Association of leptin with cardiometabolic factors in schoolchildren and adolescents with congenital adrenal hyperplasia

Jessie Nallely Zurita-Cruz¹, Miguel Ángel Villasís-Keever², Leticia Damasio-Santana³,
Leticia Manuel-Apolinar³, Rosalba Ferrusca-Ceja⁴, Elisa Nishimura-Meguro⁴,
Aleida de J. Rivera-Hernández⁴ and Eulalia Garrido-Magaña⁴

¹Unit of Research in Medical Nutrition; ²Unit of Medical Research in Clinical Epidemiology; ³Unit of Medical Research in Endocrine Diseases, Diabetes and Metabolism; ⁴Pediatrics Hospital, Department of Pediatric Endocrinology, Instituto Mexicano del Seguro Social, Centro Médico Nacional Siglo XXI, Ciudad de México, Mexico

Abstract

Introduction: In congenital adrenal hyperplasia (CAH), obesity, hyperinsulinemia and leptin levels are increased. **Objective:** To identify the frequency of cardiometabolic risk factors (CRF) in children and adolescents with CAH and to explore the relationship with leptin levels. **Method:** Cross-sectional study of 40 patients who underwent anthropometric measurements and had fasting glucose, insulin, triglycerides, 17-hidroxyprogesterone, leptin, HDL and LDL-cholesterol assessed. The patients were classified according to the number of CRFs, and leptin levels were analyzed with the Kruskal-Wallis test. Pearson's correlation was applied between leptin, body mass index (BMI) z-score and body fat percentage. **Results:** Fifty percent of the patients had obesity and overweight, 59% had hypertriglyceridemia, 40%, hypoalbuminemia, 27.5%, high LDL-cholesterol and 22.5% insulin resistance. There was positive correlation between leptin and body fat percentage ($r = 0.64$), BMI z-score ($r = 0.55$) and the number of CRFs ($r = 0.65$). In the obesity-adjusted multivariate analysis, leptin levels were associated with the number of CRFs. **Conclusion:** CAH had a high frequency of CRFs and leptin appeared to be associated with a more adverse cardiometabolic profile in subjects with obesity and overweight.

KEY WORDS: Congenital adrenal hyperplasia. Leptin. Pediatrics.

Introduction

Congenital adrenal hyperplasia (CAH) is a recessive autosomal disorder characterized by increased androgen production and cortisol deficiency.¹ The goal of treatment is to provide adequate cortisol replacement in order to prevent adrenal crises and to suppress androgen excessive production.² However, in order for this effect to be achieved, supraphysiological cortisol doses have to be administered, by means of which the line between endogenous hyperandrogenism suppression and Cushing syndrome iatrogenic induction is very thin.³

Studies in adults with CAH have demonstrated high prevalence of obesity,⁴ hypercholesterolemia, insulin resistance (IR)⁵ and arterial hypertension;⁶ cortisol supraphysiological doses can be a possible triggering factor in the development of cardiometabolic risk factors (CRF).⁷

On the other hand, Völkl et al. described 89 German children and adolescents with CAH in whom obesity was identified in 16.8 %, which is a higher proportion in comparison with that reported in children and adolescents of the German general population (2.27 %).⁸ In a study that included 22 patients with ages ranging from 7 to 22 years, higher values were observed in the BMI Z-score (BMIz) than in healthy population.⁹

Correspondence:

Jessie Nallely Zurita-Cruz
E-mail: zuritajn@hotmail.com

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Leptin is a peptide hormone¹⁰ that is produced by adipocytes, regulated by hypothalamic neuropeptides¹¹ and that is implicated in energy metabolism, thermogenesis and weight gain.¹² In human beings, leptin has been associated with obesity and is considered to be a risk factor for metabolic syndrome.¹³ Leptin plays a permissive role in pubertal development. Some studies associate puberty onset with an increase of leptin levels.¹⁴ These actions can explain why leptin levels are different according to the age, gender, body fat and puberty stage. Both obesity and puberty can be associated with leptin increase.¹⁵

Particularly in patients with CAH, high androgen serum concentrations alter leptin metabolism, which causes hyperleptinemia; on the other hand, β -adren-ergic receptors, which regulate lipid metabolism and thermogenesis, are deficient in patients with CAH.¹⁶ This induces an adverse environment, which elicits an increase in the development of CRFs.

Adults and adolescents with CAH have been described to show a higher prevalence of CRFs, with leptin levels being associated with some of them. Whether CRFs and leptin are elevated since the pre-pubertal stage has thus far not been studied, and neither has the relationship between the number of CRFs and leptin levels.

