Free triiodothyronine /free thyroxine ratio as an index of deiodinase type 1 and 2 activities negatively correlates with casual serum insulin levels in patients with type 2 diabetes mellitus

Junichi Okada¹, Atsushi Isoda², Hiroto Hoshi², Takuya Watanabe³, Eijiro Yamada⁴, Tsugumichi Saito⁴, Kazuya Okada⁵, Yasuyo Nakajima⁶, Atsushi Ozawa⁶, Kihachi Ohshima⁶, Masanobu Yamada⁴ and Shuichi Okada²,⁶

¹Department of Medicine, Division of Endocrinology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
²Hoshi-iin, Maebashi, Gunma 379-2131, Japan
³Endocrinology and Metabolism, Saku Central Hospital Advanced Care Center, Saku, Nagano 385-0051, Japan
⁴Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan
⁵Omagari Kosei Medical Center, Daisen, Akita 014-0027, Japan
⁶Hidaka Hospital, Takasaki, Gunma 370-0001, Japan

Abstract. Free triiodothyronine/free thyroxine (FT3/FT4) ratio is considered as an index of the activities of iodothyronine deiodinase types 1 and 2 (DIO1 and DIO2, respectively) and is reportedly associated with insulin resistance in euthyroid adults. Euthyroid women with polycystic ovary syndrome accompanied with insulin resistance have lesser deiodinase activities. Correspondingly, the serum insulin level in a fasted condition positively correlates with the FT3/FT4 ratio, and insulin depletion decreases the DIO2 activity in mice. Selected genetic variants in DIO1 are also associated with insulin resistance measures. Therefore, if insulin positively regulates DIO1 and DIO2, the FT3/FT4 ratio should decrease under impaired insulin action, and the casual insulin level and FT3/FT4 ratio should be negatively correlated. To evaluate this hypothesis, we conducted a single-center retrospective study between 2018 and 2021. All participants visited the selected hospitals monthly for type 2 diabetes mellitus treatment and casual plasma glucose and HbA1c level measurements. Furthermore, their casual serum insulin levels were measured annually. Meanwhile, we excluded patients treated with insulin injection. Ultimately, we evaluated 71 patients, which all exhibited euthyroid conditions. The FT3/FT4 ratio was independently associated with thyroid-stimulating hormone, casual plasma glucose, and casual insulin levels. In terms of the regression coefficients of the univariate linear regression analysis, the FT3/FT4 ratio negatively correlated with the casual serum insulin levels. Therefore, the risk of FT3/FT4 ratio underestimation should be considered when diagnosing Graves’ disease, which is often accompanied with insulin resistance.

Key words: Iodothyronine deiodinase, Type 2 diabetes mellitus, Free triiodothyronine/free thyroxine ratio, Insulin resistance
Interestingly, these women suffer from peripheral insulin resistance [7]. In mice, insulin depletion decreases the DIO2 activity [8]. Furthermore, the FT3/FT4 ratio is considered as an index of DIO1 and DIO2 activities [9], and selected genetic variants in DIO1 are associated with the measures of insulin resistance.

In this study, we aimed to conduct a single-center retrospective study between 2018 and 2021 to evaluate whether insulin positively regulates DIO1 and DIO2 and whether the FT3/FT4 ratio decreases under impaired insulin action caused by insulin resistance. If in case, the casual insulin level and FT3/FT4 ratio should show a negative correlation.

**Materials and Methods**

**Participants**

In this single-center retrospective study, we utilized the electronic records of all patients who visited Hoshi-iin (Maebashi City, Gunma Prefecture, Japan) between 2018 and 2021. Every month, all participants visited Hoshi-iin for type 2 diabetes mellitus (T2DM) treatment and casual plasma glucose (PG) and HbA1c measurements. The casual serum insulin level was also measured annually to monitor pancreatic β cell function for hyperinsulinemia as a marker of insulin resistance. Meanwhile, this study excluded patients treated with insulin injection. Ultimately, we evaluated 71 participants, which all had normal thyroid-stimulating hormone (TSH), FT3, and FT4 levels.

This study was approved as 3-1 by the ethics committees of Hoshi-iin, conforming to the principles of Declaration of Helsinki. All eligible participants provided written informed consent for clinical study participation.

**Statistical analysis**

All statistical data were analyzed using the SPSS software (version 10.0, SPSS Inc., Chicago, IL, USA). All numerical values are expressed as means ± SD. Dunnett’s test was used for the multiple comparisons of variables. Continuous variables were compared by group using the analysis of variance and Wilcoxon rank-sum test for non-normally distributed data. To estimate the linear correlation between variables, we calculated the Pearson’s correlation coefficients. All tests for significance and the resulting p values were two-sided, with a level of significance set at 5%.

