Original Article
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Obesity

High-Normal TSH Values in Obesity: Is it Insulin Resistance or Adipose Tissue's Guilt?

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Objective: Clinical evidences reported subclinical alterations of thyroid function in obesity, although the relationship between thyroid status and obesity remains unclear. We cross-sectionally investigated the influence of metabolic features on hypothalamic–pituitary–thyroid axis in obesity.

Design and Methods: We enrolled 60 euthyroid subjects with no history of type 2 diabetes mellitus and assessed the relationship of thyroid function with insulin resistance, measured using euglycemic clamp, and abdominal fat volume, quantified by computed tomography scan (CT scan). Thyroid stimulating hormone (TSH) correlated with BMI ($r = 0.46$; $P = 0.02$), both visceral ($r = 0.58$; $P = 0.02$) and subcutaneous adipose tissue volumes ($r = 0.43$; $P = 0.03$) and insulin resistance (inverse relationship with insulin sensitivity–glucose uptake: $r = -0.40$; $P = 0.04$).

Results: After performing multivariate regression, visceral adipose tissue volume was found to be the most powerful predictor of TSH ($\beta = 3.05$; $P = 0.01$), whereas glucose uptake, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, subcutaneous adipose tissue volume, and triglycerides were not. To further confirm the hypothesis that high-normal TSH values could be dependent on adipose tissue, and not on insulin resistance, we restricted our analyses to moderately obese subjects' BMI ranging 30-35 kg/m^2. This subgroup was then divided as insulin resistant and insulin sensitive according to the glucose uptake ($\leq$ or >5 mg·kg^{-1}·min^{-1}, respectively). We did not find any statistical difference in TSH (insulin resistant: 1.62 $\pm$ 0.65 μU/ml vs. insulin sensitive: 1.46 $\pm$ 0.48; P = not significant) and BMI (insulin resistant: 32.2 $\pm$ 1.6 kg/m^2 vs. insulin sensitive: 32.4 $\pm$ 1.4; P = not significant), thus confirming absence of correlation between thyroid function and insulin sensitivity *per se*.

Conclusion: Our study suggests that the increase in visceral adipose tissue is the best predictor of TSH concentration in obesity, independently from the eventual concurrent presence of insulin resistance.

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Introduction

Several hormonal abnormalities, such as hypogonadism and polycystic ovary syndrome (1), are often accompanied by obesity; among them, thyroid dysfunction is certainly the most frequent hormonal impairment causing weight changes. However, while the effects of frank hyper or hypothyroidism on weight loss or gain are clear, the relationship between subclinical thyroid alterations and obesity is still controversial. Knudsen *et al.* (2) showed a correlation between serum thyroid stimulating hormone (TSH) and BMI, a negative association between BMI and FT4 levels, and no relationship between BMI and FT3 levels in a large population of 4,082 euthyroid subjects. Michalaki *et al.* (3) confirmed that severely obese subjects have higher TSH levels when compared with nonobese subjects; conversely, Manji *et al.* (4) failed to find any association between BMI and both TSH and FT4 levels in 401 euthyroid subjects.

While the relationship between obesity and subclinical thyroid dysfunctions remains unclear, it is overall debated what could eventually be the mechanism(s) linking subclinical thyroid dysfunction to obesity; in particular, whether variations of thyroid function in obesity are related either to the fat accumulation or to insulin resistance. In support to the first hypothesis, Nannipieri *et al.* (5) found reduced gene expression of TSH and FT3 receptors in both subcutaneous and visceral fat in obese subjects. This finding led the authors to speculate

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that the increase in plasma TSH and FT3 in obesity may be an attempt to cope with the peripheral resistance, as a consequence of the hypertrophy changes of adipocytes. This hypothesis appears particularly intriguing, as it closely follows what happens at the onset of insulin resistance, where the change in size of adipocytes causes resistance to insulin binding and signaling (6). On the opposite side, it has been suggested that compensatory hyperinsulinemia (usually present with insulin resistance) may decrease the activity of type 2 deiodinase in thyrotrophic cells, simulating a hypothyroid state (7), somehow consequence of insulin resistance. In support of this link, earlier studies reported a positive association between homeostatic model assessment and TSH (8) while a negative relationship between insulin resistance and FT4 has been found by other authors (9). Besides this, it is well known that hyper and hypothyroidism are associated with changes in insulin sensitivity (10,11).

