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Serum Levels of Follistatin Are Positively Associated With Serum-Free Thyroxine Levels in Patients With Hyperthyroidism or Euthyroidism

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Abstract: Follistatin is a glycoprotein with various biologic functions that plays a role in adipocyte differentiation, muscle stimulation, anti-inflammation, and energy homeostasis. Thyroid hormones influence energy expenditure, glucose, and lipid metabolism. The association between serum follistatin level and thyroid function statuses has seldom been evaluated.

The objectives of this study were to compare serum follistatin concentrations in different thyroid function statuses and to evaluate the associations between serum follistatin and free thyroxine (fT4) levels.

In this study, 30 patients with hyperthyroidism (HY group) and 30 euthyroid individuals (EU group) were recruited. The patients of HY group were treated with antithyroid regimens as clinically indicated, whereas no medication was given to EU group. The demographic and anthropometric characteristics, biochemical data, serum levels of follistatin, and thyroid function of both groups at baseline and at the 6th month were compared. Data of all patients were pooled for the analysis of the associations between the levels of follistatin and fT4.

At baseline, the HY group had significantly higher serum follistatin levels than the EU group (median [Q1, Q3]: 1.81 [1.33, 2.78] vs 1.13 [0.39, 1.45] ng/mL, $P < 0.001$). When treated with antithyroid regimens, the follistatin serum levels in HY group decreased to 1.54 [1.00, 1.88] ng/mL at the 6th month. In all patients, the serum levels of follistatin were positively associated with fT4 levels at baseline ($\beta = 0.54$, $P = 0.005$) and at the 6th month ($\beta = 0.59$, $P < 0.001$). The association between follistatin and fT4 levels remained significant in the stepwise multivariate regression analysis, both initially and at the 6th month.

In comparison to the EU group, patients with hyperthyroidism had higher serum follistatin levels, which decreased after receiving antithyroid treatment. In addition, the serum follistatin concentrations were

positively associated with serum fT4 levels in patients with hyperthyroidism or euthyroidism.

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Abbreviations: ALT = alanine transaminase, AST = aspartate transaminase, BH = body height, BMI = body mass index, BW = body weight, Cre = creatinine, FPG = fasting plasma glucose, fT4 = free thyroxine, HDL-C = high-density lipoprotein-cholesterol, LDL-C = low density lipoprotein-cholesterol, logTSH = log transformation of thyroid stimulating hormone, T-C = total cholesterol, TG = triglycerides, TRAb = TSH receptor autoantibody, TSH = thyroid-stimulating hormone.

INTRODUCTION

Follistatin was first described as a follicle-stimulating hormone (FSH) inhibiting substance in ovarian follicular fluid in 1987.¹ As a glycoprotein with high affinity for the transforming growth factor-beta family, follistatin was initially found to suppress FSH secretion and inhibit activin A action.¹⁻³ Recent studies reveal that follistatin is widely expressed in human tissues, such as in the pituitary gland, placenta, ovaries, testes, brain, skeletal muscle, kidney, liver, pancreas, bone, heart, blood vessels, adrenal gland, and thyroid gland.¹ Nowadays, follistatin is considered as a multifunctional regulatory protein.¹ In the gonads, follistatin presents embryotropic actions by mediating oocyte competence and embryo developmental progression.⁴ In the pituitary gland, the local actions of follistatin contribute to the mechanism that generate the cyclic variations of FSH production during the estrous cycle.⁵ Daponte et al reported that activin A and follistatin could be considered as biomarkers for ectopic pregnancy and missed abortion.⁶ Follistatin has also been reported as a potent regulator of bone metabolism.⁷ Recent evidence further suggests a role for follistatin in regulating inflammation, fibrosis, and tissue repair.⁸⁻¹⁰ Effects of follistatin on metabolic parameters have been illustrated in literatures.¹¹⁻¹⁵ Follistatin promotes adipocyte differentiation, browning, and energy metabolism.^{11,12} Plasma follistatin is elevated in patients with polycystic ovary syndrome¹³ or in patients with type 2 diabetes.¹⁴ Systemic administration of follistatin increases body weight and lean muscle mass in normal mice.¹⁵ Teede et al reported significant relationships between follistatin and cardiovascular risk factors including lipids and markers of adiposity.¹³ Further, recent reports suggest that follistatin may regulate processes during tumor progression such as cell apoptosis,¹⁶ angiogenesis,¹⁷ or metastasis.¹⁸ Thus, serum follistatin level potentially acts as a marker for ovarian cancer diagnosis,¹⁹ hepatocellular carcinoma prognosis,²⁰ and prostate cancer progression.²¹ Due to its widespread biologic functions, an engineered human follistatin

