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Levels of obestatin in euthyroid patients receiving levothyroxine replacement therapy

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Abstract

To investigate the relationship between obestatin and TSH levels in patients who received thyroid hormone replacement and were in the euthyroid state. Included 30 patients who were followed up in the endocrinology outpatient clinic in 2020, who received L-thyroxine replacement, and 30 healthy individuals without any chronic disease who did not receive thyroid hormone replacement. Blood samples were taken from both groups for obestatin, thyroid-stimulating hormone (TSH) and free T4 hormone (fT4) levels. There is significant difference in TSH levels ($p = 0.007$) was found between the two groups. The obestatin levels of the L-thyroxine replacement group were lower than those of the control group ($p = 0.001$). No correlation was observed between the TSH and obestatin levels in the control and L-thyroxine replacement groups. In this study, obestatin levels were significantly lower in patients who received L-thyroxine replacement therapy and were in euthyroid status than those in the control group. No correlation was found between obestatin and TSH levels in the control and patient groups. As a result of this study, the thyroid gland may play a more important role in the synthesis or regulation of obestatin synthesis beyond our current knowledge.

Keywords: Obestatin, hypothyroidism, obesity, thyroid gland functions

Introduction

Obestatin is a peptide hormone consisting of 23 amino acids secreted from the gastric mucosa. This hormone is encoded in the gastric mucosa by a gene called preproghrelin. In addition, ghrelin hormone is a peptide hormone encoded from the same gene. Ghrelin plays a role in energy balance, appetite, weight gain and adipogenesis in the body. Obestatin has opposite effects; it reduces appetite and slows gastric emptying, leading to weight loss. The pathophysiological significance of ghrelin, which consists of 28 amino acids, was discovered in 1999 [1]. This peptide, which can stimulate growth hormone synthesis in the anterior pituitary gland, is found in the hypothalamus, pituitary gland, intestines, kidneys, heart and pancreas. Although some effects on the hypothalamic-pituitary axis, carbohydrate metabolism, fat metabolism, appetite and cardiovascular system are known, information about this peptide hormone remains insufficient [2].

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Obestatin was first detected in rat stomach and predicted to have effects opposite to those of ghrelin in terms of gastrointestinal function and energy balance. Apart from the stomach, the presence of this peptide has also been shown in the thyroid, small and large intestines, breast ducts and pancreas [3]. Obestatin increases the pancreatic beta-cell mass and decreases insulin resistance in the periphery [4]. Moreover, studies have shown that obestatin levels are low in individuals with type 2 diabetes mellitus (DM) and impaired fasting glucose [5]. The expression of obestatin and ghrelin in thyroid gland follicular epithelial cells has been reported [6]. However, the effects of these hormones associated with the thyroid gland have not yet been elucidated. The relationship between thyroid gland functions and obestatin and ghrelin is not fully known yet. Studies showing the possible relationship between these hormones and changes in appetite and weight observed in patients with thyrotoxicosis and hypothyroidism are limited. Especially patients with hypothyroidism tend to gain weight. However, patients who receive L-thyroxine replacement due to hypothyroidism and who are in the state of euthyroidism also tend to gain weight [7].

Hypothyroidism is the insufficiency of thyroid hormone in the plasma because the hormone cannot be sufficiently synthesized in

the thyroid gland. This disease is a thyroid gland disease occurring due to causes including autoimmune diseases, post-surgery and radioactive iodine intake and is common in the community [8]. Obesity is a chronic disease affecting children, adolescents and adults worldwide and is closely associated with type 2 DM, hypertension, dyslipidemia, cardiovascular disease, sleep apnea and cancer prevalence. Therefore, obesity is an important cause of mortality and morbidity [9].

In this study, we investigated the relationship between obestatin and TSH levels in patients who received thyroid hormone replacement and were in the euthyroid state.

