Abnormal Proinsulin Levels in Thyroid Dysfunction Measured by a Sensitive Proinsulin Immunochemiluminoassay*

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ABSTRACT

Abnormalities of carbohydrate metabolism in hyperthyroid patients have been long noted. Elevated proinsulin level is considered as an early marker of B-cell impairment. Proinsulin levels in hyperthyroid patients decreased after antithyroid drug therapy. However, proinsulin in hypothyroid patients was only rarely reported, and the difference was only demonstrated after glucose stimulation—there was a greater response of proinsulin secretion after thyroxine therapy—and the basal fasting proinsulin level was not different after therapy. One of the reasons might be that the assay was not sensitive enough to detect the change of basal proinsulin levels in patients with hypothyroidism after therapy. A newly developed immunochemiluminometric assay of proinsulin was used to demonstrate that the suppressed proinsulin level increased after thyroxine therapy in hypothyroid patients (4.2 ± 2.4 vs. 10.0 ± 5.6 pmol/L, p < 0.05; n = 7). On the other hand, our study also confirmed that the proinsulin levels decreased in hyperthyroid patients after antithyroid therapy by methimazole (27.8 ± 26.0 vs. 15.8 ± 15.7 pmol/L, p < 0.05; n = 12). In conclusion, proinsulin increased in hypothyroid patients after thyroxine therapy and decreased in hyperthyroid patients after methimazole therapy. The results demonstrated there is a high correlation between thyroid function and B-cell function in hypothyroid as well as hyperthyroid patients.

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Introduction

Impaired glucose tolerance in hyperthyroid patients has been recognized and studied.\(^1\)\(^2\)\(^3\)\(^4\) Hyperinsulinemia has been frequently observed in hyperthyroid patients.\(^3\)\(^4\) Hyperinsulinemia was regarded as a manifestation of insulin resistance. Recently, it was suggested that proinsulin makes up a great proportion of increased immunoreactive insulin (IRI) results. This was found by using specific proinsulin assays to measure a patient's specimens.\(^5\) The increased proinsulin has been proposed as an early marker of B-cell defect.\(^6\)\(^7\)\(^8\)\(^9\) Although insulin metabolism in hyperthyroidism was widely studied, the study of proinsulin levels in hyperthyroidism was still limited.

The proinsulin levels in hypothyroidism were even less studied. The reports of proinsulin level in hypothyroidism are rare. In addition, results from animal and human studies were rather conflicting.\(^10\)\(^11\)\(^12\)\(^13\) Searching Medline from 1966, only two articles were found to have been published. One of the studies applied proinsulin radioimmunoassay\(^14\) and the other study applied immunoradiometric assay.\(^15\) Both articles indicated that the proinsulin levels of hypothyroid patients were not different from proinsulin levels of euthyroid controls and did not increase after thyroxine therapy. However, proinsulin in response to glucose stimulation was increased after thyroxine therapy.\(^15\) The response of proinsulin increase in thyroxine treated hypothyroid patients was greater than that of untreated patients. These data suggested that the early assay might not be sensitive enough to detect the change of basal proinsulin levels in patients with hypothyroidism after therapy, but it might be sensitive enough to detect the increase after glucose stimulation. For this reason, a newly developed immunochemiluminometric assay (ICMA) of proinsulin\(^16\) was used to determine the changes of basal proinsulin levels in hypothyroid patients after thyroxine treatment. The decrease of proinsulin levels of hyperthyroid patients was determined after antithyroid treatment.

Patients and Methods

Patients

Twelve hyperthyroid patients (9 female and 3 male) and 7 hypothyroid patients (2 male and 5 female) who were treated at the National Cheng Kung University Hospital were enrolled in this study after informed consent. None of them had a family history of diabetes mellitus. Eleven of 12 thyrotoxic patients were diagnosed as Graves' disease; the other one was diagnosed as multinodular toxic goiter. The clinical diagnoses of 7 patients with hypothyroidism were atrophic thyroid in 3 and Hashimoto's thyroiditis in 4 patients. The clinical features of the patients are listed in table I. After confirming thyroid status by clinical manifestations and thyroid function tests, fasting plasma glucose, creatinine and proinsulin were measured. After methimazole treatment for hyperthyroidism or thyroxine replacement therapy for hypothyroidism, the thyroid function tests (including serum T3, T4, and TSH), fasting plasma glucose, and proinsulin levels were studied again 3 months later.

