Median-lower normal levels of serum thyroxine are associated with low triiodothyronine levels and body temperature in patients with central hypothyroidism

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Abstract

Objective: Although it has been recommended that serum free thyroxine (FT₄) levels should be targeted to middle-upper normal levels during levothyroxine (L-T₄) replacement therapy in patients with central hypothyroidism (CeH), the rationale has not been clarified.

Methods: A retrospective single-center study enrolled 116 patients with hypothyroidism (CeH, $n=32$; total thyroidectomy (Tx), $n=22$; primary hypothyroidism (PH), $n=33$; and control benign thyroid nodule (C), $n=29$). The patients had received L-T₄ therapy at the Kobe University Hospital between 2003 and 2013. They were stratified according to serum FT₄ level (≥ 1.10 or < 1.10 ng/dl), and body temperature (BT), serum free triiodothyronine (FT₃) levels, FT₃/FT₄ ratio, and lipid profiles were compared. The effect of GH replacement therapy on thyroid function was also analyzed.

Results: FT₃ levels and FT₃/FT₄ ratios were significantly lower in patients with CeH than in patients with PH ($P<0.05$) or C ($P<0.05$). In patients with FT₄ < 1.10 ng/dl, BT was significantly lower in patients with CeH ($P=0.002$) and Tx ($P=0.005$) than in patients with PH, whereas no differences were found in patients with FT₄ ≥ 1.10 ng/dl. In patients with CeH, FT₃ levels were higher in those with GH replacement therapy ($P=0.018$).

Conclusion: In CeH, patients with median-lower normal levels of serum FT₄ exhibited lower serum FT₃ levels and lower BT. These results support the target levels of serum FT₄ as middle-upper normal levels during L-T₄ replacement therapy in patients with CeH.

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Introduction

Central hypothyroidism (CeH) is caused by various hypothalamic–pituitary diseases and frequently combined with other deficiencies of pituitary hormones such as adrenocorticotrophic hormone (ACTH), growth hormone (GH), and gonadotropins (Gn). The administration of

levothyroxine (L-T₄) is standard therapy not only for patients with primary hypothyroidism (PH) but also for patients with CeH. However, despite L-T₄ replacement therapy, it has been reported that some of the patients still complain of symptoms related with hypothyroidism and

impaired quality of life (QOL) (1, 2, 3). Generally, serum thyroid-stimulating hormone (TSH) level is used as a marker for dose adjustment of L-T₄ replacement therapy (4, 5, 6). However, in patients with CeH, TSH secretion does not often accurately reflect the changes in serum free thyroxine (FT₄) levels. In patients with CeH, it has been recommended that serum FT₄ levels should be targeted within the middle to upper limit of the reference range during L-T₄ replacement therapy (7, 8). However, little evidence supports the rationale for these target FT₄ levels (9).

The replacement therapy of other pituitary hormones may affect the free triiodothyronine (FT₃)/FT₄ ratio in patients with CeH associated with hypopituitarism. GH replacement therapy promotes peripheral T₄ to T₃ conversion (10, 11), whereas high-dose glucocorticoid replacement therapy inhibits deiodinase activity (12, 13), indicating that the status of replacement therapy is also important in determining the FT₃/FT₄ ratio. Recently, it has been reported that serum FT₃ levels to FT₄ levels ratio (FT₃/FT₄ ratio) is lower in patients with CeH than in control subjects (C) (14). In addition, patients who underwent total thyroidectomy (Tx) exhibited relatively lower FT₃ levels compared with FT₄ levels (15, 16). These results suggest that T₃ secretion from the thyroid gland plays an important role in the regulation of serum FT₃ levels.

To clarify the optimal levels of serum FT₄ during L-T₄ replacement therapy in patients with CeH, we assessed serum FT₃ levels and FT₃/FT₄ ratio, and the physiological effects of thyroid hormones such as BMI, heart rate (HR), body temperature (BT), and the parameters of lipid metabolism (17) and compared these with those in patients with Tx and PH.

Subjects and methods

Subjects and design

This was a retrospective observational single-center study involving 116 consecutive patients with hypothyroidism (36 men and 80 women; mean age, 56.1 ± 17.4 years) who had been treated with L-T₄ replacement therapy at Kobe University Hospital between 2003 and 2013. These four groups were stratified (FT₄ ≥ 1.10 , or < 1.10 ng/dl) according to the median value (1.10 ng/dl) of the normal range of serum FT₄ levels (0.70–1.48 ng/dl) (FT₄ ≥ 1.10 ng/dl group: CeH, $n=12$; Tx, $n=14$; PH, $n=19$; and C, $n=17$; FT₄ < 1.10 ng/dl group: CeH, $n=20$; Tx, $n=8$; PH, $n=14$; and C, $n=12$). The following parameters

