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Association between serum thyroid hormone balance and thyroid volume in patients treated with levothyroxine monotherapy for hypothyroidism

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Abstract. Many previous studies including ours have reported that athyreotic patients on levothyroxine (LT_4) have relatively low serum free triiodothyronine (FT_3) levels, whereas patients with large goitrous diseases often have high serum FT_3 levels. Here we investigated Hashimoto thyroiditis (HT) patients on LT_4 to study the relationship between thyroid volume (TV) and thyroid hormone status in hypothyroid patients on LT_4. We retrospectively studied 408 euthyroid HT patients treated with LT_4 for hypothyroidism; divided them as per TV and compared serum levels of free thyroxine (FT_4) and FT_3 and the FT_3/FT_4 ratio in each patient group with those in euthyroid matched control group. We also evaluated the association between serum FT_3 level and FT_3/FT_4 ratio and TV among HT patients on LT_4. In patients with TV <15 mL, serum FT_3 levels were significantly lower than those in controls. In patients with TV 15–80 mL, serum FT_3 levels were equivalent to those in controls. In patients with TV ≥80 mL, the serum FT_3 levels were significantly higher than those in controls. The serum FT_3 level ($r = 0.35$, $p < 0.01$) and FT_3/FT_4 ratio ($r = 0.42$, $p < 0.01$) showed a positive correlation with TV. TVs in HT patients on LT_4 caused differences in serum thyroid hormone balance, as increasing volume increases the serum FT_3 level and FT_3/FT_4 ratio. Serum thyroid hormone balance in HT patients with smaller thyroids was similar to that in athyreotic patients. Mild thyrotropin suppression with LT_4 is needed to achieve normal FT_3 levels in such patients.

Key words: Thyrotropin, Triiodothyronine, Levothyroxine, Hashimoto thyroiditis, Hypothyroidism

THE TWO MAJOR THYROID HORMONES in the body are triiodothyronine (T_3) and thyroxine (T_4). Approximately 20% of T_3 is produced from the thyroid gland *via* two pathways, as follows: coupling of monoiodotyrosine and diiodotyrosine (DIT) and conversion of T_4 to T_3 by type 1 and type 2 iodothyronine deiodinases (D1 and D2, respectively). The remaining 80% of T_3 is derived from the conversion of T_4 to T_3 in extrathyroidal tissues. In contrast, 100% of T_4 is secreted by the thyroid gland through the coupling of two DIT moieties [1].

Some previous studies including ours have reported normal serum thyrotropin (TSH) levels associated with mildly low serum free triiodothyronine (FT_3) levels in patients on levothyroxine (LT_4) monotherapy for athyreotic or atrophic conditions after total thyroidectomy or after radioiodine treatment for Graves' disease [2-5]. In addition, we have reported that the presence of the remnant thyroid tissue was associated with normal FT_3 levels in patients treated with LT_4 who underwent hemithyroidectomy [6] or radioiodine treatment for Graves' disease [5]. In contrast, we documented that Hashimoto thyroiditis (HT) patients with increased thyroid volume (TV) tended to present with high serum FT_3 levels, low free thyroxine (FT_4) levels, and high FT_3/FT_4 ratios [7, 8]. Thus, TV may be an important factor affecting thyroid hormonal balance, including serum FT_3 levels and FT_3/FT_4 ratios.

In the present study, we investigated the thyroid hormone balance among HT patients during LT_4 monotherapy for hypothyroidism who presented with a variety of TVs, and we elucidated the relationship between TV and thyroid hormone status in hypothyroid patients on LT_4.

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Materials and Methods

Patients

We retrospectively identified 408 consecutive patients (379 women and 29 men) with HT from hospital medical records who visited the Kuma Hospital between January 2012 and May 2018. We based the diagnosis of HT on the presence of anti-thyroglobulin antibody (TgAb) positivity and/or anti-thyroid peroxidase antibody (TPOAb) positivity, and a heterogeneous hypoechoic pattern in a thyroid ultrasound examination.

The inclusion criteria were as follows: (1) underwent an ultrasound examination and TV was measured, (2) administered LT_4 before a thyroid ultrasound examination, and (3) TSH level within the laboratory reference range (0.3–5.0 μIU/mL) on a thyroid ultrasound examination. The exclusion criteria were as follows: (1) follicular adenoma and thyroid malignancies, (2) thyroid dysfunction, such as Graves' disease, thyroid dyshormonogenesis, autonomously functioning thyroid nodules, or hypothyroidism, (3) administered drugs known to affect thyroid function or thyroid hormone metabolism, such as a steroids, estrogen, amiodarone, lithium, β-blockers, sucralfate, and iron or iodine-containing drugs, (4) chronic or serious diseases, such as cardiac, pulmonary, hepatic, renal, or pancreatic diseases, diabetes or hyperparathyroidism, and (5) pregnant or lactating women. In addition to the above exclusion criteria, patients who failed to achieve the target TSH levels were also excluded from the analysis.