Biochemistry

Plasma glucose was determined by glucose oxidase reaction (Hitachi 747). Serum creatinine was measured with Hitachi 747.

Thyroid Function Tests: Commercial kits were used to measure T3 (Coat-A-Count Total T3, Abbott; Solid-phase RIA; normal range 86–187 ng/dL; intra assay CV 5.5 percent, inter assay CV 7.6 percent), T4 (Coat-A-Count Total T4, Abbott; Solid-phase RIA, normal range 4.5–12.5 µg/dL; intra assay CV 3.1 percent, inter assay 8.2 percent), and TSH levels (SPAC-S TSH kit, IRMA method, Daiichi; normal range: 0.5–5.6 µU/mL; intra assay CV 2.1 percent, inter assay CV 2.5 percent).

Immunochemiluminometric Assay (ICMA): Proinsulin levels were measured with a polyclonal immunochemiluminometric assay.\(^16\)\(^17\)\(^18\) In brief, one goat was immunized with synthetic C-peptide conjugated with bovine serum albumin (BSA). The antisera
TABLE I
Clinical Features of the Studied Patients

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism (n = 12)</th>
<th>Hypothyroidism (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean± S.D.</td>
<td>Min</td>
</tr>
<tr>
<td>Age</td>
<td>35 ± 11</td>
<td>19</td>
</tr>
<tr>
<td>BMI</td>
<td>22.0 ± 3.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Cre</td>
<td>0.6 ± 0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Glu</td>
<td>92 ± 8</td>
<td>82</td>
</tr>
<tr>
<td>Glu*</td>
<td>90 ± 8</td>
<td>80</td>
</tr>
<tr>
<td>T4</td>
<td>18.0 ± 2.7</td>
<td>15.0</td>
</tr>
<tr>
<td>T4*</td>
<td>8.0 ± 3.2a</td>
<td>1.4</td>
</tr>
<tr>
<td>T3</td>
<td>399 ± 116</td>
<td>221</td>
</tr>
<tr>
<td>T3*</td>
<td>141 ± 52a</td>
<td>59</td>
</tr>
<tr>
<td>TSH</td>
<td>0.02 ± 0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>TSH*</td>
<td>8.8 ± 25.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Min = Minimum. 
Max = Maximum. 
Age = Years. 
BMI = Body mass index [= body weight (kg)/body height (m)²]. 
Cre = Creatinine (mg/dL). 
Glu = Fasting plasma glucose (mg/dL). 
T4 = ng/dL. 
T3 = ng/dL. 
TSH = μU/mL. 
*Denotes value after therapy. 
²p < 0.0001, ³p < 0.05, and ⁴p < 0.01, comparison before and after therapy.

were immunopurified on a C-peptide affinity column. The purified antibodies were labeled with acridinium ester and used as a signal antibody. A second goat was immunized with pork insulin conjugated to BSA; the antiserum was immunopurified by human insulin affinity column. The purified insulin antibodies were immobilized on a plastic bead and used as a capture antibody, and paired with acridinium ester-labeled C-peptide signal antibody to form a “sandwich” ICMA.

After washing to remove “free” signal antibodies, chemiluminescence of the signal antibody linked on the bead vs. proinsulin was detected by luminometer. Sensitivity (determined by two standard deviations above background) was 0.2 pmol/L for proinsulin. Intra-assay and interassay coefficients of variations were 5.0 percent and 9.0 percent, respectively, for proinsulin. The assays showed no cross-reactivity with insulin or C-peptide. The reference range for proinsulin ICMA, which was determined with 120 healthy volunteers, was 3 to 20 pmol/L.

