Serum Leptin and Leptin Binding Activity in Children and Adolescents with Hypothalamic Dysfunction

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ABSTRACT

Marked disturbance in eating behaviour and obesity are common sequelae of hypothalamic damage. To investigate whether these were associated with dysfunctional leptin central feedback, we evaluated serum leptin and leptin binding activity in 37 patients (age 3.5-21 yr) with tumour or trauma involving the hypothalamic-pituitary axis compared with 138 healthy children (age 5.0-18.2 yr). Patients were subdivided by BMI <2 SDS or >2 SDS and healthy children and children with simple obesity of comparable age and pubertal status served as controls. Patients had higher BMI (mean 1.9 vs 0.2 SDS; $p <0.001$), a greater proportion had BMI >2 SDS (54% vs 8%; $p <0.001$) and higher serum leptin (mean 2.1 vs 0.04 SDS; $p <0.001$) than healthy children. Serum leptin (mean 1.1 vs -0.1 SDS; $p = 0.004$) and values adjusted for BMI (median 0.42 vs 0.23 µg/l:kg/m^2; $p = 0.02$) were higher in patients with BMI <2 SDS. However, serum leptin adjusted for BMI was similar in patients with BMI >2 SDS compared to corresponding controls (1.08 vs 0.95; $p = 0.6$). Log serum leptin correlated with BMI SDS in all subject groups but the relationship in patients with BMI <2 SDS was of higher magnitude (r = 0.65, slope = 0.29, p =0.05 for difference between slopes) than in healthy controls (r = 0.42, slope = 0.19). Serum leptin binding activity (median 7.5 vs 9.3%; $p = 0.02$) and values adjusted for BMI (median 0.28 vs 0.48 %.m^2/kg; $p <0.001$) were lower in patients than in healthy children. The markedly elevated leptin levels with increasing BMI in non-obese patients with hypothalamic-pituitary damage are suggestive of an unrestrained pattern of leptin secretion. This along with low leptin binding activity and hence higher free leptin levels would be consistent with central leptin insensitivity.

KEY WORDS

INTRODUCTION

Leptin, generated in adipocytes, is believed to reduce appetite by a central action on the hypothalamus and suppression of its primary appetite transducer, neuropeptide Y (NPY)[1]. The unregulated appetite and body weight gain commonly seen in children with hypothalamic injury[2,3] may be explained by dysfunctional leptin central feedback. Previous studies have reported an increase in serum leptin in parallel with the postoperative increase in body mass index (BMI) in patients with craniopharyngioma[4,5]. A further study showed higher leptin concentrations in relation to BMI in patients with suprasellar craniopharyngioma compared to those with an intrasellar tumour[6]. Leptin binding activity, which corresponds to the extracellular domain of the leptin receptor, reflects the amount of circulating free leptin available for inhibitory feedback. High serum

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leptin concentrations[7] but lower leptin binding activity in obese compared with lean subjects suggests that the obese have higher free levels of leptin[8-10]. However, as this does not lead to decreased caloric intake and increased energy expenditure, it is likely that obese subjects are insensitive to endogenous leptin. Serum leptin binding activity has not previously been investigated in patients with hypothalamic lesions.

The aim of this study was to evaluate (1) serum leptin and leptin binding activity in children with tumour or trauma involving the hypothalamic-pituitary axis, and (2) the influence of body composition and treatment-related factors on these biochemical variables.

PATIENTS AND METHODS

All patients (n = 37; median age 14.4 yr, range 3.5-21 yr; 15 female) under our care, aged <21 years excluding one very young patient (age 0.8 yr), with a sellar or suprasellar lesion (28 craniopharyngioma, 4 germinoma, 1 optic nerve glioma, 2 astrocytoma, 2 head trauma) were studied. Of these, all had had cranial surgery and 24 had also received radiotherapy. Patients with pituitary hormone deficiency (Table 1) were receiving appropriate hormone replacement (median doses: GH 16.8 IU/m^2/wk; hydrocortisone 9.7 mg/m^2/d; thyroxine 63.1 μg/m^2/d; desmopressin 107.1 μg/d; increasing doses of ethinyloestradiol or testosterone esters in adolescent patients) and were under review in a regional centre. Twelve of the 14 females and 18 of the 23 males were pubertal. Patients were categorised according to pubertal status (breast development for females and genital development for males) as prepubertal (B1 or G1) and pubertal (Tanner stages B2-5 or G2-5). Height, weight, four skinfold measurements (triceps, biceps, supra-iliac and sub-scapular), serum leptin and leptin binding activity were assessed at a median time of 5.8 yr (range 0.2-18 yr) after diagnosis or cranial surgery. BMI (weight/height^2), fat mass (from summed skinfold thicknesses)[11], fat free mass (weight - fat mass) and percent body fat (fat mass expressed as a percent of weight) were calculated. Height, weight and BMI were converted to standard deviation scores (SDS) using 1990 UK standards[12,13]. Blood samples for serum leptin and leptin binding activity were taken from 2-4 h after a meal, coincident with thyroid function monitoring.

