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ORIGINAL ARTICLE

Expression of thyrotropin and thyroid hormone receptors in adipose tissue of patients with morbid obesity and/or type 2 diabetes: effects of weight loss

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Objective: Increased thyroid-stimulating hormone (TSH) and FT_3 levels are often found in clinically euthyroid obese individuals. Information on thyroid gene expression in human adipose tissue is scarce. The objective of this study was to measure the expression of the TSH receptor (*TSHR*) and the thyroid hormone receptor (*TRα1*) genes in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) in obese individuals and to test the effect of weight loss on these genes.
Study Design and Participants: This study is a prospective study involving 107 obese (body mass index (BMI) $= 46 \pm 8\,kg\,m^{-2}$, 52 with type 2 diabetes or impaired glucose tolerance) and 12 lean nondiabetic participants. A total of 27 obese patients were restudied 1 year after gastric bypass surgery. Total RNA was extracted from SAT and VAT obtained at baseline from all participants, and from SAT in obese patients post surgery.
Results: Circulating TSH and FT_3 levels were 170 and 36%, respectively, higher in obese patients than in controls. In SAT, *TSHR* and *TRα1* were reduced in the obese by 67 and 33%, respectively, regardless of glucose tolerance. A similar trend was found in VAT. Post surgery, a BMI decrease of 33% was associated with a decrease in TSH and FT_3 levels and with a 150 and 70% increase in SAT of *TSHR* and *TRα1*, respectively.
Conclusion: In both subcutaneous and visceral fat, the thyroid gene expression (especially *TSHR*) is reduced in obesity. The reversal of these changes with major weight loss and the reciprocal changes in plasma TSH and FT_3 levels suggest a role for adipocytes in the regulation of TSH and thyroid hormones.
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Keywords: TSH receptor; thyroid hormone receptor; body weight

Introduction

It is now recognized that thyroid-stimulating hormone (TSH) does not exclusively target the thyroid gland, but also acts on various other tissues, including adipose tissue.[1,2] On the other hand, it is well known that obesity, especially central obesity, is associated with many hormonal changes. An assessment of thyroid function in severe obesity has shown conflicting results. In a large population study conducted in euthyroid patients from Denmark,[3] the body mass index (BMI) was found to be positively associated with serum TSH and inversely related to free tetraiodotyronine (FT_4). This finding has been confirmed in other studies,[4–6] but not in all.[7] However, in the latter report, study participants were referrals to a thyroid clinic. Furthermore, some studies were carried out in overweight individuals, others included morbidly obese individuals.[5] More recent studies have confirmed that diurnal serum TSH levels are significantly increased, if still within the normal range, in obese individuals, and that major weight loss induces a sizeable reduction in TSH and thyroid hormone levels.[8–10]

It is unknown whether TSH has a role in favoring fat deposition or, on the contrary, whether excessive accumulation of adipose tissue increases TSH secretion. Recent evidence in experimental animals and in humans has shown that adipocytes and preadipocytes possess TSH receptors (TSHRs),[11–13] and that TSH action induces the differentiation of preadipocytes into adipocytes, adipogenesis and, in children, lipolysis.[11,14,15] However, there are no data on the adipose tissue expression of the *TSHR* gene in conditions such as severe obesity and/or diabetes.

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Therefore this study was undertaken (1) to assess the separate role of obesity and type 2 diabetes (T2DM) in the expression of the thyrotropin receptor (TSHR) and thyroid hormone receptor (α1 subunit, TRα1) genes in visceral adipose tissue (VAT) and in subcutaneous adipose tissue (SAT) and (2) to explore the role of metabolic state and weight loss on receptor expression.

Methods

Participants and study design

The baseline study involved 107 severely obese participants undergoing gastric bypass surgery; 12 non-obese participants undergoing elective abdominal surgery (cholecystectomy) served as the control group. One year after bariatric surgery, 27 patients (14 with normal glucose tolerance (NGT) and 13 with T2DM at baseline) were restudied.