The purpose of this study was to identify the prevalence of CRFs in schoolchildren and adolescents with CAH and their relationship with leptin levels.

Method

This was a cross-sectional study of Mexican children and adolescents that was carried out between December 2014 and July 2015. Patients with ages ranging from 6 to 16 years, who underwent follow-up for at least 12 months by the pediatric endocrinology department and who received systemic steroid-based hormone replacement were included. Forty-six patients who met the inclusion criteria were selected; 6 were excluded: 2 had incomplete exams, and in 4, the parents denied permission for their children to participate in the study. The research protocol was approved by the hospital research committee with registry number R-2014-3603-32. Parents signed the informed consent letter and patients older than 8 years signed the letter of assent, according to recommendations of the Declaration of Helsinki.

Clinical and anthropometric assessment

Recorded somatometric variables included weight, height, waist circumference and body fat percentage (BF%) measured by the bioimpedance method (Tanita BC-568); these measurements were performed by a certified nutritionist. Pubertal status was identified by physical examination according to the Tanner scale. Blood pressure was the average of 3 measurements taken with a sphygmomanometer on three separate occasions. IR was estimated using the homeostasis model (HOMA):¹⁷

$$IR = \text{insulin (microunits per milliliter)} \times \text{glucose (millimoles per liter/22.5)}$$

Analyzed CRFs were obesity (BMI at $\geq 95^{\text{th}}$ percentile), systemic arterial hypertension (systolic pressure at $\geq 90^{\text{th}}$ percentile or diastolic pressure at $\geq 90^{\text{th}}$ percentile-for-age),¹⁸ fasting glucose alteration (plasma glucose ≥ 10 mg/dL),¹⁹ hypertriglyceridemia (plasma triglycerides [TGL] at $\geq 90^{\text{th}}$ percentile according to age and gender), hypoalphalipoproteinemia (high-density lipoprotein cholesterol [HDL-C] at $< 10^{\text{th}}$ percentile-for-age and gender),²⁰ low-density lipoprotein cholesterol (LDL-C) > 100 mg/dL and IR (HOMA score > 3.4).²¹ Body fat was considered to be abnormal according to gender: in females, when it was $> 30\%$, and in males, $> 20\%$.^{22,23}

The glucocorticoid dose was expressed as the accumulated dose per body surface area (BSA) per day (milligrams per square meter [mg/m² BSA]), taking the dose average of the last 6 months prior to the study. All glucocorticoid doses were converted to hydrocortisone (HC) doses: 1 mg prednisone = 4 mg HC and 1 mg prednisolone = 5 mg HC. CAH was regarded as being adequately controlled when 17-hydroxiprogesterone (17OHP) was < 10 ng/mL.

Biochemical assessment

Sera were obtained from blood samples taken by means of venipuncture after a 12-h fast. Glucose, TGL, LDL-C and HDL-C were determined using colorimetric enzymatic methods (Bayer diagnostics, Puteaux, France). Insulin was measured by chemoluminescence (Roche-Hitachi modular P and D), 17OHP and leptin (Human Leptin Duo Set, DY 398®) were measured with enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, MN, USA). All ELISA analyses were determined using Finstruments Multiskan EX, MTX® (Lab Systems), in duplicate, as

recommended by the manufacturer. Intra- and inter-assay variation coefficient was < 7% for all measurements. A standardization curve was also included for each assay.

Statistical analysis

Data were analyzed using the SPSS version 17.0 statistical program. Since no normal distribution was found for quantitative variables, the mean and minimum and maximum values were calculated.

Patients were classified according to the number of CRFs, and leptin levels were compared with Kruskal-Wallis test. The correlation between leptin and BMIz and BF% was determined with Pearson's coefficient. In order to identify variables associated with predicted CRF number, different linear regression models were constructed, where leptin, 17OHP, HC dose, absence or presence of puberty, BMIz and BF% were included.

Results

Forty patients (26 females), with ages ranging from 6 to 16 years (median of 11.9 years) were analyzed. The bone age/chronologic age ratio was higher than one in most cases, owing to an advanced bone age in these patients. CAH most common variant was the salt-losing type, followed by simple virilization. All patients received replacement treatment with glucocorticoids, with a median HC dose of 15.7 mg/m² BSA (Table 1).