were compared between these groups; BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), HR, BT, serum FT₃ level, FT₃/FT₄ ratio, and lipid profiles (total cholesterol (T-Chol), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides (TG)). All BT measurements were performed at 10 a.m. in the hospital during hospitalization by using an axillary digital thermometer in a single evaluation (Topnic ET-16, TOP Corporation, Tokyo, Japan). In the CeH group, patients who showed organic hypothalamic damage by tumor invasion or treatment including surgery and/or radiotherapy were excluded. In the Tx group, patients with TSH levels < 0.4 μ iu/ml who had received suppression therapy were excluded as previously described (15). Control subjects were recruited from among patients with non-functioning benign thyroid nodules (< 20 mm diameter), whose TSH levels were within the reference range (0.35–4.94 μ iu/ml). Most of the patients in the CeH group had panhypopituitarism as etiology, including GH deficiency (GHD) caused by various pituitary injuries, and some patients received GH replacement therapy. The status of pituitary function, the number of patients who had received pituitary hormone replacement therapy and etiology of CeH, including CeH subgroups, were summarized in Table 1. The hypothalamic–pituitary–thyroid axis, hypothalamic–pituitary–adrenal axis, and hypothalamic–pituitary–gonadal axis were evaluated as previously described (18). For the diagnosis of GHD, each patient was subjected to an insulin tolerance test or GH-releasing peptide 2 test. All of the patients in this study were studied after informed consent.

Biochemical assays

Serum FT₄, FT₃, and TSH levels were measured by using chemiluminescent immunoassay (ARCHITECT; CLIA, Abbott Japan). The reference ranges were 0.70–1.48 ng/dl for FT₄, 1.71–3.71 pg/ml for FT₃, and 0.35–4.94 μ iu/ml for TSH. The intra- and inter-assay coefficients of variation (CV) were 2.79–4.23% and 2.78–4.11% for FT₄, 2.29–2.49% and 2.81–4.20% for FT₃, 1.52–2.65% and 4.16–4.71% for TSH respectively. Serum T-Chol, LDL-C, HDL-C, and TG levels were measured by using enzymes or the direct method (Kyowa Medex, Tokyo, Japan). The reference ranges were 146–219 mg/dl for T-Chol, 0–139.99 mg/dl for LDL-C, 40–60 mg/dl for HDL-C, and 28–149 mg/dl for TG. The intra- and inter-assay CV were 0.64–0.65 and 1.16–1.72% for T-Chol, 0.60–0.63 and 1.72–1.76% for LDL-C, 0.42–0.43 and 1.43–1.79% for HDL-C, 0.47–0.51 and 1.32–1.51% for TG respectively.

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Table 1 Status of pituitary function and etiology of CeH. Number of patients who had received pituitary hormone replacement therapy. Among the patients with CeH, 81.3% exhibited GHD and 26.9% had GH replacement therapy.

	Total	FT ₄		GH replacement	
		≥ 1.10	< 1.10	(-)	(+)
Pituitary insufficiencies					
TSH	32 (32)	12 (12)	20 (20)	25 (25)	7 (7)
GH	26 (7)	10 (3)	16 (4)	19 (0)	7 (7)
FSH/LH	25 (5)	9 (2)	16 (3)	21 (2)	4 (3)
ACTH	28 (27)	9 (9)	19 (18)	23 (22)	5 (5)
ADH	11 (10)	5 (5)	6 (5)	8 (7)	3 (3)
Etiology					
Lymphocytic hypophysitis	6	3	3	5	1
Rathke's cleft cyst	4	0	4	4	0
Pituitary adenoma	3	1	2	3	0
Sheehan's syndrome	2	1	1	1	1
Craniopharyngioma	2	0	2	1	1
Empty sella	2	2	0	0	2
Germinoma	1	0	1	1	0
Other	12	5	7	10	2

Statistical analysis

All data were expressed as mean $\pm$ s.d. values. Comparisons between the two groups were performed by using Student's *t*-test. The χ^2 test was used to analyze differences between categorical variables. A multiple linear regression analysis was used to evaluate the following independent variables associated with BT: serum FT₄, FT₃, and TSH levels, FT₃/FT₄ ratio, age, BMI, the dose of L-T₄ replacement therapy, and the dose of L-T₄ per kilogram of body weight. The stepwise method entered the predictors sequentially. The tested factors including serum FT₄ and FT₃ levels, and FT₃/FT₄ ratio were followed by a variance inflation factor analysis. Comparisons between more than two groups were performed by using the ANOVA, followed by Bonferroni's multiple comparison test between each of the two groups. *P* values < 0.05 were considered as statistically significant. Data were analyzed by using the SPSS Software package (Dr SPSS II for Windows, Nankodo Co., Ltd, Japan).

Results

Clinical characteristics, clinical parameters, and L-T₄ doses

The clinical characteristics of the patients in the four groups are described in Table 2. No significant differences were found in age, sex distribution, blood pressure (BP), HR, and BT between the groups. BMI was higher in

patients with CeH than in those with PH or C (CeH; 26.5 ± 7.6 vs PH; 23.0 ± 5.9 kg/m²; *P* = 0.045, CeH; 26.5 ± 7.6 vs C; 21.6 ± 3.9 kg/m²; *P* = 0.003) respectively. Serum T-Chol, LDL-C, HDL-C, and TG levels were comparable between the groups (Table 2).