One possible explanation for the controversial results of the earlier studies might rely on the use of very indirect methods to estimate adipose tissue and insulin resistance, i.e., BMI, and homeostatic model assessment indexes, respectively. In fact, it is now well established that not all the subjects with high BMI are invariably insulin resistant, mostly as a consequence of varying body compositions (12). Although BMI generally correlates with insulin resistance, the link of this association is mainly represented by visceral adipose tissue (whose measurement is merely indirectly included in the BMI) and not by BMI *per se* (13).

Therefore, in order to better elucidate the possible link between adiposity, insulin resistance, and thyroid function, we assessed these parameters by direct measurements, i.e., euglycemic clamp and computed tomography scan (CT scan), in a cohort of 60 euthyroid apparently healthy subjects.

Methods and Procedures

We selected 60 euthyroid subjects (24 males and 36 females, aged 50.4 ± 17.1 years, BMI 27.6 ± 7.6 kg/m^2) among patients referred to our Division for obesity and healthy volunteers working within our hospital. All of them had negative thyroid peroxidase antibody, without any pathological change in thyroid ultrasound and did not receive LT4 replacement or suppression therapy. All participants gave their informed consent. None of the study participants had relevant endocrine or non-endocrine diseases, did not refer history of type 2 diabetes mellitus, and they did not take any chronic medication therapy. All participants had normal hepatic, cardiopulmonary, and renal functions as determined by medical history, physical examination, electrocardiogram, urinalysis, and blood chemistry measured by autoanalyzer (COBAS Integra 800; Roche diagnostics, Basel, Switzerland).

Anthropometric and hormonal evaluation

Anthropometric parameters were determined according to the standard procedure (14). Height was measured, to the nearest 0.1 cm, with a wall mounted stadiometer. Weight was recorded to the nearest 0.01 kg by using a calibrated computerized digital balance. BMI was calculated as the weight (kg) divided by the square of height (m^2). All patients had blood samples taken for hormones assessment (TSH, FT3, and FT4) and serum lipid assay (total cholesterol, high and low-density lipoprotein (HDL and LDL cholesterol)). Blood measurement was done in the morning after an 8-h overnight fast. TSH, FT3, and FT4 serum concentrations were measured by a competitive luminometric assay based on the solid-phase antigen luminescence technique principle (Liason FT3, FT4, TSH, DiaSorin, Saluggia, Italy). The normal reference range are TSH: 0.35-4.2 μUI/ml; FT3: 2.3-4.2 pg/ml; FT4: 8.5-15.5 pg/ml. The intrassay TSH, FT3, and FT4 coefficients of variation were 1.9, 2.7, and 2.4%, respectively, and the corresponding interassay coefficients of variation were 5.1, 5.7, and 4.8%, respectively.

Glucose metabolism

One week before hyperinsulinemic euglycemic clamp, all patients performed oral glucose tolerance test. In all of them, insulin sensitivity was tested with hyperinsulinemic euglycemic clamp. The clamp test was performed after a 12-h overnight fast, as described by DeFronzo *et al*. (15) and previously published (16). Before the start of the insulin clamp, a catheter was placed into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into a vein on the dorsum of the hand. A primed constant infusion of insulin was given (Actrapid HM, 40 mIU/m^2·min; Novo Nordisk, Copenhagen, Denmark). The constant velocity for the insulin infusion was reached within 10 min to achieve steady-state insulin levels; in the mean time, a variable infusion of 20% glucose was begun via separate infusion pump, and the rate was adjusted, on the basis of plasma glucose samples drawn every 5 min, to maintain the plasma glucose concentration at each participant's fasting plasma glucose level (±5%). During the last 30 min of the basal equilibration period, plasma samples were taken at 5-10 min intervals for determination of plasma glucose and insulin concentrations. Whole-body peripheral glucose utilization was calculated during the last 30-min period of the steady-state insulin infusion and was measured as glucose uptake, i.e., the mean glucose infusion rate (as mg·kg^{-1}·min^{-1}) during the second hour of the euglycemic clamp.

