

ORIGINAL ARTICLE

Relationship of thyroid function with body mass index, leptin, insulin sensitivity and adiponectin in euthyroid obese women

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Summary

Background A possible relationship between thyroid hormones and adipose tissue metabolism in humans has been suggested.

Aim of the study We sought to evaluate thyroid function and its possible relationship with body mass index (BMI), leptin, adiponectin and insulin sensitivity in euthyroid obese women.

Materials and methods Eighty-seven uncomplicated obese women (mean age 34.7 ± 9 years, mean BMI 40.1 ± 7 kg/m²) were studied. Levels of TSH, free thyroxine (FT4), free triiodothyronine (FT3), plasma adiponectin and leptin were evaluated. Insulin sensitivity was assessed by euglycaemic hyperinsulinaemic clamp (M index), fasting insulin and HOMA-IR.

Results Uncomplicated obese women with BMI > 40 kg/m² showed higher serum TSH than obese subjects with BMI < 40 kg/m² ($P < 0.01$). TSH was correlated with BMI ($r = 0.44$, $P = 0.01$) leptin ($r = 0.41$, $P = 0.01$), leptin/BMI ratio ($r = 0.33$, $P = 0.03$), body surface area ($r = 0.26$, $P = 0.05$), HOMA-IR ($r = 0.245$, $P = 0.05$) and inversely with adiponectin ($r = -0.25$, $P = 0.05$) and M index ($r = -0.223$ $P = 0.05$).

Conclusions Our data show that, although thyroid function was normal in the studied obese population, TSH and BMI were positively related. TSH has been found to be correlated also with leptin adjusted for BMI. TSH could represent a marker of altered energy balance in severe, but uncomplicated obese women.

(Received 18 August 2004; returned for revision 19 October 2004; finally revised 22 December 2004; accepted 7 February 2005)

Introduction

The relationship between obesity and thyroid function has been previously studied, but it is still not completely understood. Baseline TSH and thyroid hormone levels have been found normal or slightly

increased in obese subjects.^{1–5} A possible role of thyroid hormones in regulating adipose tissue metabolism in humans has been proposed with growing interest. Recent reports suggested that some cytokines exclusively secreted by adipocytes, such as leptin and also adiponectin, could be correlated with thyroid function. In fact, the regulation of TRH gene expression in the paraventricular nucleus of the hypothalamus by leptin has been reported to be critical for normal function of the thyroid axis in humans.⁶ Although the relationship between thyroid function and leptin levels in humans has been widely studied, the results are still controversial.⁷ Nevertheless, most of the previous studies have focused their attention on patients with thyroid dysfunction and wide range of body mass index (BMI) and obesity-related complications. Hence, no studies on thyroid function in uncomplicated obese subjects have been performed.

In this study we sought to evaluate thyroid function and its possible relationship with BMI, leptin, adiponectin and insulin sensitivity in euthyroid obese women.

Materials and methods

Subjects

We selected 87 consecutive Caucasian uncomplicated obese women (BMI > 30 kg/m²) from 127 consecutive uncomplicated obese women who were all screened at our Day Hospital over a 2-year period. The selected uncomplicated obese women had the following features: mean age of 34.7 ± 9 years, mean BMI 40.1 ± 7 kg/m² (range 30–80.1 kg/m²); duration of obesity, 15 ± 2 years (range 10–30 years). Uncomplicated obesity was defined according to the following parameters: no clinically significant abnormalities on physical examination, no lipid-lowering, hypoglycaemic or antihypertensive drugs, no history of cardiovascular and respiratory diseases, normal ECG, normal fasting glucose (< 6.10 mmol/l), normal glucose tolerance test (OGTT), systolic blood pressure (SBP) < 130 mmHg and diastolic blood pressure (DBP) < 85 mmHg for at least three measurements, normal plasma lipids [total cholesterol < 5.18 mmol/l, high density lipoprotein cholesterol (HDL-C) > 1.03 mmol/l for men and > 1.29 mmol/l for women, low density lipoprotein cholesterol (LDL-C) < 3.36 mmol/l and triglycerides < 1.69 mmol/l]. Forty-five normal weight volunteers (40 women, 5 men, mean age

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35 ± 8 years, BMI 23.5 ± 1 kg/m²), without history of thyroid disease formed the control group for thyroid function evaluation.

