Relationship between Inhibitor of Extrathyroidal 5'-Deiodinase Activity and Serum Free Fatty Acid in Children with Nonthyroidal Illness and Acute Ketosis

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Abstract. To clarify whether serum free fatty acid (FFA) is an inhibitor of extrathyroidal conversion (IEC) of thyroxine (T₄) to triiodothyronine (T₃), we measured the concentration of FFA, IEC activity and thyroid hormones in normal subjects, acute ketotic children and children with low T₃ syndrome due to nonthyroidal illness (NTI). Iodothyronine (I) 5'-deiodinase activity was assayed with reverse triiodothyronine (rT₃) as substrate and liberated ¹²⁵I- was measured. The IEC was determined by the inhibition of I 5'-deiodination by ether extract of sera or standard oleate solution. IEC values were represented as mM oleate. The serum concentration of FFA was 0.470±0.117 (SD) mM in 11 normal subjects, and it was significantly higher (1.242±0.248 mM; P<0.01) in 10 acute ketotic children and in 7 samples from 6 NTI children (0.904±0.530 mM; P<0.05). In contrast, there was no difference in IEC among three groups (normal subject, 0.451±0.069 mM; acute ketosis, 0.437±0.040 mM; NTI, 0.465±0.224 mM). No correlations were found between IEC activity and the serum FFA concentration or thyroid hormones in 28 samples from three groups. The sequential changes in serum thyroid hormones, FFA and IEC in 3 of 6 NTI children revealed no consistent relationship. Furthermore, one NTI child had significantly high IEC (>1.000 mM) but its serum FFA (1.182 mM) was below the mean value for the acute ketotic group. These results indicate that 1) many NTI patients may bear no relation to IEC and 2) IEC may not be caused by serum FFA only but includes several factors.

Key words: Inhibitor of extrathyroidal conversion, Free fatty acid, Iodothyronine 5'-deiodination, Nonthyroidal illness, Acute ketosis.

IT IS well known that marked abnormalities in the serum concentrations of thyroid hormones are seen in a variety of patients with acute and chronic nonthyroidal illness (NTI) [1–3]. Several studies indicated that the reduction of activity of iodothyronine (I) 5'-deiodinase is one of the most important factors underlying this phenomenon and a number of factors including decreased enzyme thiol cofactor in tissues [3], the presence of an enzyme inhibitor, the local release of free fatty acids, and generation of free radicals, may contribute to the inhibition of extrathyroidal conversion of thyroxine (T₄) [4–6]. However, the exact mechanism is not clear. As an enzyme inhibitor, Chopra et al. demonstrated the existence of a potent inhibitor of extrathyroidal conversion (IEC) of T₄ to triiodothyronine (T₃) in ether extracts of sera of some patients with a variety of systemic nonthyroidal illness and suggested that the nature of IEC in sera of NTI patients may be free fatty acid (FFA) [7].

In this study, to clarify the relationship between IEC activity and serum FFA, we measured the serum concentrations of FFA and thyroid hormones and IEC activity in NTI patients and acute
ketotic children. Acute ketosis in childhood is very common and often triggered by respiratory infection and physical or psychic stress. Sudden onset of vomiting, ketonemia, ketonuria and high serum FFA are characteristic. Therefore, we consider that acute ketotic children are suitable as a model of a high serum FFA concentration without severe systemic illness.

**Materials and Methods**

**Subjects and serum samples**

We selected a group of 6 patients with NTI whose serum T₃ concentrations were less than 80 ng/dl and a second group of 10 acute ketotic patients. The low T₃ group included 2 boys and 4 girls whose ages ranged from 2 to 13 yr. The clinical diagnoses of the patients were idiopathic pulmonary hypertension (1 patient), leukemia (1 patient), chronic renal failure (1 patient), diabetic ketoacidosis (1 patient) and Kawasaki disease (2 patients). Three patients with idiopathic pulmonary hypertension, leukemia and chronic renal failure died after a sample was obtained. The other three patients could be followed up and a total of seven low T₃ samples were obtained. The acute ketotic group included 5 boys and 5 girls aged from 1 to 8 yrs. Acute ketosis was diagnosed by ketonuria detected by semi-quantitative nitro-prusside reaction: 4 patients were (+), 3 patients were (+++) and 3 patients were (+++). Five patients were febrile and 5 patients had no complaint except for vomiting. The serum sample was obtained within 24 h from the onset of fever or vomiting. Control sera were obtained from 6 boys and 5 girls, 2 to 17 yr of age, with no history of primary thyroid dysfunction or severe systemic illness. Serum was immediately separated by centrifugation and stored at −20°C until assay. Serum T₄ and T₃ concentrations were determined by radioimmunoassay with commercially available kits (E test: Toso Yamaguchi, Japan, 2T4 AIA pack T4: Toso Yamaguchi, Japan, Ab tube T3: Eiken Tokyo, Japan).

