Clinica Chimica Acta 480 (2018) 225–229

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Bioactive leptin is stronger related to parameters of fat mass and distribution than conventionally measured leptin: Findings from a longitudinal study in obese children participating in a lifestyle intervention☆

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ARTICLE INFO

Keywords:
Bioactive leptin
Obesity
Children
Weight loss
Lifestyle intervention

ABSTRACT

Objective: This study analyzed the relationships between bioactive leptin, conventionally measured leptin, and parameters of fat mass and distribution in obese children before and after weight reduction.

Methods: We determined bioactive leptin (bioLep), conventional measured leptin (conLep), weight, height, body fat based on skinfold measurements and bioimpedance analyses, waist circumference (wc), and pubertal stage in 88 obese children participating in a lifestyle intervention at baseline and one year later.

Results: We identified no child with homozygous or heterozygous status for bioinactive leptin mutations. The baseline associations between bioLep and BMI (r = 0.53), BMI-SDS (r = 0.48), body fat (bioimpedance: r = 0.61, skinfold thickness: r = 0.49), wc (r = 0.42), and waist to height ratio (whr) (r = 0.39) were stronger than the associations between conLep and BMI (r = 0.50), BMI-SDS (r = 0.44), body fat (bioimpedance: r = 0.57, skinfold thickness: r = 0.41), wc (r = 0.41), and whr (r = 0.37). The changes of bioLep were stronger related to changes of BMI-SDS (r = 0.54), body fat (bioimpedance r = 0.59, skinfold thickness: r = 0.37), wc (r = 0.22), and whr (r = 0.21) than the associations between changes of conLep and changes of BMI-SDS (r = 0.48), body fat (bioimpedance: r = 0.56, skinfold thickness: r = 0.43), wc (r = 0.20), and whr (r = 0.20). The same findings were observed in multiple linear regression analyses adjusted to multiple confounders. In contrast to changes of conLep (r = 0.22), the changes of bioLep during intervention were not related to weight regain after the end of intervention. BioLep concentrations did not differ between prepubertal girls and boys, but were higher in pubertal girls compared to pubertal boys (p = 0.031).

Conclusions: Bioactive leptin was stronger related to fat mass and distribution compared to conventionally measured leptin.

1. Introduction

Leptin is a cytokine that is mainly produced in white adipocytes and secreted into the circulation [1,2]. It reflects the body's energy stores in adipose tissue and regulates body energy homeostasis as well as adipose tissue mass in a complex circuit involving central nervous pathways [1–4]. Furthermore, leptin is involved in growth [5] since its regulates growth hormone secretion and stimulates chondrogenesis [6]. Inborn errors of the leptin lead to early onset extreme obesity due to insatiable appetite and hyperphagia as well as hypogonadotropic hypogonadism at pubertal age [3,4]. A new entity of functional leptin deficiency has been described recently, which clinical presentation is similar to that of patients with classical leptin deficiency [7–9]. It has been hypothesized that most patients with bioinactive leptin are undiagnosed so far [7–9]. In these patients, circulating leptin levels measured by standard immunoassay appear to be appropriate for their fat mass. However, the mutated leptin does not bind to the leptin receptor due to a structural alteration and thus is bioinactive [8].

A new immunoassay is available to measure the bioactive form of leptin and to identify patients with functional leptin deficiency [8]. It is unclear whether this new assay has further advantages compared to the conventional leptin assay. Therefore, we performed the following longitudinal study in obese children participating in a lifestyle intervention. The aim was to compare the associations between bioactive leptin, parameters of fat mass, and growth to the associations between the conventional measured leptin, parameters of fat mass, and growth.

Abbreviations: bioLep, bioactive leptin; conLep, conventional measured leptin; BMI, body mass index; SDS, standard deviation score; Whr, waist to height ratio; ELISA, enzyme-linked immunosorbent assay; BFst, body fat based on skinfold thickness; BFbi, body fat based on bioimpedance analyses

☆ This study is registered at clinicaltrials.gov (NCT00435734).

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https://doi.org/10.1016/j.cca.2018.02.020
Received 9 January 2018; Accepted 19 February 2018
Available online 21 February 2018

Furthermore, it has been reported that leptin concentrations correlated negatively to the degree of weight loss in lifestyle interventions in children [10] and adults [11]. Therefore, we analyzed whether leptin measured with the conventional or the bioactive immunoassay or the ratio of bioactive to conventional leptin is predictive for weight changes in a lifestyle intervention in children.

2. Materials and methods

This study was approved by the local ethics committee of the University of Witten/Herdecke. Written informed consent was obtained in all children and their parents.

We chose randomly 44 obese children who participated in the lifestyle intervention "Obeldicks" with substantial weight reduction and 44 obese children without weight changes during this intervention. Details of the recruitment process have been published earlier [12]. All children did not suffer from endocrine disorders, premature adrenarche or syndromal obesity. The "Obeldicks" intervention program has been described in detail elsewhere [13,14]. Briefly, this one-year outpatient training is based on physical exercise, nutrition education, and behavior therapy including individual psychological care of the child and his or her family.

Obesity was defined by a body mass index (BMI) above the BMI 97th percentile for age and gender according to the definition of the International Task Force of Obesity [15] and using population specific data [16]. Substantial weight loss was defined as a reduction of standard deviation score of BMI (BMI-SDS) > 0.25 because with a reduction of ≤ 0.25 BMI-SDS, no improvement of cardiovascular risk factors could be measured in our cohort [17].

