Hyperthyroidism Is Associated with Higher Plasma Endothelin-1 Concentrations

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The objective of this study was to determine the change of plasma endothelin (ET)-1 concentrations and insulin resistance index after therapy for hyperthyroidism. We studied 20 patients with hyperthyroidism (15 women and 5 men; age, 34.0 ± 2.8 years), and 31 patients with euthyroid goiters as controls (27 women, 4 men; age, 37.0 ± 2.4 years). All hyperthyroid patients were treated with antithyroid drugs. The patients received evaluations before and after normalization of thyroid function. The evaluations included body mass index (BMI), body fat, and measurement of circulating concentrations of thyroid hormones, glucose, insulin, and ET-1. Hyperthyroid subjects had higher plasma ET-1 concentrations than the control group ($P < 0.001$). No significant differences in serum glucose and insulin concentrations or insulin resistance index estimated by the R value of the homeostasis model assessment (HOMA-R) were noted between the groups. Plasma ET-1 concentrations decreased after correction of hyperthyroidism compared with pretreatment ($P = 0.006$). Serum glucose concentrations decreased after correction of hyperthyroidism ($P = 0.005$). Moreover, both body weight–adjusted insulin concentrations and the HOMA-R index were also decreased after correction of hyperthyroidism compared with pretreatment ($P = 0.026$ and $P = 0.019$, respectively). Pearson's correlation revealed that plasma ET-1 levels positively correlated with serum triiodothyronine (T3) and free thyroxine (FT4) levels. Serum insulin levels and the HOMA-R index positively correlated with BMI and body fat. The HOMA-R index also positively correlated with serum T3 and FT4 levels. Neither insulin levels nor the HOMA-R index correlated with ET-1 levels.

Hyperthyroidism is associated with higher plasma ET-1 concentrations. In addition, correction of hyperthyroidism is also associated with a decrease of plasma ET-1 levels as well as the insulin resistance index calculated by HOMA-R. Exp Biol Med 231:1040–1043, 2006

Key words: endothelin-1; Graves' disease; hyperthyroidism; HOMA-R; insulin resistance

Introduction

Since the first discovery of endothelin (ET) secretion by endothelial cells (1), numerous tissues and cultured cells, including thyroid cells, have been shown to produce ET (2–6). The thyroid gland is a highly vascularized tissue. ET-1 stimulates mitogenesis in human thyroid epithelial cells derived from thyroid tissues from normal subjects and from patients with Graves' disease (7). The presence of the specific high-affinity receptor for ET on human thyrocytes has also been reported (8). However, another study found the contrary inhibition of thyrotropin-induced DNA synthesis and cell proliferation in rat thyroid epithelial cells by ET-1 (9).

Human ET is found in three isoforms: ET-1, ET-2, and ET-3 (10). In addition to being identified in many different tissues, ET-1 is also present in the plasma of human beings and animals (11, 12). Thyroid hormone status affects tissue levels but not plasma levels of ET in male rats (13). In one study, the levels of ET in hyperthyroid, hypothyroid, and euthyroid subjects were similar. However, another study found that ET-1 plasma levels are elevated in hyperthyroid patients when compared with euthyroid subjects (15).

The aim of the present study was to compare the plasma ET-1 levels before and after antithyroid drug treatment in Graves' disease hyperthyroid subjects. The change of body weight (BW), body fat (BF), glucose and insulin concentrations, and R value of the homeostasis model assessment

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(HOMA-R) index for insulin resistance were also evaluated. Correlations between plasma ET-1 levels and the parameters were also assessed.

Materials and Methods

Subjects. The study was performed on 20 patients with Graves' disease hyperthyroidism (15 women and 5 men; mean age, 34.0 ± 2.8 years). Thirty-one patients with euthyroid nodular goiter served as the control group (27 women, 4 men; mean age, 37.0 ± 2.4 years). Informed consent was obtained from all subjects after thorough explanation of the procedures. All subjects, including those in the hyperthyroid and control groups, were free of diabetes mellitus. The clinical and biochemical characteristics of the patients are summarized in Table 1.

All hyperthyroid patients were treated with one of two antithyroid drugs to treat hyperthyroidism. Of the 20 patients, 8 were treated with propylthiouracil and 12 with carbimazole. The patients were evaluated at the time of diagnosis and after thyroid function was normalized. The evaluation included physical examinations (body height, BW, and BF), measurement of fasting-state concentrations of serum free thyroxine (FT4), total triiodothyronine (T3), thyroid-stimulating hormone (TSH), glucose, insulin, and plasma ET-1. Thyroid function was normalized in 3–7 months (mean, 5.4 ± 0.3 months).

Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. BF was determined by bioimpedance spectrum analyzer (HYDRA ECF-ICF 4200; Xitron Technologies Inc., CA). Insulin resistance was estimated *via* the HOMA-R index, calculated as plasma glucose concentrations (m*M*) times the serum insulin concentrations (mU/l) divided by 22.5 (16).