The dose of L-T₄ replacement therapy was greater in patients with Tx than in those with CeH or PH (Tx; 108.0 ± 24.9 vs CeH; 68.9 ± 32.0 µg/day; *P* < 0.001, Tx; 108.0 ± 24.9 vs PH; 40.9 ± 43.7 µg/day; *P* < 0.001). The dose in patients with CeH was greater than in those with PH (CeH; 68.9 ± 32.0 vs PH; 40.9 ± 43.7 µg/day; *P* = 0.004). The dose of L-T₄ per kilogram of body weight showed similar results (Tx; 1.9 ± 0.6 vs CeH; 1.1 ± 0.6 µg/day per kg; *P* < 0.001, Tx; 1.9 ± 0.6 vs PH; 0.7 ± 0.6 µg/day per kg; *P* < 0.001, CeH; 1.1 ± 0.6 vs PH; 0.7 ± 0.6 µg/day per kg; *P* = 0.023; Table 2).

Serum FT₃ levels and FT₃/FT₄ ratios

Serum FT₄, FT₃, and TSH levels, and FT₃/FT₄ ratios were compared between the four groups. Serum FT₄ levels were comparable between patients with CeH, PH, and C (CeH; 1.04 ± 0.25 vs PH; 1.15 ± 0.20 ng/dl; *P* = 0.306, CeH; 1.04 ± 0.25 vs C; 1.13 ± 0.15 ng/dl; *P* = 0.612, PH; 1.15 ± 0.20 vs C; 1.13 ± 0.15 ng/dl; *P* = 1.000; Fig. 1A). In contrast, serum FT₃ levels were lower in patients with CeH than in those with Tx, PH, or C (CeH; 2.08 ± 0.51 vs Tx; 2.55 ± 0.51 pg/ml; *P* = 0.007, vs PH; 2.78 ± 0.40 pg/ml; *P* < 0.001, vs C; 2.80 ± 0.43 pg/ml; *P* < 0.001; Fig. 1B). In addition, the mean FT₃/FT₄ ratios were significantly lower in patients

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Table 2 Comparison of clinical characteristics between four groups. Data are expressed as mean $\pm$ s.d.

	CeH	Tx	PH	C	P value ^a
Age (y)	54.5 $\pm$ 19.3	56.9 $\pm$ 15.3	53.9 $\pm$ 16.9	62.8 $\pm$ 13.9	0.09
Gender (male/female)	10/22	9/13	6/27	11/18	0.70
BMI (kg/m ²)	26.5 $\pm$ 7.6 ^{*,†}	23.8 $\pm$ 4.2	23.0 $\pm$ 5.9 [*]	21.6 $\pm$ 3.9 [†]	0.004
sBP (mmHg)	118.8 $\pm$ 20.4	127.7 $\pm$ 20.6	125.9 $\pm$ 19.2	125.7 $\pm$ 21.6	0.27
dBp (mmHg)	69.9 $\pm$ 13.2	72.1 $\pm$ 11.3	72.2 $\pm$ 9.6	66.8 $\pm$ 9.5	0.21
HR (bpm)	72.9 $\pm$ 13.0	71.2 $\pm$ 9.7	74.6 $\pm$ 11.8	73.9 $\pm$ 12.8	0.79
BT (°C)	36.0 $\pm$ 0.7	35.9 $\pm$ 0.7	36.3 $\pm$ 0.4	36.0 $\pm$ 0.8	0.22
T-Chol (mg/dl)	216.6 $\pm$ 53.2	226.3 $\pm$ 38.0	194.0 $\pm$ 49.4	194.9 $\pm$ 47.5	0.06
LDL-C (mg/dl)	127.6 $\pm$ 37.9	138.2 $\pm$ 26.1	112.4 $\pm$ 42.5	112.9 $\pm$ 39.6	0.14
HDL-C (mg/l)	58.0 $\pm$ 20.0	66.0 $\pm$ 13.5	62.0 $\pm$ 17.1	60.1 $\pm$ 24.7	0.64
TG (mg/dl)	140.0 $\pm$ 77.3	135.8 $\pm$ 72.6	130.1 $\pm$ 63.9	152.5 $\pm$ 113.0	0.49
L-T ₄ dose (μ g/day)	68.9 $\pm$ 32.0 ^{*,‡}	108.0 $\pm$ 24.9 [‡]	40.9 $\pm$ 43.7 [†]	–	<0.001
L-T ₄ dose (μ g/day/kg) (per body weight)	1.1 $\pm$ 0.6 ^{*,‡}	1.9 $\pm$ 0.6 [‡]	0.7 $\pm$ 0.6 [*]	–	<0.001

* P <0.05, [†] P <0.01, [‡] P <0.001.

