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Plasma Free Fatty Acids, Inhibitor of Extrathyroidal Conversion of T_4 to T_3 and Thyroid Hormone Binding Inhibitor in Patients with Various Nonthyroidal Illnesses

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Abstract. In order to clarify the role of free fatty acid (FFA) in thyroid hormone abnormalities in patients with nonthyroidal illness, thyroid function, FFA, inhibitor of extrathyroidal conversion of T_4 to T_3 (IEC) and thyroid hormone binding inhibitor (THBI) were studied in 99 patients with various nonthyroidal illnesses including diabetes mellitus (DM) (n=35), liver cirrhosis (LC) (n=33), chronic obstructive pulmonary disease (COPD) (n=17) and chronic heart failure (CHF) (n=14). Patients were divided into three groups based on the level of serum T_3: Group I (T_3<50 ng/d*l*), Group II (50≦T_3<80) and Group III (80≦T_3). Serum T_4, FT_3 and the T_3/T_4 ratio decreased significantly in the order Group III, Group II and Group I (Group III>II>I). The plasma FFA level was 0.91±0.12 mmol/*l* in Group I (P<0.05, *vs.* Group III), 0.65±0.06 in Group II and 0.54±0.04 in Group III, respectively. The incidence of positive IEC was 80.0% in Group I (P<0.05, *vs.* Group III), 53.7% in Group II (P<0.05, *vs.* Group III) and 34.2% in Group III. However, IEC was not correlated with the serum T_3 concentration. The incidence of positive THBI was 80% in Group I (P<0.05, *vs.* Group III), 68.3% in Group II and 47.4% in Group III, but THBI was not correlated with the serum T_4 level. Positive correlations were observed among FFA, IEC and THBI (P<0.001). From the standpoint of the underlying illnesses, DM and LC patients with low T_3 had higher plasma FFA and higher incidence of positive IEC and THBI than those with normal T_3. In patients with COPD, plasma FFA was not increased and the incidence of positive IEC and THBI was low regardless of their T_3 levels. Patients with CHF had high plasma FFA and a high incidence of positive IEC and THBI regardless of their T_3 levels. These results suggest that FFA might act as both IEC and THBI, but the degree of the contribution of IEC and THBI to the thyroid hormone abnormalities might differ according to the type underlying illness.

Key words: Nonthyroidal illness (NTI), Thyroid hormone binding inhibitor (THBI), Inhibitor of extrathyroidal conversion of T_4 to T_3 (IEC), Free fatty acid (FFA), Low T_3 syndrome.

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NONTHYROIDAL illnesses (NTI) are frequently marked with abnormalities in the serum concentration of thyroid hormones. Decreased serum T_3 and increased rT_3 are frequently observed, sometimes in combination with low T_4. Despite these changes serum TSH levels remain within the normal range in most NTI patients. The low serum T_3 concentration is related to a reduction in the peripheral conversion of T_4 to T_3 [1], whereas the increase in rT_3 in mainly due to the reduced degradation rate of this iodothyronine. Chopra *et al.* reported that there was a circulating inhibitor of extrathyroidal conversion of T_4 to T_3 (IEC) which was extractable by ether from sera in patients with severe NTI in an intensive care unit (ICU) [2]. Gemma *et al.* also observed the presence of IEC in patients with mild to severe NTI [3]. Probably the low T_4 state may result from a

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combination of several alterations in thyroid economy, including inappropriately low serum TSH, reduced thyroidal response to TSH, the presence of thyroid hormone binding inhibitor (THBI) and reduced thyroid hormone binding proteins in serum [4–6].

Chopra *et al.* measured THBI by competitive ligand binding assay (CLBA) and reported that some fatty acids might act as both IEC and THBI [7, 8]. In this study, we classified the patients with different underlying illnesses into three groups, based on their serum concentration of T_3, and evaluated the relationships among plasma FFA, IEC and THBI.

Material and Methods

Patients

Patients with four underlying illnesses were studied. Thirty-five cases of diabetes mellitus (DM) [mean age, 51 yr (age range, 16~75 yr); 26 men and 9 women], 33 cases of liver cirrhosis (LC) [mean age, 55 yr (age range, 16~75 yr); 28 men and 5 women], 17 cases of chronic obstructive pulmonary disease (COPD) [mean age, 58 yr (age range, 28~73 yr); 7 men and 10 women] and 14 cases of chronic heart failure (CHF) [mean age 64 yr (age range 38~75 yr); 14 men]. Diabetic patients who had renal dysfunction were excluded. None of the 99 patients received glucocorticoid, dopamine or heparin which were thought to influence the peripheral metabolism of thyroid hormone. Patients were divided into three groups based on serum concentrations of T_3: Group I in whom serum T_3 levels were below 50 ng/d*l*. Group II from 50 to 80 ng/d*l* and group III above 80 ng/d*l*. Blood samples were obtained after overnight fasting and were stored at −20°C until use.

