Thyroid Function and Cardiovascular Disease: is there a Mediating Role of Coagulation?

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ABSTRACT

Introduction The mechanisms linking high and high-normal thyroid function to an increased cardiovascular risk remain unclear. We hypothesized that coagulation can play a role, and investigated: (i) the association of thyroid function with coagulation factors, (ii) whether coagulation factors mediate the association of thyroid function with cardiovascular disease.

Methods In 5918 participants (mean age, 69.1 years) from Rotterdam Study, we measured thyrotropin, free thyroxine (FT_4) and coagulation factors (von Willebrand factor antigen [VWF:Ag], ADAMTS13 activity, fibrinogen). Participants were followed for the occurrence of cardiovascular events and deaths. Associations of thyroid function with coagulation factors (standardized Z scores) and cardiovascular disease were assessed through linear regression and Cox-proportional hazard models, adjusted for potential confounders. We performed causal mediation analyses to evaluate if the effect of thyroid function on cardiovascular disease is mediated by coagulation.

Results Higher FT_4 levels were associated with higher VWF:Ag (β, 0.34; 95% confidence interval [95% CI], 0.22 to 0.47), lower ADAMTS13 activity (β,-0.22; 95% CI,-0.35 to -0.09), and higher fibrinogen (β, 0.26; 95% CI, 0.13 to 0.39). Over a median follow-up time of 11.3 (interquartile range, 10.0 to 12.7) years, 857 incident cardiovascular events and 690 cardiovascular deaths occurred. FT_4 levels were positively associated with cardiovascular disease. The effect of FT_4 on incident cardiovascular events was partly mediated by fibrinogen (1.6%), but not by VWF:Ag and ADAMTS13. VWF:Ag and fibrinogen together mediated 10.0% of the effect of FT_4 on cardiovascular deaths.

Conclusions Higher FT_4 levels were associated with higher VWF:Ag, lower ADAMTS13 activity and higher fibrinogen, indicating a procoagulant state. VWF:Ag and fibrinogen can partly explain the link of FT_4 with cardiovascular disease.

INTRODUCTION

The cardiovascular system is one of the major targets of thyroid hormone action. Thyroid hormones affect cardiomyocytes by stimulating the ion channels in the cell membranes and by binding to the nuclear thyroid hormone receptors, further promoting the expression of target genes.[1] Thyroid hormones also influence the cardiovascular system by affecting the sympathetic nervous system and the peripheral circulation.[1] To date, population-based studies among middle-aged and older adults have shown that high and high-normal thyroid hormone levels are associated with an increased risk of cardiovascular disease and mortality, independent of traditional cardiovascular risk factors as hyperlipidemia, hypertension, diabetes, and obesity.[2-4] This points towards other factors that can mediate the increased cardiovascular risk in case of excess thyroid hormones. The identification of these mediating factors is essential to better understand the role of thyroid hormones in cardiovascular disease, as well as to identify potential targets for future preventive strategies.

Hemostasis may be one mechanism through which thyroid hormones affect cardiovascular system. *In vitro*[5-8] and *in vivo*[6,9,10] studies have shown that thyroid hormones directly regulate the transcription of genes encoding coagulation proteins in the hepatic and endothelial cells. In turn, coagulation proteins, such as von Willebrand factor (VWF), ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motif repeats 13), and fibrinogen have been associated with an increased risk of coronary heart disease (CHD), ischemic stroke[11-14] and cardiovascular mortality.[15] VWF mediates platelet adhesion and aggregation, which play an important role in thrombus formation.[16] ADAMTS13 cleaves the procoagulant VWF multimers into smaller, less procoagulant multimers.[17] Fibrinogen is converted into fibrin, which strengthens the clot structure of the thrombus. Despite the clear role of coagulation factors on cardiovascular system, it has never been investigated whether and to what extent coagulation factors mediate the association of thyroid function with cardiovascular disease and mortality. To date, observational and experimental studies have established an increased risk of bleeding in hypothyroidism and an increased risk of thrombosis in hyperthyroidism.[18,19] However, previous studies have not investigated whether the anticoagulant effects of hypothyroidism and the procoagulant effects of hyperthyroidism are extended even within the normal reference range of thyroid function.

In this large prospective population-based cohort study, we aimed to: (i) assess the association of thyroid function with VWF, ADAMTS13, and fibrinogen; and (ii)

investigate whether and to what extent these coagulation factors mediate the association of thyroid function with cardiovascular events and deaths.

