Lack of effects of circulating thyroid hormone levels on serum leptin concentrations
Sabrina Corbetta, Piera Englaro 1, Salvatore Giambona, Luca Persani, Werner F Blum 1 and Paolo Beck-Peccoz

Institute of Endocrine Sciences, University of Milan, Ospedale Maggiore di Milano-IRCCS, Centro Auxologico Italiano IRCCS and Istituto Clinico Humanitas, Milano, Italy and 1University Children’s Hospital, Giessen, Germany and Lilly Germany, Bad Homburg, Germany

(Correspondence should be addressed to P Beck-Peccoz, Istituto Clinico Humanitas, Via Manzoni 56, 20089 Rozzano-Milano, Italy)

Abstract
Leptin is the protein product of the ob gene, secreted by adipocytes. It has been suggested that it may play an important role in regulating appetite and energy expenditure. The aim of this study was to evaluate a possible interaction of thyroid hormones with the leptin system. We studied 114 adult patients (65 females and 49 males): 36 were affected with primary hypothyroidism (PH), 38 with central hypothyroidism (CH) and 40 with thyrotoxicosis (TT). Patients with CH were studied both before and after 6 months of l-thyroxine replacement therapy. Body mass index (BMI; kg/m²), thyroid function and fasting serum leptin were assessed in all patients. Since BMI has been proved to be the major influencing variable of circulating leptin levels, data were expressed as standard deviation score (SDS) calculated from 393 male and 561 female controls matched for age and BMI.

No difference in SDS was recorded between males and females whatever the levels of circulating thyroid hormones. In males, no significant difference was recorded among the SDSs of PH (¹ 0.36 6 1.2), TT (¹ 0.35 6 1.2) and CH (0.01 6 1.4) patients. Females with PH had an SDS significantly lower than TT females (¹ 0.77 6 1.0 vs ¹ 0.06 6 1.2; P < 0.02), while no significant differences between CH ( 0.34 ± 0.7) and TT females or between CH and PH females were observed. SDS in CH patients after 6 months of l-thyroxine therapy significantly varied only in females (0.25 ± 1.4). In conclusion, circulating thyroid hormones do not appear to play any relevant role in leptin synthesis and secretion. However, as females with either overt hypo- or hyper-thyroidism or central hypothyroidism after l-thyroxine therapy show differences in their SDSs, a subtle interaction between sex steroids and thyroid status in modulating leptin secretion, at least in women, may occur.

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Introduction
Leptin is a 146-amino acid protein hormone encoded by the ob gene (1) and secreted by adipocytes in response to an increase in fat mass (2–4). It seems to be a key molecule in the feedback loop that regulates energy balance (5). Leptin has a dual action: it decreases the appetite and increases energy consumption, causing more fat to be burned. In fact, both ob/ob and db/db mice are characterized by numerous abnormalities, such as severe insulin resistance, infertility, decrease in lean body mass, cold intolerance and hypothermia (6, 7), while administration of leptin to ob/ob mice results in a reduction in food intake, an increase in locomotor activity (with an increase in oxygen consumption) and a reduction in body weight (8, 9). As substantial variability in serum leptin levels occurs among individuals with comparable degrees of obesity (10), other factors in addition to the amount of body fat appear to regulate leptin secretion. Recent reports suggest a role for insulin (11), glucocorticoids (12, 13), catecholamines (14, 15) and sex hormones (16).

In thyroid disorders there are changes in basal metabolic rate, oxygen consumption, appetite and body weight. Thyroid hormones are important regulators of both basal and total energy consumption, and modulate the activity of several enzymes involved in lipid metabolism (17, 18). They are therefore a major component in the regulation of energy balance and body composition in humans. In the present study, we evaluated the potential interaction of circulating thyroid hormones with the leptin system in patients with various thyroid disorders, ranging from severe primary hypothyroidism to overt thyrotoxicosis. In addition, we investigated serum leptin levels both before and after l-thyroxine (T4) replacement therapy in patients with central hypothyroidism.

Materials and Methods

Subjects
We studied 114 adult patients (65 females and 49 males) aged 48.0 ± 16.9 years (mean ± S.D.) with thyroid
immunoassay respectively, using the Delfia technique by ultrasensitive immunofluorimetric assay and fluororescence.

after an overnight fast. Reference ranges were derived from 30 patients with 6 months of T4 replacement (TSH) and anti-thyroid autoantibody levels. None of the patients had thyrotoxicosis (FT3), free thyroxine (FT4), thyrotropin (TSH) was assessed by measuring serum fasting free triiodothyronine (T3) levels were measured at 0800 h (Table 1). The difference found between PH and CH male patients was statistically significant (P < 0.05 vs controls) after adjusting for gender and BMI. Serum leptin levels correlated well with BMI in both males (Fig. 1) and females. Serum leptin levels were measured by using FT3 Amerlite MAB (Johnson & Johnson Clinical Diagnostics, Milan, Italy).