Extraction of IEC and THBI from plasma

Four volumes of diethylether were added to plasma and the mixture was swirled on a vortex mixer. A measured volume of supernatant was aspirated and evaporated to dryness at room temperature under a stream of nitrogen. The residues of the evaporate were used for THBI and IEC assay.

The measurement of IEC (%T_3 production)

We measured the *in vitro* T_3 production by the method of Chopra *et al.* [1,3]. Two residues of extraction of plasma were resuspended in the assay buffer (0.1 M Tris HCI buffer, pH 7.4, containing 5 mM dithiothreitol). Four mg of rat liver homogenate and 2.5 μM of T_4 were added in one tube and incubated for 30 min at 37°C. The reaction was stopped by the addition of 2 volumes of ethanol, and the T_3 produced was extracted into the ethanol phase in a vortex mixer. The T_3 content of the ethanol extract was quantified by RIA (a). In the other tube, rat liver homogenate was added at the end of the incubation immediately prior to extraction with ethanol. The T_3 content in this tube represented the T_3 produced not by enzymatic reaction (b). *In vitro* T_3 production was the amount of T_3 produced by enzymatic reaction. In each assay, control plasma extracts taken from three normal subjects were used. The results were expressed as a percentage of the mean amount in the control tubes (%T_3 production), and estimated as positive IEC when %T_3 production was under 72.7%, the value for 2SD below the normal mean.

The measurement of THBI by Competitive Ligand Binding Assay (CLBA)

We measured THBI by the method of Chopra *et al.* [7]. The following reagents were put into 10 ×75 mm disposable glass tubes to obtain a final volume of 0.73 m*l*: 1) 500 μl assay buffer (0.15 M phosphate buffer, pH 7.4, containing 0.25% normal rabbit serum); 2) 30 μl charcoal-treated iodothyronine-free pooled human serum as a source of T_4-binding serum proteins; 3) THBI (evaporated ether extract of plasma); 4) 100 μl of 1: 200 diluted T_4-binding antiserum; 5) 10,000 to 15,000 cpm of ^{125}I-T_4 in a volume of 100 μl. After brief mixing, the tubes were incubated at room temperature for 20 min and then in a water bath at 4°C for five min. ^{125}I-T_4 bound to anti-T_4 antibody was precipitated by adding 100 μl goat anti-rabbit gamma globulin and 600 μl 10% polyethylene glycol and centrifuged at 1000 × g for 30 min at 4°C. The supernatant was aspirated, and the radioactivity of the pellet was measured. Radioactive T_4 bound to anti-T_4 antibody in the absence of charcoal-treated serum (Control 1) was arbitrarily assigned a value of 100%. The results for other

tubes were expressed as a percent of this Control 1 value. ^{125}I-T_4 bound to anti-T_4 antibody in the presence of iodothyronine free serum (but in the absence of a THBI) has been referred to as Control 2. This value is 20~30% of Control 1. The THBI index was calculated and expressed as follows:

$$\text{THBI index} = \frac{\text{Radioactivity of each sample}}{\text{Radioactivity of Control 2}}$$

THBI was regarded as positive when the THBI index was above 1. 49, the mean value + 2SD for 16 normal controls.

Thyroid function and plasma FFA

Serum T_3, T_4, FT_3, FT_4 and the TSH concentrations were determined in duplicate by RIA with commercially available kits [T_3 (Amerlex-M T_3) FT_3 (Amerlex M Free T_3) and FT_4 (Amerlex-M Free T_4), Amersham International Ltd., Amersham, Buckinghamshire, England; T_4 (SPAC T_4) and TSH (TSH kit Daiichi II), Daiichi Radioisotope Laboratory, Tokyo, Japan].

Various methods have been used to determine FT_3 and FT_4 [9–13]. In the assay of FT_3 and FT_4 with this analogue method, the addition of free fatty acid (oleic acid) to the sample serum *in vitro* did not affect the results if the concentration of oleic acid was below 4 mmol/*l*. In this study, none of our patients have this high level of FFA.

Serum rT_3 was measured as follows. rT_3 was extracted from serum with ethanol and measured by RIA with antiserum raised by the method of Gharib *et al.* and free hormones were separated by PEG [14]. Anti rT_3 antiserum was used at a final dilution of 1: 20,000, and IC 50 22 pg/tube.