**Results**

**Subject characteristics**

Table 1 presents the subjects’ characteristics. The mean values of age, body height, body weight, and body mass index (BMI) were 66.5 ± 15 years, 164 ± 8.6 cm, 67.2 ± 12.9 kg, and 24.8 ± 3.9 kg/m², respectively. Male patients accounted for 77%. The T2DM duration was 13.8 ± 9.6 years. The casual PG level was 153.3 ± 53.9 mg/dL, while the glycated hemoglobin (HbA1c) level was 7.2% ± 1.1%. Moreover, the TSH, FT3, and FT4 levels were 1.88 ± 1.07 μIU/mL, 2.69 ± 0.4 pg/mL, and 1.11 ± 0.2 ng/dL (reference ranges: 0.35–4.94 μIU/mL, 1.88–3.18 pg/mL, and 0.70–1.48 ng/dL), respectively. Meanwhile, the FT3/FT4 ratio was 2.46 ± 0.5.

**Proportion of prescribed antidiabetic medications**

As shown in Table 2, dipeptidyl peptidase-4 inhibitors, sodium glucose cotransporter 2 inhibitors, glinide, sulfonylurea (SU), α-glucosidase inhibitors (α-GI), thiazolidinedione (TZD), biguanide, and GLP-1 receptor analog were prescribed in 41.3%, 40%, 34.9%, 28.6%, 6.3%, 1.6%, 36.6%, and 3.2% of the patients, respectively. However, 12.7% of the patients were not prescribed with antidiabetic medications. Thus, the prescribed antidiabetic medication was 1.9 ± 1.1 per patient.

**Analysis of multiple comparisons for factors affecting the FT3/FT4 ratio**

Table 3 shows that the FT3/FT4 ratio was independently associated with the TSH, casual PG, and insulin levels.
The relationship between the FT3/FT4 ratio and the casual serum insulin, TSH, and PG levels

Fig. 1 illustrates the regression coefficients of the univariate linear regression analysis between the FT3/FT4 ratio and the casual serum insulin, TSH, and PG levels. The FT3/FT4 ratio negatively correlated with the casual serum insulin ($r = -0.381$) (A) and TSH ($r = -0.221$) (B) levels but positively correlated with the PG levels ($r = 0.238$), respectively.

Discussion

Hypothyroidism and hyperthyroidism occur in 7.59% and 2.31% of patients with T2DM, respectively [10]. However, our study unintentionally found that all of our participants had euthyroid conditions. Thus, our results were obtained from a population with normal thyroid functions.

A previous study reported that the FT3/FT4 ratio increased as the TSH increased within the normal range until 40 years of age [11]. However, TSH increase was not associated with FT3/FT4 ratio increase in the older age group [11]. The influence of TSH on the FT3/FT4 ratio may be very minimal in this clinical study because the patients' mean age was 66.5 ± 15 years.

We found that the FT3/FT4 ratio negatively correlated with the TSH level (Fig. 1B), suggesting to be a compensatory mechanism to allow the FT3/FT4 ratio to resume to its normal level. Furthermore, the mean BMI was 24.8 ± 3.9 kg/m$^2$, indicating obesity tendency and possibly, mild insulin resistance.

Inconsistent with the finding of a previous report, the FT3/FT4 ratio negatively correlated with the casual serum insulin level under the abovementioned circumstances ($r = -0.381$) (Fig. 1) [1]. The opposite association between the FT3/FT4 ratio and the casual serum insulin, TSH, and PG levels.
the FT3/FT4 ratio and casual insulin levels is yet to be explained. In several previous studies, insulin positively regulated iodothyronine DIO activity [3-5, 7, 8]. Thus, impaired insulin action could consequently reduce the iodothyronine DIO activity and the conversion rate of T4 to T3 (FT3/FT4 ratio) despite having higher casual insulin levels. Although the FT3/FT4 ratio was positively associated with PG, elevated PG levels could result from β cell function impairment, causing relatively lower levels of serum insulin (Table 1).

Meanwhile, our study has limitations that warrant discussion. The sample size was small (N = 71), with a limited range for ethnicities, age, gender, and weight. Thus, the results are not fully generalizable. Hence, a larger cohort with a wider range of demographics is necessary for future studies to verify our results.

For the clinical implication, the risk of underestimating the FT3/FT4 ratio should be considered when diagnosing Graves’ disease, which is often accompanied with insulin resistance.

Acknowledgments

The study protocols were reviewed and approved by the review boards of Hoshi-iin (3-1) conducted following the Declaration of Helsinki. The subjects provided written informed consent to participate in this clinical study.

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Disclosure

None of the authors have any potential conflicts of interest associated with this clinical work. No funding was received for this study.

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