METHODS

Study population

This study was embedded within the framework of the Rotterdam Study, a large prospective population-based cohort study among the residents of Ommoord, a district of Rotterdam, the Netherlands. The objectives and study design have been extensively described elsewhere.[20] The Rotterdam Study was initiated in 1989, including 7983 participants aged 55 years or older. In 2000, the study was extended with a second cohort of 3011 subjects. Study participants undergo extensive follow-up medical examinations every 3 to 5 years. Baseline measurements for the present study were performed during the third visit of the first cohort (RS I.3, 1997-1999, n=4797) and the first visit of the second (RS II.1, 2000-2001, n=3011) cohort of the Rotterdam Study. A total of 6140 participants had data available on thyroid function measurements, VWF:Ag, ADAMTS13 activity, and fibrinogen levels. Of these, 5918 were followed for the occurrence of cardiovascular events and deaths. Participants with thyroid function measurements, coagulation data, and complete information on prevalent cardiovascular disease and mortality, were eligible. Detailed information on the selection of study participants is provided in Supplemental Figure 1.

The protocols of the Rotterdam Study have been approved by the Medical Ethics Committee of the Erasmus University and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Population Study Act Rotterdam Study. In accordance with the Declaration of Helsinki, all included participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

Assessment of thyroid function

Thyroid function was assessed during the third visit of the first cohort (RS I.3) and the first visit of the second (RS II.1) cohort using the same method and assay. Measurements of TSH and FT_4 were performed in baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay "ECLIA" Roche. The reference ranges of TSH (0.40 to 4.0 mIU/L) and FT_4 (0.86 to 1.94 ng/dL, alternatively 11 to 25 pmol/L) were determined based on national guidelines and our previous studies.[20]

Assessment of coagulation factors

Von Willebrand factor antigen (VWF:Ag) levels were measured via in-house ELISA, using polyclonal rabbit anti-human VWF antibodies (DakoCytomation, Glostrup, Denmark) for catching and tagging. ADAMTS13 activity was measured in a kinetic assay using the Fluorescence Resonance Energy Transfer Substrate VWF 73 assay.[21] Fibrinogen levels were derived from the clotting curve of the prothrombin time assay using thromborel S as a reagent in an automated coagulation laboratory (ACL 300 Instrumentation Laboratory). VWF:Ag, ADAMTS13 activity, and fibrinogen levels were measured against a reference curve of serial dilutions of normal human plasma, calibrated against the international standard (Siemens).[12,22]

Assessment of cardiovascular disease

Cardiovascular events were defined as fatal and nonfatal myocardial infarction, other CHD mortality, or stroke, as previously described.[23] Prevalent cardiovascular disease was defined as history of myocardial infarction, stroke, coronary or other arterial revascularization.[23,24] Prevalent cardiovascular disease was assessed through interview and verified in medical records. Cardiovascular mortality was defined as death due to CHD, cerebrovascular disease or other cardiovascular diseases, as previously described.[2,23] The ascertainment of cardiovascular mortality in the Rotterdam Study has been extensively described in a previous study.[23] In short, information on cardiovascular mortality was obtained from municipality, general practitioners and reports of medical specialists. The underlying cause of death was ascertained independently by two research physicians and subsequently validated by a medical specialist.

Additional measurements

The baseline home interview provided information on medical history, medication use, tobacco smoking, alcohol consumption.[20] Smoking habits were categorized as current, former and never smoking. Serum glucose and lipid levels were measured using automatic enzymatic procedures (Roche Diagnostics GmbH, Mannheim, Germany). Anthropometric measurements were performed in the research center by trained medical staff. Body mass index was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured in the sitting position on the right arm and calculated as the mean of two measurements using a random-zero sphygmomanometer. Diabetes mellitus was defined as fasting serum

glucose level of 7 mmol/L or more, non-fasting plasma glucose level of 11.1 mmol/L or more (when fasting samples were absent) or the use of antidiabetic medications. C-reactive protein was measured in nonfasting serum samples that had been kept frozen at -20°C by use of Rate Near Infrared Particle Immunoassay (Immage Immunochemistry System; Beckman Coulter). Blood group antigen phenotypes were reconstructed by haplotypes analysis of 4 single nucleotide polymorphisms rs687289, rs507666, rs8176704, and rs8176749, which served as tagging single nucleotide polymorphisms for the O, A1, A2, and B alleles. Occurrence of cancer was determined through general practitioners and by linkage with a nationwide registry of histopathology and cytopathology in the Netherlands, Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA). Two research physicians independently assessed the first date and diagnosis of cancer. Cancer events were classified according to the international classification of diseases 10^{th} edition. In case of discrepancy, consensus was sought or a cancer epidemiologist decided.

Statistical analysis

The associations of thyroid function (TSH and FT_4) with coagulation factors (ie, VWF:Ag, ADAMTS13 activity, and fibrinogen levels) were assessed by using linear regression models. βs were estimated per 1 standard deviation (sd) increase for VWF:Ag, ADAMTS13 activity, and fibrinogen levels. Associations of thyroid function and coagulation factors with incident cardiovascular events and cardiovascular deaths were assessed through Cox-proportional hazard models. We used restricted cubic splines to account for nonlinearity of the associations, but no evidence of nonlinearity was observed. Analyses were adjusted for potential confounders that were selected based on biological plausibility and previous literature. Model 1 was adjusted for age, sex, and cohort. Model 2 was additionally adjusted for smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive or lipid-lowering medications, anticoagulant medications and ABO blood group (O vs non-O). For the analyses in which coagulation factors were the exposure and cardiovascular outcomes were the outcome, we additionally adjusted for TSH and FT_4 levels.