2.1. Measurements

We measured bioactive leptin (bioLeptin) and conventional leptin (conLeptin) from stored frozen serum probes (-82°). BMI, body fat based on skinfold measurements, body fat based on bioimpedance analyses, waist circumference, and pubertal stage were determined at baseline and the end of the intervention. Furthermore, we measured these parameters one year after the end of the one-year lifestyle intervention in children with follow-up.

Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured unclothed to the nearest 0.1 kg using a calibrated balance scale.

Percentage body fat was calculated based on a skinfold-thickness equation using the following formulae [18]: Boys: body fat % = 0.783 × (skinfold-thickness subscapularis + triceps in mm) + 1.6; girls: body fat % = 0.546 × (skinfold-thickness subscapularis + triceps in mm) + 9.7. The triceps and subscapularis skinfold-thickness were measured in duplicate and averaged. Furthermore, body fat was measured based on bioelectrical using leg-leg and hand-leg systems (BC418; TANITA, Uxbridge, UK) [19]. We used estimates of percentage body fat provided by the manufacturer's software based on age, gender, height, and weight. No information regarding the formulas could be obtained from the manufacturer due to its commercially sensitive nature.

Waist circumference was measured half-way between lower rib and iliac crest [20]. The ratio of waist to height was calculated.

The pubertal development was determined according to Marshall and Tanner and categorized into two groups (prepubertal: boys with pubic hair and gonadal stage I, girls with pubic hair stage and breast stage I; pubertal: boys with pubic hair and gonadal stage ≥II and girls with pubic hair stage and breast stage ≥II).

Blood sampling was performed at 8 a.m. in the fasting state. Leptin measurements were performed for conventional leptin (conLep) with the 'E077-human leptin sensitive ELISA' and for bioleptin (bioLep) with the 'L07-human functional leptin ELISA' (both from Mediagnost, Reutlingen, Germany, www.mediagnost.de) according to the respective kit's instructions. In brief, the assays are sandwich type ELISAs. While in the conLep assay the leptin in the diluted sample is bound to an antibody coated on the microtiter plate, leptin is bound by an immobilized receptor in the bioLep assay. According to the manufactures specifications the inter- and intra-assay coefficients of variation are below 10% for both ELISAs. For internal quality control a serum-pool was analyzed for leptin concentration. The inter-assay coefficient of variation was calculated as 4% for bioLep and 3% for conLep. The analytical sensitivity of both assays yields 0.01 ng/ml. An assay comparison proved that the mean deviation of the bioLep assay is 4.4% of the mean of both test systems. The assays are calibrated according to the WHO International Standard for human leptin, NIBSC 97/594. The bioLep ELISA detects functional leptin in serum in contrast to the conLep ELISA [8]: Added functional serum leptin is bound by a recombinantly produced immobilized receptor and is subsequently detected by a highly specific polyclonal, biotin-conjugated anti-leptin antibody and a streptavidin-peroxidase conjugate in the bioLep assay. Nonreceptor-binding leptin variants give no signal. In consequence, the bioLep assay is able to detect clinical cases of bioinactive leptin in contrast to the human leptin assay [8]. Individuals heterozygous for bioinactive leptin mutations have ratios of bioactive leptin to conventional leptin (bioLep/conLep) < 0.6 in contrast to homozygous wild-type individuals, while individuals homozygous for bioinactive leptin mutations show no signal in the bioLep assay [8].

2.2. Statistics

Statistical analysis was performed using the Winstat® software package. Because the BMI is not normally distributed in childhood, we used Cole's least mean square method, which normalizes the BMI skewed distribution and expresses BMI as a standard deviation score (BMI-SDS) by the formula BMI-SDS = $(BMI / M(t)^{L(t)-1}) / (L(t) \times S(t))$ [21]. The height data were transformed into standard deviation scores (SDS) for chronological age according to the reference data for German children [22]. Kolmogorov-Smirnov test revealed normal distribution for all continuous variables at baseline and at the end of intervention. Correlations were calculated by Pearson's correlation. ANOVA, student's *t*-tests for unpaired and paired observations were used, and chi-square test for qualitative items. Backwards multiple linear regression analyses were conducted for the dependent variable anthropometrics such as BMI, BMI-SDS, waist circumference, waist to height ratio, or body fat including age, gender, pubertal stage, bioLep, and conLep as independent variables. Furthermore, backwards multiple linear regression analyses were conducted for the dependent variable changes of anthropometrics during the intervention including age, gender, pubertal stage at baseline, as well as changes of bioLep and conLep as independent variables. These multiple linear regression analyses were performed to determine which leptin assay is stronger related to parameters of fat distribution and fat mass. A p-value < 0.05 was considered as significant. Values are expressed as mean and standard deviation.

3. Results

The characteristics of the study population are demonstrated in Supplemental Table 1. None of the children demonstrated a bioLep/conLep quotient < 0.6. Children with and without substantial weight loss during the intervention did not differ significantly at baseline according to age, gender, pubertal stage, height, BMI, body fat, bioLep or conLep. However, children with substantial weight loss demonstrated a significantly greater waist circumference and waist to height ratio at baseline. One year after the end of intervention 60 children were available for follow-up measurements. These children did not differ from children without follow up according to age, gender, height, BMI at baseline or change of BMI-SDS during intervention.

In the 29 prepubertal children, bioLep levels at baseline did not differ significantly ($p = 0.957$) between boys (30.8 ± 20.0 ng/ml) and