The normal ranges (mean ± 2SD) were as follows: T_3, 80~180 ng/d*l*; T_4, 5.2~10.4 μg/d*l*; FT_3, 2.8~5.8 pg/m*l*; FT_4, 0.7~1.8 ng/d*l*; TSH, 0.5~3.8 μU/m*l*; and rT_3, 16.5~38.5 ng/d*l*.

Plasma FFA was determined by an enzyme method (normal range, 0.13~0.45 mmol/*l*).

Statistical analysis

Data were expressed as the mean ± SEM. Significance was considered to be $P<0.05$. Statistical significance was determined by ANOVA, Duncan's multiple range test, Student's *t*-test and χ^2 test, as appropriate. Regression lines and correlation coefficients were calculated by the method of least squares.

Results

Thyroid function, FFA, $\%T_3$ production and THBI index in 99 NTI patients

Table 1 and Figure 1 show thyroid function, FFA, $\%T_3$ production and the THBI index in the patients with various NTI divided into three groups based on their serum concentration of T_3. The serum T_4 and FT_3 value and the T_3/T_4 ratio were significantly different among the three groups. The serum FT_4 level was lowest in Group I, but there was no significant difference between Group II and Group III. The serum rT_3 value and plasma FFA level in Group I were high compared with those in Group III. The serum TSH value was slightly but significantly high in Group I compared with that in Group III, but it was still within the normal range. The $\%T_3$ production was significantly low in group I and Group II compared with that in Group III. In accord with this result, IEC was 80% positive in Group I, 53.7% in Group II, and 34.2% in Group III, and therefore the incidence of positive IEC increased as the T_3 level decreased. However, there was no significant correlation between $\%T_3$ production and the serum T_3 value (data not shown). The incidence of positive THBI was also high in Group I, with the lowest T_3 (85.0% in Group I, 68.3% in Group II and 47.4% in Group III). Of the 99 patients, nine had a low T_4 level, that is, the serum T_4 value was below 5.2 μg/d*l*, and their mean THBI index was high at 2.42, and THBI was positive in 8 of the 9

Table 1. Thyroid function in NTI patients

	Group I (n=20)	Group II (n=41)	Group III (n=38)
Age	55.9±3.3	59.7±1.6[b]	50.9±2.2
T_3 (ng/d*l*)	39.9±1.7[a,b]	69.3±1.2[b]	99.4±2.2
T_4 (μg/d*l*)	6.1 ±0.4[a,b]	8.3 ±0.3[b]	9.2 ±0.3
T_3/T_4 ratio	6.9 ±0.4[a,b]	8.8 ±0.3[b]	11.1±0.4
FT_3 (pg/m*l*)	1.25±0.11[a,b]	2.42±0.08[b]	3.30±0.10
FT_4 (ng/d*l*)	0.78±0.05[a,b]	1.06±0.04	1.17±0.04
TSH (μU/m*l*)	3.09±0.45[a]	1.93±0.21	2.20±0.24
rT_3 (ng/d*l*)	49.8±9.5[b]	32.9±2.1	28.4±1.5

Group I, $T_3<50$ ng/d*l*; Group II, $50 \leqq T_3<80$ ng/d*l*; Group III, 80 ng/d*l*$\leqq T_3$. a, $P<0.05$ *vs.* Group II; b, $P<0.05$ *vs.* Group III.

patients (Table 2). One patient with negative THBI was the only patient who had COPD. However, there was no significant correlation between the THBI index and the serum T_4 level. Significant correlations were noted among the plasma FFA level, $\%T_3$ production and the THBI index ($P<0.001$; Fig 2).

Thyroid function, FFA, $\%T_3$ production, and THBI index in the patients with different underlying illnesses

The patients with various underlying illnesses were divided into a low T_3 group ($T_3 < 80$ ng/d*l*) and a normal T_3 group (80 ng/d*l* $\leqq T_3$).

Table 3 shows the data for patients with DM. Among the patients with DM, cases with low T_3 (LT_3DM) were significantly older than those with normal T_3 (NT_3DM). This suggested the possibil-