To examine the robustness and applicability of our results, we performed several sensitivity analyses on the association between thyroid function and coagulation factors. (1) To account for the potential influence of inflammation and thyroid autoimmunity, we additionally adjusted our analyses for C-reactive protein levels

and thyroid peroxidase antibodies (TPOAb) positivity, respectively. Moreover, we investigated the association of TPOAb positivity with coagulation factors. (2) We additionally adjusted our analyses for prevalent cardiovascular disease. (3) Thyroid hormones have been associated with the risk of cancer, which is in turn characterized by a hypercoagulable state.[25] To exclude any potential bias caused by presence of cancer, we excluded participants with cancer at baseline (n=292). (4) We restricted the analysis to participants with thyroid function within the reference range, without past thyroid disease and not using thyroid medications. (5) To test for potential effect modification, we added product interaction terms of the exposure with covariates in the multivariable model. (6) Due to the biological interaction between VWF and ADAMTS13, we also investigated the association of thyroid function with the combination of VWF:Ag levels and ADAMTS13 activity. We grouped participants into 9 categories, based on the combinations of VWF:Ag tertiles and ADAMTS13 tertiles. Furthermore, we performed multinomial logistic regression to evaluate the association between FT_4 levels and the 9 combinations of VWF:Ag tertiles and ADAMTS13 tertiles. Individuals who were in the lowest tertile of VWF:Ag and in the highest tertile of ADAMTS13 (category with lowest thrombotic risk) were considered as reference.

We performed a causal mediation analysis, which evaluated whether the effect of FT_4 on incident cardiovascular events and cardiovascular deaths was mediated by coagulation factors (Figure 2). The following paths were tested: the direct effect (effect of the exposure on the outcome through pathways other than the mediator); the indirect effect (effect of the exposure on the outcome via the mediator); the total effect (the sum of direct effect and indirect effect); the proportion mediated (indirect effect/total effect). To test for mediation effects, we used conditional process analysis techniques as described by Hayes.[26] Statistical analyses were conducted using SPSS version 21.

RESULTS

Baseline characteristics of 5918 eligible participants are shown in Table 1. The mean age was 69.1 years (sd, 8.2) and 56.7% were women (Table 1). During a median follow-up of 11.3 (interquartile range, 10.0 to 12.7) years, 857 incident cardiovascular events and 690 cardiovascular deaths occurred.

Table 1. Baseline characteristics of 5918 participants

Age, years	69.1 ± 8.2
Women	3356 (56.7)
Smoking	
current	1068 (18.0)
former	2954 (49.9)
never	1896 (32.0)
TSH, mIU/L	1.8 [1.2-2.8]
FT_4, ng/dL	1.2 ± 0.2
TPOAb positive	817 (13.3)
Use of thyroid medication	177 (3.0)
Thyroid surgery	131 (2.2)
History of thyroid disease	498 (8.4)
BMI, kg/m^2	26.9 ± 3.9
History of diabetes	774 (13.1)
Total cholesterol, mmol/l	5.7 ± 0.9
Triglycerides, mmol/l	1.5 ± 0.7
Use of lipid-lowering medications	764 (12.9)
Systolic blood pressure, mm Hg	143.2 ± 21.2
Use of antihypertensive medications	1376 (23.3)
Use of anticoagulant medications	1214 (20.5)
Blood group O	2708 (45.8)
C-reactive protein, mg/l	1.8 [0.7-3.9]
Prevalent CHD or stroke	691 (11.7)
Prevalent cancer	289 (4.9)
Fibrinogen, g/l	3.9 ± 0.9
VWF:Ag, %	132 ± 58.4
ADAMTS13 activity, %	91.6 ± 17.6

Values for continuous variables are presented as mean ± standard deviation or median [interquartile range]. Values for categorical variables are presented as number (percentage). Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies (cutoff, 35 kU/ml); BMI, body-mass index; CHD, coronary heart disease; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13; VWF:Ag, von Willebrand factor antigen.

Thyroid function and coagulation factors

Higher FT_4 levels were associated with higher VWF:Ag (β, 0.34; 95% confidence interval [95% CI], 0.22 to 0.47, per 1 ng/dL increase in FT_4), lower ADAMTS13 activity (β,-0.22; 95% CI, -0.35 to -0.09, per 1 ng/dL increase in FT_4), and higher fibrinogen levels (β, 0.26; 95% CI, 0.13 to 0.39, per 1 ng/dL increase in FT_4) (Table 2; Supplemental Table 1). Overall, TSH was not consistently associated with any of the coagulation factors (Table 2; Supplemental Table 1). Results remained consistent after additionally adjusting for C-reactive protein, TPOAb, prevalent cardiovascular disease, and

Table 2. Association between thyroid function and Z scores of coagulation factors