Exclusion criteria

Forty patients from the 127 uncomplicated obese women initially screened were excluded for the following reasons. Twelve obese women with previous diagnosis of thyroid disorders and who were taking thyroid hormone therapy, antithyroid drugs, radio-iodine treatment, iodine tablets and any drugs that might affect thyroid hormones evaluation were not included in this study. We excluded four obese women with diagnosis of thyroid dysfunction as a consequence of thyroid hormone measurements for this study. We also excluded 24 subjects with increased serum thyroid peroxidase antibodies (AbTPO), thyroglobulin antibodies (TgAb) and TSH radio receptor antibodies (TRAb) levels. No pregnant obese women were included in this study.

Study design

Levels of TSH, FT4, FT3, plasma adiponectin, and leptin were evaluated. Insulin sensitivity was assessed by euglycaemic hyperinsulinaemic clamp, fasting insulin and homeostatic model assessment for insulin resistance (HOMA-IR) index.⁸ Resting energy expenditure (REE) was also calculated. This study was conducted in accordance with the guidelines proposed in the *Declaration of Helsinki* and has been approved by the review committee of La Sapienza University. All subjects gave their written informed consent before the study began.

Anthropometrical measurements

Weight (to the nearest 0.1 kg) and height (to the nearest 0.5 cm) were measured while the subjects were fasting and wearing only their undergarments. BMI was calculated as body weight (kg) divided by height and squared (m²) and was used as a marker of obesity.

Clamp study

We performed euglycaemic hyperinsulinaemic clamp according to previously described method⁹ in all obese subjects, after 10–12 h overnight fast. Insulin was continuously infused at the rate of 4.0 mU/kg/min for 5 min, 2.0 mU/kg/min for 5 min and 1.0 mU/kg/min for 110 min. The steady state of the test was considered the interval between 60 and 120 min. Whole body glucose utilization (M index) was calculated from the infusion rate of exogenous glucose during the second hour of the insulin clamp period after correction for changes in glucose levels in a distribution volume of 250 ml/kg. The M index was adjusted by kilograms of fat-free mass (FFM_{kg}).

Impedimentometry measurements

Fat mass (FM) and free fat mass (FFM) were estimated using a bioelectrical impedance analyser (BIA-103; Akern, Florence, Italy).

REE measurement

REE was assessed with a computerized, open-circuit, indirect calorimetry system (Deltatrac II, Datex Ohmeda, Madison, WI, USA).

Analytical procedures

Radio-immunoassay (RIA) quantitative determination of serum concentrations of FT3 (pmol/l, reference range 3.4–7.1) and FT4 (pmol/l, reference range 10–25) was performed (RADIM, Italy, intra-assay CVs of 2.5% and 3.5%, respectively, and interassay CVs of 3% and 4.5%, respectively). TSH (mU/l, reference range 0.3–4.0) levels were measured by immuno-radiometric assay (IRMA) (ADALTIS, Italy, intra- and interassay CVs 4% and 2.5%, respectively). Plasma glucose was determined by the glucose oxidase method [mmol/l, reference range: 4.16–6.10; Autoanalyser, Beckman Coulter, Inc., Fullerton, CA, USA; (CV 1.9 ± 0.2%)]. Plasma insulin concentrations were determined by RIA kit (pmol/l, reference range: 34.5–173.6; Linco Research, Inc., St Louis, MO, USA; CV, 3.0 ± 0.3%). Blood samples for plasma insulin measurements were collected in heparinized tubes. Plasma leptin concentrations were determined by RIA kit (µg/l, reference range: 1.0–7.8; Linco Research, Inc.; CV, 3.7 ± 0.5%). To evaluate day-by-day plasma leptin variation, we measured plasma leptin concentration at 24-h intervals in all subjects. Leptin concentrations had a very small interday variation (mean variation, 3.4 ± 0.6%). Plasma adiponectin concentrations were also measured by RIA kit (reference range: 1.5–100 µg/l; Linco Research, Inc.; intra- and interassay CVs 4.5% and 3%, respectively). Samples were diluted 500 times before assay.

Statistical analysis

Data in the text and in the tables are expressed as mean ± SD. Because normality was rejected for many variables, Wilcoxon two-sample test with 95% confidence interval (CI) was applied to evaluate the differences between all parameters. Linear regression analysis was performed to identify correlates of TSH, FT3 and FT4. Two-tailed $P < 0.05$ indicated statistical significance. Analysis was done using Stata 5.0 (Stata Corp., College Station, TX, USA).

Results

Anthropometric and clinical characteristics of uncomplicated obese women are summarized in Table 1. Fifty-nine subjects had a BMI < 40 kg/m² and 46 subjects showed a BMI > 40 kg/m². No differences on age, glycidic and lipid pattern and blood pressure between the two obese groups were found.

