not significantly different, indicating parallelism (Fig 1).

Leptin and Gender

In the prepubertal group, FM was not different between males and females (5.5 ± 1.7 and 5.1 ± 1.0 kg, respectively). Similarly, leptin was not different between males (10.2 ± 3.2 ng/mL) and females (8.0 ± 1.5 ng/mL). In the pubertal group, FM was higher in females (11.6 ± 1.3 *v* 4.9 ± 1.3 kg, $P = .001$). Likewise, leptin concentrations were higher in females (17.3 ± 3.5 *v* 4.0 ± 0.5 ng/mL, $P = .002$). After adjusting for FM differences, there was no significant gender difference in leptin levels. When the two groups were analyzed together in a multiple regression analysis with leptin as the dependent variable and FM and gender as independent variables, the only significant predictor of leptin was FM. The R^2 for the complete model (gender + FM) was .589, and the R^2 without gender in the model was .586 ($P < .0001$).

Leptin and Insulin Sensitivity

Plasma leptin concentrations correlated positively with fasting insulin levels (total group, $r = .79$, $P = <.001$; prepubertal, $r = .84$, $P < 0.001$; pubertal, $r = .83$, $P < 0.001$) and inversely with insulin-stimulated glucose disposal (total group, $r = -.59$, $P < .001$; prepubertal, $r = -.63$, $P = .001$; pubertal, $r = -.69$, $P < .001$). However, in a multiple regression analysis with FM and leptin as independent variables and insulin-stimulated glucose disposal as the dependent variable, the only determinant of glucose disposal was FM, explaining 57% of the variance ($P < .0001$). After controlling for FM, the correlation between leptin and fasting insulin remained significant ($r = .60$, $P < .001$).

Leptin, Energy Expenditure, and Substrate Oxidation

Plasma leptin levels correlated inversely with resting energy expenditure ($r = -.51$, $P < .001$), basal fat oxidation ($r = -.31$, $P = .014$), and glucose oxidation ($r = -.29$, $P = .02$). However, partial correlations adjusting for FM showed that there was no significant relationship between leptin and energy expenditure, as well as leptin and fat oxidation. In a multiple regression analysis with fasting glucose oxidation as the dependent variable and leptin and FM as independent variables, both leptin and FM together explained about 40% of the variance in basal glucose oxidation ($R^2 = .39$, $P < .0001$). The partial correlation coefficient (r) for FM was −.86 ($P < .0001$), and for leptin, .37 ($P = .04$).

DISCUSSION

Despite the rapidly expanding leptin literature, information in the pediatric age group remains scanty.[6,21] Our study of normal children demonstrates that (1) plasma leptin reflects body fat stores, (2) there do not appear to be gender differences in leptin independent of adiposity, (3) leptin concentrations do not seem to vary with puberty independent of adiposity, (4) leptin

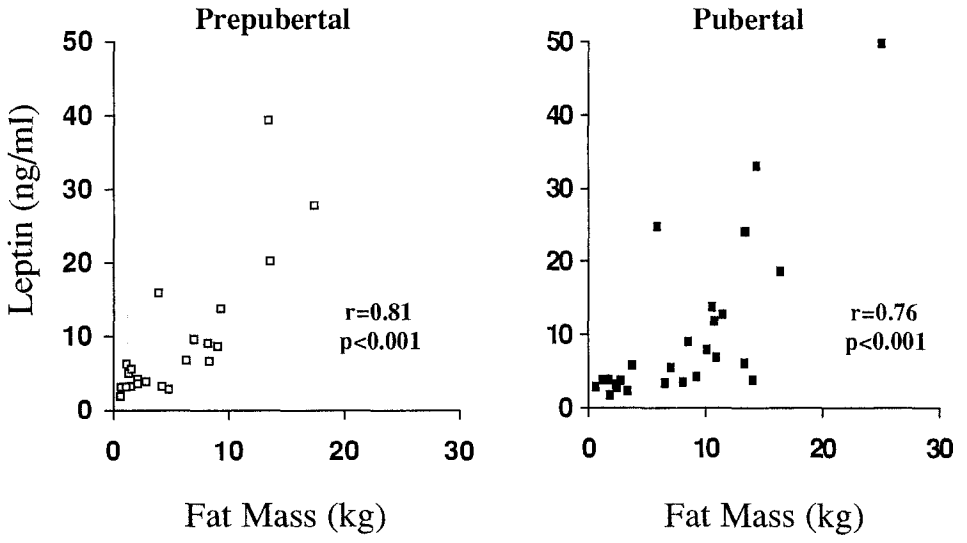

Fig 1. Correlation of leptin and FM in prepubertal children and pubertal adolescents.

correlates with fasting insulin but not insulin sensitivity independent of adiposity, and (5) leptin shows no relationship to resting energy expenditure independent of FM.