Control subjects

Overall, 1,149 consecutive euthyroid subjects (901 women and 248 men) who were examined for possible thyroid abnormalities at the Kuma Hospital during the same period as that of patients and did not have clinical or laboratory signs of thyroid diseases served as controls. Subjects with positive TPOAb or TgAb test results or with abnormal findings on ultrasound examination were excluded. Subjects with a thyroidal nodule or a goiter (TV: men ≥20 mL and women ≥18 mL) [9] on an ultrasound examination were also excluded. The other exclusion criteria were the same as those used for the selection of the patients. We balanced covariates including age, sex, and the measured year for choosing the control subjects for each patient group. Control subjects for each group of patients were chosen from 1,149 subjects selected earlier by 1:1 matching. This study was approved by the Ethical Committee at Kuma Hospital, and all patients gave written, informed consent.

Laboratory serum tests

For the patients who were taking LT_4, blood samples were obtained in the morning after the ingestion of LT_4. The patients' serum levels of TSH, FT_4, and FT_3 were measured using a chemiluminescent immunoassay (ARCHTECT i2000; Abbott Japan, Tokyo). The intra-assay coefficients of variation and the inter-assay coefficients of variation were 1.1%–5.0% and 1.7%–5.3% for the TSH assay, 2.3%–5.3% and 3.6%–7.8% for the FT_4 assay, and 1.4%–4.2% and 2.3%–5.0% for the FT_3 assay, respectively. The reference ranges in our hospital are 0.3–5.0 μIU/mL for TSH, 0.7–1.6 ng/dL for FT_4, and 1.7–3.7 pg/mL for FT_3. The serum levels of TgAb and TPOAb were measured using an electrochemiluminescence immunoassay (ECLusys 2010; Roche Diagnostics Japan, Tokyo; normal range: <40 IU/mL for TgAb, <16 IU/mL for TPOAb). A TgAb level less than 40 IU/mL was regarded as 40 IU/mL and that more than 4,000 IU/mL was regarded as 4,000 IU/mL, for the purpose of statistical calculations. A TPOAb level less than 16 IU/mL was regarded as 16 IU/mL and that more than 600 IU/mL was regarded as 600 IU/mL, for the purpose of statistical calculations. TV was measured using an ultrasound, as reported previously. First, the maximum width (W), maximum thickness (T), and maximum length (L) were measured in the right lobe (r) and left lobe (l). Second, TV was calculated by the following equation: $TV = 0.70\ (Wr \times Tr \times Lr + Wl \times Tl \times Ll)$ [10].

Statistical analysis

Grouped data are expressed as the mean ± standard deviation or the median (25^{th} to 75^{th} percentiles). Group comparisons among the HT patients stratified according to TV were analyzed using the χ^2 test (sex), Tukey-Kramer test, or Steel-Dwass. Treatment effects (control *vs.* HT patients on LT_4 for hypothyroidism) were analyzed using the paired *t*-test for data with a normal distribution and the Wilcoxon signed rank test for data with a nonparametric distribution. Significance was defined as p-value <0.05 (two-sided).

Results

Characteristics among the HT patient groups stratified according to TV

Baseline characteristics data of the HT patients are listed in the Table 1. In the present study, all patients had normal serum TSH levels. We stratified the patients into seven groups according to their TVs, as follows: <5 mL, 5–10 mL, 10–15 mL, 15–20 mL, 20–50 mL, 50–80 mL, and ≥80 mL. In the context of patients by TV, LT_4 doses tended to be higher in the atrophy group. There was an association between serum FT_3/FT_4 level and LT_4 dose in each group with 5 mL ≤TV. In contrast, there was no correlation between FT_3 level and LT_4 dose (data were not shown).