Patients were compared with 138 local healthy school children (age 5.0-18.2 yr). The latter were selected to obtain a representative distribution of males and females and children at different stages of puberty. Those with chronic medical conditions or in receipt of regular medication were excluded. However, the distribution of BMI SDS differed significantly between patients and these healthy children. Thus for further analysis patients were subdivided by BMI <2 SDS or >2 SDS. For comparison to these subgroups, a group of healthy children and children with simple obesity matched for age and pubertal status whose BMI was in a similar range served as controls (Table 2). The children with simple obesity were identified from all patients referred to the endocrine unit for obesity (BMI >2 SDS) and did not have a hypothalamic-pituitary lesion, Prader-Willi syndrome, Cushing's syndrome or other recognised obesity syndrome.

TABLE 1

Details of pituitary hormone deficiencies in patients grouped by diagnosis

	Hormone deficiency				
	GH	ACTH	TSH	LH, FSH	ADH
Craniopharyngioma (n = 28)	28	26	28	15*	24
Other tumour (n = 7)	5	5	5	3*	3
Trauma (n = 2)	2	2	2	2*	2

* Excluding prepubertal patients

TABLE 2

Clinical characteristics and biochemical values of patients with hypothalamic lesions
subdivided by BMI <2 SDS or >2 SDS and compared with healthy and obese children

	Patients	Healthy children	p
a. Body mass index <2 SDS			
total number	17	57	–
number of males	13	39	0.5
number pubertal	14	50	0.6
median age yr (IQR)	14.6 (10.9 to 17.2)	13.4 (11.6 to 15.3)	0.3
mean body mass index SDS (SD)	0.9 (0.9)	0.4 (0.7)	0.02
median serum leptin µg/l (IQR)	9.2 (4.4 to 17.6)	4.5 (2.3 to 9.4)	–
mean log serum leptin (SD)	0.94 (0.39)	0.67 (0.34)	0.02
mean serum leptin SDS (SD)	1.1 (1.3)	-0.1 (0.9)	0.004
median serum leptin/BMI µg/l:kg/m^2 (IQR)	0.42 (0.27 to 0.72)	0.23 (0.13 to 0.45)	0.02
log serum leptin vs body mass index SDS	r = 0.65 r^2 = 38% slope = 0.29 p = 0.05	r = 0.42 r^2 = 16% slope = 0.19 p = 0.001	0.03
b. Body mass index >2 SDS			
total number	17	28	–
number of males	8	14	0.8
number pubertal	13	18	0.4
median age yr (IQR)	13.8 (9.2 to 15.6)	12.6 (9.6 to 13.7)	0.1
mean body mass index SDS (SD)	2.6 (0.6)	3.1 (0.7)	0.03
median serum leptin µg/l (IQR)	26.9 (18.4 to 47.6)	26.8 (16.0 to 53.4)	–
mean log serum leptin (SD)	1.46 (0.29)	1.44 (0.36)	0.9
mean serum leptin SDS (SD)	2.9 (1.3)	6.4 (6.9)	0.01
median serum leptin/BMI µg/l:kg/m^2 (IQR)	1.08 (0.72 to 1.57)	0.95 (0.63 to 1.35)	0.6
log serum leptin vs BMI SDS	r = 0.68 r^2 = 43% slope = 0.35 p = 0.003	r = 0.66 r^2 = 42% slope = 0.34 P <0.001	0.4

Values shown are mean or median and standard deviation (SD) or interquartile range (IQR).