Samples of venous blood were obtained from an antecubital vein after an overnight fast starting at midnight. Blood samples were centrifuged immediately and the serum was stored at –20°C until assayed.

Biochemistry and Hormone Analyses. Serum FT4 was measured by chemiluminescent immunoassay (Immulite 2000; Diagnostic Product Corporation, Los Angeles, CA). The sensitivity of the assay was 0.15 ng/dl. The normal range was 0.8–1.9 ng/dl. Serum total T3 was measured by chemiluminescent immunoassay (Immulite 2000). The sensitivity of the assay was 19 ng/dl. The normal range was 84–172 ng/dl. Serum TSH was measured by chemiluminescent immunoassay (Immulite 2000). The sensitivity of the assay was 0.002 μIU/ml. The normal range was 0.4–4 μIU/ml. Serum insulin concentrations were analyzed by chemiluminescent immunoassay (Immulite 2000). The sensitivity of the assay was 2 μIU/ml. The normal range was 5–25 μIU/ml. Plasma ET-1 concentrations were measured by the quantitative enzyme immunoassay technique (R&D Systems, Inc., Minneapolis, MN). The intraassay and interassay coefficients of variation were 4.5% and 5.5%, respectively. The minimum detectable dose is typically less than 1.0 pg/ml. Serum glucose was measured by colorimetry method (Hitachi 7600–110; Hitachi Ltd., Tokyo, Japan). The normal range was 3.58–6.05 m*M*.

Statistical Analyses. Data were reported as mean ± SEM. Comparisons of means between groups were made by using Student's *t* test. The comparisons between the control group and the pretreatment or posttreatment hyperthyroid groups were made by using the independent Student's *t* test. The comparisons between the pretreatment and posttreatment groups of the 20 hyperthyroid patients were made by using the paired Student's *t* test. Chi-square analysis was used for nonparametric data. Correlations between ET-1 levels and the anthropometric parameters (BMI and %BF), thyroid hormones (T3, FT4, and TSH), and the HOMA-R index in hyperthyroidism were assessed by using Pearson's

Table 1. Characteristics of the Control Group and the Pretreatment and Posttreatment Hyperthyroid Groups[a]

		Hyperthyroidism ($n = 20$)	
	Control ($n = 31$)	Pretreatment	Posttreatment
Age (yr)	37.0 ± 2.4	34.0 ± 2.8	—
Sex (F/M)	27/4	15/5*	—
BW (kg)	56.1 ± 1.8	58.0 ± 2.9	59.5 ± 3.0**
BMI (kg/m^2)	22.0 ± 0.6	21.8 ± 0.8	22.3 ± 0.8
Net BF (kg)	14.4 ± 0.8	15.2 ± 1.4	15.0 ± 1.6
%BF	25.5 ± 0.8	26.0 ± 1.3	24.7 ± 1.6
Free T4 (ng/dl)	1.33 ± 0.05	4.98 ± 0.52*	1.16 ± 0.08****
T3 (ng/dl)	120.7 ± 4.4	457.0 ± 48.3*	127.4 ± 9.6****
TSH (μIU/ml)	1.69 ± 0.19	0.02 ± 0.00*	4.45 ± 1.87**
Glucose (m*M*)	4.83 ± 0.08	5.01 ± 0.08	4.68 ± 0.08***
ET-1 (pg/ml)	0.63 ± 0.04	0.93 ± 0.06*	0.69 ± 0.04***
Insulin (μIU/ml)	7.02 ± 0.71	8.52 ± 1.03	6.48 ± 0.93
HOMA-R index	1.53 ± 0.16	1.96 ± 0.26	1.39 ± 0.22

[a] Data are mean ± SEM. Pretreatment or posttreatment hyperthyroid group compared with control group by independent Student's *t* test: *$P <$ 0.001. Posttreatment group compared with pretreatment group by paired Student's *t* test: **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$.

Table 2. The Comparisons of BW-Adjusted Values of Serum ET-1, Insulin, and HOMA-R Index Among the Control Group and the Pretreatment and Posttreatment Hyperthyroid Groups[a]

		Hyperthyroidism ($n = 20$)	
	Control ($n = 31$)	Pretreatment	Posttreatment
ET-1/BW (kg)	0.012 ± 0.001	0.017 ± 0.001*	0.012 ± 0.001***
Insulin/BW (kg)	0.12 ± 0.01	0.15 ± 0.02	0.11 ± 0.01**
HOMA-R/BW (kg)	0.027 ± 0.002	0.035 ± 0.005	0.023 ± 0.003**

[a] Data are mean ± SEM. Pretreatment or posttreatment hyperthyroid group compared with control group by independent Student's *t* test: *$P <$ 0.01. Posttreatment group compared with pretreatment group by paired Student's *t* test: **$P < 0.05$; ***$P < 0.01$.

correlation analysis. A value of $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of the study population are shown in Table 1. The pretreatment hyperthyroid group had lower TSH and higher T3 and FT4 serum concentrations than did the control and posttreatment groups.