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variant has been considered as a novel molecule with broad therapeutic potential.²²

Changes in thyroid function are associated with changes in energy metabolism and body weight.^{23–25} Thyroid disorders potentially interact with adipocyte–myocyte crosstalk²⁶ and may affect endocrine products of adipose tissue.²⁴ Patients with hyperthyroidism had increased skeletal muscle metabolism and lipid oxidation rates.²⁷ Both hypothyroidism and hyperthyroidism affect glucose and insulin metabolism.²⁸ Patients with hyperthyroidism have a lower insulin-stimulated glucose transport than euthyroid subjects.²⁹ In patients with hyperthyroidism, the abnormal glucose metabolism and increased bone turnover normalized after antithyroid therapy.³⁰

Both follistatin and thyroid hormones levels have been reported to be associated with body weight change, energy metabolism, insulin resistance, and bone turnover.^{7,11–15,23–30} However, serum follistatin levels in patients with different thyroid function statuses have never been discussed in literatures. In this study, we compared serum follistatin concentrations in patients with hyperthyroidism and euthyroidism, and analyzed the associations between serum follistatin and free thyroxine (fT4) levels.

SUBJECTS AND METHODS

Subjects

This prospective observational study was approved by the research ethics committee at the National Taiwan University Hospital (NTUH) in accordance with the Declaration of Helsinki. The institutional review board (IRB) number for this study was 201005085R. First-visit patients to the endocrinology clinics at NTUH during 2010 and 2011 were recruited. Subjects with history of thyroid disorders or other comorbidities and those who were taking medications were excluded from the study. Consent was obtained from each of the 62 patients after providing a full explanation of the purpose, nature, and procedures of the study.

Data Collection

Sex and age of the enrolled patients were recorded. Trained staff measured patients' height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg). The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (m^2).

Blood samples were drawn with minimal trauma from an antecubital vein in the morning after a 12-h overnight fast. Levels of fasting plasma glucose (FPG), creatinine (Cre), aspartate transaminase (AST), and alanine transaminase (ALT) were measured using the Olympus AU series 680 (Beckman Coulter, Nyon, Switzerland). FPG was measured with the hexokinase method, whereas Cre, AST, and ALT were measured with the colorimetric method. Serum total cholesterol (T-C), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured by using the Olympus AU series 5800 with the cholesterol oxidase phenol 4-aminoantipyrine peroxidase method, glycerophosphate oxidase-phenol aminophenazone method, accelerator selective detergent, and liquid selective detergent, respectively (Beckman Coulter, Nyon, Switzerland). Thyroid-stimulating hormone (TSH) and fT4 levels were measured with the Siemens DPC Immulite 2000 (Siemens, Erlangen, Germany). Thyroid function values outside the laboratory measurement range (fT4 level > 5.4 ng/dL or

TSH level < 0.004 μ IU/mL) were recorded as an fT4 level = 5.4 ng/dL or a TSH level = 0.004 μ IU/mL. Serum follistatin concentrations were determined by an enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN). TSH-receptor antibody (TRAb) levels were determined by using the radioimmunoassay method (TSH receptor autoantibody coated tube kit, RSR, Cardiff, United Kingdom). A percentage inhibition of TSH binding $< 10\%$ was recorded as negative; 10% to 15% as borderline positive; and $> 15\%$ as positive. All assays were performed in accordance with the manufacturer's specifications.