Biochemical assays

Serum leptin concentrations were determined using a commercial radioimmunoassay (Linco, St Charles, MO, USA). The limit of detection of the assay was 0.5 μg/l. The intra- and interassay coefficients of variation (CVs) ranged from 3.4-8.3% and 3.6-6.2%, respectively, over a leptin concentration range of 4.9-25.6 μg/l. Values were log-transformed prior to analyses and also expressed as age, gender and puberty specific SD scores using reference values obtained from our local population of 235 healthy children (age 4.9-18.4 yr; 125 female)[14]. Serum leptin values in healthy adolescents at genitalia/breast stage 5 do not differ significantly from those in young adults aged 18-21 years and were therefore used to construct SD scores for the three patients older than 18.4 years[15].

Serum leptin binding activity was measured by an assay specific for high-affinity binding proteins and based on a method for growth hormone binding protein[16]. Serum was stripped of endogenous free leptin by preincubation with dextran-coated charcoal. Stripped serum was incubated with buffer, [^{125}I]-leptin, in the presence (nonspecific) or absence (total binding) of unlabeled leptin, and the supernatant was analysed in an automatic gamma counter. The specific binding (total binding minus nonspecific binding) obtained was expressed as a percentage of the total [^{125}I]leptin counts per minute incubated in 50 μl of serum and called leptin binding activity. The assay had a sensitivity of 0.6% specific binding. At 12 and 6% specific binding, the intra-assay CV was 3.2 and 4.1%, and the interassay CV was 6.4 and 4.8%, respectively.

Statistical analysis

Normally and non-normally distributed variables were compared using Student's *t*-test or Mann-Whitney *U* test, respectively. Values at different puberty stages were examined by analysis of variance. Pearson correlations and regression analysis were performed to investigate relationships. Backward regression analysis was used to assess the effects of independent variables (% body fat, BMI SDS, age, pubertal stage, time from diagnosis, treatment, use of radiotherapy and leptin binding activity) on leptin concentration. A p value <0.05 was considered significant.

RESULTS

Body mass index and body fat

Patients had significantly higher BMI than healthy children (mean 1.9 vs 0.2 SDS; p <0.001). Of the 37 patients, only two (5%) had BMI SDS <0 while 20 (54%) had BMI SDS ≥2.0. BMI did not differ between males (mean 1.8 SDS, SD 1.0) and females (mean 1.7 SDS, SD 1.6). Skinfold measurements could not be obtained in five patients due to extreme obesity. The correlation between BMI and body fat derived from summed skinfolds was high (r = 0.91, r² = 83%, p <0.001). Body fat was elevated in male and female patients compared to reported values in healthy children (males: mean 33.5 vs 13.2%, p <0.0001; females: mean 32.2 vs 19.8%, p = 0.02)[10].

Serum leptin and serum leptin adjusted for BMI

Serum leptin levels were higher in patients than in healthy children (mean 2.1 vs 0.04 SDS; p <0.001). Levels in patients did not differ between males and females (mean 2.3 vs 1.9 SDS). Owing to the differences in BMI SDS between patients and controls, serum leptin was adjusted for BMI and these values were also higher in patients (median 0.73 vs 0.26 μg/l:kg/m²; p <0.001) (Fig. 1). However, in the analysis of subgroups this difference was only observed between patients and controls with BMI <2 SDS but not in those with BMI >2 SDS (Table 2).

