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Serum leptin levels are not influenced by arginine and insulin infusion and by acute changes of GH

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ABSTRACT. The aim of this study was to evaluate the relationship between GH and leptin in a group of short children and adolescents. Leptin and GH serum levels were measured before and during pharmacological stimulation tests (arginine and insulin) in a group of 45 children (30 male, 15 female), mean age 8.6±3.9 yr, affected by idiopathic isolated GH deficiency (GHD), and in a group of 27 children (15 male, 12 female), age 10.9±3.3 yr, with constitutional growth delay. Results showed that basal and peak leptin levels as well as the AUC were significantly higher in GHD patients compared to controls ($p<0.05$) and correlated with BMI SDS ($p<0.0001$) in GHD patients. No change in leptin serum levels was observed during either stimulation test. No correlation was found, however, between basal leptin serum levels and basal, peak and the AUC of GH during the tests. Moreover, no correlation was found between the acute changes of serum GH concentration during both stimulation tests and leptin serum levels. The results suggest that leptin and GH secretion is not correlated and that leptin serum levels mainly reflect the amount of fat tissue, which is higher in GHD patients.

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INTRODUCTION

It is becoming clear that leptin should no longer be considered as just a hormone modulating appetite control and energy expenditure, but rather as an hypothalamic neuromodulator controlling the secretion of many pituitary hormones (1). Leptin has been shown to play a major role in sexual development in both animals (2) and humans (3, 4), and to influence spontaneous GH secretion in rats (5) and IGF-I gene expression in the liver, independently of GH (6).

It has been suggested that leptin could inhibit GH secretion in humans, the latter being inversely correlated to body fat (7). Moreover, an inverse relationship between leptin and GH has been demonstrated (8), although there is no direct evidence of the role of leptin in inhibiting GH secretion. Whether GH can influence serum leptin levels is still a matter of debate. Sudden changes of GH following arginine infusion do not seem to alter leptin serum levels (9), while acute GH treatment led to an increase in leptin levels after 24 h in elderly (10) and young patients affected by GH deficiency (GHD) (11), followed by a significant decrease after 72 h (12). Chronic GH treatment has been shown to reduce leptin levels in GHD children (13) and to have no effect in elderly GHD patients (10).

The aim of our study was thus to verify, in a group of 45 children affected by GHD and in 27 controls, 1) whether a basal leptin value could predict the response of GH to a pharmacological stimulus (arginine or insulin); 2) the relationship between GH and leptin serum levels during the test; and 3) the effect of arginine and insulin infusion on leptin serum values.

MATERIAL AND METHODS

Subjects

Seventy-two children were investigated because of short stature: 45 of them (30 boys, 15 girls) were considered to be GHD, while the remaining 27 (15 boys, 12 girls) were only growth retarded and served as controls. Their auxological data are shown in Table 1.

Key-words: Leptin, GH, GH deficiency, arginine infusion, insulin-induced hypoglycaemia.

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Table 1 - *Auxological parameters of GH-deficient patients and controls.*

	Patients	Controls
Subjects	45	27
Male/Female	30/15	15/12
Age (yr)	8.6±3.9	10.9±3.3
% pre-pubertal	75.6	59.3
Height SDS	-1.7±1.2	-1.7±1.0
BMI SDS	0.4±2.3	-0.4±1.2
Target height SDS	-0.8±1.4	-0.95±0.48

Height and Target height SDS according to Tanner (22); BMI SDS according to Rolland-Cachera (23).

The diagnosis of GHD was based on the following criteria: height <-2 SDS or <10th centile when corrected for parental target (according to J.M. Tanner's "Parents-allowed-for charts") (14); height velocity-SDS <25th centile for chronological age when measured for more than 1 yr; bone age delay >2 yr compared to chronological age; peak GH <10 µg/l in at least 2 consecutive conventional pharmacological tests (15). No patient suffered from any organic GHD, panhypopituitarism or multiple pituitary hormone deficiency, all being affected by idiopathic isolated GHD, as confirmed by a complete endocrine assessment and pituitary MRI.