Materials and Methods

Our study included 30 patients who were followed up in the endocrinology outpatient clinic in 2020, who received L-thyroxine replacement, and 30 healthy individuals without any chronic disease who did not receive thyroid hormone replacement. Patients who were found to have thyroid cancer at the end of the operation were excluded from the study. Blood samples were taken from both groups for obestatin, thyroid-stimulating hormone (TSH) and free T4 hormone (fT4) levels. Hypothyroidism was determined according to the normal ranges of TSH and fT4 used in our laboratory (TSH:0.34–5.6μIU/mL;fT4:0.58–1.38ng/dL). The study was approved by Atatürk University Ethical Board (Date: 17/12/2020, Meeting Number: 10, Decision number: 22)

Biochemical Analysis

Blood samples were taken from the antecubital area using a vacutainer from healthy controls and patients who received L-thyroxine replacement after they were allowed to rest in a sitting position. Samples were taken between 8:00 am and 10:00 am after 12 h of fasting and centrifuged for 30 min at room temperature after clotting was completed. After centrifugation, the serum samples were separated and stored until analysis by freezing at −80°C. After the serum samples were resolved under suitable conditions for analysis, all analyses were performed in a single session in the medical biochemistry laboratory. Obestatin levels in the serum samples were analyzed using Elabscience brand (Cat No: E-EL-H1989, Elabscience, Texas, USA) ELISA kits and a Dynex brand automatic ELISA reader device (Dynex Technologies Headquarters, Chantilly, USA) according to the standard protocol recommended by the manufacturer. Serum fT4 and TSH levels were measured by the chemiluminescence immunoassay method using a DxI 800 device (Beckman Coulter Inc, Brea, CA, USA).

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (version 20.0; IBM Corp., Armonk, NY, USA). The suitability of the parameters to the normal distribution was evaluated using the Kolmogorov–Smirnov test. Independent samples t-tests (independent t-test and Student's t-test) were used to compare the serum obestatin values of the patients and controls. The correlation between parameters was evaluated using the Pearson correlation analysis. Results are presented as mean ± standard deviation (SD). P values of <0.05 were used to denote statistical significance.

Results

The study included 30 patients and 30 controls. The main demographic, anthropometric, clinical and biochemical characteristics of the participants are presented in Table 1. The TSH levels were 3.96±3.27 μIU/mL in the L-thyroxine replacement group and 2.18±1.21 μIU/mL in the control group. There is significant difference in TSH levels (p=0.007) was found between the two groups. The obestatin levels were 5.67±1.36 ng/mL in the L-thyroxine replacement group and 8.20 ± 1.99 ng/mL in the control group. The obestatin levels of the L-thyroxine replacement group were lower than those of the control group (p=0.001) (Figure 1). No correlation was observed between the TSH and obestatin levels in the control and L-thyroxine replacement groups (Figure 2a, 2b).

Table 1. The main demographic, anthropometric, clinical and biochemical characteristics of the participants

Parameters	L-thyroxine replacement group (n = 30)	Control group (n = 30)	P value
Age	44.50±14.11	43.20±14.74	0.734
BMI (kg/m²)	29.12±4.46	28.16±4.82	0.432
TSH (μIU/mL)	3.96±3.27	2.18±1.21	0.007
Obestatin (ng/mL)	5.67±1.36	8.20±1.99	0.001

BMI: Body mass index, TSH: Thyroid stimulating hormone

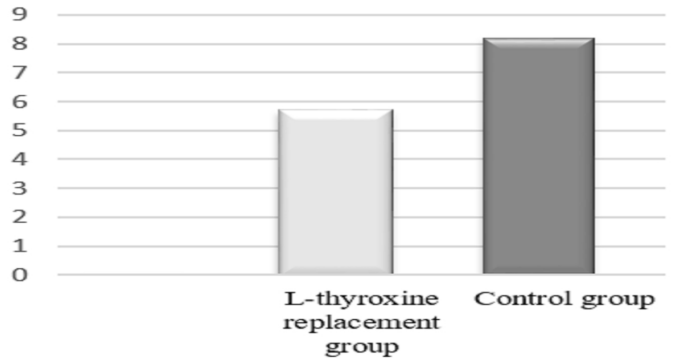

Figure 1. Obestatin levels (ng/ml, p<0.001)

