Serum leptin levels in hypo- and hyperthyroidism

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Abstract

Leptin, the product of the *ob* gene, is an important circulating signal for the regulation of body weight. In the present study the role of immunoreactive leptin (leptin-IR) was investigated in functional thyroid disease. Serum leptin-IR levels of 23 hypothyroid and 19 hyperthyroid patients were compared with 21 controls. Leptin-IR was quantified by a specific RIA. In hyperthyroid patients, leptin-IR was not different from controls. Serum leptin-IR levels were significantly increased in hypothyroid patients ($21.0 \pm 2.7 \mu\text{g/l}$ vs

controls $10.8 \pm 2.1 \mu\text{g/l}$, $P=0.0044$). When serum leptin of hypothyroid patients was compared with euthyroid controls of the same body mass index the difference was still significant ($P=0.0333$ by paired Student's *t*-test). This might indicate that elevation of the serum leptin level does not merely reflect changes in body weight secondary to hypothyroidism, but might be increased to overcome the gain of body weight caused by hypothyroidism.

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Introduction

Leptin, the protein product of the *ob* gene, is an important circulating signal for the regulation of body weight (Zhang *et al.* 1994). It interacts with specific receptors in the hypothalamus affecting appetite and satiety centres of the brain (Rohner-Jeanrenaud & Jeanrenaud 1996). It is supposed to act as a negative feedback 'adipostatic signal' to brain centres controlling energy homeostasis and food uptake (Campfield *et al.* 1995, Stephens *et al.* 1995, Rohner-Jeanrenaud & Jeanrenaud 1996, Rohner-Jeanrenaud *et al.* 1996, Wang *et al.* 1997). In *ob/ob* mice, obesity is caused by a lack of circulating leptin due to mutations in the *ob* gene, and weight loss is achieved by leptin administration (Pellemounter *et al.* 1995). In humans, sequence variations of the *ob* gene are not the cause of increased body weight in most cases (Maffei *et al.* 1996, Niki *et al.* 1996), although *ob* mutations may occur in some cases of extreme human obesity (Clement *et al.* 1996, Reed *et al.* 1996).

Thyroid status affects food intake and body mass index (BMI). Body weight is often decreased in hyperthyroidism and is increased in hypothyroid patients (Ingbar 1985). Since leptin decreases food intake and increases energy expenditure, the aim of the present study was to investigate the role of serum leptin in functional thyroid disease.

Methods

Patients

Twenty-one euthyroid controls, 19 hyperthyroid and 23 hypothyroid patients were included in the study. Induced hypothyroidism was present in 13 patients with thyroid carcinoma. In 10 patients hypothyroidism was due to autoimmune disease. Hyperthyroidism was caused by Graves' disease in 8 cases and by autonomy in 11 cases (Table 1).

Leptin RIA

A synthetic fragment consisting of amino acids 126–140 (ETLDSLGGVLEASGY) of human leptin was custom synthesised (Bachem Biochemica GmbH, Heidelberg, Germany). Antibodies were raised in seven female rabbits (strain Neuseeländer, 2.3–2.7 kg). After coupling of the fragment to albumin, initial immunisation was performed after mixing 1:1 with Complete Freund's Adjuvant, and booster injections were given at 6-week intervals with Incomplete Freund's Adjuvant. For radiolabelling, the leptin fragment was iodinated with ¹²⁵I by the chloramine T method. Labelled fragment was purified by reverse-phase HPLC using a Waters μ Bondapak C18 column. RIA was set up in 500 μl of a 0.06 M phosphate EDTA buffer with addition of sodium azide. Labelled fragment

Table 1 Age, BMI, serum leptin, TSH_b, T₃, FT₄, and insulin concentrations of eu-, hyper-, and hypothyroid patients. Data are expressed as means ± SEM.

