European Journal of Endocrinology (2011) **164** 95–100 ISSN 0804-4643

CLINICAL STUDY

Type 1 and type 2 iodothyronine deiodinases in the thyroid gland of patients with 3,5,3′-triiodothyronine-predominant Graves' disease

Mitsuru Ito, Nagaoki Toyoda[1], Emiko Nomura[1], Yuuki Takamura, Nobuyuki Amino, Toshiji Iwasaka[1], Junta Takamatsu[2], Akira Miyauchi and Mitsushige Nishikawa[1]

Kuma Hospital, Kobe, Hyogo 650-0011, Japan, [1]Internal Medicine II, Kansai Medical University, 2-3-1, Shinmachi, Hirakata City, Osaka 573-1191, Japan and [2]Takamatsu Clinic, Osaka 569-0804, Japan

(Correspondence should be addressed to N Toyoda; Email: toyoda@takii.kmu.ac.jp)

Abstract

Objective: 3,5,3′-triiodothyronine-predominant Graves' disease (T_3-P-GD) is characterized by a persistently high serum T_3 level and normal or even lower serum thyroxine (T_4) level during antithyroid drug therapy. The source of this high serum T_3 level has not been clarified. Our objective was to evaluate the contribution of type 1 and type 2 iodothyronine deiodinase (D1 (or DIO1) and D2 (or DIO2) respectively) in the thyroid gland to the high serum T_3 level in T_3-P-GD.

Methods: We measured the activity and mRNA level of both D1 and D2 in the thyroid tissues of patients with T_3-P-GD ($n=13$) and common-type GD (CT-GD) ($n=18$) who had been treated with methimazole up until thyroidectomy.

Results: Thyroidal D1 activity in patients with T_3-P-GD (492.7 ± 201.3 pmol/mg prot per h) was significantly higher ($P<0.05$) than that in patients with CT-GD (320.7 ± 151.9 pmol/mg prot per h). On the other hand, thyroidal D2 activity in patients with T_3-P-GD (823.9 ± 596.4 fmol/mg prot per h) was markedly higher ($P<0.005$) than that in patients with CT-GD (194.8 ± 131.6 fmol/mg prot per h). There was a significant correlation between the thyroidal D1 activity in patients with T_3-P-GD and CT-GD and the serum FT_3-to-FT_4 ratio ($r=0.370$, $P<0.05$). Moreover, there was a strong correlation between the thyroidal D2 activity in those patients and the serum FT_3-to-FT_4 ratio ($r=0.676$, $P<0.001$).

Conclusions: Our results suggest that the increment of thyroidal deiodinase activity, namely D1 and especially D2 activities, may be responsible for the higher serum FT_3-to-FT_4 ratio in T_3-P-GD.

European Journal of Endocrinology **164** 95–100

Introduction

The monodeiodination of thyroxine (T_4) to 3,5,3′-triiodothyronine (T_3) activates the major secretory product of the iodine-sufficient human thyroid gland, producing ~80% of the circulating T_3 in humans (1). Type 1 and type 2 iodothyronine deiodinase (D1 (or DIO1) and D2 (or DIO2) respectively) catalyze this reaction (2–4). The roles of D1 and D2 in the production of circulating T_3 in humans are unknown. Both D1 and D2 activities are demonstrated in the human thyroid, and Salvatore *et al.* (5) reported that intrathyroidal T_4 to T_3 conversion by D2 may contribute to the relative increase in thyroidal T_3 production in patients with Graves' disease (GD). On the other hand, Laurberg *et al.* (6) estimated by an indirect method using propylthiouracil (PTU) that D1-generated T_3 in the thyroid gland is the major source of plasma T_3 in hyperthyroid humans. Recently, some cases of relatively high serum T_3 levels were reported in patients with follicular thyroid carcinoma (7), GD during PTU treatment (8), thyroglobulin (*Tg*) gene mutations (9), and McCune–Albright syndrome (10). In these cases, D2 activity in the thyroid tissues was increased, and the T_4 to T_3 conversion catalyzed by D2 was assumed to be responsible for the T_3 toxicosis.

