Relation of Serum Leptin Levels to Lipid Profile in Healthy Children

Emmanuel Kavazarakis, Maria Moustaki, Dimitrios Gourgiotis, Antonios Drakatos, Apostolos Bossios, Petros M. Zeis, Androniki Xatzidimoula, and Themistocles Karpathios

The association of leptin with body fat concentration is well established. There is also experimental evidence of a direct effect of leptin on lipid metabolism. The aim of this study was to evaluate whether leptin levels are related to the corresponding serum lipid levels independently of body fat mass. The study population consisted of 294 phenotypically healthy school children aged 6 to 12 years. Age, sex, body weight, height, Tanner stage, and triceps skinfold thickness were recorded for all participating subjects. A blood sample was drawn in the morning after a 12-hour fast, and serum total, high-density lipoprotein (HDL), and low-density lipoprotein cholesterol; triglyceride; and leptin levels were determined. Multiple regression analysis showed that triglyceride values were positively correlated with the ln($\log_e$)-transformed leptin levels ($\beta = .01$, $P < .001$), whereas HDL levels were inversely associated with lnleptin values ($\beta = -.06$, $P = .05$) after controlling for age, sex, Tanner stage, and body mass index when each of the lipid parameters was tested separately in the regression model. However, the introduction of both triglycerides and HDL values in the same model eliminated the significance of association of HDL with lnleptin, and the positive relationship of triglycerides with lnleptin remained significant. Our results indicate that triglycerides are independently associated with leptin levels after controlling for any known confounder.

LEPTIN IS a recently identified protein with multiple, not fully clarified metabolic and neuroendocrine effects.[1,2] It was first described as an adipocyte-derived signaling factor which that was involved in the regulation of body weight and energy expenditure.[3] A positive correlation of circulating leptin with body fat mass either as directly measured by dual-energy x-ray or as reflected by body mass index (BMI) or skinfold thickness has been established in children and adults.[4-10] Thus the findings from different studies pointed to the same direction that circulating leptin increases with increasing adiposity.[4-7,9] Although a variation of leptin concentration depending on age does exist,[6,10] its relationship with body fat is evident throughout life, as was revealed by several studies in children, adolescents, and adults.[4-9] Despite the increasing number of studies in support of this correlation, only a few studies with rather controversial results[6,11-13] explore the possible association of leptin levels with serum lipid profile. There is also limited evidence[14,15] that elevated blood cholesterol levels in young children may precede body fat accumulation, resulting, at a second stage, in obesity.

These observations make plausible the hypothesis that there might be another factor underlying the development of both lipid abnormalities and obesity. Leptin, in addition to its role in the control of body weight homoeostasis, has important roles in multiple metabolic pathways.[1,16,17] There is enough evidence, derived from experimental studies, that leptin administration induces important changes in accumulation of triglycerides (TGs) in tissues.[18] Therefore, it is conceivable that leptin may be related not only to body fat concentration and tissue lipid metabolism, but also to lipid circulating levels. The aim of the present study was to investigate whether there is any relationship between serum lipid profile and circulating leptin levels, taking into account the effect of known regulators of leptin (age, sex, and body fat) on this relationship.

SUBJECTS AND METHODS

The study population consisted of 294 healthy children (154 boys and 140 girls) from 4 schools in the greater Athens area. The children were examined by a pediatrician participating in the study, and the following data were recorded: sex, age, height, weight, pubertal stage (following Tanner classification), and triceps skinfold thickness. Standing height was measured with a precision of 0.5 cm using a portable standard stadiometer. Weight was measured with children wearing light clothing on a digital scale. Triceps skinfold thickness was measured on the left side of the body. Two measurements were performed with a precision of 1 mm, and their mean value was recorded. BMI and skinfold thickness were used to measure adiposity because there is sufficient evidence that these parameters are valid measures of fatness.[19-21] A blood specimen was drawn from each participants. Serum was obtained by centrifugation and then stored at −70°C until the time of the assay. The clinical examination and the venipuncture for blood sampling were performed in the morning between 8 and 10 AM after a 10- to 12-hour fast. Informed parental consent was obtained, and the study was approved by the Health Committee of the Ministry of Education.

The frozen serum was used for the measurement of TG, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and leptin. Low-density lipoprotein (LDL) cholesterol was measured using the following formula: LDL = TC − HDL − TG/5.[22] TG, TC, and HDL were determined by an enzymatic colorimetric method using the BM Roche/Hitachi 717 analyzer (kits of Roche, Mannheim, Germany).

Leptin was determined in duplicate by enzyme-linked immunosorbent assay (ELISA) using commercial kits (RQD). The limit of sensitivity was 7.8 pg/mL, the maximal interassay coefficient of variation (CV) was 4.4%, and the maximal intra-assay CV was 3.2%.