Thyroid Ultrasound

All of the participants received a thyroid ultrasonographic examination at baseline. An endocrine specialist performed the sonographic examination with the use of the Toshiba Aplio Ultrasound System (SSA-790) with a PLT-805AT probe. Aspiration cytological examination was performed as clinically indicated. None of the recruited patients had lesions suspicious for malignancy.

Patient Groups, Treatment, and Follow-Up

The reference ranges for fT4 and TSH levels used in our hospital were 0.6–1.75 ng/dL and 0.1–4.5 μ IU/mL, respectively. Hyperthyroidism was defined as an fT4 level > 1.75 ng/dL and a TSH level < 0.1 μ IU/mL. Subclinical hyperthyroidism was defined as a TSH level < 0.1 μ IU/mL with an fT4 level within the reference range. Euthyroidism was defined as both fT4 and TSH levels within their reference ranges. Subclinical hypothyroidism was defined as a TSH level > 4.5 μ IU/mL with an fT4 level within the reference range. Hypothyroidism was defined as a TSH level > 4.5 μ IU/mL with an fT4 level < 0.6 ng/dL.

Thirty patients were diagnosed with hyperthyroidism (HY group). All of them had positive TRAb. Sonograms of the HY group patients revealed characteristics (hypoechoic and diffuse enlargement) compatible with autoimmune thyroiditis. The patients with hyperthyroidism were treated with antithyroid regimens (10 mg of carbimazole or 100 mg of propylthiouracil 3 times daily). They were followed up every 2 months. The dosages of the antithyroid drugs were titrated according to their clinical conditions. Follow-up laboratory data was obtained at the 6th month.

Thirty patients were classified as euthyroid (EU group). They all had negative examination results for TRAb. They were kept on follow-up without medications. Follow-up laboratory data was obtained at the 6th month.

One patient had subclinical hypothyroidism, and 1 patient had overt hypothyroidism. They (HO group) were treated with thyroxine supplementation with dose titration to attain euthyroidism. Follow-up laboratory data was obtained at the 6th month.

Statistical Analyses

This study enrolled 30 patients with hyperthyroidism (HY group), 30 patients with euthyroidism (EU group), and 2 patients with overt/subclinical hypothyroidism (HO group). The first part of our analysis was to compare the initial data between the HY group and the EU group. Due to a small sample size, we used a nonparametric method for the statistical analysis. The data for the numerical variables were presented as median values (Q1, Q3). The Mann–Whitney *U* test was used for comparisons of numerical variables between the HY group

and the EU group. Data of the 2 patients with overt/subclinical hypothyroidism were described but not included in the analysis. Categorical data were expressed as percentages. Proportions and categorical variables were tested with the use of the Fisher exact test.

Second, we compared the follow-up data of the HY group and EU group at the 6th month. The numerical variable data was presented as median values (Q1, Q3). The Mann–Whitney *U* test was used for comparisons of numerical variables between the 2 patient groups. Categorical data were expressed as percentages. Proportions and categorical variables were tested with the use of the Fisher exact test.

Third, we evaluated the possible predictive factors of serum follistatin levels. We hypothesized that serum follistatin levels varied between the different statuses of thyroid function. We also hypothesized a positive association between ft4 and follistatin levels. The initial data of the HY group and the EU group were pooled together to analyze the possible associations between serum follistatin levels and other variables. The predictive effects of demographic, anthropometric, or laboratory parameters (sex, age, BH, BW, BMI, FPG, Cre, AST, ALT, T-C, TG, HDL-C, LDL-C, and levels of ft4, TSH) for follistatin concentrations were evaluated by performing a linear regression analysis and were further tested by performing stepwise forward multivariate regression. TSH levels were converted to log form by logTSH calculation. The predictive effect of logTSH for follistatin concentration was also calculated. In a stepwise forward multivariate regression, variables with $P < 0.15$ remained in the model. Only variables with P values < 0.05 were considered as statistically significant.