In most patients with hyperthyroid GD, the elevated serum T_4 and T_3 levels decrease to within their respective normal ranges after appropriate antithyroid drug therapy is initiated. However, we (11) and other investigators (12, 13) have noted that in ~12% of patients with GD, serum T_3 levels remain raised, while serum T_4 levels become normal or even low. We have termed this phenomenon T_3-predominant GD (T_3-P-GD) (11). Previously, we reported that the T_4 5′-deiodinating activity in the thyroid tissues of patients with T_3-P-GD was higher than that in patients with common-type GD (CT-GD) (11). In our previous study, we used 5 μM T_4 as a substrate, an amount appropriate for human D1, but 100- to 1000-fold higher than that

DOI: 10.1530/EJE-10-0736
Online version via www.eje-online.org

for D2, thus obscuring the contributions of the D2 pathway to T_3 production. Therefore, to investigate the relative roles of thyroidal D1 and D2 in the establishment of a higher serum free T_3 (FT_3) relative to serum free T_4 (FT_4) in patients with T_3-P-GD, we evaluated thyroidal activities and mRNA levels of both D1 and D2 in patients with T_3-P-GD and CT-GD.

Materials, subjects, and methods

Materials

[^{125}I]T_4 and [^{125}I]T_3 (reverse T_3 or rT_3) were purchased from Perkin Elmer (Boston, MA, USA). Sephadex LH-20 was purchased from Pharmacia Biotech. All other chemicals were of the highest quality and were obtained from Sigma Chemical Co. or Nakalai Tesque (Kyoto, Japan) unless otherwise indicated.

Subjects

We studied 13 patients with T_3-P-GD and 18 patients with CT-GD who had undergone thyroidectomy between January, 2007 and October, 2007 at Kuma Hospital. GD was diagnosed on the basis of clinical findings and laboratory tests showing high serum FT_4 and FT_3 levels, low TSH concentrations, increased anti-TSH receptor antibody (TRAb) titer, and a high radioactive iodine uptake. At the time of surgery, while the serum FT_4 levels in the patients with T_3-P-GD declined to normal or low during methimazole (MMI) treatment, their serum FT_3 levels remained high or relatively high for more than 3 months. Their serum FT_3-to-FT_4 ratios were all above the normal range. In the patients with CT-GD, MMI treatment resulted in a decline in both serum FT_4 and FT_3 levels to within the normal range and a decline in FT_3-to-FT_4 ratios to within the normal range. The mean dose of MMI was 25 ± 10 mg/day in the patients with T_3-P-GD and 7 ± 3 mg/day in the patients with CT-GD. This study was approved by the ethics committee at Kuma Hospital, and all the patients gave informed consent.

Thyroid function tests

Serum concentrations of TSH, FT_4, and FT_3 were measured with a chemiluminescent immunoassay (ARCHTECT i2000; Abbott Japan). Serum TRAb titer levels were measured using a human radioreceptor assay (DYNO test; Yamasa Co., Tokyo, Japan) (14) with a reference range of <1.0 IU/l. Thyroid-stimulating antibody (TSAb) titer levels were measured in terms of the amount of cAMP produced in cultured porcine thyroid cells (Yamasa Co.) with a reference range of $<180\%$ (15). The volume of the thyroid gland was measured by ultrasonography as reported previously (16).

5′ deiodinase assays

Human thyroid tissues were homogenized, and a microsomal fraction was prepared as described previously (5). D1 and D2 activities were assayed as described previously (5). In brief, the reactions contained microsomal protein, 0.1 nM [^{125}I]T_4 purified by LH-20 chromatography, 2 nM cold T_4, 20 mM dithiothreitol (DTT), 1 mM PTU in 0.1 M potassium phosphate, and 1 mM EDTA, pH 6.9 (D2 conditions) or 0.2 nM [^{125}I]rT_3 purified by LH-20 chromatography, 1 μM rT_3, and 10 mM DTT in the presence or absence of 1 mM PTU (D1 conditions). Incubations were for 120 min (D2 conditions) or 60 min (D1 conditions) at 37 °C. $^{125}I^-$ was separated from unreacted substrate or iodothyronine products by trichloroacetic acid precipitation. Separated $^{125}I^-$ was counted with a gamma-counter. The deiodinating activity was expressed in picomoles (D1) or femtomoles (D2) of I^- released per mg protein/h. Deiodination of T_4 and rT_3 produced equimolar concentrations of labeled I^- and T_3 (from T_4) or 3,3′-diiodothyronine (from rT_3) as assessed by paper chromatographic separation of the reaction products (17).