	All participants (TN 5918)		Euthyroid participants* (TN 4646)	
	β (95% CI) Model 1	β (95% CI) Model 2	β (95% CI) Model 1	β (95% CI) Model 2
VWF:Ag				
TSH	-0.02 (-0.05; 0.01)	**-0.03 (-0.06;-0.01)**	0.00 (-0.05; 0.05)	-0.02 (-0.07; 0.04)
FT_4	**0.29 (0.16; 0.42)**	**0.34 (0.22; 0.47)**	**0.30 (0.12; 0.47)**	**0.34 (0.17; 0.50)**
ADAMTS13 activity				
TSH	0.02 (-0.01; 0.05)	0.00 (-0.03; 0.02)	**0.06 (0.01; 0.12)**	0.03 (-0.02; 0.09)
FT_4	**-0.29 (-0.42;-0.16)**	**-0.22 (-0.35;-0.09)**	**-0.42 (-0.60;-0.25)**	**-0.33 (-0.50;-0.16)**
Fibrinogen				
TSH	**-0.03 (-0.06;-0.01)**	-0.02 (-0.05; 0.01)	-0.04 (-0.09; 0.02)	-0.01 (-0.06; 0.05)
FT_4	**0.34 (0.21; 0.48)**	**0.26 (0.13; 0.39)**	**0.34 (0.16; 0.52)**	**0.25 (0.07; 0.42)**

Model 1: age, sex, cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. βs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). βs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). *Normal reference ranges of thyroid function were defined as serum TSH of 0.4-4.0 mIU/L and FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with personal history of thyroid disease. Abbreviations: TN, total number; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; VWF:Ag, von Willebrand factor antigen; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13.

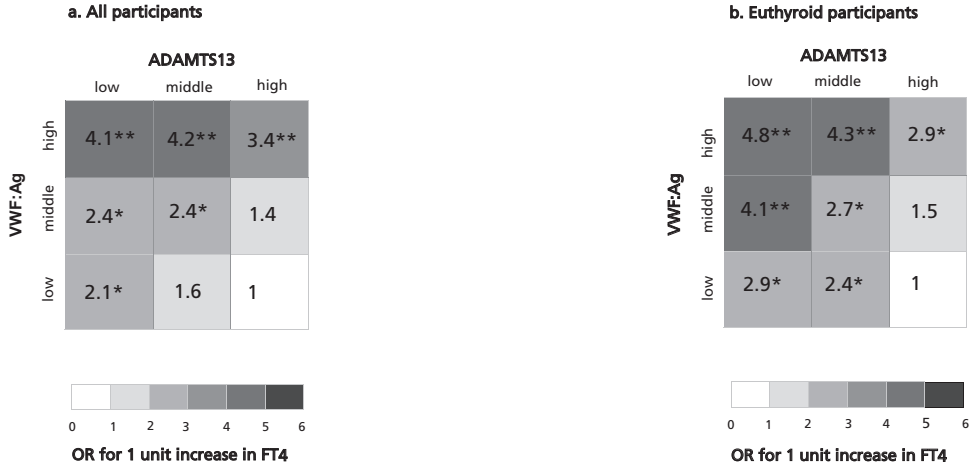
after excluding participants with cancer at baseline (Supplemental Table 2). No evidence of effect modification was observed. TPOAb positivity was not associated with the coagulation factors (Supplemental Table 3). Results remained similar or became stronger after restricting the analyses to euthyroid participants (Table 2; Figure 1).

In a sensitivity analysis, we investigated the association of FT_4 levels with the 9 combinations of ADAMTS13 tertiles and VWF:Ag tertiles. With increasing FT_4 levels, the odds of being in the highest tertile of VWF:Ag and the lowest tertile of ADAMTS13 (category with highest thrombotic risk) were 4.1 times higher than the odds of being in the reference category (category with lowest thrombotic risk) (odds ratio, 4.1 per 1 ng/dL increase in FT_4 (Figure 1).

Thyroid function, coagulation factors, and cardiovascular disease

In line with our previous data,[2] higher FT_4 levels were associated with an increased risk of incident cardiovascular events (hazard ratio [HR], 2.01, 95% CI, 1.43 to 2.82, per 1 ng/dL increase; alternatively, HR, 1.14, 95% CI, 1.07 to 1.21, per 1 sd increase in FT_4) and cardiovascular deaths (HR, 2.17, 95% CI, 1.53 to 3.09, per 1 ng/dL in-

Figure 1. Association of free thyroxine levels with combined ADAMTS 13 and VWF antigen.