All participants provided informed, written consent, and the protocol was approved by the local Ethics Committee. The obese participants were subgrouped according to their glucose tolerance status (as determined by a standard 75-g oral glucose tolerance test (OGTT) or a previous diagnosis of T2DM): 55 participants had NGT (Ob-NGT), 20 had IGT, that is, impaired glucose tolerance, (Ob-IGT) and 32 had T2DM (Ob-T2DM). All patients were asked to attend our Unit for the metabolic study 2 weeks before surgery; they were also instructed not to exercise for 48 h before the metabolic study or surgery. For the metabolic study, all patients were examined in the morning after an overnight (12–14 h) fast. Peripheral blood samples were obtained for the determination of the lipid profile, plasma glucose, insulin, TSH, FT_3 and FT_4 concentrations. A 75-g OGTT was then performed, with sampling done for every 15 min within the first hour and thereafter for every 30 min for the following 2 h, for measuring levels of plasma glucose and insulin. IGT and diabetes were classified according to the American Diabetes Association (ADA) criteria[16] Diabetic patients who were not taking insulin were considered to have T2DM; insulin-taking diabetic patients whose age at the time of onset was $\geqslant 40$ years or whose BMI was $>30\,kg\,m^{-2}$ were also considered to have T2DM. The remaining insulin-taking diabetic patients were considered to have type 1 diabetes or to be unclassifiable, and were excluded from the study.

Anthropometric measurements, height, weight, waist and hip circumferences, as well as systolic and diastolic blood pressure levels were measured as described elsewhere.[17] BMI was calculated as weight divided by height squared, and used as an index of overall adiposity. Patients with a history of binge eating, over consumption of alcohol, smoking, end-stage renal disease, cardiac failure, hepatitis B virus or hepatitis C virus chronic hepatitis, thyroid diseases (hypothyroidism or hyperthyroidism, goiter, etc) or receiving thyroid supplementation were excluded from the study.

Plasma glucose concentration was measured on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA, USA). Fasting concentrations of serum total cholesterol low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides were determined as described elsewhere (Synchron CX4, Beckman Instruments, Inc., Brea, CA, USA). Plasma insulin was measured using a radioimmumoassay (Linco Research, St Charles, MO, USA), and TSH, FT_3 and FT_4 levels were measured using a fully automated immunoenzymometric assay (AIA 600 System, TOSOH Corporation, Tokyo, Japan).

Adipose tissue biopsy samples

At baseline, adipose tissue specimens obtained from subcutaneous (SAT) and visceral (VAT) fat depots (2–3 g) were collected during abdominal surgery in RNA-Later (Ambion Inc., Applied Biosystems, Austin, TX, USA), and stored at $-20\,^{\circ}C$ for total RNA extraction. At the follow-up study, a 600-mg adipose tissue specimen was obtained from SAT in a subgroup of 14 Ob-NGT and 13 Ob-T2DM during abdominal remodeling surgery.

Adipocyte isolation

Mature adipocytes were isolated as described by Grohmann *et al*.[18] The adipose tissue was washed thrice in Hank's balanced salt solution, cut into 1-mm^3 slices and digested with 3 ml of 4 mg ml^{-1} type II collagenase in Hank's balanced salt solution for 60 min at 37 °C. Fragments of the tissue still remaining after this treatment were removed with forceps. The mature adipocytes were separated from the stromal fraction by centrifugation at $90 \times g$ for 3 min and were carefully transferred into a sterile Eppendorf tube (Eppendorf, Hamburg, Germany).

Total RNA isolation and cDNA preparation

Total RNA was isolated using a mixed protocol: Trizol and RNeasy Midi Kit (Qiagen, Hilden, Germany) to obtain a larger amount of purified RNA that was quantified by measuring absorbance at 260 and 280 nm. RNA integrity was then checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on 1% agarose gel. The average yields of total RNA were $10 \pm 1\,\mu g\,g^{-1}$ of the adipose tissue. The isolated RNA was stored at $-80\,^{\circ}C$ until quantification of the target mRNAs. For first-strand cDNA synthesis, 1 μg of total RNA was reverse-transcribed in 20-μl volume using random hexamers as primers, according to the manufacturer's instructions (First strand cDNA Synthesis Kit for reverse transcription (RT)-PCR, AMV, Roche, Indianapolis, IN, USA).

Relative quantification of TSHR; TRα1 gene expression

The relative quantification of mRNAs was performed by real-time PCR using a Light-Cycler instrument (Roche Diagnostics, Mannheim, Germany). Gene-specific primers

