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Elevated Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Antigen Levels in Overt and Subclinical Hypothyroid Patients Were Reduced by Levothyroxine Replacement

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Abstract. The influence of hypothyroidism on haemostasis is an active research area. Not only bleeding tendency but also hypercoagulable states have been reported in hypothyroid patients. Decreased and increased fibrinolytic activity in hypothyroid patients has been shown in several studies. Thrombin activatable fibrinolysis inhibitor (TAFI) is an inhibitor of fibrinolysis, which has been recently isolated from human plasma. The aim of our study was to determine plasma TAFI antigen levels in overt and subclinical hypothyroidism, and to investigate the effect of levothyroxine treatment on TAFI levels. The study was performed in age- and sex- matched 30 overt hypothyroid, 30 subclinical hypothyroid patients, and 30 healthy controls. Blood samples were obtained from patients with overt and subclinical hypothyroidism before levothyroxine replacement, and one month after achieving a euthyroid state with levothyroxine. TAFI antigen levels were measured using Enzyme-Linked ImmunoSorbent Assay kits (Affinity Biologicals; Ontario, Canada). In baseline evaluation both the overt and subclinical hypothyroid groups had higher TAFI antigen levels than control group ($p<0.05$). High levels of TAFI antigen were correlated with the degree of thyroid failure. After achieving euthyroid state with levothyroxine replacement, TAFI antigen levels decreased significantly in patients with overt and subclinical hypothyroidism ($p<0.05$). Our data suggest that there are elevated plasma levels of TAFI antigen both in overt and subclinical hypothyroidism, which may be associated with hypofibrinolysis and elevated risk of thrombosis. Normalization of thyroid state by levothyroxine replacement seems to be effective in lowering of TAFI antigen levels in hypothyroidism.

Key words: Thrombin activatable fibrinolysis inhibitor, Hypothyroidism, Levothyroxine, Fibrinolysis

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HYPOTHYROIDISM is a common endocrinopathy in the general population [1]. The relationship between overt hypothyroidism and atherosclerosis is well-known from case-control and autopsy studies [2]. However, there are few large epidemiological studies examining the effect of hypothyroidism on atherosclerosis, and it is controversial whether subclinical hypothyroidism increases cardiovascular risk or not [1, 2]. Recently, the Rotterdam Study has shown that subclinical hypothyroidism is strongly associated with aortic atherosclerosis and myocardial infarction in elderly women [3].

The pathogenesis of atherosclerosis is a complex multifactorial process. Homeostatic variables are recognized to have critical importance in the pathogenesis of atherosclerosis [4–6]. The fibrinolytic system is a part of the coagulation cascade and it may play a role in the development of atherosclerotic disease [4, 7], since decreased fibrinolytic potential is considered to be a risk factor for cardiovascular events [7].

Recently, a novel potent fibrinolysis inhibitor, thrombin-activatable fibrinolysis inhibitor (TAFI), has been isolated from human plasma [8]. TAFI inhibits fibrinolysis by removal of the carboxy-terminal lysine and arginine residues from partially degraded fibrin polymers [9]. Several studies suggest that the TAFI

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pathway may be implicated in the increased risk for cardiovascular diseases [10, 11]. Many specific conditions can influence plasma TAFI antigen levels; however, there was no data regarding thyroid dysfunction.

The aim of our study was to determine plasma TAFI antigen levels in overt and subclinical hypothyroidism and to investigate the effect of levothyroxine treatment on TAFI antigen levels.

Materials and Methods

Subjects

Thirty overt hypothyroid (24 females and 6 males, mean age 48 years, range 25–71 years) and 30 subclinical hypothyroid (29 females and 1 male, mean age 50 years, range 21–77 years) patients were enrolled in the study. Overt hypothyroidism was defined as TSH>5 mIU/L and free thyroid hormone below normal levels. Subclinical hypothyroidism was defined as TSH>5 mIU/L despite normal free thyroid hormone levels. Control group consisted of 30 age- and sex-matched euthyroid healthy hospital staff (27 females and 3 males; mean age 47 years, range 29–69 years). Informed consent was obtained in all cases, and the study was approved by the local ethics committee of Dokuz Eylul University. Exclusion criteria were known atherosclerotic disease, diabetes, morbid obesity, familial hyperlipidemia, coagulation disorders and severe systemic diseases. The patients, who were treated with lipid lowering drugs, antihypertensive agents, or any drug, which may influence the coagulation system, were not enrolled in the study. Women using oral contraceptives or hormonal replacement therapy were also excluded.