RNA preparation and real-time quantitative PCR

Total RNA from thyroid tissues was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Real-time quantitative PCR assays were performed using an Opticon 2 apparatus (Bio-Rad Lab.). Briefly, 1 μg total RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad Lab.) according to the manufacturer's instructions. Human *D1*, *D2*, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA levels were analyzed using the iQ SYBR Green Super MIX (Bio-Rad Lab.). The primers were as follows: 5′-TTAGTTCCATAGCAGATTTTCTTGTCA-3′ (sense) and 5′-CTGATGTCCATGTTGTTCTTAAAAGC-3′ (antisense) amplify the human *D1* cDNA; 5′-TCATTCTGCTCAAGCACGTG-3′ (sense) and 5′-ACCATTGCCACTGTTGTCAC-3′ (antisense) amplify the human *D2* cDNA; and 5′-GCACCGTCAAGGCTGAGAAC-3′ (sense) and 5′-TGGTGAAGACGCCAGTGGA-3′ (antisense) amplify the human *GAPDH* cDNA. Real-time PCR experiments were performed in triplicate, and mRNA levels were expressed as arbitrary units after correction for *GAPDH* mRNA level.

Statistical analysis

Group data were expressed as means ± S.D., and statistical significance was analyzed by the unpaired *t*-test or the Mann–Whitney *U* test, as appropriate. Correlations were analyzed by Pearson's correlation coefficient test. *P* values <0.05 were considered to indicate a significant difference.

Results

Clinical findings

Some basic characteristics of the 13 patients with T_3-P-GD and the 18 patients with CT-GD who completed the study are listed in Table 1. These measurements were made at the time of the thyroidectomy. The differences in the serum levels of TSH and FT_4 were not statistically significant. As expected, the patients with T_3-P-GD had a higher mean serum FT_3 level and FT_3-to-FT_4 ratio than the patients with CT-GD. The mean TRAb and TSAb levels were greater in the patients with T_3-P-GD. The mean volume of the thyroid gland was approximately seven times higher in the patients with T_3-P-GD. After thyroidectomy and an appropriate dose of L-T_4 administration, serum FT_4 and FT_3 levels in the patients with T_3-P-GD changed to within the normal range (1.21 ± 0.15 ng/dl (FT_4) and 2.12 ± 0.27 (FT_3) respectively), and the FT_3-to-FT_4 ratio declined to 1.78 ± 0.33.

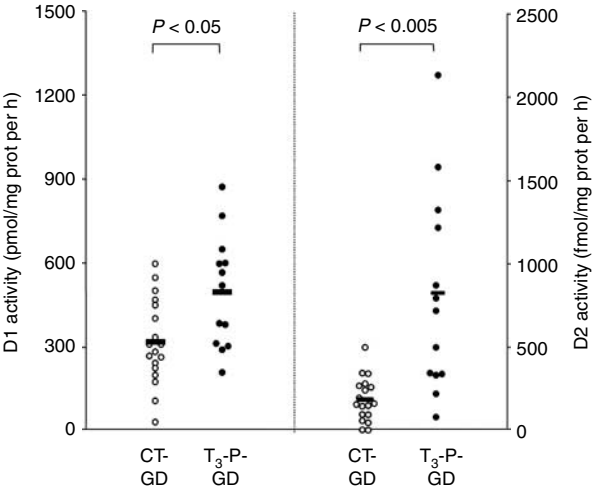

Figure 1 D1 and D2 activities in thyroid tissues. Open circles represent CT-GD patients; closed circles represent T_3-P-GD patients; solid squares represent mean value levels.

D1 and D2 activities in thyroid tissues

The D1 activity in thyroid tissues of the patients with T_3-P-GD (492.7 ± 201.3 pmol/mg prot per h) was significantly higher ($P<0.05$) than that in the patients with CT-GD (320.7 ± 151.9 pmol/mg prot per h; Fig. 1). The D2 activity in thyroid tissues of the patients with T_3-P-GD (823.9 ± 596.4 fmol/mg prot per h) was markedly higher ($P<0.005$) than that in the patients with CT-GD (194.8 ± 131.6 fmol/mg prot per h; Fig. 1).

