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Free and total leptin serum levels and soluble leptin receptors levels in two models of genetic obesity: the Prader-Willi and the Down syndromes

Caterina Proto[a], Daniela Romualdi[b], Rosa Maria Cento[a], Corrado Romano[a], Giuseppe Campagna[b], Antonio Lanzone[a,b,*]

[a]*Laboratories Department and Department of Mental Retardation, "Oasi" Institute for Research Maria SS, 94018 Troina (EN), Italy*
[b]*Department of Obstetrics and Gynaecology, Università Cattolica del Sacro Cuore, L.go Agostino Gemelli, 8-00168 Rome, Italy*

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Abstract

Alterations in energy balance and feeding behavior and the subsequent high frequency of obesity are hallmarks of 2 chromosomal diseases: the Prader-Willi syndrome (PWS) and the Down syndrome (DS). Leptin, an important regulator of food intake and energy homeostasis, circulates in 2 forms: a free, therefore active, fraction and a fraction bound to the soluble leptin receptor, whose bioavailability consequently participates in the regulation of leptin action. To investigate the possible role of the free-bound leptin balance in the pathogenesis of obesity in PWS and DS, we enrolled 7 obese women with DS, 5 obese women with PWS, 7 obese women, and 7 normal-weight healthy control women. Basal hormonal concentrations, total and free leptin levels, and leptin receptors levels were measured in plasma samples obtained from the 4 groups. No significant differences were observed in the hormonal milieu. Women with DS exhibited lower total leptin concentrations ($P < .01$), comparable leptin receptor level and, therefore, lower free leptin values ($P < .01$) when compared with obese controls, then resembling the profile peculiar to normal-weight control women. At variance, subjects with PWS did not differ from obese controls regarding both leptin and leptin receptor levels. Our data suggest that, whereas subjects with PWS have a leptin assessment corresponding to their degree of obesity, subjects with DS may have a defect in the secretion of leptin that could at least partially account for this form of syndromal obesity.

1. Introduction

Prader-Willi syndrome (PWS) is a disorder caused by the lack of expression of paternally inherited imprinted gene on chromosome 15q11-13, or to maternal uniparental disomy (2 maternal copies of 15q) [1-3]. It is characterized by mild mental retardation and distinct physical, behavioral, and psychiatric features, which are thought to originate from developmental alterations in the hypothalamus [4]. Compared with healthy persons, individuals with PWS have a reduced life expectancy due to comorbidities such as diabetes and cardiovascular diseases [5]. One of the cardinal symptoms is represented by excessive eating, which, if untreated, leads to massive obesity [6]. Patients with PWS have a peculiar body composition: the percentage of body fat is higher than normal and has a prevalent troncular distribution; on the contrary, lean body mass is scarce with a consequent low resting metabolic rate [7-10].

Alterations in energy balance and obesity are also hallmarks of the most common chromosomal disorder and genetic cause of mental retardation, the Down syndrome (DS) [11,12]. Little is known about the etiology of obesity in DS. Although the role of the metabolic rate appears as the main candidate in the development of DS-related obesity, it has been proposed that general hypotonicity, hypoactivity, and poor nutritional habits may act as contributing factors [13-15].

Recent studies identified a number of adipokines that are produced by the adipose tissue and may take part in the pathogenesis of obesity [16]. Among these, the ob protein leptin represents an important regulator of food intake and energy homeostasis in humans [17]. Leptin is able to interact with the brain centers, mainly through a feedback mechanism

* Corresponding author. Department of Obstetrics and Gynaecology, Università Cattolica del Sacro Cuore, L.go Agostino Gemelli, 8-00168 Rome, Italy. Tel.: +39 06/30577; fax: +39 06/30577.
E-mail address: alanzone@rm.unicatt.it (A. Lanzone).

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on the hypothalamus, promoting satiety and energy expenditure [18].

Serum leptin circulates in 2 fractions, a protein-bound (BL) and a free (FL) form, which is the biologically active amount able to directly interact with the central receptors [19]. The balance between the bound and the free leptin depends on the plasma concentrations of the soluble leptin receptor (SLR), which are inversely correlated with total serum leptin and body mass index (BMI) [19,20]. Leptin may bind several proteins in vitro, such as α2-macroglobulin or the sialic acid–binding lectins of the immunoglobulin superfamily [21,22]. However, their affinity for leptin binding was found to be at least a hundredfold lower compared with SLR. An elegant study, aimed to determine the molecular identity of potential leptin-binding proteins in vivo, concluded that SLR is the major leptin-binding protein in the circulating human blood [23]. The ratio between total leptin concentrations and SLR indicates the free leptin index (FLI). Recent evidence suggests that BL serves as a marker of resting energy expenditure, whereas FL reflects body fat mass [24].

