SHORT COMMUNICATION

Serum levels of glucagon-like peptide (GLP)-1 and GLP-2 in patients with Hashimoto's thyroiditis

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Background: The influence of Hashimoto's thyroiditis (HT) with subclinical hypothyroidism or euthyroid status on the alteration of glucagon-like peptide (GLP)-1 and GLP-2 levels remains uncertain. **Materials and Methods:** Twenty-four untreated HT patients with subclinical hypothyroidism, 24 euthyroid HT patients, and 24 age- and gender-matched controls were enrolled in the study. The levels of GLP-1, GLP-2, glucose, glycated albumin, insulin, thyroid hormone, and thyroid autoantibodies were measured and evaluated. **Results:** The levels of GLP-1, blood glucose, and triglyceride were higher in HT patients with subclinical hypothyroidism than in controls (all $P < 0.05$, respectively). However, the above variables, including GLP-2, were similar in euthyroid patients and controls. Neither GLP-1 nor GLP-2 was correlated with thyroid hormone, thyroid autoantibodies or metabolic parameters. **Conclusion:** The serum levels of GLP-1, not GLP-2, were increased in patients with subclinical hypothyroidism. Our data suggest that subclinical hypothyroidism affects circulating GLP-1 levels.

Key words: Glucagon-like peptide, Hashimoto's thyroiditis, subclinical hypothyroidism
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INTRODUCTION

Glucagon-like peptide-1 (GLP-1) and GLP-2 are proglucagon-derived peptides released from enteroendocrine L-cells in response to nutrient ingestion. Recently, preclinical and clinical studies have demonstrated that the role of GLP-1 is not specific to glycol metabolism; it is also involved in cardiovascular and neuroprotection effects.[1,2] GLP-2 is an intestinal trophic peptide that stimulates epithelial proliferation in the small intestine and improves intestinal barrier function. GLP-2 does not appear to regulate blood glucose to a large extent. In addition, GLP-1 and GLP-2 exhibit a diverse array of metabolic effects. The two peptides have distinct biological functions that are governed by their respective receptors.[3,4]

To the best of our knowledge, few data are available regarding the roles of GLP-1 and GLP-2 other than those in metabolism, particularly in thyroid disease. We previously reported that GLP-1 levels were increased in patients with overt hyperthyroidism, whereas no difference was observed in patients with euthyroid congenital hypothyroidism.[5] Hashimoto's thyroiditis (HT) can develop to different degrees of hypothyroidism. The roles of GLP-1 and GLP-2 in the pathophysiology of HT have not been investigated to date.

Overt or subclinical hypothyroidism has multiple pathogenic effects on the cardiovascular system. Patients with subclinical hypothyroidism show low-grade chronic inflammation and increased oxidative stress. L-thyroxine replacement therapy may improve cardiovascular risk factors in cases of subclinical hypothyroidism with HT.[6]

The homeostasis model assessment index: insulin resistance (HOMA-IR) is reportedly increased in patients with hypothyroidism when compared with euthyroid subjects.[7] It has been suggested that subclinical hypothyroidism causes disturbances in carbohydrate parameters and hormone-controlled appetite.[8] Little is known about the effect of subclinical hypothyroidism on the secretion of L-cells. GLP-1 reference values are well established in healthy populations and at various glucose tolerances;[9,10] however, the correlation of GLP-1 and GLP-2 levels with thyroid dysfunction remains uncertain. Therefore,

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we hypothesized that subclinical hypothyroidism or a euthyroid state in the presence of HT may influence circulating GLP-1 and GLP-2 concentrations. The aim of this study was to examine the changes in the two circulating peptide levels in HT patients with subclinical hypothyroidism or euthyroid patients. The study also aimed to investigate the associations of the two peptides with thyroid dysfunction and thyroid autoantibodies.

MATERIALS AND METHODS

Participant recruitment

This controlled study was performed at the endocrinology center of a medical college-affiliated hospital from May 2013 to January 2014. The study was approved by the Ethics Committee of the hospital. Informed consent was obtained from all participants.

At study entry, the participants underwent clinical evaluations. Twenty-four consecutive patients with subclinical hypothyroidism, 24 euthyroid patients, and 24 age- and gender-matched healthy controls [Table 1] participated in the study. The patients all suffered from HT without infiltrative ophthalmopathy. In addition, the patients with subclinical hypothyroidism and the euthyroid patients were newly diagnosed on the basis of serum thyrotropin (thyroid-stimulating hormone [TSH]), free thyroxine (FT4), free triiodothyronine (FT3), and thyroid peroxidase antibody (TPOAb) levels. None of the patients had received previous treatment for thyroid dysfunction.