To investigate whether thyroidal D1 and D2 contribute to the FT_3-to-FT_4 ratio, we investigated the correlation between the serum FT_3-to-FT_4 ratio and the corresponding thyroidal D1 and D2 activities. As shown in Fig. 2A, the D1 activity of patients with T_3-P-GD and CT-GD significantly correlated with the serum FT_3-to-FT_4 ratio ($r=0.370$, $P<0.05$). Meanwhile, the D2 activity of those patients strongly correlated with the serum FT_3-to-FT_4 ratio ($r=0.676$, $P<0.001$; Fig. 2B).

Furthermore, we investigated the correlation between the serum TRAb titer level and the corresponding thyroidal D1 and D2 activities. The thyroidal D1 activity of patients with T_3-P-GD and CT-GD significantly correlated with the serum TRAb titer level ($r=0.502$, $P<0.01$). The thyroidal D2 activity of those patients also significantly correlated with the serum TRAb titer level ($r=0.502$, $P<0.01$).

Table 1 Basic characteristics of patients with T_3-P-GD and CT-GD who completed the study. The normal ranges were 0.3–5.0 μIU/ml for TSH, 0.7–1.6 ng/dl for free thyroxine (FT_4), 1.7–3.7 pg/ml for free T_3 (FT_3), and 1.8–3.3 ((pg/ml)/(ng/dl)) for the FT_3-to-FT_4 ratio. A TSH concentration <0.003 μIU/ml was regarded as 0, for the purpose of statistical calculation. Values shown are means ± S.D. Values in patients with T_3-P-GD and CT-GD were compared using the unpaired *t*-test or the Mann–Whitney *U* test, as appropriate.

	T_3-P-GD ($n=13$)	**CT-GD** ($n=18$)	***P* value**
Age	36±12	49±16	0.008
Gender (F/M)	10/3	15/3	0.676
Dose of MMI (mg)	25±10	7±3	<0.001
TSH (μIU/ml)	2.50±5.05	2.03±2.05	0.754
FT_4 (ng/dl)	0.89±0.79	0.87±0.15	0.919
FT_3 (pg/ml)	4.72±3.65	2.35±0.42	0.038
FT_3/FT_4	6.6±3.0	2.7±0.5	0.001
TRAb (U/l)	206±276	5.04±4.68	<0.001
TSAb (%)	1124±582	350±338	<0.001
Thyroid volume (ml)	227±106	32±23	<0.001

D1 *and* D2 *mRNA in thyroid tissues*

We investigated the thyroidal *D1* and *D2* mRNA. The thyroidal *D1* mRNA level in the patients with T_3-P-GD (0.028 ± 0.015 arbitrary unit) was significantly higher than that in the patients with CT-GD (0.016 ± 0.014 arbitrary unit; Fig. 3). On the other hand, there was no significant difference between the thyroidal *D2* mRNA level in the patients with T_3-P-GD (0.545 ± 0.276 arbitrary unit) and that in the patients with CT-GD (0.494 ± 0.234 arbitrary unit; Fig. 3).

Next, we examined whether D1 and D2 activities correlated with the corresponding mRNA level. There was a significant correlation between the D1 activity and the *D1* mRNA level in the thyroid tissues from T_3-P-GD and CT-GD patients ($r=0.502$, $P<0.01$). On the other hand, there was no significant correlation between the D2 activity and the *D2* mRNA level in the thyroid tissues from those patients ($r=0.362$, $P=0.076$).