From the 2nd Department of Pediatrics, Athens University Medical School, "P and A" Kyriakou Children's Hospital, and the Department of Biochemistry, "P and A" Kyriakou Children's Hospital, Athens, Greece.

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Address reprint requests to Emmanuel Kavazarakis, MD, 2nd Department of Pediatrics, Athens University Medical School, "P and A" Kyriakou Children's Hospital, Thibon and Levadias, Goudi, Athens 115-27, Greece.

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Statistical Analysis

Leptin values were plotted on the ln scale because their distribution was skewed. The lnleptin values were subsequently used for the analysis of data. Partial correlation and univariate linear regression analysis were used to assess the relationship of leptin with each of the parameters of the lipid profile (TC, TG, HDL, and LDL) as well as to explore the association of leptin with age, sex, and BMI. The effect of age, sex, BMI, and lipid profile on leptin concentration was evaluated using multiple linear regression analysis. A 2-tailed P value of $<.05$ was considered significant for all the statistical analyses.

RESULTS

The anthropometric parameters of the study population are shown in Table 1, and the serum lipids and leptin levels are presented in Table 2. Univariate linear regression analysis showed that there was a significant correlation of lnleptin transformed levels with age, Tanner stage, BMI, and triceps skinfold measurement, respectively (Table 3). The ln transformed leptin levels increased significantly with age in boys ($r = .20$, $P = .013$) and girls ($r = .23$, $P = .006$), but this increase did not reach a significant level when this association was controlled for BMI and skinfold measurement.

Furthermore, controlling for these indexes, the trend of association between lnleptin values and age remained positive for girls but became negative for boys. Although sex was not significantly associated with lnleptin values in the univariate regression model, a positive association with the female sex was found after the introduction of BMI in the model (unstandardized β coefficient = 0.333, standardized β coefficient = 0.157, $P < .001$).

The correlation of lnleptin concentration with each of the lipid profile parameters (TC, TG, HDL, LDL) shows a positive association of leptin with TG and LDL ($r = .36$, $P < .001$; $r = .11$, $P = .045$, respectively) and an inverse association with HDL values ($r = -.22$, $P < .01$), whereas no association was found with TC levels. However, when these relationships were explored after controlling for age, sex, BMI, and skinfold thickness, the trend of the above-mentioned association remained constant, but it reached a significant level only with TG values ($r = .30$, $P < .001$) and marginally with serum HDL concentration ($r = -.11$, $P = .057$).

These associations were further explored by linear regression models with lnleptin values being used as dependent variable and age, sex, Tanner stage, BMI and one parameter of the lipid profile at a time as independent variables (Table 4). Entering in the same model both TG and HDL, only TG significantly related to lnleptin whereas the strength and the significance of association with the remaining independent variables did not change. The observed trends of association and the statistical significance level did not substantially change when skin fold thickness, instead of BMI, was used as an indicator of body fat concentration.

Table 1. Mean Values, SD, and SE of Age and Anthropometric Characteristics in the Study Population

	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m^2)
Mean	9.30	36.00	135.76	19.41
SD	1.66	10.70	11.40	3.68
SE	0.01	0.62	0.66	0.21

Table 2. Mean Values and Selected Centile Values of Serum Lipids and Leptin Levels in the Study Population

	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Leptin (ng/mL)
Mean	184.4	53.04	63.53	110.61	11.99
SD	28.7	18.12	15.03	27.74	11.79
SE	1.7	1.05	0.87	1.62	0.68
Percentile					
5th	141.0	27.7	40	67.5	1.12
25th	162	39	54	91.3	3.16
50th	185	50	63	108.00	8.39
75th	204	63	72.2	127.60	16.45
90th	224.6	75.5	83.0	146.40	28.79
95th	236.0	102.8	89.8	160.36	36.19

DISCUSSION

Our data showed that serum leptin levels were associated with age, sex, BMI, triceps skinfold thickness, and Tanner stage. A relationship of circulating leptin levels with the aforementioned parameters has already been suggested by several studies, and our corresponding findings are overall in line with what was previously indicated by other investigators.

Although the relationship of leptin levels with different indicators of obesity[4-10] has been thoroughly explored, the same cannot be claimed for the relationship of leptin with the serum lipid profile. Nevertheless, recently published data from experimental studies[18,23,24] justified our hypothesis that leptin may be associated with circulating lipid levels. The absence of selection bias and the elimination of possible confounders, which was attempted through the statistical analysis, ensure the validity of the associations we found. Although insulin participates in energy homeostasis, a possible confounding relation of insulin has not been explored because the study population consisted of unselected healthy schoolchildren, and therefore considerable variation of insulin levels was not anticipated. Furthermore, experimental studies indicate that TG secretion

Table 3. Univariate Linear Regression–Derived Unstandardized and Standardized Coefficients and Corresponding *P* Values for Prediction of Serum lnLeptin Levels from Age, Sex, Sexual Development, and Indicators of Adiposity