Thyroid function and obesity degree

Uncomplicated severe (BMI > 40 kg/m²) obese women showed higher serum TSH than mild/intermediate (BMI < 40 kg/m²) obese subjects, 2.30 ± 1.2 vs. 1.50 ± 0.8 mU/l ($P < 0.01$ 95% CI 0.45–1.07). We have not found significant difference in FT3 and FT4 between severe and mild/intermediate uncomplicated obese subjects. No

Table 1. Anthropometric and metabolic characteristics of uncomplicated obese women

	BMI < 40 (n = 47)	BMI > 40 (n = 40)	P
Age (years)	36.8 ± 9.0	32.4 ± 8.2	ns
FFM (kg)	49.5 ± 5.5	52.5 ± 5.4	ns
Fasting glucose (mmol/l)	4.67 ± 0.45	4.71 ± 0.45	ns
2 h OGTT glucose (mmol/l)	5.90 ± 1.05	6.10 ± 1	ns
Total cholesterol (mmol/l)	4.88 ± 0.68	4.64 ± 0.64	ns
HDL-C (mmol/l)	1.40 ± 0.3	1.35 ± 0.28	ns
LDL-C (mmol/l)	3.16 ± 0.60	3.20 ± 0.60	ns
Triglycerides (mmol/l)	0.95 ± 0.33	0.99 ± 0.33	ns
Systolic BP (mm/Hg)	122 ± 9	122 ± 10	ns
Diastolic BP (mm/Hg)	76 ± 8	77 ± 8	ns
Fasting insulin (pmol/l)	89.5 ± 45	125 ± 65	< 0.05
HOMA-IR	3.30 ± 2.5	4.55 ± 2.4	< 0.05
Leptin (µg/l)	29.5 ± 15	48.5 ± 16	< 0.01
Leptin/BMI ratio	0.52 ± 0.30	0.48 ± 0.30	ns
Adiponectin (mg/l)	41.5 ± 19	28.5 ± 16	< 0.05
FT3 (pmol/l)	5.60 ± 1.05	5.80 ± 1.05	ns
FT4 (pmol/l)	18.50 ± 5.05	16.75 ± 3.60	ns
TSH (mU/l)	1.50 ± 0.8	2.30 ± 1.2	< 0.01
REE (kCal/24 h)	1585.5 ± 257.1	1995.4 ± 356.4	< 0.01
REE/FFM _{kg} (kCal/24 h)	29.2 ± 9	37.0 ± 10.5	< 0.01

Data are expressed as mean ± SD; Comparisons between BMI groups are assessed by Wilcoxon two-sample test. BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BP, blood pressure; TSH, thyroid-stimulating hormone; FT4, free thyroxine; FT3, triiodothyronine; REE, resting energy expenditure; FFM, free fat mass.

significant differences in TSH, FT3 and FT4 between uncomplicated obese and normal weight subjects were observed.

Leptin

Plasma leptin concentration in all obese women was 36.3 ± 15 µg/l. Leptin adjusted for BMI (leptin/BMI ratio) was 0.50 ± 0.30. As expected, severe obese subjects had higher plasma leptin levels than mild/intermediate obese subjects ($P < 0.01$, 95% CI 12.5–25.3). No statistically significant difference on leptin/BMI ratio between the two groups was found (Table 1).

Adiponectin

Plasma adiponectin concentration in all obese women was 33.5 ± 19 mg/l (range 5.9–90.6). Severe obese women had lower plasma adiponectin levels than mild/intermediate obese subjects ($P < 0.01$, 95% CI –19.8.0 to –6.5; Table 1).

Insulin sensitivity

Fasting insulin average level was 117 ± 60 pmol/l (range 40.5–390.5). Plasma insulin concentration average achieved at the steady state was 975.5 ± 155 pmol/l. Whole body glucose utilization (M index) during the euglycaemic hyperinsulinaemic clamp was 9.0 ± 4 mg/FFM_{kg}/min. Coefficient of variation of blood glucose was less than 4% in each clamp study throughout the test. Severe obese women had lower M index than mild/intermediate obese women 7.40 ± 3.0 vs. 9.6 ± 3.4 mg/FFM_{kg}/min ($P < 0.01$, 95% CI 0.92–3.47).

REE

Severe obese women had higher REE and REE/FFM_{kg} than mild/intermediate obese subjects ($P < 0.01$ for both, 95% CI –422 to –397, 95% CI –11.6 to –4.1 respectively; Table 1).