for the target genes (*TSHR*: forward 5′-AGCCACTG CTGTGCTTTTAAG-3′ and reverse 5′CCAAAACCAATGAT-C TCATCC-3′, 260 bp; *TRα1*: forward 5′-GGTGCTGCATGGAG ATCATG-3′ and reverse 5′-GGAATGTTGTGTTTGCGGTG-3′, 325 bp; and the reference gene, hypoxantine-phosphorybosyl-transferase (*HPRT*): forward 5′-TGCTGACCTGCTGGATT-ACAT-3′ and reverse 5′TTGCGACCTTG-ACCATCTTT-3′, 260 bp) were designed in our laboratory. HPRT mRNA was used for sample normalization. A volume of 1 μl of cDNA was increased to a final volume of 20 μl in a glass capillary containing 1 × Light-Cycler-FastStar DNA Master SYBR Green I mix (Roche Diagnostics), 4.5 mM $MgCl_2$ and 0.3 μM of primers (Proligo, Paris, France) for TSHR, TRalfa1, respectively, and 0.5 μM for HPRT. PCR was performed with 10 min of initial denaturation and then 40 cycles with 10 s at 95 °C (denaturation), 7 s at 58 °C (annealing) and 13 s at 72 °C (extension). The last cycle was performed at 40 °C for 15 s. Fluorescent data were acquired at the end of each extension phase. After amplification, a melting curve analysis from 65 and 95 °C with a heating rate of 0.1 °C s^{-1} with a continuous fluorescence acquisition was constructed. To construct standard curves for each gene, total RNA from six control individuals (SAT and VAT) were pooled. In brief, after RNA reverse transcription of the pool sample, the cDNA was diluted into a three-fold serial dilution (1:3–1:27) and both genes (target and reference) were amplified in triplicate. The standard curve was used to assess PCR efficiency. Standard curves were accepted only if the slope for each gene was approximately −3 with an *r*-value >0.98. Each cDNA was quantified in triplicate. For the relative quantification of samples, the 'Light-Cycler Relative Quantification Software' (Roche Diagnostics, Roche Applied Science, Mannheim, Germany) was used. Results were expressed as the target–reference ratio of each sample, normalized by the target–reference ratio of the calibrator. The calibrator used for each quantification was the sample with a dilution of 10^{-3} used in the standard curve. In the present data, the coefficient of variation in measurements for the target gene in each sample was <10%.

We used *HPRT* as the reference gene because its expression was found to be relatively stable in different adipose tissue depots and showed no systematic differences between cases and controls. The mean C_t (± s.d.) of *HPRT* was 25.72 ± 1.42 in obese patients compared with 25.63 ± 1.95 in the control group for SAT, and 25.19 ± 1.24 in obese patients compared with 25.47 ± 1.73 in the control group for VAT.

Protein isolation and western blot analysis for TSHR

Adipose tissue biopsy samples (500–800 mg) were collected from six control and six obese individuals and immediately homogenized in complete Ripa lysis buffer 1 × (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004 % sodium azide (sc-24948, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) using a Tissue lyser (Qiagen). The homogenate was centrifuged at 500 × *g* for 15 min and the infranatant removed and stored at −80 °C. All steps were performed at 4 °C to minimize the risk of proteolysis. The protein assay in each sample was determined using a kit of reagents from Bio-Rad (Hercules, CA, USA). The fifth microgram per lane of total protein was then loaded onto polyacrylamide gels and separated by standard SDS-PAGE (SDS-polyacrylamide gel electrophoresis). Thereafter, the proteins were electrophoretically transferred into the nitrocellulose membrane (Bio-Rad). The blot was blocked overnight in 5% non-fat dried milk at 4 °C in phosphate-buffered solution-T (0.1% Tween-20 in phosphate-buffered solution) and then incubated for 2 h with primary monoclonal antibody against TSHRs (1:500, ab2812, Abcam Inc., Cambridge, MA, USA).

The secondary antibody conjugated to horseradish peroxidase was added at a dilution of 1:3000 (Santa Cruz). Antigen–antibody complexes were detected by chemiluminescence using a kit of enhanced chemoluminescence (ECL) reagents (Pierce, Pittsburg, PA, USA) and blots exposed to CL-XPosure films (Pierce). Films were scanned and the optical density of each specific band was analyzed using the Scion image software (Scion Corporation, Frederick, MD, USA).

Statistical analysis

Results are expressed as mean ± s.d. or median [interquartile range], for variables with normal or non-normal distribution, respectively. Group differences were compared by the χ^2 test for categorical variables and by the Kruskall–Wallis test for continuous variables. Analysis for repeated measures was carried out by ANOVA (analysis of variance) for repeated measures. Paired data were compared by the Wilcoxon signed rank test. Univariate associations were tested with Spearman's coefficient, rho. Multivariate analysis was carried out using general linear models, including both continuous and categorical variables (gender, presence of obesity and presence of diabetes); results are determined as the standardized regression coefficient (*sd.r*). For multivariate analysis, variables with non-normal distribution were log-transformed.