Table 1 Baseline characteristics sub grouped by thyroid volume in patients with Hashimoto's thyroiditis

Patient subgroups: (mL)	G1 TV <5	G2 5 ≤ TV < 10	G3 10 ≤ TV < 15	G4 15 ≤ TV < 20	G5 20 ≤ TV < 50	G6 50 ≤ TV < 80	G7 TV ≥80
n (male)	25 (1)	48 (7)	53 (4)	57 (5)	96 (6)	50 (1)	79 (5)
Age (years)	64 ± 11	62 ± 15	54 ± 17	52 ± 17	58 ± 14	61 ± 13	66 ± 10[c,d]
LT_4 dose (μg/day)	94 ± 19	80 ± 29	63 ± 30[a]	33 ± 62[a]	67 ± 28[a]	57 ± 27[a]	64 ± 29[a]
TSH (μIU/mL)	1.10 (0.62–1.62)	1.32 (0.78–2.52)	2.24[a] (1.32–3.15)	1.55 (0.90–2.70)	1.98 (1.05–3.28)	1.99[a] (1.38–3.67)	1.21[c,e,f] (0.63–1.90)
FT_4 (ng/dL)	1.16 (1.13–1.23)	1.16 (1.08–1.25)	1.08 (1.02–1.20)	1.10 (1.02–1.20)	1.06 (0.95–1.21)	0.96[a,b,c,d] (0.87–1.11)	1.04[a,b,d] (0.89–1.14)
FT_3 (pg/mL)	2.59 (2.36–2.79)	2.62 (2.43–2.84)	2.64 (2.45–2.84)	2.68 (2.48–2.93)	2.73 (2.50–2.93)	2.82[a,b] (2.57–3.00)	2.92[a,b,c,d,e] (2.74–3.16)
TgAb (IU/mL)	345.0 (93.7–2,962.0)	238.4 (115.1–592.3)	345.6 (123.6–672.8)	502.5 (322.9–1,026.0)	423.5 (217.7–788.5)	418.0 (272.7–561.5)	509.3 (385.2–744.7)
TPOAb (IU/mL)	40.9 (16.0–164.4)	64.3 (16.0–226.0)	183.9 (34.4–441.1)	160.4 (29.0–354.3)	375.6[a,b] (104.3–600.0)	337.5[a,b] (40.9–600.0)	214.9[a] (20.7–600)

Abbreviations: TV, Thyroid volume; TSH, thyroid stimulating hormone; FT_4, free thyroxine; FT_3, free triiodothyronine.
Values shown are the means ± SD in case of normal distribution and the medians (25–75% tile) in case of nonparametric distribution. Statistical significance was analyzed by the χ^2 test (sex), Tukey-Kramer test) or Steel-Dwass test for multiple comparisons.
[a] $p < 0.05$, compared with G1, [b] $p < 0.05$, compared with G2, [c] $p < 0.05$, compared with G3, [d] $p < 0.05$, compared with G4, [e] $p < 0.05$, compared with G5, [f] $p < 0.05$, compared with G6.

Serum thyroid hormone levels in HT patients stratified according to TV and those in the matched euthyroid controls

In patients with TV levels <5 mL, 5–10 mL, and 10–15 mL, the serum FT_3 levels were significantly lower than those in the matched controls ($p < 0.05$, $p < 0.001$, $p < 0.05$, respectively). In patients with TV levels 15–20 mL, 20–50 mL, and 50–80 mL, the serum FT_3 levels were equivalent to those in the matched controls ($p = 0.109$, $p = 0.111$, $p = 0.452$, respectively). In patients with TV levels ≥80 mL, the serum FT_3 levels were significantly higher than those in the matched controls ($p < 0.05$) (Fig. 1A).

In patients with TV levels <5 mL, 5–10 mL, and 10–15 mL, the serum FT_4 levels were significantly higher than those in the matched controls ($p < 0.05$, $p < 0.001$, $p < 0.05$, respectively). In patients with TV levels 15–20 mL, 20–50 mL, 50–80 mL, and ≥80 mL, the serum FT_4 levels were equivalent to those in matched controls ($p = 0.157$, $p = 0.158$, $p = 0.251$, $p = 0.844$, respectively) (Fig. 1B).

In patients with TV levels <5 mL, 5–10 mL, and 10–15 mL, the serum FT_3/FT_4 ratios were significantly lower than those in the matched controls ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). In patients with TV levels 15–20 mL, 20–50 mL, and 50–80 mL, the serum FT_3/FT_4 ratios were equivalent to those in the matched controls ($p = 0.077$, $p = 0.055$, $p = 0.094$, respectively). In patients with TV levels ≥80 mL, the serum FT_3/FT_4 ratios were significantly higher than those in the matched controls ($p < 0.05$) (Fig. 1C).

Association between serum FT_3 levels and FT_3/FT_4 ratios and TV in HT patients treated with LT_4 for hypothyroidism

The correlations between serum FT_3 levels and FT_3/FT_4 ratios and TV in HT patients treated with LT_4 for hypothyroidism were evaluated. The serum FT_3 levels showed a positive correlation with TV ($r = 0.35$, $p < 0.01$; Fig. 2A). The serum FT_3/FT_4 ratios also showed a positive correlation with TV ($r = 0.42$, $p < 0.01$; Fig. 2B).