Correlates of TSH, FT3 and FT4

Simple regression analysis with TSH, FT3 and FT4 as the prime variables of interest is reported in Table 2.

Discussion

The main finding of this study is that TSH and BMI were positively related in euthyroid obese women. Our results suggest that BMI could be the strongest determinant of TSH in metabolically healthy obese women. TSH seems to be positively related to the degree of obesity. In fact, obese subjects with BMI more than 40 kg/m² showed significantly higher TSH levels than subjects with mild obesity. Nevertheless, the clinical significance of this correlation, otherwise moderate, is still uncertain. The higher TSH observed in severe uncomplicated obese women could be a homeostatic adjustment for excessive adiposity, as previously suggested.¹ The absence of significant differences in age and metabolic parameters between the two obese groups allowed us to evaluate the correlation between degree of obesity and thyroid function without the confounding effect of other variables.

We found also a positive correlation, although weak, between leptin adjusted for BMI and TSH. The significance of this correlation remains unknown and in disagreement with previous studies.¹⁰ Leptin

Table 2. Correlates of TSH, FT3 and FT4 in uncomplicated obese women

	BMI	BSA	Leptin	Leptin/BMI	Adiponectin	HOMA	Insulin	M index
TSH	$r = 0.44$ $P = 0.01$	$r = 0.267$ $P = 0.05$	$r = 0.41$ $P = 0.01$	$r = 0.33$ $P = 0.03$	$r = -0.25$ $P = 0.05$	$r = 0.24$ $P = 0.05$	$r = 0.22$ $P = 0.06$	$r = -0.22$ $P = 0.05$
FT3	$r = -0.12$ $P = 0.23$	$r = 0.17$ $P = 0.19$	$r = 0.14$ $P = 0.32$	$r = 0.10$ $P = 0.49$	$r = 0.19$ $P = 0.11$	$r = -0.24$ $P = 0.05$	$r = -0.25$ $P = 0.05$	$r = 0.23$ $P = 0.05$
FT4	$r = -0.25$ $P = 0.06$	$r = 0.19$ $P = 0.11$	$r = 0.10$ $P = 0.52$	$r = 0.11$ $P = 0.31$	$r = 0.18$ $P = 0.10$	$r = -0.31$ $P = 0.02$	$r = -0.32$ $P = 0.01$	$r = 0.27$ $P = 0.09$

Simple regression analysis (Spearman correlations) with TSH, FT3 and FT4 as prime variables of interest. BMI, body mass index; BSA, body surface area.

has been shown to modulate TRH gene expression in the paraventricular nucleus of the hypothalamus and changes in plasma TSH have been reported to contribute to the regulation of leptin pulses.^{6,11} TSH-stimulated leptin release by a direct action on adipocyte and TSH release by leptin have been recently demonstrated.^{12,13}

Nevertheless, in spite of experimental evidence of a consistent relationship between leptin and thyroid function, many studies in humans reported extremely conflicting data.^{10,14–22} Several workers failed to find a significant role of leptin in modulating the thyroid function, while others showed a negative correlation. However, whereas most of these studies have been performed on subjects with thyroid disease and a wide range of BMIs, in this present study we analysed obese subjects without history of thyroid dysfunction and negative for thyroid antibodies. The increase in TSH and leptin observed in our severe obese women could be an adaptive response to supply the high thermogenesis due to the increased fat amount. Both thyroid hormones and leptin might be involved in the adaptive thermogenesis as previously suggested.^{23,24} Because we have not found a correlation between thyroid hormones and leptin, it could be speculated that the supposed adaptative mechanism is partially ineffective or otherwise finalized to reduce energy expenditure in subjects with severe degree of obesity. According to this latter hypothesis, TSH could represent an early marker of altered energy balance in severe obese subjects.

Finally, we found a significant relationship, albeit weak, between thyroid function and adiponectin and insulin resistance parameters, such as M index from euglycaemic clamp, fasting insulin and HOMA-IR index. However, these findings should be interpreted with caution. These correlations could be significantly influenced by the BMI. In fact, most of the insulin resistance parameters were related to the degree of obesity and also severe obese women showed lower plasma adiponectin levels.

In conclusion, our data show that, although thyroid function was normal in the studied obese population, TSH and BMI were positively related. TSH has also been found to be correlated with leptin adjusted for BMI. TSH could represent a marker of altered energy balance in severe, but uncomplicated obese women. The functional significance of these correlations remains to be determined.

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