a. All participants (TN 5918) b. Euthyroid participants (TN 4646). Due to the biological interaction between VWF:Ag and ADAMTS13, multinomial logistic regression was performed to evaluate the association between FT_4 levels and the nine combinations of VWF:Ag tertiles and ADAMTS13 tertiles, with lowest tertile of VWF:Ag and highest tertile of ADAMTS13 as the reference category. Analyses were adjusted for age, sex, cohort, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. The plots indicate the odds of being in a specific coagulation category rather than in the reference category, per one unit increase in FT_4 levels. For example, for one unit increase in FT_4 levels, the odds of being in the highest tertile of VWF:Ag and lowest tertile of ADAMTS13 (category with highest thrombotic risk) were 4.1 times higher compared with the odds of being in the lowest tertile of VWF:Ag and highest tertile of ADAMTS13 (category with lowest thrombotic risk). Normal reference ranges of thyroid function were defined as serum TSH of 0.4 to 4.0 mIU/L and FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with personal history of thyroid disease. *P value <0.05; **P value <0.0001. Abbreviations: OR, odds ratio; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13; VWF:Ag, von Willebrand factor antigen.

crease; alternatively, HR, 1.16, 95% CI, 1.08 to 1.23, per 1 sd increase in FT_4) (Table 3, Supplemental Table 4). TSH was not associated with cardiovascular outcomes (Table 3, Supplemental Table 4). Higher VWF:Ag and fibrinogen levels were associated with an increased risk of incident cardiovascular events (HR, 1.08, 95% CI, 1.01 to 1.15; HR, 1.09, 95% CI, 1.02 to 1.17, per 1 sd increase, respectively) and cardiovascular deaths (HR, 1.16, 95% CI, 1.09 to 1.24; HR, 1.23, 95% CI, 1.15 to 1.31, per 1 sd increase, respectively) (Table 3). Higher ADAMTS13 activity was associated with a decreased risk of incident cardiovascular events and cardiovascular deaths (HR, 0.92, 95% CI, 0.86 to 0.99; HR, 0.89, 95% CI, 0.82 to 0.97, per 1 sd increase) (Table 3). The effect of FT_4 on incident cardiovascular events was minimally mediated by fibrinogen (1.6%), but not by VWF:Ag or ADAMTS13 (Table 4). The effect of FT_4 on cardiovascular deaths was partly mediated by VWF (5.4%) and fibrinogen (6.4%), but not by ADAMTS13.

Table 3. Association of thyroid function and coagulation factors with incident cardiovascular events and deaths

	CV events* (857/5227) HR (95% CI)	CV deaths‡ (690/5918) HR (95% CI)
Thyroid function		
TSH	0.96 (0.89; 1.03)	0.96 (0.88; 1.04)
FT_4	**2.01 (1.43; 2.82)**	**2.17 (1.53; 3.09)**
Coagulation factors (Z scores)†		
VWF:Ag	**1.08 (1.01; 1.15)**	**1.16 (1.09; 1.24)**
ADAMTS13	**0.92 (0.86; 0.99)**	**0.89 (0.82; 0.97)**
Fibrinogen	**1.09 (1.02; 1.17)**	**1.23 (1.15; 1.31)**

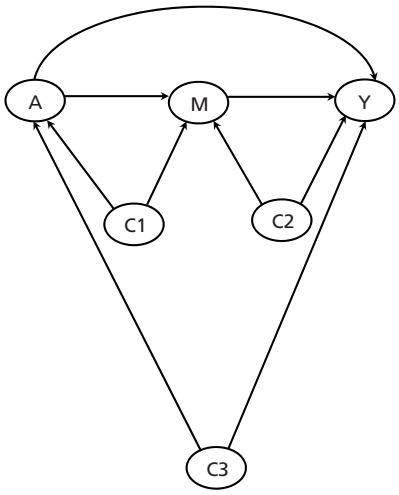
Adjusted for age, sex, cohort, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. HRs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). HRs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). *Participants with prevalent cardiovascular events were excluded from the analysis. †Additionally adjusted for TSH and FT_4. ‡Additionally adjusted for prevalent cardiovascular disease. Abbreviations: CV, cardiovascular; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; VWF:Ag, von Willebrand factor antigen; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13.

Table 4. Mediation analysis for the association of FT_4 with cardiovascular events and deaths

Potential mediator (Z scores of coagulation markers)	Direct effect β	Direct effect *P* value	Indirect effect β	Indirect effect *P* value	PM (%)
FT_4 and incident cardiovascular events (Events/TN 857/5227)*					
VWF:Ag	**0.657**	0.002	0.014	0.2	-
ADAMTS13	**0.661**	0.001	0.011	0.2	-
Fibrinogen	**0.663**	0.002	**0.011**	0.04	**1.6**
Fibrinogen and VWF:Ag	**0.651**	0.002	0.021	0.06	-
FT_4 and cardiovascular deaths† (Events/TN 690/5918)					
VWF:Ag	**0.642**	0.004	**0.037**	0.02	**5.4**
ADAMTS13	**0.674**	0.002	**0.011**	0.35	-
Fibrinogen	**0.629**	0.004	**0.043**	0.006	**6.4**
VWF:Ag and fibrinogen	**0.600**	0.007	**0.066**	0.001	**10.0**

Adjusted for age, sex, cohort, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. *Participants with prevalent cardiovascular events were excluded from the analysis. †Additionally adjusted for prevalent cardiovascular disease. Abbreviations: FT_4, free thyroxine; PM, proportion mediated; TN, total number; VWF:Ag, von Willebrand factor antigen.