**IEC activity**

Basic procedure of measurement of IEC conformed to that of Chopra et al. except for using the kidney microsomal fraction as the source of enzyme activity and rT₃ as the substrate [7]. The rat kidney microsomal fraction was prepared by differential centrifugation according to the method described by Leonard and Rosenberg [8]. Ether extracts of sera were prepared by the method described below. Two ml of diethyl ether was added to a 0.5 ml of sample serum and shaken for 30 min. The sample tubes were centrifuged at 3000 rpm at 4°C for 15 min. One ml of supernatant was aspirated and evaporated by vacuum at room temperature. The residue was suspended in 0.25 ml of assay buffer and 50 μl was added to the assay tube.

rT₃ I 5'-deiodinase activity was measured according to the method of Leonard and Rosenberg with minor modifications [9]. In brief, 0.05 mg protein of rat kidney microsomal fraction was incubated with ether extract of sera at 37°C for 30 min in duplicate in 250 μl of buffer containing 0.5 mM dithiothleitol, 0.25 μM rT₃ with a tracer amount of [125I]rT₃, 1 mM EDTA and 0.1 M potassium phosphate (pH 7.6). The reaction was
terminated by adding 100 µl of 2% BSA and 650 µl of 10% trichloracetic acid. After centrifugation at 3000 rpm for 10 min, the supernatant was applied to 1 ml of Dowex 50W-X2 column (Sigma Chemical Co., St. Louis, MO) and 125I− was eluted with 2 ml of 10% acetic acid. Separated 125I− was counted with a well-type γ-counter. In order to draw an oleate standard curve (Fig. 1), 0.03–1 mM oleate, instead of ether extracts of sera, was added to the assay and IEC activity was represented as mM oleate according to the standard curve.

Serum concentration of FFA

The serum FFA concentration was measured by a modification of the method of Nimura and Kinoshita [10–12]. Two-hundred µl of serum and 100 µl of 0.3 mM heptadecanoic acid (17:0) as an internal standard for quantification of FFA were added to 700 µl of distilled water. The resulting mixture was applied to Extrelut®-1 (Merk Co., Frankfurt, Germany), and after 30 min extracted with 10 ml of chloroform. One ml of the extracted sample was evaporated for 30 min by vacuum. Forty µl of 0.05 W/V% methanolic 9-anthryldiazomethane (ADAM; Funakoshi Chemical Co., Tokyo, Japan) solution was added to an evaporated aliquot. The resulting mixture was allowed to stand at room temperature for 2–3 h and a 10 µl aliquot of the mixture was directly subjected to high performance liquid chromatography (HPLC) and eluted with methanol-water (95:5). HPLC was performed with a chromatopack CR-1B (Shimadzu Seisakusho Co., Ltd., Kyoto, Japan) in conjugation with a Shim-pack CLC-ODS filled with a 4.6 × 250 mm column. The temperature of the column was programmed at 60°C and the flow rate was 1.5 ml/min. The fluorescence was measured at 415 nm, with excitation at 365 nm in a JASCO FP-210 spectrofluorometer (Nihon Bunkokogyo Co., Tokyo, Japan).

Statistical analysis

Statistical analysis was carried out by one way analysis of variance followed by Scheffe’s multiple comparisons, and a stepwise regression analysis was performed to evaluate the correlations between various parameters.