Study protocol

Serum leptin and GH levels were evaluated, after an overnight fast between 08:00 h and 09:00 h at basal level and 30, 60 and 90 min following an arginine (0.5 g/bw iv) and an insulin (0.1 U/bw iv) infusion.

Methods

Leptin levels were measured by RIA using a commercial kit (Human Leptin RIA Kit, Linco Research Inc., St. Louis, MO, USA). The sensitivity of the assay was 0.5 ng/ml; the intra- and inter-assay coefficients of variation (CV) were less than 8.3 and 6.2%, respectively.

GH levels were determined by using chemiluminescent enzyme immunometric assay with a commercial kit (Immulite Growth Hormone, DPC Diagnostic Products Corporation, Los Angeles, CA, USA). The detection limit of the assay was 0.01 ng/ml; the intra- and inter-assay CV were less than 6.5 and 6.2%, respectively. To eliminate the inter-assay variation, all samples were measured in duplicate in the same assay.

Statistical analysis

The results are expressed as mean and SD. Logarithmic transformation was employed to normalize the distribution of leptin. One-way analysis of variance (ANOVA) was used to determine significant differences in leptin and GH concentrations between patients and controls and a factorial model was used to adjust for the covariate BMI SDS and age. The AUC during stimulation tests was calculated according to the trapezoidal rule. Correlations between variables was performed by Pearson r coefficient. A *p* value of less than 0.05 was considered to be statistically significant. All tests were two-sided. Analyses were performed with Statistica for Windows software (StatSoft, Inc. 2000, Tulsa, OK, U.S.A.)

RESULTS

During both stimulation tests with insulin and arginine (Tables 2 and 3), basal GH was similar in GHD patients and controls, whereas peak GH and the AUC were significantly lower in patients compared to controls.

Basal and peak leptin levels were significantly higher in GHD patients compared to controls (Tables 2 and 3).

Leptin levels did not change significantly during both stimulation tests despite a significant increase in GH levels (Fig. 1).

Basal leptin significantly correlated with the BMI

Table 2 - *GH and leptin secretory pattern in GH-deficient (GHD) patients and controls during the arginine stimulation test.*

	Basal GH (ng/ml)	Peak GH (ng/ml)	AUC GH (ng/ml/30')	Basal leptin (ng/ml)	Peak leptin (ng/ml)	AUC leptin (ng/ml/30')
GHD	0.86±0.91	5.04±2.36	15.77±7.90	6.10±6.14	6.13±6.10	28.92±24.52
Controls	0.94±0.66	16.54±4.55	47.95±18.42	2.93±2.11	3.56±3.24	20.12±23.82
p	NS	<0.001	<0.001	<0.05	<0.05	NS

Table 3 - *GH and leptin secretory pattern in GH-deficent (GHD) patients and controls during the insulin stimulation test.*

	Basal GH (ng/ml)	Peak GH (ng/ml)	AUC GH (ng/ml/30')	Basal leptin (ng/ml)	Peak leptin (ng/ml)	AUC leptin (ng/ml/30')
GHD	0.81±0.81	5.59±2.39	18.14±9.92	4.71±2.36	5.58±2.48	25.43±13.71
Controls	0.86±0.89	19.72±9.10	62.48±24.43	3.23±3.82	3.82±4.12	16.84±10.78
p	NS	<0.001	<0.001	<0.05	<0.05	NS

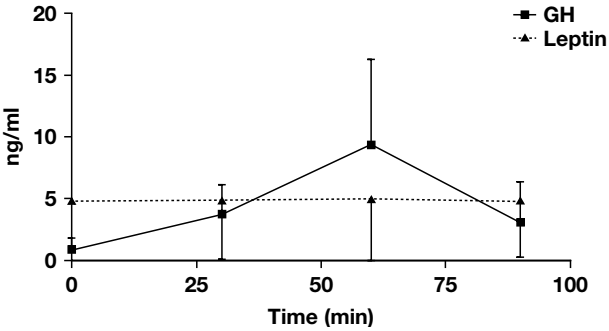

Fig. 1 - *Mean (SD) of GH and leptin serum levels during arginine and insulin stimulation tests in the 72 subjects.*

SDS in GHD patients (r=0.70; $p<0.0001$) (Fig. 2), but not in controls (r=0.35; p=NS). Furthermore, no correlation was found between basal serum leptin and basal values, peak and AUC of GH during both tests.

