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The relation of leptin and soluble leptin receptor levels with metabolic and clinical parameters in obese and healthy children

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ABSTRACT

We investigated the relation of serum leptin, soluble leptin receptor (sLR) and free leptin index (FLI) with metabolic and anthropometric parameters in obese and healthy children. Height, weight, waist circumference (WC), fasting serum glucose, insulin, lipid profile, leptin and sLR levels of 35 obese children and 36 healthy children were measured and FLI was calculated as the ratio of leptin to sLR. In obese children, serum leptin and FLI were found significantly higher, while sLR level was significantly lower than the healthy children. Comparison of obese children regarding the insulin resistance showed significantly higher serum leptin and FLI in the insulin resistant group; however sLR level was not different between the insulin resistant and non-resistant obese children. In obese children, sLR was not correlated with any of the metabolic parameters except total cholesterol, while FLI was significantly and positively correlated with BMI, WC, TC, fasting insulin, and HOMA-IR. However, regression analysis confirmed that the HOMA-IR was the only independent variable significantly correlated with FLI in obese children. Findings of this study suggest that in obese children and adolescents (i) serum leptin and FLI were found significantly higher, while sLR level was significantly lower than the healthy children, (ii) increased FLI might be a compensatory mechanism for increasing leptin effect in peripheral tissue, (iii) FLI is a more accurate marker to evaluate leptin resistance than leptin or sLR alone, and (iv) increased FLI may contribute toward the development of hyperinsulinemia and insulin resistance.

1. Introduction

Leptin, a cytokine which is secreted predominantly by the adipose tissue, is considered to be involved in satiety regulation in humans and animals [18]. In adults, circulating leptin levels are closely related to the amount of adipose tissue [16]. Leptin transports across the blood–brain barrier by a saturated system and acts within the brain regions, which regulate feeding behavior and thermogenic responses [2,5]. In addition to the above-mentioned effects, leptin also plays a role in the regulation of other body functions, including reproduction, liver and enteric metabolism, hematopoiesis, and immunity via leptin receptors, which are shown to be present in many peripheral tissues [5,26]. In humans, four different mRNA splice variants of the leptin receptor, including a membrane-bound receptor with a long cytoplasmic domain and three membrane-bound receptors with a short cytoplasmic domain have been identified. A circulating soluble form of the leptin receptor [soluble leptin receptor (sLR)], which is the main leptin-binding protein and determinant of free leptin index (FLI), also exists [3]. Although the source of sLR in the plasma is not known definitely, it (sLR) is generated by the proteolytic cleavage of membrane-anchored receptors, indicating that the leptin receptor might have other functions besides signal transduction [6,17,24]. In blood, leptin is suggested to circulate both in the free form as well as bound to sLR and possibly also to other as yet unidentified binding proteins [24]. Whilst binding of leptin with sLR has been suggested to increase the bioavailability of leptin in the plasma and to elongate its circulatory half-life [10,12,14,24,30], it was also proposed to decrease the free fraction of leptin, resulting in decreased binding of free leptin to the membrane-bound leptin receptors [15]. However, the role of the sLR in the regulation of the physiological function of leptin is until now unclear.

Currently, limited data exists regarding the relation of sLR and FLI with metabolic and anthropometric parameters in childhood

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obesity. In this study, we aimed to investigate the relation of serum leptin, sLR and FLI with clinical and metabolic parameters in obese and healthy children.

2. Subjects and methods

The study included 35 obese children with a body mass index (BMI) above 95th percentile, who applied to our department with the complaint of weight gain and 36 healthy children with a BMI below 85th percentile, who had similar age and gender distribution. Obesity was defined according to the data from the Centers for Disease Control and Prevention (CDC), 2000, growth charts. For calculation of BMI-standard deviation score (SDS), data from the CDC were used [13].

Before the outset of the study, all of the obese and healthy children underwent a thorough physical examination and laboratory evaluation including thyroid function tests and serum cortisol measurement for probable endocrine pathology. Those with chronic diseases (cardiovascular, gastrointestinal, and respiratory), a history of drug use (steroids and antipsychotics), endocrine pathology (Cushing syndrome and hypothyroidism), or syndromes associated with obesity (Prader–Willi and Laurence–Moon–Biedle syndromes) were excluded from the study.

Height was measured using a Harpenden stadiometer with a sensitivity of 0.1 cm and weight was measured using a SECA scale with a sensitivity of 0.1 kg. The weight of each subject was measured with all clothing removed except undergarments. BMI was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (WC) was measured between the lowest rib and the iliac crest, horizontally through the narrowest part of the torso. The percentage of body fat and fat mass were measured using bioelectric impedance analysis (Tanita BC-418, Tokyo, Japan). Findings for pubertal development were evaluated according to Tanner staging [25]. A testicular volume of $\geq$4 mL in males and breast development of stage 2 and over in females were considered to be findings of puberty.