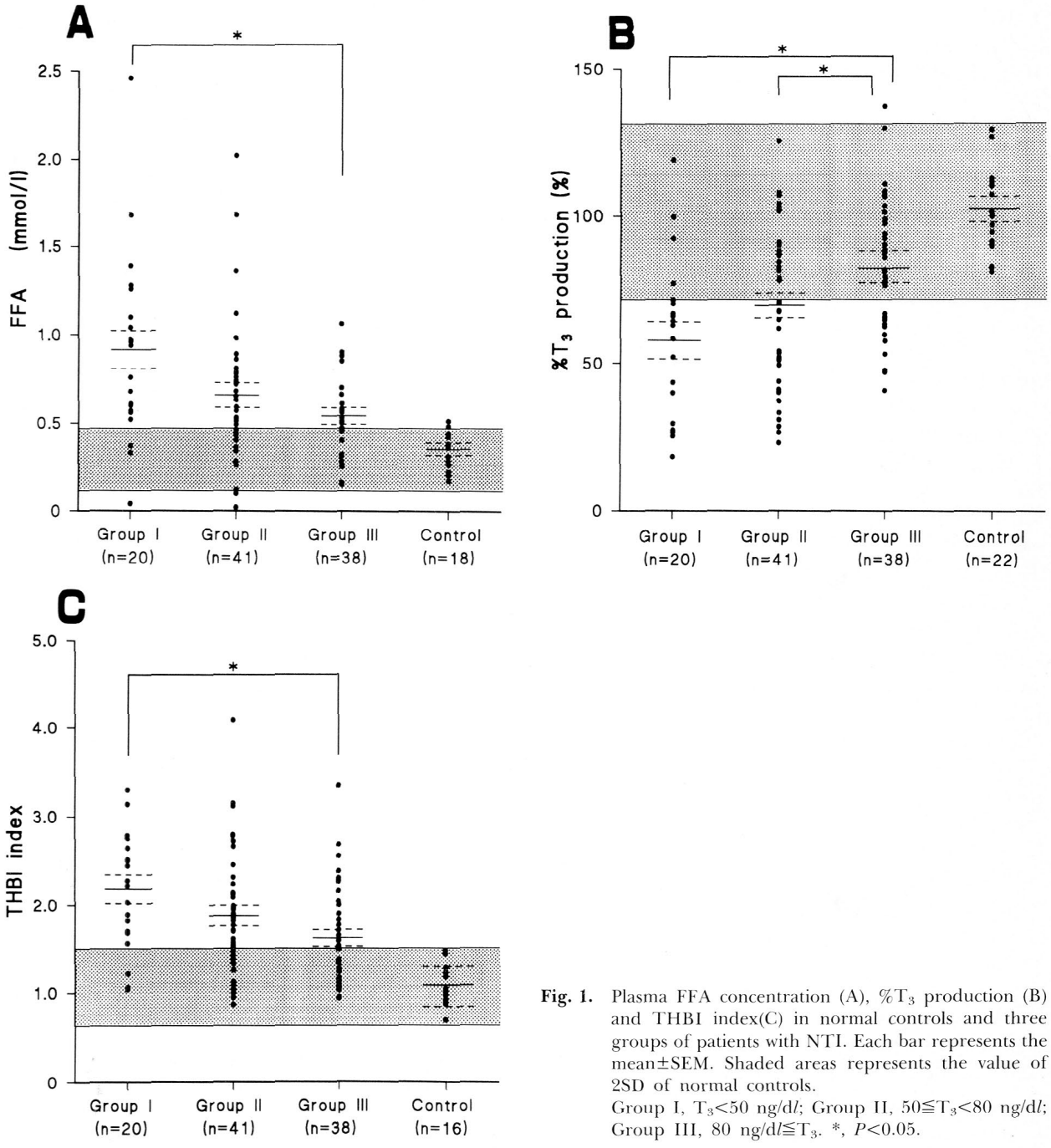

Fig. 1. Plasma FFA concentration (A), $\%T_3$ production (B) and THBI index(C) in normal controls and three groups of patients with NTI. Each bar represents the mean±SEM. Shaded areas represents the value of 2SD of normal controls.
Group I, $T_3<50$ ng/d*l*; Group II, $50 \leqq T_3<80$ ng/d*l*; Group III, 80 ng/d*l* $\leqq T_3$. *, $P<0.05$.

Table 2. Thyroid function, FFA, $\%T_3$ production and THBI index in NTI patients

	LT_4NTI (n=9)	LT_4NTI (n=90)
Age	48.1±5.0	56.4±1.4
T_3 (ng/dl)	57.4±8.9	76.5±2.5
T_4 (μg/dl)	4.7 ±0.2[a]	8.5 ±0.2
T_3/T_4 ratio	12.3±4.1	9.0 ±0.2
FT_3 (pg/ml)	1.74±0.31[a]	2.59±0.09
FT_4 (ng/dl)	0.71±0.06[a]	1.08±0.03
TSH (μU/ml)	1.79±0.27	2.26±0.17
rT_3 (ng/dl)	40.5±7.2	34.2±2.4
FFA (mmol/l)	0.86±0.15	0.65±0.04
$\%T_3$ production	49.3±9.6[a]	74.2±2.7
IEC positive ratio (%)	77.8(7/9)	48.9(44/90)
THBI index	2.42±0.25[a]	1.82±0.07
THBI positive ratio (%)	88.9(8/9)	61.1(55/90)