Results

Table 1 shows the serum concentrations of T₄, T₃, T₃/T₄, IEC activities and the FFA in the 3 groups. In acute ketotic patients, the mean serum concentration of FFA was significantly higher than that in normal subjects (P<0.01). Although the T₃/T₄ ratio in the acute ketotic group was significantly lower than that in the control (P<0.01), affected by a higher T₄ value (P<0.05) and a lower T₃ value (P<0.05), the serum T₃ concentrations in acute ketogenic patients corresponded in general to the range of normal values. The IEC activity in the acute ketotic group was slightly lower than that in the control in spite of significantly high serum FFA values and a low T₃/T₄ ratio. In the low T₃ group, the IEC activity did not increase despite a significant decrease in serum T₃ (P<0.01) and the T₃/T₄ ratio (P<0.01). The mean serum FFA concentration in the low T₃ group was higher than that in the control in spite of significantly high serum FFA values and a low T₃/T₄ ratio. In the low T₃ group, the IEC activity did not increase despite a significant decrease in serum T₃ (P<0.01) and the T₃/T₄ ratio (P<0.01). In comparison with the acute ketotic group, there was a significant decrease in T₄ (P<0.01), T₃ (P<0.01) and the T₃/T₄ ratio (P<0.01) in the low T₃ group. However, the mean serum FFA value and IEC activity

<table>
<thead>
<tr>
<th>Group</th>
<th>T₄ ng/dl</th>
<th>T₃ µg/dl</th>
<th>T₃/T₄ ratio (×10⁻⁵)</th>
<th>IEC (mM oleate)</th>
<th>FFA (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Ketosis (n=10)</td>
<td>137.4±21.5&lt;sup&gt;a1&lt;/sup&gt;</td>
<td>11.4±2.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.3±2.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.437±0.040</td>
<td>1.242±0.246&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low T₃ (n=7)</td>
<td>54.4±10.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.2±2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0±1.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.465±0.224</td>
<td>0.904±0.530&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>158.3±15.9</td>
<td>9.1±1.2</td>
<td>17.7±2.4</td>
<td>0.451±0.069</td>
<td>0.470±0.117</td>
</tr>
</tbody>
</table>

Values are the mean ±SD.  
<sup>a</sup> P<0.05 vs. Control;  
<sup>b</sup> P<0.025 vs. Control;  
<sup>c</sup> P<0.01 vs. Control;  
<sup>d</sup> P<0.01 vs. Acute Ketosis.
were not increased.

Table 2 demonstrates the individual serum T₄, T₃ values, T₃/T₄ ratio, IEC activity and serum FFA in the low T₃ group. IEC activity in case 1 was significantly high and beyond the range of assay. The four-fold diluted case 1 sample demonstrated 0.502 mM of IEC activity. However, the case 1 serum FFA concentration was 1.182 mM, which was lower than the mean FFA value for the acute ketotic group. Furthermore, case 4 IEC activity was 0.302 mM in spite of a high serum concentration of FFA (2.002 mM). In addition, IEC activity in low T₃ patients, except for case 1, was relatively low in comparison with that in the other 2 groups and the serum FFA concentration in 3 of 7 samples was within the normal range.

Figure 2 demonstrates the sequential changes in the serum T₃ concentration, the T₃/T₄ ratio, IEC activity and the serum FFA concentration in 3 of 6 low T₃ patients whom we could follow up. In these three patients, the serum FFA concentration decreased to various degrees as the serum T₃ value increased. However, no significant change in IEC activity could be found.

Table 3 shows the serum concentrations of FFA fractions in the 3 groups. In the acute ketotic group, all FFA fractions significantly increased. As reported previously, high serum FFA was predominantly unsaturated FFA, especially oleate. In the low T₃ group, FFA fractions were intermediate values between those for control subjects and acute ketotic patients, but individual variance was very wide. Serum concentrations of palmitic, palmitoleic, oleic and arachidonic acids were significantly higher than in control subjects. However, there were no significant differences between low T₃ and acute ketotic patients in FFA fractions.

Figure 3 shows no correlation between IEC and serum FFA in 28 samples from the three groups. There were no significant correlations between serum T₃ concentrations and serum FFA concentrations, unsaturated FFA and IEC activities. And

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### Table 2. Individual values for thyroid hormones, serum FFA concentrations and IEC activities in the low T₃ group