Fourth, follow-up data collected at the 6th month was used for validating our hypothesis concerning the associations between ft4 and follistatin levels. The data from the HY group and the EU group at the 6th month were pooled together. The predictive effects of demographic, anthropometric, or laboratory parameters at the 6th month for follistatin concentrations were evaluated by performing a linear regression analysis and were further tested by performing a stepwise forward multivariate regression.

Using the Kolmogorov–Smirnov test, we assessed the normality of all the models; none of the models exhibited any collinearity problems. All analysis was performed using SAS version 9.1 statistical package for Windows (SAS, Cary, NC).

RESULTS

The anthropometric and laboratory characteristics of the HY group and the EU group are shown in Table 1. At the first visit, the HY group had higher ft4, AST, and ALT levels, but lower TSH, BMI, Cre, T-C, and LDL-C levels than the EU group. The HY group had higher follistatin levels than the EU group (1.81 [1.33, 2.78] ng/mL vs 1.13 [0.39, 1.45] ng/mL, $P < 0.001$) (Table 1, a vs c).

In the HY group, ft4 levels declined where TSH levels elevated significantly after the antithyroid treatment (Table 1). Among the HY group, 4 (13.3%) remained hyperthyroid, 15 (50%) patients reverted to subclinical hyperthyroidism, 10 (33.3%) patients returned to euthyroid status, whereas 1 (3.3%) changed to hypothyroid status at the 6th month. All patients of the EU group remained at euthyroid status at the 6th month. The HY group had higher ft4 (1.14 [0.89, 1.58] vs 0.96 [0.86, 1.06] ng/dL, $P = 0.02$), ALT (23.5 [18.0, 27.0] vs 15.0 [13.0, 20.0] U/L, $P = 0.003$) than the EU group at the 6th month.

The follistatin levels of the HY group declined after antithyroid regimens. However, the follistatin levels of the HY patient group at the 6th month was still higher than that of the EU group (1.54 [1.00, 1.88] vs 1.16 [0.58, 1.46] ng/mL, $P = 0.01$). The anthropometric parameters, FPG, Cre, AST, lipid profiles, and TSH levels were not statistically different between the HY and the EU group at the 6th month (Table 1, b vs d).

At baseline, univariate linear regression analysis revealed that the levels of follistatin were positively associated with ft4 ($\beta = 0.54$, $P = 0.005$) and ALT ($\beta = 0.03$, $P = 0.04$) and negatively associated with TSH ($\beta = -0.65$, $P = 0.04$) or logTSH ($\beta = -0.63$, $P = 0.005$). At the 6th month, ft4 levels ($\beta = 0.59$, $P < 0.001$), AST ($\beta = 0.06$, $P = 0.002$), and ALT ($\beta = 0.03$, $P = 0.001$) had positive associations with the levels of follistatin (Table 2). The association between levels of follistatin and TSH was not significant at the 6th month. The negative association between logTSH and serum follistatin levels remained significant ($\beta = -0.24$, $P = 0.008$) at the 6th month.

At baseline, stepwise multivariate regression analysis in all subjects revealed that the levels of ft4 ($\beta = 0.60$, $P = 0.005$) were significantly associated with follistatin concentrations. At the 6th month, ft4 ($\beta = 0.62$, $P < 0.001$) and gender (female vs male, $\beta = -0.57$, $P = 0.02$) were significantly associated with the concentration of follistatin (Table 3).