Patients were treated with oral levothyroxine. Thyroid function tests were measured at the beginning and repeated monthly. Anthropometric measurements, clinical findings, serum lipid levels and TAFI Ag levels were measured at the time of diagnosis and a month after achieving euthyroidism.

Methods

Height (m), weight (kg), waist (cm), and hip (cm) were measured under fasting conditions with subjects in light clothing and without shoes. Waist circumference (WC) was recorded as the minimum between the costal margin and iliac crest. Hip circumference (HC) was the maximal circumference over the buttocks as seen from the side. All measurements were taken with the subject standing upright. Body mass index (BMI) was calculated as body weight divided by square height and waist-hip ratio (WHR) was calculated by dividing waist circumference by hip circumference for each subject. Blood pressure was measured using a sphygmomanometer in the sitting position after 5 min rest.

Fasting blood samples for TAFI Ag assessment were obtained during hypothyroid status and a month after euthyroidism was achieved between 8:00 am and 9:00 am from the cannulated antecubital vein in tubes containing buffered citrate. Platelet-poor plasma was obtained by centrifugation at 2000 bpm for 15 min at 4°C. The plasma samples were stored at –80°C until use.

TAFI Ag levels were measured using ELISA kits (Affinity Biologicals; Ontario, Canada). Triglycerides, total cholesterol and HDL cholesterol were measured by Roche/ Hitachi D/P Modular System Autoanalyzer (Roche Diagnostics, Basel, Switzerland). LDL cholesterol was calculated from the results of cholesterol, triglycerides and HDL cholesterol using the Friedewald's Equation.

Statistical analysis

Data were analyzed using one-way ANOVA with Bonferroni correction used as a post hoc test and paired samples t-tests using the SPSS 11.0 for Windows software package. Mann-Whitney U test was used in statistical analysis to compare differences between subgroups. Linear regression analysis with Pearson coefficients was employed to assess correlations between studied parameters. Statistical significance was accepted at $p<0.05$. Data are expressed as means ± SD.

Results

The most common cause of hypothyroidism was autoimmune thyroid disease in our study (68.3%). Other cases had hypothyroidism due to thyroidectomy (25%), radioactive iodine (5%) and lithium treatment (1.7%). Baseline characteristics of hypothyroid patients and controls are given in Table 1. There was no significant difference between overt hypothyroid, sub-

Table 1. Baseline characteristics of patients and controls

	Overt Hypothyroid (n = 30)	Subclinical Hypothyroid (n = 30)	Control (n = 30)
Age	48.07 ± 11.97	50.67 ± 13.91	47.87 ± 11.60
Gender (F/M)	24/6	29/1	27/3
Smoking	7 (23.3%)	3 (10%)	6 (20%)
Weight (kg)	75.07 ± 13.19	69.6 ± 10.93	67.68 ± 10.93
BMI (kg/m^2)	28.15 ± 4.48	27.01 ± 3.77	26.5 ± 4.57
Waist (cm)	88.87 ± 13.79*	84.06 ± 12.65	77.9 ± 11.25
Systolic BP (mmHg)	120.33 ± 11.29	118.67 ± 11.36	119.67 ± 11.59
Diastolic BP (mmHg)	73.67 ± 5.56	75.67 ± 6.79	77.66 ± 9.71
FT_3 (pg/ml)	1.72 ± 1.68*†	2.56 ± 0.59	2.76 ± 0.33
FT_4 (ng/dl)	0.55 ± 0.22*†	1.24 ± 0.18*	1.36 ± 0.16
TSH (mIU/L)	80.06 ± 57.47*†	17.9 ± 15.57*	1.48 ± 0.97
Total-Cholesterol (mg/dl)	280.1 ± 69.73*†	193.72 ± 36.32	186.33 ± 30.23
Triglyceride (mg/dl)	152.03 ± 101.49	118.17 ± 53.46	117.07 ± 57.46
LDL-Cholesterol (mg/dl)	182.46 ± 62.01*†	108.72 ± 30.04	108.67 ± 24.6
HDL-Cholesterol (mg/dl)	67.23 ± 16.42*	58.03 ± 13.30	54.27 ± 11.5
TC/HDL	4.32 ± 1.18*†	3.42 ± 1.04	3.57 ± 0.94
LDL/HDL	2.81 ± 0.96*†	2.03 ± 0.71	2.1 ± 0.7
TAFI Ag (μg/ml)	13.39 ± 1.47*†	12.4 ± 1.66*	10.99 ± 1.3