^a P values are for the comparisons between all groups by ANOVA, except for sex distribution (χ^2 test), followed by Bonferroni's multiple comparison test between each of the two groups.

with CeH than in those with PH, whereas no difference was found between patients with CeH and Tx (CeH; 2.10 ± 0.67 vs PH; 2.49 ± 0.52 ; $P=0.045$, vs Tx; 2.12 ± 0.44 ; $P=1.002$; Fig. 1C).

In patients with CeH and PH, the remaining function of TSH secretion from the pituitary, and the T₃ and/or T₄ secretion from the thyroid, which may affect the FT₃/FT₄ ratio, had to be considered. Therefore, the subjects were stratified according to L-T₄ dose per kilogram of body weight (≥ 1.0 or <1.0 μ g/day per kg), and the serum thyroid hormone levels were compared between the groups. The serum FT₄ levels were comparable between patients with CeH and PH, whereas the serum FT₃ levels were lower in patients with CeH than in those with PH, both in the L-T₄ ≥ 1.0 and L-T₄ <1.0 μ g/day per kg groups (data not shown).

Association between serum thyroid hormone levels and clinical parameters

Next, we analyzed the association between serum thyroid hormone levels and clinical parameters such as HR and BT, which are regulated by thyroid hormone action. In the multiple linear regression analysis, no significant association was observed between the serum thyroid hormone levels and HR (data not shown). Serum FT₃ value was an independent factor associated with BT ($\beta=1.051$, 95% CI 0.012 to 2.089, $P=0.045$; Table 3). However, no significant association was observed between serum FT₄ levels and BT ($\beta=-2.027$, 95% CI -4.146 to 0.093, $P=0.07$; Table 3).

Therefore, we stratified the subjects according to the median value of the normal range of serum FT₄ levels (≥ 1.10 or <1.10 ng/dl), and analyzed the association

between serum thyroid hormone levels and BT. Clinical characteristics are summarized in Table 4. The dose of L-T₄ was greater in patients with Tx than in those with CeH or PH, both in the FT₄ ≥ 1.10 and FT₄ <1.10 ng/dl groups. Intriguingly, in the FT₄ <1.10 ng/dl group, patients with CeH and Tx exhibited lower BT than those with PH (CeH;

Figure 1

Serum FT₄ (A) and FT₃ (B) levels, FT₃/FT₄ ratios (C), and serum TSH levels (D) between four groups. Serum FT₄ levels were comparable between patients with CeH, PH, and C. Serum FT₃ levels were lower in patients with CeH than in those with Tx, PH, and C. FT₃/FT₄ ratios were lower in patients with CeH than in those with PH, whereas no difference was found between patients with CeH and Tx. Data are expressed as mean $\pm$ s.d. P values are for the comparisons between all groups by ANOVA, followed by Bonferroni's multiple comparison test between each of the two groups. * P <0.05, ** P <0.01, *** P <0.001, NS, not significant.

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Table 3 Associations between BT and serum thyroid hormone levels by multiple linear regression analysis. Serum FT₃ level was independently associated with BT. However, there was no significant association between serum FT₄ level and BT.

	β (95% CI)	P value
FT ₄	-2.027 (-4.146 to 0.093)	0.07
FT ₃	1.051 (0.012 to 2.089)	0.045
FT ₃ /FT ₄ ratio	-1.021 (-2.086 to 0.037)	0.06
TSH	0.004 (-0.034 to 0.047)	0.89
Age	-0.001 (-0.009 to 0.008)	0.77
BMI	0.019 (-0.009 to 0.049)	0.19
L-T ₄ dose	-0.004 (-0.013 to 0.008)	0.37
L-T ₄ dose (per body weight)	0.107 (-0.473 to 0.683)	0.74

35.9±0.5 vs PH; 36.4±0.3 °C; $P=0.002$, Tx; 35.8±0.4 vs PH; 36.4±0.3 °C; $P=0.005$; Table 4), whereas no significant differences were found in the FT₄ ≥ 1.10 ng/dl group (CeH; 36.0±0.7 vs PH; 36.3±0.5 °C, Tx; 36.0±0.8 vs PH; 36.3±0.5 °C; Table 4). These results suggest that the activity of the thyroid hormone is not sufficient in patients with CeH, whose serum FT₄ levels were lower than the median value of the normal range.