Variable	β Coefficient	β Standardized Coefficient	*P*
Age (1 year)	0.135	0.214	<.001
Sex			
Male	Reference		
Female	0.07	0.03	NS
Tanner stage (stage I)	0.44	0.20	<.001
BMI (kg/m^2)	0.20	0.69	<.001
Triceps skinfold thickness (mm)	0.09	0.63	<.001

Table 4. Statistically or Marginally Significant Unstandardized and Standardized Coefficients Derived From Multiple Regression Models for the Prediction of lnLeptin Values Using as Independent Variables Sex, Age, Tanner Stage, BMI, and Either TG or HDL Serum Levels

	TG Model			HDL Model		
Variable	β Coefficient	β Standardized Coefficient	*P*	β Coefficient	β Standardized Coefficient	*P*
Gender						
Male	Reference					
Female	0.25	0.12	.03	0.29	0.14	.013
BMI (m/kg^2)	0.19	0.69	<.001	0.12	0.71	<.001
TG (mg/dL)	0.01	0.22	<.001			
HDL (mg/dL)				−0.05	−0.07	.057

NOTE. Adjusted R^2 for the core model (age, Tanner stage, sex, and BMI) = 0.5. Adjusted R^2 for the TG model = 0.55. Adjusted R^2 for the HDL model = 0.53.

rate is associated with leptin independent of insulin, whereas short-term insulin infusion does not alter leptin concentration.[25,26] The possible mechanisms through which leptin and serum lipids are interrelated were not identified because this information is not within either the aim or the ability of this study.

Leptin is the afferent loop that informs the hypothalamus about the state of fat stores with hypothalamic efferents, activating responses that regulate energy expenditure and deposition of fat.[27] However, experimental studies indicate that peripheral actions of leptin may also directly alter lipid metabolism. Nevertheless, body fat accumulation is a major contributor to leptin as well as to TG production; thus the hypothesis that TG concentration participates in the regulation of leptin secretion is biologically possible. For these reasons, we adopt a regression model with leptin as the dependent variable and lipid levels as well as anthropometric parameters as the independent variables. This model explained 55% of the variance of circulating leptin levels, revealing an independent association of leptin with TGs. However, TG and HDL, although statistically significant, contributed only 5% and 3%, respectively, to the explained variance. Another clinical study, with a limited number of patients, pointed out that leptin levels were elevated out of proportion to BMI in patients with familial combined hyperlipedemia,[11] a lipid disorder characterized by significant elevation of TG levels, a moderate increase in TC, and a reduction in HDL cholesterol. The investigators suggest that an underlying mechanism responsible for the elevation of TGs may also be associated with increased leptin levels. This assumption has not been verified by other investigators who either did not find any relationship between the lipid profile and the circulating leptin levels[6,13] or found an association only with HDL cholesterol in patients with lipid metabolism disorders.[12]

Because leptin resistance was not assessed, only speculation is possible about the underlying mechanism of the association between leptin and TG levels. It seems biologically more plausible to interpret this relationship as an indication of leptin resistance. Evidence derived from experimental studies is in favor of this explanation because leptin administration reduced the TG content of islets cells by inducing oxidation of free fatty acids and preventing ATG formation from free fatty acids.[18] However, this function was not observed in rats who had a phenotype of leptin resistance with obesity despite hyperleptinemia.[18]

Our findings also showed an inverse association of HDL with the circulating leptin levels. The trend of this association should have been expected, irrespective of the level of significance, because it is well known that HDL levels are inversely related to BMI, which is positively associated with leptin levels. However, Haluzik et al[12] showed either a positive or a negative trend of association between leptin and serum HDL values in patients with hyperlipidemia, depending on the type of lipid disorder and the sex of the studied group. On the other hand, a 2-fold elevation of plasma HDL cholesterol levels was seen in a ob/ob mouse model of obesity, accompanied by a substantial increase of ApoA-I and ApoA-II.[23] These abnormalities were reversed after the administration of a low dose of leptin, indicating that the defective catabolism of HDL apolipoproteins may be regulated in part by leptin signaling.[23] This evidence was further supported by the same research group in another experimental study showing decreased binding, association, degradation, and resecretion of HDL apoproteins by ob/ob hepatocytes.[24] However, there are many differences in lipoprotein metabolism between mice and humans[23] that discourage the extrapolation of similar conclusions for humans.

In conclusion, we suggest that serum lipid profile parameters are related to circulating leptin levels after controlling for known possible confounders. The fact that the results of other relevant clinical studies are not unidirectional could be attributed to the diversity of the metabolic defects responsible for different types of hyperlipidemias. Further studies are needed to explore the correlation of leptin with the lipid profile in patients with different types of lipid disorders and to elucidate the underlying causative mechanisms.

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