Figure 2. DAG for the association of free thyroxine, coagulation factors and cardiovascular outcomes.



A, free thyroxine; M, coagulation factor; Y, cardiovascular outcome; C1, C2, C3, potential confounders.

Taken together, VWF:Ag and fibrinogen mediated 10.0% of the effect of FT_4 on cardiovascular deaths (Table 4).

DISCUSSION

In this large population-based cohort study, higher FT_4 levels were associated with higher VWF, lower ADAMTS13 activity, and higher fibrinogen levels, which indicate a procoagulant state. Participants with higher FT_4 levels had an increased thrombotic risk. The associations were independent of cardiovascular risk factors, markers of inflammation and thyroid autoimmunity. Results were consistent and more pronounced within the normal range of thyroid function. The association of FT_4 with cardiovascular outcomes was partly mediated by VWF and fibrinogen, but not by ADAMTS13.

To date, few population-based studies have investigated the association between categories of thyroid function and coagulation factors. High thyroid function has been linked to elevated levels of VWF and fibrinogen.[27,28] One cohort study showed that high FT_4 levels are associated with elevated VWF concentrations,[28] and another cohort study reported an association of low TSH levels with elevated fibrinogen concentrations.[27] However, both studies were based on arbitrary categorizations of

thyroid function, thus not being able to account for potential risk variations within categories. Besides VWF and fibrinogen, an additional factor of coagulation that could be influenced by thyroid function is the metalloprotease ADAMTS13. Yet, to our knowledge, no other cohort studies have explored the potential link between thyroid function and ADAMTS13 activity. Against this background, our study provides novel evidence on the association of thyroid function with coagulation, by focusing on the continuous range of TSH and FT_4 levels, beyond the thyroid status categories. Our results support the hypothesis that the procoagulant effects of high thyroid hormones and the anticoagulant effects of low thyroid hormones are extended even within the normal reference range of thyroid function, as a continuum of effects.

We accounted for several mechanisms that could explain the positive association of thyroid function with coagulation. Among others, cancer and/or thyroid autoimmunity could alter both the circulating levels of thyroid hormones and coagulation factors.[29,30] However, the exclusion of cancer patients and the adjustment for TPOAb did not affect our results. Inflammation is another mechanism through which thyroid function could influence coagulation, but additional adjustments for levels of C-reactive protein did not change our results. In line, a randomized crossover study found no effect of thyroid hormones on the expression of inflammation-related genes.[31] Taken together, these data suggest that cancer, thyroid autoimmunity and inflammation, do not explain our results.

Several plausible mechanisms may explain the link between thyroid hormones and VWF. Low thyroid function is a well-known cause of acquired von Willebrand disease, which is characterized by low VWF antigen and/or activity. When induced by hypothyroidism, acquired von Willebrand disease is reversed after thyroid hormone replacement therapy, indicating a direct influence of thyroid hormones on VWF.[32] Most likely, thyroid hormones downregulate the synthesis of VWF in the endothelial cells, via controlling the transcription of the VWF gene.[10,33] In particular, an experimental study found that modulation of the VWF gene requires a prolonged exposure to triiodothyronine (ie, two weeks). This suggests that thyroid hormones can influence the synthesis of VWF not only via the nuclear thyroid hormone receptors, but also via affecting intermediate transcriptional receptors and/or via other mechanisms than receptor mediated gene expression.[10] Previous studies indicate that thyroid hormones can induce the release of VWF via stimulation of the sympathetic nervous system.[34,35] Furthermore, our data suggests that thyroid hormones

can attenuate the role of ADAMTS13 in cleaving the procoagulant VWF multimers into less procoagulant forms. Future studies need to confirm our results and further unravel the potential underlying mechanisms.

The association of thyroid hormones with fibrinogen can be explained by the direct action of thyroid hormones on thyroid hormone receptors and corresponding response elements in the promoter region of the fibrinogen gene.[10,33] In a recent study, the administration of triiodothyronine resulted in a rapid modulation of fibrinogen gene, thus indicating that thyroid hormones have immediate effects on the synthesis of fibrinogen.[10] Variations in circulating thyroid hormone levels can also alter fibrin clot structure and lysis.[36] Hypothyroidism has been associated with less compact fibrin networks, enhanced fibrinolysis and low fibrinogen levels; whereas hyperthyroidism has been associated with compact fibrin networks, resistance to fibrinolysis and high fibrinogen levels.[36,37]

Our study showed that VWF and fibrinogen partly mediate the association of FT_4 with cardiovascular disease. The observed proportion of mediation was 10%, which is quite considerable given the multiple mechanisms through which thyroid hormones affect cardiovascular health. We found no evidence for a mediating role of ADAMTS13, which could indicate a lack of mediation by ADAMTS13. Besides VWF and fibrinogen, other coagulation factors including factors VII, VIII, IX, X, XII, can also play a mediating role.[19,28,38,39] Unfortunately, data on these factors were not available in our study. If all relevant coagulation factors could be taken into account in our mediation analyses, the expected proportion of mediation related to coagulation may have been even higher than what we observed in our study.