* vs. control group, p<0. 05; † vs. subclinical hypothyroid group, p<0.05
TC: Total cholesterol, FT_3: Free 3.5.3'-triiodothyronine, FT_4: Free thyroxine
Conversion factor (CF) for lipid levels (CF × C = SI): 0.02586
CF for FT_3 (CF × C = SI): 1.54, CF for FT_4 (CF × C = SI): 12.9

Table 2. TAFI antigen and lipid levels in overt and subclinical hypothyroid groups before and after levothyroxine replacement therapy

	Overt Before	Overt After	Subclinical Before	Subclinical After
TAFI Ag (μg/ml)	13.39 ± 1.47*	11.7 ± 1.81	12.4 ± 1.66†	11.67 ± 1.23
Total-Cholesterol (mg/dl)	280.1 ± 69.73*	195.8 ± 31.69	193.72 ± 36.32	209.1 ± 113.73
Triglyceride (mg/dl)	152.03 ± 101.49	117.3 ± 72.23	118.17 ± 53.46	125.7 ± 51.30
LDL-Cholesterol (mg/dl)	182.46 ± 62.01*	115.6 ± 25.95	108.72 ± 30.04	109.3 ± 22.79
HDL-Cholesterol (mg/dl)	67.23 ± 16.42*	56.9 ± 11.50	58.03 ± 13.30	57.2 ± 13.69
TC/HDL	4.32 ± 1.18*	3.57 ± 0.9	3.42 ± 1.04	3.86 ± 2.39
LDL/HDL	2.81 ± 0.96*	2.12 ± 0.67	2.03 ± 0.71	2.04 ± 0.74

* before treatment vs. after treatment for overt hypothyroid group, p<0. 05;
† before treatment vs. after treatment for subclinical hypothyroid group, p<0. 05.
TC: Total cholesterol, Conversion factor (CF) for lipid levels (CF × C = SI): 0.02586

clinical hypothyroid and control groups regarding gender, age, body mass index and smoking habits. Baseline systolic and diastolic blood pressures were similar.

Overt hypothyroid patients had, compared to controls, elevated total cholesterol, LDL cholesterol and HDL cholesterol levels before levothyroxine replacement (p<0.05). However, lipid measurements were similar between subclinical hypothyroid patients and controls. Total cholesterol/HDL and LDL/HDL ratios were significantly higher among subjects in the overt hypothyroid group than in subjects in the subclinical hypothyroid and control groups (p<0.05). Subclinical hypothyroid patients had similar total cholesterol/HDL and LDL/HDL ratios to controls (Table 1).

Both overt and subclinical hypothyroid patients had significantly increased baseline TAFI antigen levels than controls (p = 0.000 and p = 0.001, respectively). Overt hypothyroid subjects had the highest TAFI antigen levels and the difference in TAFI antigen levels between overt and subclinical hypothyroid groups was statistically significant (p = 0.032; Table 1).