Statistical methods
Age, BMI, serum TSH, FT4, FT3 and leptin levels were compared among the various groups of patients using Student’s t-test and within each group by linear regression analysis. Data are expressed as means ± S.D.

Leptin levels were adjusted for gender and BMI by calculating the standard deviation scores (S.D.) according to the following formula (20): for males, S.D. = [ln leptin – ln 0.0237 – (0.1985 × BMI)]/0.63859; for females, S.D. = [ln leptin – ln 0.3204 – (0.1448 × BMI)]/0.52483.

Results
As seen in the control group, serum leptin levels in males with thyroid disorders were in general lower than those recorded in females. Leptin values correlated well with BMI in both male (Fig. 1) and female (Fig. 2) patients, with no evident relationship with the thyroid status, the various slopes of the correlation lines being similar in the different groups of patients. Serum leptin levels in PH and CH male patients were measured as means ± s.d. and ranges are in parentheses.

Table 1 Clinical and biochemical data of patients with various thyroid disorders. Data are expressed as means ± s.d. and ranges are in parentheses.

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<sup>a</sup> P < 0.01 vs controls. <sup>b</sup> P < 0.02 vs PH males; <sup>c</sup> P < 0.02 vs PH females; <sup>d</sup> P < 0.04 vs CH basal.

Assays
Serum leptin levels were determined by RIA with an inter- and intra-assay coefficients of variation of 6.5 and 0.8% respectively and a sensitivity of 0.03 μg/l (19). Circulating levels of TSH and FT4 were measured by ultrasensitive immunofluorimetric assay and fluorimunoassay respectively, using the Delfia technique (Pharmacia, Milan, Italy). Serum FT3 levels were measured by using FT3 Amerlite MAB (Johnson & Johnson Clinical Diagnostics, Milan, Italy).

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<sup>a</sup> P < 0.01 vs controls. <sup>b</sup> P < 0.02 vs PH males; <sup>c</sup> P < 0.02 vs PH females; <sup>d</sup> P < 0.04 vs CH basal.
females was mainly due to the significantly different BMI in the two groups (25.0 ± 6.3 vs 29.0 ± 4.8 kg/m²; P < 0.015), and possibly to the concomitant untreated GH deficiency in the latter group. Leptin levels after T₄ treatment in CH patients were not significantly different from those recorded in pretreatment conditions (males: 7.7 ± 6.0 µg/l; females: 27.5 ± 16.9 µg/l), and a complete lack of correlation with FT₄ levels was seen. Serum leptin levels in male and female TT patients were 6.3 ± 7.6 and 10.9 ± 8.1 µg/l respectively. Interestingly, no significant differences in basal leptin concentrations were seen between TT and PH patients, as documented by the lack of correlation between leptin and FT₄ concentrations in PH and TT patients.

In order to avoid possible interference of BMI variations in the evaluation of serum leptin concentrations in the various groups of patients, we used the SDS calculated from normal controls matched for sex and BMI. Values of SDS higher than +2 or lower than −2 were seen in a minority of cases and were equally distributed among hyper- and hypothyroid patients. The correlations of SDS values and serum FT₄ concentrations in male and female patients with various thyroid disorders are reported in Figs 3 and 4 respectively.

The means of SDS values were similar in all groups of patients, with the exception of female CH patients before and after T₄ treatment (−0.34 ± 0.66 vs 0.25 ± 1.35, P < 0.04) and PH and TT females (−0.77 ± 1.00 vs −0.06 ± 1.20, P < 0.02) (Table 1). However, as depicted in Fig. 4, the SDS values overlapped greatly.
Discussion

The present results clearly suggest that circulating thyroid hormones do not play a major role in the regulation of leptin synthesis and secretion. Thus the ability of thyroid hormones to regulate energy expenditure does not operate through variations in serum leptin levels. Our results confirm and extend previous data showing that hypo- and hyperthyroidism in patients of both sexes (21) and short-term hyperthyroidism in males (22) do not alter circulating leptin concentrations. In contrast, they do not support previous studies in humans showing decreased leptin concentrations in hypothyroid patients (23) and in Zucker rats, demonstrating a decrease in leptin mRNA in response to T₄ administration (24). In the latter case, the significant loss of animal weight and the consequent decrease in adipose stores probably account for the reduced leptin gene expression.

In agreement with other reports (10, 11, 25, 26), we found that serum leptin concentrations recorded in both normal controls and patients with thyroid disorders were characterized by high variability, the major determinants of which are BMI and gender. This figure is documented by the significant correlation of which are BMI and gender. This figure is documented by the significant correlation

Acknowledgements

The authors are indebted to APREC (Milan, Italy) for financial support for this study.

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