Discussion

In the present study, in patients with small or normal TVs (<15 mL), the serum FT_3 levels significantly lower than those of controls. Thus, these data were similar to those from previous studies of athyreotic patients on LT_4 who underwent total thyroidectomy [2] or atrophic thyroid patients on LT_4 who underwent radioiodine treatment for Graves' disease [5]. In contrast, in patients with slight or moderate goiter (TV, 20–80 mL), serum FT_3 levels were equivalent to those of the controls. These data were consistent with those from previous studies of patients on LT_4 who underwent a hemithyroidectomy [6] or of a certain volume of thyroid gland (TV >10 mL) patients on LT_4 who underwent radioiodine treatment for

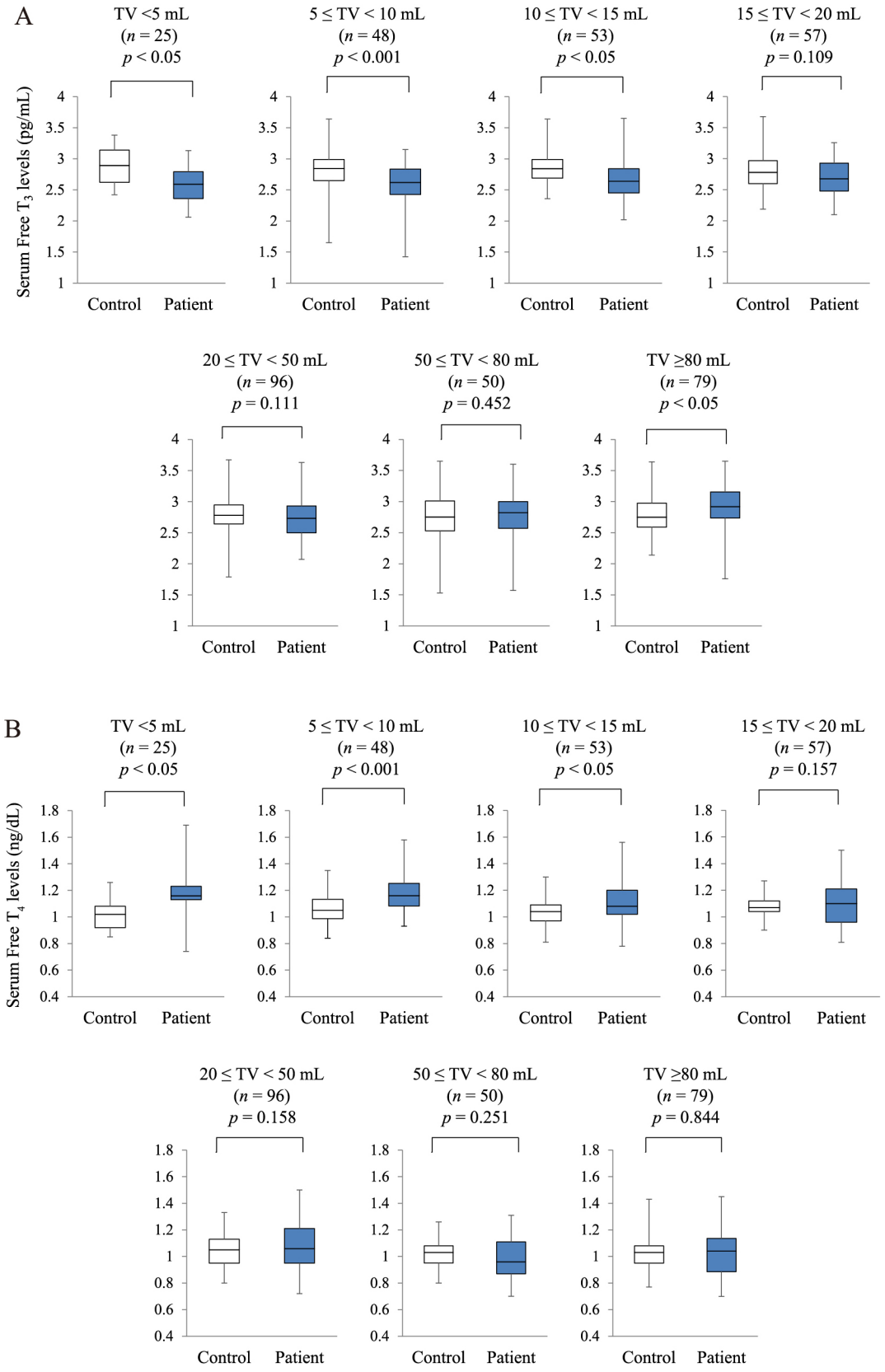

Fig. 1 Serum levels of FT_3 (A), FT_4 (B) and FT_3/FT_4 (C) in patients with Hashimoto thyroiditis (HT) and in euthyroid controls with intact thyroid matched by age, sex, and the measured year. The HT patients were divided into seven groups stratified by TV levels. The top, bottom, and middle lines of the boxes correspond to the 75th, 25th, and 50th percentiles (median), respectively. The whiskers extend from the minimum to the maximum. TV, Thyroid volume; FT_3, free triiodothyronine; FT_4, free thyroxine; TSH, thyrotropin.