CT scan

Body composition in total, subcutaneous, and visceral abdominal adipose tissue were determined by CT. All CT measurements were performed by trained staff in the Department of Radiology by using a GE CT scanner. All scans were performed in the supine position (120 kV, 200 mA, section thickness of 5 mm, scanning time of 0.5 s, field of view of 400 mm) measured on one cross-section scan obtained at the level of the L4-L5 (approximately the level of umbilicus), using the GE Volume Viewer Vox tool, version 3.0.64w software (GE Medical Systems, New York, NY). We used a fixed attenuation range from −190 to −30 HU, as the standard reference, to exclude bone, lean and mixtures of stool and air within the colon from the fat measure. An outer line was drawn manually to define the total fat. A region of interest of the subcutaneous fat layer, easily distinguishable by its density on the scan, was defined by tracing its contour using a cursor. The software calculated the volume of subcutaneous, visceral, and total fat in the region of interest and a histogram for fat tissue was computed on the basis of number of pixel with the attenuation values in the selection range.

Statistical analysis

Statistical analysis was carried out by using SPSS, version 9 (SPSS, Chicago, IL). $P < 0.050$ was considered significant. Data are expressed as mean ± s.d. Patients were stratified in two groups according to the BMI: we included subjects with BMI >25 in the overweight/obese group and ≤25 in the lean group. Wilcoxon signed rank test was used to evaluate hormonal and metabolic differences between the two groups.

TABLE 1 Clinical and biochemical characteristics of subjects in the entire cohort and based on BMI

Parameters	All	Overweight/obese	Lean	*P* value
n	60	32	28	NA
Age (year)	50.4 ± 17.1	52.9 ± 17.4	47.3 ± 17.2	NS
Gender (M/F)	24/36	13/19	11/17	
BMI (kg/m^2)	27.6 ± 7.64	31.8 ± 8.87	23.2 ± 0.98	<0.01
Glucose uptake (mg·kg^{-1}·min^{-1})	4.73 ± 1.83	4.25 ± 1.59	5.18 ± 1.95	0.02
Systolic blood pressure (mm Hg)	121 ± 20.5	125 ± 21.4	117 ± 19.6	NS
Diastolic blood pressure (mm Hg)	78 ± 19.5	81 ± 20.6	75 ± 18.4	NS
Total cholesterol (mg/dl)	191 ± 49.6	183 ± 40.7	198 ± 57.1	NS
LDL cholesterol (mg/dl)	125 ± 33.9	111 ± 33.6	137 ± 29.1	<0.01
HDL cholesterol (mg/dl)	49.1 ± 11.9	47.8 ± 14.5	50.2 ± 8.25	NS
Triglycerides (mg/dl)	132 ± 76.2	154 ± 99.6	112 ± 35.8	0.01
TSH (μUI/ml)	1.95 ± 1.11	2.58 ± 1.09	1.36 ± 0.76	<0.01
FT3 (pg/ml)	3.29 ± 0.72	3.45 ± 0.63	3.11 ± 0.78	NS
FT4 (pg/ml)	11.9 ± 2.13	11.8 ± 2.31	12.1 ± 1.98	NS
Total adipose tissue (cc)	10,250 ± 4,760	12,440 ± 3,762	8,202 ± 4,731	0.0001
Visceral adipose tissue (cc)	2,918 ± 2,091	4,146 ± 1,907	1,769 ± 1,545	<0.01
Subcutaneous adipose tissue (cc)	7,116 ± 3,489	8,139 ± 3,816	6,158 ± 2,895	0.01

Data are expressed as mean ± s.d.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable; NS, not significant; TSH, thyroid stimulating hormone.

In the entire cohort of patients, Spearman and Pearson correlation analysis were carried out to assess correlation between variables.

Multivariate regression analysis was used to investigate the relationship of TSH levels with insulin resistance, total, LDL, HDL cholesterol, triglycerides, visceral and subcutaneous adipose tissue. The sample size was calculated using ''Power and Sample Size'' software, version 2.1.25 (Department of Biostatistics, Vanderbilt University, Nashville, TN). On the basis of the research design and strategy, sample size was calculated using two means formula with 90% power of study. The minimum requirement for each group was 14 patients. To evaluate that significant differences would be found to evaluate each of the seven parameters in linear regression analysis, we performed power analysis finding that sample size of 60 will provide 90% power with the criterion for significance (α) set at $P = 0.05$.