DISCUSSION

Follistatin is a multifunctional regulatory protein.¹ In normal adults, the serum follistatin level increase gradually with age.³¹ More specifically, mean follistatin levels do not change during puberty but are higher in adult and postmenopausal women.³² Moreover, serum follistatin levels are markedly elevated during pregnancy.³³ Previous studies have also reported that serum follistatin concentrations are significantly elevated in patients with chronic liver disease, chronic renal failure, advanced solid cancer, hematological malignancies, or septicemia.^{33,34} However, serum follistatin levels in patients with different thyroid function statuses have never been discussed in the literature. Our study is the first to investigate the effect of different statuses of thyroid function on follistatin levels. Our analysis revealed that the HY group had significantly higher serum follistatin levels than the EU group. In the HY group, follistatin levels decreased after the administration of the antithyroid regimens. Our analysis also revealed that levels of follistatin were positively associated with serum ft4 levels, both initially and at the 6th month. Due to a small patient number, we did not recruit patients with hypothyroidism in our analysis. The follistatin levels of the 2 patients with subclinical/overt hypothyroidism increased after levo-thyroxine supplement (0.738–1.858 ng/mL and 0.892–1.383 ng/mL, respectively). Those data were comparable with our findings that serum follistatin levels are positively associated with ft4 levels.

Patients with hyperthyroidism usually present with insulin resistance and an increased basal metabolic rate, energy expenditure, and rate of lipolysis.^{23,24,28,29} In this study, the HY group initially had a lower BMI, T-C and LDL-C levels than the EU group. These findings are comparable to those reported by previous studies.^{25,30,35} The differences in BMI, T-C, and LDL-C levels between the HY and EU groups became insignificant at the 6th month. Thyroid hormones may influence renal hemodynamics, glomerular filtration rate, and sodium and water homeostasis.^{36,37} Patients with hyperthyroidism usually have an increase in their glomerular filtration rate and reduced serum Cre levels.^{37,38} In this study, the HY group had lower

TABLE 1. Characteristics of Subjects With Hyperthyroidism or Euthyroidism

	Hyperthyroidism (N = 30)		Euthyroidism (N = 30)		P*
	Initial ^a	The Sixth Month ^b	Initial ^c	The Sixth Month ^d	
Male: Female	9: 21		4: 26		
Age (year)	37 (29, 43)		43 (32, 52)		0.21
BH (cm)	161 (158, 170)		160 (157, 165)		0.11
BW (kg)	56.6 (49.8, 61.0)	57.5 (54.0, 67.1)	60.5 (55.0, 67.0)	60.0 (55.0, 67.0)	0.27
BMI	21.7 (19.5, 23.2)	22.7 (20.3, 23.9)	23.1 (21.2, 26.0)	23.0 (21.8, 26.0)	0.15
Cre (mg/dL)	0.6 (0.5, 0.8)	0.7 (0.6, 0.9)	0.7 (0.7, 0.8)	0.8 (0.7, 0.9)	0.02†
AST (U/L)	26.5 (22.0, 32.0)	21.5 (17.0, 24.0)	18.5 (17.0, 22.0)	19.0 (16.0, 22.0)	<0.001†
ALT (U/L)	35.5 (28.0, 49.0)	23.5 (18.0, 27.0)	14.5 (12.0, 18.0)	15.0 (13.0, 20.0)	<0.001†
FT4 (ng/dL)	3.19 (2.22, 3.92)	1.14 (0.89, 1.58)	0.99 (0.87, 1.06)	0.96 (0.86, 1.06)	<0.001†
TSH (μIU/mL)	0.004 (0.004, 0.006)	0.006 (0.004, 1.450)	1.105 (0.651, 1.370)	0.904 (0.490, 1.420)	<0.001†
FPG (mg/dL)	88.5 (82, 93)	88 (79, 95)	86 (79, 89)	84.5 (79, 89)	0.05
T-C (mg/dL)	146.5 (121, 171)	181.5 (158, 207)	194 (182, 228)	191 (178, 227)	<0.001†
TG (mg/dL)	80 (60, 100)	86.5 (69, 103)	79.5 (61, 125)	92 (69, 154)	0.62
HDL-C (mg/dL)	49 (40, 58)	53 (47, 63)	56 (47, 65)	51 (43, 69)	0.05
LDL-C (mg/dL)	82.8 (66, 99.8)	110.2 (88.0, 130.0)	129 (105.4, 140.6)	107.4 (89.0, 136.4)	<0.001†
Follistatin (ng/mL)	1.81 (1.33, 2.78)	1.54 (1.00, 1.88)	1.13 (0.39, 1.45)	1.16 (0.58, 1.46)	<0.001†

Numerical data were presented as median (Q1, Q3).