Several reports in the literature suggest that total leptin levels are not altered in subjects with PWS, who exhibit circulating levels of this protein reflecting total adipose tissue mass, as in the general population [25-28]. On the contrary, the only study on leptin levels in DS seems to indicate that affected subjects may have lower total leptin levels than expected based on the degree of obesity [25].

The leptin system exerts a paramount role in the pathogenesis of human obesity. Nonetheless, many aspects are still controversial: the reciprocal roles of total and free leptin, the complex leptin receptor mechanisms, and the regulatory influence of other endocrine signals on leptin secretion and action. To improve our understanding of such dynamics in genetic obesity, we investigated for the first time the possible alterations in the plasma levels of total and free leptin in DS and PWS, in relation to the anthropometric and hormonal parameters.

2. Subjects and methods

Twelve female subjects were enrolled in the study: 7 were affected by DS (mean age, 22.7 ± 5.6 years; BMI, 29.00 ± 1.40 kg/m^2) and 5 by PWS (mean age, 20.4 ± 7.4 years; BMI, 31.40 ± 5.03 kg/m^2) as confirmed by chromosomal analysis. Data obtained from these 2 groups of patients were compared with those from a control group including 7 obese women (mean age, 36.28 ± 3.98 years; BMI, 29.70 ± 2.04 kg/m^2) and 7 normal-weight women (mean age, 30.9 ± 11.24 years, BMI, 21.30 ± 3.07 kg/m^2). Obesity was defined as BMI of 27 kg/m^2 or greater.

Normal adrenal and thyroid function had been previously ascertained. Two patients with PWS showed a deficit of growth hormone (GH) secretion, detected by pharmacologic stimuli, insulin-induced hypoglycemia (0.1 U/kg intravenously) and clonidine test, as previously described [39]. Peak serum GH concentration less than 10 ng/mL after both tests was considered indicative of GH deficiency.

None of the study subjects took any medication or had any evidence of metabolic disease except obesity. They were not in any active weight loss program or taking any drug for the treatment of obesity. The study protocol was approved by our ethical committee, and written informed consent was provided by each subject or by the parents, where appropriate.

Blood samples were collected early in the morning after a 12-hour fasting. The following were assayed in each sample: leptin, SLRs, insulin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, prolactin, testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), GH, and insulinlike growth factor 1.

Serum leptin concentrations were measured (in nanograms per milliliter) by a commercially available specific radioimmunoassay (Linco Research, St Charles, MO). The intra- and interassay coefficients of variation were 4.2% and 4.5%, respectively; the sensitivity of the assay was 0.5 ng/mL. Leptin receptor serum levels were detected with an enzyme-linked immunosorbent assay kit (Bio Vendor Laboratory Medicine, Brno, Czech Republic). For the receptor assay, standards, controls, and samples were diluted 1:3 with dilution buffer, then 100 μL diluted standard, controls, and samples were pipetted into 96-well microtiter plates coated with antileptin receptor monoclonal antibody. After incubation at room temperature for 1 hour, the wells were washed and incubated for 1 hour with horseradish peroxidase–labeled monoclonal antibody. The wells were again washed and incubated for 5 minutes with tetramethylbenzidine reagent. Then 100 μL of 0.2 mL per liter H_2SO_4 was added to each well to stop the reaction and the absorbance at 450 and 655 nm was measured. The limit of detection was 0.4 U recombinant leptin receptor per milliliter sample. The standard material used in this kit was recombinant human IgG–Fc fragment human leptin receptor dimeric chimera, which is different from the native SLR that was measured in serum; 2 ng of the recombinant standard is equivalent to 1 U soluble native human leptin receptor. Intra- and interassay coefficients of variation were 2.6% to 4.7% and 6.3% to 7.2%, respectively. Minimum detection limits for SLR were 2 U/mL (4 ng/mL). The interassay coefficient of variation was 5.4%, and the intra-assay coefficient of variation was less than 5%. Insulin serum levels were evaluated by using a solid-phase ^{125}I radioimmunoassay (Diagnostic Products, Billerica, MA). Analytical sensitivity was 1.2 μIU /mL; intra- and interassay precision was 3.1% and 4.9%, respectively. LH, FSH, prolactin, estradiol, progesterone, testosterone, androstenedione, DHEAS, and GH serum concentrations were evaluated in all samples with a radioimmunoassay kit (Nichols Institute Diagnostic, San Juan Capistrano, CA); intra- and interassay coefficients of variation were less than 8% and 15%, respectively.