We excluded patients with autoimmune disorders (with the exception of thyroid autoimmune disorders) and those suffering from chronic renal failure, congestive heart failure, hepatic insufficiency, inflammatory conditions, ischemic events, and diabetes. Both glycated hemoglobin A1c ≥6.5% and fasting plasma glucose ≥7.0 mmol/L were used to exclude diabetes.[11,12] Controls without clinical evidence of a major disease underwent a routine medical check-up.

Biochemical measurements

After a 10-h fast, blood samples were drawn from the subjects by venipuncture into Vacutainer tubes. The levels of serum thyroid hormone and autoantibodies were determined by electrochemiluminescence immunoassays (Roche Corporation, USA). The following reference ranges were used: TSH 0.34-5.44 mIU/mL, FT3 2.92-5.93 pmol/L, FT4 7.91-20.59 pmol/L, anti-thyroglobulin antibody (TgAb) 0-34.00 IU/mL, and anti-TPOAb 0-12.00 IU/mL. Thyroid ultrasound was performed for the hypothyroid and hyperthyroid patients.

The blood samples were stored at −80°C until analysis. High-sensitivity GLP-1 and GLP-2 active chemiluminescent ELISA kits (EMD Millipore Corporation, USA) were used for nonradioactive quantification in serum. The laboratory tests were measured by routine clinical assays in the hospital laboratory. HOMA-IR = fasting plasma insulin (U/L) ×plasma glucose (mmol/L)/22.5.[13]

Table 1: The demographic and clinical characteristics of the participants

Variables	Controls (*n* = 24)	Euthyroid (*n* = 24)	Subclinical hypothyroidism (*n* = 24)
Female/male (*n*/*n*)	23/1	21/3	22/2
Age (years)	34±10	36±13	38±12
BMI (kg/m^2)	20.59±5.47	22.96±2.49	23.82±2.20
SBP (mmHg)	116±12	122±11	125±10
DBP (mmHg)	71±10	81±7	85±6*
TSH (mIU/mL)	2.35 (1.28-3.83)	2.75 (0.69-5.32)	13.70**§§ (5.68-100.00)
FT3 (pmol/L)	4.99±0.67	4.89±0.93	4.27±0.99*§
FT4 (pmol/L)	16.16±2.14	15.66±2.70	11.10±3.74**§§
TgAb	24.98 (10.00-65.18)	323.45** (10.00-4000.00)	578.4**§§ (50.00-4000.00)
TPOAb	11.2 (2.84-26.41)	229.2** (42.14-800.00)	418.65**§§ (125.90-1000.00)
Plasma glucose (mmol/L)	4.62±0.34	4.82±0.39	4.95±0.45§
Glycated albumin (mmol/L)	1.96±0.12	2.02±0.25	1.99±0.16
Triglyceride (mmol/L)	0.80 (0.42-1.91)	0.88 (0.55-2.75)	1.02* (0.67-4.38)
Cholesterol (mmol/L)	4.38±0.70	4.54±1.13	4.89±1.18
Lg (insulin [U/mL])	1.06±0.24	1.11±0.24	1.25±0.28
Lg (HOMR-IR)	2.43±0.54	2.55±0.71	2.74±0.59^{D}
Lg (GLP-1 [pmol/L])	1.06±0.16	1.16±0.19	1.22±0.23*
GLP-2 (ng/mL)	9.25±1.67	9.24±1.33	10.05±1.98

Comparison to control group: *P < 0.05; **P < 0.001; comparison to euthyroid group: $^{\S}P$ < 0.05, $^{\S\S}P$ < 0.001; ^{D}P = 0.052. Data shown are the real case numbers or the median (minimum-maximum) of each group. The normal clinical range for TSH: 0.27-4.2 mIU/mL; FT4: 12.0-22.0 pg/mL; FT3: 3.1-8.6 pg/mL; TPOAb: <35.0 IU/mL; TgAb: <115.0 IU/mL. BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; TSH = Thyroid-stimulating hormone; FT3 = Free triiodothyronine; FT4 = Free thyroxine; TgAb = Anti-thyroglobulin antibody; TPOAb = Thyroid peroxidase antibody; HOMR-IR = Homeostasis model assessment: Insulin resistance; GLP = Glucagon-like peptide

Statistical analyses

Quantitative data are presented as the mean ± standard deviation or as the median when appropriate. Parameters with nonnormal distributions were log-transformed and were statistically analyzed using the log-transformed values. Statistical analyses were conducted with the SPSS 18.0 package for Windows (SPSS Inc., Chicago, IL, USA). A pair-wise comparison among the three groups was conducted using ANOVA, followed by the *post-hoc* Tukey or Kruskal-Wallis nonparametric test. Mann-Whitney U analysis was used for nonparametric comparisons of the two subgroups. Chi-squared tests were utilized to compare the other clinical features. The correlations were examined using Pearson's correlation analysis. A two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the subjects are shown in Table 1. A total of 72 cases were included in the study. With respect to age and gender, there were no significant differences, as shown in Table 1. As expected, thyroid antibodies (TgAb and TPOAb) were significantly higher in the patients with HT than in the controls (all $P < 0.001$). The thyroid function variables and diastolic blood pressure were significantly altered in the patients with subclinical hypothyroidism when compared with the controls ($P < 0.05$ or $P < 0.001$). Regarding the thyroid function variables, there was no difference between the euthyroid HT patients and the controls.

