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ORIGINAL ARTICLE

Leptin sexual dimorphism, insulin resistance, and body composition in normal weight prepubescent

Dimorfismo sexual de la leptina, resistencia a la insulina y composición corporal en prepúberes normopeso

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What do we know about the subject matter of this study?

Leptin and insulin resistance have been associated in the pre-pubertal stage, a critical period of fat development, however, there are few studies in normal-weight prepubertal children.

What does this study contribute to what is already known?

Sex differences in adiposity and leptin levels are confirmed in normal-weight prepubertal children; however, these are not linked to differences in BMI or insulin resistance.

Abstract

The prepubertal stage is a critical period of body fat development, in which leptin and insulin resistance has been associated, however, there are few studies in normal-weight prepubescents. **Objective:** To assess the relationship between leptin and body composition and insulin resistance in a group of normal-weight prepubescents. **Patients and Method:** Analytical cross-sectional study with 128 healthy prepubescents of normal weight, aged between 6 and 10 years. Height, weight, body mass index (BMI), body fat percentage (BFP), waist circumference (WC), and hip circumference (HC) were measured. Plasma leptin (ng/mL) and insulin (mU/L) were evaluated by immunoassay and glycemia (mmol/L) by enzymatic method. HOMA-IR was calculated. A comparison study and correlation analysis by sex were performed. **Results:** Females presented higher values than males of leptin (6.8 ± 5 vs 3.3 ± 3.7; p = 0.000), insulin (7.1 ± 4.5 vs 5.2 ± 2.5; p = 0.016), BFP (22.4 ± 4.3 vs 18.6 ± 3.9; p = 0.000), and HC (67 ± 5.7 vs 65.0 ± 4.5; p = 0.019), and a lower waist/hip ratio (0.84 ± 0.04 vs 0.88 ± 0.04; p = 0.000). Leptin correlations with anthropometric variables were significant in

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both sexes, with greater association in females. The association of HOMA-IR with leptin was similar in both sexes. **Conclusions:** in normal-weight prepubescents aged between 6-10 years, there are sex differences in adiposity and leptin levels not associated with differences in BMI or insulin resistance. The greater association of leptin with adiposity in girls could be related to a high rate of adipogenesis induced by this hormone.

Introduction

During the prepubertal stage, around age 6, adiposity rebound (AR) occurs. The onset of this rebound can be defined as the age at which the Body Mass Index (BMI) increases after reaching its lowest value. This increase in BMI is mainly attributed to the gain of adipose tissue[1,2] that occurs between 6 and 10 years old, where the concentration of sexual hormones is incipient[3] so its effect is not determinant at this stage. AR before 5.5 years of age or a higher BMI in the prepubertal stage have been associated with obesity in adulthood[2,4,5].

Fat gain in prepubertal children occurs predominantly by adipogenesis[3,6]. One of the factors associated with this process is leptin, a peptide hormone produced mainly by subcutaneous adipocytes, described as a marker of adipogenesis[7]. Since one of its functions is to decrease satiety in the hypothalamus and increase peripheral energy expenditure, its dysfunction has been associated with obesity[7] and insulin resistance in adults[3]. In addition, the increased size of the adipocyte has been related to the increase in its concentration which correlates with the total body fat mass[3,8].

Leptin has been studied mainly in overweight and obese prepubertal patients[9,10], characteristics where there is a predominance of adipocyte hypertrophy. In normal-weight prepubertal patients, where adipogenesis is a determining factor[2], the sex-specific dimorphism of leptin concentration and its relationship with insulin resistance and body composition has been little explored[11,12].

The objective of this study was to evaluate by sex the relationship between circulating leptin concentration with body composition and insulin resistance in a group of normal-weight prepubertal children.

Patients and Method

Analytical cross-sectional study conducted on prepubertal school children in Cali, Colombia, between October 2017 and March 2018. To calculate the sample size, we assumed power of 80% and a leptin effect size by age of 0.115, estimating n = 80 subjects. This guaranteed the functional relationship between the variables of the study, however, 128 prepubertal patients were finally recruited, improving the power to 95%. Normal weight was considered a BMI between -1SD and +1SD[13].