Several studies in adults have demonstrated a positive relationship between leptin and body fat using a variety of adiposity measures, including the body mass index (BMI),[5,22,23] skinfold thickness,[7] bioelectrical impedance,[3,4] hydrodensitometry,[24] and dual-energy x-ray absorptiometry.[8,25] These investigations have included healthy normal-weight and obese men and women[3,5,7,8,22] and patients with diabetes, anorexia, and polycystic ovary syndrome.[4,5,21,23] Studies in children have yielded conflicting results, showing a positive correlation between leptin and the BMI in obese and non-obese children,[6] or no such relationship.[21] In the present study, careful assessment of body composition reveals that leptin concentrations correlate positively with total FM and percent body fat in normal children. However, contrary to previous findings,[6] our results do not show gender-related differences in children. The most likely reason for these contrasting findings compared with previous observations is that the BMI in the growing child, even though epidemiologically acceptable, does not provide a sensitive measure of adiposity. The advantage of the current investigation is the assessment of body composition with a reliable method that provides information on FM and fat-free mass. In prepubertal children, males and females had comparable FM and leptin levels. In pubertal adolescents, after adjusting for the higher FM in females, leptin levels were not different between the genders. It could be argued, though, that the $H_2{}^{18}O$ method for calculating body composition during puberty may not be the gold standard, because of puberty-associated changes in hydration of the lean body mass. Since there appears to be a sexual dimorphism in the relationship between circulating leptin concentrations and adiposity in adults,[7,22,24,25] whereas none exists in prepubertal children, it remains to be determined at which stage of puberty this dimorphism becomes evident. Longitudinal studies are needed.

Studies in mice suggest that leptin may have a role in triggering the onset of reproductive function.[9-11] In ob/ob mice that are genetically deficient in leptin, exogenous leptin results in weight loss and restoration of fertility.[9,10] In normal mice, exogenous leptin results in subnormal weight gain and advanced estrus.[11] However, a recent study in Sprague-Dawley rats suggests that leptin is not the primary signal initiating puberty, but instead is a permissive factor that allows puberty to proceed.[12] In contrast to the latter proposal, the only human study shows that leptin increases transiently just before the onset of puberty in boys, but decreases to baseline values after initiation of puberty.[21] Our cross-sectional study of healthy normal children negates any difference in leptin levels between prepubertal and pubertal subjects. However, longitudinal studies may be more sensitive in detecting within-subject changes in leptin with progression of puberty. Moreover, the slope and intercept of the regression between leptin and FM are not significantly different in prepubertal children versus adolescents, suggesting that the leptin versus FM relationship is not affected by pubertal status. A study in highly trained athletes with and without menstrual cyclicity demonstrates that leptin levels are equally reduced in the two groups irrespective of menstrual status.[26] Additional longitudinal studies with careful assessment of body compositional changes and pubertal hormonal changes are needed to better define the role of leptin in human reproduction.

In the rodent model, hyperinsulinemia is associated with overexpression of ob mRNA, and insulin has been reported to directly stimulate leptin mRNA in rat adipocytes.[27,28] Therefore, few studies have addressed the interrelationship between leptin and insulin in humans. These studies demonstrated that obesity and hyperinsulinemia are associated with elevated leptin levels.[29] Fasting insulin correlated with plasma leptin in normal postmenopausal women but not in women with impaired glucose tolerance, and leptin did not correlate with insulin sensitivity independent of body fat content.[30] In dexamethasone-treated healthy women, the correlation between insulin sensitivity and leptin levels pretreatment and posttreatment, was dependent on the BMI.[31] Our study in normal children, in agreement with the adult studies, demonstrates that the correlation between leptin and insulin sensitivity is secondary to adiposity and disappears once differences in FM are adjusted for. On the other hand, despite controlling for FM, the positive correlation between fasting insulin and leptin remains significant. Whether this is secondary to a direct effect of leptin on β-cell secretory function as proposed previously[30] or an effect of insulin on leptin remains to be elucidated. Data in humans as to whether insulin acutely regulates plasma leptin remain confusing. In some studies, no changes in leptin levels were detected after 3 to 5 hours of supraphysiologic hyperinsulinemia,[32,33] whereas in others, hyperinsulinemia increased leptin levels.[22,34] It remains to be determined if the degree of insulinemia or the duration is the factor responsible for the different findings.

Because leptin appears to be involved in energy homeostasis by conserving energy during periods of food deprivation and preventing obesity during periods of energy surplus, we investigated the relationship of leptin to resting energy expenditure during childhood, which is a constantly changing state of energy balance. Our results show no relationship between leptin and energy expenditure after controlling for body composition. This holds true whether energy expenditure data are expressed as kilocalories per 24 hours, or per unit of body weight (kcal/24 h/kg), or per unit of metabolically active fat-free mass (kcal/24 h/kg FFM). Similar to our findings, resting energy expenditure did not correlate with leptin levels in obese postmenopausal white women.[35]

In summary, the present cross-sectional study in normal children demonstrates that leptin levels most strongly reflect FM, with no puberty- or gender-related differences. Leptin levels do not correlate with resting energy expenditure or with insulin sensitivity, but do correlate positively with fasting insulin. Further investigations are needed to elucidate the nature of the insulin and leptin interaction in childhood and the chronology of sexual dimorphism in the leptin versus FM interaction between the genders.

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