<table>
<thead>
<tr>
<th></th>
<th>T₃ ng/dl</th>
<th>T₄ μg/dl</th>
<th>T₃/T₄ ratio (×10⁻³)</th>
<th>IEC (mM oleate)</th>
<th>FFA (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1*</td>
<td>52.3</td>
<td>6.6</td>
<td>7.9</td>
<td>&gt;1.000</td>
<td>1.182</td>
</tr>
<tr>
<td>Case 2*</td>
<td>40.5</td>
<td>3.6</td>
<td>11.3</td>
<td>0.370</td>
<td>0.566</td>
</tr>
<tr>
<td>Case 3*</td>
<td>55.6</td>
<td>9.5</td>
<td>5.9</td>
<td>0.376</td>
<td>0.251</td>
</tr>
<tr>
<td>Case 4</td>
<td>72.4</td>
<td>10.3</td>
<td>7.0</td>
<td>0.302</td>
<td>2.002</td>
</tr>
<tr>
<td>Case 5</td>
<td>58.2</td>
<td>7.7</td>
<td>7.6</td>
<td>0.332</td>
<td>0.786</td>
</tr>
<tr>
<td>Case 6</td>
<td>61.0</td>
<td>8.1</td>
<td>7.5</td>
<td>0.409</td>
<td>0.551</td>
</tr>
<tr>
<td>Case 7</td>
<td>40.5</td>
<td>4.5</td>
<td>9.0</td>
<td>0.464</td>
<td>0.989</td>
</tr>
</tbody>
</table>

* nonsurvivor; Case 1, idiopathic pulmonary hypertension; Case 2, chronic renal failure; Case 3, leukemia; Case 4, diabetic ketoacidosis; Case 5, 6, Kawasaki disease.
Table 3. Serum concentrations of various free fatty acids in the three groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n=11)</th>
<th>Acute Ketosis (n=10)</th>
<th>Low T₃ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Conc. (mM)</td>
<td>Serum Conc. (mM)</td>
<td>Increment vs. Control (%)</td>
<td>Serum Conc. (mM)</td>
</tr>
<tr>
<td>Myristic acid (14:0)</td>
<td>0.020±0.005</td>
<td>0.041±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>205.0</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>0.073±0.017</td>
<td>0.172±0.041&lt;sup&gt;a&lt;/sup&gt;</td>
<td>235.6</td>
</tr>
<tr>
<td>Palmitoleic acid (16:1)</td>
<td>0.019±0.013</td>
<td>0.077±0.030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>405.3</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>0.138±0.028</td>
<td>0.210±0.056&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152.2</td>
</tr>
<tr>
<td>Olenic acid (18:1)</td>
<td>0.125±0.054</td>
<td>0.469±0.109&lt;sup&gt;a&lt;/sup&gt;</td>
<td>375.2</td>
</tr>
<tr>
<td>Linoleic acid (18:2)</td>
<td>0.076±0.036</td>
<td>0.240±0.078&lt;sup&gt;a&lt;/sup&gt;</td>
<td>315.8</td>
</tr>
<tr>
<td>Linolenic acid (18:3)</td>
<td>0.004±0.004</td>
<td>0.016±0.011&lt;sup&gt;a&lt;/sup&gt;</td>
<td>400.0</td>
</tr>
<tr>
<td>Arachidonic acid (20:4)</td>
<td>0.009±0.002</td>
<td>0.014±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.6</td>
</tr>
</tbody>
</table>

Total Unsaturated FFA 0.234±0.093 | 0.861±0.212<sup>a</sup> | 348.7 | 0.580±0.376<sup>a</sup> | 247.9 |

Total FFA 0.470±0.117 | 1.242±0.247<sup>a</sup> | 264.3 | 0.902±0.513<sup>a</sup> | 191.9 |

<sup>a</sup>P<0.05 vs. Control; <sup>a</sup>P<0.025 vs. Control; <sup>a</sup>P<0.01 vs. Control.

Discussion

A previous study reported that patients with a low serum T₃ concentration are exceedingly common and estimated to be about 70% of NTI patients [2]. On the other hand, the serum concentration of total FFA increases in patients with a variety of NTI and a good correlation is found between increased FFA values and poor patient outcome [13-15]. Interestingly, serum FFA, especially oleate, plays the role of thyroid hormone binding inhibitor (THBI) [13, 14]. Chopra et al. demonstrated that a potent IEC activity of T₄ to T₃ existed in ether extracts of sera of 8 or 40% of 20 NTI patients and the nature of IEC might be serum FFA because unsaturated FFAs inhibited the hepatic conversion of T₄ to T₃ in vitro and there was a significant correlation between the serum concentration of FFA and IEC activity in 6 IEC-negative and 4 IEC-positive patients. They also reported that a significant correlation was observed between IEC and THBI activity [7].