Results

Clinical and hormonal characteristics of all subjects are summarized in Table 1.

Among all patients, an impaired fasting plasma glucose was found in 16.6%, impaired glucose tolerance in 20%, and impaired fasting plasma glucose/impaired glucose tolerance in 5% of our population. Unknown type 2 diabetes was diagnosed in 8.3% of the patients.

Twenty-eight patients were lean (BMI $\leq$25 kg/m^2) and 32 patients were overweight/obese (BMI >25 kg/m^2). The two groups were similar in age and proportion of male/female subjects. Although the overweight/obese group displayed a higher values of TSH compared with the lean group ($P < 0.01$), there was no difference regarding to the FT3 and FT4 levels.

Taking into account the metabolic status, the overweight/obese group showed a higher visceral ($P < 0.01$) and subcutaneous adipose tissue volume ($P = 0.01$) and an increase in insulin resistance ($P = 0.02$). The plasma insulin and glucose concentrations were higher during the oral glucose tolerance test in overweight/obese than in lean patients, but levels of statistical significance were not attained. We found a higher LDL cholesterol ($P < 0.01$) and triglycerides levels ($P = 0.03$) in the overweight/obese group, but we did not find any difference regarding to the total cholesterol and HDL cholesterol.

In the entire cohort of patients, serum TSH was positively associated with BMI ($r = 0.46$; $P = 0.02$), visceral adipose tissue volume ($r = 0.58$; $P = 0.02$) (Figure 1), and subcutaneous adipose tissue volume ($r = 0.43$; $P = 0.03$); instead an inverse relationship was found with glucose uptake ($r = -0.40$; $P = 0.04$) (Figure 2). No correlation was found between the values of TSH with lipid profile. In addition, FT3 and FT4 levels were not associated with any metabolic parameter.

Given the close relationship of TSH with several metabolic parameters, we decided to perform multivariate regression analysis ($P < 0.01$) to identify which metabolic factor had a major role in determining the changes in TSH in a model including subcutaneous and visceral adipose tissue, triglycerides, LDL, HDL cholesterol, triglycerides, and glucose uptake. The most powerful predictor of TSH concentration was visceral adipose tissue volume ($P = 0.01$), whereas glucose uptake, HDL cholesterol, LDL cholesterol, subcutaneous adipose tissue volume, and triglycerides were not powerful predictors (Table 2).

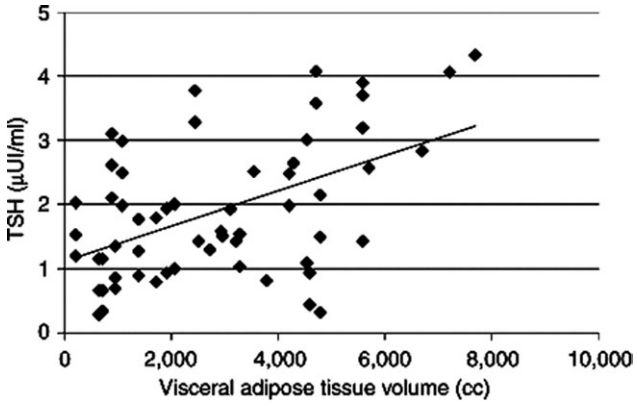

FIGURE 1 Correlation between TSH and visceral adipose tissue volume. R = 0.58, P = 0.02. TSH, thyroid stimulating hormone.

Since obesity is not invariably associated with insulin resistance (12), as normal insulin sensitivity can be present in some obese subjects as well, and to further confirm the hypothesis, already demonstrated by linear regression that increase in TSH was mostly dependent on visceral adipose tissue more than insulin resistance, we restricted our analyses to moderately obese subjects. We therefore selected 15 obese insulin-resistant and 14 obese insulin-sensitive subjects with BMI >30 kg/m^2, but <35 kg/m^2 among the obese/overweight group. This population was then divided into two groups: insulin resistant and insulin sensitive based on glucose uptake (glucose uptake $\leq$ or >5 mg·kg^{-1}·min^{-1}, respectively) as earlier reported (16). By definition, glucose uptake was highly statistically different (2.91 $\pm$ 0.75 vs. 5.88 $\pm$ 2.45 mg·kg^{-1}·min^{-1}) between the two groups while we did not find any statistical difference in TSH concentration (insulin resistant: 1.62 $\pm$ 0.65 μU/ml vs. insulin sensitive: 1.46 $\pm$ 0.48; P = not significant) and BMI (insulin resistant: 32.2 $\pm$ 1.6 kg/m^2 vs. insulin sensitive: 32.4 $\pm$ 1.4; P = not significant).