Results

Baseline study

The clinical characteristics of the study population are reported in Table 1. The three obese groups generally had similar clinical phenotypes, but blood pressure, serum triglycerides, fasting and 2-h plasma glucose levels, as well as fasting plasma insulin concentrations were higher in patients with T2DM than in NGT patients, whereas IGT patients showed intermediate values. Type 2 diabetic patients were on the following treatment: 67% with metformin and 33% with sulfonylureas; one-third of obese patients, 70% of whom had T2DM, were on antihypertensive treatment (angiotensin-converting enzyme inhibitors, calcium-channel blockers, β-blockers and diuretics).

Table 1 Baseline clinical and metabolic characteristics

Obese-NGT	*Obese-IGT*	*Obese-T2DM*	*Controls*	P-*value*
Subjects (*n*)	55	20	32	12	
Sex (F/M)*	49/6	14/6	23/9	8/4	
Age (years)*	40 ± 11	39 ± 10	48 ± 8	45 ± 10	
BMI (kg m^{-2})*	47.4 ± 9.7	46.9 ± 7.1	45.4 ± 6.4	23.6 ± 3.0	
Waist girth (cm)*	124.2 ± 15.0	132.1 ± 9.9	130.0 ± 15.1	84.6 ± 8.2	
SBP (mm Hg)*	126 ± 15	126 ± 17	139 ± 14	123 ± 10	
DBP (mm Hg)*	77 ± 11	80 ± 11	86 ± 9	77 ± 7	
TG (mg per 100 ml)*	112 [44]	135 [90]	186 [153]	121 [11]	
T-chol. (mg per 100 ml)	195 ± 33	207 ± 56	215 ± 52	192 ± 6	
HDL-chol. (mg per 100 ml)	49 ± 10	43 ± 11	45 ± 12	47 ± 2	
FPG (mg per 100 ml)*	88 [11]	97 [16]	153 [40]	88 [14]	
2-h PG (mg per 100 ml)*	112 [27]	166 [18]	243 [23]	113 [11]	
FPI (μU ml^{-1})*	16.8 [12]	15.4 [16]	20.7 [25]	7.7 [4]	
2-h PI (μU ml−1)	64 [79]	100 [63]	94 [115]	73 [42]	
TSH (μUI ml^{-1})*	1.49 [1.46]	1.43 [1.78]	2.00 [1.54]	0.80 [0.32]	
FT_3 (pg ml^{-1})*	3.7 [1.5]	3.8 [1.0]	3.5 [0.9]	2.7 [0.5]	
FT_4 (pg ml^{-1})*	10.2 [2.6]	9.6 [4.7]	10.7 [4.3]	12.5 [4.2]	
FT_4/FT3	2.76	2.52	3.10	4.63	

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; F, female; FGP, fasting plasma glucose; FPI, fasting plasma insulin; FT3, free threeiodothyronine; FT_4, free tetraiodotyronine; HDL-chol., high-density lipoprotein-cholesterol; M, male; PG, plasma glucose; PI, plasma insulin; SBP, systolic blood pressure; T-chol., T-cholesterol; TG, triglyceride; *TSH*, thyroid-stimulating hormone. *$P \leqslant 0.05$ by Kruskall–Wallis test. Data are expressed as mean ± s.d. or median [interquartile range].

Table 2 *TSHR* and thyroid hormone receptor (*TRα1*) gene expression, in subcutaneous (SAT) and visceral adipose tissues (VAT)[a]

Subjects (n)	*Obese-NGT* 55	*Obese-IGT* 20	*Obese-T2DM* 32	*Controls* 12	P-*value*
Subcutaneous adipose tissue					
TSHR	0.22 (0.28)	0.22 (0.32)	0.16 (0.25)	0.63 (0.41)	0.005
TRα1	0.67 (0.46)	0.80 (0.43)	0.79 (0.36)	1.09 (0.65)	0.07
Visceral adipose tissue					
TSHR	0.23 (0.24)	0.18 (0.14)	0.17 (0.16)	0.36 (0.40)	0.006
TRα1	0.48 (0.26)	0.49 (0.28)	0.43 (0.34)	0.59 (0.18)	0.15

Abbreviations: *HPRT*, hypoxantine-phosphorybosyl-transferase; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; *TSHR*, thyroid-stimulating hormone receptor; T2DM, type 2 diabetes. [a]Gene expression data are given as ratio of target gene to reference gene (*HPRT*). *P*-values are obtained by Kruskal–Wallis.