We next analyzed whether the decreased BT in patients with CeH was associated with serum FT₃ levels. As shown in Fig. 2, in the FT₄ < 1.10 ng/dl group, patients with CeH and Tx exhibited lower serum FT₃ levels and FT₃/FT₄ ratios than those with PH (FT₃ levels; CeH; 2.18±0.56 vs PH; 2.76±0.35 pg/ml; $P=0.001$, Tx; 2.26±0.53 vs PH; 2.76±0.35 pg/ml; $P=0.041$; Fig. 2A) (FT₃/FT₄ ratios; CeH; 2.41±0.64 vs PH; 2.87±0.41; $P=0.040$, Tx; 2.39±0.54 vs PH; 2.87±0.41; $P=0.039$; Fig. 2B). In contrast, in the FT₄ ≥ 1.10 ng/dl group, no significant differences in serum FT₃ levels and FT₃/FT₄ ratios were observed between these groups (FT₃ levels; CeH; 2.48±0.46 vs PH; 2.82±0.47 pg/ml, Tx; 2.71±0.44 vs PH; 2.82±0.47 pg/ml; Fig. 2C) (FT₃/FT₄ ratios; CeH; 1.85±0.36 vs PH; 2.20±0.45, Tx; 1.97±0.29 vs PH; 2.20±0.45; Fig. 2D). Taken together, these data suggest that lower BT in patients with CeH is associated with relatively lower serum FT₃ levels.

CeH patients with GH replacement therapy

To clarify the effect of GH replacement therapy on the pituitary–thyroid axis, serum FT₄ and FT₃ levels, and the dose of L-T₄ in CeH patients with GH replacement therapy (GH (+)) or without (GH (-)) were compared. In the GH (+) group, serum FT₃ levels were significantly higher than those in the GH (-) group (GH (-); 2.01±0.53 vs GH (+); 2.48±0.42 pg/ml; $P=0.018$; Fig. 3A). In contrast, no

significant difference in serum FT₄ levels was found between the groups (GH (-); 1.04±0.26 vs GH (+); 1.13±0.26 ng/dl; $P=0.150$; Fig. 3B). Furthermore, the dose of L-T₄ tended to be higher in the GH (+) group than in the GH (-) group (GH (-); 69.4±26.5 vs GH (+); 80.9±36.0 µg/day; $P=0.075$; Fig. 3C).

We also analyzed the influence of obesity, menopause, and other pituitary hormone deficiency on BT or serum thyroid hormone levels in CeH. We stratified the CeH subjects according to BMI (BMI ≥ 25 kg/m²: $n=15$; BMI < 25 kg/m²: $n=17$), the presence or absence of menopause (menopause (-): $n=14$; menopause (+): $n=18$), ACTH deficiency (ACTH deficiency (-): $n=4$; ACTH deficiency (+): $n=28$), and hypogonadism (hypogonadism (-): $n=7$; hypogonadism (+): $n=25$), and compared the following parameters between these groups: BT, serum FT₄ and FT₃ levels, FT₃/FT₄ ratios, the dose of L-T₄ replacement therapy, and the dose of L-T₄ per kilogram of body weight (Table 5). However, no significant differences were observed between these groups.

Discussion

In this study, we demonstrated that patients with CeH, who exhibited median-lower normal levels of serum FT₄, revealed low BT with relatively low serum FT₃ levels. Although serum FT₄ levels have been recommended to target within the middle to upper limit of the reference range in patients with CeH, few evidences have been shown to support the rationale of this target. Considering that a low BT represents a state of hypothyroid (17), the relatively lower serum FT₃ levels with decreased BT in the present study suggest that median-lower normal levels of serum FT₄ are not sufficient to maintain normal thyroid hormone action in patients with CeH. We also compared serum FT₃ levels or FT₃/FT₄ ratios in four different etiologies of hypothyroidism including patients with CeH, Tx, PH, and C simultaneously, and showed that serum FT₃ levels were lower in patients with CeH compared with the other three groups.

Physiologically, serum T₄ is secreted by the thyroid gland (~100%), and serum T₃ is derived from both the thyroid gland (20%) and extra-thyroidal tissues, where T₄ is converted to T₃ (80%) (19). Meanwhile, owing to the lack of TSH stimulation, T₃ produced by the thyroid gland will decrease in patients with CeH, resulting in lower serum FT₃ levels.

Several factors can affect serum FT₃ and FT₄ levels, and the FT₃/FT₄ ratio in patients with CeH, including other pituitary hormone deficiency, hormone replacement

Table 4 Clinical characteristics (subjects stratified by the median-normal value of serum FT₄). Data are expressed as mean $\pm$ s.d.