Based on the negative feedback mechanism, the production of FT_4 is tightly regulated by the hypothalamic-pituitary-thyroid axis, with a unique set point for each individual.[40] Our study consistently found an association of FT_4 with coagulation factors. Though not statistically significant, the association of TSH with coagulation factors was generally in the expected opposite direction of FT_4. Other population-based studies among middle-aged and older adults have also reported that FT_4, rather than TSH, is associated with an increased risk of clinical outcomes.[2,4] This may be attributable to the aging process, which reduces the sensitivity of the pituitary gland to thyroid hormones.[41] In order to maintain the same FT_4 levels, older subjects (such as the Rotterdam Study participants) may need different TSH levels compared with younger subjects.

To our knowledge, this is the first population-based cohort study investigating the association of thyroid function with ADAMTS13, and the largest study investigating the association of thyroid function with VWF and fibrinogen. Moreover, this is the first study investigating the potential role of several coagulation factors in mediating the association between thyroid function and cardiovascular disease. Other major strengths are the long term follow-up (maximum follow-up time of almost 15 years), the comprehensive adjudication of events, and the extensive information on potential confounders. Multiple sensitivity analyses provided consistent findings.

Several limitations should also be mentioned. Thyroid function and coagulation measurements were performed at the same time, and we had no information on the temporal relationship of the association. Nevertheless, current evidence supports an effect of thyroid hormones on coagulation rather than vice-versa.[19] Furthermore, we had no data available on serum triiodothyronine levels. TSH and FT_4, however, represent the most commonly used measurements in clinical practice. Though we adjusted our analyses for multiple potential confounders, we cannot exclude the possibility of residual or unmeasured confounding. Lastly, the majority of our participants were white middle-aged and older adults. Therefore, the generalizability of our findings to other populations remains unclear.

Conclusions

Among middle-aged and older adults, high and high-normal FT_4 levels were associated with a procoagulant state. VWF and fibrinogen partially explained the association of FT_4 with cardiovascular disease. The potential mediating role of additional coagulation factors needs to be further explored. Future strategies against cardiovascular diseases might need to evaluate the potential predictive value of measuring coagulation factors, in addition to thyroid function.

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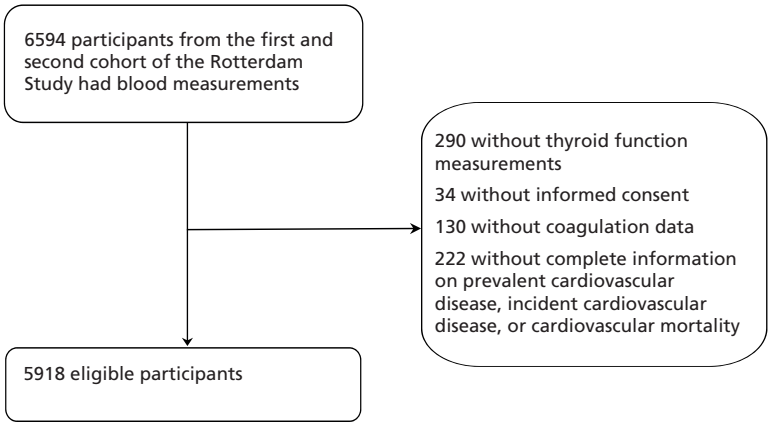
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Flow chart for the selection of study participants.



This study included participants from the third visit of the first cohort (RS I.3) and the first visit of the second cohort (RS II.1) of the Rotterdam Study. vWF:Ag, von Willebrand factor antigen; ADAMTS13 indicates a disintegrin and metalloprotease with thrombospondin motif repeats 13.

Supplemental Table 1. Association between thyroid function and coagulation factors

	All participants (TN 5918)		Euthyroid participants* (TN 4646)	
	β (95% CI) Model 1	β (95% CI) Model 2	β (95% CI) Model 1	β (95% CI) Model 2
VWF:Ag				
TSH	-1.10 (-2.79; 0.58)	**-2.00 (-3.60;-0.40)**	0.09 (-3.20; 3.38)	-1.13 (-4.32; 2.06)
FT_4	**16.8 (9.03; 24.6)**	**20.0 (12.6; 27.5)**	**17.3 (7.22; 27.4)**	**19.8 (10.1; 29.3)**
ADAMTS13 activity				
TSH	0.31 (-0.18; 0.81)	-0.01 (-0.50; 0.48)	**1.09 (0.10; 2.10)**	0.52 (-0.47; 1.51)
FT_4	**-5.18 (-7.49;-2.88)**	**-3.81 (-6.09;-1.53)**	**-7.45 (-10.5;-4.37)**	**-5.88 (-8.91;-2.85)**
Fibrinogen				
TSH	**-0.03 (-0.06;-0.01)**	-0.02 (-0.04; 0.01)	-0.03 (-0.09; 0.02)	-0.01 (-0.06; 0.05)
FT_4	**0.32 (0.20; 0.45)**	**0.24 (0.12; 0.37)**	**0.32 (0.15; 0.49)**	**0.23 (0.07; 0.40)**