Relationship between log serum leptin, BMI SDS and other variables

Log serum leptin correlated significantly with BMI SDS (r = 0.81, r² = 64%, p <0.001) and body fat % (r = 0.76, r² = 54%, p <0.001) in patients. The relationship between log serum leptin and BMI SDS was of higher magnitude in patients (r = 0.65, r² = 38%, slope = 0.29) than in controls (r = 0.42, r² = 16%, slope = 0.19; p = 0.03 for difference between slopes) in the subgroups with BMI <2 SDS,
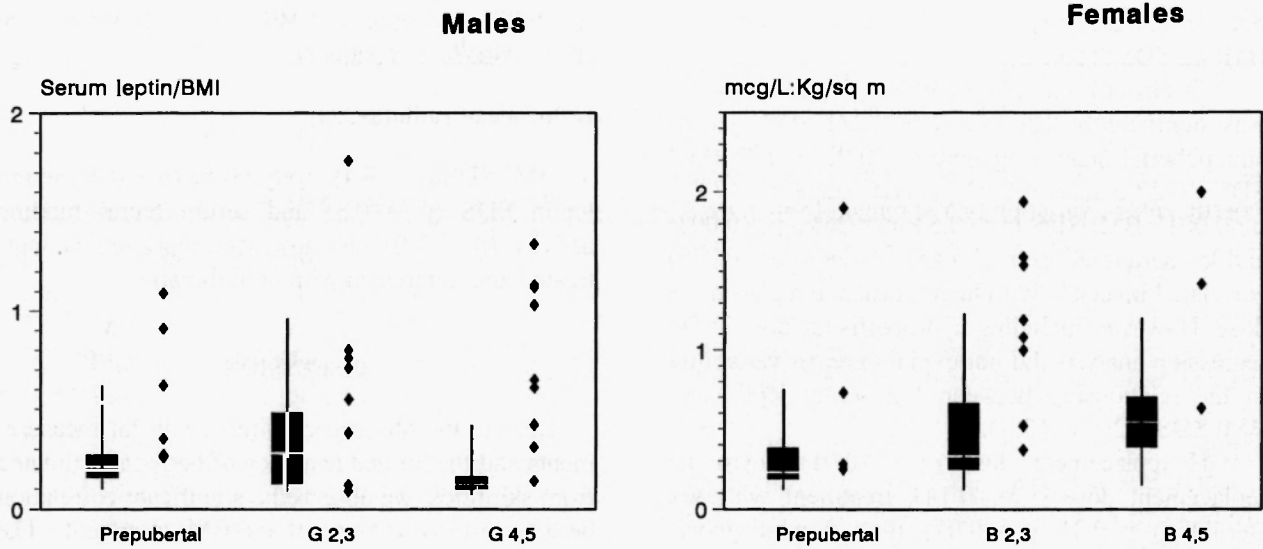
Males

Serum leptin/BMI

Females

mcg/L:Kg/sq m

Fig. 1: Serum leptin adjusted for BMI ($\mu g/l:kg/m^2$) at different pubertaal stages (genitalia and breast development for males and females, respectively) in patients compared with those from healthy children. Box-whisker plots represent median, inter-quartile range and range for healthy children, and points represent values for individual patients. Values for patients were higher than for healthy children (p <0.001; 95% CI 0.27 to 0.60).
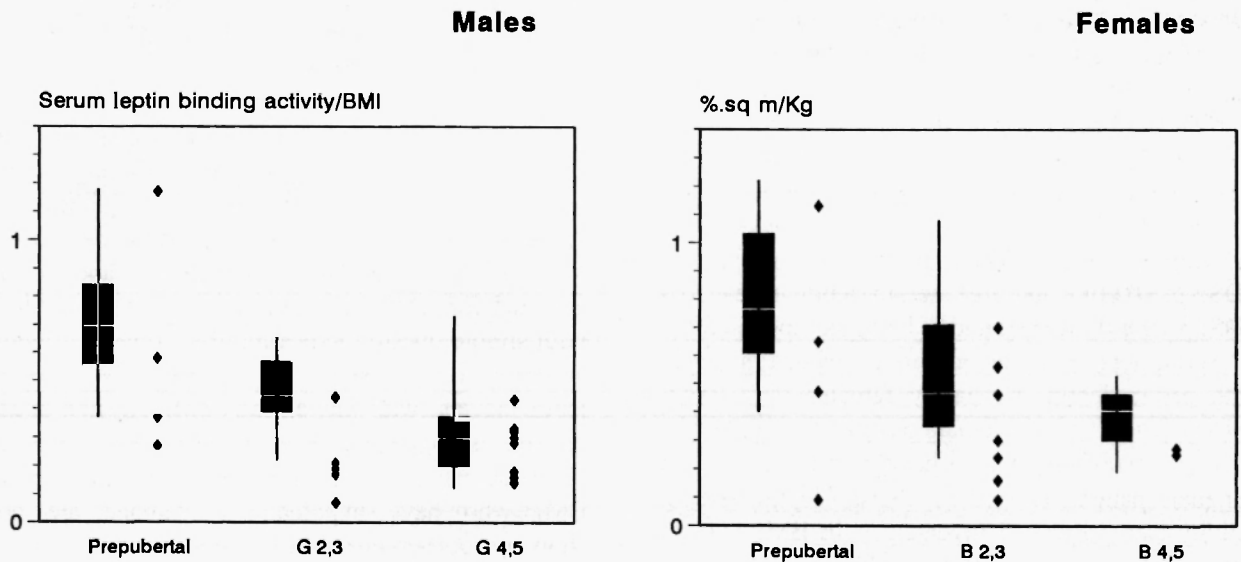
Males