Table 3. Plasma TAFI antigen levels according to thyroid subgroups determined by TSH level (TSH< >50 mU/L) before and after levothyroxine replacement

	TSH>50 mU/L Before (n = 23)	TSH>50 mU/L After (n = 23)	TSH≤50 mU/L Before (n = 37)	TSH≤50 mU/L After (n = 37)	Control (n = 30)
TAFI Ag (μg/ml)	13.23 ± 1.65*	12.07 ± 1.83*	12.68 ± 1.61*	11.45 ± 1.28	10.99 ± 1.3

vs. control group, p<0.05

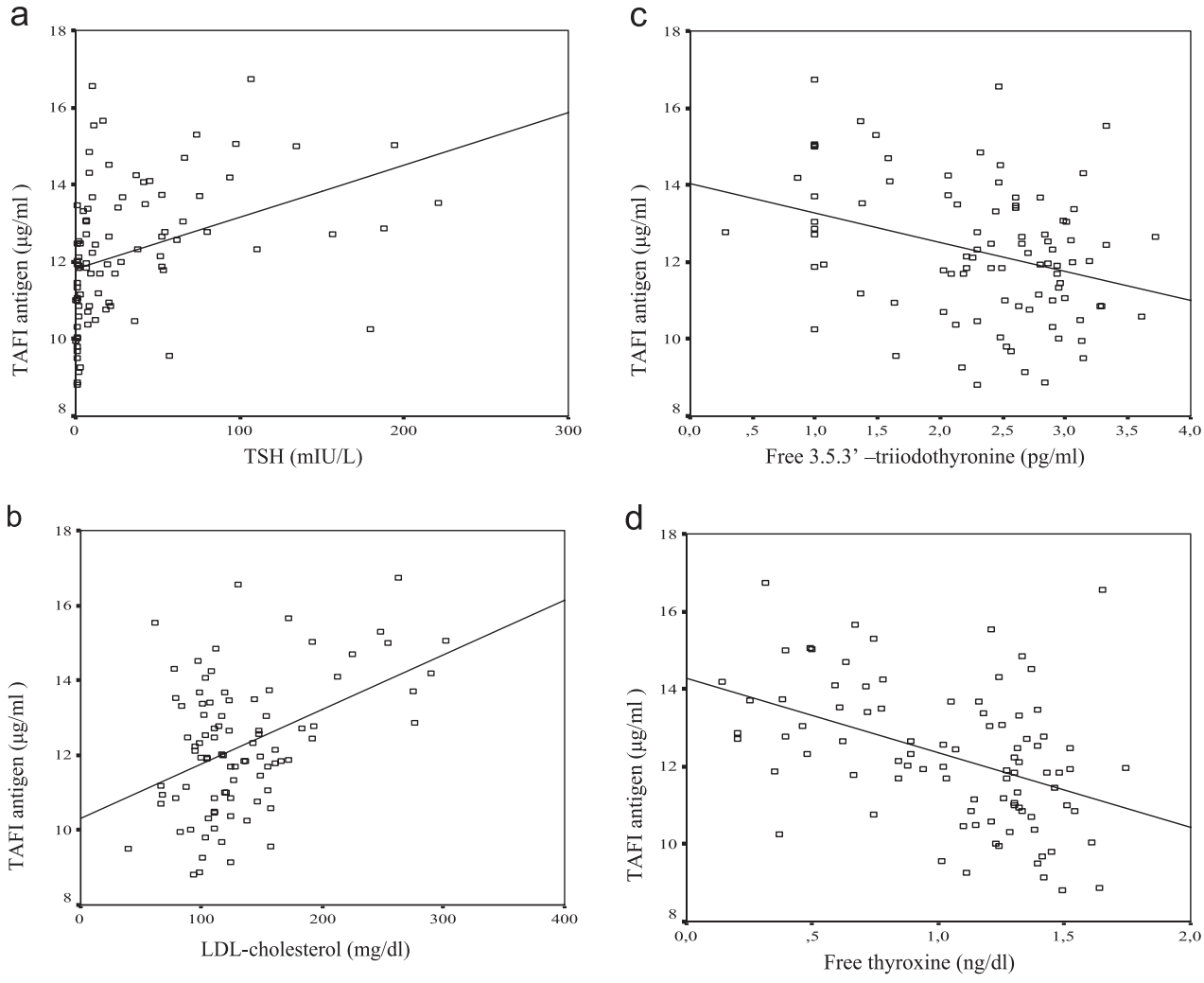


Fig. 1. Significant correlations of plasma TAFI antigen levels with thyroid hormones, and LDL-cholesterol. a. TSH: r = 0.367, p = 0.000; b. LDL- Cholesterol: r = 0.436, p = 0.000; c. FT_3: r = –0.325, p = 0.002; d. FT_4: r = –0.438, p = 0.000
FT_3: Free 3.5.3'-triiodothyronine, FT_4: Free thyroxine