Plasma TSH and FT_3 concentrations were significantly higher in association with obesity (by 1.3 μUI ml^{-1} and 0.8 pg ml^{-1} on average, $P<0.001$ and $P=0.01$, respectively), whereas FT_4 concentrations were significantly lower (by 2.3 ng ml^{-1}, $P=0.02$), with no independent effect of diabetes (Table 1). In a multiple regression analysis, a positive correlation was found between plasma TSH and BMI (*sd.r* = 0.32, $P=0.001$) after adjusting for sex, age and fasting plasma glucose.

In the entire dataset, *TSHR* expression was higher in SAT than in VAT ($P=0.01$) (Table 2) as was its protein expression on western blot (1.70 ± 0.7 vs 1.4 ± 0.6, $P<0.05$) (Figure 1). Similarly, *TRα1* expression was higher in SAT than in VAT (0.70 [0.47] vs 0.48 [0.29], $P<0.0001$).

In SAT, the expression of *TSHR* was depressed markedly, and the *TRα1* expression tended to be lower, in association with obesity, with no separate effect of diabetes (Table 2).

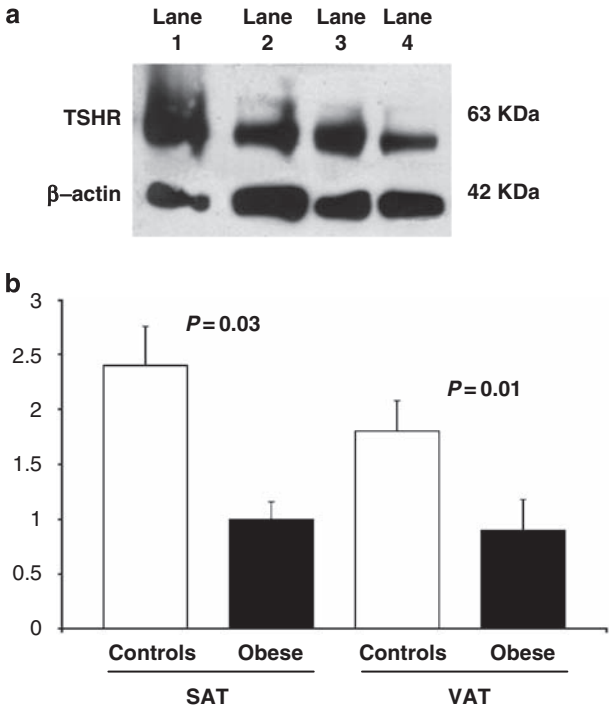

Figure 1 (**a**) Thyroid-stimulating hormone receptor (TSHR) and beta-actin protein expression by western blot analysis. Lane 1, subcutaneous adipose tissue (SAT) of a control subject; lane 2, SAT of an obese subject; lane 3, visceral adipose tissue VAT of a control subject; lane 4, VAT of an obese subject. (**b**) Mean ± s.d. TSHR protein expression (ratio between TSHR and β-actin) in SAT and VAT from six control subjects and 6 obese patients (Ob).

The protein expression of TSHR was also significantly lower in obese than in control individuals (Figure 1). Within this depot, expression levels of *TSHR* and *TRα1* were positively interrelated. In VAT, the *TSHR* expression was significantly lower in association with obesity, as confirmed by protein expression (Figure 1), whereas *TRα1* expression did not differ among groups (Table 2).

In a univariate analysis of the entire dataset, *TSHR* and *TRα1* expressions in VAT were each reciprocally related to BMI (with rho values of −0.28 and −0.21, respectively, $P \leqslant 0.02$). Similarly, in SAT, expression levels were inversely related to BMI (with rho values of −0.20 and −0.32, respectively, $P \leqslant 0.04$). In multivariate models adjusting for sex, age and fasting plasma glucose, *TSHR* and *TRα1* expressions remained significantly associated with BMI in an inverse manner both in SAT and in VAT (with *sd.r*s ranging from −0.18 to −0.28).

Follow-up study

For the 27 obese patients who underwent gastric bypass surgery (14 with NGT and 13 with diabetes at baseline), the clinical characteristics at baseline and 1 year after surgery are reported in Table 3. For an average 32% decrease in BMI, all clinical and metabolic parameters were significantly improved, the changes in HbA_{1c} (hemoglobin A1c) and fasting glucose concentrations being more pronounced in