DISCUSSION

The results of our study, in agreement with a previous report (9), fail to support the hypothesis of a relationship between GH and leptin secretion, at least over a short-term period. In fact, we could not find either a correlation between basal leptin levels and various parameters of GH secretion during the stimulation tests, or between the profiles of GH and leptin during the tests.

Acute GH administration has been previously shown to affect leptin serum levels in absence of a change in body composition (10-12); changes were observed however only 24 h after GH administration.

Long-term studies failed to show a direct influence of GH on leptin secretion. The decrease in leptin levels observed after one year of GH treatment in a group of GHD children was not apparent anymore when expressed per unit fat mass (13); also in acromegalic subjects the reduction in leptin appears to be more the result of a reduced percentage of body fat rather than a direct consequence of GH excess (16).

We found a significant correlation between leptin and BMI in GHD patients, suggesting that the increased leptin levels merely represent the increased fat mass of these subjects lacking the lipolitic action of GH (17).

Leptin therefore appears to be an unlikely modulator of GH secretion. In a recent paper (18), however, where leptin and GH secretion were analyzed and time-cross-correlated over 24 h in a group of short-normal pre-pubertal children, it was shown that GH secretion leads leptin secretion in a positive way by 5 and 2 h for boys and girls, respectively, suggesting a positive direct leptin releasing effect of GH on adipocytes. The same Authors indicate in that paper that there is a significant correlation over time with leptin leading GH at lag times 11 and 8.5 h for boys and girls, respectively. Thus, it seems that leptin might still be a possible candidate for modulation of GH secretion; this however could not be demonstrated by our work, since the study period was too short.

Moreover, serum leptin was not influenced either by arginine or insulin administration, confirming that neither arginine (9) nor insulin (18) are able to stimulate leptin secretion in the short term. A longer period of exposure to insulin is probably needed in order to evoke a response; this is supported by clinical studies reporting a correlation between insulin and leptin serum concentration (19), and by *in vitro* studies of fat cell cultures showing that insulin directly stimulates leptin secretion (20).

In conclusion, our findings obtained from a large group of children, seem to suggest that leptin and GH secretion are not correlated and that leptin serum levels mainly reflect the amount of fat tissue, which is increased in GHD patients.

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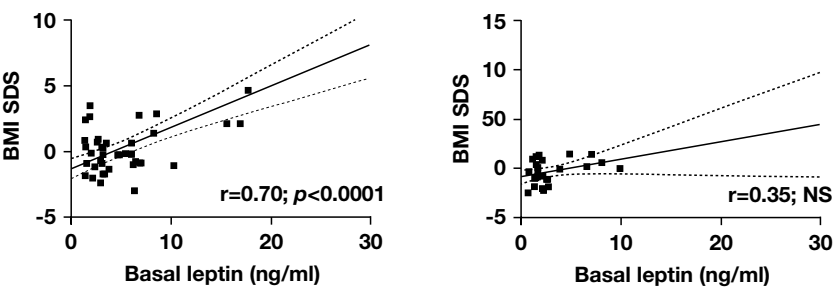

Fig. 2 - *Correlation between BMI SDS and basal leptin in GHD patients and controls.*