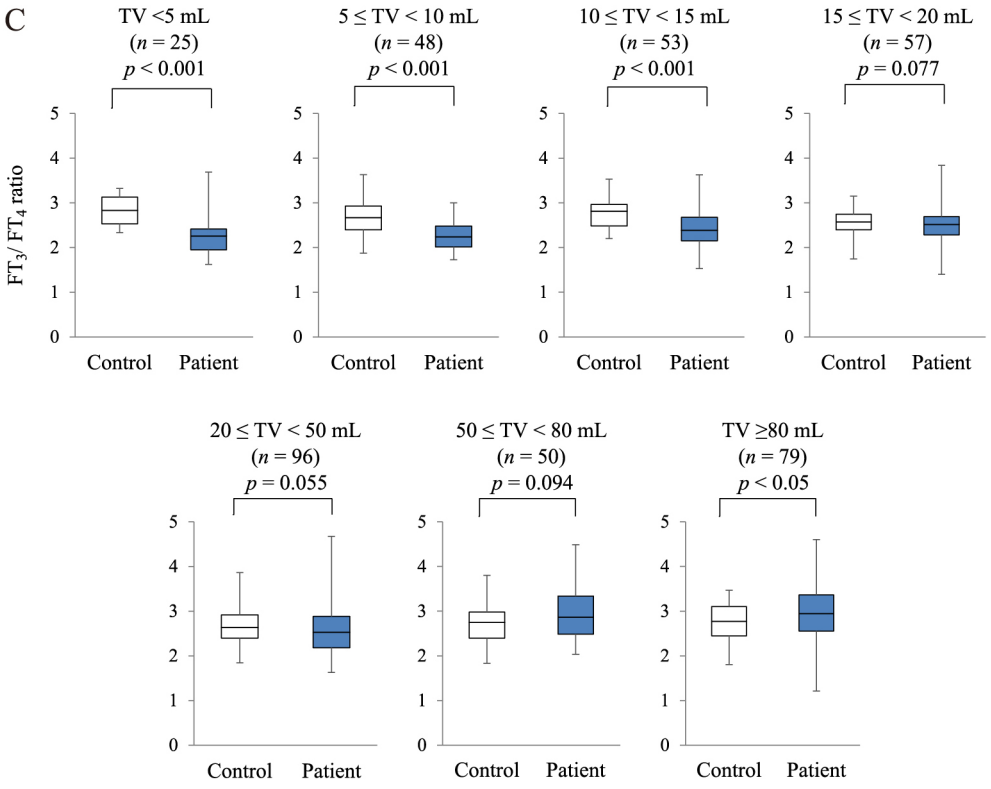

Fig. 1 Cont.

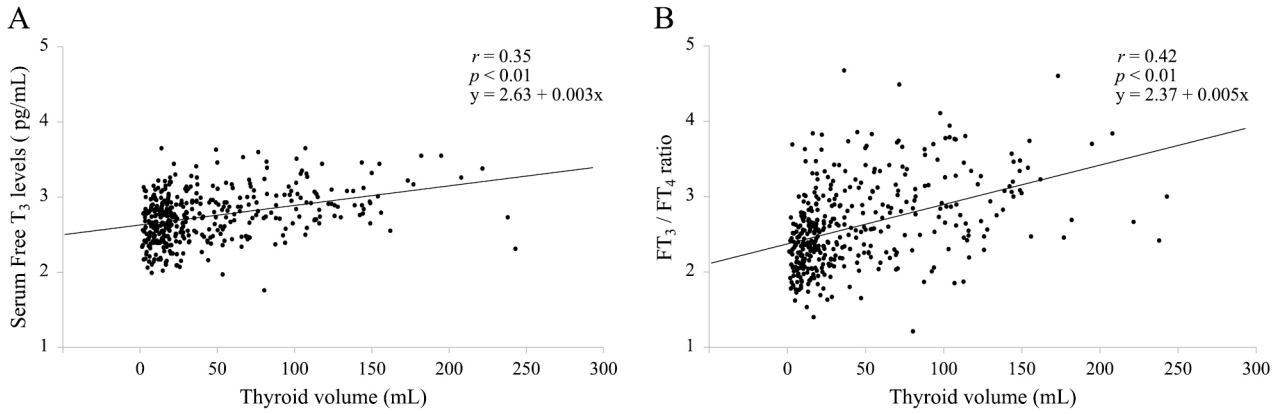

Fig. 2 (A) Association between serum free triiodothyronine and thyroid volume using the Pearson's correlation coefficient test among Hashimoto thyroiditis patients. (B) Association between serum free triiodothyronine/free thyroxine ratio and thyroid volume using the Pearson's correlation coefficient test among Hashimoto thyroiditis patients.