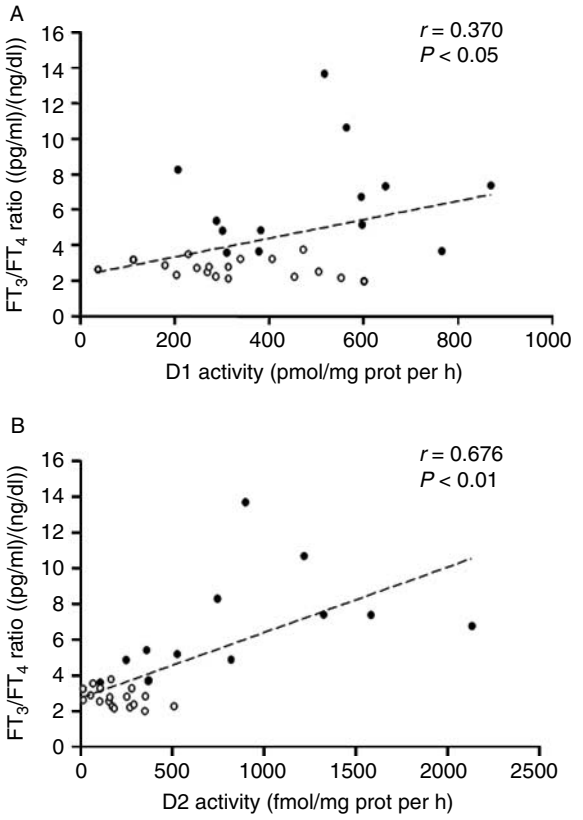

Figure 2 Correlation between thyroidal D1 (A) and D2 (B) activities and FT_3-to-FT_4 ratio. Open circles represent CT-GD patients; closed circles represent T_3-P-GD patients.

Furthermore, we investigated the correlation between the serum TRAb titer level and the corresponding thyroidal *D1* and *D2* mRNA level. There was a significant correlation between the thyroidal *D1* mRNA level and the serum TRAb titer level in the patients with T_3-P-GD and CT-GD ($r=0.651$, $P<0.01$). On the other hand, there was a significant but weak correlation between the thyroidal *D2* mRNA level and the serum TRAb titer level in those patients ($r=0.489$, $P<0.05$).

Discussion

The serum FT_4 and FT_3 levels and FT_3-to-FT_4 ratio in the patients with T_3-P-GD changed to within the almost normal range after thyroidectomy and administration of the appropriate dose of L-T_4. Therefore, it was suggested that T_3 production from the thyroid mainly contributed to the elevated serum T_3 level in T_3-P-GD. T_3 production from the thyroid is thought to originate from deiodination of T_4 in the thyroid and from the hydrolysis of Tg. It is thought that the thyroid gland deiodinates both T_4 released from Tg and T_4 taken up from the vascular bed (18).

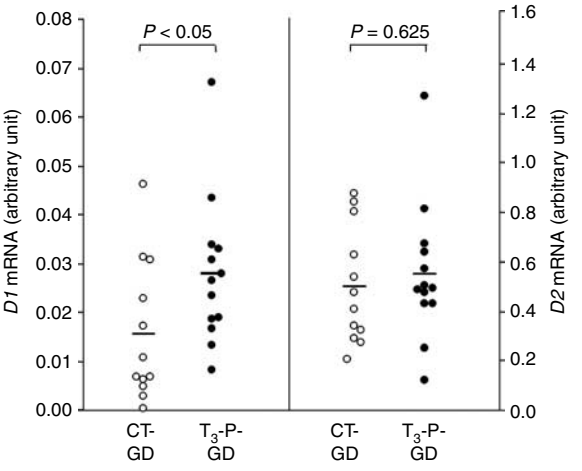

Figure 3 *D1* and *D2* mRNA in thyroid tissues. Open circles represent CT-GD patients; closed circles represent T_3-P-GD patients; solid squares represent mean value levels.

We demonstrated that the deiodinase activities of D1 and especially D2 in the thyroid tissues of T_3-P-GD were significantly higher than those of CT-GD. Increased D2 activity of the thyroid tissues was also observed in some cases of T_3 thyrotoxicosis such as follicular thyroid carcinoma, GD during PTU treatment, *TG* gene mutations, and McCune–Albright syndrome (7–10). Laurberg *et al.* suggested that D1-generated T_3 in the thyroid was a major part of the total T_3 production in untreated GD (6). However, in T_3-P-GD patients who were treated with MMI, the serum FT_4 level was within the normal range, while the serum FT_3 level was elevated. Therefore, we suggest that the mechanism(s) by which elevated serum FT_3 levels are maintained in the patients with T_3-P-GD probably differ from those in the patients with untreated GD. Although we have neither direct nor indirect results to indicate which deiodinase is responsible for the elevated serum T_3 level in T_3-P-GD, the closer correlation between thyroidal D2 activity and serum FT_3-to-FT_4 ratio favors thyroidal D2 as the cause, but this is not definitive.