Table 3 Clinical and metabolic characteristics after 1-year of follow-up

	NGT		*T2DM*	
	Pre	*Post*	*Pre*	*Post*
Subjects	14	14	13	13
Sex (F/M)	14/0	—	7/6	—
Age (years)	40 ± 8	—	47 ± 5	—
BMI (kg m^{-2})*	46.4 ± 6.3	30.6 ± 5.1	44.9 ± 4.6	30.7 ± 5.0
SBP (mm Hg)*	136 ± 23	121 ± 5	148 ± 11	128 ± 7
DBP (mm Hg)*	82 ± 17	75 ± 6	91 ± 5	81 ± 5
TG (mg per 100 ml)*	97 [18]	62 [35]	142 [97]	80 [88]
T-chol. (mg per 100 ml)*	198 ± 43	174 ± 31	236 ± 31	186 ± 35
HDL-chol. (mg per 100 ml)*	50 ± 19	58 ± 19	43 ± 8	50 ± 12
FPG (mg per 100 ml)*	90 [18]	86 [7]	133 [45]	86 [22]
FPI (μU ml^{-1})*	21 [23]	11 [4]	23 [16]	10 [9]
HbA_{1c} (%)*	5.2 [0.6]	5.1 [0.2]	6.0 [1.7]	5.7 [0.7]
TSH (μU ml^{-1})*	1.4 [0.7]	1.1 [1.0]	2.0 [0.9]	0.9 [1.0]
FT_3 (pg ml^{-1})*	2.9 [0.6]	2.5 [0.3]	2.8 [1.0]	2.6 [0.3]
FT_4 (pg ml^{-1})*	10.9 [4.0]	14.5 [6.9]	10.3 [3.9]	12.6 [4.5]

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; DBP, diastolic blood pressure; F, female; FGP, fasting plasma glucose; FPI, fasting plasma insulin; FT3, free threeiodothyronine; FT_4, free tetraiodotyronine; HDL-chol., high-density lipoprotein-cholesterol; M, male; NGT, normal glucose tolerance; PG, plasma glucose; PI, plasma insulin; SBP, systolic blood pressure; T-chol., T-cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; T2DM, type 2 diabetes. *$P \leqslant 0.05$ for the time factor by repeated measures ANOVA.

Table 4 *TSHR* and thyroid hormone receptors (*TRα1*) gene expression, in subcutaneous adipose tissue (SAT) after 1-year follow-up[a]

	Obese-NGT Pre	*Obese-NGT Post*	*Obese-T2DM Pre*	*Obese-T2DM Post*	P-*value*
Subjects (n)	*14*	*14*	*13*	*13*	
Subcutaneous adipose tissue					
TSHR	0.22 (0.3)	0.35 (0.6)	0.10 (0.4)	0.46 (0.8)	0.01
TRα1	0.78 (0.3)	1.21 (0.6)	0.69 (0.9)	1.33 (0.4)	0.0003

Abbreviations: ANOVA, analysis of variance; *HPRT*, hypoxantine-phosphorybosyl-transferase; NGT, normal glucose tolerance; TSHR, thyroid-stimulating hormone receptor; T2DM, type 2 diabetes. [a]Gene expression data are given as ratio of target gene to reference gene (*HPRT*). *P*-values are obtained by ANOVA for repeated measures.

diabetic than in NGT patients. Plasma TSH and FT_3 concentrations decreased in both groups post surgery, whereas FT_4 concentrations increased (Table 3). In SAT, the expression of *TSHR* and *TRα1* each was increased significantly regardless of glucose tolerance status (Table 4).

Discussion

The major findings of this study are that: (1) the expression levels of *TSHR* and *TRα1* are higher in SAT than in VAT; (2) in both adipose tissue depots, *TSHR*—and, marginally, *TRα1*—is reduced in obesity (and in proportion to the degree of obesity) regardless of the glucose tolerance status; (3) serum TSH and FT_3 concentrations are higher in obese than in lean individuals; and (4) after major weight loss, *TSHR* and *TRα1* expression in SAT increases significantly, with a concomitant reduction in circulating TSH and FT_3 levels.