Graves' disease [5]. These findings suggest that the reason underlying the decreased serum T_3 levels in such patients is the lack of intra-thyroidal T_3 production caused by atrophy or loss of the thyroid gland.

There are two types of deiodinases (D1 and D2) that contribute to T_3 production. The serum FT_3/FT_4 ratio reflects the activity of the deiodinase enzyme, which converts T_4 to T_3 by 5'-deiodination [11]. Maia *et al.* estimated that D2 is the major contributor of extrathyroidal T_3 production in euthyroid subjects [12]. While, Hoermann *et al.* indicated that LT_4-treated patients with a post-interventional lower residual volume (<5 mL) have significantly reduced deiodinase activity and lowered T_3 levels, as compared with patients with a higher residual TV [13]. Such atrophic or athyreotic patients have reduced thyroidal deiodinase activity and T_3 production from the thyroid gland, resulting in a relatively low serum FT_3 level and FT_3/FT_4 ratio [6]. It is necessary to

clarify whether D1 and/or D2 activity in the thyroid tissues of such patients contributes to the patients' lower serum FT_3 level and serum FT_3/FT_4 ratios.

Our present study revealed that HT patients with large goiter (TV ≥80 mL) had relatively high serum FT_3 levels and a high FT_3/FT_4 ratio. We also demonstrated a positive correlation between the serum FT_3/FT_4 ratio and TV, findings consistent with those from our previous report [7, 8]. Elevation of thyroidal deiodinase activity, predominantly that of D2 activity, was reported as the cause of serum FT_3 level and FT_3/FT_4 ratio elevation in several large goitrous thyroid diseases, such as those involving thyroglobulin gene mutations, McCune-Albright syndrome, and T_3-predominant Graves' disease [14-16]. Recently, we reported the elevation of thyroidal deiodinase activity (especially D2 activity) at the posttranslational level in seven HT patients with large goiters; this increase in enzyme activity may be responsible for the relatively high serum FT_3/FT_4 ratio observed in these patients [7]. We also demonstrated a positive correlation between deiodinase activities and TV [8]. These findings suggest that intra-thyroidal T_3 production by increased deiodinase activity is a substantial factor influencing the relatively high serum T_3 levels in HT patients with large goiters. These may also be related to higher LT_4 doses when the goiter was small and lower LT_4 doses when the goiter was large in the present study. These results suggest that thyroid tissue capacity plays a significant role in the physiological process of T_3 homeostasis in humans; this contention fits well with the results from the present study.

In atrophic or normal thyroid size patients on LT_4, patients with normal TSH levels had relatively low serum FT_3 levels. The question arises as to whether such a patient is in a euthyroid condition. In the athyreotic patients with normal TSH and low T_3 levels, the relatively higher serum T_4 levels that accompany LT_4 monotherapy seems to result in normal T_3 receptor occupancy and TSH in pituitary thyrotrophs. In contrast, in peripheral tissues, the relatively higher serum T_4 levels could impair intracellular T_3 production *via* downregulation of a D2 pathway [17]. In fact, an animal study has shown that LT_4 alone administered in thyroidectomized rats at doses to normalize plasma TSH levels does not normalize T_3 contents in some tissues [18]. In another study of rats, Werneck *et al.* reported that a combination of high serum T_4 and low serum T_3 levels during T_4 monotherapy in rats had consequences of thyroid hormone action, as reflected in the brain, liver, and skeletal muscle, all of which exhibited indications of hypothyroidism despite normal serum TSH level [17]. In a previous study conducted in humans, we compared biochemical markers reflecting thyroid function before and after thyroidectomy. Therefore, the biochemical markers suggest that the patients with mildly suppressed TSH levels were closest to euthyroid, whereas those with normal TSH levels were mildly hypothyroid [19]. Recently, in a large LT_4-treated population with normal serum TSH, participants exhibited lower serum T_3 levels and differed in terms of both objective and subjective measures [20]. In addition, a meta-analysis performed by McAninch *et al.* showed that serum total cholesterol and low-density lipoprotein levels remain high in LT_4-treated euthyroid patients in meta-analysis [21].