Serum leptin binding activity/BMI

Females

%.sq m/Kg

Fig. 2: Serum leptin binding activity adjusted for BMI (%.m^2/kg) at different pubertal stages (genitalia and breast development for males and females, respectively) in patients compared with those from healthy children. Box-whisker plots represent median, inter-quartile range and range for healthy children, and points represent values for individual patients. Values for patients were lower than for healthy children (p <0.001; 95% CI 0.29 to -0.11).

but did not differ (p = 0.4) in the subgroups with BMI >2 SDS (Table 2).

In backward multiple regression, log serum leptin was significantly determined by BMI SDS, gender and pubertal stage (log leptin = 0.97 + 0.29 BMI SDS - 0.29 gender + 0.07 pubertal stage; r^2 = 70%, p <0.001). BMI SDS (r = -0.53, r^2 = 26%, p = 0.002) and log serum leptin (r = -0.45, r^2 = 18%, p = 0.009) correlated inversely with hydrocortisone replacement dose. However, including hydrocortisone dose in the regression analysis did not explain greater variability in the relationship between log serum leptin and BMI SDS (62% vs 63%).

GH replacement dose (r = -0.01), thyroxine replacement dose (r = -0.14), treatment with sex steroids (r = 0.31, p = 0.07), time from diagnosis (r = 0.22) and use of radiotherapy (r = 0.24) had no significant influence on serum leptin. Log serum leptin did not correlate significantly with height SDS (r = 0.35).

Serum leptin binding activity

Serum leptin binding activity (median 7.5 vs 9.3%; p = 0.02) and values adjusted for BMI (median 0.28 vs 0.48 %.m²/kg; p <0.001) were lower in patients than in healthy children (Fig. 2). Serum leptin binding activity was lower in pubertal (median 6.9%) than prepubertal patients (median 10%; p = 0.01) and also in pubertal (median 7.5%) than prepubertal healthy children (median 11.8%; p <0.001). Bound and free serum leptin were estimated from measured values of serum leptin and leptin binding activity and expressed as age-, gender- and puberty-specific SD scores using values estimated from healthy children. Both bound (mean 5.5 SDS; p <0.001) and free serum leptin (mean 6.0 SDS; p <0.001) were found to be higher in patients.

Serum leptin binding activity correlated inversely with serum leptin in healthy females (r = -0.42, r² 16%, p <0.001) and female patients (r = -0.73, r² = 49%, p = 0.005) but not in healthy males (r = 0.03) nor male patients (r = -0.12). Serum leptin binding activity also correlated inversely with BMI SDS in female patients (r = -0.69, r^2 = 43%, p = 0.009) but not male patients (r = 0.06). The significant inverse relationship between serum leptin binding activity and age-puberty subgroups in healthy children

(p <0.001 and values at B/G 2 to 5 lower than at B/G 1) was lost in patients.

Influence of radiotherapy

BMI SDS (p = 0.4), body fat % (p = 0.4), serum leptin SDS (p = 0.3) and serum leptin binding activity (p = 1.0) did not differ between patients treated and not treated with radiotherapy.

DISCUSSION

Despite the absence of direct body fat measurements and the limited accuracy of body fat estimated from skinfolds, we observed a significant correlation between estimated body fat and BMI in patients. The altered body composition in this group of patients with hypothalamic-pituitary lesions was associated with elevated leptin concentrations, in keeping with previous observations[4-6]. Possible explanations for elevated leptin concentrations include unrestrained secretion from defective negative feedback mechanisms or reduced leptin clearance. The latter may be influenced by leptin binding activity and associated with more leptin being bound or by hormone replacement treatment. In our study, we found no evidence in support of either of these. Within the group of 37 patients, 54% had BMI SDS ≥2.0. Nevertheless, the remainder had a BMI within the normal range. The relationship between leptin and BMI SDS in these patients had a steeper slope than that seen in normal children. This indicates that non-obese patients with hypothalamic damage were generating more leptin for each unit of BMI than controls with the same BMI. For those with BMI >2.0, however, leptin concentrations and levels adjusted for BMI were similar to those in children with simple obesity, indicating that control of leptin secretion is comparable. The markedly elevated leptin levels with increasing BMI in non-obese patients is suggestive of an unrestrained pattern of leptin secretion. Thus those children with normal BMI who have hypothalamic damage are not showing appropriate control of leptin.