LT_4NTI, $T_4<5.2$ μg/dl; NT_4NTI, 5.2 μg/dl≦T_4. a, $P<0.05$ *vs.* NT_4NTI.

ity that the duration of illness was longer in the LT_3DM, although the onset of DM was not necessarily clear. Between LT_3DM and NT_3DM, there was no difference in the fasting blood glucose (FBG) value, but the HbAlc value was significantly higher in LT_3DM than in NT_3DM. This fact suggests that low T_3 states tend to occur when poor glycemic control has persisted. In LT_3DM, the serum levels of T_4, FT_3, FT_4 and the T_3/T_4 ratio were significantly low compared with those in NT_3DM. The incidence of positive IEC and THBI was higher in LT_3DM than in NT_3DM. In some patients IEC and THBI became undetectable when their glycemic control improved (Table 4).

Table 5 shows the data for the patients with LC. In cases with low T_3 (LT_3LC), the T_3/T_4 ratio, T_4, FT_3, FT_4 and albumin level were low, while the

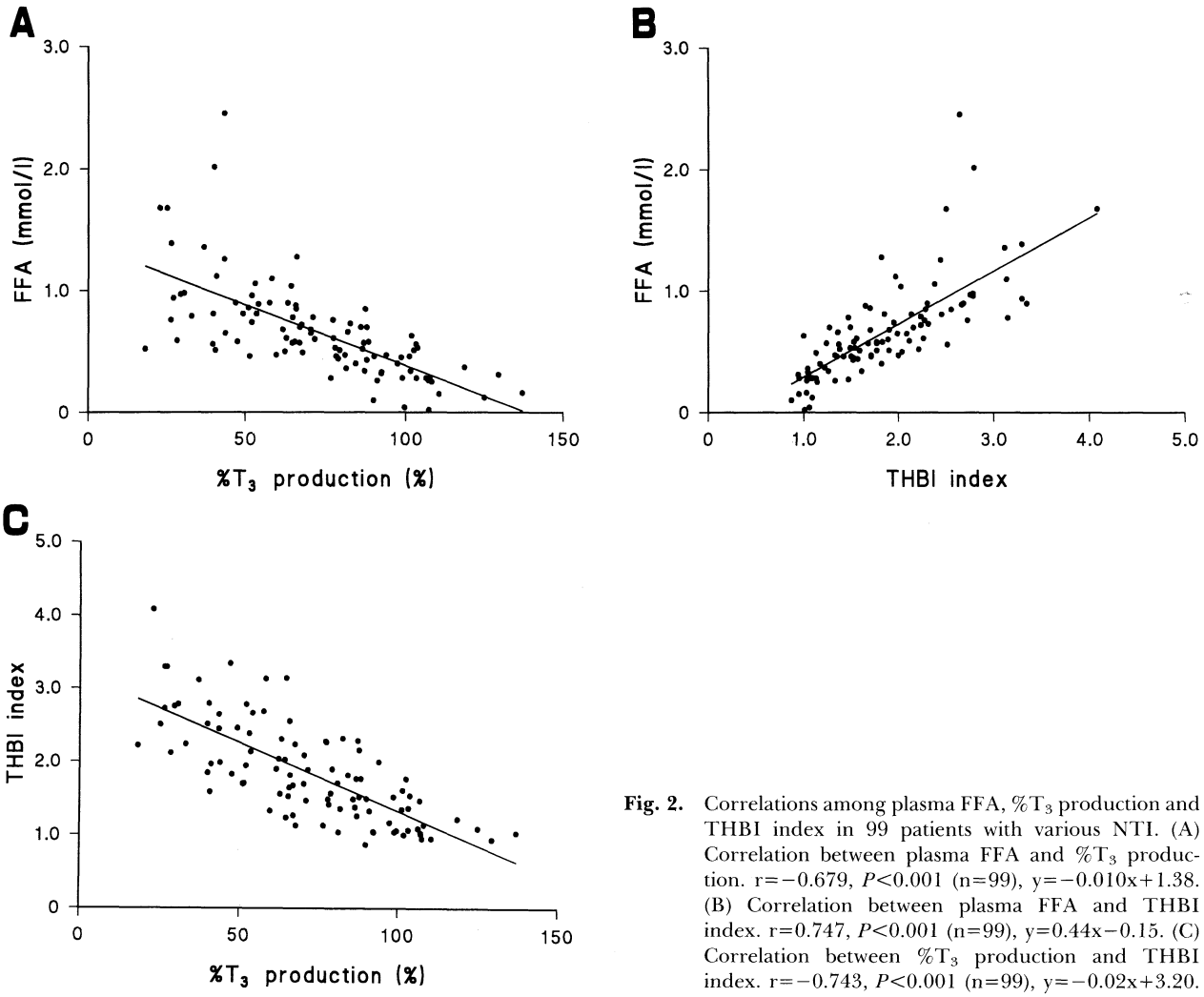

Fig. 2. Correlations among plasma FFA, $\%T_3$ production and THBI index in 99 patients with various NTI. (A) Correlation between plasma FFA and $\%T_3$ production. r=−0.679, $P<0.001$ (n=99), y=−0.010x+1.38. (B) Correlation between plasma FFA and THBI index. r=0.747, $P<0.001$ (n=99), y=0.44x−0.15. (C) Correlation between $\%T_3$ production and THBI index. r=−0.743, $P<0.001$ (n=99), y=−0.02x+3.20.

Table 3. Thyroid function, FBG, HbA1c, FFA, $\%T_3$ production and THBI index in Diabetes Mellitus

	LT_3DM (n=19)	NT_3DM (n=16)
Age	56.1 ±3.2[a]	45.8 ±3.3
T_3 (ng/d*l*)	61.8 ±3.8[a]	98.4 ±3.5
T_4 (μg/d*l*)	7.6 ±0.5[a]	9.8 ±0.4
T_3/T_4 ratio	8.4 ±0.4[a]	9.8 ±0.4
FT_3 (pg/m*l*)	2.28 ±0.18[a]	3.54 ±0.12
FT_4 (ng/d*l*)	1.06 ±0.06[a]	1.30 ±0.06
TSH (μU/m*l*)	1.73 ±0.19	1.76 ±0.28