ALT = alanine transaminase, AST = aspartate transaminase, BH = body height, BMI = body mass index, BW = body weight, Cre = creatinine, FPG = fasting plasma glucose, FT4 = free thyroxine, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, T-C = total cholesterol, TG = triglyceride, TSH = thyroid stimulating hormone.

^aHyperthyroid patients, initial data.

^bHyperthyroid patients, data at the sixth month.

^cEuthyroid patients, initial data.

^dEuthyroid patients, data at the sixth month.

* Fisher exact test for comparisons of categorical variables between hyperthyroid and euthyroid patients. Mann-Whitney U tests for comparisons of numerical variables between hyperthyroid and euthyroid patients.

† P < 0.05.

TABLE 2. Univariate Regression Model With Concentrations of Follistatin as Dependent Variables, and Demographic, Anthropometric, and Laboratory Parameters as Independent Variables in All Subjects (N = 60)

Independent Variable	Follistatin (0)		Follistatin (6)	
	β(95% CI)	P	β(95% CI)	P
Sex	-0.11 (-1.41, 1.19)	0.86	-0.32 (-0.83, 0.19)	0.21
Age	-0.03 (-0.07, 0.02)	0.26	0.00 (-0.02, 0.02)	0.73
BH	0.00 (-0.07, 0.07)	0.92	0.01 (-0.02, 0.04)	0.47
BW	-0.00 (-0.06, 0.06)	0.93	0.01 (-0.01, 0.04)	0.34
BMI	-0.02 (-0.20, 0.17)	0.86	0.01 (-0.07, 0.09)	0.79
FT4	0.54 (0.17, 0.90)	0.005*	0.59 (0.34, 0.85)	<0.001*
TSH	-0.65 (-1.25, -0.04)	0.04*	-0.01 (-0.04, 0.02)	0.50
LogTSH	-0.63 (-0.15, -0.21)	0.005*	-0.24 (-0.41, -0.64)	0.008*
FPG	-0.04 (-0.09, 0.02)	0.22	0.01 (-0.00, 0.03)	0.15
Cre	-3.03 (-6.35, 0.29)	0.07	-0.24 (-1.62, 1.14)	0.73
AST	0.06 (-0.00, 0.13)	0.06	0.06 (0.02, 0.09)	0.002*
ALT	0.03 (0.00, 0.06)	0.04*	0.03 (0.01, 0.04)	0.001*
T-C	-0.01 (-0.02, 0.00)	0.17	0.00 (-0.01, 0.00)	0.28
TG	-0.00 (-0.01, 0.01)	0.82	0.00 (-0.00, 0.01)	0.63
HDL-C	-0.02 (-0.05, 0.02)	0.26	0.00 (-0.01, 0.01)	0.88
LDL-C	-0.01 (-0.02, 0.01)	0.21	-0.01 (-0.01, 0.00)	0.16

95% CI: 95% confidence interval.

Follistatin (0): levels of follistatin at baseline.

Follistatin (6): levels of follistatin at the 6th month.

For follistatin (0): sex, age, and anthropometric and laboratory data at baseline were used as independent variables.

For follistatin (6): sex, age, and anthropometric and laboratory data at the 6th month were used as independent variables.

ALT = alanine transaminase, AST = aspartate transaminase, BH = body height, BMI = body mass index, BW = body weight, Cre = creatinine, FPG = fasting plasma glucose, FT4 = free thyroxine, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, LogTSH: log transformation of TSH levels, Sex = female vs male, T-C = total cholesterol, TG = triglyceride, TSH = thyroid-stimulating hormone. linear regression, *P* < 0.05.

serum Cre levels than the EU group, where this difference became insignificant at the 6th month. Thyroid hormones can also regulate the basal metabolic rate of hepatocytes and affect hepatic function.^{39,40} In the setting of hyperthyroidism, the prevalence of liver test abnormalities ranged from 15% to 76%.⁴⁰⁻⁴³ In this study, the HY group had higher AST and

ALT levels than the EU group. The difference in AST levels between the 2 patient groups became insignificant at the 6th month, whereas the HY group still had higher ALT levels than EU patients at this time.