It is predicted that D1 activity in the liver and kidney might be increased in T_3-P-GD, as D1 is positively regulated by T_3 (19). Maia *et al.* (21) estimated that D2 is the major contributor of extrathyroidal T_3 production in euthyroid subjects, and peripheral T_3 production may switch from D2 to D1 dependency in thyrotoxic patients, since the D2 activity decreases due to the posttranslational substrate-induced inactivation of D2 (20, 21). In this study, since the serum FT_4 level in the patients with T_3-P-GD was within the normal range, we considered that D2-generated T_3 mainly contributes to the extrathyroidal T_3 production in the patients with T_3-P-GD. Therefore, it is suggested that any change in D1 activity in the liver and kidney of T_3-P-GD patients would contribute little to the peripheral T_3 production, even if D1 activity is increased in T_3-P-GD.

D2 mRNA is positively regulated by cAMP via cAMP-responsive element in the human *D2* gene (22, 23) and negatively regulated by T_3 at pretranslational level (24, 25). In this study, the correlation between the thyroidal *D2* mRNA level and the TRAb titer in the patients with T_3-P-GD and CT-GD was weak. These results suggest that not only positive regulation by cAMP, which is produced in the thyroid cells by TRAb stimulation, but also negative regulation by T_3 may also regulate the thyroidal *D2* mRNA level in those patients. Further investigations are necessary to clarify the mechanism(s) by which thyroidal *D2* mRNA is regulated.

Although the thyroidal D2 activity in the patients with T_3-P-GD was significantly higher than that in the patients with CT-GD, there was no significant difference between the thyroidal *D2* mRNA level in the patients with T_3-P-GD and that in the patients with CT-GD. Furthermore, there was no significant correlation between the thyroidal D2 activity and the *D2* mRNA level in those patients. It is well known that D2 activity is negatively regulated at the posttranslational level by its preferred substrate T_4 via the stimulation of the ubiquitin-mediated proteasome degradation of the enzyme (20). There was no significant difference between the serum FT_4 level in the patients with T_3-P-GD and that in the patients with CT-GD in this study. Therefore, it is suggested that translational and/or posttranslational mechanism(s), which are not induced by T_4, may be involved in the higher thyroidal D2 activity in the patients with T_3-P-GD.

Interestingly, the correlation between the TRAb titer level and the thyroidal D2 activity was stronger than that between the TRAb titer level and the thyroidal *D2* mRNA level in the patients with T_3-P-GD and CT-GD. These results suggest that TRAb may regulate the D2 activity not only at pretranslational level, but also at translational and/or posttranslational level(s).

Of note is the fact that the volume of the thyroid gland was greater in the patients with T_3-P-GD. It is likely that the large goiter size is related to stimulation by higher TRAb in the patients with T_3-P-GD. Interestingly, higher thyroidal D2 activities have been observed in some large goitrous thyroid diseases such as *TG* gene mutations or McCune–Albright syndrome (9, 10). These findings suggest that a large goiter itself or any stimulating factor(s) that enlarge the thyroid volume may induce higher thyroidal D2 activity in the patients with T_3-P-GD. Further investigations are necessary to clarify the mechanism(s) by which higher thyroidal D2 activity is induced in the patients with T_3-P-GD.

Both the thyroidal D1 activity and the *D1* mRNA level in the patients with T_3-P-GD were significantly higher than those in the patients with CT-GD. Furthermore, there was a significant correlation between the thyroidal D1 activity and the *D1* mRNA level in the patients with T_3-P-GD and CT-GD. Significant positive correlation between the thyroidal *D1* mRNA level and the serum TRAb titer level was present in those patients. These results suggest that the thyroidal D1 activity may be mainly regulated at pretranslational level by cAMP, which is produced in the thyroid cells by TRAb stimulation.

On the other hand, in our previous study, both TG and the iodine content in thyroid tissues of patients with T_3-P-GD were lower than those of patients with CT-GD (26). Therefore, an enhanced iodine metabolism and possibly a higher rate of TG hydrolysis with prompt release of thyroid hormones, mostly T_3, may also contribute to the higher serum FT_3-to-FT_4 ratio in the patients with T_3-P-GD.