In this study, we demonstrate that no significant differences in IEC activity could be found among
low T₃, acute ketotic and control group, and IEC activities of low T₃ patients, except for case 1, were relatively low in comparison with those in the other two groups. These findings suggest that serum T₃ concentrations in many NTI patients bear no relation to in vitro IEC activity in ether extract of sera. Moreover, in vitro IEC activity did not correlate with serum concentration of T₃, T₄ and T₃/T₄ ratio. This is consistent with the findings of Gemma et al.; they demonstrated that there was no correlation between IEC activities and serum T₃ concentrations in 140 NTI patients [19]. However, we consider that IEC activity is closely related to a reduction in the serum T₃ concentration in some NTI patients because one patient in our low T₃ group had strong IEC activity. Gemma et al. also demonstrated that patients with liver cirrhosis had a significant negative correlation between IEC activities and serum T₃ concentrations despite even though there was no correlations in the patients with diabetes mellitus, respiratory failure and chronic renal failure [16]. This indicates that IEC may play an important role in reducing serum T₃ in some illnesses.

From the standpoint of the severity of the illness, our patient with high IEC activity was a nonsurvivor, and all survivors had low IEC activities. However, other two nonsurvivors had also relatively low IEC. Gemma et al. revealed that the incidence of positive IEC of nonsurvivors (62.5%) was higher than that of survivors (37.5%) [16]. These suggest that IEC activity may depend on the severity of illness.

Next, we investigate the relationship between IEC activity and serum FFA concentration. In the acute ketotic group, the patients with significantly higher serum FFA concentrations had relatively lower IEC activities than the mean value of control group and there was no correlation between IEC activity and serum FFA concentration in 28 samples of the three groups. Furthermore, case 1 and 4 in low T₃ group demonstrated the marked discrepancy between IEC activity and serum FFA concentration and follow-up of three patients in low T₃ group revealed that there was no relationship between the sequential change in IEC activity and the serum FFA concentration. These findings suggest that IEC may not be caused by serum FFA only and includes several factors other than FFA.

In comparison with the mean ± SD FFA concentration of 1.55 ± 0.08 mM in IEC positive subjects reported by Chopra, that of 1.24 ± 0.248 mM in our acute ketotic patients was slightly lower than that in IEC positive patients, but about 2.5 times as high as those in our normal subjects, IEC negative (0.51 ± 0.08 mM) and normal subjects (0.50 ± 0.08 mM) in Chopra’s report [7]. Therefore, we consider that our acute ketotic group was suitable as a model to investigate the relationship between IEC activity and serum FFA. On the other hand, it is unlikely that the discrepancy between IEC activity and the FFA concentration in this study is affected by the different FFA components because serum FFA fractions in our patients with high serum FFA consist of almost the same FFAs as previously reported [13, 14]. However, it may be that the differences in the severity and quality of illness or the ages of subjects in our study and previous reports account for the results of this study. In addition, the number of IEC positive patients included in our study was only one and the size of the low T₃ group was small. In view of these facts, we cannot negate the possibility that serum FFA acts as IEC in some situations which include patients with more severe illness and an extremely high serum FFA concentration.

Finally, we discuss whether the serum FFA concentration affects the thyroid hormone metabolism or not. In the acute ketotic group, patients with a significantly high serum FFA concentration had significantly low T₃, high T₄ and a low T₃/T₄ ratio compared with control subjects. In addition, FFAs inhibited I 5'-deiodination in vitro. These suggest that serum FFA may inhibit the extrathyroidal conversion of T₄ to T₃. However, we consider that a serum concentration of FFA less than about 1.7 mM, which was the highest value in acute ketotic patients, may not cause a significant reduction in serum T₃ because 1) serum T₃ concentrations in the acute ketotic group were within the normal range, 2) there was no negative correlation between the serum FFA and T₃ concentration in 28 samples. However, we cannot rule out the possibility of I 5'-deiodinase being inhibited by the locally released FFA in peripheral tissues.

In conclusion, this study indicates that 1) IEC activities of ether extracts of sera do not seem to offer an explanation for many patients with low T₃ syndrome and 2) IEC may include several factors...
reducing the serum T₃ concentration in some illnesses or patients, and serum FFA appears to be one of these factors only in some situations.

References