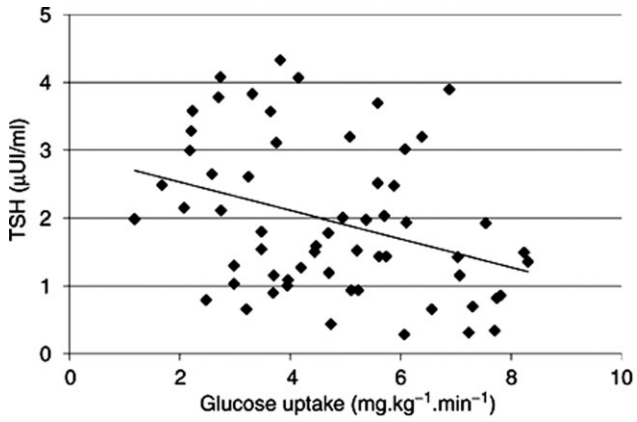

FIGURE 2 Correlation between TSH and glucose uptake. R = -0.40, P = 0.04. TSH, thyroid stimulating hormone.

TABLE 2 Multivariate regression analysis for metabolic parameters predicting thyroid stimulating hormone levels

	β	P
Visceral adipose tissue (cc)	3.05	0.001
Subcutaneous adipose tissue (cc)	0.28	NS
LDL cholesterol (mg/dl)	–1.25	NS
HDL cholesterol (mg/dl)	0.67	NS
Total cholesterol (mg/dl)	–0.583	NS
Tryglicerides (mg/dl)	1.47	NS
Glucose uptake (mg·kg^{-1}·min^{-1})	–1.15	NS

The only significant parameter was body visceral adipose tissue.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant.

Discussion

These data suggest that TSH level is related to visceral adipose tissue volume in overweight/obese subjects, and raise the interesting possibility of a functional link between the pituitary-thyroid axis and adiposity.

The relationship between thyroid hormones and body mass is well known: weight loss is a typical sign of hyperthyroidism, instead hypothyroidism is associated with weight excess (10,11). Several authors found an increase in serum TSH levels, although in the upper limit of the normal range, in euthyroid obese individuals (17-19), leading to different hypotheses regarding to the link between thyroid function and the metabolic syndrome features. In fact, it is not clear whether obesity *per se* may influence thyroid function (through the increase in adipose tissue and/or insulin resistance) or if small differences in thyroid function, even in the range of normality, might chronically play a role in modulating body weight. This important question could appear apparently answered through an interventional study: studying the effects of a losing weight program on thyroid function would validate the first hypothesis, administrating thyroid hormones up to subclinical hyperthyroidism to reduce body weight and/or visceral fat would confirm the latter. However, both losing weight and subclinical hyperthyroidism have significant confounding effects (e.g., reducing inflammation for the first and intermediate metabolism, such as for cholesterol, for the latter) that do not guarantee a clear answer to this important clinical question. We therefore decided to re-examine this query establishing visceral fat and insulin sensitivity with state-of-the-art methods, i.e., CT scan and hyperinsulinemic clamp. In fact, although these two parameters usually correlate, they do not invariably coexist; since increased visceral adipose tissue is considered the major cause (and not the consequence) of insulin resistance in obesity, if thyroid function correlates better with visceral obesity than insulin resistance, thyroid alterations would become the suspect primary event of this dangerous vicious circle.

In agreement with earlier studies (2,3), our data showed a significant increase in TSH levels in obese subjects compared with lean, but no differences regarding to FT3 and FT4 levels. Strictly speaking, higher TSH together with nondifferent FT3 and FT4 cannot be defined as subclinical hypothyroidism; attempting to understand the

cause of higher TSH in the presence of normal FT3 and FT4 makes our observations even more stimulating.