Table 1
Clinical and hormonal features of the 4 study groups

	DS (n = 7)	PWS (n = 5)	Obese controls (n = 7)	Normal-weight controls (n = 7)
Age (y)	22.7 ± 5.6	20.4 ± 7.4	36.3 ± 4.0	30.4 ± 9.9
BMI (kg/m^2)	29.00 ± 1.53	31.4 ± 5.03	29.7 ± 2.04	21.30 ± 3.07 *
FSH (IU/mL)	5.13 ± 3.08	6.75 ± 1.52	9.26 ± 3.30	8.11 ± 3.07
LH (IU/mL)	5.52 ± 2.68	3.54 ± 1.81	6.06 ± 2.81	6.37 ± 2.56
Androstenedione (ng/mL)	1.46 ± 0.65	1.19 ± 0.55	1.65 ± 0.75	2.11 ± 0.74
Testosterone (ng/mL)	0.41 ± 0.27	0.38 ± 0.10	0.31 ± 0.22	0.43 ± 0.24
DHEAS (μg/mL)	1.80 ± 0.39	1.36 ± 0.33	1.82 ± 0.83	1.97 ± 0.97
Prolactin (μg/L)	16.45 ± 8.29	11.03 ± 4.44	12.72 ± 3.87	17.15 ± 5.61
Estradiol (pg/mL)	41.13 ± 3.60	42.28 ± 6.85	37.14 ± 7.49	40.14 ± 9.93
Progesterone (ng/mL)	0.96 ± 0.46	0.59 ± 0.30	0.36 ± 0.09	0.68 ± 0.27
Insulin (μUI/mL)	10.31 ± 6.92	10.47 ± 4.74	8.46 ± 4.5	8.71 ± 3.24

* $P < .01$ vs other groups.

Data were stored and analyzed using the Statistical Package for Social Sciences release 5.0 (SPSS, Chicago, IL) on an IBM-compatible computer. The Kolmogorov-Smirnov test was performed to assess differences in the general slopes of distribution. The means and standard deviation were calculated, and the individual parameters detected in the study groups were compared using 1-way analysis of variance. The significance of differences between the study groups was confirmed by the Wilcoxon-Mann-Whitney test. P values less than .05 were regarded as significant. The relationships between the individual parameters were evaluated with the Spearman correlation test.

3. Results

The clinical and hormonal characteristics of the 4 groups of patients are presented in Table 1. According to the study design, the mean value of BMI was lower in the normal-weight control group ($P < .01$ vs other groups). No statistically significant differences were observed in the hormonal milieu.

As shown in Table 2, obese control subjects showed significantly higher total leptin values and FLI than did lean controls ($P < .01$ for both comparisons).

Obese women affected by DS exhibited leptin plasma concentrations significantly lower than those found in the control subjects with comparable BMI ($P < .01$). Because leptin receptor levels of these 2 groups were very similar, the mean FLI values resulted much higher in obese controls compared with women with DS ($P < .01$). Interestingly, this last group showed a profile resembling that observed in the normal-weight control subjects: we were not able to observe any significant difference either in total and free leptin levels or in leptin receptor concentrations between these 2 metabolically heterogeneous types of patients.

This peculiar assessment of leptin secretion and action was not evidenced in PWS. In accordance with their degree of obesity, patients with PWS showed high levels of total leptin, low levels of leptin receptors, and, consequently, elevated levels of free leptin. Such data matched those obtained from the obese control group and the 3 evaluated parameters gave significantly different results when compared with both normal-weight control and DS subjects ($P < .01$ for the all the contrasts, except $P < .05$ for leptin receptors in DS vs PWS).

4. Discussion

Except for rare genetic mutations leading to leptin deficiency, human obesity is characterized by high circulating leptin levels, which are unable to exert an adequate anorexigenic effect. This condition is known as leptin resistance and seems to play a paramount role in the pathogenesis of obesity. The mechanisms underlying leptin resistance are still not clarified, although a defect in protein transport across the blood-brain barrier as well as a decreased hypothalamic signaling may have a putative role [29].