The symptoms of thyroidal dysfunction have also been reported by several prior studies. Recently, we compared reported subjective symptoms reflecting thyroid function before and after thyroidectomy. Therefore, the symptoms suggest that the patients with mildly suppressed TSH levels were closest to euthyroid, whereas those with normal TSH levels were mildly hypothyroid [22]. Larisch *et al.* conducted a retrospective longitudinal study including patients with differentiated thyroid carcinoma on LT_4. Therefore, 26% of patients expressed hypothyroid and 9.7% hyperthyroid complaints at any one visit. Hypothyroid symptoms correlated well with FT_3 levels and were observed when TSH levels were below the reference range [23]. The American Thyroid Association stated in its guidelines that for the treatment of hypothyroidism such as in athyreotic patients, there is insufficient evidence of benefit to recommend LT_4 treatment for achieving low-normal TSH values or high-normal T_3 values [24]. Overall, the presence of biochemical markers and symptoms of thyroid function in animal and human studies of LT_4-treated athyreotic conditions suggests that patients with normal TSH might not be euthyroid in all tissues, and mild TSH suppression with LT_4 might be needed to achieve euthyroidism.

In the present study, the patients on LT_4 with large goitrous thyroid had relatively high serum FT_3 levels. It is unclear currently whether relatively high serum FT_3 in such patients presents with thyrotoxicosis. The clinical significance of the relative high serum FT_3 levels in patients with large goiter should be evaluated in the future.

There are some limitations in the present study. First, the limited number of study patients, unequal group distribution, and single time point reduced the internal validity of the study. Second, we did not evaluate biochemical markers and the symptoms reflecting thyroid function. In addition, we did not evaluate echo pattern (low or normal) of HT patients. Studies including measures of these clinical parameters are thus needed to clarify the best method of managing HT patients' thyroid function on LT_4.

LT_4 has been considered the standard of care for treatment of hypothyroidism for many years. LT_4

replacement therapy has three main goals: (i) provide resolution of the patients' symptoms and hypothyroid signs, including biological and physiologic markers of hypothyroidism, (ii) achieve normalization of serum TSH with improvement in thyroid hormone concentrations, and (iii) avoid overtreatment [24]. This study suggests that one of the three main goals has a pitfall. Patients with hypothyroidism due to HT are treated with LT_4 and live in a chronic condition of abnormal thyroid hormone status for their lives. Therefore, even if the thyroidal dysfunction may be subtle, its long-term effects cannot be overlooked. We analyzed a large number of HT patients on LT_4 and demonstrated that their TVs caused differences in serum thyroid hormone balance. The patients with normal TSH levels had relatively high serum FT_3 levels and FT_3/FT_4 ratios as their goiter size increased. Thus, serum low FT_3 levels in HT patients on LT_4 with relatively small thyroid were consistent with those in athyreotic patients on LT_4, and serum high FT_3 levels in HT patients on LT_4 with relatively large thyroid were consistent with those in patients with large goitrous thyroid disease. In the former, the possibility of mild hypothyroidism has been suggested, and mild TSH suppression with LT_4 may be needed to achieve normal FT_3 levels. The clinical significance of the latter is for further study. Our findings may provide novel information that could assist in the management of a large number of patients treated with LT_4 for hypothyroidism.

Acknowledgments

Author contributions: M. Ito constructed the study design. S. Takahashi analyzed the data. The other coauthors contributed by administering patient care.

Disclosure Statement

The authors declare no competing financial interests.