	<i>n</i>	Age (years)	Leptin (μg/l)	TSH _b (mU/l)	T ₃ (ng/l)	FT ₄ (ng/l)	Insulin (mU/l)	BMI (kg/m ²)
Euthyroid patients								
Female	13	49.9 ± 4.9	14.2 ± 2.9	1.23 ± 0.17	12.46 ± 0.41	0.13 ± 0.01	16.6 ± 3.7	23.7 ± 1.8
Male	8	54.8 ± 5.2	5.2 ± 1.2	0.95 ± 0.20	12.84 ± 0.55	0.11 ± 0.01	8.4 ± 1.5	24.0 ± 1.1
Total	21	51.7 ± 3.6	10.8 ± 2.1	1.13 ± 0.13	12.60 ± 0.33	0.12 ± 0.01	13.5 ± 2.5	23.8 ± 1.1
Hyperthyroid patients								
Female	14	53.7 ± 4.7	18.8 ± 4.4	0.01 ± 0.00	28.19 ± 2.55	0.50 ± 0.01	25.3 ± 6.0	23.7 ± 4.7
Male	5	61.6 ± 5.9	5.6 ± 0.6	0.01 ± 0.00	25.42 ± 2.10	0.48 ± 0.01	11.2 ± 0.3	24.7 ± 1.1
Graves' disease	8	43.5 ± 4.7	13.0 ± 2.8	0.01 ± 0.00	30.50 ± 4.16	0.51 ± 0.01	19.5 ± 7.5	21.4 ± 0.9
Autonomy	11	64.7 ± 3.9	17.0 ± 5.7	0.01 ± 0.00	25.25 ± 1.35	0.47 ± 0.01	23.1 ± 6.0	25.7 ± 1.2
Total	19	55.8 ± 3.8	15.3 ± 3.5	0.01 ± 0.00	27.46 ± 1.95	0.49 ± 0.01	21.6 ± 4.6	23.9 ± 0.9
Hypothyroid patients								
Female	19	49.8 ± 2.9	23.8 ± 2.8	72.40 ± 11.10	4.16 ± 0.62	0.04 ± 0.03	15.1 ± 2.9	26.9 ± 1.2
Male	4	57.8 ± 9.0	8.1 ± 2.6	54.87 ± 18.36	4.45 ± 1.29	0.04 ± 0.02	21.9 ± 13.2	26.6 ± 1.6
Induced hypothyroidism (thyroid carcinoma)	13	45.8 ± 3.2	20.6 ± 3.2	86.05 ± 13.80	3.35 ± 0.73	0.04 ± 0.04	13.8 ± 3.1	26.5 ± 1.4
Primary hypothyroidism	10	58.2 ± 4.2	21.6 ± 4.7	47.64 ± 10.08	5.33 ± 0.72	0.04 ± 0.05	19.3 ± 5.9	27.3 ± 1.5
Total	23	51.2 ± 2.8	21.0 ± 2.7	69.35 ± 9.66	4.21 ± 0.55	0.04 ± 0.03	16.0 ± 3.0	26.8 ± 1.0

(50 µl) was added, and antibody was added to all tubes except for non-specific binding tubes. Synthetic human leptin (Tebu, Frankfurt, Germany) was used as an assay standard. Bound and free antibody were separated by addition of charcoal, subsequent centrifugation and separation of supernatant and pellet. All samples were assayed in duplicate. Plasma or gel chromatography samples were measured in a single assay.

For crossreactivity studies, insulin, tri-iodothyronine (T_3), free serum thyroxine (FT_4) and thyroid-stimulating hormone (TSH) were assayed at 1, 5, 10, 50 and 100 nmol/tube.

Gel chromatography

Gel chromatography of selected serum samples was performed on Sephadex G75 columns (length 26.5 cm, diameter 3.5 cm). ^{125}I , Blue Dextran and cytochrome C were used as markers for molecular size. Recovery was expressed as per cent of the amount of peptide found in assayed fractions related to the amount of peptide loaded onto the column.

Quantification of insulin, TSH, T_3 and FT_4

Blood samples were collected in Sarstedt monovettes and centrifuged immediately for 15 min at 4 °C. Serum was aliquoted and frozen on solid carbon dioxide. Insulin was quantified using a commercial RIA (Pharmacia, Uppsala, Sweden), T_3 and FT_4 by competitive immunoassays (Ciba Corning Diagnostics, Fernwald, Germany) and TSH by a two-sided chemiluminometric immunoassay (Ciba Corning Diagnostics).