As a result of levothyroxine replacement, serum levels for total cholesterol, LDL cholesterol and HDL cholesterol decreased in overt hypothyroid group (p<0.05). Lipid parameters remained stable in subclinical hypothyroid group. Despite the reduction in HDL cholesterol levels among subjects in the overt hypothyroid group, total cholesterol/HDL and LDL/HDL ratios decreased significantly after levothyroxine (p<0.05; Table 2).

One month after achieving euthyroidism by levothyroxine replacement, TAFI antigen levels decreased in patients with overt and subclinical hypothyroidism (p = 0.000 and p = 0.028; respectively; Table 2). Mean reduction of plasma TAFI antigen levels after achieving euthyroidism was 1.7 μg/ml in overt hypothyroid

group and 0.7 μg/ml in subclinical hypothyroid group.

We divided patients into two subgroups, which were determined as moderate and severe hypothyroidism, defined by serum TSH levels below 50 mU/L and above 50 mU/L. Basal plasma TAFI antigen levels were similar between severe hypothyroid (TSH>50 mU/L) and moderate hypothyroid (TSH≤50 mU/L) subgroups. Plasma TAFI antigen levels decreased significantly in both subgroups after levothyroxine ($p<0.05$). However, patients with severe hypothyroidism had higher TAFI antigen levels compared to controls after euthyroidism was achieved ($p = 0.023$; Table 3).

Positive correlations between serum TSH ($r = 0.367$, $p = 0.000$; Fig. 1a), LDL cholesterol ($r = 0.436$, $p = 0.000$; Fig. 1b) and plasma TAFI antigen levels, and inverse correlations between free thyroid hormone levels ($r = –0.325$, $p = 0.002$ for FT_3; Fig. 1c, and $r = –0.438$, $p = 0.000$ for FT_4; Fig. 1d) and plasma TAFI antigen levels were found. Multiple regression analysis showed that low serum free thyroxin and serum LDL-cholesterol levels were significant predictors of high plasma TAFI Ag levels. TAFI antigen levels were not associated with sex, anthropometric measurements, and menopausal status.

Discussion

The influence of hypothyroidism on homeostasis is complex and still not very well understood [12]. Both bleeding tendency and hypercoagulable states have been reported. Decreased and increased fibrinolytic activity in hypothyroidism has been shown in several studies [13–16]. Thrombin activatable fibrinolysis inhibitor (TAFI) is a zymogene that potently inhibits fibrinolysis [8]. The direct action of TAFI involves removal of carboxy-terminal lysyl and arginyl residues from fibrin clot. Removal of carboxy-terminal amino acids provides elimination of plasminogen binding sites. This prevents plasminogen from activation into plasmin and retards the lysis of a fibrin clot [17].

Plasma TAFI antigen levels have been measured in various conditions [18]. To our knowledge, this is the first study to evaluate TAFI antigen levels in patients with thyroid dysfunction. In the present study, patients with hypothyroidism had elevated plasma TAFI antigen levels, which may suggest an inhibition of fibrinolysis in hypothyroidism. Both overt and subclinical hypothyroid groups had significantly higher TAFI antigen levels compared to controls. In addition, the difference in TAFI antigen levels between clinical hypothyroid group and subclinical hypothyroid group was statistically significant, and a positive correlation between the degree of thyroid failure and plasma TAFI antigen levels was found. Multiple regression analysis revealed that low levels of free thyroxine lead to high plasma TAFI antigen levels. After achieving euthyroid state with levothyroxine, TAFI antigen levels decreased to levels similar to controls. Normalization of thyroid state by levothyroxine replacement seems to be effective in reducing plasma TAFI antigen levels.