However, there was no significant difference between either pretreatment or posttreatment and the control groups in terms of BMI, net BF, %BF, glucose and insulin serum concentrations, and HOMA-R index. Nevertheless, significantly higher ET-1 plasma concentrations were observed in the pretreatment hyperthyroid group than in the control group ($P < 0.001$). No significant differences of ET-1 concentrations were found between the control and posttreatment groups ($P > 0.05$).

Compared with the hyperthyroidism pretreatment group, the posttreatment group had higher BWs ($P < 0.05$) but lower concentrations of serum glucose ($P < 0.01$) and plasma ET-1 ($P < 0.01$). However, there was no difference between the pretreatment and posttreatment groups in BF or in serum insulin concentrations, or in the HOMA-R index ($P > 0.05$).

In the eight patients receiving propylthiouracil treatment, the ET-1 concentration decreased by 0.24 ± 0.11 pg/ml, as compared with a decrease of 0.25 ± 0.11 pg/ml in the 12 patients receiving carbimazole treatment. The change of ET-1 concentrations was not different ($P = 0.479$) between these two groups.

Because BW was higher in the posttreatment group than the pretreatment group ($P < 0.05$), we adjusted the ET-1 and insulin circulating concentrations as well as the HOMA-R index for BW (Table 2). The posttreatment group had lower adjusted ET-1 ($P < 0.01$) and insulin ($P < 0.05$) concentrations, as well as a lower adjusted HOMA-R index ($P < 0.05$) than the pretreatment group.

Correlation analyses (Table 3) show that plasma ET-1 levels positively correlated with serum T3 and FT4 levels. Insulin serum levels only positively correlated with the anthropometric parameters (BMI and %BF). No correlation was found between insulin and thyroid hormones (T3, FT4, and TSH) levels. The HOMA-R index positively correlated with both anthropometric parameters (BMI and %BF) and thyroid hormone (T3, FT4, and TSH) levels. However, no correlation was found between plasma ET-1 and serum insulin levels or the HOMA-R index.

Discussion

We compared plasma ET-1 concentrations between Graves' disease hyperthyroidism patients and euthyroid subjects. We also evaluated the ET-1 concentration change after hyperthyroidism treatment in Graves' disease patients. The results showed higher ET-1 plasma concentrations in hyperthyroidism status, which could be decreased after hyperthyroidism treatment.

The direct effect of Graves' disease hyperthyroidism on plasma ET-1 concentrations is still lacking elucidation. Investigators previously found that, in Graves' disease, cytokines, such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α are increased (17–20). ET-1 levels are also found to closely correlate with levels of proinflammatory cytokines, such as P-selectins, E-selectins, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and IL-1a, IL-1b, IL-6, IL-8, and IL-10 (21). The indirect stimulation by immunomodulating factors involved in Graves' disease should, therefore, be considered. However, higher ET-1 levels that are not associated with immunomodulating factors are also found in toxic thyroid adenoma patients (15). In addition, our study showed a positive correlation between plasma ET-1 levels and thyroid hormone (T3 and FT4) levels in hyperthyroid patients. Hence, we

Table 3. Correlation Analyses in the Hyperthyroid Subjects[a]

Factors	ET-1	Insulin	HOMA-R
BMI	0.064	0.411***	0.398**
%BF	0.117	0.311**	0.296*
T3	0.556***	0.203	0.244*
FT4	0.474***	0.227	0.271*
TSH	−0.022	−0.188	−0.180
ET-1	1	0.118	0.147
Insulin	0.118	1	0.990***
HOMA-R	0.147	0.990***	1

[a] Pearson's correlation coefficients with level of significance.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

demonstrated that thyroid hormones are important determinants of plasma ET-1 concentrations in humans.

In the hyperthyroid group, all 20 patients were treated with antithyroid drugs, 8 patients with propylthiouracil and 12 patients with carbimazole. No significant difference in ET-1 concentration changes were observed between these two groups. Therefore, the influence of the treatment drug can be excluded in this case.

Thyrotoxicosis is associated with a state of insulin resistance (22). In our study, however, there were no changes in insulin serum levels or the insulin resistance index calculated by HOMA-R after treatment of hyperthyroidism. However, the BW adjustment for these two values was decreased. The adjusted value can exclude the influence of BW because it increased significantly after the hyperthyroidism treatment. These findings, combined with the decreased serum glucose concentrations, seem compatible with the idea that correction of hyperthyroidism may be associated with improvement of insulin resistance. However, the insulin levels and HOMA-R index did not correlate with ET-1 levels in hyperthyroid patients. These observations suggest that insulin resistance associated with hyperthyroidism is not mediated by the levels of plasma ET-1.

In summary, our results demonstrate that Graves' disease hyperthyroidism is associated with higher plasma ET-1 concentrations. In addition, correction of hyperthyroidism is also associated with a decrease of plasma ET-1 levels as well as the insulin resistance index calculated by HOMA-R. However, the insulin resistance is not mediated by the levels of plasma ET-1.

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