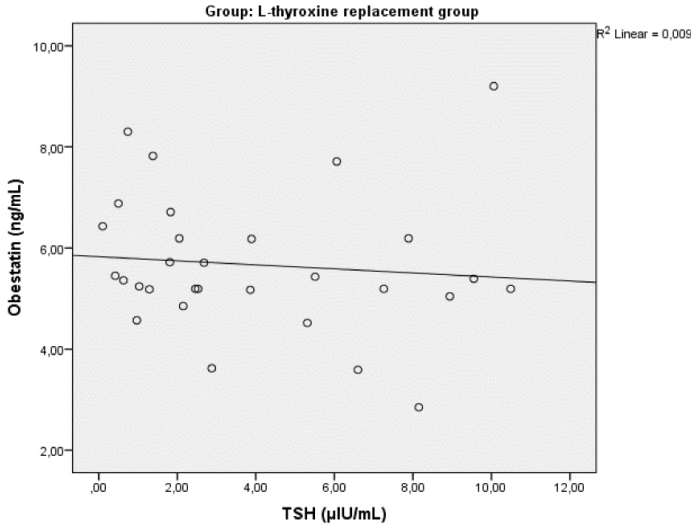

Figure 2a. Relationship between TSH and obestatin levels in L-thyroxine replacement group

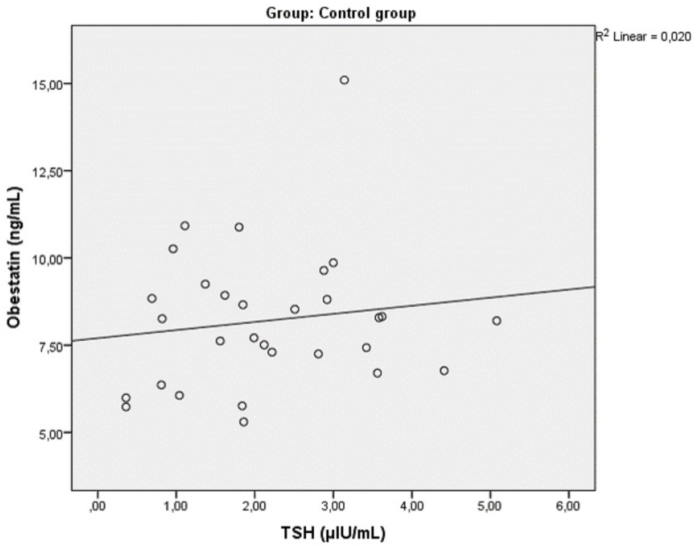

Figure 2b. Relationship between TSH and obestatin levels in control group

Discussion

In this study, where we examined the obestatin levels of patients with hypothyroidism for any reason and receiving thyroid hormone replacement therapy, obestatin levels were significantly lower than those of the control group. No correlation was observed between the TSH and obestatin levels in both groups.

Hypothyroidism is the insufficiency of thyroid hormones that the body needs. This situation occurs due to the surgical removal of the thyroid gland, its ablation with radioactive iodine therapy or the inability to synthesis sufficient hormones due to atrophy after thyroiditis. Thyroid hormone synthesis occurs by organizing the iodine taken and combining it with thyroglobulin. All stages including iodine uptake, thyroid hormone synthesis, and storage and release of thyroid hormones are regulated by the hypothalamic-pituitary-thyroid axis. Thyroid hormones are metabolic hormones closely related to basal metabolic rate, thermogenesis, food intake, fat oxidation and appetite [10]. Weight gain occurs in patients with hypothyroidism. However, weight gain continues even in patients who become euthyroid with thyroid hormone replacement [11]. This situation has been attempted to be explained in many studies [12, 13] Although the cause is not fully explained, especially in patients with thyroidectomy, somehow permanent metabolism slowdown occurs even if euthyroidism is achieved [14].