Statistics: Paired t-test was used to analyze the results. A p < 0.05 was considered significant.

Results

Eleven out of 12 hyperthyroid patients had their proinsulin levels decreased after antithyroid therapy.
roid drug—methimazole therapy (average reduction of mean ± S.D. from 27.8 ± 26.0 to 15.8 ± 15.7 pmol/L, p < 0.05). Five of the 12 hyperthyroid patients had their proinsulin levels greater than the upper limit of reference range, 20 pmol/L. After a 3-month methimazole treatment, all of the 5 patients had decreased proinsulin levels; however, 2 patients still had proinsulin levels greater than the upper limit of normal range. In total, 11 of the 12 patients had decreased proinsulin levels but one patient had an increased proinsulin level after therapy (figure 1). In hypothyroid patients, three of the 7 had lower proinsulin levels than the lower limit of reference range (3 pmol/L). All 7 patients had significantly

![PROINSULIN IN THYROID DYSFUNCTION](image)

**Figure 1.** Changes of proinsulin levels before and after 3 months of therapy. Hyperthyroidism by the antithyroid drug, methimazole; hypothyroidism by thyroxine. Shaded areas indicate reference range of proinsulin.
increased proinsulin levels after thyroxine replacement therapy (4.2 \pm 2.4 vs 10.0 \pm 5.6 pmol/L, p < 0.05) (figure 1).

Discussion

Using our immunochemiluminometric assay, it was demonstrated that the results of increased proinsulin levels in hyperthyroidism were consistent with the results measured by radioimmunoassay or by immunoradiometric assay. The results suggested impaired B-cell function in hyperthyroidism. A study of carbohydrate metabolism in hypothyroidism was more limited. Since 1966, there were only two studies published; one by Sestoft et al and the other by Beer et al. They both indicated that there was no change in proinsulin levels after thyroxine treatment in hypothyroid patients. Beer et al reported that the basal (fasting) proinsulin levels were not changed; however, there was a significant increase in proinsulin levels after glucose stimulation performed by glucose tolerance test in the group of thyroxine-treated hypothyroid patients. These data suggested that the no difference in basal proinsulin level of hypothyroid patients was due to their assays which were not sensitive enough to measure the minute change in the proinsulin level after thyroxine therapy.

In contrast to both studies, the decrease in basal proinsulin levels in our hypothyroid patients was statistically significantly lower than the control. In comparison with previous studies, our assay had an improved sensitivity (0.2 pmol/L vs. 10 pmol/L and 0.5 pmol/L), which may contribute the difference. Since insulin sensitivity reported no decrease in hypothyroidism, our result suggested that decreased B-cell activity may have occurred in hypothyroidism.

Two of the hyperthyroid patients showed marked elevated proinsulin levels, which decreased after methimazole therapy but were still greater than the upper limit of reference range (figure 1). One of these two patients was overweight. Obesity, which was currently considered to be related to insulin resistance, might be a contributing factor of the elevated proinsulin level. However, proinsulin decreased after 3 months of methimazole therapy, when body weight was increased slightly (from 66 kg to 67.5 kg) after therapy. Oral glucose tolerance test after therapy showed normal (not shown) indicating insulin resistance was unlikely. Apparently the decrease in proinsulin level after therapy in this patient was due to effect of improved thyroid status.

The other patient with marked elevated proinsulin, who was lean (body mass index 19.3), had a history of hyperthyroidism for more than 2 years and did not receive any treatment until the study. After 3 months of methimazole therapy, the thyroid function test still revealed mild T3 toxicosis. It was suspected that the long-term hyperthyroid disease had a profound effect on B-cell function, which led to a much increased proinsulin level. The mechanisms of proinsulin level changes in thyroid dysfunction should be studied further.

Conclusion

Increased proinsulin levels in patients with hyperthyroidism and decreased proinsulin levels in hypothyroid patients were demonstrated in this study. After therapy, the patients showed decreased and increased proinsulin, respectively. The results imply there is a high correlation between B-cell function and thyroid function.

References