Many previous studies have reported increased TSH and FT_3 levels in obese, clinically euthyroid patients;[3–6] our series confirms these findings. On the other hand, this study is one of the few investigations that have unequivocally shown that weight loss leads to a reduction in both TSH and FT_3 levels.[9,10,19,20] Several explanations have been proposed to account for the influence of obesity on thyroid hormones. One suggests that the increased TSH level could reflect pituitary adaptation to increased adiposity through adipokines. In morbidly obese patients with subclinical hypothyroidism, bariatric surgery led to changes in obesity-related adipokines believed to influence the synthesis and secretion of thyroid hormones.[9,19] However, in a recent study from our laboratory,[10] the direct relationship between 24-h mean concentrations of TSH and leptin in euthyroid obese patients was lost after adjusting for fat mass, suggesting that other mechanisms modulate TSH concentrations in morbidly obese patients. Another explanation is that the thyroid hormone profile of the obese individuals reflects pituitary resistance to thyroid hormones, with a variable degree of secondary impairment of thyroid hormone peripheral metabolism.[19] It has also been suggested that the impaired control of TSH release by thyroid hormones in obesity may represent an adaptive thermogenic phenomenon. Finally, it is possible that obesity leads to a state of TSH and thyroid hormone resistance in peripheral tissues.[20] However, information on the tissue expression of genes encoding for TSH and thyroid hormone receptors, and activity of the respective proteins in relation to body weight or diabetes is scarce. TSHRs have been found in rats and in human adipose tissues.[21,22] In the post-neonatal period and in children, an important lipolytic effect of TSH on adipose tissue has been described, rapidly declining with increasing age.[15] Other studies have shown that TSH has a role in the regulation of several adipokines synthetized in mature adipocytes[23,24,25] and in the induction of preadipocyte proliferation and differentiation.[26]

To our knowledge, this is the first study addressing the expression of *TSHR* and *TRα1* in adipose tissue obtained from obese patients, and evaluating the effect of weight loss on these receptors. The findings, of a reduced expression which reverses with weight loss and those of reciprocal changes in expression and circulating TSH and FT_3 concentrations, suggest a role of obesity—and the expanded adipose mass in particular—in regulating TSHRs and, indirectly, also in circulating TSH, rather than a contribution of thyroid axis to the causation of obesity. Thus, one could speculate that hypertrophy changes the cellular phenotype of the adipocyte, reducing the expression of these receptors in much the same way as with adiponectin receptors[27] or insulin sensitivity.[28] As a consequence of the downregulation in the thyroid hormone and TSHR expression, plasma TSH and FT_3 concentrations could increase to cope with peripheral hormone resistance. This sequence of events would be

reversed by weight loss, which restores the size and function of mature adipocytes. Unfortunately, we could not obtain functional data on the activity of these receptors before and after surgery in adipose tissue, which would support this hypothesis. Further studies determining peripheral indices of thyroid function (thyroid hormone metabolites, deoidinase activity and iodine intake) as well as receptor activity on adipose cells will be required to further characterize the clinical significance of increased TSH and low TSHR in morbidly obese patients.

It is of interest that, in our series the presence of diabetes had no effect on the thyroid hormone system independently of obesity. Although a low T_3 syndrome has been reported in some patients with decompensated diabetes,[29] adipose tissue mass has not been taken into account. Our finding relates to relatively well-controlled diabetes, which essentially resolved with major weight loss.