	FT ₄ $\geq$ 1.10					FT ₄ < 1.10				
	CeH	Tx	PH	C	P value ^a	CeH	Tx	PH	C	P value ^a
Age (y)	46.6 $\pm$ 16.9 [*]	55.9 $\pm$ 14.4	51.9 $\pm$ 18.9	65.7 $\pm$ 11.0 [*]	0.006	56.5 $\pm$ 20.6	58.6 $\pm$ 17.8	56.6 $\pm$ 13.9	58.8 $\pm$ 16.9	0.07
Gender (male/female)	3/9	4/10	1/18	5/12	0.81	7/13	5/3	5/9	6/6	0.66
BMI (kg/m ²)	25.8 $\pm$ 4.7 ^{*,†}	21.7 $\pm$ 2.7 [*]	22.1 $\pm$ 4.5 [†]	22.3 $\pm$ 3.0	0.01	27.0 $\pm$ 8.1	27.4 $\pm$ 4.1	24.0 $\pm$ 7.3	20.7 $\pm$ 4.9	0.06
sBP (mmHg)	112.5 $\pm$ 16.9	128.7 $\pm$ 24.1	121.4 $\pm$ 19.9	128.1 $\pm$ 21.5	0.13	121.3 $\pm$ 21.7	126.0 $\pm$ 13.8	131.0 $\pm$ 17.7	122.0 $\pm$ 22.2	0.63
dBp (mmHg)	69.9 $\pm$ 9.7	72.9 $\pm$ 12.9	69.9 $\pm$ 9.9	69.3 $\pm$ 8.8	0.78	69.1 $\pm$ 14.5	70.8 $\pm$ 8.5	74.7 $\pm$ 9.0	62.9 $\pm$ 9.8	0.12
HR (bpm)	71.0 $\pm$ 15.8	68.4 $\pm$ 9.8	74.8 $\pm$ 10.4	74.2 $\pm$ 11.1	0.46	74.5 $\pm$ 14.3	76.1 $\pm$ 7.7	74.5 $\pm$ 13.6	73.5 $\pm$ 15.3	0.98
BT (°C)	36.0 $\pm$ 0.7	36.0 $\pm$ 0.8	36.3 $\pm$ 0.5	36.0 $\pm$ 0.6	0.54	35.9 $\pm$ 0.5 [*]	35.8 $\pm$ 0.4 ^s	36.4 $\pm$ 0.3 ^{*,†}	35.9 $\pm$ 0.8	0.048
T-Chol (mg/dl)	213.8 $\pm$ 73.9	219.7 $\pm$ 29.9	204.9 $\pm$ 55.1	204.6 $\pm$ 51.7	0.83	219.5 $\pm$ 58.4	237.6 $\pm$ 49.6	177.7 $\pm$ 35.4	179.7 $\pm$ 37.4	0.16
LDL-C (mg/dl)	125.8 $\pm$ 30.8	139.0 $\pm$ 26.1	117.9 $\pm$ 46.7	125.3 $\pm$ 41.5	0.68	130.2 $\pm$ 45.2	135.7 $\pm$ 31.9	106.5 $\pm$ 38.8	98.0 $\pm$ 33.2	0.13
HDL-C (mg/dl)	61.6 $\pm$ 24.7	66.8 $\pm$ 15.2	68.8 $\pm$ 19.5	62.3 $\pm$ 19.7	0.84	56.1 $\pm$ 17.9	63.7 $\pm$ 8.5	53.9 $\pm$ 18.4	57.5 $\pm$ 30.6	0.88
TG (mg/dl)	138.9 $\pm$ 71.3	126.1 $\pm$ 81.8	124.6 $\pm$ 78.0	156.1 $\pm$ 127.8	0.78	157.1 $\pm$ 104.2	150.3 $\pm$ 73.4	137.8 $\pm$ 53.4	147.6 $\pm$ 94.8	0.78
L-T ₄ dose (μ g/day)	79.8 $\pm$ 35.1 [*]	108.9 $\pm$ 30.4	51.3 $\pm$ 46.2 ^{*,}	—	<0.001	58.4 $\pm$ 25.8 ^{*,}	106.3 $\pm$ 11.6 ^{,†}	26.8 $\pm$ 37.0 ^{*,}	—	<0.001
L-T ₄ dose (μ g/day per kg) (per body weight)	1.2 $\pm$ 0.7 [*]	2.1 $\pm$ 0.7 ^{*,}	0.9 $\pm$ 0.6	—	<0.001	0.8 $\pm$ 0.4 ^{*,s}	1.5 $\pm$ 0.3 ^{*,}	0.4 $\pm$ 0.6 ^{*,}	—	<0.001

^aP values are for the comparisons between all groups by ANOVA, except for sex distribution (χ^2 test), followed by Bonferroni's multiple comparison test between each of the two groups. ^{*}P<0.05, [†]sP<0.01, ^{||}P<0.001.

therapy, and the degree of TSH deficiency. Other pituitary hormone deficiency and their replacement therapy have been shown to affect the T₄ to T₃ conversion (14). In particular, GH replacement therapy promotes peripheral T₄ to T₃ conversion (10, 11). Indeed, in patients with CeH, serum FT₃ levels were higher in subjects with GH replacement therapy than those without it in our study. Coexisting GHD might decrease serum FT₃ levels because of decreased deiodinase activity (20, 21). Furthermore, it is well known that high-dose glucocorticoid replacement therapy inhibits deiodinase activity (12, 13). However, there were few patients who had received such high-dose glucocorticoid treatment in our study and we did not find any differences in serum thyroid hormone levels between CeH patients with and those without ACTH deficiency. In addition, we should consider an influence of Gn on BT in patients with CeH. It has been reported that central estradiol upregulates Brown adipose tissue (BAT) thermogenesis via inactivating hypothalamic AMPK (22). However, we did not find any differences in BT or serum thyroid hormone levels between CeH patients with and those without menopause and hypogonadism.

