

SHORT COMMUNICATION

Free and bound leptin in prepubertal children with Down's syndrome and different degrees of adiposity

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Objective: To evaluate plasma total, free (FL) and protein-bound (BL) leptin in children with Down's syndrome (DS) and different degrees of adiposity and its relationship with thyroid stimulating hormone (TSH), free thyroxine (FT₄), and free triiodothyronine (FT₃).

Subjects: A total of 24 prepubertal clinically euthyroid DS children.

Methods: Plasma leptin, TSH, FT₄, and FT₃ concentrations were determined by immunometric/radioimmunologic assays. FL and BL were evaluated by fast protein liquid chromatography.

Results: In DS children, leptin circulates in two fractions, corresponding to BL and FL. The amount of BL and FL is negatively and positively correlated to body mass index (BMI), respectively. Plasma leptin concentrations correlate with BMI, but not with TSH, FT₄, and FT₃.

Conclusions: In prepubertal DS children, leptin circulates as both BL and FL, correlates with adiposity and its concentration appears independent of thyroid function.

Sponsorship: MIUR, Università degli Studi di Milano, Banca Popolare di Milano Foundation.

European Journal of Clinical Nutrition (2004) 58, 1547–1549. doi:10.1038/sj.ejcn.1602000

Published online 26 May 2004

Keywords: free leptin; bound leptin; prepubertal children; Down's syndrome; thyroid function

Introduction

The adipose-borne hormone leptin participates in the regulation of food intake and energy metabolism by stimulating satiety and energy expenditure (Ahima *et al*, 2000). Increased plasma leptin concentrations have been reported in human obesity (Maffei *et al*, 1995), a condition more frequent in Down's syndrome (DS) than in normal subjects (Sharav & Bowman, 1992). Leptin concentrations correlate with adiposity and body mass index (BMI) in lean and obese subjects (Ahima *et al*, 2000), and in adult obese women with DS (Cento *et al*, 1999). Leptin circulates in two

fractions, free (FL) and protein-bound (BL) leptin, which is bound to a specific binding protein (sOB-R) (Sinha *et al*, 1996). A greater proportion of leptin circulates as BL in lean subjects, whereas, in obese subjects, FL is quantitatively the major plasma fraction (Sinha *et al*, 1996). The relationship between leptin and thyroid function is controversial in adults and has been rarely examined in children. In this study, we have evaluated the partitioning of plasma leptin between FL and BL and the relationship between leptin and thyroid function in DS prepubertal children.

Methods

In all, 24 prepubertal (Tanner stage 1) children with DS (10 females, 14 males; age: 2–14 y), diagnosed according to clinical and genetic evaluations, clinically euthyroid, and with a wide range of body mass index (BMI) (weight(kg)/height²(m²)), were studied. Informed parental consent was obtained. Plasma leptin concentrations were measured by radioimmunoassay (RIA) kit (Linco Research, St Louis, MO, USA). FL and BL were studied in six (five males, one female;

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Contributors: PM and MMC were responsible for the study concept and writing of the manuscript. MR and ED were responsible of the biochemical studies and the literature search. FL was responsible for the clinical management. ER and MM were responsible for the critical revision of the manuscript.

Received 12 November 2003; revised 3 March 2004; accepted 5 April 2004; published online 26 May 2004

classified as normal weight, overweight, and obese (Cole *et al*, 2000)) out of 24 DS children and not in the whole group, since this procedure requires relatively high amounts of plasma, that were difficult to collect for every DS child. FL and BL were studied using a FPLC system (Amersham Biosciences Italia, Milan, Italy), followed by RIA for leptin (Magni *et al*, 2000) and are expressed as both percent of the sum of the peak areas, and absolute values (ng), calculated as follows: FL (or BL)(ng) = total leptin (ng/dl)*%FL (or %BL)/100. Plasma TSH, FT₄, and FT₃ concentrations were determined by immunometric assays. Data were analyzed using the Systat statistical package.

Results

Analysis of FL and BL revealed the presence of two peaks of leptin-like IR in plasma samples of DS children (Figure 1, a–f)

and normal children (taken as reference; not shown). The first eluted peak (molecular weight (mw): ≥ 200 kDa) corresponds to BL, whereas the other peak corresponds to FL (mw: 16 kDa). FL fraction was lower than BL in normal weight DS children (subjects a–c), and, as BMI increased toward overweight (subjects d–e) and obesity (subject f), total plasma leptin and FL increased, relatively to BL. Percent BL was negatively correlated with BMI ($r = -0.83$; $P < 0.05$) and FL ($r = -0.87$; $P < 0.05$). Percent FL was negatively correlated with %BL ($r = -0.99$; $P < 0.001$).

The relationship between total leptin and thyroid hormones was studied in the remaining 18 DS children, classified as normal weight (five females, six males), overweight (four females, one male), and obese (two males) (Table 1). Leptin concentrations were low and no subjects had TSH levels below the lower limit of the normal range (0.3 mU/l); four females and two males had elevated serum