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. βs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). βs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). *Normal reference ranges of thyroid function were defined as serum TSH of 0.4 to 4.0 mIU/L and FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with personal history of thyroid disease. Abbreviations: TN, total number; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; VWF:Ag, von Willebrand factor antigen; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13.

Supplemental Table 2. Sensitivity analyses for the association between thyroid function and Z scores of coagulation factors (TN 5918)

	β (95% CI) Model 1	β (95% CI) Model 1a	β (95% CI) Model 1b	β (95% CI) Model 1c	β (95% CI) Model 1d
VWF:Ag					
TSH	-0.02 (-0.05; 0.01)	-0.02 (-0.05; 0.00)	-0.02 (-0.05; 0.01)	-0.02 (-0.05; 0.01)	-0.02 (-0.05; 0.00)
FT_4	**0.29 (0.16; 0.42)**	**0.30 (0.16; 0.43)**	**0.29 (0.16; 0.42)**	**0.28 (0.15; 0.42)**	**0.29 (0.16; 0.43)**
ADAMTS13 activity					
TSH	0.02 (-0.01; 0.05)	0.02 (-0.01; 0.04)	0.02 (-0.01; 0.05)	0.02 (-0.01; 0.04)	0.02 (-0.01; 0.04)
FT_4	**-0.29 (-0.42;-0.16)**	**-0.30 (-0.42;-0.17)**	**-0.29 (-0.42;-0.16)**	**-0.30 (-0.43;-0.17)**	**-0.30 (-0.44;-0.17)**
Fibrinogen					
TSH	**-0.03 (-0.06;-0.01)**	**-0.04 (-0.07;-0.02)**	**-0.03 (-0.06;-0.01)**	-0.03 (-0.06; 0.00)	**-0.04 (-0.07;-0.01)**
FT_4	**0.34 (0.21; 0.48)**	**0.37 (0.26; 0.48)**	**0.35 (0.21; 0.48)**	**0.34 (0.21; 0.48)**	**0.38 (0.25; 0.52)**

Model 1: age, sex, cohort, smoking, and alcohol intake. Model 1a: Model 1, C-reactive protein; Model 1b: Model 1, prevalent cardiovascular disease; Model 1c: Model 1, thyroid peroxidase antibody positivity (cutoff 35 kU/ml); Model 1d: Model 1, after excluding participants with prevalent cancer (n=289). βs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). βs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: TN, total number; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; VWF:Ag, von Willebrand factor antigen; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13.

Supplemental Table 3. Association between TPOAb positivity and Z scores of coagulation factors (TN 5918)

	β (95% CI) Model 1	**β (95% CI) Model 2**
VWF:Ag	-0.05 (-0.12; 0.03)	-0.01 (-0.08; 0.06)
ADAMTS13 activity	-0.03 (-0.10; 0.04)	-0.02 (-0.10; 0.05)
Fibrinogen	-0.01 (-0.09; 0.06)	-0.03 (-0.10; 0.04)

Model 1: age, sex, cohort, and lnTSH. Model 2: Model 1, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. Abbreviations: TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml); VWF:Ag, von Willebrand factor antigen; TSH, thyroid-stimulating hormone; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13.

Supplemental Table 4. Association of thyroid function (Z scores) and coagulation factors (Z scores) with incident cardiovascular events and deaths

	CV events* (857/5227) HR (95% CI)	**CV deaths‡ (690/5918) HR (95% CI)**
Thyroid function (Z scores)		
TSH	0.96 (0.90; 1.03)	0.96 (0.88; 1.03)
FT_4	**1.14 (1.07; 1.21)**	**1.16 (1.08; 1.23)**
Coagulation factors (Z scores)†		
VWF:Ag	**1.08 (1.01; 1.15)**	**1.16 (1.09; 1.24)**
ADAMTS13	**0.92 (0.86; 0.99)**	**0.89 (0.82; 0.97)**
Fibrinogen	**1.09 (1.02; 1.17)**	**1.23 (1.15; 1.31)**

Adjusted for age, sex, cohort, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. HRs of TSH are denoted per 1 sd increase of natural log transformed TSH. HRs of FT_4 are denoted per 1 sd increase in FT_4. *Participants with prevalent cardiovascular events were excluded from the analysis. †Additionally adjusted for TSH and FT_4. ‡Additionally adjusted for prevalent cardiovascular disease. Abbreviations: CV, cardiovascular; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; VWF:Ag, von Willebrand factor antigen; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13.