In conclusion, in obese individuals, *TSHR* is less expressed than in lean individuals in the face of higher plasma TSH levels; both these changes are reversed after major weight loss.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Davies TF, Andi T, Lin RY, Tomer Y, Latif R. Thyrotropin receptor-associated diseases: from adenoma to Graves disease. *J Clin Invest* 2005; **115**: 1972–1983.
2 Davies TF, Marians R, Latif R. The TSH receptor reveals itself. *J Clin Invest* 2002; **110**: 161–164.
3 Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L *et al*. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *J Clin Endocrinol Metab* 2005; **90**: 4019–4024.
4 Nyrnes A, Jorde R, Sundsfjord J. Serum TSH is positively associated with BMI. *Int J Obes* 2006; **30**: 100–105.
5 Michalaki MA, Vagenakis AG, Leonardou AS, Argentou MN, Habeos IG, Makri MG *et al*. Thyroid function in humans with morbid obesity. *Thyroid* 2006; **16**: 73–78.
6 De Pergola D, Ciampolillo A, Paolotti S, Trerotoli P, Giorgino R. Free triiodothyronine and thyroid stimulating hormone are directly associated with waist circumference, independently of insulin resistance, metabolic parameters and blood pressure in overweight and obese women. *Clin Endocrinol* 2007; **67**: 265–269.
7 Manji N, Boelaert K, Sheppard MC, Holder RL, Gough SC, Franklyn JA. Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects. *Clin Endocrinol* 2006; **64**: 125–128.
8 Kok P, Roelfsema F, Langendonk JG, Frölich M, Burggraaf J, Meinders AE *et al*. High circulating thyrotropin levels in obese women are reduced after body weight loss induced by caloric restriction. *J Clin Endocrinol Metab* 2005; **90**: 4659–4663.
9 Chikunguwo S, Brethauer S, Nirujogi V, Pitt T, Udomsawaengsup S, Chand B *et al*. Influence of obesity and surgical weight loss on thyroid hormone levels. *Surg Obes Relat Dis* 2007; **3**: 631–635.
10 Camastra S, Manco M, Frascerra S, Iaconelli A, Mingrone G, Ferrannini E. Daylong pituitary hormones in morbid obesity: effects of bariatric surgery. *Int J Obes* 2009; **33**: 166–172.
11 Sorisky A, Bell A, Gagnon A. TSH receptor in adipose cells. *Horm Metab Res* 2000; **32**: 468–474.
12 Bell A, Gagnon A, Grunder L, Parikh SJ, Smith TJ, Sorisky A. Functional TSH receptor in human abdominal preadipocytes and orbital fibroblasts. *Am J Physiol Cell Physiol* 2000; **279**: C335–C340.
13 Schaffler A, Binart N, Schölmerich J, Büchler C. Hypothesis paper brain talks with fat–evidence for a hypothalamic-pituitary-adipose axis? *Neuropeptides* 2005; **39**: 363–367.
14 Valyasevi RW, Harteneck DA, Dutton CM, Bahn RS. Stimulation of adipogenesis, peroxisome proliferator-activated receptor-gamma (PPARgamma), and thyrotropin receptor by PPARgamma agonist in human orbital preadipocyte fibroblasts. *J Clin Endocrinol Metab* 2002; **87**: 2352–2358.
15 Janson A, Karlsson FA, Micha-Johansson G, Bolme P, Brönnegård M, Marcus C. Effects of stimulatory and inhibitory thyrotropin receptor antibodies on lipolysis in infant adipocytes. *J Clin Endocrinol Metab* 1995; **80**: 1712–1716.
16 The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 2002; **25**: S5–S20.
17 Nannipieri M, Manganiello M, Pezzatini A, De Bellis A, Seghieri G, Ferrannini E. Polymorphisms in the hANP (human atrial natriuretic peptide) gene, albuminuria, and hypertension. *Hypertension* 2001; **37**: 1416–1422.
18 Grohmann M, Sabin M, Holly J, Shield J, Crowne E, Stewart C. Characterization of differentiated subcutaneous and visceral adipose tissue from children: the influences of TNF-alpha and IGF-1. *J Lipid Res* 2005; **46**: 93–103.
19 Krotkiewski M. Thyroid hormones in pathogenesis and treatment of obesity. *Eur J Pharmacol* 2002; **440**: 85–98.
20 Reinehr T, Andler W. Thyroid hormones before and after weight loss in obesity. *Arch Dis Child* 2002; **87**: 320–323.
21 Shimura H, Miyazaki A, Haraguchi K, Endo T, Onaya T. Analysis of differentiation-induced expression mechanisms of thyrotropin receptor gene in adipocytes. *Mol Endocrinol* 1998; **12**: 1473–1486.
22 Endo T, Ohno M, Kotani S, Gunji K, Onaya T. Thyrotropin receptor in non-thyroid tissues. *Biochem Biophys Res Commun* 1993; **190**: 774–779.
23 Antunes TT, Gagnon A, Chen B, Pacini F, Smith TJ, Sorisky A. Interleukin-6 release from human abdominal adipose cells is regulated by thyroid-stimulating hormone: effect of adipocyte differentiation and anatomic depot. *Am J Physiol Endocrinol Metab* 2006; **209**: E1140–E1144.
24 Mendez C, Baldelli R, Camina JP, Escudero B, Peino R, Dieguez C *et al*. TSH stimulates leptin secretion by a direct effect on adipocytes. *J Endocrinol* 2003; **176**: 7–12.
25 Marcus C, Ehren H, Bolme P, Arner P. Regulation of lipolysis during neonatal period. Importance of thyrotropin. *J Clin Invest* 1988; **82**: 1793–1797.
26 Haraguchi K, Shimura H, Kawaguchi A, Ikeda M, Endo T, Onaya T. Effects of thyrotropin on the proliferation and differentiation of cultured rat preadipocytes. *Thyroid* 1999; **9**: 613–619.
27 Nannipieri M, Bonotti A, Anselmino M, Cecchetti F, Mancini E, Baldi S *et al*. Pattern of expression of adiponectin receptors in human adipose tissue depots and its relation to the metabolic state. *Int J Obes* 2007; **31**: 1843–1848.
28 Foley JE, Laursen AL, Sonne O, Gliemann J. Insulin binding and hexose transport in rat adipocytes. Relation to cell size. *Diabetologia* 1980; **19**: 234–241.
29 Mouradian M, Abourizk N. Diabetes mellitus and thyroid disease. *Diabetes Care* 1983; **6**: 512–520.