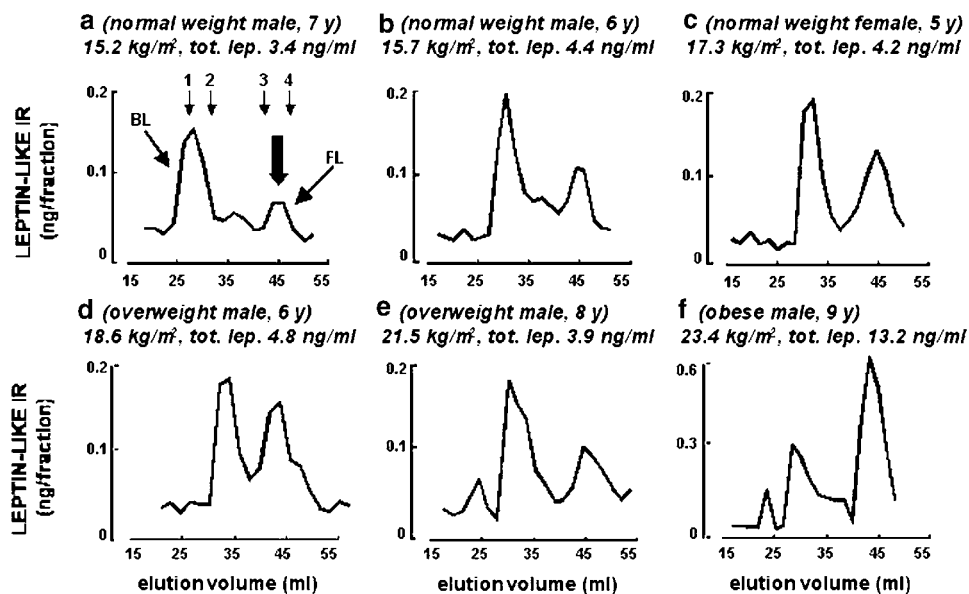


Figure 1 Fast protein liquid chromatography FPLC elution profiles of plasma leptin-like IR of six DS children. a, b, c correspond to normal weight subjects, d and e to overweight subjects, and f to an obese subject. Note the different ordinate scale of profile f. Individual age (y), BMI (kg/m^2) and total leptin are indicated. In panel a, the numbered arrows indicate the elution volumes of molecular weight markers: (1) Blue dextran (2000 kDa); (2) β -Amylase (200 kDa); (3) Carbonic anhydrase (29 kDa); (4) Cytochrome c (12.4 kDa). The thick arrow indicates the elution volume of recombinant human leptin (standard reference); the peaks relative to BL (in this case, 71% of the total leptin-like IR eluted) and FL (29%) are indicated.

Table 1 Age, BMI, total leptin and thyroid parameters of the DS children included in the study of leptin and thyroid function

	Age (y)	BMI (kg/m^2)	Leptin (ng/ml)	TSH (mU/l)	FT ₄ (pg/ml)	FT ₃ (pg/ml)
Females (n=9)						
Mean \pm s.d.	6.3 \pm 2.3	16.7 \pm 1.7	1.4 \pm 0.6	5.5 \pm 3.9	12.7 \pm 1.7	3.7 \pm 0.5
Range	3–10	14.3–19.1	0.3–2.5	1.4–13.4	11.2–15.6	2.7–4.5
Males (n=9)						
Mean \pm s.d.	8.3 \pm 3.8	17.4 \pm 3.4	1.6 \pm 1.4	4.3 \pm 1.2	11.5 \pm 2.0	3.7 \pm 0.4
Range	2–14	15.4–23.3	0.2–4.2	2.74–6.8	9.4–15.7	3.1–4.1

The female and the male groups did not differ for any parameter (ANOVA).

TSH concentrations (above 5.0 mU/l), with normal free thyroxine (FT₄) and free triiodothyronine (FT₃) values. No gender differences were found and BMI, thyroid stimulating hormone (TSH), FT₄, FT₃, and leptin values of all subjects were pooled. Leptin correlated with BMI ($r = 0.77$; $P < 0.001$), also after correction of leptin for BMI ($r = 0.67$; $P < 0.01$), but not with age, TSH, FT₄, and FT₃ values.

Discussion

This study reports plasma concentrations of total leptin and its partitioning in free and bound fractions in DS prepubertal children with different degrees of adiposity. Leptin values did not differ according to gender, in agreement with studies in normal prepubertal children (Garcia-Mayor et al, 1997), and were very low, falling in most cases below 1.85 ng/ml, a proposed threshold for the permissive effect of the hormone on puberty onset and reproduction (Ballauff et al, 1999). In DS children, leptin correlated with BMI, also after correction of leptin values by BMI, suggesting that fat mass might not be the only factor in determining plasma leptin concentrations at this age. To our knowledge, this is the first report on plasma FL and BL in prepubertal DS children, and, although the number of DS subjects is small because of methodological limitations, the results obtained may add new information on this topic. This study indicates that both BL and FL are present in the plasma of DS prepubertal children and that FL concentrations increase, relatively to BL, together with adiposity and become prevalent once obese levels are reached, like in obese adults (Sinha et al, 1996; Magni et al, 2000). The significance of FL and BL partitioning is not completely understood: at least in adults, BL correlates with resting energy expenditure, whereas FL reflects adiposity (Brabant et al, 2000). The observation of elevated plasma sOB-R levels concentrations in children (Kratzsch et al, 2002) and the increased BL fraction, here reported, may contribute to the temporary blockade of the reproductive axis in prepubertal children, which may be overcome, once an increase of FL occurs, in association with an increased adiposity and possibly other factors, giving rise to pubertal development. No relationship between thyroid function and leptin concentrations were found in DS children, as also shown in subjects with congenital hypothyroidism (Asami et al, 2000) and obesity (Reinehr & Andler, 2002), whereas an inverse correlation between leptin and TSH was found in normal children of short stature (Ghizzoni et al, 2001). These discrepancies are similar to those observed in adults with or without thyroid diseases (Corbetta et al, 1997; Valcavi et al, 1997; Korbionits, 1998). In summary, in DS prepubertal children, leptin is correlated with the degree of adiposity and circulates as BL and FL. Moreover, the lack of correlation between TSH, FT₄, and FT₃ and leptin suggests that the latter is independent of thyroid function in these subjects.

Acknowledgements

We thank Ms Paola Assi and Giovanna Miccichè for expert technical collaboration.

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