Physiologic leptin effects are mediated by the interaction with specific receptors (LRs) belonging to the class I cytokine receptor superfamily [30,31]. The shortest isoform of LRs, which lack the hydrophobic transmembrane domain, is probably responsible for the transport of leptin across the blood-brain barrier and the production of circulating LR extracellular domain to complex with leptin [23,32-34]. The secreted form contains only the extracellular domain that binds circulating leptin and is probably involved in the

Table 2
Leptin profiles in the 4 study groups

	DS (n = 7)	PWS (n = 5)	Obese controls (n = 7)	Normal-weight controls (n = 7)
Total leptin (ng/mL)	16.33 ± 7.26	37.36 ± 3.73 [a, b]	33.38 ± 7.75 [a, b]	11.04 ± 5.41
LR (IU/mL)	18.43 ± 4.46	12.80 ± 2.61 [a, c]	15.30 ± 7.05	19.38 ± 2.77
FLI	0.98 ± 0.61	2.85 ± 0.37 [a, b]	2.49 ± 1.39 [a, b]	0.57 ± 0.28

[a] $P < .01$ vs normal-weight controls.
[b] $P < .01$ vs subjects with DS.
[c] $P < .05$ vs subjects with DS.

regulation of the free, therefore active, fraction of the protein [34].

The main finding of the present study is that leptin levels in obese patients with DS are in the range of normal-weight controls and significantly lower than those found in both obese controls and subjects with PWS. This tendency concerns either total and free leptin levels, as no significant differences were found in soluble receptor levels between DS subjects and control groups. These results seem to give evidence against a deficit in receptor synthesis in DS. Furthermore, previous in vitro studies evidenced that subcutaneous adipose tissue secretes FL and BL in the same proportion both in lean and in obese subjects [35]. Hence, the observation of low leptin levels in obese individuals affected by DS could depend on an impaired secretory activity of adipocytes. Because leptin is released by the fat mass, it should be borne in mind that, BMI being similar, DS patients could have a lower percentage of fat compared with control subjects. However, previous studies have documented a normal body composition in persons with DS [36,37], and some authors even found a higher percentage of body fat in subjects with DS [38], thus remarking on the peculiarity of the lower leptin levels found in this group of patients.

Data from the literature suggest that the origin of the decrease of leptin release in DS could be identifiable already during the intrauterine life. Fetuses with DS, in fact, have significantly lower plasma leptin levels than control euploid fetuses matched for gestational age, leading to the hypothesis that damage beginning from this stage may compromise the synthesis and secretion of this protein [39]. Although an impaired secretory activity of the adipocytes might be the most likely physiopathologic mechanism, a defect in the connection system between the central mechanism and adipocytes could take place in subjects with DS.

The percentage of body fat is well recognized as the main regulator of the absolute leptin levels and of the ratio between the bound and free form: human obesity is characterized by a relative deficiency of bound leptin and a predominant increase of the free form of the protein [40]. Nevertheless, other factors are able to influence this parameter and could be responsible for the low leptin serum concentrations found in our patients with DS. Among these factors, the hormonal environment is considered an important regulator of the adipocyte endocrine function. Testosterone, for instance, exerts an inhibitory activity over leptin secretion [41], whereas estrogens are considered positive regulators of its levels in women [42]. Before puberty, leptin values are very low both in normal and in DS children [43,44], thus exerting a permissive effect on the onset of puberty and reproduction [45]. To rule out possible confounding factors related to the menstrual cycles, in this study, all women were evaluated during the follicular phase. In accordance with previous reports in the literature [46], we did not observe significant differences in the hormonal environment of women with DS compared with control subjects. Based on the evidence, it seems unlikely that a disturbance in the hormonal pattern may account for the low leptin levels seen in our DS patients. Moreover, the FL and BL partitioning were previously demonstrated to be independent of the endocrine milieu in DS [44], relying exclusively on the presence of excessive body fat.

Hyperphagia, obesity, and hypogonadism observed in PWS are also features of subjects with leptin deficiency and leptin receptor defects [47-49], suggesting that defects in leptin pathways in the brain may account for these stigmata in PWS patients. In accordance with this hypothesis, a similar proclivity to disturbed eating behavior and obesity was found in subjects with hypothalamic damage, although in the presence of markedly high free leptin levels [50]. In a previous study, we reported about 3-fold higher circulating leptin values in PWS compared with DS [25]. Such results are confirmed by present data documenting that women affected by PWS as well as obese controls exhibit leptin values comparable to those of nonsyndromal obesity: all components, LR, FL, and total leptin are influenced by the degree of adiposity. In line with several reports in the literature [25-28], the production of leptin as well as of its soluble receptor seems to be in the normal range of physiology in PWS. As in most cases of nonsyndromal human obesity, a state of leptin resistance could account for the hyperphagia and massive adiposity of these subjects.

Concerning the intricate question of the endocrine regulation of energy balance, several other factors, such as ghrelin, neuropeptide Y, and agouti-related protein, need to be investigated to gain insight into the metabolic disorders of these 2 complex syndromes.

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