A relevant finding in our study is the strong correlation between thyroid function and abdominal adipose tissue, in particular, with visceral adipose tissue. In agreement with us, Westerink *et al*. (20) already observed a relationship between TSH and visceral adipose tissue, much more than subcutaneous adipose tissue, even though the limit of the study was the poor accurate measurement of adipose tissue (ultrasound scans) and no accurate estimation of insulin sensitivity was performed. A further explanation of this link may lie in the ability of adipose tissue to express TSH receptors, which is highest in the early stages of differentiation and declines later in time (21). In fact, TSH may also promote adipogenesis in embryonic stem cells, in absence of other proadipogenic factors (22). Obesity may play an important role in modifying the thyroid hormones and TSH receptor gene expression in both subcutaneous and visceral adipose tissue, as reported by Nannipieri *et al*. (5) who measured their expression in subjects undergoing gastric bypass. The weight loss was associated with an increase in thyroid hormones and TSH receptor gene expression, leading to hypothesize that hypertrophy modifies the cellular phenotype of adipocyte reducing the expression of these receptors in much the same way as with insulin receptors (6). However, according to our results, visceral adipose tissue seems to be a more powerful predictor than subcutaneous adipose tissue in determining TSH levels; one explanation could be that the increase in visceral adipose tissue may be responsible for creating a vicious cycle. Furthermore, as previously hypothesized (7), the increase in insulin resistance (and the compensatory hyperinsulinemia arising from the enlargement of visceral adipose tissue's reservoir) may exert a direct effect on the pituitary−thyroid axis. In our study, we found an inverse linear correlation between TSH and glucose uptake during hyperinsulinemic euglycemic clamp, i.e., greater was the increase in TSH levels, greater was the decrease in insulin sensitivity. However, this relationship lost its significance in the multivariate regression analysis, allowing us to hypothesize that the main effect of insulin resistance on TSH was not independent, but it was mediated by adipose tissue. In agreement with our hypothesis, Brüning *et al*. (23) observed the expression of insulin receptors both in hypothalamic and pituitary murine samples; furthermore, there are *in vitro* evidences that insulin may induce type 2 deiodinase (D2) expression in hepatocytes from fasted rats (24). Thus, it was hypothesized that in severe obesity, due to insulin resistance, the activity of D2 in the pituitary is decreased (7). This may cause a reduced intracellular availability of T3 in thyrotrophic cells resembling a ''state of hypothyroidism'' and, as a consequence, an increase in TSH levels.

In turn, the increase of TSH may promote the release of adipokines by adipose tissue. Adding TSH to differentiated subcutaneous adipose tissue increased interleukin-6 production by adipocytes (25). This inflammatory response may be regulated through the activation of the nuclear factor-κB pathway by TSH receptor on the p65-p50 dimer (26). These data suggest that TSH may promote a proinflammatory state that may be responsible of the increase of insulin resistance.

Another intriguing hypothesis to explain higher TSH levels in obesity identifies leptin as the main link. As well known, leptin secretion is increased in obesity, as a compensatory mechanism to leptin resistance, and it has been shown to up regulate thyrotropin releasing hormone expression in rat hypothalamus, suggesting that the increased leptin feedback may enhance TSH output (27). However, since leptin is produced by both subcutaneous and visceral adipose tissue, if TSH changes are due to leptin secretion, one would expect to find TSH correlating with both visceral and subcutaneous adipose tissues together. Since our data demonstrate a clear correlation only with visceral fat, they do not support (although leptin concentration is unfortunately missing) this hypothesis.

In conclusion, the increase of adipose tissue, mostly the visceral adipose tissue, and the consequent insulin resistance may play the pivotal role in the changes of thyroid homeostasis, but in turn the impairment of pituitary-thyroid axis may worsen the features of obesity. The main limit of our study is to be cross-sectional, allowing us to make only assumptions; further studies are needed to clarify whether the mechanism through the visceral adipose tissue reset the thyrostat at higher levels. Although we cannot ascertain whether the primary dysfunction relies on thyroid or visceral fat, the existence of this intriguing vicious cycle is strongly suggested, compelling for further research and possible solutions.

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