In healthy individuals, the circulating concentrations of TSH and thyroxine are tightly regulated.⁴⁴ Small changes in

TABLE 3. Forward Stepwise Regression Models in All Subjects (N = 60) With Levels of Follistatin as Dependent Variables, and Sex, Age, Anthropometric and Laboratory Parameters as Independent Variables

Dependent Variables	Independent Variables	Parameter Estimate	Standard Error	Partial R ²	Model R ²	F Value	P
Follistatin (0)	ft4 (0)	0.60	0.18	0.15	0.15	8.52	0.005
	FPG (0)	-0.05	0.03	0.06	0.21	3.67	0.06
Follistatin (6)	ft4 (6)	0.62	0.13	0.29	0.29	19.78	<0.001
	Sex	-0.57	0.23	0.08	0.37	6.19	0.02

Forward stepwise regression analysis, variables left in the models are significant at the levels of 0.15.

Follistatin (0): levels of follistatin at baseline.

Follistatin (6): levels of follistatin at the 6th month.

ft4 (0): ft4 at baseline.

ft4 (6): ft4 at the 6th month.

FPG (0): fasting plasma glucose at baseline.

Sex: female vs male.

For follistatin (0): sex, age, and anthropometric and laboratory data at baseline were used as independent variables.

For follistatin (6): sex, age, and anthropometric and laboratory data at the 6th month were used as independent variables.

FPG = fasting plasma glucose, ft4 = free thyroxine.

circulating thyroxine concentrations can result in large relative changes in TSH.⁴⁴ Spencer et al reported an inverse linear relationship between the logTSH and the fT4 index.⁴⁵ In contrast, large population studies reported that the relationship between TSH and fT4 is complex and nonlinear.^{44,46,47} Of note, the relationship between TSH and fT4 differs by age and sex.⁴⁴ In this study, the serum follistatin level was negatively associated with the TSH level initially, but the association between the levels of follistatin and TSH became insignificant at the 6th month. Instead, the negative association between serum follistatin and logTSH occurred initially and at the 6th month. TSH had effects on thermogenesis in rat brown adipocytes.⁴⁸ Whether TSH has a direct influence on follistatin levels warrants future study.

The interactions between follistatin and the liver have been discussed in the literature.^{49–60} Zhang et al reported that the expression of mRNA for follistatin is positively controlled by activin A, tumor transforming growth factor-beta, and other hormones or neurotransmitters in the rat hepatocyte.⁴⁹ Elevations in follistatin levels have been reported in patients with chronic liver disease,³³ acute liver failure,^{50,51} nonalcoholic fatty liver disease,⁵² and hepatocellular carcinoma.^{20,53} Follistatin may regulate the immune system, as well as liver biology and pathophysiology.⁵⁴ Patella et al reported that follistatin could attenuate early events in fibrogenesis by constraining hepatic stellate cell proliferation and inhibiting hepatocyte apoptosis.⁵⁵ After massive hepatectomy, follistatin markedly accelerated liver regeneration but reduced the function of the remnant liver.⁵⁶ Animal studies demonstrated that follistatin expression is necessary for the proliferation of small hepatocytes.⁵⁷ Kanamoto et al reported that follistatin had a beneficial role after hepatic ischemia-reperfusion injuries in rats.⁵⁸ Patients with chronic hepatitis C had decreased follistatin levels.⁵⁹ Ashshi et al reported that thyroid disorders were common during the treatment of chronic hepatitis C of genotypes 1 and 4.⁶⁰ Activin-A and/or follistatin could be involved in the induction/aggravation of TSH receptor antibodies.⁶⁰ In our study, the HY group initially had higher AST, ALT, and follistatin levels than the EU group. At the 6th month, the HY group still had higher ALT and follistatin levels than the EU group. The follistatin levels were positively correlated with fT4 and ALT levels initially. At the 6th month, the levels of follistatin were positively associated with fT4, AST, and ALT levels. The underlying pathogenesis between the interactions of thyroid, liver, and follistatin remains to be investigated.