As shown in Table 1, the mean Lg GLP-1 (pmol/L) level was 1.06 ± 0.16 pmol/L in the control group and 1.22 ± 0.23 pmol/L in the subclinical hypothyroidism group ($P = 0.024$). In addition, the levels of plasma glucose and triglyceride were significantly higher in the subclinical hypothyroidism group than in the control group (all $P < 0.05$). Unfortunately, homeostasis model assessment: insulin resistance showed only an insignificant trend toward significance between the subclinical hypothyroidism and control groups ($P = 0.052$). No differences in the levels of cholesterol, glycated albumin, GLP-2, or insulin were observed among the three groups. GLP-1 levels were positively correlated with GLP-2 levels in patients with subclinical hypothyroidism ($r = 0.431$, $P = 0.035$). No other clinical correlations were observed within the two patient groups.

DISCUSSION

The present study demonstrated that the changes in GLP-1 and GLP-2 levels in HT patients with subclinical hypothyroidism or in a euthyroid state. The levels of GLP-1, not GLP-2, were significantly elevated in the subclinical hypothyroidism patients. Although the exact mechanism behind this elevation is unclear, it can be concluded that the most significant factor influencing fasting GLP-1 levels might be clinical thyroid status. Subclinical hypothyroid status was associated with increased GLP-1 secretion or impairment of insulin sensitivity and glycol metabolism. The available data in support of this study showed that the plasma glucose and triglyceride levels were increased in patients with subclinical hypothyroidism. Furthermore, insulin resistance was increased in patients with subclinical hypothyroidism, which is in accordance with the existing literature.[7]

The incremental levels of GLP-1 and GLP-2 were increased after food intake. An inconsistent or inverse relationship between GLP-1 and GLP-2 secretion and insulin sensitivity has been reported.[4,14] In normal subjects, GLP-1 and glucose-dependent insulinotropic polypeptide are responsible for 70% of the insulin response during a meal, but in diabetic subjects and those with other insulin-resistant conditions, the incretin effect is impaired.[15,16] Hypothyroidism is associated with insulin resistance, and the pathophysiologic mechanisms underlying hypothyroidism resemble those of type 2 diabetes; therefore, patients with hypothyroidism may have alterations in the incretin hormone response. Thus, the incremental secretion of GLP-1 in the fasting state may compensate in subclinical hypothyroidism for insulin resistance. The elevated GLP-1 levels in patients with subclinical hypothyroidism were not correlated with the FT3 and FT4 levels. The factors influencing GLP-1 and GLP-1 secretion may be clustered, including increased thyroid hormone production, oxidative stress, and rates of nutrient metabolism. The pathology of subclinical hypothyroidism may also include amplified activity of the enteroinsular axis. Thus, a correlation between GLP-1 and GLP-2 levels was observed in subclinical hypothyroidism.

Thyroid hormone withdrawal altered the lipid concentrations significantly, in a short period of time. The levels of both low-density lipoprotein-cholesterol and high density lipoprotein-cholesterol particles increased concurrently.[17] In the present study, the euthyroid patients showed no metabolic alteration, including in GLP-1 or GLP-2 levels. However, the clinical significance and potential hormonal effects of these alterations remain to be clarified, particularly in patients with subclinical hypothyroidism. We have previously reported that euthyroid status was important for maintaining glucose metabolism and insulin sensitivity. This conclusion aligned with our hypotheses, just as some changes in erythrocyte deformability features improved during L-thyroxine treatment.[18]

In summary, our study showed that circulating GLP-1 levels were elevated in patients with subclinical hypothyroidism, whereas GLP-2 levels remained unaltered. The GLP-1

levels in subclinical hypothyroidism should be interpreted with caution in relation to the deterioration of glucolipid metabolism. Further studies are focusing on the role and effect of GLP-1 and GLP-2 in ameliorating metabolic disorders in patients with thyroid dysfunction are needed.

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AUTHOR'S CONTRIBUTION

SM contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. YJ, HL, JC and KZ contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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