rT_3 (ng/d*l*)	39.9 ±10.0	29.9 ±2.4
FBG (mg/d*l*)	359.4±114.4	198.6±16.6
HbA1c (%)	10.2 ±0.5[a]	8.4 ±0.5
FFA (mmol/*l*)	0.73 ±0.07	0.57 ±0.06
% T_3 production IEC	66.4 ±5.1	79.0 ±6.0
positive ratio (%)	73.7(14/19)	43.8(7/16)
THBI index THBI	1.83 ±0.11	1.60 ±0.17
positive ratio (%)	73.7(14/19)[a]	31.3(5/16)

LT_3DM, T_3<80 ng/d*l*; NT_3DM, 80 ng/d*l*≦T_3. a, $P<0.05$ *vs.* NT_3DM.

Table 5. Thyroid function, Albumin, FFA, $\%T_3$ production and THBI index in Liver Cirrhosis

	LT_3LC (n=23)	NT_3LC (n=10)
Age	55.5±2.5	55.1±1.1
T_3 (ng/d*l*)	56.5±3.3[a]	99.7±1.5
T_4 (μg/d*l*)	7.6 ±0.4[a]	9.2 ±0.3
T_3/T_4 ratio	7.7 ±0.5[a]	11.4±0.5
FT_3 (pg/m*l*)	1.74±0.14[a]	2.95±0.07
FT_4 (ng/d*l*)	0.85±0.04[a]	1.03±0.03
TSH (μU/m*l*)	3.06±0.36	2.26±0.20
rT_3 (ng/d*l*)	41.5±3.4[a]	32.0±1.3
Alb. (g/d*l*)	3.2±0.1[a]	3.7 ±0.1
FFA (mmol/*l*)	0.74±0.11[a]	0.44±0.02
$\%T_3$ production IEC	59.1±6.1[a]	88.5±3.3
positive ratio (%)	65.2(15/23)	30.0(3/10)
THBI index THBI	2.15±0.15[a]	1.58±0.05
positive ratio (%)	82.6(19/23)	70.0(7/10)

LT_3LC, T_3<80 ng/d*l*; NT_3LC, 80 ng/d*l*≦T_3. a, $P<0.05$ *vs.* NT_3LC.

Table 4. Thyroid function, FBG, HbA1c, FFA, $\%T_3$ production and THBI index in 10 Diabetic Patients

Glycemic control	Uncontrolled	Controlled
T_3 (ng/d*l*)	68.1 ±4.1[a]	80.9 ±3.6
T_4 (μg/d*l*)	7.7 ±0.6	7.9 ±0.4
T_3/T_4 ratio	9.0 ±0.4[a]	10.2 ±0.4
FT_3 (pg/m*l*)	2.50 ±0.11	2.76 ±0.17
FT_4 (ng/d*l*)	1.10 ±0.06	1.10 ±0.05
TSH (μU/m*l*)	1.50 ±0.28[a]	1.90 ±0.35
rT_3 (ng/d*l*)	29.2 ±2.0[a]	24.3 ±1.1
FBG (mg/d*l*)	276.6±36.6[a]	146.4±14.7
HbA1c (%)	10.6 ±0.7[a]	8.0 ±0.6
FFA (mmol/*l*)	0.82 ±0.08	0.55 ±0.04
$\%T_3$ production	64.4 ±5.9[a]	85.3 ±4.1
THBI index	1.67 ±0.13[a]	0.99 ±0.11

a, $P<0.05$.