Statistical analysis

Data are shown as mean values $\pm$ S.E.M. Statistical analysis was performed by an unpaired Student's *t*-test (two tailed *P* values, 95% confidence intervals) unless otherwise indicated.

Results

Leptin RIA

Chloramine T iodination of leptin fragment resulted in a ^{125}I incorporation of 84.3%. HPLC purification showed a peak eluting at 37% acetonitrile comprising more than 90% of incorporated radioactive iodine (Fig. 1). Our antibody XC71 gave a binding of 37.4% after incubation for 5 days. The antiserum used in the RIA at a final dilution of 1:720 000 was collected after the fourth boost. The sensitivity of the antibody was 1.5 fmol/tube. It did

Figure 1 HPLC purification of radioiodinated leptin fragment, using a Waters HPLC system and an acetonitrile (ACN)/water gradient, showing a peak eluting at 37% ACN comprising more than 90% of incorporated radioiodine.

Figure 2 Gel chromatography (Sephadex G75) of a serum sample. The major peak of leptin (87% plasma radioactivity) eluted at the position of human leptin.

not show any crossreactivity with insulin, T_3 , FT_4 or TSH up to 0.1 µmol/tube. Intra-assay variation was 4.7%, interassay variation 11.4%.

Gel chromatography

Mean recovery of pooled plasma samples was 56% ($n=6$, range 51 to 60%). The major peak of leptin plasma radioactivity (87%) eluted at the position of human leptin (Fig. 2). Thirteen per cent of immunoreactive leptin (leptin-IR) eluted at 2000 Da.

Serum leptin-IR levels in thyroid disease

Basal TSH (TSH_b) was 1.13 ± 0.13 mU/l (mean $\pm$ S.E.M.) in euthyroid patients, 69.35 ± 9.66 mU/l in hypothyroid, and 0.01 ± 0.00 mU/l in hyperthyroid patients. T_3 levels were 12.60 ± 0.33 ng/l in the euthyroid,

Figure 3 Correlation of serum leptin levels and TSH_b in hypo- and euthyroid patients.

27.46 ± 1.95 ng/l in the hyperthyroid, and 4.21 ± 0.55 ng/l in the hypothyroid group. FT₄ was 0.12 ± 0.01 ng/l in euthyroid controls, 0.49 ± 0.01 ng/l in the hyperthyroid, and 0.04 ± 0.03 ng/l in the hypothyroid group (Table 1).

Leptin levels in hyperthyroid patients were not different from euthyroid controls. Leptin-IR in Graves' disease ($n=8$, 13.0 ± 2.8 µg/l) was similar to that in thyroid autonomy (17.0 ± 5.7 µg/l, $n=11$, $P=0.5826$).

Serum leptin-IR in hypothyroid patients was significantly higher than in euthyroid controls (21.0 ± 2.7 µg/l vs 10.8 ± 2.1 µg/l, $P=0.0044$) (Fig. 4). When serum leptin of hypothyroid patients was compared with euthyroid controls of the same BMI (differences of BMI ≤ 0.5 kg/m²) the difference was still significant ($P=0.0333$ by paired Student's *t*-test, $n=12$). No difference was found in hypothyroid patients with thyroid carcinoma and in non-malignant hypothyroid disease (20.6 ± 3.2 µg/l vs 21.6 ± 4.7 µg/l; $P=0.8574$). The correlation coefficient for leptin-IR and TSH_b concentrations in eu- and hypothyroid patients was $r=0.3847$ ($P=0.0099$, $n=44$) (Fig. 3). When eu- and hypothyroid patients were analysed separately it was $r=0.3463$ ($P=0.1240$, $n=21$) in euthyroid controls and $r=0.1547$ ($P=0.4810$, $n=23$) in hypothyroid patients.