In conclusion, this study suggests that both thyroidal D1 and, especially, D2 may at least partly contribute to the higher serum FT_3-to-FT_4 ratio in the patients with T_3-P-GD. Further studies are needed to clarify the mechanism(s) by which the higher serum FT_3-to-FT_4 ratio and higher thyroidal D1 and D2 activities are induced in the patients with T_3-P-GD.

Declaration of interest

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

Funding

This study was supported in part by the Smoking Research Foundation of Japan.

References

1 Larsen PR, Silva JE & Kaplan MM. Relationships between circulating and intracellular thyroid hormones: physiological and clinical implications. *Endocrine Reviews* 1981 **2** 87–102. (doi:10.1210/edrv-2-1-87)
2 St Germain DL & Galton VA. The deiodinase family of selenoproteins. *Thyroid* 1997 **7** 655–668. (doi:10.1089/thy.1997.7.655)
3 Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* 2002 **23** 38–89. (doi:10.1210/er.23.1.38)
4 Kuiper GGJM, Kester MHA, Peeters RP & Visser TJ. Biochemical mechanisms of thyroid hormone deiodination. *Thyroid* 2005 **15** 787–798. (doi:10.1089/thy.2005.15.787)
5 Salvatore D, Tu H, Harney JW & Larsen PR. Type 2 iodothyronine deiodinase is highly expressed in human thyroid. *Journal of Clinical Investigation* 1996 **98** 962–968. (doi:10.1172/JCI118880)
6 Laurberg P, Vestergaard H, Nielsen S, Christensen SE, Seefeldt T, Helleberg K & Pedersen KM. Source of circulating 3,5,3′-triiodothyronine in hyperthyroidism estimated after blocking type 1 and type 2 iodothyronine deiodinses. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 2149–2156. (doi:10.1210/jc.2007-0178)
7 Kim BW, Daniels GH, Harrison BJ, Price A, Harney JW, Larsen PR & Weetman AP. Overexpression of type 2 iodothyronine deiodinase in follicular carcinoma as a cause of low circulating free thyroxine levels. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 594–598. (doi:10.1210/jc.2002-020921)
8 Weetman AP, Shepherdley CA, Mansell P, Ubhi CS & Visser TJ. Thyroid over-expression of type 1 and type 2 deiodinase may account for the syndrome of low thyroxine and increasing triiodothyronine during propylthiouracil treatment. *European Journal of Endocrinology* 2003 **149** 443–447. (doi:10.1530/eje.0.1490443)

9 Kanou Y, Hishinuma A, Tsunekawa K, Seki K, Mizuno Y, Fujisawa H, Imai T, Miura Y, Nagasaka T, Yamada C, Ieiri T, Murakami M & Murata Y. Thyroglobulin gene mutations producing defective intracellular transport of thyroglobulin are associated with increased type 2 iodothyronine deiodinase activity. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 1451–1457. (doi:10.1210/jc.2006-1242)

10 Celi FS, Coppotelli G, Chidakel A, Kelly M, Brillante BA, Shawker T, Cherman N, Feuillan PP & Collins MT. The role of type 1 and type 2 5′-deiodinase in the pathophysiology of the 3,5,3′-triiodothyronine toxicosis of McCune–Albright syndrome. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 2383–2389. (doi:10.1210/jc.2007-2237)

11 Takamatsu J, Sugawara M, Kuma K, Kobayashi A, Matsuzuka F, Mozai T & Hershman JM. Ratio of serum triiodothyronine to thyroxine and the prognosis of triiodothyronine-predominant Graves' disease. *Annals of Internal Medicine* 1984 **100** 372–375.