Table 6. Thyroid function, PaO_2 $PaCO_2$, FFA, $\%T_3$ production and THBI index in Chronic Obstructive Pulmonary Disease

	LT_3COPD (n=9)	NT_3COPD (n=8)
Age	63.2±2.5	52.3 ±5.6
T_3 (ng/d*l*)	65.3±3.8[a]	101.0±4.5
T_4 (μg/d*l*)	7.7 ±0.5	8.9 ±0.6
T_3/T_4 ratio	8.6 ±0.5[a]	11.6 ±0.5
FT_3 (pg/m*l*)	2.44±0.19[a]	3.36 ±0.24
FT_4 (ng/d*l*)	1.10±0.09	1.18 ±0.09
TSH (μU/m*l*)	1.58±0.24[a]	3.36 ±0.61
rT_3 (ng/d*l*)	29.9±4.0	24.4 ±1.7
PaO_2 (torr)	68.6±3.3	75.1 ±6.0
$PaCO_2$ (torr)	44.2±2.7[a]	36.6 ±1.2
FFA (mmol/*l*)	0.44±0.13	0.42 ±0.06
$\%T_3$ production IEC	89.8±9.4	90.5 ±5.3
positive ratio (%)	22.2(2/9)	12.5(1/8)
THBI index THBI	1.47±0.21	1.37 ±0.16
positive ratio (%)	44.4(4/9)	25.0(2/8)

LT_3COPD, T_3<80 ng/d*l*; NT_3COPD, 80 ng/d*l*≦T_3. a, $P<0.05$ *vs.* NT_3COPD.

serum rT_3 and plasma FFA value were very high compared with those with normal T_3 (NT_3LC). In LT_3LC, the $\%T_3$ production was low and the THBI index was very high compared with those in NT_3LC.

Table 6 shows the data for the patients with COPD. In patients with COPD, there was no difference in the serum T_4 level between patients with low T_3 (LT_3COPD) and normal T_3 (NT_3COPD). The TSH level was lower in LT_3COPD than NT_3COPD. Arterial blood gas analysis showed that the $PaCO_2$ was higher in LT_3COPD than in LT_3COPD suggesting that respiratory function was worse in LT_3COPD. In the COPD patients, the plasma FFA level was lower than that in other groups of patients

Table 7. Thyroid function, FFA, $\%T_3$ production and THBI index in Chronic Heart Failure

	LT_3CHF (n=10)	NT_3CHF (n=4)
Age	65.4±2.9	58.8±7.0
T_3 (ng/d*l*)	57.6±5.7[a]	99.0±11.1
T_4 (μg/d*l*)	7.2 ±0.8	7.6 ±1.0
T_3/T_4 ratio	8.3 ±0.8[a]	13.8±2.0
FT_3 (pg/m*l*)	1.89±0.24	3.08±0.53
FT_4 (ng/d*l*)	0.94±0.09	1.03±0.11
TSH (μU/m*l*)	2.35±0.80	1.45±0.38
rT_3 (ng/d*l*)	36.2±6.0[a]	21.2±1.1
E.F. (%)	49.7±4.8[a]	74.6±0.5
FFA (mmol/*l*)	1.01±0.16	0.88±0.07
$\%T_3$ production	57.5±6.8	73.0±8.8
IEC positive ratio (%)	70.0 (7/10)	50.0 (2/4)
THBI index	2.34±0.25	2.29±0.05
THBI positive ratio (%)	80.0 (8/10)	100.0 (4/4)

LT_3CHF, $T_3<80$ ng/d*l*; NT_3CHF, 80 ng/d*l*$\leqq T_3$. a, $P<0.05$ *vs.* NT_3CHF.

regardless of their serum T_3 values, and the incidences of positive IEC and THBI were also low.

Table 7 shows the data for the patients with CHF. All the patients with CHF had underlying ischemic heart disease. In cases of patients with acute myocardial infarction (AMI), more than one month had passed since the onset of the infarction. All these patients underwent cardiac catheterization, and the left ventricular ejection fraction was calculated by an area-length method. The left ventricular ejection fraction was lower in patients with low T_3 (LT_3CHF) than in those with normal T_3 (NT_3CHF). There was no difference in the serum T_4, FT_3 and FT_4 levels between LT_3CHF and NT_3CHF. The plasma FFA value in patients with CHF was higher than that in other groups of patients regardless of the T_3 level. Furthermore, the incidences of positive IEC and THBI were high in patients with CHF regardless of the serum T_3 level.