In order to identify the normal-weight children, we measured and weighed 1,076 apparently healthy schoolchildren aged between 6 and 10 years from six basic primary education schools, from a middle socioeconomic stratum. Although 345 were selected, 128 subjects (62 boys and 66 girls) in Tanner stage I (prepubertal), diagnosed by medical evaluation were normal weight[14,15]. Subjects with weight control treatment, endocrine and/or neurological disease, with inflammatory processes diagnosed by ultra-sensitive C-reactive protein (Us-PCR) in plasma (> 0.43 mg/L)[4], or who voluntarily withdrew from the study were excluded. The parents and the selected children accepted and signed the informed consent and assent, respectively.

This study was approved by the Human Ethics Committee of the Universidad del Valle - Colombia (act No. 005-017), according to the Declaration of Helsinki and the regulations of the participating educational institutions.

Sociodemographic characterization and anthropometric measurements

Sociodemographic information was obtained through a survey. The anthropometric measurement was carried out on the 128 subjects according to ISAK (International Society for the Advancement of Kinanthropometry) guidelines[16].

The anthropometric measurements were obtained with the subjects in light clothing, without shoes, and after bladder emptying. For the weight (kg), a mechanical scale was used (SECA model 761 with a dial, ±100g), and a wall-mounted stadiometer (SECA 240 ±1 mm), was used for measuring height (cm). With these measurements, the BMI (kg/m^2) was calculated.

To measure waist and hip circumference (cm), we used a flexible and inextensible steel tape measure (Harpenden ± 0.1cm). The waist circumference (WC) was measured at the end of the exhalation, placing the tape measure at the midpoint between the lower edge of the tenth rib and the iliac crest. The hip circumference (HC) was measured while standing with feet together, placing the tape measure around the largest part of the hip (the widest part of the buttocks) and the pubic symphysis[16].

The body fat percentage (BFP) was obtained by measuring the triceps and subscapular skinfolds with a skinfold caliper (Harpenden ±0.2mm, with constant pressure of 10g/mm^2)[17]. The BFP was estimated with the Slaughter equation[18]. The technical error associated with the measurement was 2.5%, lower than that accepted by this equation.

Biochemical determinations

Plasma concentrations of leptin, insulin, glucose, and Us-PCR of 110 subjects were measured in blood samples obtained by venipuncture, after a 12-hour fast. The plasma obtained by centrifugation was stored at -20°C. For the measurement of insulin by immunoassay (Architect Insulin Reagent Kit, Abbott Laboratories), 24μL of plasma with a kit's sensitivity of < 1.0 μU/mL[19] was used. In the determination of leptin (Leptin ELISA LDN-kit), 20μL of duplicate plasma sample was used, with a detection limit of 0.5ng/mL[20]. Blood glucose was determined by the glucose oxidase technique (Glucose, Abbott, Clinical Chemistry, USA) using 2μL of the sample, and Us-PCR determination was made by turbidimetry (Multigen CRP Vario®; Abbott, Wiesbaden, Germany) with 2μL of the blood sample with a detection limit of 0.1mg/L. The degree of insulin resistance (IR) was determined by the HOMA-IR index, calculated as HOMA-IR= [insulin (μU/mL) x glucose (mmol/L)] / 22.5[21].

Statistical analysis

The description according to sex of the continuous variables (age, body composition [height, weight, BMI, BFP, WC, HC, ICC]) and the biochemical ones (blood glucose, insulin, HOMA-IR), was made through the mean and the median as measures of central tendency, and the standard deviation (±SD) or the 95% confidence interval (CI) were used as measures of dispersion. The normal distribution was evaluated with the Shapiro-Wilk test. For comparison analysis, variables that did not fit a normal distribution were evaluated using the Mann-Whitney U test and, otherwise, using the Student T-test. Bivariate correlations between anthropometric and biochemical variables according to their distribution were made through Spearman's test (ρ) or Pearson's test (r). A $p \leq 0.05$ value was considered significant differences. The analyses were made with the SPSS software version 22.

Results

The characterization by sex showed similarity in age, BMI, and HOMA-IR. Girls presented significantly higher BFP, HC, ICC, and leptin concentration values, while the waist/hip ratio was higher in boys (table 1).