In our univariate regression analysis, gender was not significantly associated with serum follistatin levels, both initially and at the 6th month. In forward stepwise multivariate regression analysis, the fT4 levels acted as a significant predictor for initial follistatin levels. At the 6th month, fT4 levels and gender acted as significant predictors for the serum level of follistatin in the stepwise regression analysis. Kettle et al reported that men have lower follistatin levels than normal cycling or postmenopausal women.³² Refaat et al conducted a study in patients with chronic hepatitis C and reported that follistatin levels were significantly higher in males than in females.⁵⁹ It is important to note that we had more number of female than male patients in this study and gender had no association with the levels of follistatin in the univariate regression analysis. The true impact of gender on the levels of follistatin remains to be clarified in future studies.

Our study is the first report to demonstrate that thyroid function status may affect follistatin levels. However, it has

several limitations. First, this is an observational study. The study subjects were enrolled through Endocrinology clinics. In this study, we did not recruit volunteers who were euthyroid and had no goiter as control group. The hypothesis of our study is that thyroid function status may influence serum follistatin levels. Patients with hyperthyroidism usually have thyroid enlargement. To enroll euthyroid subjects with goiter as comparative group may minimize the possible influencing effect of thyroid size. Furthermore, the sample size was small. We analyzed data from patients with hyperthyroidism or euthyroidism, wherein the data of 2 patients with hypothyroidism were described but not included in the analysis. Thus, the association between fT4 and follistatin levels in the whole thyroid function spectrum remains to be investigated. Second, thyroid function values outside the laboratory measurement range (fT4 level > 5.4 ng/dL or TSH level < 0.004 μ IU/mL) were recorded as an fT4 level = 5.4 ng/dL or a TSH level = 0.004 μ IU/mL, and therefore the true effects of advanced thyrotoxicosis on follistatin levels would be biased. Third, the effects of antithyroid medications, not changes in thyroid function, on follistatin levels were not investigated in this study. Fourth, severity, duration, and treatment response varied in the patients with hyperthyroidism. We collected initial and follow-up data at the 6th month. The serial change of follistatin levels in a shorter follow-up period during the treatment course was not evaluated in this study. Fifth, given that our study was performed in 1 medical center in Taiwan, the data may not be a true representative of the entire population. Sixth, follistatin is a multifunctional regulatory protein.¹ Wada et al reported that follistatin was immunostained in the thyroid follicular cells.⁶¹ Follistatin has been reported as an adipokine important for adipogenesis.⁶² Thyroid dysfunction may influence endocrine products of adipose tissue²⁴ and interact with adipocyte-myocyte crosstalk.²⁶ To our knowledge, serum follistatin levels in patients with different thyroid function statuses have never been discussed in literatures. Our study revealed a positive correlation between follistatin and fT4 levels. This preliminary study raised further interesting topics to be investigated: Does follistatin play a role in follicular cell metabolism? Will follistatin affect thyroid function statuses and/or the treatment prognosis of thyroid dysfunction? Is follistatin just a bystander serum parameter of thyroid dysfunction? Do changes of energy consumption in thyroid disorders induce changes of serum follistatin levels? The true mechanism and the impact of the association between serum levels of follistatin and fT4 remained to be studied.

In conclusion, our findings demonstrated that: (1) patients with hyperthyroidism had higher serum follistatin levels than subjects with euthyroidism, (2) the serum follistatin levels in patients with hyperthyroidism declined after administration of the antithyroid regimens, and (3) among patients with hyperthyroidism and euthyroidism, serum levels of follistatin were positively associated with serum fT4 levels. Whether the associations between fT4 and follistatin levels persist in the whole thyroid function spectrum warrants future investigation.

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