Regarding CRFs, 14 (35 %) and 8 (20 %) subjects had obesity and overweight, respectively; hypertriglyceridemia was identified in 20 (59 %), and 16 (40 %) had hypoalphalipoproteinemia, 11 (27.5 %), LDL-C increase, 9 (22.5 %), IR, 3 (7.5 %), systemic arterial hypertension and one had altered fasting glucose. Of the 40 subjects, 33 (77.5 %) had on or more CRFs; specifically, 25 % had one factor, 20 %, two, 17.5 %, three, 7.5 %, four, and 7.5 % had five. BF% was found to be above normal in 65 % (n = 26) of patients (Table 2).

In total, only 25 % of patients (n = 10) had no CRFs, 17.5 % (n = 7) had one factor and 57.5 % (n = 23) had more than two. The number of CRFs was compared between patients with and without obesity: 92.8 % (n = 13) of patients with obesity had two or more CRFs; of the patients without obesity, only 38.46 % (n = 10) had two or more, with statistical significance (p = 0.013).

Table 1. General data of 40 pediatric patients with congenital adrenal hyperplasia

	n	%
Gender		
Female	26	65
Male	14	35
CAH classification		
Salt-losing	30	75
Simple virilization	2	5
Late	8	20
Glucocorticoid therapy		
Dexamethasone	7	17.5
Prednisolone	9	22.5
Prednisone	23	56.5
Hydrocortisone	1	2.5
CAH adequate control	16	40
Tanner stage		
I	9	25
II	8	22.2
III	4	11.1
IV	10	27.8
V	5	13.9
	Median	Minimum-maximum
Chronologic age (years)	12	6-16
Bone age (years)	11	6-16
17-hydroxiprogesterone (ng/mL)	11.11	0.20-444.7
Glucocorticoid dose (mg/m ² BSA)	15.7	4.9-33.1

CAH, congenital adrenal hyperplasia; m² BSA, square meter of body surface area.

To identify if patients had CRFs since pre-pubertal stage and their prevalence, a sub-analysis was carried out of 14 subjects with ages ranging from 6 to 9 years, where 10 (71.4%) had overweight and obesity (28.5 % overweight and 42.8 % obesity), 7 (50 %) had hypertriglyceridemia, 6 (42.8 %) had hypoalphalipoproteinemia, 3 (21.4 %) had increased LDL-C and 3 (21.4 %) had IR; 12 (85.7 %) had at least one CRF.

There was positive correlation between leptin values, BF% and BMIz (r = 0.55) (Fig. 1). Leptin levels were also compared and correlated with the number of CRFs, where the higher the number of factors, the higher the leptin level (Fig. 2).

A linear regression model was constructed to identify patient characteristics that might be related to the number of CRFs; leptin, BMIz, BF%, 17OHP and HC dose were included. The model showed that leptin and 17OHP were significantly associated with the number of CRFs. Subsequently, a model adjusted for puberty status and presence of overweight and obesity was constructed, where leptin levels were independently related to the number of CRFs (Table 3).

Figure 1. Correlation between leptin levels, body fat percentage (A) and BMI Z-score (B) in patients with congenital adrenal hyperplasia.

Table 2. Somatometric data and biochemical parameters in 40 patients with congenital adrenal hyperplasia

	Median	Maximum-minimum
BMI Z-score	0.81	-1.62-3.26
BMIz percentile	82	5-99
Waist circumference (cm)	68	49-108
Waist circumference percentile	50	10-90
Body fat percentage	30.75	18.3-48.8
Female	31.7	21.5-48.8
Male	26	18.3-45
Fasting glucose (mg/dL)	85.15	66.6-109
LDL-C (mg/dL)	82.7	11-143
HDL-C (mg/dL)	53.2	26-77
Triglycerides (mg/dL)	94	32-204
Systolic pressure (mmHg)	100	70-135
Systolic pressure percentile	67	50-80
Diastolic pressure (mmHg)	60	50-70
Diastolic pressure percentile	50	50-90
Fasting insulin (μ U/mL)	9.85	1.02-37.2
HOMA	2.01	0.21-9.09

BMI, body mass index; BMIz, body mass index Z-score; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Discussion

In this research, patients with CAH had a high prevalence of CRFs, both at the pre-pubertal and pubertal stage. In addition, leptin levels were established to increase according to the number of CRFs. Particularly, in patients with overweight and obesity, leptin levels were associated with an adverse cardiometabolic profile.