The sex correlations of leptin with anthropometric variables were significant in both sexes, with a greater degree of association in girls for BMI, BFP, WC, and HC (table 2). The Waist/Hip ratio was negatively correlated but not significantly so in both sexes. The

Table 1. Biochemical and anthropometric variables by sex of prepubertal children

	Boys (n = 54)		Girls (n = 56)	
	Mean ± SD	CI 95%	Mean ± SD	CI 95%
Age (years)	7.8 ± 1.3	7.4 - 8.1	7.9 ± 1.2	7.6 - 8.3
Height (cm)	126 ± 7	124 - 128	126 ± 8	124 - 128
Weight (kg)	25.8 ± 3.6	24.8 - 26.7	26.2 ± 5.2	24.8 - 27.6
BMI (kg/m^2)	16.1 ± 1.2	15.7 - 16.4	16.4 ± 1.8	15.9 - 16.8
BFP (%)**	18.4 ± 3.5	17.5 - 19.3	22.4 ± 4.1	21.3 - 23.5
WC (cm)	57.1 ± 3.4	56.2 - 58.1	56.2 ± 4.4	55.1 - 57.4
HC (cm)*	65.0 ± 4.5	63.8 - 66.2	67 ± 5.8	65.8 - 68.9
WHI**	0.88 ± 0.04	0.87 - 0.89	0.84 ± 0.04	0.83 - 0.85
FBG (mmol/L)	4.9 ± 0.3	4.8 - 5.0	4.8 ± 0.4	4.7 - 4.9
FI (μU/L)	5.2 ± 2.5	4.6 - 6.0	7.1 ± 4.5	5.9 - 8.3
HOMA-IR index	1.2 ± 0.6	0.9 - 1.3	1.5 ± 1.0	1.2 - 1.8
Leptin (ng/ml)**	3.3 ± 3.7	2.3 - 4.3	6.9 ± 5.0	5.5 - 8.2

Values are presented as mean ±SD and Confidence Interval (CI) of BMI: Body mass index, BFP:Body Fat Percentage, WC:Waist Circunference, HC: HipCircumference, W/H-R: Waist/Hip- ratio, FBG: Fasting blood glucose; FI: Fasting Insulin. A *p < 0,05 and **p < 0,01 value was considered significant differences between girls and boys, calculated with test de la U de Mann Whitney association of leptin with insulin and the HOMA-IR index was significant and moderate in both sexes (table 2).

Correlations of anthropometric variables by age according to sex were also analyzed, which were significant with height and weight, but in girls, the degree of association of BMI with HC (r = 0.86 p = 0.000) and WC (r = 0.82 p = 0.000) was higher. The correlation of BMI with age was significant only in girls (table 3). There was no significant correlation between age and BMI in boys as opposed to girls.

Discussion

In this study, the relationship of circulating leptin concentration with body composition and insulin resistance in normal-weight prepubertal children was evaluated according to sex.

We found that between 6 and 10 years old, despite having no difference in BMI, girls presented higher values of BFP, HC, leptin, and insulin concentration and a lower Waist/Hip ratio than boys.

The difference in adiposity by sex found has been reported in other studies with similar populations, such as that of Benjumea et al.[22], who found that after the age of 6, adiposity increases significantly in both sexes, but more so in girls (p = 0.000). Garnett et al.[23] also reported a higher fat composition in girls in prepubertal age 7-8 years. Sexual dimorphism of fat mass in our study may explain the significant correlation between age and BMI only in girls, a finding described from earlier ages[11,12,24].

The concentration of circulating leptin was significantly higher in girls, similar to that found by Poveda et al.[9] who reported higher leptin concentrations in normal-weight girls. Although Thomsen et al.[25] also obtained similar results, it is important to emphasize that the study population was aged 6 to 18 years and the analysis included both obese and non-obese subjects, unlike our study that only included normal-weight children aged 6 to 10 years, and without inflammation that can alter leptin concentration.

The finding of increased BFP and its association with significant leptin concentration in girls could be explained by an increased rate of adipogenesis[3,23], probably due to incipient levels of estrogens of insulin-like growth factor type I (IGF-I)[23] and other hormones such as leptin and insulin[3,8,26] at these ages. These prepubertal changes may be anticipating the development of the pubertal stage, in which leptin as an indicator of energy reserves in girls has been linked to preparation for reproduction and breastfeeding, while in boys, such energy reserve may be lower to ensure spermatogenesis[27,28].