12 Hegedüs L, Hansen JM, Bech K, Kampmann JP, Jensen K, Andersen E, Hansen P, Karstrup S & Bliddal H. Thyroid stimulating immunoglobulins in Graves' disease with goiter growth, low thyroxine and increasing triiodothyronine during PTU treatment. *Acta Endocrinologica* 1984 **107** 482–488. (doi:10.1530/acta.0.1070482)

13 Chan JJS & Ladenson PW. Discordant hypothyroxinemia and hypertriiodothyroninemia in treated patients with hyperthyroid Graves' disease. *Journal of Clinical Endocrinology and Metabolism* 1986 **63** 102–106. (doi:10.1210/jcem-63-1-102)

14 Costagliola S, Morgenthaler NG, Hoermann R, Badenhoop K, Struck J, Freitag D, Poertl S, Weglöhner W, Hollidt JM, Quadbeck B, Dumont JE, Schumm-Draeger PM, Bergmann A, Mann K, Vassart G & Usadel KH. Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 90–97. (doi:10.1210/jc.84.1.90)

15 Inui T, Kouki T, Yamashiro K, Hachiya T, Ochi Y, Kajita Y, Sato Y & Nagata A. Increase of thyroid stimulating activity in Graves' immunoglobulin-G by high polyethylene glycol concentrations using porcine thyroid cell assay. *Thyroid* 1998 **8** 319–325. (doi:10.1089/thy.1998.8.319)

16 Murakami Y, Takamatsu J, Sakane S, Kuma K & Ohsawa N. Changes in thyroid volume in response to radioactive iodine for Graves' hyperthyroidism correlated with activity of thyroid-stimulating antibody and treatment outcome. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3257–3260. (doi:10.1210/jc.81.9.3257)

17 Bellabarba D, Peterson RE & Sterling K. An improved method for chromatography of iodothyronines. *Journal of Clinical Endocrinology and Metabolism* 1968 **28** 305–307. (doi:10.1210/jcem-28-2-305)

18 Laurberg P. Thyroxine entering the thyroid gland via the vascular bed may leave the gland as triiodothyronines. Studies with perfused dog thyroid lobes. *Endocrinology* 1986 **118** 895–900. (doi:10.1210/endo-118-3-895)

19 Toyoda N, Zavacki AM, Maia AL, Harney JW & Larsen PR. A novel retinoid X receptor-independent thyroid hormone response element is present in the human type 1 deiodinase gene. *Molecular and Cellular Biology* 1995 **15** 5100–5112.

20 Gereben B, Goncalves C, Harney JW, Larsen PR & Bianco AC. Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. *Molecular Endocrinology* 2000 **14** 1697–1708. (doi:10.1210/me.14.11.1697)

21 Maia AL, Kim BW, Huang SA, Harney JW & Larsen PR. Type 2 iodothyronine deiodinase is the major source of plasma T_3 in euthyroid humans. *Journal of Clinical Investigation* 2005 **115** 2524–2533. (doi:10.1172/JCI25083)

22 Bartha T, Kim SW, Salvatore D, Gereben B, Tu HM, Harney JW, Rudas P & Larsen PR. Characterization of the 5′-flanking and 5′-untranslated regions of the cyclic adenosine 3′,5′-monophosphate-responsive human type 2 iodothyronine deiodinase gene. *Endocrinology* 2003 **140** 229–237. (doi:10.1210/en.141.1.229)

23 Canettieri G, Celi FS, Baccheschi G, Salvatori L, Andreoli M & Centanni M. Isolation of human type 2 deiodinase gene promoter and characterization of a functional cyclic adenosine monophosphate response element. *Endocrinology* 2000 **141** 1804–1813. (doi:10.1210/en.141.5.1804)

24 Burmeister LA, Pachucki J & St Germain DL. Thyroid hormones inhibit type 2 iodothyronine deiodinase in the rat cerebral cortex by both pre- and posttranslational mechanisms. *Endocrinology* 1997 **138** 5231–5237. (doi:10.1210/en.138.12.5231)

25 Kim SW, Harney JW & Larsen PR. Studies of the hormonal regulation of type 2 5′-iodothyronine deiodinase messenger ribonucleic acid in pituitary tumor cells using semiquantitative reverse transcription-polymerase chain reaction. *Endocrinology* 1998 **139** 4895–4905. (doi:10.1210/en.139.12.4895)

26 Takamatsu J, Hosoya T, Naito N, Yoshimura H, Kohno Y, Tarutani O, Kuma K, Sakane S, Takeda K & Mozai T. Enhanced thyroid iodine metabolism in patients with triiodothyronine-predominant Graves' disease. *Journal of Clinical Endocrinology and Metabolism* 1988 **66** 147–152. (doi:10.1210/jcem-66-1-147)

Received 20 September 2010
Accepted 11 October 2010