References

1. Pilo A, Iervasi G, Vitek F, Ferdeghini M, Cazzuola F, *et al.* (1990) Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis. *Am J Physiol* 258: E715–E726.
2. Ito M, Miyauchi A, Morita S, Kudo T, Nishihara E, *et al.* (2012) TSH-suppressive doses of levothyroxine are required to achieve preoperative native serum triiodothyronine levels in patients who have undergone total thyroidectomy. *Eur J Endocrinol* 167: 373–378.
3. Gullo D, Latina A, Frasca F, Le Moli R, Pellegriti G, *et al.* (2011) Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One* 6: e22552.
4. Hoermann R, Midgley JE, Giacobino A, Eckl WA, Wahl HG, *et al.* (2014) Homeostatic equilibria between free thyroid hormones and pituitary thyrotropin are modulated by various influences including age, body mass index and treatment. *Clin Endocrinol (Oxf)* 81: 907–915.
5. Ito M, Kawasaki M, Danno H, Kohsaka K, Nakamura T, *et al.* (2019) Serum thyroid hormone balance in levothyroxine monotherapy-treated patients with atrophic thyroid after radioiodine treatment for Graves' disease. *Thyroid* 29: 1364–1370.
6. Ito M, Miyauchi A, Kang S, Hisakado M, Yoshioka W, *et al.* (2015) Effect of the presence of remnant thyroid tissue on the serum thyroid hormone balance in thyroidectomized patients. *Eur J Endocrinol* 173: 333–340.
7. Harada A, Nomura E, Nishimura K, Ito M, Yoshida H, *et al.* (2019) Type 1 and type 2 iodothyronine deiodinases in the thyroid gland of patients with huge goitrous Hashimoto's thyroiditis. *Endocrine* 64: 584–590.
8. Kawasaki M, Ito M, Danno H, Kousaka K, Nakamura T, *et al.* (2019) The association between thyroid hormone balance and thyroid volume in patients with Hashimoto thyroiditis. *Endocr J* 66: 763–768.
9. Kitaoka M, Suzuki S (ed) (2016) Thyroid Ultrasound – A guidebook for diagnosis and management (3rd edition). Japan Association of Breast and Thyroid Sonology. Nankodo, Tokyo, Japan (In Japanese).
10. Murakami Y, Takamatsu J, Sakane S, Kuma K, Ohsawa N (1996) Changes in thyroid volume in response to radioactive iodine for Graves' hyperthyroidism correlated with activity of thyroid-stimulating antibody and treatment outcome. *J Clin Endocrinol Metab* 81: 3257–3260.
11. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* 23: 38–89.
12. Maia AL, Kim BW, Huang SA, Harney JW, Larsen PR (2005) Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. *J Clin Invest* 115: 2524–2533.
13. Hoermann R, Midgley JE, Larisch R, Dietrich JW (2015) Integration of peripheral and glandular regulation of triiodothyronine production by thyrotropin in untreated and thyroxine-treated subjects. *Horm Metab Res* 47: 674–680.
14. Kanou Y, Hishinuma A, Tsunekawa K, Seki K, Mizuno Y, *et al.* (2007) Thyroglobulin gene mutations producing defective intracellular transport of thyroglobulin are associated with increased thyroidal type 2 iodothyronine deiodinase activity. *J Clin Endocrinol Metab* 92: 1451–1457.
15. Celi FS, Coppotelli G, Chidakel A, Kelly M, Brillante BA, *et al.* (2008) The role of type 1 and type 2 5'-deiodinase in the pathophysiology of the 3,5,3'-triiodothyronine

toxicosis of McCune-Albright syndrome. *J Clin Endocrinol Metab* 93: 2383–2389.
16. Ito M, Toyoda N, Nomura E, Takamura Y, Amino N, *et al.* (2011) Type 1 and type 2 iodothyronine deiodinases in the thyroid gland of patients with 3,5,3'-triiodothyronine-predominant Graves' disease. *Eur J Endocrinol* 164: 95–100.
17. Werneck de Castro JP, Fonseca TL, Ueta CB, McAninch EA, Abdalla S, *et al.* (2015) Differences in hypothalamic type 2 deiodinase ubiquitination explain localized sensitivity to thyroxine. *J Clin Invest* 125: 769–781.
18. Escobar-Morreale HF, Obregón MJ, Escobar del Rey F, Morreale de Escobar G (1995) Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J Clin Invest* 96: 2828–2838.
19. Ito M, Miyauchi A, Hisakado M, Yoshioka W, Ide A, *et al.* (2017) Biochemical markers reflecting thyroid function in athyreotic patients on levothyroxine monotherapy. *Thyroid* 27: 484–490.
20. Peterson SJ, McAninch EA, Bianco AC (2016) Is a normal TSH synonymous with "euthyroidism" in levothyroxine monotherapy? *J Clin Endocrinol Metab* 101: 4964–4973.
21. McAninch EA, Rajan KB, Miller CH, Bianco AC (2018) Systemic thyroid hormone status during levothyroxine therapy in hypothyroidism: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 103: 4533–4542.
22. Ito M, Miyauchi A, Hisakado M, Yoshioka W, Kudo T, *et al.* (2019) Thyroid function related symptoms during levothyroxine monotherapy in athyreotic patients. *Endocr J* 66: 953–960.
23. Larisch R, Midgley JEM, Dietrich JW, Hoermann R (2018) Symptomatic relief is related to serum free triiodothyronine concentrations during follow-up in levothyroxine-treated patients with differentiated thyroid cancer. *Exp Clin Endocrinol Diabetes* 126: 546–552.
24. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, *et al.* (2014) Guidelines for the treatment of hypothyroidism. *Thyroid* 24: 1670–1751.