Figure 2

Serum FT₃ levels (A and C) and FT₃/FT₄ ratios (B and D) between four groups (subjects stratified according to the median-normal value of serum FT₄). Patients with CeH and Tx exhibited lower serum FT₃ levels (A) and FT₃/FT₄ ratios (B) than those with PH when serum FT₄ levels were below the median value of the normal range. Meanwhile, there were no significant differences in patients whose serum FT₄ levels were in the median-upper normal range (C and D). Data are expressed as mean $\pm$ s.d. P values are for the comparisons between all groups by ANOVA, followed by a non-paired t-test between each of the two groups. ^{*}P<0.05, ^{**}P<0.01, NS, not significant.

Figure 3

Serum FT₃ (A) and FT₄ (B) levels, and the dose of L-T₄ (C) in CeH patients with or without GH replacement therapy. In patients with CeH, serum FT₃ levels were significantly higher in patients with GH replacement therapy (GH (+)) than those without it (GH (-)), whereas serum FT₄ levels did not differ between these two groups. The dose of L-T₄ tended to be higher in the GH (+) patients than in the GH (-) patients. Data are expressed as mean $\pm$ s.d. P values are for the comparisons between two groups by non-paired *t*-test. **P* < 0.05, NS, not significant.

It is also well known that hypothalamic damage can contribute to BT via the autonomic nervous system. However, in our study, patients who might suffer with hypothalamic damage by tumor invasion or treatment including surgery and/or radiotherapy were excluded. Furthermore, it has been reported that obesity is associated with BT or serum thyroid hormone levels (23, 24).

Table 5 BT and thyroid parameters according to BMI, menopausal status, and other pituitary hormone deficiency in CeH. Data are expressed as mean $\pm$ s.d. P values are for the comparisons between two groups by using a non-paired *t*-test.

	BMI				Menopause				ACTH deficiency				Hypogonadism			
	<25		≥25		(-)		(+) P value		(-)		(+) P value		(-)		(+) P value	
BT (°C)	36.1 $\pm$ 0.8	36.0 $\pm$ 0.6	0.62	36.2 $\pm$ 0.5	35.9 $\pm$ 0.8	0.20	36.0 $\pm$ 0.2	36.0 $\pm$ 0.8	36.0 $\pm$ 0.2	36.0 $\pm$ 0.8	0.91	35.9 $\pm$ 0.5	36.1 $\pm$ 0.8	0.43		
FT ₄ (mg/dl)	1.09 $\pm$ 0.25	0.98 $\pm$ 0.25	0.21	1.12 $\pm$ 0.23	0.96 $\pm$ 0.25	0.07	1.14 $\pm$ 0.29	1.02 $\pm$ 0.25	1.14 $\pm$ 0.29	1.02 $\pm$ 0.25	0.46	1.12 $\pm$ 0.22	1.01 $\pm$ 0.26	0.30		
FT ₃ (mg/dl)	2.25 $\pm$ 0.53	1.94 $\pm$ 0.47	0.09	2.30 $\pm$ 0.51	1.91 $\pm$ 0.47	0.053	2.35 $\pm$ 0.26	2.04 $\pm$ 0.54	2.35 $\pm$ 0.26	2.04 $\pm$ 0.54	0.11	2.09 $\pm$ 0.45	2.08 $\pm$ 0.54	0.98		
FT ₃ /FT ₄ ratio	2.19 $\pm$ 0.80	2.04 $\pm$ 0.56	0.55	2.16 $\pm$ 0.77	2.07 $\pm$ 0.61	0.72	2.16 $\pm$ 0.53	2.10 $\pm$ 0.70	2.16 $\pm$ 0.53	2.10 $\pm$ 0.70	0.85	1.89 $\pm$ 0.38	2.17 $\pm$ 0.73	0.19		
L-T ₄ dose (µg/day)	78.3 $\pm$ 26.5	61.8 $\pm$ 35.8	0.14	78.6 $\pm$ 37.8	62.5 $\pm$ 26.4	0.19	93.8 $\pm$ 51.5	66.1 $\pm$ 28.4	93.8 $\pm$ 51.5	66.1 $\pm$ 28.4	0.36	66.1 $\pm$ 51.4	70.5 $\pm$ 26.2	0.83		
L-T ₄ dose (µg/day per kg)	1.0 $\pm$ 0.3	1.2 $\pm$ 0.7	0.21	1.1 $\pm$ 0.8	1.1 $\pm$ 0.4	0.82	1.6 $\pm$ 1.4	1.0 $\pm$ 0.4	1.6 $\pm$ 1.4	1.0 $\pm$ 0.4	0.46	1.2 $\pm$ 1.1	1.1 $\pm$ 0.4	0.78		

We analyzed the influence of obesity on BT or thyroid parameters in CeH but found no significant differences between CeH patients with and those without obesity.