Blood samples for glucose, insulin, lipids, thyroid function tests, cortisol, leptin and sLR levels were drawn after 10–12 h of overnight fasting. The plane tubes were centrifuged at 1200 × *g* 10 min and serum samples were removed from clots into the Eppendorf tubes using plastic Pasteur pipettes. They were stored at −80 °C until analysis.

Serum glucose, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured enzymatically using DP Modular Systems (Roche Diagnostic Corp., Indianapolis, IN). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula when plasma TG's were <400 mg/dL. Serum insulin was measured according to the electro chemiluminescence immunoassay method, using an automated immunoassay analyzer (Immulite 2500 Insulin, Diagnostic Products Corporation, Los Angeles, CA). Cut-off points above the 95th percentile of healthy children were used to define dyslipidemia and impaired fasting glucose, according to the international recommendations [1,9]. Insulin resistance was evaluated according to the homeostasis model assessment-insulin resistance (HOMA-IR) index. Different cut-off values for prepubertal and pubertal stages were used to determine the status of insulin resistance (prepubertal > 2.5, pubertal > 4) [27].

Serum leptin and sLR concentrations were measured by Enzyme Immunoassay (EIA) kit based on the principle of standard sandwich enzyme immunoassay (Catalog No. EK-0437, Boster Biological Tech., China and Catalog No. CSB-E04647h, CUSABIO Biotech Coop., USA, respectively). Standards and samples were pipetted into the wells, which were precoated with analyte specific antibody. The analytes were bound by the immobilized antibody. After removing unbound conjugates, biotinylated detection antibodies were added to the wells subsequently and then followed by washing with wash buffer. Avidin conjugated Horseradish Peroxidase (HRP) were added to the wells and then unbound conjugates were washed away. A substrate solution was added to the wells and color develops in proportion to the amount of bounded analyte and the intensity of the color was measured. The ELISA assays had a sensitivity of 10 pg/mL and 0.78 pg/mL; a detection range of 62.5–4000 pg/mL and 3.12–200 pg/mL, respectively; intraaasay CV of <10% and <8%, interassay CV of <15% and <10%, respectively. Free leptin index (FLI) was calculated as the ratio of leptin to sLR.

Blood pressure was measured by one of the investigators using a validated protocol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10-min rest in the supine position using a calibrated sphygmomanometer. Hypertension was defined as blood pressure values above the 95th percentile for height, age, and gender [23].

The study was initiated upon approval of the local ethics committee of Dokuz Eylul University, Faculty of Medicine in light of the Helsinki Declaration. A written informed consent of the parent(s) of each subject was also obtained before the study.

3. Statistical analyses

Statistical analysis of the data was conducted with SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA). Homogeneity of the data was evaluated with the Kolmogorov–Smirnov test. Between study groups, the obtained data were compared by using Student's *t*-test (for normally distributed data) and Mann–Whitney *U* test (for non-normally distributed data) Categorical variables were compared using Chi-square test. The correlations between the independent variables were investigated with Pearson's correlation analysis. Variables with a p-value < 0.05 in univariate correlation analysis were included in a multivariate linear regression analysis model to assess the independent determinants of FLI. $p < 0.05$ was considered statistically significant.

4. Results

A total of 35 obese children (22 male; 16 prepubertal; mean age, 11.0 ± 3.2 years) and 36 healthy children (17 male; 19 prepubertal, mean age, 13.0 ± 2.5 years) were included in this study. The groups were similar for age and gender. There were significant differences between obese and healthy children in terms of BMI, BMI-SDS, WC, insulin, TG, HDL-C, HOMA-IR, leptin, sLR levels, FLI, and leptin/BMI ratio ($p < 0.05$), while TC and LDL-C levels were not different ($p > 0.05$). Obese children had significantly higher SBP and DBP values than those of the healthy children ($p < 0.05$) (Table 1).

Comparison of the obese children regarding the insulin resistance showed statistically significant differences for age, pubertal status, BMI, WC, fat mass (kg), percentage of body fat (%), insulin, HOMA-IR, leptin, FLI, and leptin/BMI ratio ($p < 0.05$); however serum sLR, glucose, LDL-C, HDL-C, TG and TC levels were not different between the insulin resistant and non-resistant obese children ($p > 0.05$) (Table 2).

In healthy children, FLI was positively correlated with BMI, BMI-SDS, and WC while in obese children FLI was positively correlated with BMI, WC, insulin, HOMA-IR, and TC levels ($p < 0.05$) (Table 3). In multivariate regression analysis, HOMA-IR ($\beta = 0.289$; $p = 0.042$) was the only independent variable significantly correlated with FLI in obese children, which explained 28.8% of the variance (Table 4).

In obese children, sLR significantly correlated only with TC ($r = -0.416$, $p = 0.013$), while sLR had a weak negative correlation with leptin, insulin and TG levels which failed to reach statistical significance ($r = -0.224$, $p = 0.195$; $r = -0.334$, $p = 0.06$; $r = -0.260$,