Comparison with other studies

In this study, included patients showed a high prevalence of overweight and obesity (55 %) in comparison with that what is expected for normal pediatric population in Mexico (34.4 %).²⁴ CRF prevalence was compared with that reported by Arlt et al.,⁶ who describe adult subjects, out of which those with CAH had a higher prevalence of hypoalphalipoproteinemia (40 versus 14 %), lower prevalence of LDL-C increase (27.5 versus 39 %) and similar prevalence of IR (22.5 versus 29 %), which draws the attention because high prevalence of IR and presence of dyslipidemia are observed since early pediatric stages.

The pathophysiology of CRF development in patients with CAH is multiple, with supraphysiological steroid doses and hyperandrogenemia standing out, both chronically. The age at which patients develop the incipient clinical and biochemical changes that determine the presence of these cardiovascular and metabolic factors is not known. However, the results suggest that since 6 years of age there are already manifestations.

Puberty is a confounding variable with regard to CRFs.^{25,26} IR increase is related to puberty onset owing to a physiological increase of counter-regulatory hormones that are necessary for maturation and growth.²⁷ For this reason, a sub-analysis of CRFs was performed in pre-pubertal patients, where they were observed to have a high prevalence of CRFs; however, in the multivariate analysis, puberty was not shown to be associated with an increase in CRFs; therefore, puberty was not a variable related to CRFs.

Obesity-associated mechanisms (BF% increase,¹⁶ IR and leptin elevation) are diverse, particularly in this

Figure 2. Comparison (A) and correlation (B) of leptin levels and cardiometabolic risk factors.

Table 3. Linear regression analysis to predict the number of cardiometabolic risk factors

	Initial model		Adjusted model	
	β^*	95% CI	β^{**}	95% CI
Leptin serum concentrations (ng/mL)	0.269 ∞	0.097-0.441	0.322 ∞	0.057-0.606
17-hydroxiprogesterone (ng/mL)	0.005 ∞	0.0001-0.010	0.004	-0.003-0.012
Glucocorticoid dose (mg/m ² BSA)	-0.074	-0.166-0.011	0.099	0.099-0.09
BMI Z-score	-0.037	-0.451-0.376	-0.219	-0.861-0.423
Body fat (%)	-0.003	-1.96-3.68	-0.014	-0.19-0.16

$\infty p < 0.5$

*Linear regression coefficient.

**Multiple linear regression coefficient adjusted for obesity/overweight and puberty stage.

BMI, body mass index; m² BSA, square meter of body surface area.

group of subjects. Excess of androgens, such as dehydroepiandrosterone and testosterone, can interfere in leptin metabolism via its signaling, which causes a decrease in leptin OB receptor, thus originating peripheral resistance and, consequently, hyperinsulinism, insulin resistance and obesity, which in turn alters steroidogenesis and increases the production of adrenal²⁸⁻³⁰ and ovarian³¹ androgens.

Obesity and CAH are associated with high levels of leptin and deficiency of α -adrenergic receptors, which modulate lipid metabolism and thermogenesis, which in turn stimulates the development of CRFs.^{32,33} This induces a pro-inflammatory environment, increased risk for metabolic syndrome and polycystic ovary, as well as a decrease in glucocorticoid treatment efficacy. This explains the high prevalence of CRFs identified in the studied patients.

According to observations, patients with CAH require early initiation of dietary-environmental and pharmacological intervention since school-age. For example, pharmacological treatment might focus on improving IR by means of sensitizers, such as

metformin,³⁴ since in these patients there is no way to discontinue the treatment with glucocorticoids or to modify androgen concentration fluctuations, in spite of being controlled.

In this group of patients, which showed a high prevalence of obesity, overweight (55 %) and elevated BF% (65 %), these variables were observed to be confounding with regard to the number of CRFs and leptin levels. Although leptin has been described to be directly related to BF% and BMIz,³¹ patients with CAH comprise a particular group of subjects in whom hyperandrogenemia²⁸⁻³² alters the metabolism, including that of leptin, which favors cardiometabolic alterations. This is explained because leptin was an independent variable in the number of CRFs in patients with CAH and obesity.²⁸

Study limitations

Sample size was small and, therefore, the results should be interpreted as a trend rather than conclusions. Furthermore, since this is a cross-sectional

study, carrying out a longitudinal analysis would be advisable in order for the age at which CRFs start appearing to be identified. Adipocytokines, in addition to leptin and other anthropometric markers, should be studied in future investigations.

In conclusion, it can be asserted that patients with CAH have a high prevalence of CRFs and that leptin levels are independently associated with a more adverse cardiometabolic profile in patients with obesity.

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