Thyroid hormone is one of the key regulators of thermal homeostasis (25). T₃ induces uncoupling protein-1 (*UCP-1*) expression and mitochondrial biogenesis in human adipocytes, and the effects of T₃ on *UCP-1* induction are dependent on the thyroid hormone receptor- β (26). BAT is specialized for energy expenditure through thermogenesis (27), mediated by *UCP-1* expression (28). It is also known that the type 2 iodothyronine deiodinase (DIO2), an important enzyme to convert T₄ to T₃, is essential for adaptive thermogenesis in BAT (29). These findings along with our results indicate that BT might be reduced by relatively low serum FT₃ levels through low *UCP-1* expression levels in BAT.

The effect of combined therapy with L-T₄ and liothyronine (L-T₃) has been investigated for patients with hypothyroidism, whose QOL has been impaired with L-T₄ monotherapy. Several studies have compared the effect of L-T₄/L-T₃ combined therapy and that of L-T₄ monotherapy in patients with hypothyroidism. Bunevicius *et al.* (30) reported that partial substitution of L-T₃ for L-T₄ might improve mood and neuropsychological function in patients with both PH and Tx, suggesting the significance of additional L-T₃ administration. However, L-T₄/L-T₃ combined therapy demonstrated no beneficial changes in body weight, lipid profiles, and symptoms of hypothyroidism compared with L-T₄ monotherapy in patients with PH and Tx (31). Overall, L-T₄/L-T₃ combined therapy provided no advantage when compared with standard L-T₄ monotherapy in a meta-analysis of randomized controlled trials (32). However, because patients with CeH have not been included in these studies, L-T₄/L-T₃ combined therapy in patients with CeH is worth investigating.

To date, no consensus guidelines on the management of patients with CeH have been established. Several studies have been performed to investigate the optimal dose of L-T₄ in patients with CeH. It has been shown that L-T₄ dose based on body weight and aiming at serum FT₄ levels in the upper reference range is superior to aiming at the middle of normal FT₄ levels (2). In clinical practice, it has been recommended that serum FT₄ levels should be targeted within the middle to upper limit of the reference range in patients with CeH (7, 8, 9). However, there has been little evidence supporting the target levels in patients with CeH by using clinical markers of hypothyroidism, such as HR, BT, and lipid profiles during L-T₄ replacement therapy. Our results show that median-lower normal

levels of serum FT₄ are associated with both low serum FT₃ levels and low BT, suggesting that these parameters could be clinically useful markers in patients with CeH, in addition to serum FT₄ levels. To confirm these findings, further investigations are required based on the other clinical markers such as basal metabolic rate measured by using an expiration gas analyzer or patient well-being assessed by using hypothyroid specific QOL questionnaires.

Our study has several limitations. First, the sample size, especially after stratification, was relatively small. Therefore, we could not exclude coincidental results. However, the association between low serum FT₃ levels and BT in patients with CeH strongly suggests a functional relevance. Second, various L-T₄ doses were used for replacement therapy, suggesting that patients with varied degrees of the remaining function of the pituitary's TSH secretion and the thyroid's T₃ or T₄ secretion were included, which might have affected the FT₃/FT₄ ratio. Third, although significant differences in serum FT₃ levels and FT₃/FT₄ ratios were observed in the patients with CeH and FT₄ <1.10 ng/dl, a similar tendency was found in the patients with CeH and FT₄ $\geq$ 1.10 ng/dl, suggesting that the threshold may not be the median-normal value of serum FT₄. In this aspect, the optimal replacement dose should be considered based on individual conditions. Fourth, several other confounding factors, including DIO2 polymorphism, may influence BT or serum thyroid hormone levels (33). Finally, the selection bias in each group needs to be considered. In particular, higher serum TSH levels were observed in patients with Tx because of the exclusion of subjects with TSH levels <0.4 μ iu/ml, in whom TSH suppression therapy for thyroid cancer was performed.

In conclusion, patients with CeH who exhibited median-lower normal levels of serum FT₄ revealed low BT with relatively low serum FT₃ levels. GHD might have contributed to the serum FT₃ attenuation in these patients. These data support the previously recommended target levels of serum FT₄ at the middle to upper limit of the reference range in patients with CeH. It is not known whether the middle-upper normal levels of serum FT₄ would improve low BT and maintain well-being during L-T₄ replacement therapy in patients with CeH. Further large-scale, prospective, interventional studies are needed to determine the optimal replacement therapy in patients with CeH.

Declaration of interest

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Y Hirata drafted the manuscript, and assembled and analyzed the data. H Fukuoka and Y Takahashi were responsible for the conception and design of the study. The other coauthors contributed by collecting the data or caring for the patients. H Fukuoka was responsible for the critical revision of the manuscript for important intellectual content.

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