Exogenous glucocorticoid administration is known to increase leptin levels and have an inhibitory effect on central leptin action. However, hydrocortisone replacement for ACTH deficiency is

not likely to explain the high leptin levels in our patients as they showed an inverse relationship between hydrocortisone dose and leptin concentration. One explanation for this may be relative underdosing in the obese with a replacement dose based on surface area. GH deficiency (GHD) was not likely to account for the high leptin levels in our patients as all were on appropriate replacement treatment and levels were higher than those previously observed in untreated and treated patients with GHD[17]. Adolescent patients with gonadotrophin deficiency received treatment with ethinyloestradiol or testosterone esters. Although the timing and dosage increments of this treatment may be far from physiological it did not have a significant effect on serum leptin concentrations. However, owing to the small number of patients involved in analysis of subgroups by treatment as well as gender, pubertal status and BMI, caution is required in interpreting our results as a type II error cannot be excluded.

Obesity, predominantly in females[18] and associated with hyperleptinaemia[19], has been reported in young adults with hypothalamic damage following cranial irradiation for childhood leukaemia. A proposed explanation for the leptin resistance in these patients is damage to hypothalamic neurons which mediate leptin action. Simple obesity has also been considered a state of leptin resistance, in which increased body fat is matched by increased leptin. The leptin resistance is not due to increased binding of leptin to circulating proteins, and levels of free leptin are higher in obese individuals[8-10]. From our results, this situation appears to hold in patients with hypothalamic damage. Serum leptin binding activity for BMI, not previously reported with hypothalamic damage, was low in our patients. This in conjunction with high leptin levels indicates increased levels of free leptin and lends further support to the central leptin resistance theory. The mechanism for reduction in leptin binding activity in these children is not clear. However, low leptin binding activity when adjusted for BMI implies that the central hypothalamic damage may be having an effect on the peripheral generation of soluble truncated leptin receptor.

Investigation of serum and cerebrospinal fluid leptin concentrations suggests that leptin enters the brain by a saturable transport system[20]. The mecha-

nism of leptin resistance in simple obesity is not yet understood but one possibility is reduced efficacy of brain leptin transport[21,22]. Leptin transport into the cerebrospinal fluid in patients with hypothalamic dysfunction has not been investigated. However, it may be postulated that the saturable mechanism operates, but that leptin which is available in the central nervous system has no hypothalamic target, as this has been destroyed by tumour, surgery and/or radiotherapy. Some support for this theory comes from the reported association between the severity of hypothalamic damage identified on MRI scanning after surgery for craniopharyngioma and weight gain[23]. All tumour patients had had cranial surgery and we therefore were not able to examine the effect of the lesion itself or of radiotherapy alone. However, we did not find any differences in serum leptin concentrations between those who had had surgery alone compared to surgery and radiotherapy.

NPY is the primary physiological appetite transducer in the hypothalamus and is reduced in rodent models of hyperphagia and obesity experimentally induced by lesions in and around the hypothalamus[24]. Disturbances in NPY expression and signalling remain to be investigated in patients with hypothalamic lesions associated with tumours, surgery, radiotherapy or trauma. Modulation of the leptin-hypothalamic-adipose tissue axis to improve leptin sensitivity or alter NPY secretion, in order to affect satiety and reduce obesity, may not be achievable in patients with structural damage to the hypothalamus.

Hyperinsulinaemia associated with hyperphagia and obesity has been reported in patients with craniopharyngioma[5,25] and other hypothalamic insults[26]. Although desirable, serum insulin levels were not measured in our patients, and information about calorie intake and energy expenditure was not available. Increased secretion of insulin owing to disinhibition of vagal tone to pancreatic β-cells, secondary to hypothalamic damage, has been proposed as another explanation for hypothalamic obesity[26]. Reduction in insulin secretion was associated with weight loss and fall in leptin levels in a study of eight children with hypothalamic obesity treated with octreotide[26]. Treatment with this somatostatin receptor agonist analogue which attenuates insulin secretion requires further study.

We conclude that children with hypothalamic damage have a significant incidence of obesity. Despite raised serum leptin levels, they retain the relationship between leptin and BMI SDS. This along with low leptin binding activity suggests that leptin secretion in those with hypothalamic damage increases unchecked by any central negative feedback.

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