The mean serum leptin-IR in females was higher than in males (Table 1). This was true in euthyroid ($P=0.0304$), in hyperthyroid ($P=0.0967$), and in hypothyroid patients ($P=0.0213$). Leptin in hypothyroid females was significantly higher than in euthyroid females ($P=0.0284$), whereas the leptin-IR in hyperthyroid and euthyroid females did not differ ($P=0.3954$).

The overall correlation coefficient of the BMI and the serum leptin concentrations was $r=0.6678$ ($P<0.0001$, $n=63$); and when analysed separately, $r=0.7340$ ($P=0.0002$, $n=21$) in euthyroid control subjects, $r=0.6717$ ($P=0.0004$, $n=23$) in hypothyroid, and $r=0.6407$

Figure 4 Mean serum leptin concentration in eu-, hyper-, and hypothyroid patients. Mean values $\pm$ S.E.M.

($P=0.0031$, $n=19$) in hyperthyroid patients. Mean serum insulin concentrations in euthyroid, in hyperthyroid and in hypothyroid patients were not different.

Discussion

Serum leptin levels are known to increase with the percentage of body fat and, to a lesser degree with the BMI. This might indicate insensitivity to endogenous leptin in obesity (Considine *et al.* 1996a,b). This was confirmed in our study. Since we showed a strong correlation of serum leptin and BMI, when hyper- or hypothyroid patients were analysed separately, thyroidal dysfunction does not attenuate weight related changes of leptin.

Higher leptin levels in women are in agreement with a recently published study demonstrating the involvement of sex hormones in the regulation of leptin synthesis (Saad *et al.* 1997). In addition, *ob* gene messenger RNA expression is increased in obese women compared with obese man (Lonnqvist *et al.* 1995).

Similar to the findings of Valcavi *et al.* (1997), an effect of hyperthyroidism on serum leptin was not detected. This is in agreement with the observation that administration of T₃ to healthy volunteers did not alter circulating leptin, although cardiovascular and metabolic effects were present (Mantzoros *et al.* 1997).

The increased leptin levels in hypothyroidism are not due to a direct effect of thyreostatic medication since leptin was increased both in drug-induced hypothyroidism and in autoimmune disease. In contrast to our finding, Valcavi *et al.* (1997) described a decrease of the serum leptin-IR levels in primary hypothyroidism. There is no obvious explanation for the discrepancy, although the TSH levels in hypothyroidism were two-fold lower and

the BMI was higher in our study. The differences cannot be attributed to crossreactivity of the antibody since we demonstrated that the antibody used did not bind thyroid hormones or TSH or insulin. Gel chromatographic runs of serum samples indicated that our antibody detected the complete and intact protein.

In our study we could exclude that changes in leptin were secondary to altered insulin levels, since mean insulin was not different in eu-, hyper-, and hypothyroid patients. Insulin is known to stimulate *ob* gene expression and to increase serum leptin concentration in non-insulin-dependent diabetes mellitus (NIDDM) (Cusin *et al.* 1995). In non-obese NIDDM patients, leptin synthesis is resistant to the acute effect of insulin, whereas testosterone may contribute to the regulation of leptin synthesis in these patients (Tuominen *et al.* 1997, Widaja *et al.* 1997).

The significant increase of leptin in hypothyroidism cannot be explained by differences in body weight because leptin levels remained significantly increased when compared with controls with an identical BMI. This might indicate that elevation of the serum leptin level does not merely reflect changes in body weight secondary to hypothyroidism, but might be increased to overcome the gain of body weight caused by hypothyroidism.