Table 2. Correlations of concentrations of leptin, by sex, with anthropometric and biochemical variables

	Total (n = 110)	Boys (n = 54)	Girls (n = 56)
Age (years)	0.37**	0.31*	0.44**
Height (cm)	0.35**	0.41**	0.42**
Weight (kg)	0.54**	0.52**	0.69**
BMI (kg/m2)	0.56**	0.35**	0.72**
PZ- BMI	0.44**	0.29*	0.56**
BFP (%)	0.79**	0.61**	0.79**
WC (cm)	0.47**	0.45**	0.69**
HC (cm)	0.69**	0.56**	0.78**
WHI	-0.35**	-0.20	-0.23
FBG (mmol/L)	0.05	0.13	0.10
FI (mU/L)	0.65**	0.62**	0.65**
HOMA-IR	0.62**	0.61**	0.62**

Bivariate correlations coefficients were calculated with the Spearman test (ρ); BMI: Body mass index, PZ_BMI: score Z-BMI, BFP: Body Fat Percentage, WC: Waist Circumference, HC: Hip Circumference, W/H-R: Waist/Hip-ratio, FBG: Fasting blood glucose; FI: Fasting Insulin. A *p < 0.05 **p < 0.01 values were considered significant.

Table 3. Correlations of Age and BMI by sex

	Girls (n = 56)		Boys (n = 54)	
	Age	BMI	Age	BMI
Age (years)	0.79**		0.73**	
Height (cm)	0.87**		0.85**	
BMI (kg/m²)	0.40**		0.14	
BFP (%)	0.43**	0.79**	0.24	0.45**
WC (cm)	0.51**	0.86**	0.45**	0.65**
HC (cm)	0.72**	0.82**	0.65**	0.56**
FI (mU/L)	0.24	0.25	0.19	0.28*
FBG (mmol/L)	0.39**	0.15	0.28*	0.008
Leptin (ng/ml)	0.41**	0.65**	0.25	0.39**
HOMA- IR	0.28	0.24	0.20	0.26

Bivariate correlations coefficients were calculated with the Pearson test. BMI: Body mass index, BFP:Body Fat Percentage,WC:Waist Circunference, HC: Hip Circumference, FI: Fasting Insulin, and FBG: Fasting blood glucose. A *p < 0.05 and **p < 0.001 values were considered significant.

Considering these results according to sex, the differences found could not be explained by the degree of IR, measured with the HOMA-IR index, nor by insulin in blood, factors that in adult obesity have been associated with leptin resistance and hyperleptinemia[29].

In normal-weight animal models, it has been described that elevated leptin levels in the presence of high BFP in females could be explained by reduced transport through the blood-brain barrier[8,29,30]. This could be related to a higher concentration of circulating leptin in prepubertal girls that stimulates adipogenesis, without changes in the central effects of leptin, such as decreased intake and increased energy expenditure, all of which would contribute to the higher BFP found in girls.

Since the prepubertal stage, levels of sex steroids such as 17ß-estradiol and estrone are higher in girls[31]. In vitro, estrogen in women has been reported to induce increased leptin secretion in adipocytes. Increased estrogen concentration and high sensitivity to these hormones may favor increased leptin levels in girls[32]. It is not ruled out that other mechanisms such as sex differences in the clearance, transport, peripheral sensitivity, and autocrine self-regulation of leptin may be related to sexual dimorphism, processes that are worth further investigation.

The strengths of this study were the diagnosis of Tanner stage by medical examination, the homogeneous distribution by sex and age of the sample, and also that only subjects evaluated without systemic inflammation were included. These conditions favor the reliability of our results. A weakness of the study was that no factors regulating adipogenesis and leptin secretion were measured, which could help explain the results associated with their sexual dimorphism.

Conclusions

In prepubertal normal-weight children aged between 6 and 10 years, there are differences by sex in adiposity and leptin levels, which do not seem to be associated with differences in BMI or IR.

The high degree of association of circulating leptin with adiposity in girls may be related to a higher rate of adipogenesis induced by this hormone.

The higher adiposity in girls could make them more susceptible to obesity in the pubertal stage or adulthood, therefore, it is important to emphasize in this group the incorporation of healthy lifestyles from an early age.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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