Discussion

In the present study, we classified the patients with various NTI into three grades according to their serum T_3 levels, and studied the relationships between IEC and a low T_3 state, and between THBI and a low T_4 state in patients with mild to moderate NTI.

In group I, which was defined as the group with a serum T_3 concentration less than 50 ng/d*l*, T_4, FT_3 and FT_4 were also lower than those in the other two groups. The rT_3 level was higher in this group than in the other two groups. These results coincided with many reports.

The incidence of positive IEC or THBI was higher in the group with lower T_3 levels, and IEC and THBI were considered to be causative factors in low T_3 and low T_4 level.

The nature of IEC and THBI has not been completely clarified, but it has been proposed that FFA was the most possible candidate for both IEC and THBI [2,5]. The present study showed a significant correlations among FFA, IEC and THBI and this result indicates that FFA might act as both IEC and THBI at least *in vitro*. However, there was no significant correlation between IEC and the serum T_3 level or between the THBI index and the T_4 level. These results suggested that there might be factors other than IEC and THBI playing a causal role in low T_3 and low T_4 levels *in vivo*.

In patients with DM, a comparison between the low T_3 group and the normal T_3 group showed that there was no difference in the FBG level, but the HbAlc level was significantly higher in the low T_3 group. The incidence of positive IEC and THBI was higher in the low T_3 group, and in some patients followed IEC and THBI became negative when the FBG, HbAlc and FFA levels were improved. These results suggested that the thyroid hormone abnormalities detected in these diabetic patients were related to the degree of glycemic control. The plasma FFA level in LT_3DM was higher than that in NT_3DM but not statistically significant. These results supported the concept that the increased plasma FFA in poorly controlled diabetic patients acts as IEC and THBI.

In LC patients, the plasma FFA level was much higher in the low T_3 group than in the normal T_3 group and the incidence of positive IEC and THBI was also high in the low T_3 group. From these results, it is considered that thyroid hormone abnormalities in LC patients are strongly related to the existence of IEC and THBI. The serum albumin level was much lower in the low T_3 group than in the normal T_3 group. Although serum thyroid hormone binding protein levels were not measured, these proteins are also considered to be

decreased in the low T_3 LC patients. The decrease in thyroid hormone binding protein may contribute further to the low thyroid hormone level in LC.

It has been reported that there was no difference in serum T_3 levels between elderly patients with severe COPD and healthy volunteers [15], but in our study, 9 of 17 patients with COPD had a low T_3 level. Plasma FFA was low and the incidence of positive IEC and THBI was also low in our patients with COPD regardless of their T_3 levels. Crum *et al.* reported that patients with chronic respiratory disease often have pulmonary infection and this infection leads to the consumption of plasma FFA to produce surfactant, resulting in a low plasma FFA level [16]. These results suggest that IEC and THBI may not play a major role in the low T_3 level seen in patients with COPD. The serum TSH level was much lower in the low T_3 group than in the normal T_3 group with COPD, but the meaning of low TSH in the low T_3 group is not clear.

Our results showing that the low T_3 group with CHF was associated with a low ejection fraction coincided with the report that a low FT_3 index/rT_3 ratio was associated with poor ventricular function [17]. But, the positive ratio of THBI and IEC was high in both the low T_3 and normal T_3 groups and the plasma FFA level was also high in both groups. These results suggest that IEC and THBI may not play a major role on low T_3 level in patients with CHF. However, Tanaka *et al.* reported that a significant negative correlation was observed between serum T_4 concentrations and THBI in the patients with AMI during the month immediately following the onset [18]. The difference between Tanaka's study and our study might be attributable to the difference in the timing after the onset of AMI, but plasma FFA levels were almost identical in both studies.

Thyroid hormone abnormalities in patients with NTI are thought to be an adaptation mechanism to maintain homeostasis, and it is reported that the serum TSH level is normal although the peripheral thyroid hormone level is low. In this study, the TSH level in Group I with the lowest T_3 level was high but within the normal range compared with that in Group III. The meaning of this result is not clear, but one possibility is that the secreted TSH in NTI has altered glycosylation which is associated with reduced biological activity as reported by Lee *et al.* [19]. Another possibility is that the nocturnal surge of TSH may be abolished in these patients such as those with NTI observed by Romijn *et al.* [20]. This topic and the mechanism of the regulation of TSH secretion in NTI, especially in association with IEC, THBI and FFA, seem to deserve further investigation.

In summary, our results suggest that FFA might act as both IEC and THBI, but the degree of contribution of IEC and THBI to thyroid hormone abnormalities might differ according to the underlying illness.

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