Impaired fibrinolysis is an important predictor of increased risk for stroke and myocardial infarction. Increased concentrations of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen have been found in subjects with cardiovascular disease [4, 5]. The PRIME Study has shown that high levels of PAI-1 and fibrinogen are predictors of new cardiovascular events [7]. Although TAFI is implicated in the increased risk of cardiovascular disease, there is inadequate data to establish that high plasma TAFI Ag levels lead to any cardiovascular event. Recently, Morange *et al*. [19] showed that plasma TAFI antigen levels are not associated with the risk of coronary heart disease. Moreover several studies suggest that elevated TAFI antigen levels may be protective against coronary events [20, 21].

On the other hand, there is some evidence that plasma TAFI levels are related to atherosclerotic disease. Silveira *et al.* [22] showed elevated TAFI antigen plasma levels in men with symptomatic coronary artery disease. High plasma levels of TAFI were found in patients with stable angina pectoris and angiographically verified coronary artery disease [11, 22, 23]. Malyszko *et al.* [24] showed that renal transplant recipients with coronary artery disease had significantly elevated TAFI antigen concentrations and activity when compared to patients without coronary artery disease. In another study, increased TAFI activity was associated with an almost fourfold higher risk of coronary artery disease [25]. TAFI is overexpressed after ischemic stroke and increased TAFI antigen levels and activity have been proposed as a risk factor for stroke [26–28]. Consequently, we speculate that increased TAFI antigen levels in hypothyroidism may be associated with an elevated risk of cardiovascular disease and levothyroxine treatment seems to be effective in reducing plasma TAFI antigen levels.

Dyslipidemia observed in overt hypothyroidism is

characterized by increased serum concentrations of total and LDL cholesterol, similar to our findings [29]. Depletion of thyroid hormones leads to a reduced number of LDL receptors in the liver and to decreased biliary excretion of cholesterol resulting in elevated serum LDL. The relationship between LDL cholesterol and TAFI antigen levels have been reported in several studies [30–32]. Aso *et al.* [32] found that serum LDL cholesterol is an independent predictor of plasma TAFI levels in type 2 diabetes. Similarly, our results indicate that elevated serum LDL-cholesterol levels due to thyroid failure result in increased TAFI antigen levels.

In one study, the degree of hypothyroidism was found to be associated with different effects on coagulation. Chadarevian *et al.* [33] reported a different pattern of fibrinolytic abnormality according to the severity of hypothyroidism. Fibrinolytic activity was decreased in subjects with moderate hypothyroidism (TSH<50 mU/L); however, it was increased in severe hypothyroid patients (TSH>50 mU/L). In our study, both severe and moderate hypothyroid patients had elevated TAFI antigen levels, and TAFI antigen levels were correlated with TSH levels. In addition, after treatment with levothyroxine, TAFI antigen levels decreased significantly in cases with severe and moderate hypothyroidism. However, severe hypothyroid patients had higher TAFI antigen levels than controls after euthyroidism was restored. Increased post-treatment TAFI antigen levels in patients with severe hypothyroidism compared to controls may be associated with the degree of thyroid failure. The other reason for the increased post-treatment TAFI antigen levels in severe hypothyroidism may be the short duration of euthyroid state. We obtained plasma samples for TAFI antigen assay one month after achieving euthyroid state. Assessment at the end of a longer euthyroid period may suggest additional information.

Increased TAFI antigen levels in hypothyroidism may be related to decreased clearance of TAFI or increased production by adipose tissue and endothelium. Decreased clearance of some coagulation factors have been shown in hypothyroidism [13]. Increased TAFI antigen levels have been reported in patients with diabetic nephropathy [34] and in patients undergoing peritoneal dialysis [35]. However, there is no study that investigated TAFI clearance in humans. TAFI is mainly synthesized in the liver, but also TAFI mRNA was detected in adipose tissue and endothelial cells [36]. Endothelial dysfunction may be a source for increased TAFI antigen levels in hypothyroidism [37].

In conclusion, we found that TAFI antigen levels are elevated in overt and subclinical hypothyroidism, and that these increased levels are related to the degree of thyroid dysfunction. Achieving euthyroidism by levothyroxine replacement therapy reduces plasma TAFI antigen levels, and may be associated with a decrease in risk of thrombosis in patients with hypothyroidism. Further studies investigating TAFI pathway and the relationship with other components of fibrinolysis are required in hypothyroid patients.

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