References

- Campfield LA, Smith FJ, Guisez Y, Devos R & Burn P 1995 Recombinant mouse *ob* protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269** 546–549.
- Clement K, Garner C, Hager J, Philippi A, LeDuc C, Carey A, Harris TJ, Jury C, Cardon LR, Basdevant A, Demenais F, Guy-Grand B, North M & Froguel P 1996 Indication for a linkage of the human *ob* gene region with extreme obesity. *Diabetes* **45** 687–690.
- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian J, Marco CC, McKee LJ, Bauer TL & Caro JF 1996a Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine* **334** 292–295.
- Considine RV, Considine EL, Williams CJ, Hyde TM & Caro JF 1996b The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the *db/db* mouse and *fa/fa* mutations. *Diabetes* **45** 992–994.
- Cusin I, Sainbury A, Doyle P, Rohner-Jeanrenaud F & Jeanrenaud B 1995 The *ob* gene and insulin. A relationship leading to clues to the understanding of obesity. *Diabetes* **44** 1467–1470.
- Ingbar SH 1985 The thyroid gland. In *Textbook of Endocrinology*, pp 975–1170. Eds JD Wilson & DW Foster. Philadelphia: Saunders.
- Lonnqvist F, Arner P, Nordfors L & Schalling M 1995 Overexpression of the obese (*ob*) gene in adipose tissue of human obese subjects. *Nature Medicine* **1** 950–953.
- Maffei M, Stoffel M, Barone M, Moon B, Dammerman M, Ravussin E, Bogardus C, Ludwig DS, Flier JS, Talley M, Auerbach S & Friedman JM 1996 Absence of mutations in the human *ob* gene in obese/diabetic subjects. *Diabetes* **45** 679–682.
- Mantzoros CS, Rosen HN, Greenspan SL, Flier JS & Moses AC 1997 Short-term hyperthyroidism has no effect on leptin levels in man. *Journal of Clinical Endocrinology and Metabolism* **82** 497–499.
- Niki T, Mori H, Tamori Y, Kishimoto-Hashimoto M, Ueno H, Araki S, Masugi J, Sawant N, Majithia HR, Rais N, Hashimoto M, Taniguchi H & Kasuga M 1996 Human obese gene: molecular screening in Japanese and Asian Indian NIDDM patients associated with obesity. *Diabetes* **45** 675–678.
- Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T & Collins F 1995 Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science* **269** 540–543.
- Reed DR, Ding Y, Xu W, Cather C, Green ED & Price RA 1996 Extreme obesity may be linked to markers flanking the human *ob* gene. *Diabetes* **45** 691–694.
- Rohner-Jeanrenaud F & Jeanrenaud B 1996 Obesity, leptin, and the brain. *New England Journal of Medicine* **334** 324–325.
- Rohner-Jeanrenaud F, Cusin I, Sainsbury A, Zakrewska KE & Jeanrenaud B 1996 The loop system between neuropeptide Y and leptin in normal and obese rodents. *Hormone and Metabolic Research* **28** 642–648.
- Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, Jinagouda SD, El-Tawil K, Rude RK & Kamdar V 1997 Sexual dimorphism in plasma leptin concentration. *Journal of Clinical Endocrinology and Metabolism* **82** 579–584.
- Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, MacKellar W, Rostek PR, Schoner B, Smith D, Tinsley FC, Zhang XY & Heiman M 1995 The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* **377** 530–532.
- Tuominen JA, Ebeling P, Stenman UH, Heiman ML, Stephens TW & Koivisto VA 1997 Leptin synthesis is resistant to the acute effects of insulin in insulin-dependent diabetes mellitus patients. *Journal of Clinical Endocrinology and Metabolism* **82** 381–382.
- Valcavi R, Zini M, Peino R, Casanueva FF & Dieguez C 1997 Influence of thyroid status on serum immunoreactive leptin levels. *Journal of Clinical Endocrinology and Metabolism* **82** 1632–1634.
- Wang Q, Bing C, Al-Barazanji K, Mossakowaska DE, Wang XM, McBay DL, Neville WA, Taddayon M, Pickavance L, Dryden S, Thomas MEA, McHale MT, Gloyer IS, Wilson S, Buckingham R, Arch JRS, Trayhurn P & Williams G 1997 Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* **46** 335–341.
- Widaja A, Stratton IM, Horn R, Holmann RR, Turner R & Brabant G 1997 UKPDS 20: Plasma leptin, obesity, and plasma insulin in type 2 diabetic subjects. *Journal of Clinical Endocrinology and Metabolism* **82** 654–657.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* **372** 425–432.

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