Furthermore, some gastric mucosal peptide hormones including obestatin play a regulatory role in the body's nutrition and energy balance along with thyroid hormones (3). These hormones modulate the central pathways in the brain, including the hypothalamus, to maintain energy homeostasis [15]. The relationship between thyroid hormones and intestinal peptides has not been elucidated yet [16]. Obestatin released from the gastric mucosa is found in various parts of the body and tissues, especially in the hypothalamus, pituitary gland, heart, pancreas, kidneys, spleen and gonads. In addition, obestatin, a peptide with 23 amino acids, has an effect opposite to that of ghrelin, a similar protein derivative [17]. In this study, obestatin levels were lower in euthyroid patients who received L-thyroxine replacement. Few studies in the literature have focused on the relationship between thyroid gland functions and obestatin levels. In these studies, obestatin levels were studied in patients with hyperthyroidism or hypothyroidism. However, the results in patients with hypothyroidism and hyperthyroidism are contradictory. For example, in a study by Emami et al., while obestatin levels were significantly lower in patients with subclinical hypothyroidism, they were significantly higher in patients with subclinical hyperthyroidism. Besides, the significant correlation between the results obtained in both groups and TSH levels was also shown in this study [16]. However, in another study, obestatin levels were similar in patients with hypothyroidism and the control group [18]. In our study, although the euthyroid status was achieved with L-thyroxine replacement, obestatin levels were significantly lower in patients who received L-thyroxine replacement than those in the control group.

Obesity is a multifactorial disease in which many underlying causes such as genetic, environmental, lifestyle and hormonal factors coexist [19]. In a study, the obestatin levels in obese children were lower than those in the non-obese control group [20]. In another study, obestatin levels were significantly higher in obese children in proportion to the body mass index. Since the role of obestatin in obesity is not fully understood, the detection of high obestatin levels in obese individuals has been attempted to be explained as a compensatory mechanism by researchers [21]. In the review by Zhang et al., nine studies published in PubMed were compiled, and as a result, it was stated that obestatin levels were lower in obese patients than those in normal individuals [22].

The relationship between hormones such as TSH and TRH, which are involved in the regulation of thyroid functions, and intestinal peptides, which play a role in weight gain and energy balance, has not been adequately explained today. In Gurgul's study, obestatin expression has been shown in the thyroid tissue itself and some types of thyroid cancer [6]. Considering the results obtained from the Aly's study, there may be a cross-talk between the hypothalamic-pituitary-thyroid axis and the intestinal peptides whose effects on the metabolism have been shown [20]. In our study, hypothyroidism in patients occurred after conditions such as thyroidectomy, radioactive iodine treatment or thyroiditis. The results of our study regardless of the disease causing hypothyroidism, even in patients with euthyroid status after L-thyroxine replacement, suggest that the thyroid gland may play a role in obestatin metabolism due to the significantly lower levels of obestatin. Although sufficient thyroid hormone was administered and the state of euthyroidism was achieved, the low levels of obestatin support this theory. Today, the thyroid gland classically secretes thyroid hormones and calcitonin from parafollicular C cells. However, as result of the data of our study, it suggests that the thyroid gland either synthesizes obestatin itself or produces a substance that takes part in the regulation of tissues known to synthesize obestatin. Another issue supporting our theory is that no correlation was found between obestatin and TSH levels in both the control group with intact thyroid tissue and the patient group where the gland was surgically removed or damaged in some way. The possible relationship between the thyroid gland and obestatin metabolism seems to be independent of the classical hypothalamic-pituitary-thyroid axis. Currently, obestatin seems to be a potential new drug to control insulin resistance, appetite and weight gain and to prevent complications in patients with diabetes [23]. However, there are no data in the literature regarding

obestatin treatment or replacement in patients whose thyroid gland has been removed or damaged. In the future, considering obestatin treatment in patients who are prone to gain weight is possible, especially those in the euthyroid state, just as in patients with DM. As a limitation; the number of patients we included in this study was relatively small and in addition to obestatin, examining the relationship between ghrelin hormone may provide more detailed information. However, this study was designed to shed light on more comprehensive studies on this subject in line with the results we obtained.

Conclusion

In this study, obestatin levels were significantly lower in patients who received L-thyroxine replacement therapy and were in euthyroid status than those in the control group. No correlation was found between obestatin and TSH levels in the control and patient groups. As a result of this study, the thyroid gland may play a more important role in the synthesis or regulation of obestatin synthesis beyond our current knowledge. Because of more comprehensive and well-designed studies, the possibility of using obestatin for weight control, regardless of thyroid hormone replacement, will be better understood in patients whose thyroid gland has been removed or damaged in any way.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was approved by Atatürk University Ethical Board (Date: 17/12/2020, Meeting Number: 10, Decision number: 22)

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