REFERENCES

1. Blum W.F. Leptin. The voice of the adipose tissue. Horm. Res. 1997, *48:* 2-8.
2. Chehab F.F, Lim M.E., Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat. Gen. 1996, *12:* 318-320.
3. Montague C.T., Farooqi I.S., Whitehead J.P., *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 1997, *387:* 903-908.
4. Clement K., Vaisse C., Lahlou N., *et al.* A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998, *392:* 398-401.
5. Carro E., Senaris R., Considine R., Casanueva F.F., Dieguez C. Regulation of in vivo growth hormone secretion by leptin. Endocrinology 1997, *138:* 2203-2206.
6. Thomas M.J., Pitts S.H., Siwitz W. Leptin stimulates hepatic insulin-like growth factor (IGF)-I gene expression in vivo. In: Endocrine Society, 79th Annual Meeting of the Endocrine Society, Minneapolis, Minnesota, 1997, OR 40-42, p. 123.
7. Veldhuis J.D., Iranmanesh A., Ho K.K.Y., Waters M.J., Johnson M.L., Lizzaralde G. Dual defects in pulsatile GH secretion and clearance subserve the hyposomatotropism of obesity in man. J. Clin. Endocrinol. Metab. 1991, *72:* 51-59.
8. Gill M.S., Toogood A.A., O'Neill P.A., *et al.* Relationship between growth hormone status, serum leptin and body composition in healthy and GH-deficient elderly subjects. Clin. Endocrinol. (Oxf.) 1997, *47:* 161-167.
9. Bennek C., Gehrt I., Keller A., *et al.* Arginine infusion and/or acute changes of growth hormone levels do not acutely alter leptin serum levels. J. Pediatr. Endocrinol. Metab. 1999, *12:* 847-851.
10. Gill M.S., Toogood A.A., Jones J., Clayton P.E., Shalet S.M. Serum leptin response to the acute and chronic administration of growth hormone (GH) to elderly subjects with GH deficiency. J. Clin. Endocrinol. Metab. 1999, *84:* 1288-1295.
11. Nørrelund H., Gravholt C.H., Englaro P., *et al.* Increased levels but preserved diurnal variation of serum leptin in GH-deficient patients: lack of impact of different modes of GH administration. Eur. J. Endocrinol. 1998, *138:* 644-652.
12. Lissett C.A., Clayton P.E., Shalet S.M. The acute leptin response to GH. J. Clin. Endocrinol. Metab. 2001, *86:* 4412-4415.
13. Matsuoka H., Fors H., Bosaeus I., Rosberg S., Albertsson-Wikland K., Bjarnason R. Changes in body composition and leptin levels during growth hormone (GH) treatment in short children with various GH secretory capacities. Eur. J. Endocrinol 1999, *140:* 35-42.
14. Tanner J.M. Foetus into man: physical growth from conception to maturity, 2nd ed. Castelmead Publications, Ware, 1989.
15. Cowell C.T. Short stature. In: Brook C.G.D. (Ed.), Clinical paediatric endocrinology, 3rd ed. Blackwell Science, Oxford, 1994, p. 136.
16. Miyakawa M., Tsushima T., Murakami H., Isozaki O., Demura H., Tanaka T. Effect of growth hormone (GH) on serum concentrations of leptin: study in patients with acromegaly and GH deficiency. J. Clin. Endocrinol. Metab. 1998, *83:* 3476-3479.
17. Wabitsch M., Hauner H., Heinze E., Teller W.M. The role of growth hormone/insulin-like growth factors in adipocyte differentiation. Metabolism 1995, *44:* 45-49.
18. Ghizzoni L., Mastorakos G., Street M.E., *et al.* Leptin, cortisol, and GH secretion interactions in short normal prepubertal children. J. Clin. Endocrinol. Metab. 2001, *86:* 3729-3734.
19. Dagogo-Jack S., Fanelli C., Paramore D., Brothers J., Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes 1996, *45:* 695-698.
20. Considine R.V., Sinha M.K., Heiman M.L., *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 1996, *334:* 292-295.
21. Wabitsch M., Jensen P.B., Blum W.F., *et al.* Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 1996, *45:* 1435-1438.
22. Tanner J.M., Whitehouse R.H., Takaishi M. Standards from birth to maturity for height, weight, height velocity and weight velocity: British children. Arch. Dis. Child. 1966, *41:* 613-635.
23. Rolland-Cachera M.F., Cole T.J., Sempé M., Tichet J., Rossignol C., Charraud A. Body mass index variations: centile from birth to 87 years. Eur. J. Clin. Nutr. 1991, *45:* 13-21.